# Climate Smart Brachiaria Grasses for Improving Livestock Production in East Africa – Kenya Experience

**Proceedings of the workshop held in Naivasha, Kenya, 14 - 15 September, 2016**


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## Foreword

The agricultural sector is the main driver of Kenya's economy and livelihood for the majority of Kenyans. The sector accounts for 65% of Kenya's total exports and provides more than 70% of informal employment in the rural areas. Livestock contributes about 30% of the agricultural gross domestic product. One of the research agenda of Kenya Agricultural and Livestock Research Organization (KALRO) is development of improved technologies for increased agricultural production. Therefore as the country focuses on poverty alleviation, employment creation and food security in the rural areas, improved livestock technologies contributes towards this goal. Improvement of livestock productivity through access to high quality feeds can significantly contribute to the Kenya's Vision 2030 through 'equity and wealth creation opportunities for the rural poor.'

On the anticipation that Brachiaria grasses can be integrated into the agricultural systems to address increased livestock productivity, a collaborative research project was initiated between KALRO and Biosciences eastern and central Africa - International Livestock Research Institute (BecA-ILRI) Hub in 2012. Laboratory and field research were conducted simultaneously. The objective of the laboratory studies was to identify role of endophytes and associated microbes in enhancing adaptation of Brachiaria grass cultivars to drought and low fertility soils. The field research focused on the integration of the grasses into smallholder mixed crop-livestock system and assessing their contribution to milk and meat production.

Studies were conducted in Kwale and Kilifi Counties in coastal lowlands, Machakos and Makueni Counties in mid-altitude eastern region, Nyandarua, Nyeri and Embu Counties in central highlands and in Trans-Nzoia, Uasin Gishu, Elgeyo Marakwet and Busia Counties in western Kenya.

At the end of the project, a workshop was held which brought together researchers from diverse backgrounds, from KALRO and BecA-ILRI Hub. The research findings presented in the workshop are important in; (i) elucidating roles of endophytes in enhancing adaptation of Brachiaria grasses to drought and low fertility soils, (ii) demonstrating the role of Brachiaria in enhancing livestock productivity in terms of milk and meat, (iii) assessment of contribution of the Brachiaria to soil health and mitigation of climate change, and (iv) evaluating the potential of Brachiaria seed production in Kenya.

The information presented in these proceedings will benefit farmers, researchers and development partners in Kenya and East Africa. This document will have an instrumental role in promoting forage research and development programs in the region for sustainable development of the livestock sector.

**E. K. Kireger (PhD)**
Director General, KALRO

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The Director General, Kenya Agricultural and Livestock Research Organization (KALRO) and Director, Biosciences eastern and central Africa - International Livestock Research Institute (BecA-ILRI) Hub are acknowledged for providing enabling environment during implementation of the project. The Deputy Director General, Livestock–KALRO is thanked for providing guidance during project implementation and organization of the workshop. The commitment of KALRO and BecA-ILRI Hub collaborating scientists in the preparation of papers and participation in the workshop is appreciated. We thank partners from Rwanda Agriculture Boards, International Centre for Tropical Agriculture, Colombia; Grasslanz Technology Limited and AgResearch of New Zealand for their contribution in the project. Special thanks also go to staff from BecA-ILRI Hub and KALRO institutes and centres that assisted in many ways to make the workshop successful. Farmers who participated in the implementation of field activities reported in the proceedings are highly appreciated. The editorial committee that organised and facilitated the review, editing and publishing of the proceedings is also gratefully acknowledged. Ms Ruth N. Mutua is thanked for typesetting and formatting the proceedings. The research outputs that culminated to the workshop and proceedings were from a collaborative project between KALRO and BecA-ILRI Hub with financial support of the Swedish International Development Cooperation Agency (Sida). The opinions expressed in the proceedings are those of the authors and do not necessarily reflect the views of Sida.

# Setting the Scene

# Overview of the climate smart Brachiaria grass programme

**J. G. Mureithi[1] and A. Djikeng[2]**

[1]KALRO-Nairobi, [2]BecA-ILRI Hub, Nairobi

This overview provides the rationale and genesis for conducting research on Brachiaria grasses for improving livestock productivity in Kenya.

**Importance of livestock in Kenya**

Livestock contributes about 30% of the agricultural gross domestic product (GDP) and up to 10% of Kenya's total GDP. About 40% of the total labour force in the agricultural sector is employed in livestock production. Over 70% of all the livestock in the country are found in the arid and semi-arid lands (ASALs) and the sub-sector employ 90% of population (7 million people) who live in these regions and contribute 95% of their income and livelihoods. In the humid and sub-humid areas where 80% of smallholder crop-livestock farmers are located, livestock are source of cash, provide milk, meat, manure and draught power in crop production.

In the smallholder mixed crop livestock system, Napier grass (*Pennisetum purpureum* Schum.), the most widely grown fodder for the cut-and-carry production system, is threatened Napier stunt and smut diseases. Rhodes grass (*Chloris gayana* L.), one of the cultivated pastures has a narrow genetic base and limited ecological adaptation. Crop residues principally maize stovers which are highly lignified form the bulk of livestock feed during the dry season. Their crude protein is generally low; below 7%, the minimum required for animal production and are not fortified with minerals or vitamins during feeding. In the extensive livestock production systems, common with the pastoral communities, within ASALs region, livestock subsist mainly on natural pastures that are of low quality and productivity decline rapidly during the dry season. Lack of adapted forages, frequent and prolonged drought and low rainfall are major factors contributing to inadequate quantity and quality of feeds. Increased population and opening of pastoral land for crop production and other non-agricultural uses has aggravated the situation on feed scarcity. In both mixed crop-livestock farming and pastoral systems, livestock are characterised by a low productivity.

The rising interest in livestock development fueled by increased demands of animal products has led to the demand for productive and high quality forages to bridge feed deficit. Consequently, there is need for research to develop forage options to increase livestock productivity in order to meet the growing demand for livestock products. A collaborative research project between Kenya Agricultural and Livestock Research Organization (KALRO) and Biosciences eastern and central Africa - International Livestock Research Institute (BecA-ILRI) Hub was initiated in 2012 with financial support from the Swedish International Development Cooperation Agency (Sida). The aim was to explore superior feeds resources for increasing animal productivity and for generation of income from smallholder farmers through the use of Brachiaria grass.

## Research on Climate smart Brachiaria grass

Interest on Brachiaria research was spurred by the exceptional performance of livestock production on Brachiaria pasture in South America. Millions of hectares of Brachiaria species have been sown as improved pastures in South and Central America with estimated acreage of 99 million hectares in Brazil alone (Jank *et al*., 2014), supporting a highly vibrant beef industry.

Grasses in the genus Brachiaria have advantage over those in other genera including adaptation to drought and low fertility soils, ability to sequester carbon; increase nitrogen use efficiency through biological nitrification inhibition (BNI) and arrest greenhouse gas emissions. The genus Brachiaria consists of about 100 species distributed across tropical and sub-tropical region (Renvoize *at al*., 1996). Africa is the centre of origin of Brachiaria grasses and are thus adaptable in Kenya and can be well integrated in the existing farming systems. Despite the immense benefits demonstrated of these grasses in South America, the potential of improved Brachiaria grass in Kenya to address the challenge of livestock feed scarcity remain unexploited.

The climate smart Brachiaria programme is a larger initiative of BecA-ILRI Hub in partnership with KALRO, Rwanda Agriculture Board, International Centre for Tropical Agriculture, of Colombia, Grasslanz Technology Limited and AgResearch of New Zealand that focus on integrating improved Brachiaria grass into smallholder mixed crop-livestock systems for increasing livestock productivity (milk and meat) and seed production in East Africa. The objectives of the BecA-ILRI Hub - KALRO component of the program were to; (a) investigate the role of endophytes and plant associated microbes in enhancing adaptation to drought and low fertility soils, (b) increase the production of milk by 30% and/or meat by 20% in integrated smallholder crop-livestock systems while improving soil quality and (c) develop seed production systems for increased availability of seeds and generation of income.

Laboratory and greenhouse experiments on endophytes were conducted at BecA-ILRI Hub, and field evaluations of the grasses in four regions with distinct agro-ecological zones; coastal lowlands (Kwale and Kilifi Counties), mid-altitude eastern region (Machakos and Makueni), central highlands (Nyandarua Nyeri and Embu) and western (Trans-Nzoia, Uasin Gishu, Elgeyo Marakwet and Busia) Kenya. The project ended in 2016 culminating in a two days' end of project workshop whose proceedings are reported.

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# Biophysical environment and farming systems of selected regions for integrating Brachiaria grasses in Kenya

**D. M. G. Njarui, E. M. Gichangi and M. Gatheru**

KARLO – Katumani,

**Abstract**

The focus of this paper is to provide background information on the biophysical environment and farming systems of four regions where research on Brachiaria grasses was conducted in Kenya. The project, 'Climate-smart Brachiaria grasses for improving livestock production in East Africa' aim was to alleviate feed shortage and increase animal productivity and generate income in small-holder farms. The regions targeted are located in diverse agro-ecological zones and include; coastal lowlands, mid-altitude eastern region, central highlands and north western highlands. The altitude ranges from sea level in the coastal lowlands to 4000 m above sea level in the central and north western highlands. Annual rainfall ranges from 700 mm in the mid-altitude eastern region 2200 mm in the highlands. Soils vary from shallow sandy clay of low fertility in the coastal lowlands and mid-attitude eastern region to very deep high fertility Andosols and Nitosols in the highlands. The smallholder farmers, who account for 80% of the farming community, predominantly practice mixed crop-livestock farming, with livestock and crop production being an integral component of the system. Natural and cultivated pastures are the main source of livestock feed while crop residues principally maize stover form the bulk of livestock feed during the dry season. Seasonal feed scarcity is widespread and has been reported in 79 - 99% of farms. This paper sets the scene for subsequent papers that describe in details the specific sites where the on-station and on farm research was conducted using participatory approach.

**Key words**: Agro-ecological zones, Brachiaria cultivars, climate, livestock feeds, soil types

## Introduction

The total area of Kenya is approximately 582, 646 km$^2$ of which 11, 230 km$^2$ or about 1.9% is covered by water. Based on moisture index the country can be divided into seven agro-ecological zones (AEZ) (Sombroek *et al.,* 1982) (Figure 1). Annual rainfall is variable in the different zones and ranges from <200 mm in the very arid zone to >2500 mm in the humid zones. Annual evaporation exceeds the amount of rainfall in all the zones except in the humid zone where rainfall is higher than evaporation. Annual evapotranspiration is higher in the very arid zones (2100 - 2500 mm) than in the humid zone (1200 – 2000 mm) (KARI, 2001). The altitude ranges from sea level in the eastern side to over 5000 m above sea level with the highest altitude in Mount Kenya.

The arid and semi-arid lands (ASAL) constitute 83% of the total land area while the rest is made up of the humid and sub-humid zones. The humid zones are mainly located in the highlands east and west of Rift Valley while the sub-humid comprises the Lake Victoria region, coastal lowlands and part of western Kenya. Due to high rainfall, (1000-2700 mm) the humid and sub-humid zones are regarded as medium to high potential for crop production. Dairy cattle and other livestock farming account for utilization of 30% of the land in the humid and sub-humid zones (KARI, 2001). Rainfall in the ASAL is low (annual rainfall, 150 - 900 mm) and erratic with a large part of the year being dry. Consequently, these areas have marginal to low potential for crop production. Nevertheless, livestock thrive in these zones and are important source of livelihoods. In 2009 census, the total population of ruminants (cattle, sheep, goats, camels and donkeys) stood at about 67 million (KNBS, 2010) with about 60% of all these livestock being in the ASALs. The sub-sector utilises about 81% of the ASALs, employs 90% of the 7 million inhabitants and contributes 95% of the family income and livelihood security. About 80% of the farming communities are smallholders, predominantly practising mixed crop-livestock farming, with livestock and crop production being an integral component of the sustainable system. The farming communities selected in the regions represented the mixed crop-livestock production system in Kenya. Livestock production is constrained by inadequate quantity and quality of feeds and thus integrating of Brachiaria grasses is likely to bridge this gap, improve ruminant livestock productivity and livelihoods. The focus of this paper is to provide background information on biophysical environment and farming systems of four regions where research on Brachiaria grasses was conducted. The regions targeted for integrating Brachiaria grass cultivars were; the coastal lowlands, mid-altitude eastern region, central highlands and north western highlands of Kenya (Figure 2).

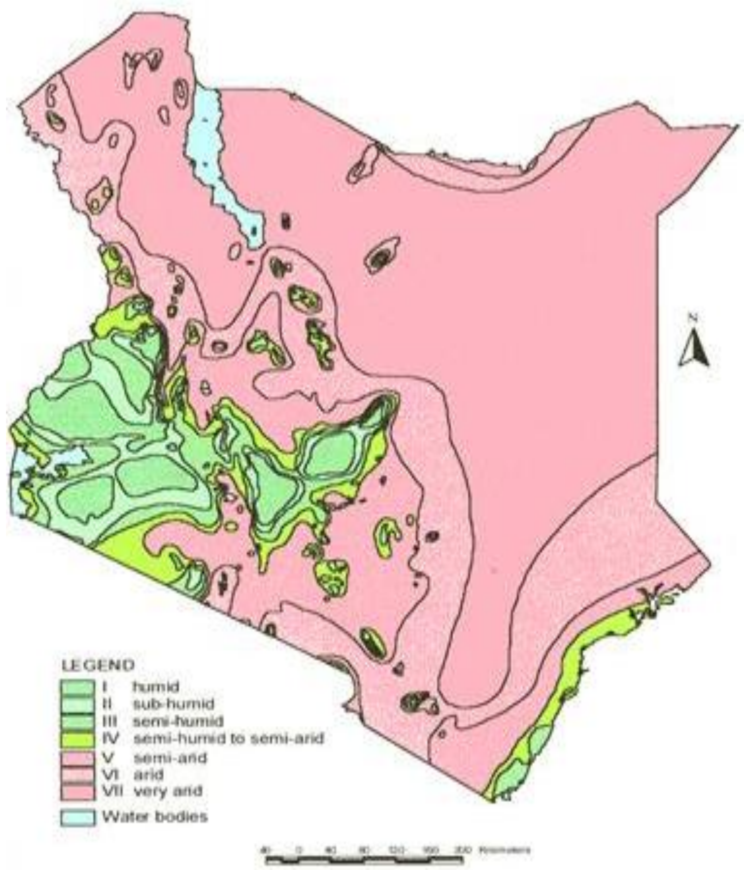


**Figure 1** Map of Kenya showing the agro-ecological zones

*Geographical location on selected region for Brachiaria research*

In the coastal lowlands research was implemented between latitudes 03°22′S to 03°40′S and stretched from longitudes 39°48′E and 39°12′E, in Malindi, Kilifi and Kwale Counties, in CL3, 4 and 5 AEZ. The mid-altitude eastern region stretches from latitudes 0°45′S to 1°35′S and longitudes 36°45′E and 38°30′E within Machakos and Makueni Counties, in Upper Midland (UM) 3 and Lower Midland (LM) 4 AEZ. In the central highlands, it covered areas between latitudes 0°8′S and 0°50′S and 35° 13′E and 36° 42′ E in Nyandarua County mainly in Upper Highland (UH) 2 and 3 and Lower Highland (LH) 3 and 4 AEZ. In north western highlands, the study concentrated on the area between latitudes 0°7′N to 0°20′N and longitudes 34°05′E and 35°59′E.

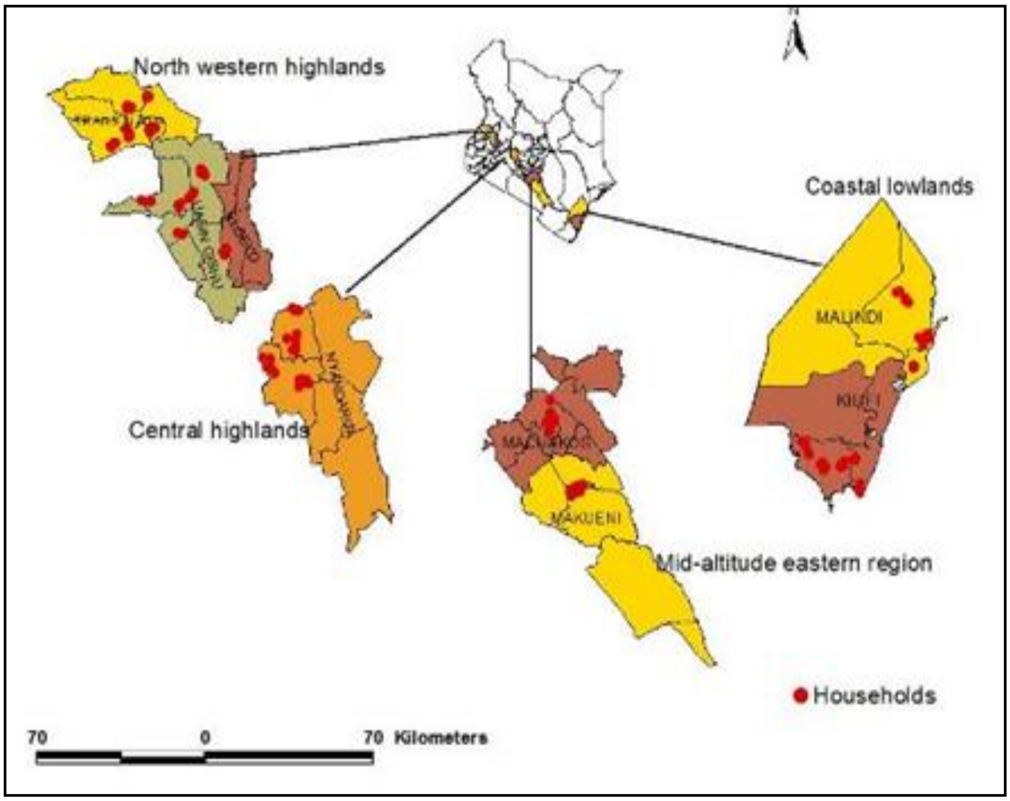


**Figure 2** Map showing the coastal lowlands, mid-altitude eastern, central highlands and north western highlands of Kenya

## Biophysical characteristics

*Coastal lowlands*

The coastal lowlands (CL), that extends for about 30 km into the hinterland from sea is divided into five AEZ; CL2, CL3, CL4, CL5 and CL6 based on rainfall (Jaetzold *et al*., 2006). The annual rainfall ranges from 700 mm in CL6 to 1200 mm in CL3, with a bimodal pattern, the long rains season occurring from April to June and the short rains from October to December (Figure 3).

Potential evapo-transpiration ranges from 1900 to 2300 mm per annum and exceeds annual precipitation, thus resulting in water deficit. Mean annual temperature ranges from 22 to 35$^{o}$C and relative humidity from 70 to 90%.

The topography is low lying, from the sea level on the eastern side, rising to 300 m asl toward the hinterland on the western side. The Coastal plain, the foot plateau to the north, the Coastal Range and the Nyika Plateau are some of the key physical features dotting the region. Major soils include Ferralsols which are sandy clay, strongly weathered and red brown in colour, and Cambisols, which are shallow to moderately deep. They are deficient in nitrogen (N); phosphorus (P) and potassium (K) with soil P being below the critical level of 20 ppm (Njarui and Mureithi, 2004).

*Mid-altitude eastern region*

Typically the mid-altitude eastern region is semi-arid and the mean annual rainfall is around 700 mm but in the hill masses it increases to about 1050 mm in the hill masses. The rainfall is bimodal with two distinct rainy seasons; the long rains occurring from March to May and short rains from October to December (Figure 3). Annual evapo-transpiration exceeds the amount of rainfall and ranges from 1200 to 1800 mm (KARI, 2001). Minimum mean annual temperature vary from 14 to 22$^{o}$C while maximum mean annual temperature vary from 26 to 34$^{o}$C.

The region is characterized by low to medium altitude, rising from 800 to1800 m asl. The soils are derived from the pre-Cambrian 'basement-complex' rocks consisting of mainly granites, gneisses and sometimes schist's sandstones or phyllitic shales (Simpson *et al.,* 1996). They are often shallow and contain low organic matter and high sand content (Kusewa and Guiragossian, 1989). The predominant soils are Luvisols, Acrisols and Ferralsols. Numerous other soil types occur but are of less significance in terms of the agricultural area they occupy.

*Central highlands*

The central highlands also receive bi-modal rainfall, the long rains occurring from March to May with a maximum annual rainfall of 1600 mm and the short rains from September to December with annual rainfall of 700 mm (Figure 3). Temperature ranges from as low as 2°C to a maximum of 25°C. Night frosts are common in AEZ of Afro-Alpine highlands and Upper Highlands (Jaetzold *et al.,* 2006). The main physical features is the Aberdare ranges which consist mainly of the Kinangop Plateau, Ol joro Orok Plateau and the Ol Kalou Salient which have slopes that are interrupted by low undulating hills. In most parts, the soils are of volcanic deposits, mainly basaltic lava are dominant. Major soils are the Nitosols, Andosols and Phaeozems (Jaetzold *et al*., 2006) which are highly fertile.

*North western highlands*

The north western highlands experience primarily uni-modal rainfall distribution, which starts in April and continues through to October/November with peak in May and August (Figure 3). Rainfall ranges from 1200 to 2200 mm and the average annual temperature from 14 to 28$^{o}$C. The elevations range from about 900 m  asl in the Kerio valley to 2700 m asl in the cool highlands of Elgeyo escarpment and Cherangani hills with some higher ridges of up to 3365 m asl on the eastern part. On the western boundary the extinct volcano, Mt. Elgon is an outstanding landmark which rises to 4000 m asl. Apart from the volcanic rocks of the Mt. Elgon area, most of the regions are underlain by acid to intermediate rocks of the basement system. The major soils are Humic Ferralsols in Trans Nzoia, Ferralic Cambisols and Orthic Ferralsols in Uasin Gishu (FAO-UNESCO, 1994). There are pockets of Acrisols which are not widely distributed. These soils have low fertility with a weak to moderate sub-angular block structure and are well drained with high moisture storage capacity.

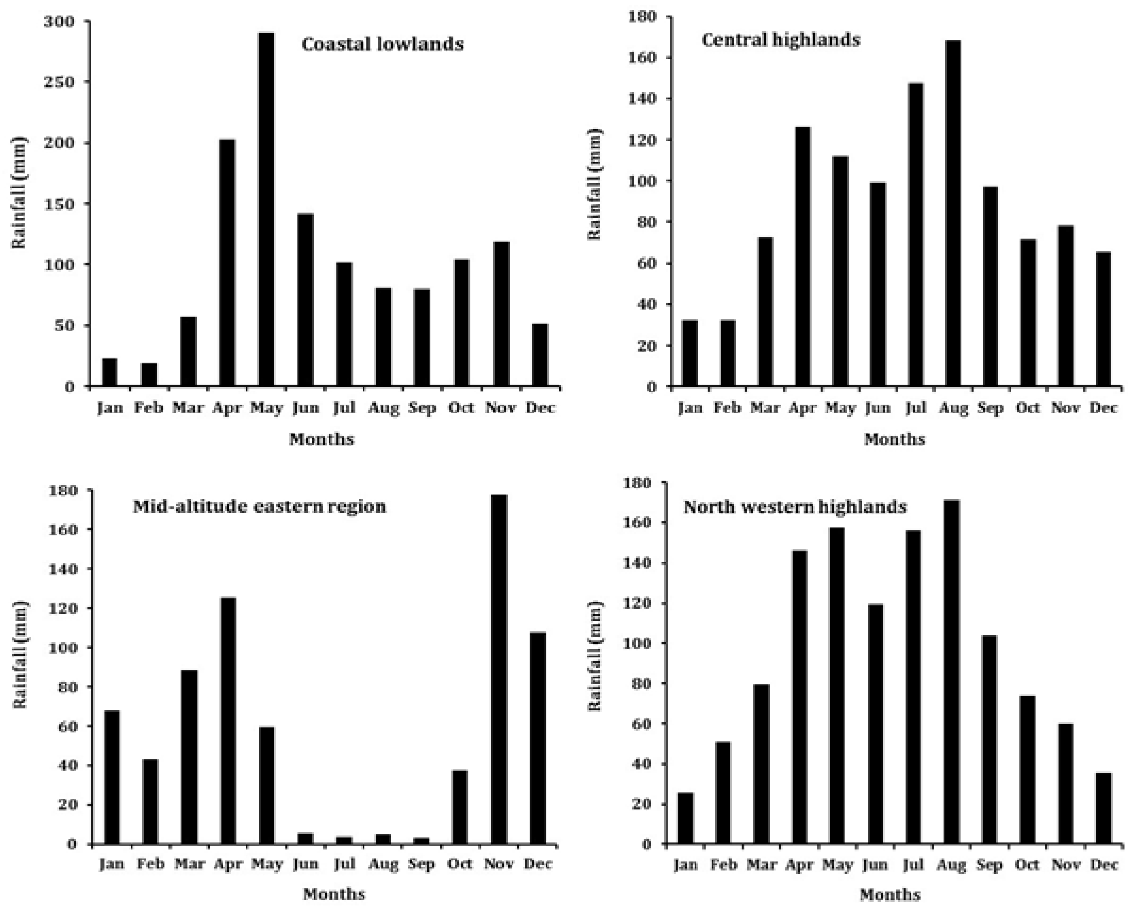


**Figure 3** Rainfall in coastal lowlands, mid-altitude, central and north western highlands

## Farming system

*Coastal lowlands*

A description of farming system of the coastal lowlands has been given in details by Njarui and Mureithi (2004). The rural households engage in diverse agricultural activities which include crops and livestock farming. Maize (*Zea mays* L.) and cassava (*Manihot esculenta* Crantz.) are the main staple foods followed by cowpea (*Vigna unguiculata* [L.] Walp). They are almost always cultivated as intercrops under trees cash crops of coconuts (*Cocos nucifera* L.), cashew nuts

(*Anacardium occidentale* L.) and horticultural crops such as mango ((*Mangifera indica* L.) and citrus (*Citrus* spp.) as reported by Njarui and Mureithi (2004). Crop yields are generally low due to low soil fertility and poor crop husbandry. For example, maize yield is less than 1 t/ha (Wekesa *et al*., 2003) compared with over 5 t/ha achieved from research (Mwamachi *et al*., 2005) and the situation has remained relatively the same. Livestock kept includes cattle, goats, sheep and poultry. Most of the cattle are the local zebus (*Bos indicus*) and are kept as a source of cash and security against crop failure.

*Mid –altitude eastern region*

The farming systems are characterized by mixed crop-livestock production. Maize is the most important cereal and is commonly intercropped with beans (*Phaseolus vulgaris* L.), cowpea and pigeon pea (*Cajanus cajan* L.). Horticultural fruit trees such as citrus, mango, avocado (*Persea Americana* Mill.) and pawpaw (*Carica papaya* L.) are important source of cash. Maize, beans and cowpea are planted in both long and short rains while pigeon pea is planted only during the short rains. The yields are generally low because of low soil fertility, limited use of fertilizer and lack of adoption of recommended agronomic practices. Due to erratic and poor rainfall, crop failure usually occurs in 7 out of 10 seasons. Major livestock kept include cattle, sheep and goats. A large proportion of cattle kept are the Zebu (*Bos indicus*) mainly under extensive grazing system while in the wetter hill masses adoption of European breeds (*Bos Taurus*) of cattle is high.

*Central highlands*

Farming is highly commercialized and is predominantly crop-livestock farming system. The main crops grown in Nyandarua County are wheat (*Triticum aestivum* L.), maize and vegetables which include potato (*Solanum tuberosum* L.), cabbage (*Brassica oleracea* L.), peas (*Pisum sativum* L.) and carrot (*Daucus carota* L.). These crops are not exclusively meant for subsistence as they also provide significant income for most of the households. Nevertheless, pyrethrum (*Chrysanthemum cinerariifolium* [Trevir.] Vis.), cut flowers and horticulture are the main cash crops grown in the County. Livestock farming is a major activity and the main animals reared are exotic European breeds and their crosses for milk production. Goats, sheep, rabbits and poultry are also important in the region.

*North western highlands*

Economic activities in the north western highlands are characterized by mixed crop-livestock farming system. The agricultural sector varies widely from predominantly small scale (<10 ha) with low external inputs to highly mechanized large scale (>20 ha) farming with high levels of external inputs. However, small scale farmers account for more than 80% of the farming community and the number is increasing due to sub-division of large scale farms. The land is highly fertile and ideal for maize, wheat and barley (*Hordeum vulgare* L.) production and due to high yield the region is regarded as the grain basket for the country. Horticultural crops grown include tomato (*Solanum lycopersicum* L.), mangoes and vegetables. The major cattle production system is the open grazing system where dairy cows are kept for commercial purposes. Zero grazing is also practiced in the small scale farms. In some farms, beef cattle are also kept for local market.

**Livestock production**

Livestock play an important role in the livelihoods of rural households in all the regions. They are important source of food, milk, manure, draft power for crop production and are sold to for cash. They also provide investment, employment, and risk reduction opportunities. In semi-arid region animals represent a "saving account", providing an economic security against frequent crop failure (Muhammad, 1996). The major ruminant livestock kept include, cattle, sheep and goats. The cattle comprise of the local Zebu, European breeds mainly the Friesian, Ayrshire, Jersey, Guernsey and their crosses with local Zebu. Dairy cattle are not popular in the coastal lowlands compared with Zebu cattle because they are more susceptible to tsetse flies and tick-borne disease (Maloo *et al*., 1994; MoLD, 2012). A recent study in coastal lowlands indicates that about 71.3% of the respondents owned Zebu compared with 34.3% who owned dairy cattle (Njarui *et al*., 2016). The exotic dairy cattle are widely kept in the other regions because of the high demand for milk and favourable milk prices. The small ruminants, sheep and goats are widely kept in all the regions because they are resilience to drought and can easily be sold off to provide cash in the event of crop failure. Livestock are either kept under stall feeding/zero-grazing or grazing but the combination of the two systems is common. Grazing is more common in the coastal lowlands where farmers relatively large number of local zebu and do not have sown pastures.

The dairy cattle are milked manually under the smallholder system usually twice per day; in the morning and afternoon. There are differences in milk production among the different region. Average daily milk production was reported to be about 11.8 litres/cow in central highlands (Muia *et al*., 2011), 6 - 9 litres/cow/day in mid-altitude eastern region (Mungube et al., 2014) and 7.8 litres/cow/day in coast lowlands (Njarui *et al*., 2016). Low milk production has been attributed to poor nutrition and lack of supplementation with high protein feeds (Lanyasunya *et al*., 2006; Lukuyu *et al*., 2011). The Zebu also provide milk for household consumption but normally produces comparatively less milk (<4 litres/cow/day) than the dairy cattle. There are several marketing channels for milk which include catering services located in the urban centres, neghbouring households and well established milk processing factories while beef cattle are sold in local markets.

**Livestock feed resources**

Farmers normally depend on natural pastures, cultivated forages and crop residues from cereals and grain legumes after grain harvest (Figure 4). However in the coast lowlands the proportion of farmers with sown forages is low and livestock depend on natural pastures. Over 90% of farmers in mid-altitude region, central highlands and north western highlands grow Napier grass compared with 145 in coastal lowlands (Njarui *et al*., 2016). Napier grass is the most important forage crop for cut-and-carry feeding where animals are confined in stalls under zero-grazing system. It is preferred because it is high yielding and larger amount of herbage can be obtained from a relatively small area. However, the survival of Napier grass in central highlands is threatened by emergence of smut disease. The disease has been reported to cause yield loss of 25-46% (Mwendia *et al*., 2006). Browse legume species such as Sesbania (*Sesbania sesban* L.), Leucaena (*Leucaena leucocephala* Lam.), Calliandra (*Calliandra calothyrus* Meissn.) and Gliricidia (*Gliricidia sepium* [Jacq.] Steud.) are planted but generally only a few trees are found in the farm. Farmers purchase small quantities of commercial concentrate and mineral supplements, particularly for high yielding dairy cattle. Kikuyu grass (*Pennisetum clandistenum* Hochst.) is an important natural pasture for grazing in central highlands. Generally there is overgrazing and poor management of pastures which lead to low productivity. During the dry season, the grasses are deficient in minerals, crude protein (CP) and energy with CP dropping to less than 4% (Thairu and Tessema, 1987). On the other hand, the stovers are usually high in roughage, but low in nutritive value (3-5% CP) and do not provide adequate nutrients required for optimal production (Njarui *et al*., 2011).

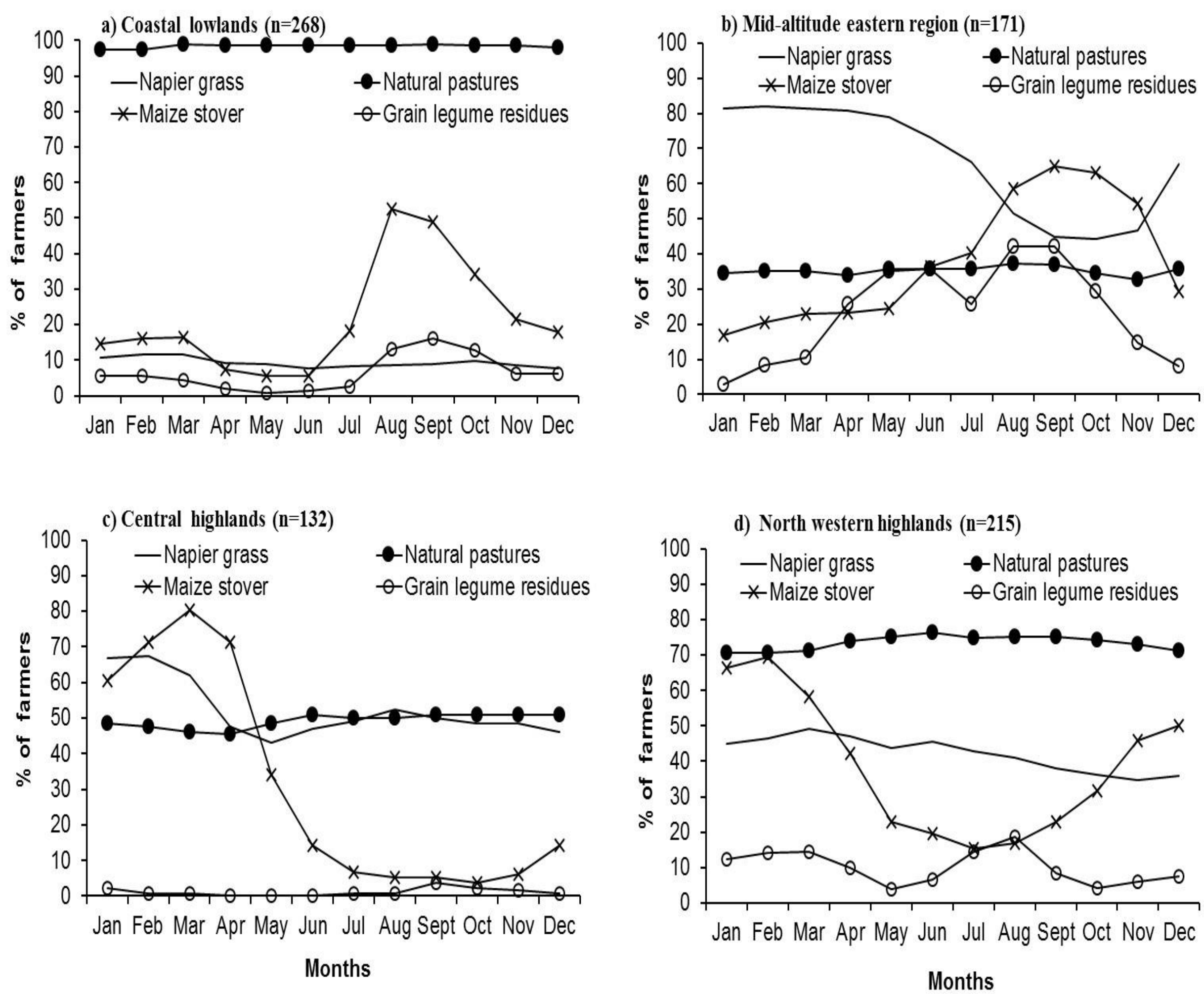


**Figure 3** Main type of feeds used for feeding livestock by months across four regions (Source: Njarui *et al*., 2016)

Seasonal fluctuation in feed availability is widespread with low feed availability being felt during the dry season across all the regions. The low and erratic rainfall and frequent drought has been attributed to seasonal shortfall of feed resources. Feed availability follow the rainfall pattern closely with relatively adequate feed reported during the wet season. In most of the regions, feed shortage occurs from January to May while in mid-altitude eastern region it is in July to October. The proportion of farmers who experience feed shortage is relatively high (79-99%) in this region (Njarui *et al*., 2016). Low availability of feed has a direct effect on performance of livestock productivity (meat or milk).

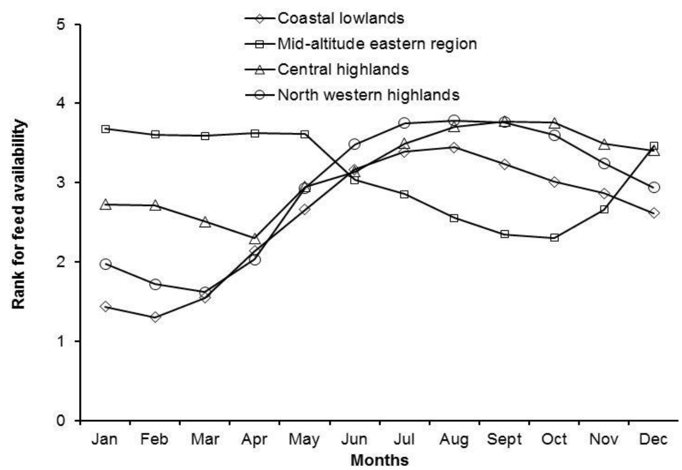


**Figure 4** Level of feeds availability by month (all type of feed considered); ≤3 inadequate and >3 adequate (Adapted from: Njarui *et al*., 2016).

**Conclusions**

The biophysical and farming systems characterisation presented in this paper show a large diversity across the regions where the study was conducted. While the coastal lowlands is low lying from sea level and rising to 300 m asl in the other regions have the altitude ranging from 800 m to above 4000 m asl in the mountain areas. Soils are sandy and shallow in the coast lowlands whereas soil the in the highlands are fertile and deep. In all the regions, farming system is complex and farmers keep different types of livestock and cultivate a variety of crops. Livestock generally depend on natural pastures, cultivated forages and crop residues Napier grass is the most widely cultivated fodder due to its high yield. However, livestock feed scarcity is widespread and is attributed to low and erratic rainfall and frequent drought. While in the coastal lowlands there is limited cultivation of forages farmers have adopted methods of improving their livestock feed base in the other regions.

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# Theme 1

# Genetic Diversity and Production

*Brachiaria brizantha* cv. Xaraes

# Genetic diversity of Brachiaria grass ecotypes in Kenya

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**Abstract**

Brachiaria grass is an emerging forage option for livestock production in Kenya. Kenya lies within the center of diversity for Brachiaria species therefore a high genetic diversity is expected in the natural populations of Brachiaria is expected. Overgrazing and clearing of natural vegetation for crop production and non-agricultural uses and climate change continue to threaten the natural bio-diversity. In this study we assembled 79 Brachiaria ecotypes collected from different parts of Kenya and examined their genetic variations along with eight commercial varieties. A total of 120 different alleles were detected by 22 markers in the 79 ecotypes. Markers were highly informative in differentiating ecotypes with average diversity and polymorphic information content of 0.623 and 0.583, respectively. Five sub-populations (ILRI, Kitui, Kisii, Alupe and Kiminini) differed in sample size, number of alleles, number of private alleles, diversity index and percentage polymorphic loci. The contribution of within the individual difference to total genetic variation of Kenyan ecotype population was 81%, and the fixation index ($F_{ST}$ =0.021) and number of migrant per generation (Nm=11.58) showed negligible genetic differentiation among the populations. The genetic distance was highest between Alupe and Kisii populations (0.510) and the lowest between ILRI and Kiminini populations (0.307). The unweighted NJ tree showed test ecotypes into three major clusters: ILRI ecotypes were present in all clusters; Kisii and Alupe ecotypes and improved varieties grouped in cluster I and II; and ecotypes form Kitui and Kiminini grouped in cluster I. This study confirms high genetic diversity in Kenyan ecotypes than eight commercial varieties (Basilisk, Humidicola, Llanero, Marandu, MG4, Mulato II, Piata and Xaraes) that represent three species and one three way cross hybrid Mulato II. There is a need for further collection of local ecotypes and perform morphological, agronomical and genetic characterizations to support Brachiaria grass breeding and conservation programs.

**Keywords**: AMOVA; breeding; fixation index; genetic conservation; private allele

## Introduction

Brachiaria grass is one of the most important tropical grasses distributed throughout the tropics especially in Africa (Renvoize *et al*., 1996). The genus Brachiaria consists of about 100 documented species of which seven perennial species of African origin have been used for pasture production in South America, Asia, South Pacific and Australia (Miles *et al*., 1996). It has high biomass production potential and produces nutritious herbage thus increase livestock productivity **(**Holmann *et al*., 2004**;** Jotee, 1988). Brachiaria is adapted to drought and low fertility soils, sequesters carbon through its large roots system, enhance nitrogen use efficiency and subsequently minimize eutrophication and greenhouse gas emissions (Subbarao *et al*., 2009; Arango *et al*., 2014; Moreta *et al*., 2014; Rao *et al*., 2014). Brachiaria plays important roles in soil erosion control and ecological restoration. Brachiaria species have been important component of sown pastures in humid low lands and savannas of tropical America with current estimated acreage of 99 million hectare in Brazil alone (Jank *et al*., 2014).

In Africa, the evaluations of Brachiaria species for pasture improvement started during 1950s. This research focused on *B. brizantha*, *B. decumbens*, *B. mutica* and *B. ruziziensis* for forage production, agronomy (establishment, drought, cutting intervals and fertilizers), compatibility with herbaceous and tree legumes, nutritive values and their benefit to ruminant production. These studies concluded broader adaptation of multiple Brachiaria species in different agro-ecological zones of Africa (Ndikumana and de Leeuw, 1996). However, these practices were not widespread because of ample communal pastures, limited realization on roles of sown pasture in the livestock production, subsistence animal farming and low government priority to pasture development. Recently, the mounting demand for livestock products in Africa has renewed interest of farmers, researchers, and development and government agencies on forages particularly to climate resilient forages like Brachiaria grass. Therefore, there have been multiple repatriations of Brachiaria grass to Africa as hybrids and improved landraces (Maas *et al*., 2015; Ghimire *et al*., 2015). These materials have shown positive performance in terms of improved forage availability and livestock productivity in Kenya and Rwanda. These results have revealed Brachiaria as an ideal forage option for the livestock farmers in East Africa.

Despite high popularity, the Brachiaria acreage in Africa is low, and only a few varieties that were developed for tropical Americas and Australia are cultivated. Some of these varieties have shown susceptibility to pests and diseases thus limiting future expansion of the Brachiaria grass in Africa. There is therefore a need for an Africa based Brachiaria improvement program to develop varieties that are tolerant to biotic and abiotic stresses for different production environments. The prerequisite for any crop improvement program is germplasm with broad genetic base. The best approach to increase genetic variations in apomictic species like Brachiaria is by tapping natural variations from the center of diversity. Since 1950s, multiple missions were undertaken in Africa to collect Brachiaria germplasm with current inventory of 987 accessions of 33 known Brachiaria species (Keller-Grein *et al*. 1996). Considering distribution of Brachiaria in Africa and size of the continent the number of samples available in collection is definitely non-exhaustive and warrant further collection efforts. However, the existence of these genetic resources in Africa is continuously threatened by overgrazing and clearing of vegetation for crop production and non-agricultural uses and adverse effect of climate change.

Kenya is located within region that represents a center of diversity for genus Brachiaria. Therefore, high natural variation is expected among Brachiaria populations in Kenya. The objectives of this study were to: i) assemble a collection of local Brachiaria ecotypes in Kenya, ii) assess genetic diversity using microsatellite markers and iii) examine genetic relationships of ecotypes with selected commercial cultivars. The study will broaden geographical coverage and/or genetic base of global Brachiaria collection and provide invaluable information for Brachiaria improvement and conservation programs.

## Materials and methods

*Source of plant materials*

Whole plant samples of 79 Brachiaria ecotypes were collected from five different parts of Kenya: Alupe, ILRI Campus, Kiminini, Kisii and Kitui in 2013 and 2014, and maintained in the field at forage research plots of International Livestock Research Institute (ILRI), Nairobi, Kenya. Seeds of *B. decumbens* cv. Basilisk, *B. brizantha* cvs. Marandú, Xaraes, Piatá, and MG4, *B. humidicola* cvs. Humidicola and Llanero were obtained from Marangatu Sementes, Brazil, whereas Mulato II seeds were purchased from Tropical Seeds, USA and planted at forage research plots at ILRI, Headquarters. About 4 week old leaves were harvested; freeze dried and stored at -80° prior to DNA extraction. Ecotypes from all location except ILRI Campus were collected jointly by Biosciences eastern and central and Africa-International Livestock Research Institute (BecA-ILRI) Hub and Kenya Agricultural and Livestock Research Organization (KALRO).

*Genomic DNA extraction*

The DNA was extracted using cetyl-trimethyl ammonium bromide (CTAB) (Doyle and Doyle, 1990) method with slight modifications. About 150 mg of the young leaves were cut into small pieces, ground in liquid nitrogen and added with 800 µl of 2% CTAB buffer. The suspension was transferred into a clean microfuge tubes and incubated at 65 °C for 30 minutes, followed by incubation at room temperature for 5 minutes and centrifuged at 3,500 rpm for 10 minutes. After centrifugation, 400 µl of supernatant was transferred into new microfuge tubes and 400 µl of chloroform iso-amyl alcohol (24:1) were added to each tube and mixed by inversion for 10 minutes. Tubes were spun at 3,500 rpm for 10 minutes, and aqueous phase was transferred to clean microfuge tubes, and 400 µl of chloroform iso-amyl alcohol (24:1) were added again to each tube and spun for 10 minutes at 1,100 rpm and the process was repeated twice. After the final centrifugation, the DNA was precipitated in 300µl of cold isopropanol (100%) and inverted about 50 times to facilitate the mixing and precipitation, and incubated overnight at -20°C.The following day the microfuge tubes were removed from the freezer, thawed and spun at 3,500 rpm at 4 °C for 20 minutes. The isopropanol was decanted and the genomic DNA pellet was air dried. The DNA pellet was rinsed with 300µl of 70% (w/v) ethanol and dissolved in 100µlof low salt TE buffer containing 3µl of 10 mg/ml of 1% RNase solution and incubated in water bath at 45°C for 90 minutes. DNA quality and quantity were checked in 0.8% agarose gel (w/v) and NanoDrop Spectrophotometer. The genomic DNA was adjusted to the final concentration of 20 ng/µl and stored at 4°C for PCR amplification.

*PCR Amplification and genotyping*

The genomic DNA was amplified using AccuPower®PCRPreMix (BIONEER Negative dye). A reaction volume of 10µl containing 0.4 µl MgCl2 (final concentration of 2mM MgCl2), 0.4 µl each of forward and reverse primers labeled with different fluorescent dyes: 6-FAM (blue), VIC (green), NED (black) and PET (red), 2 µl template DNA (20 ng/µl) and 6.8 µl of sterile distilled water was used for PCR amplification. A total of 22 SSR markers (Table 1) initially developed for *B. ruziziensis* with the proven transferability to other species were used in this study (Silva *et al*., 2013). The PCR conditions were, initial denaturation for 5 minutes at 94 °C followed by 35 cycles at 94 °C for 30 sec, 57 °C for 60 sec, 72 °C for 2 minutes and final extension at 72 °C for 10 minutes. The amplicons integrity was checked using agarose gel electrophoresis in 2% agarose gel (w/v) stained with 2.5 µl of GelRed solution. The agarose gel images were visualized under Ultra-Violet and the digital image was captured. The size of amplified fragments was estimated comparing with1kbDNAladder (Thermo Fisher Scientific). The SSR fragment sizes and allele variations in the repeats were assessed by capillary electrophoresis of amplicons and sequencing the amplified loci. The multiplexed PCR products were mixed with 8.87µlHi-Di-formamide and 0.135 µl Fluorescent-labeled GeneScan™ LIZ size standard (Applied Biosystems, USA) in a 96-well microtiter plate. The mixed products were denatured at 95°C for 3 minutes and snap-chilled on ice for 5 minutes to avoid the formation of double strand DNA. The products were loaded to Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, USA).

**Table 1** Microsatellite primers, primer sequences, annealing temperature (Ta), allele sizes and number of repeat motifs (adapted from Silva *et al*., 2013)

| Marker | Forward primer | Reverse primer | Ta (°C) | Allele size bp) | Repeat moti |
|---|---|---|---|---|---|
| Brz0012 | ACTCAAACAATCTCCAACACG | CCCACAAATGGTGAATGTAAC | 59 | 160 | $(AT)^{8}$ |
| Brz0028 | CATGGACAAGGAGAAGATTGA | TGGGAGTTAACATTAGTGTTTTT | 57 | 158 | $(TA)^{8}$ |
| Brz0029 | TTTGTGCCAAAGTCCAAATAG | TATTCCAGCTTCTTCTGCCTA | 56 | 150 | $(AG)^{14}$ |
| Brz0067 | TTAGATTCCTCAGGACATTGG | TCCTATATGCCGTCGTACTCA | 51 | 156 | $(AT)^{9}$ |
| Brz0076 | CCTAGAATGCGGAAGTAGTGA | TTACGTGTTCCTCGACTCAAC | 58 | 151 | $(AT)^{7}$ |
| Brz0087 | TTCCCCCACTACTCATCTCA | AACAGCACACCGTAGCAAGT | 60 | 243 | $(GA)^{9}$ |
| Brz0092 | TTGATCAGTGGGAGGTAGGA | TGAAACTTGTCCCTTTTTCG | 54 | 251 | $(AT)^{6}$ |
| Brz0100 | CCATCTGCAATTATTCAGGAAA | GTTCTTGGTGCTTGACCATT | 56 | 256 | $(AT)^{11}$ |
| Brz0115 | AATTCATGATCGGAGCACAT | TGAACAATGGCTTTGAATGA | 59 | 252 | $(AT)^{6}$ |
| Brz0117 | AGCTAAGGGGCTACTGTTGG | CGCGATCTCCAAAATGTAAT | 60 | 260 | $(TA)^{5}$ |
| Brz0118 | AGGAGGTCCAAATCACCAAT | CGTCAGCAATTCGTACCAC | 57 | 252 | $(CT)^{11}$ |
| Brz0122 | CATTGCTCCTCTCGCACTAT | CTGCAGTTAGCAGGTTGGTT | 57 | 253 | $(CA)^{6}$ |
| Brz0130 | TCCTTTCATGAACCCCTGTA | CATCGCACGCTTATATGACA | 57 | 248 | $(CT)^{14}$ |
| Brz0149 | GCAAGACCGCTGTTAGAGAA | CTAACATGGACACCGCTCTT | 57 | 245 | $(AT)^{11}$ |
| Brz0156 | GCCATGATGTTTCATTGGTT | TTTTGCACCTTTCATTGCTT | 58 | 260 | $(AC)^{7}$ |
| Brz0203 | CGCTTGAGAAGCTAGCAAGT | TAGCCTTTTGCATGGGTTAG | 57 | 301 | $(GA)^{8}$ |
| Brz0212 | ACTCATTTTCACACGCACAA | CGAAGAATTGCAGCAGAAGT | 57 | 301 | $(CA)^{5}$ |
| Brz0213 | TGAAGCCCTTTCTAAATGATG | GAACTAGGAAGCCATGGACA | 57 | 296 | $(CA)^{7}$ |
| Brz0214 | TCTGGTGTCTCTTTGCTCCT | TCCATGGTACCTGAATGACA | 57 | 309 | $(AT)^{8}$ |
| Brz0235 | CACACTCACACACGGAGAGA | CATCCAGAGCCTGATGAAGT | 57 | 298 | $(TC)^{9}$ |
| Brz3002 | GCTGGAATCAGAATCGATGA | GAACTGCAGTGGCTGATCTT | 57 | 160 | $(AAT)^{7}$ |
| Brz3009 | AGACTCTGTGCGGGAAATTA | ACTTCGCTTGTCCTACTTGG | 55 | 151 | $(AAT)^{10}$ |

## Data analysis

The allele sizes generated by all 22 SSR markers on 79 ecotypes and eight commercial varieties were scored using GeneMapper v4.1 software (Applied Biosystems, USA). ALS-Binary and Allelobin software (http://www.icrisat.org/bt-software-d-allelobin.htm) were used to convert allelic data to binary data (0, 1) where 0 and 1 represent absent and presence of an allele, respectively. Statistical analysis of allelic and binary data were performed using PowerMarker v.3.25 (Liu and Muse, 2005) to obtain total number of alleles per locus, allele size range, genetic diversity and heterozygosity, and frequency based genetic distances were calculated using shared alleles distance matrix. The population diversity indices e.g. number of allele, private allele, and effective allele per locus, Shannon Information index, observed and expected heterozygosity were calculated using GenAIEx v.6.5 (Peakall and Smouse, 2012). The same software was used to compute analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) and matrix of genetic distance. The Dice binary similarity co-efficient (Dice, 1945) was used to generate the Unweighted Neighbor-Joining tree (NJT) showing relationships among test genotypes in Darwin Software v6.0 (Perrier and Jacquemound, 2006).

## Results

*Descriptive statistics for SSR markers*

Descriptive statistics for all marker sets were computed (Table 2). The major allele frequency ranged from 0.2405 (Brz3002) to 0.8228 (Brz0076) with mean of 0.5184. The number of different alleles ranged from 3 (Brz0029) to 10 (Brz0130) with mean of 5.45. The genetic diversity averaged to 0.6225 with range of 0.3169 to 0.8021. Similarly, the polymorphic information content ranged from 0.3087 (Brz0076) to 0.8384 (Brz3002) with mean of 0.5825.

*Population diversity indices*

The population diversity indices for five ecotype populations from Kenya were summarized (Table 3). The ILRI population had highest number of different alleles and the least for Alupe population. The number of private allele was highest for ILRI population and the lowest for Kisii population. The information index ranged from 0.408 to 0.887 with mean of 0.599. The observed heterozygosity was higher than expected for all populations. The percentage polymorphic loci ranged from 46.47% (Kitui) to 86.87% (ILRI).

*Genetic diversity and relationships*

The pairwise genetic distance and population matrix of Nie genetic identity were calculated (Table 4). The genetic distance was highest between Alupe and Kitui populations (0.510) whereas the least between ILRI and Kiminini populations (0.307). Similarly, genetic identity was the highest between ILRI and Kiminini populations (0.636) and the lowest between Alupe and Kitui populations (0.235). The PCoA plot of ecotypes from five populations showed no distinct clustering pattern (Figure 1). The first two principal coordinate explained 18.27% of the total genetic variation within the test ecotypes. Specifically, the first and second coordinates explained 10.85% and 7.42% of the total genetic variation, respectively. However, an Unweighted Neighbor Joining tree of 79 ecotypes and eight commercial cultivars showed them into three distinct clusters (Figure 2).

**Table 2** Descriptive statistics for microsatellite markers

| Marker | MAF | $N_{DA}$ | I | PIC |
|---|---|---|---|---|
| Brz0012 | 0.4304 | 5 | 0.7101 | 0.6670 |
| Brz0028 | 0.4304 | 5 | 0.6521 | 0.5892 |
| Brz0029 | 0.6203 | 3 | 0.5124 | 0.4327 |
| Brz0067 | 0.4051 | 5 | 0.7419 | 0.7061 |
| Brz0076 | 0.8228 | 3 | 0.3169 | 0.3087 |
| Brz0087 | 0.481 | 8 | 0.6983 | 0.6649 |
| Brz0092 | 0.8101 | 5 | 0.3352 | 0.3240 |
| Brz0100 | 0.4684 | 4 | 0.6614 | 0.6052 |
| Brz0115 | 0.3671 | 7 | 0.8021 | 0.7829 |
| Brz0117 | 0.6076 | 6 | 0.5371 | 0.4676 |
| Brz0118 | 0.5063 | 4 | 0.5573 | 0.4613 |
| Brz0122 | 0.4557 | 6 | 0.6739 | 0.6225 |
| Brz0130 | 0.3418 | 10 | 0.7947 | 0.7706 |
| Brz0149 | 0.7722 | 5 | 0.3874 | 0.3679 |
| Brz0156 | 0.6456 | 4 | 0.5365 | 0.497 |
| Brz0203 | 0.3671 | 7 | 0.7685 | 0.7379 |
| Brz0212 | 0.5823 | 8 | 0.6195 | 0.5906 |
| Brz0213 | 0.7468 | 4 | 0.4192 | 0.3932 |
| Brz0214 | 0.4304 | 7 | 0.7432 | 0.7138 |
| Brz0235 | 0.4051 | 4 | 0.7438 | 0.709 |
| Brz3002 | 0.2405 | 5 | 0.854 | 0.8384 |
| Brz3009 | 0.4684 | 5 | 0.6313 | 0.5643 |
| Mean | 0.5184 | 5.45 | 0.6225 | 0.5825 |

MAF = minor allele frequency, $N_{DA}$ = number of different alleles, I = Shannon's genetic diversity and PIC = polymorphic information content

**Table 3** Summary of population diversity indices averaged over 22 SSR markers

| Population | N | Na | Np | Ne | I | Ho | He | PL (%) |
|---|---|---|---|---|---|---|---|---|
| ILRI | 60 | 3.633 | 0.833 | 2.21 | 0.887 | 0.76 | 0.499 | 86.67 |
| KITUI | 3 | 1.233 | 0.133 | 1.171 | 0.408 | 0.417 | 0.261 | 46.67 |
| KISII | 5 | 1.567 | 0.067 | 1.396 | 0.498 | 0.537 | 0.315 | 56.67 |
| ALUPE | 4 | 1.6 | 0.0133 | 1.486 | 0.524 | 0.544 | 0.333 | 60.00 |
| KIMIN | 7 | 2.133 | 0.1 | 1.833 | 0.678 | 0.647 | 0.41 | 70.00 |
| Mean | 15.8 | 2.033 | 0.22926 | 1.619 | 0.599 | 0.581 | 0.364 | 64.00 |

N= number of samples, Na= number of different Alleles, Np= number of private alleles, Ne= effective population size, I= Shannon's information index, Ho= observed heterozygosity, He= expected heterozygosity and PL=Percentage polymorphic loci

**Table 4** Pairwise genetic distance based on shared allele (left) and population matrix of Nei genetic identity (right) among the Brachiaria ecotype population from Kenya

| Population | Alupe | ILRI | Kiminini | Kisii | Kitui |
|---|---|---|---|---|---|
| Alupe | - | 0.462 | 0.388 | 0.323 | 0.235 |
| ILRI | 0.393 | - | 0.636 | 0.440 | 0.327 |
| Kiminini | 0.448 | 0.307 | - | 0.399 | 0.299 |
| Kisii | 0.467 | 0.392 | 0.446 | - | 0.247 |
| Kitui | 0.510 | 0.441 | 0.413 | 0.503 | - |

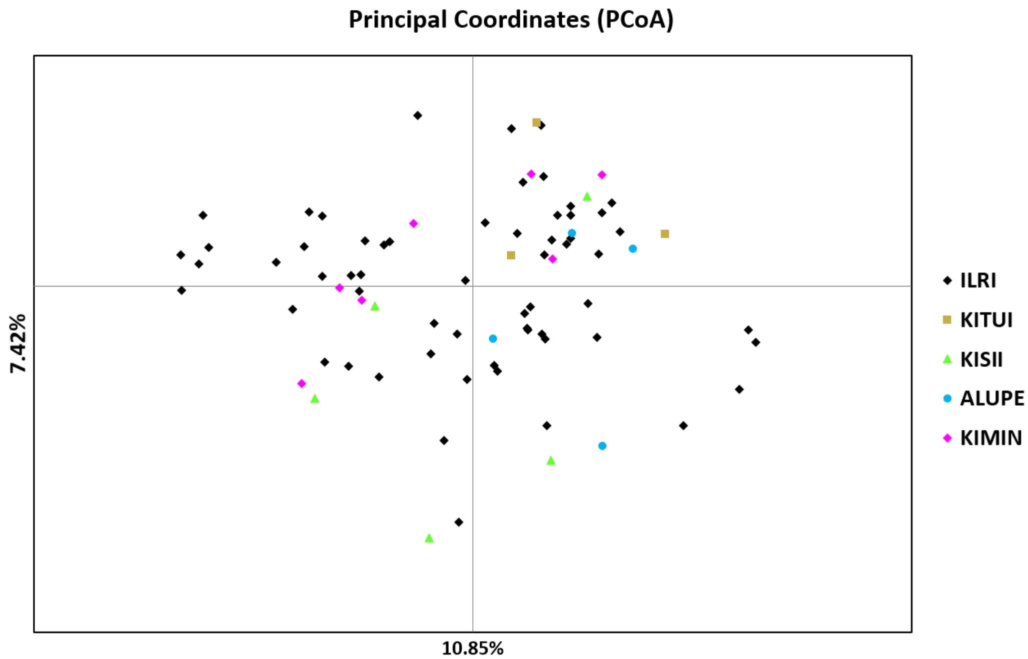


**Figure 1** Principal coordinates analysis (PCoA) bi-plot showing the clustering of the 79 Brachiaria ecotypes from different parts of Kenya (Orange= Kitui, Black= ILRI Farm, Green=Kisii, Blue=Alupe and Purple=Kiminini).

*Analysis of molecular variance (AMOVA)*

The partitioning of the total variation in population at different levels was estimated with AMOVA (Table 5). Within the individual difference contributed highest (81%) to total variation followed by among individual difference (17%) and among population differences (2%). The fixation index ($F_{ST}$) and number of immigration per generation (Nm) for study populations were 0.021 and 11.585 respectively.

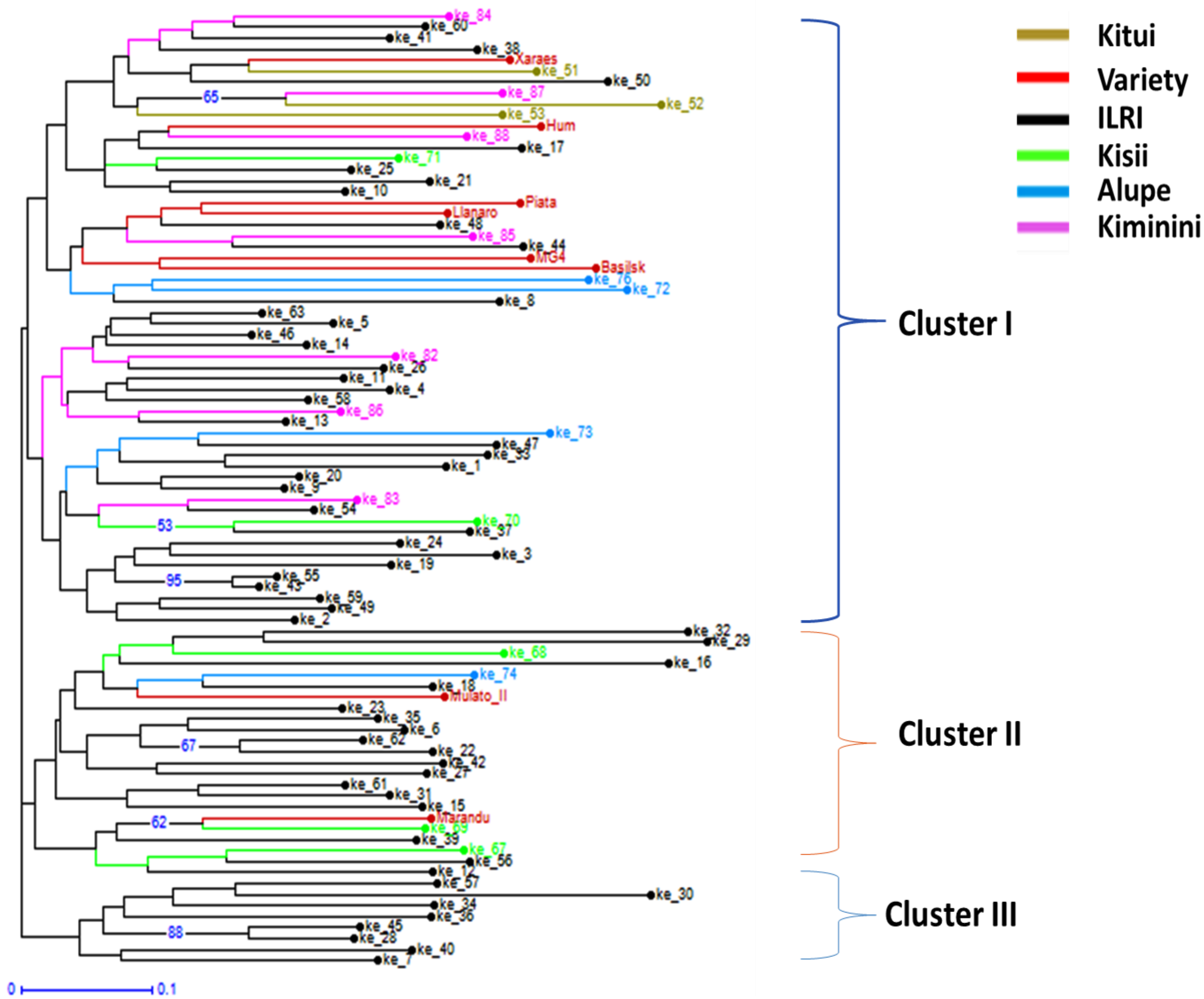


**Figure 2** Unweighted Neighbor-joining tree using the simple matching dissimilarity coefficient based on 22 micro-satellite loci for the total of 79 Brachiaria ecotypes (within ke-1 to ke_88) collected from different parts of Kenya (orange= Kitui, red= commercial cultivars, black= ILRI Farm, green=Kisii, blue=Alupe and purple=Kiminini), and seven commercial cultivars (*B. brizantha* cv. Marandu, MG4, Piata and Xaraes, *B. decumbens* cv. Basilisk, *B. humidicola* cvs. Humidicola and Llanero, and three species ways cross hybrid Mulato II.

**Table 5** Analysis of molecular variance among and within populations, and within individuals Brachiaria accessions based on 22 SSR loci

| Source | Degree of Freedom | Sum of Squares | Mean Squares | Estimated Variance | Variation (%) | P values |
|---|---|---|---|---|---|---|
| Among Populations | 4 | 43.440 | 10.860 | 0.155 | 2% | 0.023 |
| Among Individual | 74 | 619.649 | 8.374 | 1.215 | 17% | 0.001 |
| Within Individual | 79 | 469.500 | 5.943 | 5.943 | 81% | 0.001 |
| Total | 157 | 1132.589 | | 7.313 | 100% | |

$F_{ST}$ = 0.021 and Nm= 11.580

$F_{ST}$= Fixation index; Nm = Number of migration per generation

## Discussion

Brachiaria grass has shown a great potential as preferred forage option to supply quality forage to livestock especially during the dry seasons in East Africa. Current efforts are focused on repatriation in the form of hybrids and improved varieties developed mostly in South America and Australia. These repatriated materials have shown excellent performance in terms of biomass yields and livestock productivity but challenges of pests and diseases are observed in some varieties indicating potential threat of pests and diseases as acreage under Brachiaria continue to grow in Africa. Moreover, all repatriated commercial varieties were developed outside Africa in the absence of natural pests and enemies. Therefore upscaling of repatriated material requires caution and Africa based Brachiaria improvement program is imperative to develop varieties considering agro-climatic diversity of World's second largest continent and natural pests and disease. The genetic complexity, primarily apomictic mode of reproduction, and abundant natural variations in Africa urge for two pronged approach (selection and breeding) for improving Brachiaria grass in Africa. All-inclusive germplasm base with documented variations are prerequisite for the effective breeding programs. This study collected 79 Brachiaria ecotypes in Kenya and documented their genetic variations using microsatellite markers.

The PIC values for 22 SSR markers averaged to 0.5825 suggesting markers were capable in differentiating 79 Kenyan Brachiaria ecotypes. The PIC value in this study is within the range reported by Silva *et al*. (2013), Jungmann *et al*. (2009a) and Vigan *et al*. (2011) but was lower than found by Jungmann *et al*. (2009b) and Pessoa-Filho *et al*. (2015). Similarly, the average numbers of allele detected per loci (5.45) was in the range reported by Silva *et al*. (2013), Jungmann *et al*. (2009a), and Vigan *et al*. (2011) but was about half and one third of that reported by Jungmann *et al*. (2009b) and Pessoa-Filho *et al*. (2015), respectively. However, these comparisons may not be conclusive due to differences in markers and germplasm (in terms of type and number) and germplasm species compositions.

The analysis of the distributions of alleles across populations is important for explaining genetic diversity and population relationships (Szpiech *et al*., 2008). Private alleles are important in plant breeding and conservation as they are present only in a single population among a broader collection of populations (Kalinowski, 2004). Five ecotypes populations of Kenya were different for private alleles with highest number of private alleles in ILRI population and least in Kiminini population. Such variations in the private alleles among populations moist likely was the effect of population size ranging from 3 to 60 individual per population. Although no information available on species composition of each population, it is likely the presence of multiple species resulting into high number of private alleles in some population. Irrespective of populations $H_O$ was higher than $H_E$ indicating some degree of gene flow in each population. It is consistent with the human involvement in moving planting materials and out crossing nature of some Brachiaria species for example *B. ruziziensis*.

The study population varied for genetic distance and genetic identity coefficients. The highest genetic distance between Alupe and Kitui populations can be explained by the wider geographical distance between these two locations (675 km) but the genetic distance between other populations could not be associated to geographical proximity. Reports are available on forage research including seed production of *B. ruziziensis* in Kitale, Kenya (Boonman 1971; Boonman 1993), and involvement of Kenya Agricultural Research Institute and Kenya Seed Company in the past in production and trading *B. ruziziensis* seeds (Wandera 1997). It is likely that some of these Brachiaria seeds might have reached to farmers' field and other research stations in Kenya including ILRI and afterwards naturalized in the wild. If this hold true a low genetic distance (0.307) between ILRI and Kiminini (20 km away from Kitale) populations could be because of shared genetic materials in early days.

The contribution of within individual difference to total variation was 81%, whereas among the individual and among populations differences contributed 17% and 2%, respectively (Table 5). These observations were in agreement with Vigna *et al.* (2011) and Pessoa-Filho *et al*. (2015), who reported high contribution of within the accession/individual differences to total variation in *B. brizantha* (84%) and *B. ruzi*ziensis (88%) populations. Similarly, Garcia *et al*. (2013) and Azevedo *et al*. (2011) reported 73% and 65% of total variation attributed to within species or cluster, respectively. However, Jungmann *et al*. (2010) reported 44% of the variation in *B. humidicola* accessions due to the subdivision of the germplasm into five groups. The $F_{ST}$ and effective number of migrants per generation (Nm) values of 0.021 and 11.580 indicated relatively a negligible genetic differentiation among populations (Wright 1951) and relatively high level of gene flow among the Kenyan ecotypes populations (Vicetich and Waite, 2000) respectively. A low genetic differentiation among the study populations could be associated with apomictic mode of reproduction, variable ploidy causing meiotic anomalies leading to reduced pollen fertility and dispersal of seeds by migratory herbivorous and humans activities such as hay transportation and feeding animals (do Valle and Savidan 1996; Harrington *et al*., 2011; Leitch and Leitch 2008; Jungmann *et al*. 2010; Malo and Saurez, 1995; Vigna *et al*. 2011). Polyploid plants are effective colonizers that can occupy pioneer habitats and generate individuals that are able to exploit new niches or out-compete progenitor species, whereas apomictic polyploid plants can fix heterosis (Leitch and Leitch 2008; Jungmann *et al*. 2010; Vigna *et al*. 2011).

This study represents among the very first studies of this century in sub Saharan Africa that involved collection of local Brachiaria ecotypes from different part of Kenya and examination of their genetic differences using microsatellite markers. The genetic diversity data revealed that ecotypes though represented a few locations of Kenya contained much diversity than currently available eight improved Brachiaria varieties which represent three species (*B. brizantha*, *B. decumbens* and *B. humidicola*) and three way cross hybrid Mulato II (*B. brizantha* x *B. decumbens* x *B. ruziziensis*). These results clearly indicate (I) need of further collection of local ecotypes in Kenya and other east and central African countries that represent center of diversity of Brachiaria species to enrich the Brachiaria gene pool in the gene bank collections (II) genetic characterization of local ecotypes and currently available gene bank materials to understand diversity and ascertain the need for further collection, and (III) morphological characterization of available genetic resource to identify/develop varieties suitable for different production environment.

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# Agro-morphological characterisation of Brachiaria grass accessions

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**Abstract**

Information of existing genetic diversity of Brachiaria grass is important in selection for pasture development. Forty seven accessions of Brachiaria grass consisting of eight different species obtained from gene bank of International Centre of Tropical Agriculture in Columbia were characterised using a set of 22 agronomical and morphological characters. Most of the accessions originated from Eastern Africa. The accessions were planted in December 2013 at Katumani within the semi-arid region of eastern Kenya. Twelve seedlings were transplanted in single row plots at a spacing of 10 cm between plants for each accession. Agro-morphological data was collected from the middle 10 plants for each accession. Multivariate analysis was applied to cluster the accessions with similar agronomical and morphological traits. Principal component analysis revealed 4 components with eigenvalues greater than 1 with first and second components accounting for 23.8 and 20.2%, respectively. The cluster analysis showed high variation among the Brachiaria accessions and identified five main groups differentiated largely by day to 50% flowering, flowering duration and plant spread. Leafiness, growth habit, culm thickness and stigma colour did not show significance difference among the clusters. The results provided useful information on the diversity that exist and is important for pasture development and for future evaluation and collections.

**Key words:** Accessions, agro-morphological characters, cluster analysis, principal component

## Introduction

The genus Brachiaria consists of over 100 species and 33 species have been reported in Kenya. The Eastern and Central Africa is the centre of origin and diversity of the Brachiaria grasses where they occur in natural habitat (Boonman, 1993). However, a few of the species have been selected for forage production and are widely cultivated in South America, Australia and South East Asia. The most common and extensively cultivated Brachiaria species for pastures are *B. brizantha, B. ruziziensis, B. decumbens* and *B. mutica* (Ndikumana and de Leeuw,1996). Some of the germplasms were collected in the 1940s in Africa and are held in gene banks across the world under the International Treaty on Plant Genetic Resources for Food and Agriculture and have not been exploited for pasture production.

Despite the diversity of *Brachiaria* spp. in Eastern and Central Africa, comparatively little information is available on their agro-morphological characteristics. In Kenya, past evaluations identified and selected Congo Signal (*B. ruziziensis*) for commercialisation in the Western region (Wandera, 1997). However, the demand for seeds was comparatively low compared with other preferred grass, such as *Chloris gayana* (Rhodes grass) and therefore, the seed production was discontinued. Other species, *B. brizantha*, *B. decumbens*, and *B. humidicola* were evaluated in small-plot agronomic trials in the 1990s (Ndikumana and de Leeuw, 1996) but none of them found its way into commercial uses.

In recent years, there has been renewed interest in Kenya to develop high yielding and nutritious forages to support growing livestock industry using Brachiaria grasses. Grasses in the genus Brachiaria have advantage over grasses in other genera including adaptation to infertile acid soils and produce high dry matter (DM) yield (Rodrigues *et al*., 2014). The recent programme on pasture development in Kenya commenced with introduction of selected landraces improved cultivars and hybrids from South America and Australia to assess their adaptation and production in different agro-ecological zones. Unfortunately, some of these grasses have shown susceptibility to pest and diseases (Njarui *et al*., 2016). Consequently there is need to explore other germplasms either through collection or acquisition of material maintained by different research institutions and gene bank across the world.

The Kenya Agricultural and Livestock Research Organization (KALRO) obtained several Brachiaria accessions from the Genetic Resource Program of International Centre for Tropical Agriculture (CIAT). To exploit these germplasms for forage, it is important to understand the agro-morphological characteristics and variations that exist among them. Past evaluation of various plant germplasm has indicated considerable diversity in growth habit (Veasey *et al*., 2001; van de Wouw *et al*., 2009). Morphological and agronomical classification methods have been widely used to group accessions with similar characters (Pengelly *et al*., 1992). Successful classification has been carried out on large number of accessions of Buffel grass, Panicum and Indigofera (Jorge *et al*., 2008: van de Wouw *et al*., 2008; Hassen *et al*., 2006) using cluster and principal component analysis and identified distinct groups. The objective of the study was to characterise the variations Brachiaria grasses accessions obtained from CIAT and compare their diversity and morphological and agronomic traits which can be exploited for possible integration in different farming systems of Kenya.

**Materials and methods**

*Site*

The experiment was conducted from December 2013 to August 2014 at KALRO- Katumani (37°28′E; 1°58′S), Kenya. The climate and soil characteristic have been described by Njarui and Wandera (2004). Elevation is 1600 m asl and mean annual total rainfall is 717 mm, with a bimodal pattern; the long rains occurs from March to May and the short rains from October to December with peaks in April and November, respectively. There are two distinct dry seasons; from January to February and June to September. Evapo-transpiration rates are high and exceed the amount of rainfall in all the months except in November in which total rainfall exceeds total evaporation. The mean temperature is 19.6°C with March (21°C) and July (16.6°C) being the warmest and coolest months, respectively. The soils are chromic luvisols (Aore and Gitahi, 1991) and are generally low in nitrogen and phosphorus (Okalebo *et al*., 1992), with a pH of 6.5.

*Origin of material*

Eighty accessions of Brachiaria grass were obtained from Genetic Resource Program of CIAT, Columbia. Most of these accessions originated from Eastern Africa countries; Ethiopia, Kenya, Burundi, Rwanda and Uganda and a few from central Africa, Zaire and Zimbabwe. The rest of the accessions were from Togo in West Africa and Oman in Asia. Information regarding the origin of nine accessions was not available.

*Treatment and design*

Individual seeds of all 80 Brachiaria accessions were sown in polybags in a greenhouse using forest soil, sand and manures at a ratio of 3:2:1. At about four weeks after seedling emergence, 12 uniform and health seedlings from each accession were transplanted to the field in December 2013. The seedlings were planted in single rows at spacing of 10 cm between seedlings. The row to row spacing between accessions was maintained at 2 m. The spacing between rows was 2 m while the space between different accessions within the row was 1 m. Triple super phosphate (TSP 46% $P_2O_5$) fertilizer was applied at a rate of 40 kg P/ha only during planting. The plots were kept weed free by hand weeding.

*Data collection*

Data was collected from 47 accessions comprising 8 species (Table 1) as the rest were lost due to termite damage and/or poor establishment. The data was collected on 22 agro-morphological characters (Table 2). The characters selected were based on their agronomic relevance and expected variation among the accessions. Twelve qualitative characters were recorded for 10 plants of each accession as suggested by van de Wouw *et al*. (1999b) in the middle plants leaving one plant on either side of the row to avoid any border effect. Other attributes were recorded from whole plot (Table 2). All plants were evaluated once at 50% flowering stage to minimize differences due to stage of growth.

*Data analysis*

The correlations among the observed variables were calculated using the Pearson's correlation coefficient. When pairs of variables had a high correlation coefficient ($r \geq 0.7$), one of these variables was omitted to avoid indirect weighting in cluster analysis according to criteria applied by Hassen *et al*. (2006) and van de Wouw *et al*. (2009). After standardizing the variables to a mean of 0 and a variance of 1, a principal component analysis was carried out using the program Statistical Analysis System (SAS) software (SAS Inc. 2001). Hierarchical cluster analysis was carried out using the complete linkage method according to criteria recommended by van de Wouw *et al*. (2009). Variations between the groups of accessions for the different characteristics were assessed by one-way analysis of variance considering groups as treatments and individual accessions within a group as replications.

**Table 1** List of Brachiaria accessions used in the characterisation study

| Accession No [†]. | Species | Origin | Location | Latitude | Longitude | Altitude (m asl) |
|---|---|---|---|---|---|---|
| 660 | *Brachiaria. brizantha* | Unknown | - | - | - | - |
| 664 | *B. decumbens* | Unknown | - | - | - | - |
| 667 | *B. brizantha* | Unknown | - | - | - | - |
| 6130 | *B. ruziziensis* | Kenya | Rift Valley | 0.6167 | 35.1667 | 2030 |
| 6241 | *B. ruziziensis* | Uganda | - | - | - | 909 |
| 6369 | *B. humidicola* | Unknown | - | - | - | - |
| 6370 | *B. decumbens* | Unknown | - | - | - | - |
| 6084 | *B. brizantha* | Kenya | Rift Valley | -0.0667 | 34.6833 | 1400 |
| 6384 | *B. brizantha* | Kenya | Rift Valley | -0.0667 | 34.6833 | 1400 |
| 6385 | *B. brizantha* | Kenya | Rift Valley | 0.6 | 35.5333 | 2120 |
| 6399 | *B. brizantha* | Kenya | Rift valley | - | - | 2130 |
| 6419 | *B. ruziziensis* | Zaire | - | - | - | - |
| 6426 | *B. brizantha* | Kenya | Rift Valley | 0.5833 | 35.3667 | 2300 |
| 6674 | *B. brizantha* | Unknown | - | - | - | - |
| 6684 | *B. brizantha* | Kenya | Rift Valley | 0.35 | 34.8167 | 1606 |
| 6686 | *B. brizantha* | Uganda | East Mengo | 1.4333 | 32.0167 | 1061 |
| 6711 | *B. ruziziensis* | Unknown | - | - | - | - |
| 6735 | *B. brizantha* | Malawi | Central | -13.6833 | 33.75 | 1300 |
| 16097 | *B. brizantha* | Zimbabwe | - | - | - | - |
| 16106 | *B. brizantha* | Ethiopia | Shoa | 8.9833 | 37.3333 | 1900 |
| 16118 | *B. brizantha* | Ethiopia | Welega | 9.0833 | 35.9 | 1890 |
| 16122 | *B. brizantha* | Ethiopia | Welega | 9.55 | 35.45 | 1990 |
| 16150 | *B. brizantha* | Ethiopia | Sidamo | 7.15 | 37.95 | 2040 |
| 16158 | *B. brizantha* | Ethiopia | Sidamo | 6.8167 | 37.7167 | 1990 |
| 16169 | *B. brizantha* | Ethiopia | Harerge | 9.4 | 42.0333 | 1970 |
| 16289 | *B. brizantha* | Ethiopia | Kaffa | 8.1 | 37.4667 | 1850 |
| 16320 | *B. brizantha* | Ethiopia | Welega | 8.9333 | 35.5333 | 1640 |
| 16324 | *B. brizantha* | Ethiopia | Gojjam | 10.9667 | 36.4833 | 1690 |
| 16339 | *B. brizantha* | Ethiopia | Gonder | 12.5167 | 37.0339 | 2080 |
| 16482 | *B. brizantha* | Kenya | Uashin Gishu | 0.5333 | 35.0333 | 1700 |
| 16483 | *B. brizantha* | Kenya | Nandi | 0.35 | 35.05 | 1900 |
| 16514 | *B. jubata* | Kenya | Trans Nzoia | 1.1167 | 35.0667 | 1920 |
| 16536 | *B. jubata* | Kenya | Trans Nzoia | 1.0667 | 34.8833 | 1800 |
| 16539 | *B. jubata* | Kenya | Trans Nzoia | 0.8833 | 35.9333 | 1640 |
| 16541 | *B. jubata* | Kenya | Nandi | 0.35 | 35.05 | 1900 |
| 16903 | *B. nigropedata* | Zimbabwe | Murewa | -17.7 | 31.8 | 1360 |
| 16906 | *B. nigropedata* | Zimbabwe | Mazowe | -17.6333 | 30.95 | 1240 |
| 26107 | *B. brizantha* | Burudi | Rutana | -4.0167 | 30.0833 | 1220 |
| 26129 | *B. brizantha* | Burudi | Rutana | -3.9667 | 30.15 | 1170 |
| 26133 | *B. brizantha* | Burudi | Rutana | -4.0167 | 30.0833 | 1200 |
| 26302 | *B. decumbens* | Rwanda | Byumba | -1.3333 | 30.3 | 1410 |
| 26353 | *B. jubata* | Rwanda | Byumba | -1.4667 | 30.2833 | 1470 |
| 26646 | *B. brizantha* | Unknown | - | - | - | - |
| 26647 | *B. brizantha* | Burudi | Karuzi | -3.05 | 30.15 | 1640 |
| 26886 | *B. lata* | Oman | - | 17.1667 | 54.5 | 200 |
| 26894 | *B. subquadripara* | Togo | Maritime | 6.1667 | 1.25 | 10 |
| 26991 | *B. brizantha* | Unknown | - | - | - | - |
| 36083 | *B. humidicola* | Ethiopia | Sidamo | 5.86 | 39.1 | 1790 |

[†]CIAT accession numbers

**Table 2** List and definition of characters used in the agronomic and morphological study

| Characters | Definition | No. of observation | Unit |
|---|---|---|---|
| Date of first flowering[1] | Appearance of first flower | full plot score | day |
| Date to 50% flowering | Half of plants plots have flowered | full plot score | day |
| Date to full flowering[1] | All plants have flowered | full plot score | day |
| Flowering duration | Day from first flower to full flowering | full plot score | day |
| Plant height | Average height from ground to flag leaf at 50% flowering | 10 plants | cm |
| Leafiness | An estimate of the amount of leaves (1=no leaves 10=very leafy at full flowering | full plot score | 1.-10 |
| Growth habit | Angle of the culm to the ground (1=Prostrate to 5=erect) taken at 50% flowering | full plot score | 1.-5 |
| Culm thickness | Average diameter of culm at lowest internode at 50% flowering | 10 observations | mm |
| Rhizomes[1] | Presence of rhizomes (1=no rhizomes to 10 prolific rhizomes) 2 weeks after harvest | full plot core | 1.-10 |
| Leaf length | Length from ligule to tip of leaf (second leaf from flag leaf) | 10 observation | cm |
| Leaf width | Width of leaf of widest point( second leaf from flag leaf) | 10 observation | mm |
| Leaf ratio | Leaf length divided by leaf width | | |
| Ligule length[1] | Length of the ligule | 10 observation | mm |
| Leaf hairiness-Adaxial[1] | Hairiness of adaxial surface of the leaf (1=glabrous to 5-medium dense hairs) | 10 observations | 1.-5 |
| Leaf hairiness-Abaxial[1] | Hairiness of abaxial surface of the leaf (1=glabrous to 5=medium dense hairs) | 10 observations | 1.-5 |
| Leaf sheath hairiness | Hairiness of the leaf sheath (1=glabrous to 5=medium dense hairs) | 10 observations | 1.-5 |
| *Inflorescence* | | | |
| Inflorescence length[1] | Length of the main rachis from the lowest branch to the top spikelet/bristles | 10 observation | cm |
| Inflorescence width | Width of widest point | 10 observation | cm |
| Inflorescence ratio[1] | Inflorescence length divided by width | - | - |
| Raceme length[1] | Longest primary branch of inflorescence | 10 observations | cm |
| Stigma colour | 1=no purple to 5 =entire stigma purple | full plot score | 1.-5 |
| Plant spread | Width of widest braches of the plant | 10 observation | cm |

[1]Characters excluded from agro-morphological analysis due to high correlation (Pearson's coefficient ≥0.7) with other characters

## Results

The principal component (PC) analysis revealed four components with eigenvalues greater than 1 (Table 3). The first principal component which explained 23.8% of the total variation was positively associated with agro-morphological characters: leaf width, plant height, days to 50% flowering, plant spread and flowering duration, and was also negatively associated with leaf ratio. The second PC which explained 20.2% of the total variation was strongly and positively associated with leaf length, inflorescence width and leaf ratio. The third PC which explained 14.5% of the total variation was positively associated with leaf width while the fourth PC which explained only 9.9% of the total variation was strongly and positively associated with culm thickness. The first and second principal components are plotted in Figure 1 and described 44% of the variation. They revealed separation of groups across the PC1 axis. Accessions with higher values for PC1 (16320, 6674, 6686, 6369, 6130, 26133, 6419, 667 and 660) had a prostrate growth habit, higher plant spread and were taller and late flowering. Accessions with higher values for PC2 had an erect growth habit, large leaflets (length to width ratio) and higher inflorescence width.

**Table 3** Eigenvector coefficients of 13 characteristics for the first 4 principal components with eigenvalue, individual and cumulative percentage of the total variance.

| Characteristics | Principal Component | | | |
|---|---|---|---|---|
| | First | Second | Third | Fourth |
| Days to 50% flowering | 0.422 | -0.117 | -0.409 | -0.106 |
| Flowering duration | 0.300 | -0.036 | -0.496 | -0.306 |
| Plant height | 0.425 | 0.237 | 0.002 | 0.020 |
| Leafiness | -0.006 | 0.118 | 0.254 | -0.505 |
| Growth habit | -0.018 | 0.234 | -0.387 | 0.377 |
| Culm thickness | 0.182 | 0.006 | 0.265 | 0.627 |
| Leaf length | 0.178 | 0.546 | 0.030 | 0.029 |
| Leaf width | 0.432 | 0.028 | 0.341 | -0.099 |
| Leaf ratio | -0.208 | 0.419 | -0.264 | 0.157 |
| Leaf sheath hairiness | 0.263 | -0.284 | 0.223 | 0.149 |
| Inflorescence width | 0.097 | 0.467 | 0.224 | -0.117 |
| Stigma colour | -0.027 | 0.290 | 0.119 | -0.120 |
| Spread | 0.419 | -0.053 | -0.043 | 0.137 |
| Eigenvalue | 3.094 | 2.627 | 1.880 | 1.286 |
| Individual percentage | 23.80 | 20.21 | 14.46 | 9.88 |
| Cumulative percentage | 23.80 | 44.01 | 58.47 | 68.35 |

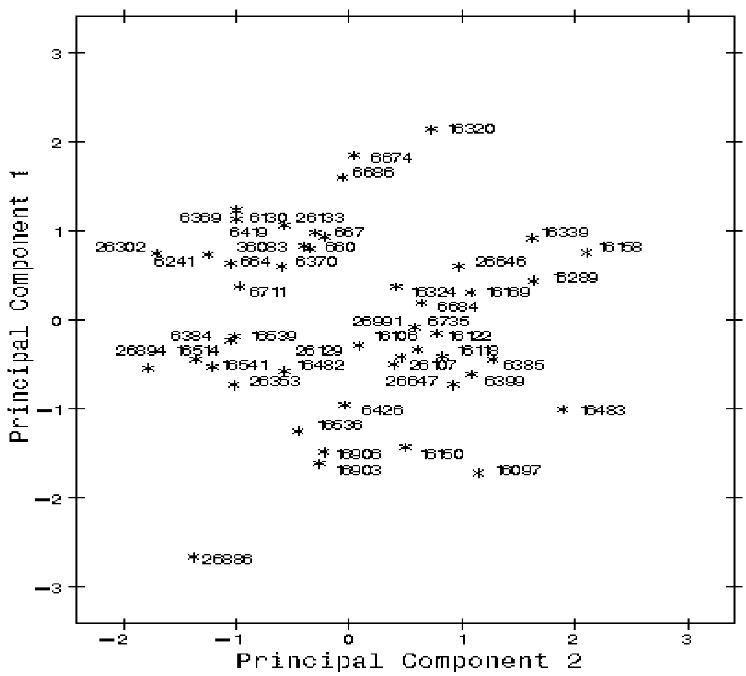


**Figure 1** Scatter diagram of 47 Brachiaria accessions plotted against the first two principal components of the correlation matrix explaining 44% of the total variation

Most of the plant characters recorded showed significant ($P<0.05$) variations (Table 4). The difference in days to 50% flowering was large and varied from 91-194 days while difference in height was small and ranged from 53-86 cm. Difference in plants spread was large (115-216 cm) with plants in group V having the largest spread and those in group 1 with the lowest spread. On the other hand, the difference between groups on leaf width, leaf ratio, leaf sheath hairiness and inflorescence was small. However, groups were not different for leafiness, leaf length, growth habit, culm thickness and stigma colour.

**Table 4** Means of agro-morphological characteristics showing differences among clusters of 47 Brachiaria accessions

| Characteristics | Cluster group | | | | |
|---|---|---|---|---|---|
| | 1 | II | III | IV | V |
| Number of accessions included | 13 | 16 | 11 | 5 | 2 |
| Days to 50% flowering (days) | 129.0$^{b}$ | 91.4$^{c}$ | 138.2$^{b}$ | 126.6$^{b}$ | 194.0$^{a}$ |
| Flowering duration (days) | 49.0$^{b}$ | 25.1$^{c}$ | 59.1$^{b}$ | 25.0$^{c}$ | 104.0$^{a}$ |
| Plant height (cm) | 68.0$^{b}$ | 52.7$^{b}$ | 85.6$^{a}$ | 69.5$^{ab}$ | 70.6$^{ab}$ |
| Leafiness | 6.6$^{a}$ | 7.0$^{a}$ | 7.2$^{a}$ | 6.6$^{a}$ | 5.5$^{a}$ |
| Growth habit | 3.7$^{a}$ | 3.9$^{a}$ | 3.9$^{a}$ | 3.2$^{a}$ | 6.5$^{a}$ |
| Culm thickness (cm) | 2.9$^{a}$ | 3.0$^{a}$ | 3.6$^{a}$ | 6.4$^{a}$ | 2.9$^{a}$ |
| Leaf length (cm) | 20.3$^{a}$ | 21.7$^{a}$ | 29.0$^{a}$ | 16.8$^{a}$ | 19.7$^{a}$ |
| Leaf width (mm) | 13.1$^{bc}$ | 10.4$^{cd}$ | 14.7$^{ab}$ | 15.0$^{a}$ | 9.9$^{d}$ |
| Leaf ratio | 15.8$^{b}$ | 22.2$^{a}$ | 21.3$^{a}$ | 11.1$^{b}$ | 20.6$^{ab}$ |
| Leaf sheath hairiness | 2.3$^{b}$ | 1.9$^{b}$ | 2.5$^{b}$ | 4.3$^{a}$ | 1.6$^{b}$ |
| Inflorescence width (mm) | 8.8$^{b}$ | 10.2$^{b}$ | 13.4$^{a}$ | 8.5$^{b}$ | 4.5$^{b}$ |
| Stigma colour | 3.2$^{a}$ | 3.6$^{a}$ | 3.9$^{a}$ | 2.6$^{a}$ | 2.0$^{a}$ |
| Spread (cm) | 115.5$^{c}$ | 107.4$^{c}$ | 178.8$^{b}$ | 225.6$^{a}$ | 216.0$^{a}$ |

Within a row, means followed by different letters differ significantly at $P<0.05$

Cluster analysis based on agro-morphological characters highlighted five main groups as shown in the dendrogram (Figure 2). The first level of separation (Group V *vs*. others) was mainly on the basis of days to 50% flowering and flowering duration. The two accessions classified in group V, both from *B. humidicola* (6369 and 36083) were late in flowering (Table 4) and took 194 days to flower. The accession 36083 originated from Ethiopia while the origin for 6369 is not known. The next separation (groups IV and III) occurred due to plant spread, leaf width, leaf size (length to width ratio) leaf sheath hairiness, inflorescence width, and plant height. Accessions in group IV had higher spread, broad leaves and more hairiness while accessions in group III were taller and had higher inflorescence width. The five accessions in group IV were evenly distributed; from Zaire, Kenya, Uganda, Rwanda and unknown origin and represented *B. ruziziensis* and *B. decumbens*. Group III memberships were *B. brizantha* and included accessions mainly from Ethiopia and others from unknown origin. Separation of groups I and II was mainly due to days to 50% flowering, flowering duration, leaf width and leaf size (length to width ratio). The majority of accessions in group II originated from Kenya, Ethiopia and Zimbabwe and represented four species: *B. brizantha, B. nigropedata, B. jubata* and *B. lata*. Accessions in group I were late flowering had broader and larger leaves than accessions in Group II. Five out 13 accessions from Group I were from Kenya and represented *B brizantha, B. decumbens, B. ruziziensis, B. subquadripara, B. jubata* and *B. lata*.

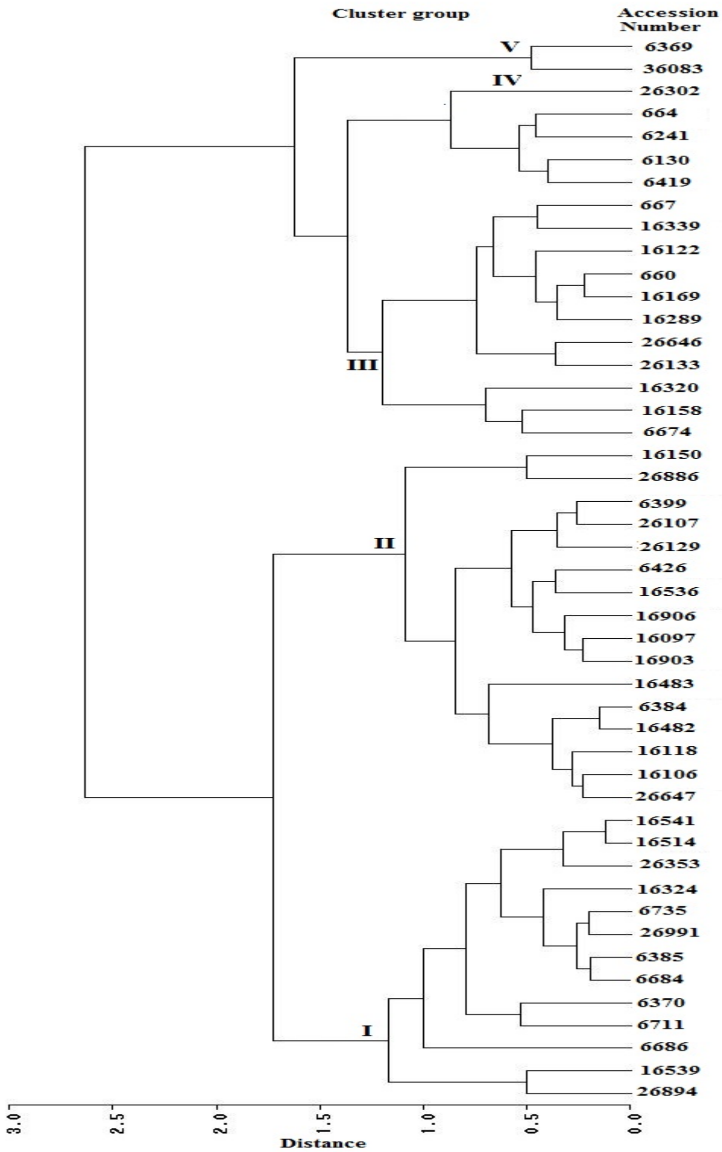


**Figure 2** Dendrogram of agro-morphological classification of 47 Brachiaria accessions obtained from complete clustering on 13 characters. Accessions numbers belongs to CIAT.

**Discussion**

The classification using agro-morphological technique is useful in defining group based on agronomic characters (Pengelly *et al*., 1992). In this study, 13 plant characters were selected from a total of 22 morphological and agronomical characters. The principal component and cluster analysis showed existing genetic variability among and between Brachiaria species. Approximately 60% of the accessions evaluated were *B. brizantha* and majority of these originated mainly from eastern Africa which is regarded as the centre of genetic diversity of

Brachiaria. Ethiopia and Kenya accounted for about 51% of the origin of the tested Brachiaria accessions. A good number of accessions were from Burundi, Rwanda and Zimbabwe while the rest from Uganda, Zaire, Oman and Togo were poorly represented. The origin of the member of group was not limited to one country with materials from same region classified in different groups indicating great diversity among the Brachiaria grass. The 11 accessions originating from Ethiopia were distributed in 4 out of the 5 clusters while 13 accessions from Kenya belonged to 3 clusters. It is also possible that the different accessions which are genetically similar occur across different countries. However, genetically closely related accession can have a very different morphology and therefore a very different prospective use and agronomic value (van de Wouw *et al*., 1999a).

The agro-morphological characters were variable in determining the groups with 50% flowering, flowering duration and plant spread being key determinant of the group. These characters are important and can form the basis of selection for different environment and utilization. Flowering data is important adaptive characteristics (Hassen *et al*., 2006). Early flowering ensure survival and sustainability in areas with short growing period. On the other hand, accessions 6369 and 36083 that flowered late would be useful in areas with long growing season. Those that have a wide spreading habit in cluster IV are useful for ground cover to reduce soil erosion while tall accessions which occurred in cluster III are suitable for cut-and-carry livestock feeding system.

The variance accounted by the first and second component for agro-morphological data was 44%, a relatively low percentage of the total variation compared with >75% obtained by Hassen *et al*. (2006) and Veasey *et al*. (2001). This may not explain satisfactory the variability expressed by the individual accessions. Normally variation of >75% is required to satisfactory explain the variability expressed between individual accessions (Veasey *et al*., 2001). However it is important to note that most of the accessions originated from only eight countries in Africa and from each country only a few accessions were classified and this may not reflect the total Brachiaria diversity that exists within the region. Further, even within the country of origin the accessions were from similar agro-ecological zone. For example, all the 13 accessions evaluated from Kenya were collected from areas within the Rift Valley region. There is need to expand collection to cover wider agro-ecological zones in addition to exploring germplasm from other countries in Africa. Nevertheless, the results provided useful information on the diversity among Brachiaria accession. This information would be important for pasture development and future evaluation and collections of Brachiaria species in Africa.

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# Establishment and growth of Brachiaria grass cultivars in the coastal lowlands of Kenya.

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**Abstract**

Livestock production in the coastal lowlands of Kenya depends mainly on natural pastures which are of poor quality and the yield is generally low during the dry season. There is therefore a need to introduce alternative forages of high quality that are adapted to the region. The objective of this study was to assess the establishment and early growth of Brachiaria grass cultivars in Mtwapa and Msabaha in the coastal lowlands of Kenya. The experiment was conducted between November 2013 and April 2014 at Mtwapa and Msabaha in the coastal lowlands of Kenya. The grass cultivars were *Brachiaria decumbens* cv. Basilisk, *B. brizantha* cvs. Marandu, MG4, Piatã and Xaraes, *B. humidicola* cv. Llanero and *B. hybrid* cv. Mulato II. These were compared with Rhodes grass (*Chloris gayana* cv. ex-Tozi), a locally cultivated grass. The treatments were laid out in a randomized complete block design with four replications. Plant and tiller numbers, height, spread and plot covers were monitored at 4, 8, 12 and 16 weeks after seedlings emergence (WAE). In addition the grasses were harvested at 16 WAE for determination of dry matter (DM) yield. Plant established successfully at both sites but the numbers were higher at Mtwapa (17 - 22 plants/m$^2$) than at Msabaha (6.3 - 13.6 plants/m$^2$) at end of establishment period. Mulato II had the highest number of tillers at both Mtwapa (44 tillers/plant) and Msabaha (53.6 tillers/plant). The DM yield was low at Msabaha (1700 - 3600 kg/ha) compared with Mtwapa (5300 - 8700 kg/ha). Xaraes had the highest yield at Mtwapa while Basilisk and MG4 had the highest at Msabaha.

**Keywords**: Dry matter yield, height, plant number, spread, tiller numbers

## Introduction

Forages play an important role in agricultural economy of developing countries by providing the cheapest source of feed for the livestock. In coastal lowlands of Kenya, one of the most important challenges to livestock production is scarcity of feeds during the dry season. The feed resources available in the smallholder mixed farms are inadequate in quantity and low in quality mainly due to lack of suitable grasses adapted to environmental conditions of the region. Farmers depend on natural pasture for livestock and more often give low priority to pasture establishment. Past attempts to improve dairy production focused mainly on promotion of Napier grass (Mureithi *et al.*, 1998) and other grasses and legumes (Njunie and Ogora, 1990). Despite these efforts, cultivated forages account for less than 15% of dairy cattle feed in all the month within a year (Njarui *et al.*, 2016). In addition, land sub-division has also contributed to feed shortage through limited available land for pasture establishment (Jones *et al.*, 2004). To address the challenges of feed shortage in the region, there is need to select high quality forages that are adapted to the region.

Brachiaria grasses are widely grown in South America with over 99 million hectares in Brazil (Jank *et al.*, 2014). Brachiaria grasses are adapted to low soil fertility and grow in a range of environmental conditions and have potential to mitigate climate change (Miles *et al*., 2004). In Kenya, Brachiaria grows well in areas where annual rainfall is above 700 mm and mean temperatures exceeding 19$^{o}$C. It also requires well drained and deep soils (Njarui *et al*., 2015). There have been previous efforts to improve the productivity of these grasses (Ndikumana and de Leeuw., 1996), but however there is little information on the establishment and growth of these grasses in the coastal region. The objective of this study was therefore to assess the establishment and initial growth of Brachiaria grass cultivars in the coastal lowlands of Kenya.

**Materials and methods**

*Description of the study sites*

The study was conducted at the Kenya Agricultural Research and Livestock Organisation (KALRO), research centres, Mtwapa and Msabaha in coastal lowlands (CL) of Kenya. Mtwapa lies in CL 3 agro-ecological zone while Msabaha falls in CL4, (Jaetzold *et al*., 2006). The sites experience bimodal rainfall, the long rains (LR) occurring from March to August and the short rains (SR) from October to December with a short dry spell in January and February. The total rainfall recorded during the experiment period was 193.7 mm at Msabaha and 369.9 mm at Mtwapa. The mean temperatures in both sites was 28 $^{0}$C. Prior to commencement of the study, the soils were sampled at 0-20 cm depth and the chemical analyses determined. In Mtwapa, the soil pH was 6.7 (1:2.5 soil water), total 0.03%, organic carbon (OC) 0.3%, phosphorus (p) 18.3ppm, potassium (K) 0.22 (me) %, magnesium (mg) 0.95 and calcium (Ca) 1.5. In Msabaha, the soil pH was 5.3, total N 0.03%, OC 0.26%, phosphorus 15 ppm and magnesium Mg 0.55).

**Table 1** Location, elevation, temperatures, rainfall and soils for the two experimental sites, Mtwapa and Msabaha.

| Site | Mtwapa | Msabaha |
|---|---|---|
| Latitude | 3$^{o}$56'S | 3$^{o}$16'S |
| Longitude | 39$^{o}$44'E | 40$^{o}$03'E |
| Altitude (m asl) | 15 | 45 |
| Mean temperature ($^{o}$C) | 22 | 32 |
| Annual rainfall (mm) | 1200 | 1000 |
| Soil type | Orthic Ferralsols | Orthic Luvisols |
| pH | 6.7 | 5.3 |

*Experimental design and treatments*

The experiment was conducted from November, 2013 to April 2014. The grass cultivars were *Brachiaria decumbens* cv. Basilisk, *B. brizantha* cvs. Marandu, MG4, Piatã and Xaraes, *B. humidicola* cv. Llanero and *B. hybrid* cv. Mulato II. Rhodes grass (*Chloris gayana* cv. ex-Tozi) was included at Mtwapa as a control but not at Msabaha due to limited seeds. The treatments were laid out in a randomized complete block design with four replications. The plot sizes were 5 m x 4 m with a 1 m path between plots and 1.5 m between replications. The seeds were drilled by hand in furrows of about 2 cm deep on a well prepared seed bed at the seed rate of 5 kg ha$^{-1}$ with inter-rows spacing of 0.5 m and covered with a thin layer of soil. Triple superphosphate (TSP, 46%

$P_2O_5$) fertilizer was applied in the furrows prior to sowing of seeds at a rate of 40 kg P/ha. The experiments were kept weed free during the experimental period by hand weeding.

*Data collection and analysis*

Plant number and growth parameters (plant height, plot cover, tiller number and plant spread) were recorded at 4, 8, 12 and 16 weeks after seedling emergence (WAE) in both sites. At the end of establishment period, 16 WAE, the plants were harvested for dry matter (DM) yield determination. Plant numbers were determined by counting within 1 m x 1 m fixed quadrat frame placed randomly over two rows within the plots. Plant height was determined by measuring the primary shoots from the base of the plant to the topmost flag leaf of four tagged plants as described by Rayburn and Lozier (2007). The percentage plot cover was determined from a 1 m x 1 m quadrat sub-divided into 25 squares as described by Njarui and Wandera (2004). Tillers were counted for tagged plants while plant spread was determined by measuring the width of grass stool from one edge to the other for the four tagged plants. During the DM yield determination, the plants were cut to a stubble height of 5 cm in an area of 4 $m^2$. Fresh herbage was harvested, weighed and a sub-sample taken, oven dried at of 65$^oC$ to a constant weight and dry weights recorded. The values on growth parameters and dry matter yields were statistically evaluated by analysis of variance (ANOVA) using general linear model (GLM) procedure of Statistical Analysis System (SAS) package (SAS, 2001). Means were separated using the Tukey's HD test.

**Results**

*Plant numbers*

Plant numbers increased slightly after 4 WAE and were highest at 12 WAE and then remained fairly stable for all the Brachiaria cultivars at both sites. However plant numbers were more at Mtwapa than at Msabaha (Table 2). Rhodes grass had more plant at Mtwapa in all the observations and were more ($P < 0.05$) than all Brachiaria cultivars at 4 WAE while in the other observations the plant numbers were not significantly ($P < 0.05$) different from some of the Brachiaria grasses. At Msabaha plant numbers were higher among the Brachiaria cultivars at 4 and 12 WAE and MG4, Basilisk, Llanero and Xaraes were the only Brachiaria grasses which attained ≥10plant/$m^2$ (Table 2).

*Plant tiller number*

The average number of tillers increased gradually over time for all the cultivars in both sites (Table 3). At Mtwapa the number increased from 10 - 23 tillers/plant at 4 WAE to 17 - 44 tillers/plant at 16 WAE. Mulato II had the highest number of tillers at both 4 and 16 WAE while Piata had the lowest. At Msabaha the number increased from 5.7 - 13.4 tillers/plant at 4 WAE to 17.8 - 53.6 tillers/plant at 16 WAE. Basilisk had the highest number of tillers at 4 WAE while Mulato II had the highest number at 16 WAE.

**Table 2** Mean plant number (plants $m^{-2}$) of the Brachiaria and Rhodes grass during establishment period at Mtwapa and Msabaha, coastal lowlands

| | Mtwapa | | | | Msabaha | | | |
|---|---|---|---|---|---|---|---|---|
| Grass | weeks | | | | weeks | | | |
| cultivars | 4 | 8 | 12 | 16 | 4 | 8 | 12 | 16 |
| Piata | 20.0 | 22.0 | 22.0 | 22.0 | 9.5 | 8.5 | 8.3 | 8.3 |
| MG4 | 19.5 | 24.8 | 24.8 | 24.8 | 8.5 | 10.0 | 10.0 | 9.8 |
| Llanero | 15.8 | 18.8 | 19.0 | 19.0 | 8.3 | 11.0 | 13.6 | 13.6 |
| Basilisk | 15.3 | 19.5 | 19.3 | 19.3 | 8.0 | 10.5 | 10.3 | 10.3 |
| Xaraes | 14.8 | 19.0 | 22.0 | 19.0 | 7.5 | 8.0 | 10.0 | 10.0 |
| Marandu | 12.5 | 17.8 | 17.8 | 17.3 | 5.0 | 6.3 | 7.0 | 7.0 |
| Mulato II | 12.5 | 16.5 | 18.3 | 18.3 | 7.0 | 7.5 | 6.3 | 6.3 |
| Rhodes grass | 27.8 | 28.0 | 28.0 | 28.0 | 8.0 | 10.5 | 10.3 | 10.3 |
| Mean | 17.3 | 20.8 | 21.4 | 21.0 | 8.8 | 10.3 | 10.8 | 10.8 |
| LSD ($p < 0.05$) | 7.3 | 7.1 | 7.2 | 7.0 | 3.5 | NS | 4.4 | NS |

**Table 3** Mean plant tiller number/$m^2$ of Brachiaria and Rhodes grass during establishment at Mtwapa and Msabaha, coastal lowlands

| | Mtwapa | | | | Msabaha | | | |
|---|---|---|---|---|---|---|---|---|
| Grass | weeks | | | | weeks | | | |
| cultivars | 4 | 8 | 12 | 16 | 4 | 8 | 12 | 16 |
| Mulato II | 22.8 | 25.0 | 33.0 | 44.0 | 8.1 | 21.9 | 33.3 | 53.6 |
| Basilisk | 18.4 | 19.5 | 24.3 | 24.3 | 15.9 | 19.9 | 20.6 | 24.4 |
| Xaraes | 18.0 | 13.3 | 20.4 | 24.9 | 11.5 | 13.2 | 14.9 | 24.1 |
| MG4 | 17.1 | 19.8 | 23.5 | 28.1 | 13.4 | 22.5 | 28.6 | 18.0 |
| Llanero | 16.0 | 24.1 | 33.1 | 39.2 | 12.5 | 16.8 | 21.0 | 23.9 |
| Marandu | 13.4 | 23.7 | 31.0 | 31.1 | 6.8 | 10.0 | 18.0 | 24.7 |
| Piata | 9.9 | 15.4 | 16.4 | 16.8 | 5.7 | 9.9 | 12.1 | 17.8 |
| Rhodes grass | 16.8 | 18.9 | 19.8 | 28.3 | - | - | - | - |
| Mean | 16.6 | 20.0 | 25.2 | 29.6 | 10.6 | 16.3 | 12.1 | 26.6 |
| LSD ($p < 0.05$) | 9.1 | 7.2 | 15.2 | 15.5 | 4.4 | 7.9 | 14.9 | 17.5 |

*Plant heights*

At Mtwapa plant height increased steadily in all the Brachiaria cultivars from around 10 cm at 4 WAE to >50 cm at 16 WAE with Mulato II being the tallest both at 12 WAE (44.9 cm) and 16 WAE (92.5 cm) and was the only Brachiaria with similar height to Rhodes at 16 WAE. (Table 4). At Msabaha growth was relatively slow and by 16 WAE none of the cultivar had reached 50 cm in height. Basilisk and Llanero were the tallest while MG4 and Marandu had the lowest height.

**Table 4** Mean plant height (cm) of Brachiaria and Rhodes grass during establishment at Mtwapa and Msabaha, coastal lowlands

| Grass cultivars | Mtwapa weeks 4 | 8 | 12 | 16 | Msabaha weeks 4 | 8 | 12 | 16 |
|---|---|---|---|---|---|---|---|---|
| Basilisk | 10.6 | 14.2 | 24.3 | 54.5 | 14.6 | 27.2 | 39.5 | 46.9 |
| Llanero | 8.0 | 13.7 | 33.1 | 66.0 | 13.6 | 20.6 | 37.3 | 43.8 |
| Marandu | 9.0 | 16.0 | 31.1 | 47.6 | 7.4 | 10.4 | 19.8 | 23.7 |
| MG4 | 9.6 | 17.0 | 23.5 | 54.7 | 9.5 | 17.4 | 21.8 | 24.2 |
| Mulato-II | 7.9 | 15.4 | 44.9 | 92.5 | 5.2 | 8.1 | 18.2 | 30.2 |
| Piata | 9.1 | 17.9 | 19.4 | 63.9 | 9.3 | 13.4 | 30.1 | 27.9 |
| Xaraes | 11.5 | 19.3 | 20.4 | 55.7 | 11.0 | 14.3 | 18.9 | 32.5 |
| Rhodes grass | 9.3 | 20.6 | 27.8 | 102.4 | - | - | - | - |
| Mean | 9.4 | 16.8 | 28.1 | 67.2 | 10.1 | 15.9 | 26.5 | 32.5 |
| LSD (p < 0.05) | 5.03 | 9.1 | 8.1 | 13.1 | 6.9 | 11.3 | 16.1 | 16.4 |

*Plant spread*

There was significant difference among the cultivars on plant spread at both sites (Table 5). At Mtwapa, Llanero spread more than the other cultivars but was only significantly more than Marandu, Piata and Xaraes at 4 WAE while at 16 WAE it spread more ($P<0.05$) than Marandu and Piata. Rhodes grass showed greater spread than all the Brachiaria cultivars at both 4 and 16 WAE. At Msabaha significant difference ($P<0.05$) was recorded only at 4 WAE where Llanero had the lowest spread compared to the other cultivars.

**Table 5** Mean plant spread (cm) of Brachiaria and Rhodes grass during establishment period at Mtwapa and Msabaha, coastal lowlands

| Grass cultivars | Mtwapa weeks 4 | 8 | 12 | 16 | Msabaha weeks 4 | 8 | 12 | 16 |
|---|---|---|---|---|---|---|---|---|
| Llanero | 44.8 | 71.0 | 79.1 | 88.1 | 34.0 | 58.6 | 59.1 | 82.9 |
| Basilisk | 42.1 | 72.2 | 80.3 | 84.2 | 57.9 | 67.2 | 69.1 | 75.0 |
| Marandu | 27.1 | 59.5 | 62.6 | 71.9 | 53.5 | 56.6 | 66.5 | 77.3 |
| MG4 | 34.1 | 57.0 | 67.6 | 84.7 | 55.6 | 60.3 | 69.6 | 70.7 |
| Mulato-II | 39.7 | 63.7 | 73.6 | 79.3 | 41.7 | 56.8 | 66.7 | 73.0 |
| Piata | 21.4 | 52.9 | 55.2 | 73.7 | 52.5 | 65.3 | 66.3 | 76.4 |
| Xaraes | 30.1 | 43.5 | 65.5 | 76.8 | 57.5 | 60.5 | 80.6 | 83.5 |
| Rhodes grass | 59.9 | 60.3 | 64.7 | 105.2 | - | - | - | - |
| Mean | 37.4 | 60.0 | 68.6 | 83.0 | 50.4 | 60.8 | 68.3 | 77.0 |
| LSD p<0.05 | 11.5 | 15.5 | 21.2 | 13.8 | 16.5 | NS | NS | NS |

*Plot cover*

Plot cover increased steadily from around 20% to over 55% at 8 WAE for most of the Brachiaria and thereafter the increase was slow (Table 6). The cv. MG4 consistently recorded the highest plot cover in all the observations although it was not higher ($P < 0.05$) than most of the

Brachiaria cultivars. At Msabaha, all cultivars showed slow growth recording mean plot cover of 16.8 at 4 WAE and 57.4% at 16WAE. Xaraes attained the highest at 16 WAE (80%) and was higher that most of the other Brachiaria cultivars. The low cover attained at Msabaha could probably be due to the low rainfall recorded during the experimental period.

**Table 6** Mean plot cover (%) of Brachiaria and Rhodes grass during establishment period at Mtwapa and Msabaha, coastal lowlands

| Grass cultivars | Mtwapa | | | | Msabaha | | | |
|---|---|---|---|---|---|---|---|---|
| | weeks | | | | | | | |
| | 4 | 8 | 12 | 16 | 4 | 8 | 12 | 16 |
| Piata | 25.0 | 68.0 | 72.0 | 72.0 | 20.0 | 39.0 | 47.0 | 57.0 |
| MG4 | 28.0 | 79.0 | 92.0 | 96.5 | 19.5 | 33.8 | 47.0 | 61.5 |
| Llanero | 20.0 | 63.0 | 75.0 | 81.3 | 13.0 | 25.0 | 42.0 | 47.0 |
| Basilisk | 19.0 | 61.0 | 80.0 | 90.3 | 18.0 | 43.0 | 49.0 | 55.0 |
| Xaraes | 20.0 | 57.0 | 71.0 | 91.8 | 14.0 | 25.0 | 62.0 | 80.0 |
| Marandu | 17.0 | 44.0 | 54.0 | 59.0 | 17.0 | 38.0 | 35.0 | 58.0 |
| Mulato-II | 20.0 | 59.0 | 80.0 | 80.8 | 16.0 | 31.0 | 37.0 | 43.0 |
| Rhodes grass | 31.0 | 44.0 | 81.0 | 82.0 | - | - | - | - |
| Mean | 22.5 | 59.4 | 75.6 | 81.7 | 16.8 | 33.5 | 45.6 | 57.4 |
| LSD ($p<0.05$) | 8.1 | 22.6 | 21.1 | 15.5 | 7.0 | 13.6 | 16.6 | 20.8 |

*Dry matter yield*

Figures 1and 2 shows the dry matter yield of the cultivars at week 16. Generally DM yield for all the Brachiaria cultivars was higher at Mtwapa than at Msabaha. At Mtwapa, there were significant ($P < 0.05$) differences among the Brachiaria cultivars on DM yields in kg/ha. Xaraes (8700 kg/ha) recorded the highest yield followed by Marandu (7847 kg/ha) while Piata (5000 kg/ha) and Llanero (5300 kg/ha) had the lowest. Rhodes grass cv. Ex-Tozi had similar yield to Llanero and Piata but was lower than the other cultivars. At Msabaha, DM yield ranged between (1700 - 3600) with Basilisk and MG4 having higher yield than the other cultivars.

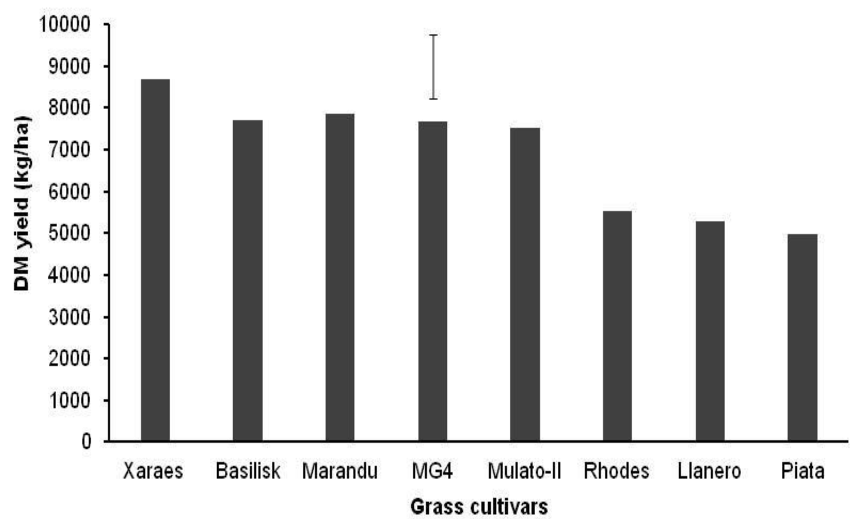


**Figure 1** Dry matter yields of Brachiaria grass cultivars at 16 WAE at Mtwapa

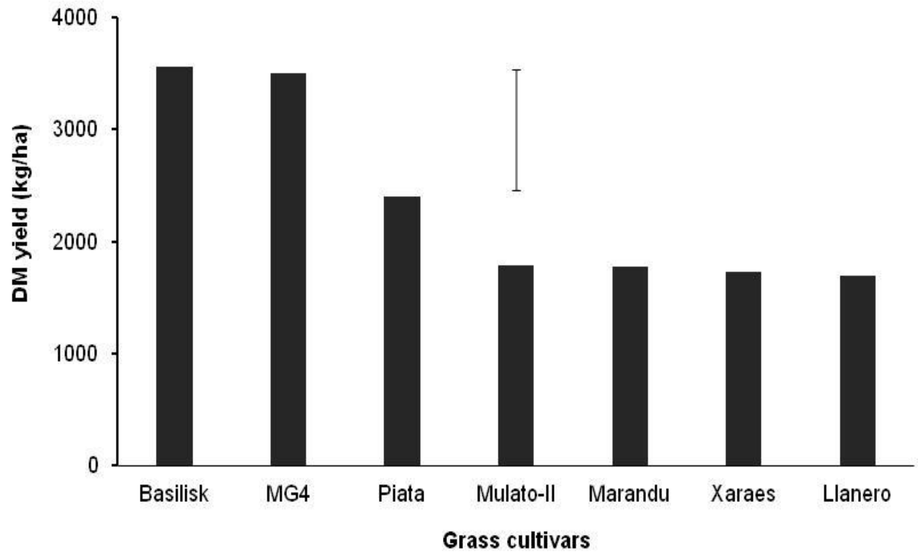


**Figure 2** Dry matter yields of Brachiaria grass cultivars at 16 WAE at Msabaha

**Discussion**

Plant establishment was good at both sites considering that the grass cultivars with the lowest had 6.3 plants$^{-2}$ at Msabaha and 17.5 plants$^{-2}$ at Mtwapa 16 WAE. However, plant numbers and growth was better at Mtwapa than at Msabaha. The difference in establishment and growth between the two sites is attributed to amount rainfall received. Total rainfall during the establishment period was 194 mm compared with 370 mm at Mtwapa. Further, the soils at the coastal lowlands are generally sandy and with high temperatures (38$^{o}$C) evapo-transpiration is high. Poor rainfall distribution at Msabaha resulted in low seedling emergence.

Surprising the difference in growth habit that would have contributed to difference in height and spread was not expressed. Llanero which has a prostrate growth habit was even taller than the other cultivars with erect growth habit such as Basilisk and Marandu. However it can be speculated that the high temperature influenced plant spread more than height. The difference in cover among the cultivars attributed to differences in seedling emergence and growth rate among the grasses. Marandu was slow to establish and therefore took time to build reasonable cover. According to Cook *et al*., (2005), Llanero has strongly stoloniferous growth habit that enables it to have higher cover than the other cultivars. Mulato II also showed a sharp increase in plot cover at 8 and 12 weeks enabling the grass achieve 80% plot cover.

Llanero, Mulato II, Marandu and MG4 had higher tillering ability among the cultivars at Mtwapa. The tiller numbers increased the chances of survival for most grasses and that large number of tillers produced allowed grasses to attain relatively high DM at an early age. This concurs with Mganga 2009, reporting that tillering ability complements yield and resilience of grass stand under defoliation. The results are also in agreement with those of Cook (2005) who found out that tillering ability of Llanero is as a result of its growth habit. As grasses ages, forage yield is increased due to the increases in tissues of the plant (Minson, 1990). Wolfson (2000), also reported that shoots or tillers that remain undefoliated for long decline and may cease to produce other stems especially where the grass have spread and covered the ground. Xaraes attained higher yields among the cultivars followed by Marandu, Basilisk, MG4 and Mulato-ll. The yields concurs with findings by Mutimura *et al*., (2012) showing that Mulato II produced high primary DM yield. Also, Guiot and Melendez, (2003) reported high DM yield of Mulato II as a result of large size leaves (15.2′ long and thick stems (1-1.5′ width). The low yield recorded at Msabaha could be due to moisture stress experienced during establishment. Finally, Xaraes and Basilisk can be grown as alternative grass to Rhodes grass in Mtwapa and Msabaha respectively.

### Conclusions

Brachiaria species performed well in terms of plot cover, plant tiller number, plant height and plant spread and had appreciable dry matter yield. The information on persistence and productivity after establishment period is reported elsewhere in these proceedings.


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# Effects of seasons and cutting intervals on productivity and nutritive value of Brachiaria grass cultivars in semi-arid eastern Kenya

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**Abstract**

The livestock production in semi-arid Kenya is based on a few cultivated forage species. There is need for diversification and develop forages that are nutritious with wide ecological adaptation. The productivity and nutritive quality of improved Brachiaria grass cultivars were evaluated in two diverse semi-arid environments in Kenya in 2014 and 2015. Eight Brachiaria grass cultivars (*Brachiaria decumbens* cv. Basilisk, *B. brizantha* cvs. Marandu, MG4, Piatá and Xaraes, *B. humidicola* cvs. Humidicola and Llanero and *B. hybrid* cv. Mulato II) were evaluated along with two controls; Napier grass (*Pennisetum purpureum*) cv. Kakamega 1 and Rhodes grass (*Chloris gayana*). At Katumani, all the Brachiaria cultivars remained productive up to long rains of 2015 after which over 90% of plants died in all plots due to the dry season. The herbage dry matter (DM) yields were highest in the first wet season (long rains 2014) and declined considerably over time with MG4, Xaraes, Piatá and Basilisk consistently producing the highest DM yield. At Ithookwe, all the plants survived during the two years of evaluation and generally the cvs. Piatá, Xaraes, Basilisk, MG4 and Llanero produced the highest yield whereas Mulato II had the lowest. The yields of top performing cultivars were similar to those of Napier and Rhodes grass in most of the seasons. The DM yield increased with increasing cutting interval from 6 to 12 weeks at Ithookwe but at Katumani there was no yield advantage beyond 8 weeks. The crude protein (CP) declined with increased cutting interval, with Mulato II having the highest CP (12.8% of DM) at 6 weeks cutting interval. Harvesting at 8 weeks interval offered a better compromise for nutritive value and DM yield. There is need to determine if the high nutritive value can be translated into animal production.

**Key words:** Brachiaria grass, crude protein, dry matter yield, *in-vitro* dry matter digestibility, plant numbers.

## Introduction

Livestock feed scarcity is a salient feature in semi-arid regions of Kenya (Njarui *et al*., 2011) and it is a major constraint to livestock production during the dry seasons when the quality also declines. Seasonal fluctuation of feed availability is widespread among smallholder crop-livestock farmers and between 79 - 99% of experience feed shortage within a year (Njarui *et al*., 2016). The demand for productive and high quality forages to bridge feed deficit is high. The rising interest in livestock development fuelled by rising demands of animal products has led to research in identifying drought tolerant, more productive and persistence forages to support livestock productivity.

Livestock farming is based on a few cultivated pastures species that have a narrow genetic base. Rhodes grass (*Chloris gayana* L.) which constitutes the main commercial pastures has limited ecological adaptation. On the other hand, Napier grass (*Pennisetum purpureum* Schum.), the most widely grown fodder for dairy cattle in hill masses for cut-and-carry production system, is susceptible to stunting and head smut diseases. Napier stunt causes herbage yield reduction of 40-90% (Lusweti *et al*., 2004; Mulaa *et al*., 2004) and 35% milk reduction while head smut causes yield loss of 25-46% (Mwendia *et al*., 2006). Consequently, there is need for diversification of pastures species that are high yielding, have wide agro-ecological adaptation and for insurance against emerging pests and diseases triggered by recent climate change.

Grasses in the genus Brachiaria have advantage over those in other genera including adaptation to infertile acid soils and produce high dry matter (DM) yield (Rodrigues *et al*., 2014). These grasses are the most widely grown forages in South America (Miles *et al*., 2004) with estimated acreage of 99 million hectares in Brazil alone (Jank *et al*., 2014), supporting a highly vibrant beef industry. Signal grass (*Brachiaria decumbens* cv. Basilisk) which was developed in Australia is adapted to a wide range of soil types and environments and grows at a range of altitudes, from 500 to 2300 m asl. It is a highly productive tropical grass that is widespread in South America, Australia, Indonesia, Vanuatu and Malaysia (Low, 2015). Brachiaria grasses produce high biomass, enhance soil fertility and reduce greenhouse gas emission (Peters *et al*., 2012) and contribute to carbon sequestration (Djikeng *et al*., 2014). Research efforts in South America led to development of high yielding and nutritious grasses and eventually release of several cultivars from Brachiaria genus. For example, *Brachiaria hybrid* cv. Mulato II was developed from three way crosses between *B. ruziziensis* (sexual tetraploid), *B. decumbens* and *B. brizantha* (apomitic tetraploid) (Miles *et al*., 2006). It has superior nutritive value to other warm season grasses and is suitable for grazing (Inyang *et al*., 2010a). Dry matter yield of up to 19 t/ha has been recorded from Mulato II over 8 months growth period (Argel *et al*., 2007). Hare *et al*. (2013a) recorded annual yield of up to 20 t/ha from Mulato II in Thailand. Cattle fed with Mulato II produced 11% more milk during the dry season and 23% more during the rainy season compared with those fed on cv. Basilisk and *B. brizantha* cv. Xaraes (CIAT, 2004). In Brazil, livestock fed on *B. brizantha* cv. Piatá showed average daily weight gain of 0.44 kg/head (do Valle *et al*., 2013).

Although Africa is the centre of origin and diversity of Brachiaria grasses, their contribution to livestock productivity has been negligible in Kenya because there has been limited selection of suitable species for cultivation. It is therefore imperative to introduce suitable high quality species and develop management practices for high yield and quality. Cutting interval is a key management aspect that affects yield and nutritive value of grasses. It is well known that, sown pasture differs in growth rate and productivity in response to defoliation regimes in different environments. A number of studies have been conducted on cutting management on productivity and quality of grasses e.g. Hare *et al*. (2013a; 2013b); Tessema *et al*. (2010). Hare *et al*. (2013a) recorded highest CP content of *Brachiaria hybrid* cvs. Cayman and Mulato II at a cutting frequency of 30 days while the highest yield was recorded at cutting frequency of 90 days. On the other hand, Tessema *et al*. (2010) found out that increasing cutting frequency reduced productivity whereas long intervals between harvests led to increased fibre and reduction in quality of Napier grass. The objective of the study was to evaluate the seasonal DM yield and nutritive value of Brachiaria grass cultivars under different cutting intervals in semi-arid tropics of Kenya.

## Material and methods

*Study sites*

The study was conducted at Katumani and Ithookwe in the semi-arid mid–altitude tropics of eastern Kenya (Table 1). Katumani is located at higher altitude but mean temperature is lower than Ithookwe. Ithookwe receives higher mean annual rainfall (1010 mm) than Katumani (700 mm). The rainfall in both sites is bimodal, with the long rains (LR) occurring from March to May and short rains (SR) from October to December with peaks in April and November, respectively. Evapo-transpiration is high and exceeds the amount of rainfall except in November when rainfall is higher than evapo-transpiration. Total annual evapo-transpiration ranges from 1600 to 2300 mm (KARI, 2001). The soils are generally low in nitrogen and phosphorus (Okalebo *et al.*, 1992).

**Table 1** Geographical position, elevation, temperature, rainfall and soils at the experimental sites, Katumani and Ithookwe.

| Site | Katumani | Ithookwe |
|---|---|---|
| Latitude | 1°35′S | 1°37′S |
| Longitude | 37°14′E | 38°02′E |
| Altitude (m asl) | 1600 | 1160 |
| Mean temperature (°C) | 19.6 | 22.5 |
| Annual rainfall (mm) | 710 | 1010 |
| Soil type | Chromic luvisols | Red sandy earth |

The soil chemical analysis determined using method of Horwitz and Latimer (2005) showed a pH of 5.9 (1:2.5 soil: water); total nitrogen (N), 0.12%; organic carbon (OC), 1.21%; phosphorus (P) 15.0 ppm; other minerals (me%) potassium (K), 0.36; calcium (Ca), 3.7 and magnesium (Mg), 5.77 at Katumani. In Ithookwe, the pH was 5.5 ; total N, 0.12%; OC, 1.14%; P, 10.0 ppm; other minerals (me %); K, 0.16; Ca, 2.0; Mg, 4.48 and Na, 0.16.

*Experimental design and treatments*

The grasses evaluated were *Brachiaria decumbens* cv. Basilisk (CIAT 606), *B. brizantha* cvs. Marandú (CIAT 6294), Xaraes (CIAT 26110), Piatá (CIAT 16125) and MG4 (CIAT 26646), *B. humidicola* cvs. Humidicola (CIAT 679) and Llanero (CIAT 6133) and B. *hybrid* cv. Mulato II (CIAT 36087). These were compared with commonly cultivated forages; Napier grass cv. Kakamega 1 and Rhodes grass. The design of the experiment was a randomized complete block in a split-plot arrangement, with four replications. The main plots were grass cultivars and the sub-plots were the regrowth ages after cutting (6, 8 and 12 weeks). Plot sizes were 4 m x 5 m with a 1 m alley between plots and replications. The plots were established during the SR 2013 in November using a seed rate of 5 kg/ha except for Napier grass. The seeds were drilled in furrows at a depth of 0.5-1.0 cm on a well prepared seedbed with an inter-row spacing of 0.5 m and covered with a thin layer of soil. For Napier grass, a single split was planted per hole at a spacing of 1m x 1m apart. Triple super phosphate (TSP 46% $P_2O_5$) fertilizer was applied in planting furrows prior to sowing of seeds at a rate of 40 kg/ha P.

A standardization cut was made in March 2014 at the start of the LR 2014 wet season in all plots to stimulate uniform plant growth. Calcium ammonium nitrate (CAN, 26% N) was applied at 100 kg N/ha per year in 2 splits, during the LR and SR seasons and commenced after the standardisation cut. The fertilizer was broadcasted in the plots and covered slightly using hand hoes, approximately one week after onset of each rainy season. The plots were kept weed free throughout the experimental period by weeding using hand hoes.

*Data collection*

The first wet season (SR 2013) was regarded as the establishment phase up to 16 weeks after seedling emergence and subsequent seasons as production phase. During the production phase, the data was collected on plant numbers, pest and disease damage and DM yield were recorded. Number of plants was determined by counting plants within a 1m x 1m frame, randomly placed over the 2 central rows at 4 weeks interval. The pest damage was rated on a 1-5 scale (0=no damage and 5=highest damage) every 4 weeks. The plants were repeatedly sampled at re-growth ages of 6, 8 and 12 weeks intervals to determine DM yield. At each harvest, an area of 2 m x 1 m was sampled and the plants were cut to around 5 cm stubble height using hand held sickles. For Napier grass harvesting was made after every 8 weeks since this is the recommended harvest regime and the plants were cut at 10-15 cm stubble height in an area of 2 m x 2 m (4 stools/plot). The fresh material was weighed; a sub-sample was taken where necessary then dried at 105$^{o}$C for 48 hours and weighed for DM determination. The herbage samples for forage quality analysis which consisted of all the stem and leaves harvested were dried at 65°C for approximately 72 hours.

*Forage quality analysis*

Due to relatively high cost of analysis, only samples harvested during the LR 2014 season at Katumani were analysed. The herbage was ground to pass through a 1-mm screen in a Willey mill (Udy Corporation, Fort Collin, CO). Analysis was conducted for crude protein (CP), fibres, lignin, calcium, phosphorus and *in-vitro* dry matter digestibility (IVDMD). Ash was determined by heating the samples at 600$^{o}$C for 2 hours in a muffle furnace. Crude protein was determined using micro–Kjeldahl according to the method of the Association of Official Analytical Chemist (AOAC 2000). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined using the Ankom method of Van Soest *et al.* (1991). The IVDMD was determined according to the procedure of Goering and Van Soest (1970).

**Data analysis**

Analysis of variance was carried out on plant numbers, DM yield and forage quality composition. The data for Humidicola were not included in the analysis due to poor plant population in all the plots. For the DM yield and quality composition of Napier grass, only the cutting interval at 8 weeks was used for comparison. The plant numbers for Napier grass were not included because only four splits were planted within the sampling area and this was expected to remain constant. The DM yield for each season was obtained by combining the harvest made in the respective seasons. At Katumani, plants died after the LR 2015 season and therefore, it was not possible to analyse data across sites and season. Consequently, an analysis was conducted separately for each site using the Statistical Analysis System (SAS, 1987) model;

$$A_{ijk} = \mu + R_i + C_j + E_k + (C*E)_{jk} + \varepsilon_{ijk},$$

Where; A is the measurement (observation response), $\mu$ = overall mean, $R_i$ is the effect of $i^{th}$ replicate ($i$ = 1 to 4), $C_j$ is the effect of $j^{th}$ grass cultivars ($j$ = 1 to 9), $E_k$ is the effect of $k^{th}$ cutting interval, $(C*E)_{jk}$ is the interaction between $j^{th}$ cultivar and $k^{th}$ cutting interval and $\varepsilon_{ijk}$ is the experimental error. The means of plant numbers, DM yields and forage quality composition were separated using the least significant difference (LSD) test at $P < 0.05$ (Steel and Torrie, 1981). #

## Results

### *Climatic conditions*

Rainfall and temperature during the experimental period (2014 - 2015) and average the rainfall are given in Figure 1. At Katumani, rainfall was around the average in LR 2014 but in SR 2014 and LR 2015, it was below the average. At Ithookwe rainfall was above the average in all the seasons except in LR 2014 where it was below average. The temperature ranged from 17.8 - 22.9°C at Katumani and 20.3 - 22.7°C at Ithookwe.

### *Plant persistence*

There was no significant ($P > 0.05$) interaction between the Brachiaria cultivars and cutting intervals on plant numbers in both sites. Significance difference ($P < 0.05$) on plant numbers occurred among the Brachiaria cultivars in both sites. At commencement of production phase, plant numbers ranged from 9 - 19 plants/$m^2$ at Katumani, (Table 2a) and 7 - 20 plants/$m^2$ and Ithookwe (Table 2b) with Marandu and MG4 having highest plant numbers at both sites. Plant number declined over time for all the Brachiaria cultivars except Llanero which maintained a constant plant number (10.6 plants/$m^2$) at Ithookwe. The decline was higher at Katumani with all the cultivars having less than 9 plants/$m^2$ in July 2015 except Marandu which had 10.4 plants/$m^2$. Over 90% of the plants died following the long dry season of 2015 at Katumani. At Ithookwe, all the Brachiaria cultivars survived and maintained a reasonable number of plants in all the plots during the two years of evaluation. The control, Rhodes grass maintained the highest plant numbers (32 - 54 plants/$m^2$) throughout the experimental period in both sites

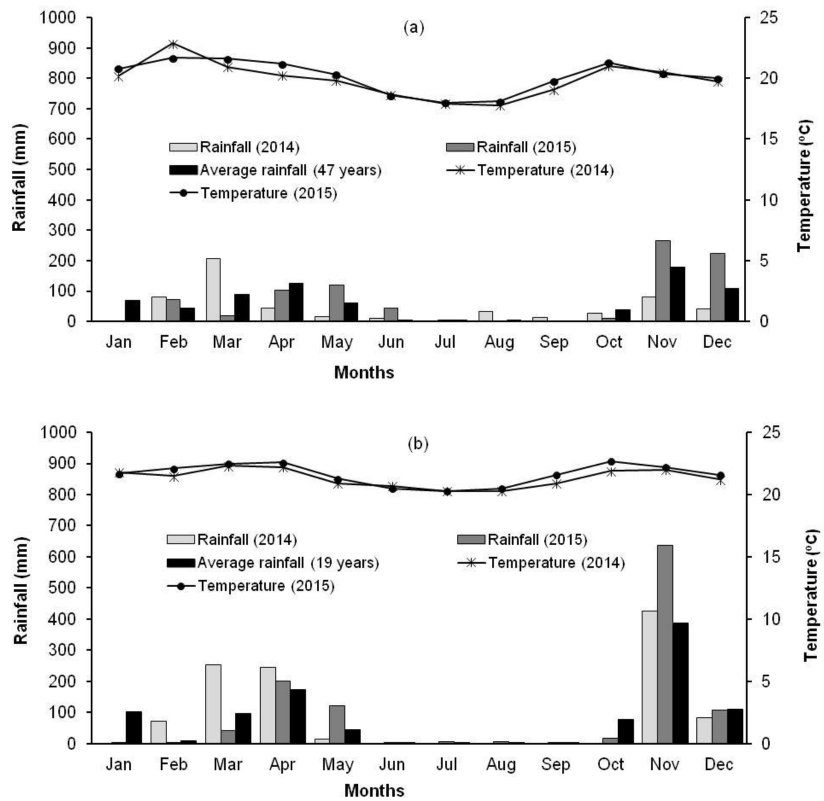


**Figure 1** Total monthly and average rainfall and mean monthly temperatures at a) Katumani and b) Ithookwe during the experimental period (2014 – 2015)

**Table 2** Plant population changes of Brachiaria grass cultivars during the production phase at (a) Katumani and (b) Ithookwe

**(a) Katumani**

| Observation dates / Brachiaria cultivars | 2014 | | | | 2015 | | |
|---|---|---|---|---|---|---|---|
| | 21/3 | 19/6 | 9/9 | 9/12 | 13/2 | 7/5 | 4/7 |
| | Plants number /m$^2$ | | | | | | |
| Basilisk | 14.9 | 16.3 | 11.8 | 11.8 | 10.0 | 9.1 | 8.3 |
| Marandu | 15.1 | 12.6 | 11.9 | 11.9 | 10.0 | 9.0 | 8.7 |
| MG4 | 19.6 | 19.6 | 17.9 | 17.9 | 13.3 | 11.0 | 10.4 |
| Mulato II | 14.0 | 14.0 | 10.4 | 10.4 | 9.0 | 7.1 | 6.6 |
| Piatá | 9.1 | 9.1 | 8.9 | 8.9 | 7.2 | 6.7 | 6.3 |
| Xaraes | 10.3 | 8.1 | 8.1 | 8.1 | 6.3 | 6.3 | 5.3 |
| Llanero | 15.2 | 15.3 | 15.3 | 15.3 | 11.8 | 9.6 | 9.6 |
| Rhodes grass | 33.9 | 34.7 | 31.9 | 51.9 | 44.6 | 54.4 | 48.7 |
| Mean | 16.5 | 16.2 | 14.5 | 17.0 | 14.0 | 14.2 | 13.0 |
| LSD (P<0.05) | 3.5 | 3.7 | 3.9 | 5.9 | 7.1 | 12.2 | 5.0 |
| CV (%) | 2.6 | 2.1 | 2.7 | 12.7 | 11.1 | 17.8 | 17.7 |

**(b)Ithookwe**

| Observation dates | 2014 | | | | 2015 | | | | 2016 |
|---|---|---|---|---|---|---|---|---|---|
| Brachiaria cultivars | 18/3 | 17/6 | 11/9 | 11/12 | 17/1 | 24/4 | 14/7 | 13/12 | 29/1 |
| | Plants number /$m^2$ | | | | | | | | |
| Basilisk | 12.6 | 12.6 | 11.8 | 11.8 | 11.1 | 10.6 | 10.6 | 10.6 | 10.4 |
| Marandu | 13.7 | 13.7 | 13.1 | 13.1 | 12.7 | 11.4 | 11.3 | 11.3 | 11.2 |
| MG4 | 19.1 | 19.1 | 16.9 | 16.9 | 13.67 | 13.6 | 13.6 | 13.6 | 14.9 |
| Mulato II | 7.1 | 7.1 | 6.6 | 6.6 | 6.6 | 5.4 | 5.4 | 5.4 | 5.3 |
| Piatá | 9.2 | 9.2 | 8.7 | 8.7 | 8.4 | 8.4 | 8.4 | 8.4 | 8.2 |
| Xaraes | 12.8 | 12.8 | 11.3 | 11.3 | 9 | 9.0 | 9.0 | 9.0 | 9.0 |
| Llanero | 10.6 | 10.6 | 10.6 | 10.6 | 10.6 | 10.6 | 10.6 | 10.6 | 10.6 |
| Rhodes grass | 11.8 | 11.8 | 13.7 | 43.7 | 33.9 | 27.9 | 21.8 | 21.8 | 32.4 |
| Mean | 12.1 | 12.1 | 11.6 | 15.3 | 13.2 | 12.0 | 11.4 | 11.4 | 12.8 |
| LSD (P<0.05) | 4.2 | 4.2 | 3.5 | 5.6 | 3.4 | 11.9 | 4.4 | 4.4 | 8.4 |
| CV (%) | 10.3 | 10.3 | 10.4 | 20.4 | 20.9 | 21.1 | 5.2 | 5.2 | 17.4 |

*Dry matter production*

**Katumani**

There was no significant (P > 0.05) interaction between the Brachiaria cultivars and cutting intervals on DM yield in all the seasons at Katumani. Significant differences (P<0.05) on yield occurred between cutting intervals and among the grass cultivars. During the LR 2014 season, yield ranged from 4382 to 7283 kg/ha with MG4 and Piatá attaining the highest yield while Llanero had the lowest (Figure 2a). Significant (P < 0.05) difference was recorded between cutting interval of 6 and 12 weeks (Figure 2b). In SR 2014, the yield declined to between 1521 and 3617 kg/ha (Figure 2c). During this season, the 6 and 8 weeks gave similar yields and were higher than at 12 weeks cutting interval (Figure 2d). In LR 2015 season, DM yield declined further to <2000 kg/ha for all the Brachiaria cultivars (Figure 2e). However increasing cutting interval from 6 to 8 weeks increased yield but at 12 weeks there was no further yield benefit (Figure 2f). The most productive cultivars in all the seasons were Basilisk, MG4, Piatá and Xaraes. The control, Napier grass had highest (P < 0.05) yield than all the Brachiaria cultivars in the first harvest (LR 2014 season) while in SR 2014 season, the yield was not different and in LR 2015 it yielded less than Basilisk and Xaraes.

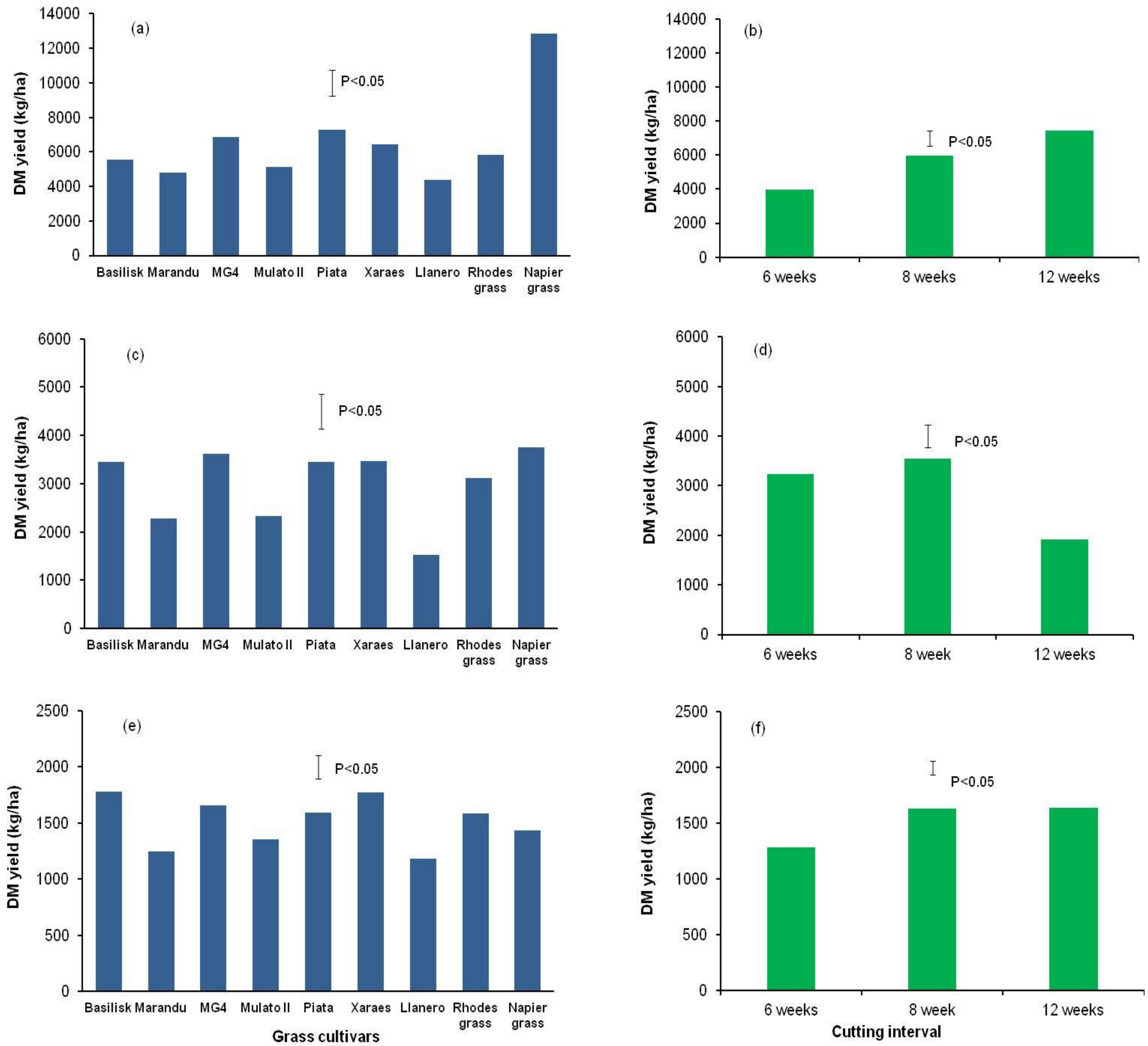


**Figure 2** Dry matter yield of Brachiaria grass cultivars during; (a, b) LR 2014, (c, d) SR 2014 and (e, f) LR 2015 at Katumani

### Ithookwe

At Ithookwe, significant (P < 0.05) interaction between Brachiaria cultivars and cutting intervals on DM yield occurred in SR 2014 and SR 2015 seasons. In LR 2014 season both main effects (cultivars and the cutting intervals), had a significant effect on yield while in LR 2015 season only the cutting interval had a significant effect on yield. In LR 2014 season, yield ranged from 1738 to 5672 kg/ha with Xaraes, Piatá and Basilisk having the highest yield while Mulato II had the lowest (Figure 3a). The control, Napier grass produced more yield (12766 kg/ha) than all the Brachiaria cultivars while Rhodes grass out yielded Mulato II only. Increasing cutting interval from 6 to 8 weeks increased the yield but a further increase to 12 weeks was not significant (Figure 3b). In SR 2014 season, generally increasing cutting interval resulted to increase in yield for all the Brachiaria cultivars (Figure 3c). In LR 2015 season, increasing cutting interval from 6 to 12 weeks tripled the DM yield (Figure 3d). However, in SR 2015 none of the cultivar consistently produced higher yield than the others at all cutting intervals but yields were lowest at 6 week (1400 - 4300 kg/ha) and highest at 12 weeks cutting interval (3500 - 7100 kg/ha) (Figure 3e). Mulato II had poor yield at 6 and 8 weeks but production improved at 12 weeks cutting interval. Generally, Piatá, Xaraes, Basilisk, MG4 and Llanero tended to produce the highest DM yield whereas Mulato II had the lowest.

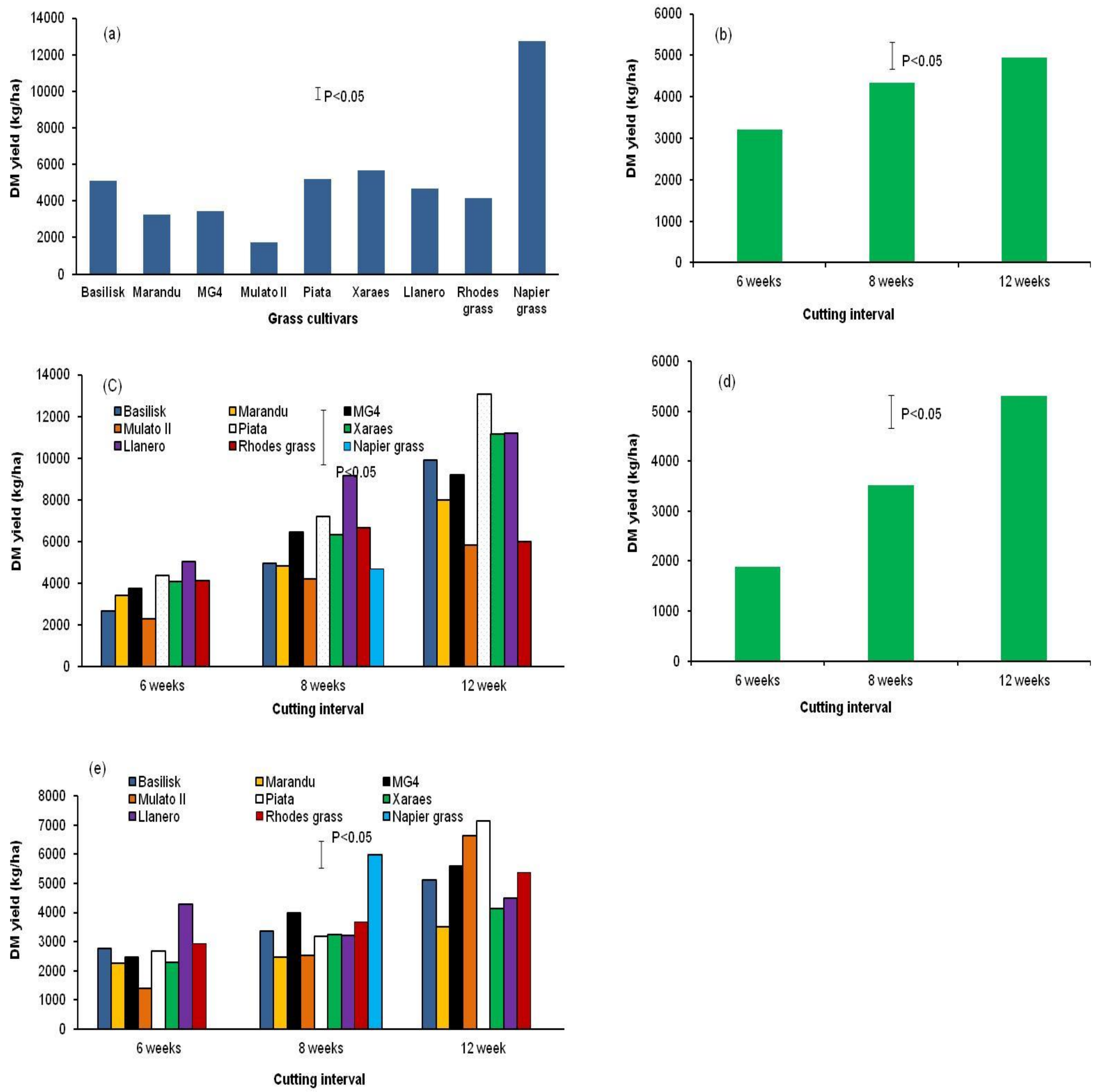


**Figure 3** Dry matter yield of Brachiaria grass cultivars during; (a, b) LR 2014; (c) SR 2014, (d) LR 2015 and (e) SR 2015 at Ithookwe.

*Forage quality composition*

There were significant interactions between Brachiaria cultivars and cutting interval on CP, fibres, ash, digestibility, Ca and P (Table 3). For the lignin only the main effect (cultivars and cutting interval) had a significant effect. The CP declined with increasing cutting interval from 6 weeks (CP 9.8 - 12.9% of DM) to 12 weeks (6.1 - 8% of DM). Mulato II had the highest CP content at both 6 and 8 weeks cutting intervals. The NDF ranged from 56.1 to 66.4% of DM and ADF from 32.7 to 48.6% of DM and increased with cutting interval. The control, Rhodes grass had higher NDF at each cutting interval. The ash was highest at 6 weeks (12.3-15.0%) and declined with increased cutting interval for all the grasses. Lignin content was highest at 12 weeks cutting interval with Marandu (3.0%) having the lowest and Mulato II (5.30%) the highest. The IVDMD ranged from 46.0 to 61.1% and declined with increased cutting interval. The level of Ca and P increased with cutting interval from 6 to 8 weeks while at 12 weeks they either remained the same, increased or declined depending on the cultivars (Table 3). The control; Rhodes grass had the highest Ca at 8 and 12 weeks (0.35 and 0.36%) while MG4 had the highest P (0.13%) at 8 week cutting interval.

*Pest and diseases damage*

Insect pests were recorded in all the Brachiaria cultivars in most of the months except on Llanero and the control; Rhodes grass (Table 4). The common pests were the red spider mites (*Tetranychus urticae* Koch.), grass midge (*Oscella frit* L.) and to some extent sorghum shoot fly (*Atherigona soccata* Rondani). The red spider mites attacked the underside of the leaves while the grass midge and shoot fly attacked the young growing tillers. The damage from the spider mites was the most devastating particularly for Mulato II and Marandu which recorded a total index of 4.1 and 5.0, respectively (Table 4). The highest attack was in March, October and November with indices of 0.5 to 1.0, implying that between 50 and 100% of plants were damaged. Xaraes, Piatá, Marandu and Mulato II were susceptible to red spider mites while MG4 and Basilisk showed some level of resistance. When attached by the mites, Basilisk and MG4 produced some purple pigmentation (probably anthocyanin) on leaves limiting insect infestation. The diseases recorded were leaf rust, leaf spot and blight mainly at Ithookwe. Rust attack was widespread in MG4 and Rhodes grass and was concentrated on the old mature leaves. Blight infestation was minor on all the Brachiaria cultivars and occurred during the dry season between July and September only.

**Table 3** Herbage chemical composition (% of DM) and digestibility of Brachiaria cultivars at three cutting interval at Katumani, semi-arid tropics of Kenya

| Brachiaria cultivars | Cutting interval (weeks) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6 | 8 | 12 | 6 | 8 | 12 | 6 | 8 | 12 | 6 | 8 | 12 |
| | †CP | | | ‡NDF | | | ±ADF | | | Ash | | |
| Basilisk | 9.8 | 8.1 | 4.9 | 63.9 | 68.1 | 71.3 | 38.6 | 42.2 | 42.4 | 13.3 | 12.1 | 8.5 |
| Llanero | 10.7 | 9.5 | 6.6 | 66.4 | 66.6 | 68.7 | 40.6 | 40.8 | 40.4 | 12.3 | 11.4 | 11.1 |
| MG4 | 11.5 | 8.0 | 7.0 | 60.6 | 64.7 | 69.1 | 36.1 | 38.8 | 42.9 | 13.3 | 12.5 | 9.6 |
| Marandu | 11.9 | 9.2 | 6.2 | 60.3 | 65.5 | 65.6 | 35.3 | 38.6 | 38.0 | 14.6 | 13.9 | 12.0 |
| Mulato II | 12.8 | 10.7 | 7.0 | 56.1 | 60.6 | 63.3 | 32.8 | 36.9 | 37.5 | 15.0 | 15.0 | 11.4 |
| Piatá | 10.5 | 9.5 | 6.1 | 63.6 | 64.1 | 69.0 | 37.1 | 48.6 | 41.1 | 13.1 | 12.0 | 10.2 |
| Xaraes | 11.1 | 9.6 | 8.0 | 64.6 | 65.2 | 67.5 | 39.2 | 35.4 | 39.9 | 12.6 | 12.6 | 10.6 |
| Rhodes grass§ | 9.7 | 6.9 | 4.4 | 72.5 | 72.3 | 73.8 | 45.6 | 48.5 | 50.2 | 9.5 | 8.6 | 8.3 |
| Napier grass§ | | 7.6 | | | 68.1 | | | 45.1 | | | 15.2 | |
| LSD (P<0.05) | | 1.4 | | | 2.6 | | | 3.9 | | | 1.4 | |

| Brachiaria cultivars | Mean | Cutting interval (weeks) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 8 | 12 | 6 | 8 | 12 | 6 | 8 | 12 |
| | ADL+ | ⸆IVDMD | | | Ca | | | P | | |
| Basilisk | 3.9 | 57.5 | 54.3 | 46.0 | 0.11 | 0.11 | 0.20 | 0.06 | 0.05 | 0.06 |
| Llanero | 4.7 | 54.1 | 55.4 | 47.4 | 0.07 | 0.17 | 0.10 | 0.06 | 0.10 | 0.07 |
| MG4 | 3.6 | 59.2 | 56.9 | 48.3 | 0.12 | 0.22 | 0.17 | 0.09 | 0.13 | 0.08 |
| Marandu | 3.0 | 58.3 | 58.2 | 52.3 | 0.16 | 0.19 | 0.27 | 0.08 | 0.08 | 0.09 |
| Mulato II | 5.3 | 57.4 | 58.6 | 54.6 | 0.14 | 0.13 | 0.28 | 0.09 | 0.08 | 0.09 |
| Piatá | 3.4 | 55.8 | 52.7 | 48.6 | 0.12 | 0.22 | 0.14 | 0.07 | 0.10 | 0.06 |
| Xaraes | 3.8 | 61.1 | 56.1 | 50.5 | 0.10 | 0.27 | 0.18 | 0.07 | 0.10 | 0.07 |
| Rhodes grass† | 6.1 | 50.5 | 45.3 | 30.2 | 0.16 | 0.35 | 0.36 | 0.05 | 0.07 | 0.05 |
| Napier grass† | 3.2 | | 54.8 | | | 0.11 | | | 0.05 | |
| LSD (P<0.05) | 1.4 | | 5.0 | | | 0.05 | | | 0.02 | |

§Rhodes and Napier grasses were included as control; †CP=Crude protein; ‡NDF=Neutral detergent fibre, ±ADF=Acid detergent fibre; +ADL=Acid detergent lignin; ⸆IVDMD=*in-vitro* dry matter digestibility

**Table 4** Average insect pest damage index on Brachiaria grass for Katumani and Ithookwe in 2014. Index is the total of overall replications expressed as a proportion of the maximum possible total [number of observation (n=3) with each observation having a maximum score of 5]

| Grass cultivars | 2014 | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Total |
| Xaraes | 0.1 | 0.2 | 0.1 | 0.1 | 0 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.6 | 0.2 | 2.1 |
| MG4 | 0.2 | 0.3 | 0.2 | 0.1 | 0 | 0.2 | 0.1 | 0.1 | 0 | 0.5 | 0 | 0.2 | 1.9 |
| Basilisk | 0.1 | 0.1 | 0.8 | 0.3 | 0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.6 | 0.4 | 0.2 | 2.6 |
| Piatá | 0 | 0 | 0.3 | 0.2 | 0.1 | 0.2 | 0.0 | 0.1 | 0.1 | 0.7 | 0.5 | 0.3 | 2.5 |
| Marandu | 0.1 | 0.3 | 0.5 | 0.3 | 0.1 | 0.3 | 0.3 | 0.4 | 0.4 | 1.0 | 0.8 | 0.5 | 5.0 |
| Mulato II | 0.2 | 0.1 | 0.8 | 0.5 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.5 | 0.6 | 0.6 | 4.1 |
| Llanero | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhodes | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

## Discussion

This study has provided important information on effect of cutting interval on the productivity of Brachiaria grass cultivars which could be applied as guidelines to maximise quality and yield in semi-arid tropics of Kenya.

*Plant persistence*

The Brachiaria cultivars had a high plant numbers and showed variation in adaptation to different environment within the semi-arid tropics of Kenya. The higher plant numbers for MG4 and Marandu than other Brachiaria cultivars was attributed to their high germination level (Nguku *et al*., 2015). These grasses are perennial but nevertheless the plant numbers continued to decline over time. Although they flowered, they did not produce seeds due to low rainfall thus there was no new plant numbers through seedlings recruitment. The control, Rhodes grass produced viable seeds resulting in increased plant numbers. The poor survival of the Brachiaria grass at Katumani was due to low precipitation and the prolonged dry season experienced from May – October 2015. Difference in amount of rainfall received has been attributed to grass survival in the same environment (Njarui *et al*., 2015).

*Dry matter yield*

There were variations in DM yield among the Brachiaria cultivars and they exhibited seasonality in yield. The decline in yield after the LR 2014 season was attributed to relatively depressed rainfall in SR 2014 and LR 2015 which were below the long term average. The DM yields of 2- 6 t/ha obtained at Katumani were low compared with that recorded by Hare *et al*., (2009) (16.3 t/ha annual DM yield) for cv. Xaraes in Thailand. At Ithookwe yield remained relatively the same in LR 2014 and SR 2014 and were higher than at Katumani due to favourable rainfall but declined slightly in SR 2015. The top performing Brachiaria cultivars produced similar yield as the control; Napier and Rhodes grass.

Increasing cutting interval from 6 to 12 weeks increased yield in the first harvest but in second and third harvest there was no significant yield gains at Katumani. These findings are similar to those of Hare *et al*. (2013b) who reported that DM yield of guinea grass cv. Mombasa increased from 9.8 to 12 t/ha by delaying cutting interval from 30 to 60 days but at 90 days the DM yield did not increase in Thailand. However, this trend was not manifested at Ithookwe where longer cutting interval of 12 weeks resulted in increased yield. Hare *et al*. (2013a) also observed increased herbage accumulation in Mulato II from about 11 to 20 t/ha in Thailand as regrowth interval increased from 30 to 90 days. Similarly, Inyang *et al*. (2010b) reported lower herbage accumulation at 2 weeks regrowth than at cutting at 6 weeks interval for the Mulato II. A study by Njarui and Wandera (2004) showed higher yield for Basilisk at longer cutting interval than at short cutting interval in the same region. The difference in yield due to cutting intervals was attributed to moisture availability. At Katumani, rainfall was low compared with Ithookwe and was not sufficient to sustain addition growth beyond 8 weeks. Moreover, evapo-transpiration is high in the semi-arid resulting in reduced moisture in the soil. Stewart and Hash (1982) recorded evapo-transpiration rate of 8.2 mm per day in the semi-arid. The poor performance of Mulato II and Marandu at 6 and 8 weeks cutting interval at Ithookwe was attributed to infestation by the red spider mites. However, it seemed to improve in SR 2015 under the 12 weeks cutting interval as population of mites tended to decline. Mulato II is hybrid developed from three species of Brachiaria for resistance against spittle bugs in South America but its susceptibility to native pests in the Africa was not a surprise.

*Forage quality composition*

Overall, the CP and digestibility were reasonably high, for all the Brachiaria cultivars when cut at 6 and 8 weeks of growth which is an important aspect for livestock production. Though Mulato II had the highest CP at 6 and 8 weeks (9.8 - 12.9%) it was lower than the values of 13-16% recorded by Vendramini *et al*. (2014) but were similar to values reported by Hare *et al*. (2009). Hare *et al*. (2009) recorded average CP over 3 years ranging from 9.8 to 11.8% (leaf) and 6.7 to 7.3% (stem) of several Brachiaria grasses including Mulato II, Marandu, Xaraes and Basilisk.

Among the most productive cultivars, Piatá and Xaraes were more nutritious in terms of CP than Napier and Rhodes grass. Basilisk and MG4 and had similar CP, fibres and digestibility to Napier and Rhodes grass and thus can be regarded as equally nutritious. This implies that they can complement Napier and Rhodes grass as livestock feed and meet the minimum CP content of 7%, the critical for animal production. The decline of CP and INVDMD with increased cutting intervals is attributed to accumulation of fibres with time. Inyang *et al*. (2010b) reported higher nutritive value of Mulato II when harvested at 2 week re-growth than at 6 weeks. Cutting Mulato II at 30 days interval produced CP level of 3 - 4% greater than cutting interval of 45 to 60 days interval (Hare *et al*., 2013a). In another study, Hare *et al*. (2013b) also showed that increasing cutting interval significantly reduced the CP and increased the fibres concentration in guinea grass**.**

## Conclusions

The Brachiaria cultivars evaluated differed in performance within the semi-arid tropical Kenya. They showed low adaptation at Katumani and failed to tolerate the dry season of 2015 implying that they are unlikely to survive in areas that receives less than 800 mm annual rainfall with dry seasons exceeding four months. The DM yield was highest in the first harvest and declined progressively with seasons at Katumani with Xaraes, Piatá, Basilisk and MG4 consistently producing the highest yield. At Ithookwe, yields were fairly stable across all seasons with Xaraes, Llanero and Piatá having the highest yield in most of the seasons. These top performing cultivars yielded as much as the controls; Napier and Rhodes grass thus could be considered as suitable grasses for inclusion in the local farming system. Although Mulato II had higher CP than the other cultivars, high DM yield is more appealing to farmers and due to its being susceptible to pest, it is not recommended for cultivation in the region. Cutting interval of 6 weeks had the highest CP and digestibility but had the lowest DM yield. On the other hand, the quality was low at 12 weeks but yield was highest although in some instances it was not significantly higher than at 8 weeks interval. In view of the fact that at 12 weeks, the nutritive quality declined considerably, it is recommended that the best option for harvesting be 8 weeks interval as the yield is high and the nutritive quality is not compromised. However, there is need to evaluate their feeding value to livestock.

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# Production and nutritive quality of Brachiaria grass cultivars subjected to different cutting intervals in the cool sub-humid highlands of central Kenya

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## Abstract

Inadequate quantity and quality of feeds is the major constraints to livestock production in majority of smallholder farms in Kenya. Eight Brachiaria cultivars were evaluated for dry matter (DM) yield and nutritive quality at three cutting intervals (6, 8 and 12 weeks) in the cool sub-humid highlands of central Kenya. The grass treatments were; *Brachiaria brizantha* cvs. Marandu, Xaraes, Piata, and MG4, *B. decumbens* cv. Basilisk, *B. humidicola* cv. Humidicola and Llanero, *B. hybrid* cv. Mulato II and two controls; Napier and Rhodes grass. The treatments were laid out in a randomized complete block design in a split plot arrangement with four replications. The main plots contained the grasses while the defoliation frequencies, 6, 8 and 12 weeks formed the sub-plots. Measurements were carried out on herbage dry matter (DM) yield and nutritive quality of the grasses. During the wet season (May - Oct 2014), DM yield of Basilisk and Piata were similar to that of Napier grass, but significantly ($P < 0.05$) higher than for Rhodes grass. During the dry season, Napier grass out-yielded all the Brachiaria grasses while MG4 and Piata produced higher yield than Rhodes grass. Increasing the cutting interval from 6 to 12 weeks resulted in increased DM yield in the wet season while in the dry season yield declined in the order 12 > 6 > 8 weeks cutting interval. Increasing the cutting interval from 6 to 12 weeks decreased ($p < 0.05$) CP and Ca, but the dry matter digestibility remained the same. Mulato, Xaraes, Humidicola and Llanero had the highest CP content although were similar to that of Napier grass, but higher ($P < 0.05$) than for Rhodes grass. The results of this study indicate that Brachiaria grasses have potential of increasing quality feeds for livestock production in the cool sub-humid highlands of central Kenya.

**Keywords***:* Brachiaria, herbage yield, cutting frequency, nutritive value

## Introduction

Napier grass and Rhodes grass are the major cultivated forage grasses in Kenya due to their relatively high herbage yield, ease of propagation and management. In the intensive market oriented smallholder livestock production systems of central Kenya, Napier and Rhodes grass constitute between 40 and 80% of forages used by smallholder dairy farmers (Romney *et al*., 2004). Although there are efforts to evaluate new more adaptive cultivars of Napier grass (Wamalwa, 2013) there has been limited research on other grasses to widen the genetic base of fodders for the region. In addition, the emerging lethal diseases such as Napier smut (Farrel *et al*., 2001) and Napier stunt (Jones *et al*., 2004) reduces the yield of Napier grass by 40 to 90% (Orodho, 2007) and therefore the urgent need to identify and promote other high quality forages that are adapted to cool sub-humid highlands of central Kenya.

Brachiaria grasses are productive warm-season perennial grasses with superior nutritive value to other warm-season grasses (Vendramini *et al.*, 2014), and can be used for grazing (Inyang *et al*., 2010a) or harvested and conserved for feeding when needed (Vendramini *et al*., 2010). Brachiaria grasses are indigenous to eastern central and southern Africa (Ndikumana and de Leeuw, 1996) and have revolutionized the livestock industry as the most adaptable and widely cultivated in South America (Miles *et al*., 2004). The potential of improved Brachiaria grass in its native land Africa remains largely unexploited and yet they offer opportunities to address the challenge of livestock feed scarcity.

A few Brachiaria grass cultivars were introduced to improve forage production, broaden the range of adapted grasses, and ensure high nutritive value in Kenya. The introduction of new cultivars should be based on adequate understanding of physiological processes and growth potential under a range of management practices. Cutting frequency is an important management practice that affects herbage accumulation, nutritive value and persistence of warm-season grasses (Inyang *et al*., 2010). Hare *et al*. (2013) compared Mulato II, Cayman and BR02/1794 in Thailand and concluded that cutting at 30 days intervals would produce CP levels of 3 − 4% higher than cutting at 45 and 60 days intervals, but herbage accumulation was 20% lower than cutting at the longer intervals. Inyang *et al*. (2010b) studied the effects of regrowth interval and stubble height on herbage accumulation, nutritive value and persistence of Mulato II. While herbage harvested at 2 week regrowth intervals had greater nutritive value, herbage accumulation was less than observed for longer regrowth intervals, supporting results reported with other warm-season grasses. However, there is little information on herbage accumulation and nutritive value of these new hybrids in tropical regions and in particular Kenya. Consequently, there is need to understand the production of these grasses under different management option. The specific objective of this study was to determine the herbage yield and nutritive value of selected Brachiaria cultivars under different cutting frequency in the cool sub-humid highlands of central Kenya.

**Materials and methods**

*Site description*

The experiment was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO) Ol joro Orok Centre, in the cool central highlands of Kenya from December 2013 to March 2016. The site is located at latitude, $00^0$ 22' S and longitude, $36^0$ 46' W at altitude of 2393 m asl in Upper Highlands 2-3 (UH 2-3) agro ecological zone (Jaetzold *et al*., 2006. Average annual rainfall is about 950 mm is bi-modal with the long rains from March to May and the short rains from September to December. Temperature ranges from 8 and 22 °C with a mean of 13$^o$C. The soils are classified as verto-luvic and chromo-luvic Phaeozems (Sombroek *et al.*, 1982). These are well drained with good permeability. Prior to initiation of the experiment, the pH was 4.7 (1:2.5 soil: water), 0.24% total nitrogen (adequate), 10 ppm phosphorus (low), 1.72 me potassium (adequate) and 2.4 % total organic carbon (moderate).

*Experimental design and treatment*

The grass treatments were *Brachiaria brizantha* cvs. Marandu, Xaraes, Piata, and MG4, *B. decumbens* cv. Basilisk, *B. humidicola* cvs. Humidicola and Llanero and *Brachiaria hybrid* cv. Mulato II while Napier grass (*Pennisetum purpureum* Schum.) and Rhodes grass (*Chloris gayana*) were included as control. The treatments were laid out in a randomized complete block design in a split plot arrangement with four replications. The main plots contained the grasses while the defoliation frequencies, 6, 8 and 12 weeks formed the sub-plots. The plot size measured 4m by 5 m. Phosphorus was applied to the soil prior to sowing of the seeds at a rate of 40 kg P $ha^{-1}$ in the planting rows. The grass seeds were drilled by hand in furrows about 1-2 cm deep in a well prepared seed bed with an inter row spacing of 0.5 m and covered with a thin layer of soil. The plots were kept weed free by hand weeding. All the plants were cut back to a 5 cm stubble height at the end of the establishment phase (20 weeks after seedling) to stimulate uniform growth. Plots were top-dressed with 50 kg N $ha^{-1}$ per season and the application commenced after standard cut.

*Measurements*

Measurements were made on dry matter yield at 6, 8 and 12 weeks intervals during the wet and dry seasons per schedule in Table 1. Dry matter yield was determined from a net harvest area of 2 m x 2 m. The harvesting was done by cutting the grasses at a stubble height of 5 cm above the ground and the fresh herbage weights recorded and dried at 60°C to a constant weight for dry matter determination and forage quality analysis.

**Table 1** Seasons for sampling during evaluation of Brachiaria cultivars

| | |
|---|---|
| First wet season | 20th May 2014 to 20th October 2014 |
| First dry season | 20th October 2014 to 6th April 2015 |
| Second wet season | 6th April 2015 to 27th September 2015 |
| Second dry season | 28th September 2015 to 30th March 2016 |

## Statistical Analysis

The data on DM yield and nutritive value parameters were analysed using PROC MIXED General Linear model of SAS (SAS Institute Inc., 2001) with cultivars and cutting interval, year and their interactions as fixed effects. Block and its interactions were random effects. The means were separated using the least significant difference (LSD) test at $P < 0.05$ (Steel and Torrie, 1981).

## Results and Discussion

*Seasonal condition*

The rainfall received during the experiment period was below the medium term average of 11 years during the long rains (March - May) while during the short rains season it was around the medium term average (Figure 1).

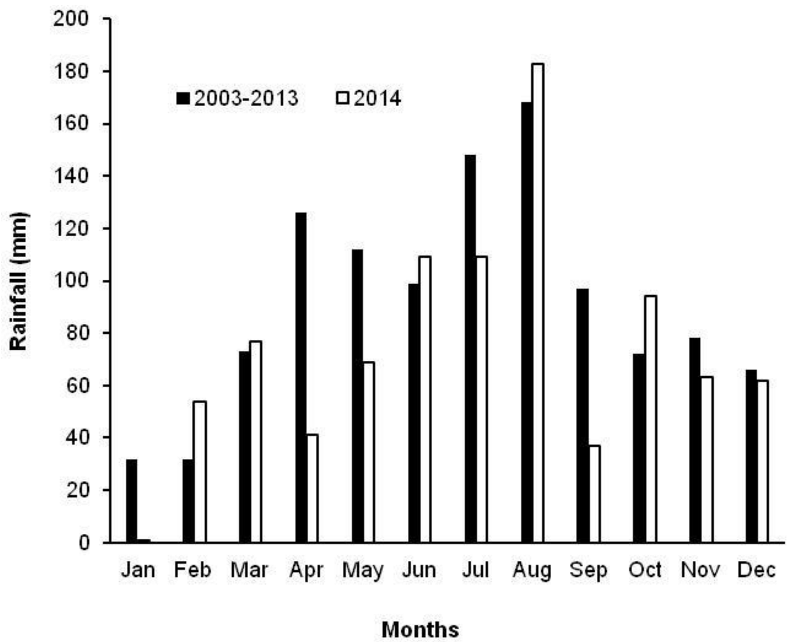


**Figure 1** Rainfall received at Ol joro Orok, during the experiment period and the 11-years average (2003 - 2013)

*Dry matter yield*

Only data for the first west season (May - Oct 2014) and first dry season (Oct 2014 - Apr 2015) is reported. There was no significant ($P > 0.05$) interaction between the Brachiaria cultivars and cutting interval on DM yield in both seasons. However, significant difference occurred among the Brachiaria and the cutting interval in both seasons. During the wet season, Basilisk and Piata had the highest DM yield but were similar to Napier grass but higher than Rhodes (Figure 2a). Marandu, MG4, and Xaraes had similar yield to Rhodes. Llanero had the lowest yield followed by Mulato II. Increasing cutting interval from 6, 8 and 12 weeks resulted in increased DM yield (Figure 2b). During the dry season, Napier grass out yielded all the Brachiaria grass cultivars. Among the Brachiaria cultivars, Piata and MG4 were the most productive and had higher ($P < 0.05$) yield than Rhodes grass (Figure 2c). Llanero had the lowest yield and was the only cultivar that produced lower yield than Rhodes grass. Reducing cutting interval from 8 to 6 weeks and increasing cutting interval from 8 to 12 weeks resulted in increasing DM yield (Figure 2d).

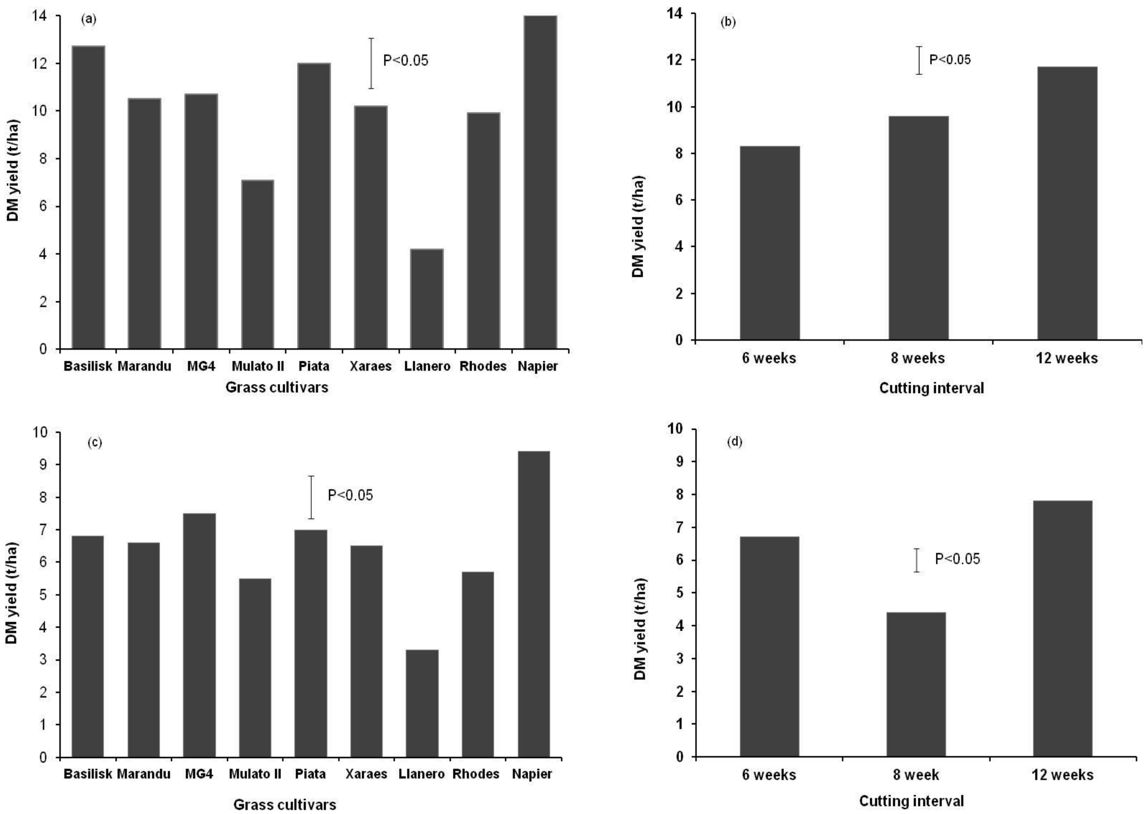


**Figure 2** Dry matter yield of Brachiaria grass cultivars during; (a, b) long rains and (c, d) dry season at Ol joro Orok.

*Herbage nutritive quality*

There were no significant interaction between the cultivars and the cutting interval on any of the parameters analysed for chemical composition. Significant effects occurred on both the main effects (cultivars and cutting interval). All the Brachiaria grass cultivars except Piata had CP similar to Napier grass (Table 2). The mean CP of 14% recorded among the Brachiaria grasses was higher than that reported by Hare *et al*. (2015) for Mulato II and Mendonca *et al*. (2013) for *B. decumbens*. All Brachiaria cultivars had similar NDF to Napier grass but were lower ($P < 0.05$) than Rhodes grass. The mean NDF of 64% among the Brachiaria grasses was higher than that reported by Hare *et al*. (2015). Napier grass had higher DMD than the Brachiaria cultivars except MG4 while Rhodes grass had similar DMD to all the Brachiaria grasses. Xaraes had highest Ca content and was more than Mulato II, Piata and Rhodes grass.

The mean CP content at 6 to 8 weeks cutting interval was similar but further increase of cutting interval to 12 weeks resulted in decreased CP (12.6%) (Table 3). The decrease in CP with increasing cutting interval is consistent with findings by Vendramini *et al*. (2014) on Brachiaria hybrids. The CP of Brachiaria grasses, was about double the minimum CP concentration of 60 to 80 g $kg^{-1}$ dry matter (DM) required for optimum rumen microbial activity (Minson and Milford, 1967), suggesting that the Brachiaria grasses could be used to supplement livestock feeding on low quality roughages. Varying the cutting interval did not affect dry matter digestibility and this could be attributed to low fibre content at both 6 and 12 weeks. However, increasing cutting intervals from 6 to 8 weeks resulted in declining Ca concentration in the herbage but on the contrary the P content increased significantly ($P < 0.05$).

**Table 2** Chemical composition (% of DM) of Brachiaria grass, Rhodes and Napier grass at Ol joro Orok in the cool sub-humid highlands of central Kenya

| Cultivar | CP | NDF | ADF | ADL | OMD | DMD | Ca | P |
|---|---|---|---|---|---|---|---|---|
| Napier grass | 14.7 | 64.6 | 43.7 | 3.86 | 55.7 | 55.9 | 0.36 | 0.36 |
| MG4 | 14.1 | 64.9 | 39.4 | 4.05 | 51.4 | 52.2 | 0.40 | 0.41 |
| Basilisk | 13.6 | 64.2 | 40.8 | 3.59 | 53.5 | 51.1 | 0.38 | 0.38 |
| Mulato II | 15.4 | 59.6 | 39.7 | 3.79 | 50.5 | 48.3 | 0.36 | 0.35 |
| Rhodes grass | 12.6 | 70.3 | 44.1 | 5.11 | 51.9 | 52.4 | 0.40 | 0.42 |
| Piata | 12.6 | 64.5 | 41.2 | 4.04 | 52.5 | 50.2 | 0.36 | 0.32 |
| Marandu | 13.8 | 64.9 | 41.7 | 3.92 | 53.6 | 50.7 | 0.39 | 0.39 |
| Xaraes | 14.8 | 64.4 | 41.3 | 3.95 | 51.1 | 48.4 | 0.45 | 0.40 |
| Llanero | 14.6 | 64.1 | 41.3 | 3.97 | 53.1 | 49.2 | 0.35 | 0.28 |
| LSD (P<0.05) | 1.6 | 3.96 | 1.8 | 0.92 | NS | 4.22 | 0.08 | 0.05 |
| CV (%) | 13.7 | 7.6 | 5.2 | 28.0 | 11.7 | 10.2 | 25.1 | 17.9 |

**Table 3** Effects of cutting interval on minerals and chemical composition (% of DM) of Brachiaria grass at Ol joro Orok in the cool sub-humid highlands of central Kenya

| Weeks | CP | NDF | ADF | ADL | OMD | DMD | Ca | P |
|---|---|---|---|---|---|---|---|---|
| 6 | 14.7 | 65.6 | 42.6 | 4.04 | 52.1 | 49.6 | 0.46 | 0.34 |
| 8 | 15.2 | 61.5 | 40.6 | 3.66 | 53.8 | 51.2 | 0.38 | 0.32 |
| 12 | 12.6 | 65.1 | 41.1 | 4.49 | 52.2 | 51.8 | 0.32 | 0.42 |
| LSD | 0.9 | 2.17 | 1.0 | NS | NS | NS | 0.05 | 0.03 |
| CV (%) | 13.7 | 7.6 | 5.2 | 28.0 | 11.7 | 10.2 | 25.1 | 17.9 |

## Conclusions

Among the Brachiaria cultivars, Piata consistently produced high DM yield in both wet and dry season while Basilisk had high yield in wet season and MG4 in the dry season. During the wet season, the most productive Brachiaria had similar yield to Napier grass but during the dry season, Napier grass out yielded all the Brachiaria grass. The performance of Llanero was generally poor. The nutritive quality in term of CP for most of the Brachiaria was similar to Napier and Rhodes grass. It is important to examine the data for the other two seasons to conclusively determine the performance of the Brachiaria grass cultivars in the cool highlands of central Kenya.

## Acknowledgements

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# Effects of cutting frequency on forage production and nutritive value of Brachiaria grass cultivars in coastal lowlands of Kenya

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## Abstract

Feed shortage during the dry season limits livestock productivity in coastal lowlands of Kenya. A study was conducted to evaluate the effect of cutting frequency on productivity and nutritive value of Brachiaria grass cultivars. Seven (7) Brachiaria grass cultivars: *Brachiaria brizantha* cvs. Marandu, Xaraes, Piata and MG4, *B. decumbens* cv. Basilisk, *Brachiaria hybrid* cv. Mulato II, *B. humidicola* cv. Llanero were evaluated along *Chloris gayana* cv. ex-Tozi as a control at Mtwapa and Msabaha in the coastal lowlands of Kenya. Plant numbers, tiller numbers and dry matter (DM) yield were monitored at 6, 8 and 12 weeks intervals in 2014 and 2015 in both long and short rain seasons. At Mtwapa, generally increasing cutting interval from 6 to 8 weeks resulted to increased DM yield but further increase to 12 weeks, the yield either remained the same or declined. At Msabaha, there was no distinct trend on DM yield by increasing cutting interval. Mulato II, Marandu, MG4 and Xaraes had the highest yield in most of the seasons. The crude protein was generally low (5.3 - 7.7% of DM) and was similar among the Brachiaria grass cultivars and also to Rhodes grass and Napier grass. The CP content at 6 and 8 weeks cutting interval was similar (7.12 and 7.24% of DM) and by increasing cutting interval to 12 weeks it declined to 4% of DM. Similarly increasing cutting interval from 6 to 12 weeks resulted to decline in digestibility. Based on high nutritive quality at 8 week cutting interval and relatively high DM; it can be concluded that harvesting Brachiaria at 8 weeks cutting interval is appropriate in coastal lowlands of Kenya.

**Key words**: Brachiaria, cutting interval, dry matter yield, forage; nutrient; yield

## Introduction

Seasonal feed shortage and inadequate nutrient supply are major constraints to livestock production in coastal Kenya (Mburu, 2015). Ruminant livestock are a predominant component of mixed farming in the region. Dairy production contributes to both improved household nutrition and income (Nicholson *et al*., 2002). Dairy cattle are mainly fed on natural pastures since Napier grass, the recommended fodder is grown by only 10% of the farmers (Njarui *et al*., 2016). Knowledge on the effects of harvesting frequency on foliage yield and quality is essential for development of successful livestock year round feeding strategies. The interval between harvests of grasses affects herbage production, nutritive value and re-growth ability. According to Ball *et al*, (2009), forage quality is influenced by forage species, stage of maturity at harvest, soil fertility and climatic factors. Young re-growth is characterized by high protein, low cellulose and lignin and high digestibility (Wijiphans *et al*., 2009). Various grass harvest intervals and intensity studies revealed that the cutting interval influence growth, yield and persistence of the sward (Probst *et al*., 2011). Slow re-growth of the forages was observed immediately after cutting as the plants had few leaves to intercept light for photosynthesis. To ensure improved and sustainable livestock production under the global influence of climate change, forage management strategies that optimizes the quantity and quality of fodder supplies is necessary. A study was therefore conducted to assess the seasonal dry matter (DM) production and nutritional value of seven (7) *Brachiaria* grasses under different cutting intervals in coastal lowlands of Kenya.

## Materials and methods

*Sites*

The study was conducted at Mtwapa and Msabaha in the coastal lowlands. The location, detailed climatic condition and soil characteristics of these sites are given by Ondiko *et al*. (2016), in these proceedings.

## Experimental design and treatments

Seven (7) *Brachiaria* grass cultivars: *B. brizantha* cvs. Marandu, Xaraes, Piata and MG4, *B. decumbens* cv. Basilisk, *B. hybrid* cv. Mulato II, *B. humidicola* cv. Llanero were evaluated. Rhodes grass (*Chloris gayana*) cv. ex-Tozi was included as a control. The experimental design was completely randomized block in a split plot arrangement with four replications. The main plots were cultivars and the sub plots were the cutting frequencies (6, 8 and 12 weeks). The plot size was 5 x 4 m with a 1 m path between plots and 1.5 m between replicates. The seeds were sown in November 2013 in furrows of about 2 cm deep on well prepared seed bed after ploughing and disc harrowing. The inter row spacing was 0.5 m, giving 10 rows in each plot. Triple super phosphate (TSP, 46% $P_2O_5$) was applied at the rate of 200 kg/ha prior to sowing of the seeds. The trials were kept free from weeds by hand weeding and slashing within the plots. A standardization cut was carried out in April 2014 at onset of rains which marked the end of the establishment phase. The nitrogenous fertilizer (26% N) was applied at the rate of 100 kg/ha which was done after the standard cut at the onsets of rains.

*Data collection*

The data recorded were number of plants, tiller number and dry matter at a regrowth period of 6, 8 and 12 weeks after standardization cut. The plot was sub-divided into three plots, and 2 inner rows were sampled leaving a guard row at each side. The number of plants per unit was determined by counting the plants within a 1 x 1 m frame randomly placed over the two rows. Four plants within the central rows were randomly selected for tiller number determination. Immediately after the measurements, fresh biomass production for the re-growth at 6, 8 and 12 weeks were harvested and weighed; sub samples were taken and oven dried at 65°C to constant weight for DM determination.

*Herbage chemical analysis*

Crude protein (CP) was determined using micro-Kjeldahl according to the method of Association of Official Analytical Chemist (AOAC, 2000). The acid detergent fibre (ADF), neutral detergent fibre (NDF) digestibility and lignin were analysed according to Van Soest and Robertson, 1980.

Ash was determined by heating the samples at 600°C for 2 hours in a muffle furnace. Total P and Ca were determined according to the methods described by Okalebo *et al.* (2002).

**Statistical analyses**

The forage DM yields were grouped into LR and SR seasons for each cultivar and the three harvesting intervals (6, 8 and 12 weeks). Data on plant population, tiller number and mean DM yield per season were statistically evaluated using analysis of variance (SAS, 2010). The means were separated using Tukey's HSD at 5% level of significance.

**Results**

*Seasonal condition*

Rainfall and temperature during the experimental period (2014-2015) at Mtwapa and Msabaha are given in Figure 1. At Mtwapa, the total rainfall was highest in LR 2014 (851 mm) and LR 2015 (855 mm) and lowest in SR 2015 (289 mm). The minimum and maximum temperatures were 23 and 29°C, respectively. Similarly the rainfall at Msabaha was highest in LR 2014 season (592 mm) and LR 2015 (607 mm) and lowest in SR 2015 (136 mm). The minimum and maximum temperatures were similar to that of Mtwapa.

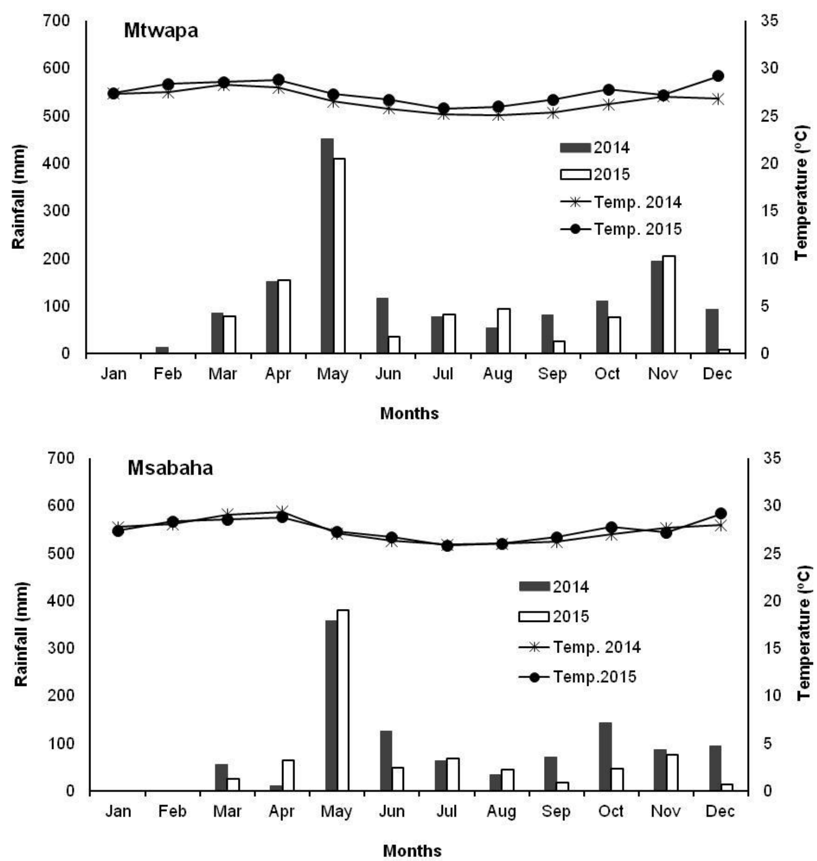


**Figure 1** Total monthly rainfall and average temperature during the experimental period 2014- 2015 at Mtwapa and Msabaha

**Plant number**

There were differences (P < 0.05) on plant numbers among the Brachiaria grasses in both sites. Generally, the Brachiaria cultivars had more plant numbers at Mtwapa than at Msabaha. At Mtwapa, Llanero had consistently the highest number of plants in all the seasons but differed (P < 0.05) from all the other Brachiaria cultivars only in LR 2014 (Table 1). In LR 2014 it had 40 plants/$m^2$ and declined to 20.5 plants/$m^2$ in SR 2014 and further to 13.3 plants/$m^2$ in SR 2015. Mulato II had the lowest plant numbers in all the seasons. At Msabaha, Llanero had the highest plant numbers in LR 2014 while in the other seasons none of the cultivars had consistently the highest plant numbers (Table 1). Like in Mtwapa plant number declined over time for all Brachiaria cultivars except Mulato II where they remained fairly stable. However, plant number declined with increasing cutting interval from 6 to 12 weeks at both sites (Table 2).

**Table 1** Number of surviving plants /$m^2$ during the long and short rainy seasons at Mtwapa and Msabaha, coastal lowlands**.**

| Grass cultivars | Mtwapa | | | | Msabaha | | | |
|---|---|---|---|---|---|---|---|---|
| | LR 2014 | SR 2014 | LR 2015 | SR2 015 | LR 2014 | SR 2014 | LR 2015 | SR 2015 |
| Llanero | 40.8a | 19.5ab | 19.2a | 13.3a | 20.5a | 14.7a | 11.6a | 11.0a |
| Basilisk | 28.4b | 19.1abc | 16.3a | 9.3a | 16.0ab | 16.1a | 10.1a | 8.5ab |
| MG4 | 21.7bc | 18.3abc | 14.5b | 9.9a | 16.5ab | 16.8a | 11.3a | 11.0a |
| Piata | 20.4cd | 18.3abc | 16.4a | 9.3a | 12.2ab | 16.7a | 10.0ab | 9.6ab |
| Xaraes | 19.5cd | 15.8bc | 15.9a | 9.2a | 8.0ab | 12.9ab | 9.8ab | 8.3ab |
| Marandu | 19.4cd | 17.4abc | 15.1b | 9.3a | 10.5ab | 12.5ab | 10.7a | 10.2a |
| Mulato II | 14.3d | 13.3c | 13.4b | 8.7a | 7.6ab | 14.3a | 12.9a | 12.3a |
| Rhodes grass | 37.6a | 23.1a | 17.2a | 11.3a | - | - | - | - |
| Mean | 25.3 | 18.1 | 16.2 | 10.0 | 13.0 | 14.8 | 10.9 | 10.1 |
| CV% | 11.6 | 13.9 | 12.4 | 19.7 | 83.5 | 47.3 | 33.4 | 33.4 |

Means followed by a different letter within a column are significantly (P<0.05) different

**Table 2** The effects of cutting frequency on plant numbers/$m^2$ at Mtwapa and Msabaha, coastal lowlands.

| Cutting interval (weeks) | Mtwapa | | | | Msabaha | | | |
|---|---|---|---|---|---|---|---|---|
| | LR 2014 | SR 2014 | LR 2015 | SR 2015 | LR 2014 | SR 2014 | LR 2015 | SR 2015 |
| 6 | 29.8 a | 28.3a | 21.2a | 9.7b | 18.9a | 15.6b | 15.6a | 13.9a |
| 8 | 26.5b | 18.3b | 13.7b | 8.6b | 11.0b | 19.9a | 10.7b | 14.0a |
| 12 | 19.4c | 7.72c | 13.4b | 10.8a | 8.1b | 6.9c | 5.0c | 6.6b |

Means followed by a different letter within a column are significantly (P<0.05) different

*Tiller number*

There was variation in the numbers of tillers among the Brachiaria grass cultivars in all seasons. Overall, the Brachiaria cultivars had more tillers during the LR seasons than in the SR seasons at both sites. At Mtwapa, Mulato II maintained the highest number of tillers in all the seasons (Table 3). Rhodes grass recorded lowest number of tillers compared with all the other Brachiaria grasses except in SR 2015. At Msabaha, Mulato II had consistently the highest number of tillers in LR 2014, SR 2014 and LR 2015 and was among the Brachiaria with the highest number of tillers in SR 2015 (Table 3). Piata and Llanero tended to have the lowest number of tillers in most of the seasons. There was no definite trend on number of tillers by increasing cutting interval from 6 to 12 weeks; the number either increased on declined in different season (Table 4).

**Table 3** Average seasonal cumulative tiller numbers per plant during the production phase (2014-2015) at Mtwapa and Msabaha.

| Grass cultivars | Mtwapa | | | | Msabaha | | | |
|---|---|---|---|---|---|---|---|---|
| | LR 2014 | SR 2014 | LR 2015 | SR 2015 | LR 2014 | SR 2014 | LR 2015 | SR 2015 |
| Mulato II | 78.6a | 43.3a | 83.0a | 57.1a | 75.8[a] | 34.3[ab] | 48.3[a] | 32.6[ab] |
| MG4 | 48.9b | 29.6b | 53.7bc | 42.0ab | 48.4[ab] | 28.1[ab] | 36.8[ab] | 35.0[a] |
| Marandu | 43.8b | 29.5b | 45.9b | 37.0b | 53.8[ab] | 31.8[ab] | 35.6[ab] | 27.9[ab] |
| Basilisk | 39.3b | 28.6b | 39.6bc | 41.0ab | 48.3[ab] | 28.8[ab] | 32.8[ab] | 21.4[ab] |
| Xaraes | 36.7b | 24.9b | 40.0bc | 29.5b | 44.7[b] | 28.3[ab] | 31.3[ab] | 26.6[ab] |
| Piata | 36.5b | 24.5b | 38.8bc | 35.1b | 37.3[bc] | 24.0[b] | 27.8.[b] | 22.0[ab] |
| Llanero | 35.7b | 26.4b | 28.1c | 29.6b | 36.8[bc] | 33.6[ab] | 28.6[b] | 16.5[bc] |
| Ex-Tozi | 33.4b | 23.1b | 26.1c | 37.7ab | - | - | - | - |
| Mean | 44.1 | 28.7 | 44.4 | 38.6 | 49.3 | 29.8 | 33.6 | 26.8 |
| CV% | 10.6 | 19.7 | 20.7 | 18.9 | 72.2 | 41.8 | 47.7 | 38.8 |

Means followed by different letter within same column are significantly (P<0.05) different

**Table 4** The effects of cutting frequency on tiller numbers/m$^2$ at Mtwapa and Msabaha.

| Cutting interval (weeks) | Mtwapa | | | | Msabaha | | | |
|---|---|---|---|---|---|---|---|---|
| | LR 2014 | SR 2014 | LR 2015 | SR 2015 | LR 2014 | SR 2014 | LR 2015 | SR 2015 |
| 6 | 45.5a | 31.2a | 46.6b | 33.9b | 49.8ab | 23.8b | 30.1b | 35.5a |
| 8 | 44.7a | 28.6ab | 45.8b | 33.6b | 41.3b | 32.6a | 30.3b | 12.8c |
| 12 | 42.1a | 26.26b | 51.3a | 40.8a | 52.9a | 34.8a | 41.2a | 28.3b |

Means followed by different letter within same column are significantly (P<0.05) different

## Dry matter yield

*Mtwapa*

There was a significant interaction between Brachiaria cultivar and cutting interval on DM yield at Mtwapa. The DM yield increased for all the Brachiaria cultivars with increased cutting interval from 6 to 8 weeks during LR 2014 season except for Xaraes and Rhodes grass where yield declined. At 12 weeks the yield remained relatively the same or declined marginally for some cultivars (Figure 2). In SR 2014 season, DM yield were highest at 6 and 8 week cutting interval and declined at 12 weeks cutting interval. In both LR 2015 and SR 2015, yield increased with increasing cutting interval from 6 to 8 weeks but at 12 weeks the yield either remained relatively the same or declined significantly for some of the Brachiaria grasses. None of the Brachiaria out yielded the others in LR 2015 but in SR 2015 Llanero had highest yield at 12 weeks cutting interval. Mulato II, Piata, MG4, and Marandu tended to have the attained the highest yield in most of the season.

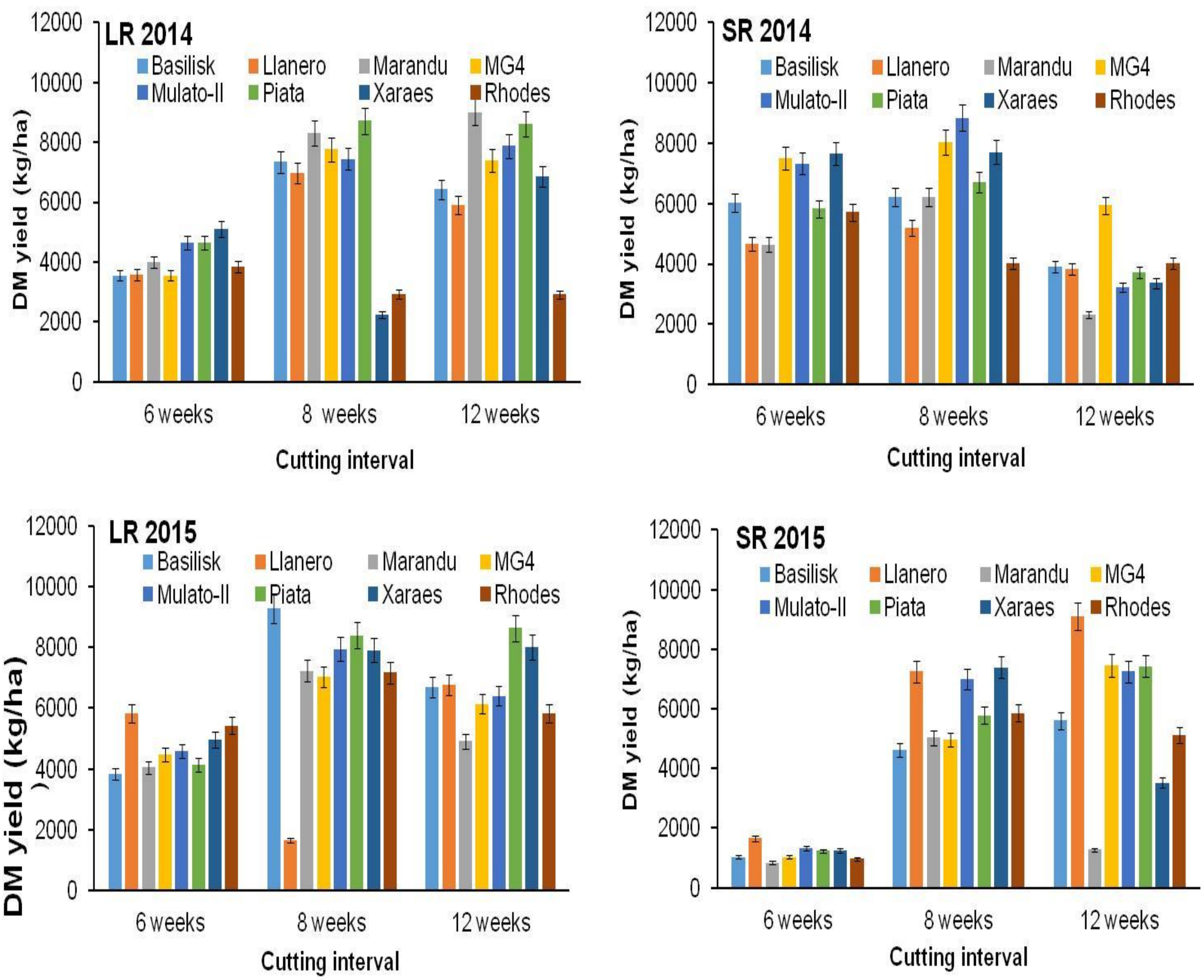


**Figure 2** Seasonal DM yield (kg ha$^{-1}$) of grasses at Mtwapa in coastal lowlands of Kenya.

*Msabaha*

Like in Mtwapa, there was also a significant interaction between Brachiaria cultivars and cutting interval on DM yield at Msabaha. However there was no distinct trend on DM yield by increasing cutting interval across seasons. In LR 2014, there were large variation in yield among the Brachiaria grasses at 6 and 8 weeks cutting interval but at 12 weeks yield were almost similar (Figure 3). In SR 2014 yield tended to higher at 6 and 8 weeks cutting interval and declined at 12 weeks for all the Brachiaria grasses. In LR 2015, increasing cutting interval from 6 to 8 week resulted in increased DM yield for all the Brachiaria but at 12 weeks cutting interval, yield declined considerably. In SR 2015 yield were relatively low (<2000 kg/ha) for all the Brachiaria in all cutting interval.

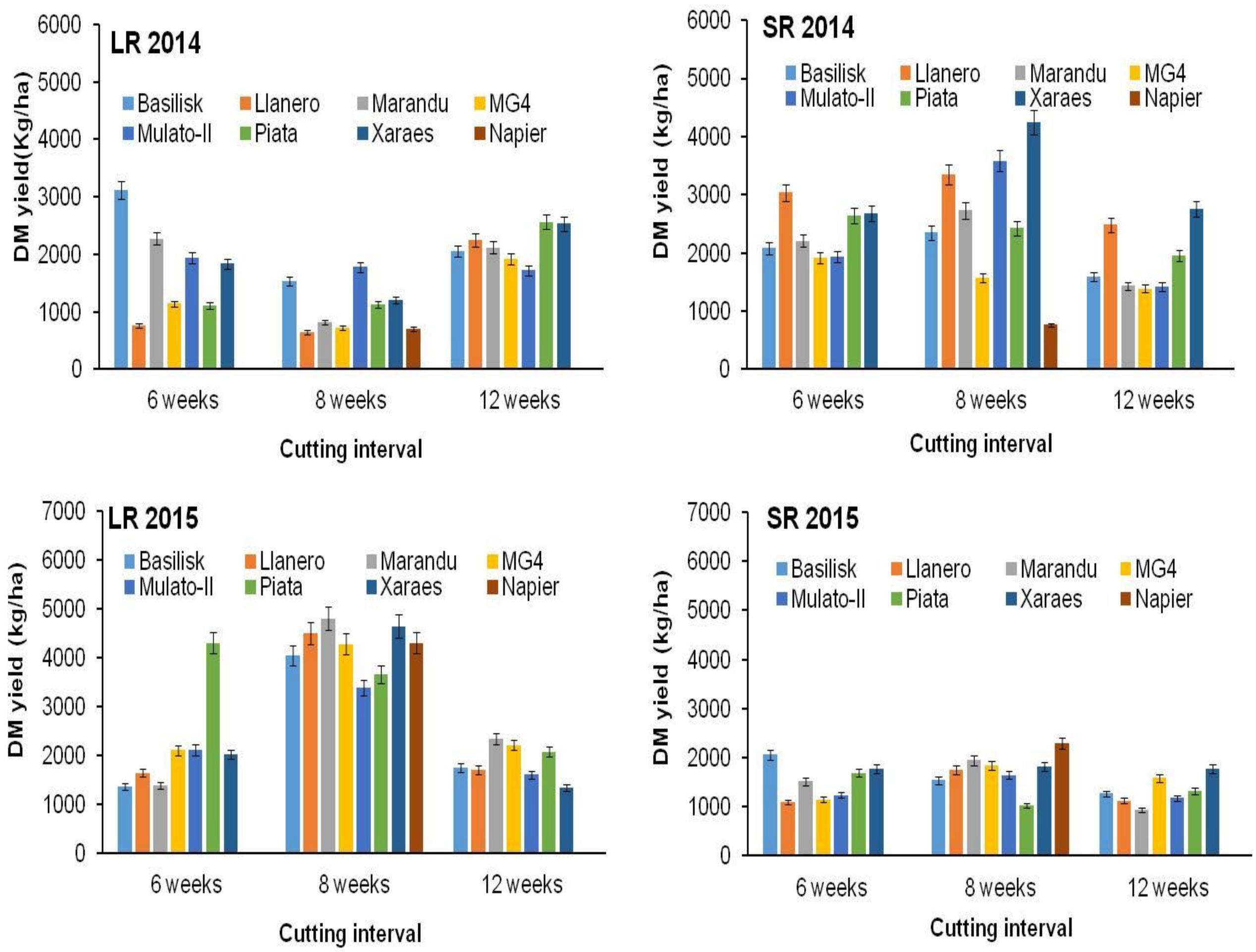


**Figure 3** Seasonal DM yield (kg ha$^{-1}$) of grasses at Msabaha in coastal lowlands of Kenya.

*Chemical composition*

The crude protein was generally low (5.3 - 7.7% of DM) and was similar among the Brachiaria grass cultivars and also to Rhodes grass and Napier grass. There were differences ($P < 0.05$) in ADF, NDF, digestibility, ash and Ca content. Rhodes grass had the highest ADF and NDF compared with the other the Brachiaria grasses but was only significantly (P<0.05) different from Mulato II (Table 5). Napier grass had the highest DMD (57.6%) but was only higher (P < 0.05) than Basilisk, Xaraes and Rhodes grass. Rhodes grass had higher Ca than Piata, MG4, Basilisk and Llanero. Increasing cutting interval from 6 to 12 weeks resulted in decreasing CP content and DMD but the fibres increased (Table 6).

**Table 5** Chemical composition (% of DM) for Brachiaria cultivars and controls in coastal Kenya

| Grass cultivars | CP | ADF | NDF | ADL | DMD- | Ash | Ca | P |
|---|---|---|---|---|---|---|---|---|
| Piata | 5.4a | 48.9abc | 66.6ab | 2.5a | 50.2ab | 8.3ab | 0.3b | 0.3a |
| Marandu | 6/0a | 49.0abc | 65.7ab | 3.2a | 51.2ab | 8.4ab | 0.4ab | 0.3a |
| Mulato-II | 5.8a | 46.2c | 61.7b | 2.1a | 52.2ab | 9.4ab | 0.4ab | 0.3a |
| MG4 | 6.2a | 49.5abc | 65.7ab | 3.3a | 48.3abc | 8.9ab | 0.3b | 0.3a |
| Basilisk | 7.7a | 49.7abc | 65.1ab | 4.0a | 46.0bc | 7.8b | 0.3b | 0.3a |
| Llanero | 6.5a | 48.5abc | 67.4ab | 3.0a | 52.1ab | 8.7ab | 0.3b | 0.3a |
| Xaraes | 4.9a | 50.6ab | 63.7ab | 2.8a | 43.8bc | 8.1ab | 0.4ab | 0.3a |
| Rhodes grass | 5.3a | 52.4a | 69.4a | 3.4a | 40.4c | 6.9b | 0.5a | 0.3a |
| Napier grass | 6.9a | 48.2bc | 61.1b | 2.9a | 57.6a | 11.0a | 0.4ab | 0.3a |
| Mean | 6.1 | 49.2 | 65.1 | 3.0 | 49.1 | 8.6 | 0.4 | 0.31 |
| CV (%) | 26.0 | 2.9 | 3.9 | 38.4 | 6.7 | 12.1 | 14.2 | 12.3 |
| SE | 1.6 | 1.4 | 2.6 | 1.2 | 3.3 | 1.0 | 0.1 | 0.04 |

CP= Crude protein, ADF- Acid detergent fibre, NDF-Neutral detergent fibre, ADL-acid detergent lignin, , DMD%-, Ca-calcium, P- phosphorus, CV%- coefficient variation, se- standard error

**Table 6** Effects of cutting interval; 6, 8 and 12 weeks on nutritive quality (% of DM) of Brachiaria grass at Mtwapa.

| Cutting interval | CP | ADF | NDF | ADL | DMD- | Ash | Ca | P |
|---|---|---|---|---|---|---|---|---|
| 6 | $7.12^{a}$ | $46.36^{b}$ | $62.28^{b}$ | $3.08^{ab}$ | $55.63^{a}$ | $10.39^{a}$ | $0.39^{a}$ | $0.36^{a}$ |
| 8 | $7.24^{a}$ | $49.89^{a}$ | $63.34^{b}$ | $2.02^{b}$ | $47.82^{b}$ | $9.346^{b}$ | $0.35^{ab}$ | $0.20^{b}$ |
| 12 | $3.99^{b}$ | $51.41^{a}$ | $69.84^{a}$ | $3.92^{a}$ | $48.80^{c}$ | $6.11^{c}$ | $0.33^{b}$ | $0.25^{c}$ |

CP= Crude protein, ADF- Acid detergent fibre, NDF-Neutral detergent fibre, ADL-acid detergent lignin, , DMD%-, Ca- calcium, P- phosphorus, Means followed by a similar letter with the same row are not different (P>0.05) within a column

## Discussion

The study demonstrated variation in tiller development and productivity among the Brachiaria grasses. Basilisk and Llanero produced high plant number among the cultivars in all the cutting intervals. The number of tiller increased with frequent cutting interval as observed by Onyeonagu *et al*. (2005a). The effect of rainfall on forage yields in the coastal lowlands was demonstrated with higher yield recorded during the LR season when the rainfall was high. The DM yields at Msabaha were 46% lower than those obtained at Mtwapa and this was attributed to the lower rainfall at that site. Msabaha is located in CL4 which receives less rainfall than CL3 where Mtwapa is located. The total rainfall at Mtwapa during the LR 2014 was 851 mm compared to 592 mm at Msabaha while in SR 2015 rainfall at Mtwapa (289 mm) was twice that of Msabaha (136 mm).

Among the tested grasses Mulato II, Piata, Marandu and Xaraes showed outstanding potential as a forage plant in coastal lowlands since it has shown that it can grow under low rainfall maintaining high yields. More tillers were reported for Mulato II at Mtwapa and in Msabaha. The grasses at the 8 week interval have had developed stems and leaf photosynthetic area, resulting into higher dry matter production (Vinther, 2006). The current results are in agreement with Vinther (2006) who found that harvest interval affects productivity, partly through changes in their morphological development. DM production is thus related to harvest frequency.

**Nutritional composition**

The CP content was general low in all the Brachiaria grasses (5.3 - 7.7%) compared with mean of 7-10% reported by Nguku *et al.* (2015) in the semi-arid region of eastern Kenya. However, the CP of 7.7% content from Basilisk was relatively higher compared with that reported by Evitayani *et al*. (2004a). The high temperatures at Mtwapa could have contributed to plants having lower CP than expected as temperatures have been reporded to have effect of quality of grasses (Njarui *et al*., 2015). The ADF content of Rhodes grass was higher was less digestible than Brachiaria indicating that Brachiaria grasses are superior to Rhodes grass. As ADF increased, the DMD declined and this is consistent with work of Albayrak *et al*. (2011) who reported that as the ADF increases the digestibility of the forage usually decrease causing consumption of the forage by animal to decrease. However, the stage at which the grass is harvested may have positive or negative impact on quality. As reported by Bruinenberg *et al*. (2002), at a given harvest date, the differences in digestibility of DM of grasses may occur because of differences in the phenological stage.

**Conclusions**

Based on this research, it can be concluded that the cutting intervals can affect the forage DM yield and nutritive values of Brachiaria grasses. Cutting 8 weeks interval could be the optimal level for harvesting Brachiaria grass since the quality forage is high and yield are not compromised. The forages should be fed to ruminant livestock to determine animal responses in terms of production of milk, meat and animal health.

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# Establishment and early growth of Brachiaria grass cultivars in acidic soils of western Kenya

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KALRO - Kitale

### Abstract

Successful establishment determines productivity and long term persistence of pastures. The objective of this study was to evaluate the establishment and early growth of eight Brachiaria grass cultivars at three sites in the humid highlands of western Kenya. The grass cultivars were; *Brachiaria brizantha* cvs. Marandu, Xaraes, Piata, and MG4, *B. decumbens,* cv. Basilisk, *B. humidicola* cvs. Humidicola and Llanero and *Brachiaria hybrid* cv. Mulato II. Rhodes grass (*Chloris gayana*) and Napier grass (*Pennisetum purpureum* cv. Kakamega 1) were included as controls. Growth parameters (height, cover and spread) were monitored at 8, 12 and 14 weeks after seedling emergence (WAE). In addition, dry matter yield (DMY) was determined at 14 WAE. Growth parameters and DMY varied significantly ($p < 0.05$) among the cultivars in all the sites. Among the Brachiaria cultivars, MG4, Basilisk and Xaraes recorded the highest mean height and plot cover. All Brachiaria cultivars gave significantly ($p<0.05$) lower DMY than Napier grass in Eldoret and Kitale while Basilisk and Xaraes recorded similar DMY with Napier grass at Alupe. In Eldoret, Xaraes recorded the highest DMY (2.54 t $ha^{-1}$) while in Kitale and Alupe the highest DMY was recorded in MG4 (3.7 t $ha^{-1}$) and Basilisk (4.72 t $ha^{-1}$), respectively. Among the Brachiaria cultivars, Basilisk, MG4, Xaraes and Piata established successfully and attained the highest spread, cover and were tallest.

**Key words**: Brachiaria cultivars, dry matter yield, plot cover, plant height, tillers, spread

### Introduction

Dairy farming is an important enterprise for the livelihoods of many households in Kenya, as a source of income and employment (Thorpe *et al*., 2000). In western Kenya, cattle also play an important role, as a source draught power and provide manure for crop production. Unfortunately, in many small-scale farms in this region, inadequate and poor quality feeds are among major constraints to dairy production in the zero-grazing system (Orodho, 2007). In most cases particularly during the dry seasons, the cut and carry systems in the integrated crop-livestock systems becomes unsustainable and cattle are left to graze freely. One approach to achieve increased livestock production in western Kenya where the soils have inherent low soil fertility (Okalebo *et al*., 2006) is through introduction of high quality forages. Such forages must be adapted to biotic and abiotic factors such as soil fertility, climatic conditions and resilience to continuous defoliation. Brachiaria grass a perennial grass native to East and Central Africa is widely grown in South America to sustain the dairy and beef industries (Maass *et al*., 2015). Improved Brachiaria grasses are exceptionally tolerant to aluminum toxicity due to acidity and drought (Miles *et al*., 2004) and could play a role in integrated crop-livestock systems in the humid highlands of western Kenya where soils are acidic.

Previous studies reported by Njarui and Wandera, 2004; Nguku *et al*., 2016 on adaptability of Brachiaria cultivars in semi-arid regions showed that a number of Brachiaria cultivars produced more dry matter than commonly cultivated Rhodes grass. Elsewhere in Kiboko, Kenya, cv. Mulato-II was found to be superior to native range grasses such as Buffel (*Cenchrus ciliaris*) and horsetail grass (*Chloris roxburghiana*) in both primary dry matter production and subsequent regrowth under irrigation (Machogu, 2013). Additionally the grass had higher nutritive quality but was heavily infested by red spider mites. However, little information is available on their adaptability of this grass in the humid region of western Kenya. The objective of the study was therefore to evaluate the establishment and early growth of selected Brachiaria grass cultivars in the humid highlands of western Kenya.

## Materials and methods

### *Study site description*

The experiments were conducted in three sites, Kitale, Alupe and Eldoret in the western region of Kenya. They lie in different agro-ecological zones with different rainfall and temperature regimes (Table 1). Rainfall in Kitale is unimodal and occurs from April to November while in Alupe and Eldoret is bimodal. The dominant soils in Alupe and Eldoret are classified as Rhodic Ferralsols that are well drained, shallow to moderately deep with very low water retention capacity (WRB, 2006). In Kitale the soil are characterized by weak to moderate structure, low fertility and low organic matter content (WRB, 2006). Selected initial physical and chemical soil properties in the study sites are presented in Table 2.

**Table 1** Location, elevation, temperatures, rainfall and soils for the three experimental sites, Kitale, Alupe and Eldoret.

| Site | Kitale | Alupe | Eldoret |
|---|---|---|---|
| Latitude | 1°0'6.6''N | 0°28'N | 1°0' 6.6''N |
| Longitude | 34°59'E | 34°07'E | 34°59' 10''E |
| Agro-ecological zone | UM3 | LM3 | LH3 |
| Altitude (m asl) | 1890 | 1189 | 2073 |
| Mean temperature (°C) | 10 - 27 | 22 | 7 - 29 |
| Annual rainfall (mm) | 1000 - 1200 | 1100 – 1450 | 1103 |
| Soil type | Acrisols | Rhodic Ferralsols | Rhodic Ferralsols |

UM=Upper Highlands; LM=Lower midlands; LH=Lower Highlands

### *Treatments and experimental design*

The treatments consisted of seven Brachiaria grass cultivars; *Brachiaria decumbens* cv. Basilisk, *B. humidicola* cvs. Llanero and Humidicola, *B. brizantha* cvs. Marandu, MG4, Piatã, Xaraes and *B. hybrid* cv. Mulato II., Two commonly cultivated local grasses, Rhodes grass (*Chloris gayana*) and Napier grass (*Pennisetum purpureum* cv. Kakamega 1) were included as control. The treatments were laid out in a randomized complete block design with three replications. Prior to sowing of the seed, triple super phosphate (TSP, 46 % $P_2O_5$) fertilizer was applied in the furrows at a rate of 40 kg P $ha^{-1}$. The grasses were sown in June 2014 in plot sizes of 4 m x 5 m. The seeds were manually drilled in the furrows at an inter row spacing of 0.5 m, at rate of 5 kg $ha^{-1}$ while 3 root splits of Napier grass were planted in holes 15 cm deep at a spacing of 1 m within and between rows. All the plots were kept weed free throughout the experimental period by hand weeding.

**Table 2** Soil physical and chemical properties in Kitale, Eldoret and Alupe sites

| | Kitale | Eldoret | Alupe* |
|---|---|---|---|
| Parameters | 0-30cm | 0-30cm | 0-30cm |
| Soil pH | 5.76 | 5.86 | 5.7 |
| Total N (%) | 0.03 | 0.03 | 0.12 |
| Organic C (%) | 0.3 | 0.3 | 1.12 |
| Available P Mehlick (ppm) | 18 | 15 | - |
| Available P Olsen (ppm) | - | - | 2 |
| Potassium (me %) | 1.17 | 2.01 | 0.16 |
| Calcium (me %) | 4.5 | 6.6 | 2.11 |
| Magnesium (me %) | 1.2 | 1.22 | 0.92 |
| Texture | Clay loam | Sandy clay | Sandy clay |

*Source: Omondi (2013).

*Data collection*

In this study, the establishment period was considered to be the 14 weeks after seedling emergence (WAE). Plant parameters (plant height, plot cover and plant spread) were recorded at 8, 12 and 14 WAE. The spread was measured from one edge to the other of the four plants using a meter ruler while the plant height was measured on the primary shoots from the base of the plant top-most leaf. Plot cover was determined using a quadrat of 1 m x1 m subdivided into 25 squares of 0.2 m x 0.2 m as described by Njarui and Wandera (2004) while Napier grass cover was determined using the dot method as described by Sarrantonio (1991). At the end of establishment period, the grasses were harvested for dry matter yield determination. Harvesting of plant was carried out from 2 m x 2 m net plots at a cutting height of 5 cm above ground. Samples of fresh shoots biomass were recorded, and approximately 500g subsamples were dried at 65°C to constant weight in forced-air drier and weighed.

**Statistical analysis**

Data on height, plot cover, spread and dry matter yield were subjected to analysis of variance (ANOVA) to determine the effects of grass cultivars at different growth stages using a general linear model (SAS, 2003) separately for each site. The grass cultivars and replications were considered as fixed factors. Mean differences were evaluated by computing least signifcance difference (LSD). Pearson correlation was performed to determine the relationships between the shoots dry matter yield and growth parameters (tiller numbers, plant height, spread and cover) using statistix 10 package (Statistix, 2003).

## Results and Discussion

*Seasonal condition*

Figure 1 below shows the rainfall received in the study sites during the establishment period. The rainfall was 366, 1275 mm and 898 mm for Busia, Kitale and Eldoret, respectively. The wettest months in Eldoret, Kitale and Busia were August, October and September respectively while the month of December received the lowest rainfall in all the sites.

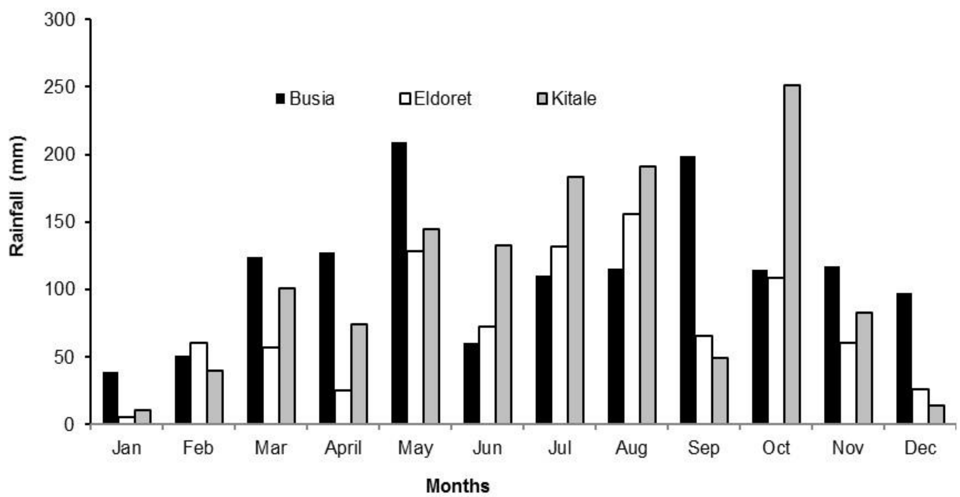


**Figure 1** Monthly rainfall during establishment period (year 2014) in Busia, Eldoret and Kitale sites

## Growth characteristics

*Eldoret*

The means of the growth parameters (height, cover and spread) during the establishment period in Eldoret are presented in Table 3. There were significant ($p < 0.05$) differences in spread, cover and height among the grasses. Napier grass recorded significantly higher mean plant heights, spread and cover than all the Brachiaria cultivars. Napier grass being a fodder crop and gigantic in nature would naturally show greater advantage in terms of growth characteristic than other grasses when the environment is favorable (Orodho, 2007; Nguku *et al*., 2016). However, plant height did not differ significantly ($P < 0.05$) among the Brachiaria grasses but Basilisk spread more than Xaraes at 12 WAE and had higher cover than MG4 at 12 WAE.

*Kitale*

The growth parameters (tillers, spread, cover and height) during establishment stage varied significantly ($p<0.05$) among the grass cultivars in Kitale. Napier grass recorded higher ($P<0.05$) plant heights and cover than all the Brachiaria cultivars (Table 4). At 8 and 12 WAE, Brachiaria cultivars gave similar plant heights which were not significantly different from Rhodes grass. However, at the end of establishment period, MG4 and Basilisk recorded significantly higher plant heights than Rhodes grass and most of the Brachiaria cultivars. The cv. Llanero had the lowest plant height (3.5 cm) and this was attributed to its spreading growth characteristic (Nguku *et al*., 2016) unlike the other cultivars that are more erect. The highest cover was recorded in cv. MG4 (51.1%) at 12 WAE and Xaraes (58.5%) at 4 WAE which and was significantly ($p<0.05$) higher than that of all the other Brachiaria cultivars. The cvs. MG4, Basilisk and Xaraes gave significantly higher cover than Rhodes grass while Marandu, Mulato II, Llanero and Humidicola recorded similar cover with Rhodes grass. The erect growth habit of MG4 and decumbent growth characteristic of Basilisk explains why they had greater heights and cover during the establishment.

*Alupe*

During the early growth stages (8WAE), the heights of all Brachiaria cultivars except Basilisk did not differ significantly with the height of Rhodes grass (Table 5). However, at 12 and 14 WAE all Brachiaria cultivars recorded significantly lower heights than Rhodes and Napier grass. The cvs. Llanero, Humidicola, Marandu and Mulato II recorded the lowest heights while Basilisk, MG4, Piata and Xaraes were the tallest. Plant spread differed significantly ($p < 0.05$) among the Brachiaria cultivars. Napier grass recorded the highest spread compared to all the Brachiaria cultivars at 8 and 12 WAE but at 14 WAE, plant spread in all Brachiaria cultivars except Xaraes and Mulato II were similar to that of Napier grass. Basilisk recorded the highest plant spread at 14 WAE while Rhodes grass recorded the lowest plant spread. At 8 and 12 WAE, Napier grass recorded significantly higher plant cover than all Brachiaria cultivars but at 14 WAE, the plant cover for Napier grass was not significantly different from that of Basilisk, Xaraes and Marandu. Piata, MG4, Mulato II, Humidicola and Llanero showed low plot cover during this period and were similar to that of Rhodes grass at the end of establishment period.

**Table 3** Mean plant height, spread and cover of grass cultivars during establishment stage in Eldoret site

| Brachiaria cultivars | Plant height (cm) | | | Plant spread (cm) | | | Plot cover (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 8 WAE | 12 WAE | 14 WAE | 8 WAE | 12 WAE | 14 WAE | 8 WAE | 12 WAE | 14 WAE |
| Basilisk | 4 | 8.4 | 9.5 | 8.4 | 32.7 | 51.3 | 5.5 | 51.1 | 53 |
| MG4 | 5.7 | 7.5 | 8.8 | 9.7 | 26.9 | 36.2 | 7.3 | 37.8 | 50.5 |
| Marandu | 4.1 | 5.7 | 13.2 | 9.9 | 29 | 26.8 | 7.3 | 42 | 43.5 |
| Piata | 3.5 | 7.1 | 7.9 | 9.5 | 30.6 | 37.4 | 6.5 | 37 | 41 |
| Xaraes | 5.3 | 8.6 | 8.8 | 11.1 | 24.9 | 32.6 | 8 | 47.8 | 58.5 |
| Napier grass | 9.2 | 45.2 | 60.2 | 21 | 63.6 | 79.5 | 57.3 | 91.8 | 94.3 |
| Mean | 5.30 | 13.91 | 18.1 | 11.6 | 34.6 | 43.9 | 15.3 | 51.3 | 56.8 |
| LSD (P<0.05) | 1.85 | 8.48 | 13.7 | 3.65 | 7.6 | 20.83 | 9.23 | 9.16 | 15.4 |

**Table 4** Mean plant height, spread and cover of the grass cultivars during establishment stage in Kitale site.

| Brachia Cultivars | Plant height (cm) | | | Plant spread (cm) | | | Plot cover (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 8 WAE | 12 WAE | 14 WAE | 8 WAE | 12 WAE | 14 WAE | 8 WAE | 12 WAE | 14 WAE |
| Basilisk | 6.7 | 10.6 | 24.9 | 25 | 36.1 | 48.6 | 8 | 24 | 51 |
| MG4 | 7.3 | 11.3 | 29.2 | 21.5 | 30.1 | 45.6 | 11.5 | 42.5 | 77.5 |
| Marandu | 5 | 7.4 | 7.8 | 10.1 | 20.3 | 31.8 | 9.5 | 16.8 | 34 |
| Piata | 7.2 | 11.3 | 15.8 | 19.7 | 28.3 | 41.1 | 8.5 | 21.5 | 45 |
| Xaraes | 6.3 | 8.7 | 10.6 | 13.2 | 21.5 | 26.1 | 9.5 | 24.5 | 51 |
| Mulato II | 3.7 | 4.3 | 4.5 | 6.3 | 13.3 | 17.2 | 4.5 | 8 | 11.5 |
| Llanero | 3.3 | 3.3 | 3.4 | 23.6 | 17 | 18.6 | 5.6 | 7.6 | 17 |
| Napier grass | 20.6 | 67.8 | 120 | 31.2 | 58.2 | 32.7 | 62.5 | 87.2 | 82.4 |
| Rhodes grass | - | 4.2 | 4.6 | - | 10.3 | 15.5 | - | 8 | 18.5 |
| Mean | 7.52 | 14.3 | 24.5 | 18.8 | 26.1 | 30.79 | 14.8 | 26.7 | 43.1 |
| LSD (P<0.05) | 4.78 | 12.64 | 17.3 | 13.1 | 16.65 | 13.94 | 10.6 | 13.9 | 16.14 |

**Table 5** Mean plant height, spread and cover of the grass cultivars during establishment stage in Alupe site.

| Brachiaria cultivars | Height (cm) | | | Plant spread (cm) | | | Plot cover (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 8 WAE | 12 WAE | 14 WAE | 8 WAE | 12 WAE | 14 WAE | 8 WAE | 12 WAE | 14 WAE |
| Basilisk | 22 | 24.2 | 33.7 | 18.2 | 12.8 | 25.2 | 38 | 66 | 68.5 |
| MG4 | 15.7 | 16.2 | 24.5 | 17.7 | 14.0 | 22.3 | 23 | 46 | 41 |
| Marandu | 11.9 | 12.1 | 17.4 | 15.4 | 14.0 | 19.2 | 27 | 50.5 | 50 |
| Piata | 13.1 | 18 | 25 | 19.3 | 12.9 | 19.0 | 27 | 43.5 | 42 |
| Xaraes | 15.9 | 23.9 | 26.6 | 15.9 | 11.3 | 14.5 | 33 | 77 | 61.5 |
| Mulato II | 7.6 | 8 | 8.2 | 9.8 | 13.1 | 12.9 | 18 | 34.5 | 38.5 |
| Humidicola | 3.2 | 1.9 | 2.9 | 20.1 | 15.1 | 19.6 | 4.5 | 15 | 20.8 |
| Llanero | 5.2 | 3.3 | 3.4 | 17.6 | 12.3 | 19.9 | 6.3 | 31 | 20 |
| Napier grass | 34.4 | 67.3 | 95.3 | 28.6 | 24.7 | 23.1 | 73 | 94.5 | 65 |
| Rhodes grass | 13.1 | 75.2 | 74.9 | 9.5 | 7.8 | 11.6 | 15.5 | 22.5 | 30 |
| Mean | 14.2 | 25 | 31.2 | 17.2 | 13.8 | 18.7 | 26.5 | 48.1 | 43.7 |
| LSD (P<0.05) | 7.54 | 16.48 | 14.8 | 8.6 | 4.6 | 7.2 | 9.54 | 15.9 | 18.3 |

*Dry matter yield*

The mean DMY for the grasses are presented in Figure 2, 3 and 4. Dry matter yield varied significantly ($p < 0.0001$) among the grass cultivars in all the sites. In Eldoret, Brachiaria cultivars had significant ($p < 0.05$) lower DMY than Napier grass (Figure 2). The cv. Xaraes gave significantly ($p < 0.05$) higher DMY (2.54 t $ha^{-1}$) than Marandu (1.56 t $ha^{-1}$) and Piata (1.83 t $ha^{-1}$). In Kitale, cv. MG4 had the highest DMY (3.7 t $ha^{-1}$) while Mulato II had the lowest (0.37 t $ha^{-1}$) but the yield were significantly lower than those of Napier grass (9.9 t $ha^{-1}$). However, cv. MG4 yielded more than Rhodes grass (Figure 3). In Alupe site, Basilisk and Xaraes were the most productive grasses and yielded 4.7 and 3.9 t $ha^{-1}$, respectively. However DMY were not significantly different from that of Napier grass (5.2 t $ha^{-1}$) but were higher than that of Rhodes grass (1.8 t $ha^{-1}$) (Figure 4). The higher DMY in Alupe was attributed to a warmer (23.8 - 30.7 $^{o}C$), humid and high rainfall during establishment (97.5 - 198.6 mm) between July - December 2014 during the establishment period (Figure 1). At Alupe and Kitale most Brachiaria cultivars showed upright growth characteristics, whereas in Eldoret the same cultivars showed a spreading growth habit which possibly had a positive effect on DMY production in Alupe and Kitale.

A significant linear relationship ($R^2$= 0.60 - 0.96; $P < 0.001$) occurred between the plant height and DMY in Kitale and Alupe site while in Eldoret DMY was positively and strongly correlated with plot cover ($R^2$ = 0.94; $P < 0.001$). Mganga, 2009 and Nguku *et al*., 2016 observed that pasture species which grow fast and tall are more efficient in utilization of resources and therefore, are more competitive and likely to have higher biomass production. Among the Brachiaria cultivars, Basilisk, MG4 and Xaraes were the tallest and had the largest cover (> 50%) at the end of establishment period. Basilisk has extensive roots system (Bulo *et al*., 1994; Guenni *et al*., 2002), aggressive growth habit, dense cover and utilizes nitrogen efficiently (Loch, 1997). Xaraes is reported to have greater leaf and stem elongation rates and higher leaf blade which results to higher biomass production since the stem is the structural component with higher weight than leaves (Rodrigues *et al*., 2014). The cv. MG4 spread more and this contributed to higher DMY. The low yield of Humidicola and Mulato II was attributed to the slow establishment. Several authors (Bauer *et al*., 2010, Mutimura and Everson, 2012; Nguku *et al*., 2016) have previously reported high DMY production in Mulato II and attributed it to large leaves sizes. However, the range of DMY of 0.4 - 1.6 t $ha^{-1}$ obtained in this study for Mulato II was far below the DMY (4.1 t $ha^{-1}$) reported by Nguku *et al*., (2016) from trials in the semi-arid eastern Kenya. Observations made in all sites showed that Mulato II had higher incidences of spider mites attack as was previously reported in trials in Kiboko by Machogu, (2013). This infestation probably contributed to reduced biomass. Among the Brachiaria cultivars, Basilisk, MG4, Xaraes and Piata established successfully and attained the highest spread, cover and were tallest. The productivity of these grasses after establishment is presented elsewhere in this proceeding.

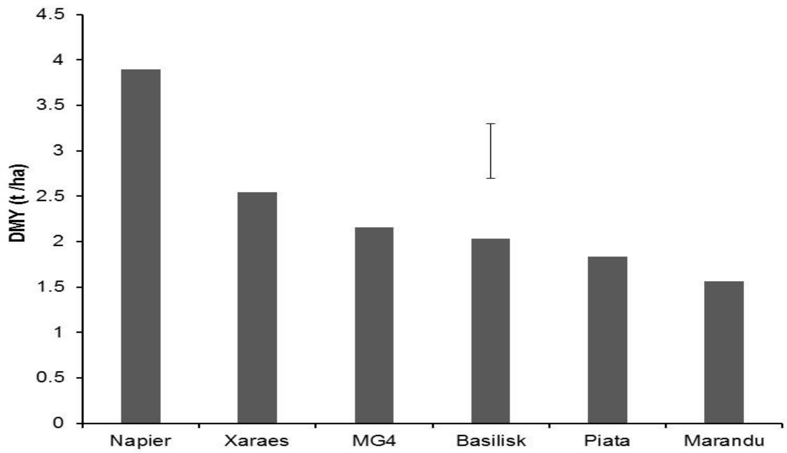


**Figure 2** Dry matter yields of grass cultivars during establishment stage in Eldoret (Bar represents the $LSD_{P<0.05}$)

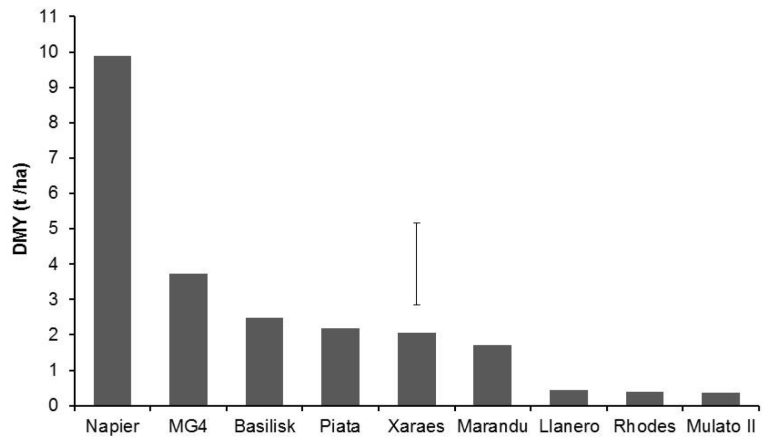


**Figure 3** Dry matter yields of grass cultivars during establishment stage in Kitale (Bar represents the $LSD_{P<0.05}$)

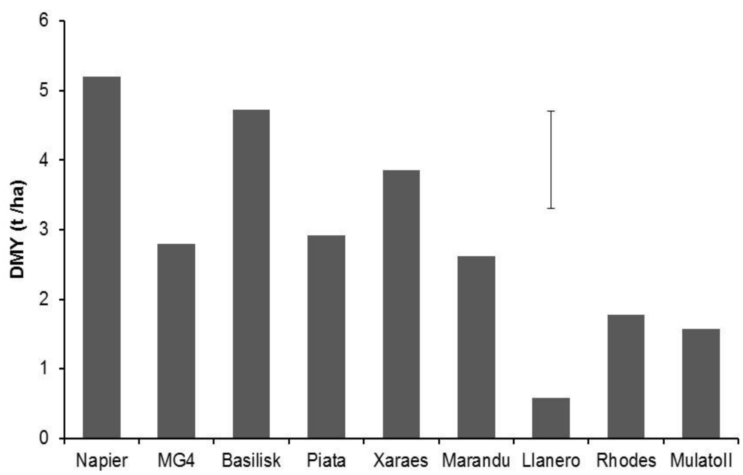


**Figure 4** Dry matter yields of grass cultivars during establishment stage in Alupe (Bar represents the $LSD_{P<0.05}$)

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# The effects of cutting frequency on the dry matter yield and nutritive quality of Brachiaria grass cultivars in acidic soils of western Kenya

**M. N. Kifuko-Koech, K. W. Ndung'u-Magiroi and M. Kamidi**

KALRO – Kitale

**Abstract**

Subjecting forages to different cutting intervals influence productivity and quality. The objective of this study was to evaluate the effects of cutting frequency on nutritive quality and dry matter yield (DMY) of Brachiaria grass in western Kenya. Eight Brachiaria grass cultivars: *Brachiaria brizantha* cvs. Marandu, Xaraes, Piata, and MG4, *B. decumbens* cv. Basilisk, *B. humidicola* cvs. Llanero and Humidicola and *Brachiaria hybrid* cv. Mulato II were evaluated at Kitale, Eldoret and Alupe in western Kenya in 2014 and 2015. One commonly cultivated grass, Rhodes grass (*Chloris gayana*) was included as control. The plants were repeatedly harvested for DMY at 6, 8 and 12 weeks intervals. Significant ($P<0.05$) differences on DMY among Brachiaria cultivars were recorded in all sites. In the dry season, Basilisk gave the highest DMY in Eldoret and Kitale while Llanero and Xaraes recorded the highest DMY in Alupe. In the wet season, Basilisk, Llanero and Xaraes recorded the highest DMY in Eldoret, Kitale and Alupe respectively. The DMY was positively influenced by plot cover, spread, height and tillers while plant number had no effect in all sites. At Eldoret, increasing the cutting intervals to 12 weeks reduced yield in the dry season but increased yield in the wet season. At Kitale, increasing cutting interval resulted to increased yield during the wet season but during the dry season, there was no definite trend. At Alupe, there was no definite trend by increasing or reducing cutting interval on DMY. Crude protein (14.2-15.0%) and *in- vitro* organic matter digestibility (65-67%) were highest at 6 and 8 weeks cutting interval and were significantly higher than at 12 weeks cutting interval. This study clearly demonstrated that Brachiaria cultivars have higher nutritive value and are more productive than Rhodes grass. To maximize production and quality, cutting Brachiaria at 8 weeks intervals is recommended.

**Key words**: Crude protein, cutting interval, dry matter yield, organic matter digestibility

## Introduction

Poor nutrition caused by inadequate quantity and low quality of feeds is a major constraint to livestock productivity not only in the semi-arid Kenya (Njarui and Wandera, 2004) but also in the humid regions of western Kenya (Amiani, 2011). Due to the high population in western Kenya (360 inhabitants $km^{-2}$), land holding per household is declining and natural pastures are diminishing at an alarming rate (Amiani, 2011; De Groote *et al*., 2008). In addition, most soils in this region are acidic and highly deficient in plant nutrients (Kifuko- Koech *et al*., 2012) which when combined with fluctuating rainfall due to climate change negatively affect crop-livestock production systems (Amiani, 2011; GOK, 2013). With exception of few large scale farmers who graze their livestock on natural pastures, most small scale farmers have adopted cut-and-carry feed systems (Franzel and Wambugu, 2007). In Uasin Gishu and Trans Nzoia counties, the common practice is feeding ground maize stalks after the grains are harvested and this accelerates soil nutrient mining. Further, the quality of natural grasses (CP-2-4%) (Thairu and

Tessema, 1987) and maize stover (4-7%) (Hancock, 2009) is below the reported minimum CP requirement of 6-8% for livestock maintenance (Minson, 1981). Napier grass which is the most commonly grown fodder grass by dairy farmers in western Kenya is at risk of Napier stunting disease which is causing a big economic loss (Orodho, 2006). Despite the challenges of limiting livestock feed in this region, most farmers are shifting to livestock production due to declining maize yields as a result of maize lethal necrosis menace (Mahuku *et al*., 2015). Therefore, to alleviate the problem of feed shortage, inclusion of alternative high quality grass cultivars with high dry matter production is key in enhancing livestock production in western Kenya.

One such promising grass is Brachiaria which originate from African origin (Boonman, 1993). Despite Africa being the centre of origin and diversity, use of Brachiaria species in pasture improvement particularly in Kenya has been limited to few cultivars that are susceptible to biotic and abiotic challenges (Maass et *al*., 2015). Past studies have identified Brachiaria cultivars that are well adapted to semi arid agro-ecological zones and their best cutting strategies (Njarui and Wandera, 2004). However, productivity and persistence of some of these promising cultivars under different cutting regimes in humid regions of western Kenya have not been evaluated. The objective of this study was therefore to assess the effects of cutting frequency on, dry matter yield (DMY) and quality of Brachiaria cultivars in different agro ecological zones in western Kenya.

## Materials and methods

### *Study site*

The experiments were conducted at three sites; Kitale, Eldoret and Alupe in western Kenya. The geographical location, detailed climatic conditions and soils charateristics of these sites are given by Kifuko-Koech *et al*. (2016), in these proceedings.

### *Treatments and experimental design*

Eight Brachiaria grasses: *Brachiaria brizantha* cvs. Marandu, Xaraes, Piata, MG4, *B. decumbens,* cv. Basilisk, *B. humidicola* cvs. Llanero and Humidicola and *Brachiaria hybrid* cv. Mulato II were evaluated. They were compared with one control grass; Rhodes grass. The experimental design was completely randomized block in a split plot arrangement with four replications. The main plots were grass cultivars and the sub-plots were the cutting frequency (6, 8 and 12 weeks). Plot sizes were 4 m x 5 m with a 1 m space between plots and replications.

### *Management and data collection*

A fine seed bed was prepared and furrows of about 2 cm for sowing seeds were dug using hand hoes. Triple super phosphate (TSP, 46 % $P_2O_5$) fertilizer was applied to the furrows at a rate of 40 kg P ha$^{-1}$. Thereafter, seeds were manually drilled in the furrows at a rate of 5 kg ha$^{-1}$ with an inter row spacing of 0.5 m, giving 10 rows in each plot and covered with a thin layer of soil. The plots were kept weed free throughout the experiment by hand weeding. After standardization cut which was conducted at 14 weeks after seedling emergency, the plots were split into three equal portions and allocated the three cutting frequency at random i.e. 6, 8 and 12 weeks intervals. Data collection was conducted during two dry and one wet seasons (Table 1.)

**Table 1** Sampling period and weeks after standardization allocated to dry and wet seasons

| Site | Dry season 1 | Wet season | Dry season 2 |
|---|---|---|---|
| Eldoret/ Kitale | 6-24WAS ( November 2014-April 2015) | 24-48 WAS (May- October, 2015) | 54-72 WAS (November 2015-March 2016). |
| Alupe | 6 -18 WAS (January 2015-April 2015) and 32-36 WAS (July-August 2015). | 24-30 WAS (May 2015-June 2015) and 40- 48 WAS (September-October 2015) | 54 -72 WAS. December 2015 to April 2016 |

Plant number, tillers, height, spread and plot covers were monitored at all cutting intervals from 1 m x 1 m quadrant. In addition DMY was determined at all cutting intervals from an effective area of 2m$^2$. At each harvest, samples of shoots biomass were weighed fresh, and a subsample taken and dried at 60°C to a constant weight and weighed. Dry matter yield for each season was determined by summing up all the harvest within each season for each cut, separately.

*Chemical composition analysis*

At harvest, the herbage was analysed for nutritive quality. Subsamples were dried at 60◦C for 48 h and ground to pass a 1-mm sieve. Ash was determined by heating the samples at 550$^o$C for 2 hours in a muffle furnace. Crude protein (CP) was determined using micro-Kjeldahl according to the method of Association of Official Analytical Chemist (AOAC, 2000). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were determined using Ankom method of Van Soest *et al.* (1991). The organic matter digestibility (OMD) was determined according to the procedure of Goering and Van Soest (1970).

**Statistical analysis**

The DMY data were subjected to analysis of variance (ANOVA) to determine the effects of grass cultivars and cutting frequency using a general linear model (SAS, 2001) with treatments and replications considered as fixed factors separately for each site. Mean differences were evaluated by computing least significance difference (LSD at $P < 0.05$). Pearson correlation was performed to determine the relationships between the herbage DMY and growth parameters using statistix 10 package (Statistix, 2003).

**Results**

*Seasonal condition*

Figure 1, 2 and 3 shows rainfall and temperature for Eldoret, Kitale and Alupe during establishment (year 2014) and production (year 2015) periods. Rainfall and temperature for year 2015 is given for 9 months (January-December) while in Kitale only year 2015 temperatures were captured. During the two years, Alupe received the highest mean annual rainfall followed by Kitale while the least amount was recorded in Eldoret. The hottest months were March in Eldoret and Kitale and September in Alupe. Temperature fluctuation was observed in Alupe while less variation was observed in Eldoret and Kitale.

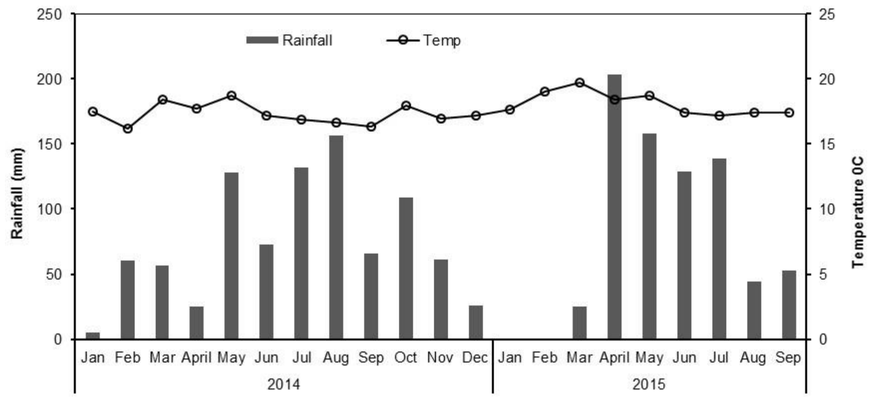


**Figure 1** Monthly rainfall and mean temperatures at Eldoret from January 2014-August 2015.

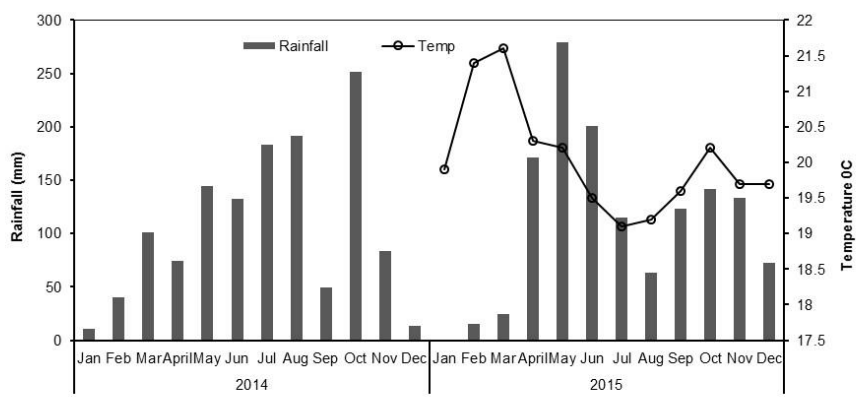


**Figure 2** Monthly rainfall and mean temperatures at Kitale from January 2014-December 2015.

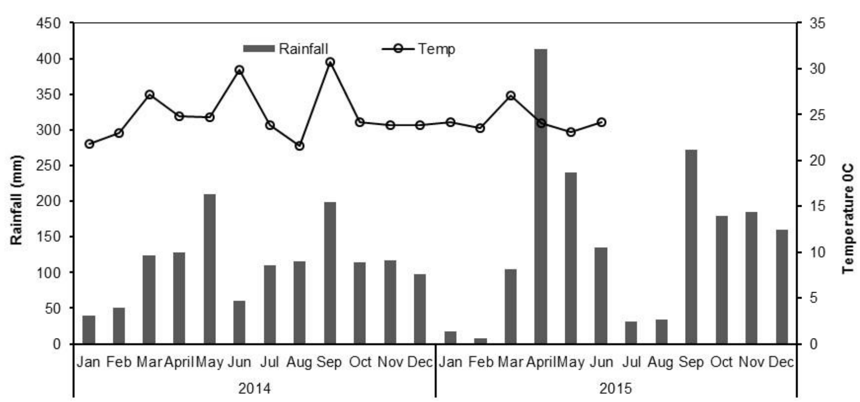


**Figure 3** Monthly rainfall and mean temperature at Alupe from January 2014-December 2015.

*Dry matter yield*

**Eldoret**

There was no significant interaction between grass cultivars and cutting frequency on DMY at Eldoret. However significant difference was recorded among the cultivars and on cutting frequency. Establishment of Rhodes grass was slow and no DMY was recorded during the first dry season. During the first dry season, Basilisk, MG4 and Xaraes gave comparable and significant higher DMY than Piata and Marandu. In the wet season, all Brachiaria cultivars except Mulato II recorded significant higher DMY (10.3-14.3 t $ha^{-1}$) than Rhodes grass (7.6 t $ha^{-1}$). At the end of second dry season, all Brachiaria cultivars except Mulato II and Marandu accumulated significantly more DMY than Rhodes grass. Basilisk and MG4 gave the highest DMY (8.2-10.1 t $ha^{-1}$). During the wet and second dry season, Marandu had the highest DMY while Mulato II consistently produced the lowest yield in all the seasons.

Significant effects on cutting interval on DMY occurred in the second dry and wet seasons. Increasing cutting interval from 6 to 12 reduced DMY during the dry season (Figure 4). However, in the wet season, delaying harvesting to 8 weeks did not show significant gain but delaying further to 12 weeks resulted to increased DMY.

**Kitale**

There were significant interaction between Brachiaria cultivars and cutting interval on DMY in all the season. During the first dry season, yield of the Brachiaria cultivars were similar in all cutting interval (Table 3). However Rhodes grass produced more yield than all the cultivars at 8 week cutting interval. In the wet season, generally increasing cutting interval increased yield for all the Brachiaria cultivars. In the second dry season, increasing cutting interval from 6 to 8 week increased the DMY yield marginally for most of the cultivars and was not significant. Mulato II had the highest yield in all the cutting intervals.

## Alupe

Like Kitale, there were significant interaction between Brachiaria cultivars and cutting interval on DMY in all the season. However, during the second dry season, Rhodes grass succumbed to drought and no DMY was recorded. In first dry season, there was no definite trend on cutting interval on yield while in the wet season and second dry season, increasing cutting interval increased the DMY marginally (Table 4). Llanero recorded higher DMY when cut at 8 intervals than other cultivars including Rhodes grass during the first wet season while Xaraes had the highest yield when cut at 12 week interval.

**Table 2** Dry matter yield (t ha$^{-1}$) of Brachiaria cultivars and Rhodes grass during the dry and wet seasons in Eldoret

| | Dry season 1 | Wet Season | Dry season 2 |
|---|---|---|---|
| Basilisk | 4.13 | 14.3 | 8.19 |
| MG4 | 3.67 | 12.68 | 10.13 |
| Marandu | 2.74 | 10.34 | 6.09 |
| Piata | 2.82 | 12.46 | 6.58 |
| Xaraes | 3.66 | 11.45 | 6.82 |
| Mulato II | 1.83 | 8.81 | 5.26 |
| Rhodes | - | 7.6 | 5.36 |
| Mean | 3.14 | 11.09 | 6.92 |
| LSD ($p < 0.05$) | 0.68 | 2.43 | 1.05 |

LSD- Least significant difference, - not estimated, Means with the same letter within the same column are not significantly different

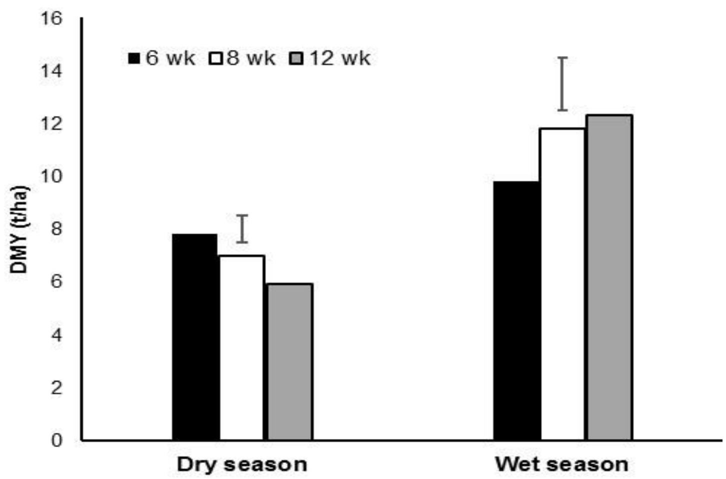


**Figure 4** Mean dry matter yield of Brachiaria cultivars at three cutting frequency (6, 8 and 12 weeks) during dry and wet seasons in Eldoret.

**Table 3** Dry matter yield (t ha$^{-1}$) of grass cultivars subjected to varying cutting intervals in Kitale

| | Dry season 1 | | | Wet season 1 | | | Dry season 2 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cutting interval | | | | | | | | |
| Grass cultivars | 6 | 8 | 12 | 6 | 8 | 12 | 6 | 8 | 12 |
| Basilisk | 1.2 | 3.1 | 4.3 | 8.9 | 8.2 | 17.9 | 6.3 | 16.5 | 8.7 |
| MG4 | 2.1 | 2.1 | 2.8 | 8.3 | 9.6 | 18.9 | 5.6 | 7.7 | 10.7 |
| Marandu | 1.9 | 2.7 | 3.3 | 10.7 | 9.8 | 15.1 | 7.0 | 7.7 | 6.3 |
| Piata | 1.2 | 1.2 | 4.0 | 9.9 | 10.4 | 19.2 | 6.2 | 7.4 | 7.7 |
| Xaraes | 1.4 | 1.2 | 2.6 | 11.3 | 11.2 | 15.5 | 7.1 | 8.6 | 9.6 |
| Mulato II | 0.4 | 2.3 | 2.1 | 11.1 | 10.1 | 14.5 | 11.6 | 10.6 | 10.8 |
| Llanero | | | | 13.3 | 10.0 | 19.5 | 5.2 | 6.3 | 7.4 |
| Rhodes | 2.0 | 9.4 | 3.7 | 7.7 | 10.0 | 14.3 | 4.3 | 5.1 | 7.3 |
| LSD $_{cultivars}$ | 2.2 | | | 1.8 | | | 3.7 | | |
| LSD $_{cuing\ frequency}$ | 1.5 | | | 1.1 | | | 2.1 | | |
| LSD$_{cultivar*cut\ frequency}$ | 5.3 | | | 6.05 | | | 6.0 | | |

LSD-Least significance difference (P<0.05), WK- weeks,

**Table 4** Dry matter yield (t $ha^{-1}$) of grass cultivars subjected to varying cutting intervals in Alupe

| | Dry season 1 | | | Wet season 1 | | | Dry season 2 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cutting interval | | | | | | | | |
| Grass cultivars | 6 | 8 | 12 | 6 | 8 | 12 | 6 | 8 | 12 |
| Basilisk | 2.6 | 2.9 | 1.9 | 3.2 | 3.6 | 4.7 | 1.6 | 2.2 | 4.1 |
| MG4 | 2.0 | 2.8 | 2.1 | 2.3 | 2.3 | 3.9 | 1.7 | 1.9 | 3.0 |
| Marandu | 2.3 | 2.6 | 2.0 | 2.8 | 2.4 | 4.3 | 1.0 | 1.8 | 2.4 |
| Piata | 2.5 | 2.7 | 2.5 | 3.5 | 2.7 | 3.9 | 1.9 | 2.3 | 2.9 |
| Xaraes | 3.3 | 3.3 | 2.8 | 4.3 | 4.9 | 6.5 | 2.8 | 3.9 | 5.0 |
| Mulato II | 1.9 | 2.4 | 1.7 | 2.4 | 2.8 | 2.7 | 0.7 | 1.3 | 1.6 |
| Llanero | 2.1 | 5.6 | 3.9 | 5.4 | 1.2 | 1.4 | 1.9 | 4.3 | 5.0 |
| Rhodes | 1.1 | 1.3 | 0.8 | 0.6 | 0.8 | 0.6 | | | |
| Mean | 2.2 | 3.0 | 2.2 | 3.1 | 2.6 | 3.5 | 1.7 | 2.4 | 3.1 |
| $LSD_{cultivars}$ | 0.8 | | | 1.1 | | | 0.8 | | |
| $LSD_{cutting\ freq}$ | 0.5 | | | 0.7 | | | 0.5 | | |
| $LSD_{cultivar*cutting\ freq}$ | 2.0 | | | 2.5 | | | 1.8 | | |

LSD-Least significance difference ($P<0.05$), WK- weeks, DM- Dry matter yield

*Effects of growth parameters on dry matter yield*

The relationships between growth parameters and total DMY in Eldoret are shown in Table 5. Total DMY was strongly and positively influenced by cover ($R^2 = 0.60$, $p < 0.001$) and height ($R^2 = 0.59$, $p < 0.001$). The total DMY was however weakly but positively correlated with plant tillers and spread while plant number had no effect on DMY. In Kitale, total DMY was positively and significantly correlated with plant spread ($R^2=0.48$, $p<0.001$) and plant cover ($R^2=0.42$, $p<0.001$). A weak but positive correlation between DMY with height and tillers was observed but plant number did not significantly influence DMY. In Alupe, total DMY was significantly and positively correlated with, cover, tillers and spread ($R^2$ = 0.43 - 0.63, $p<0.001$) in order of importance while plant height had a significant but negative effect on DMY production. No significant relationship occurred between DMY and plant number.

**Table 5** Pearson correlation coefficients showing relationship between cultivars growth parameters and dry matter yield during the dry and wet seasons in Eldoret

| Growth parameters | Dry matter yield | Plant number | Tillers | Height | Spread | Cover |
|---|---|---|---|---|---|---|
| Plant number | 0.16 | 1 | | | | |
| Tillers | 0.35** | -0.27 | 1 | | | |
| Height | 0.59*** | -0.08 | 0.57*** | 1 | | |
| Spread | 0.35** | -0.33 | 0.54*** | 0.68*** | 1 | |
| Cover | 0.60*** | -0.004 | 0.35** | 0.66*** | 0.73*** | 1 |

Where * - P≤0.05; ** - P≤0.01; ***- P≤0.001

**Table 6** Pearson correlation coefficients showing relationship between cultivars growth parameters and dry matter yield during the dry season in Kitale

| | Dry matter yield | Plant number | Tillers | Height | Spread | Cover |
|---|---|---|---|---|---|---|
| Plant number | -0.05 | 1 | | | | |
| Tillers | 0.36** | -0.36 | 1 | | | |
| Height | 0.25* | 0.58*** | -0.27 | 1 | | |
| Spread | 0.48*** | -0.2301 | 0.79*** | -0.05 | 1 | |
| Cover | 0.42*** | 0.1761 | 0.60*** | -0.002 | 0.71*** | 1 |

Where * - P≤0.05; ** - P≤0.01; ***- P≤0.001

**Table 7** Pearson correlation coefficients showing relationship between cultivars growth parameters and total dry matter yield in Alupe.

| | Dry matter yield | Plant number | Tillers | Height | Spread | Cover |
|---|---|---|---|---|---|---|
| Plant number | 0.0998 | 1 | | | | |
| Tillers | 0.54*** | -0.19 | 1 | | | |
| Height | -0.35 | -0.14 | -0.4 | 1 | | |
| Spread | 0.43*** | -0.16 | 0.53*** | -0.06 | 1 | |
| Cover | 0.63*** | 0.15 | 0.40*** | -0.05 | 0.64*** | 1 |

Where * - P≤0.05; ** - P≤0.01; ***- P≤0.001

*Nutritional value*

There was no significant interaction between cultivars and cutting interval on the parameter analyzed for the nutritive quality. However, the CP, ADF, NDF and OMD differed among the cultivars. All the Brachiaria cultivars except MG4 had significantly higher CP content (12.9-16.2%) than Rhodes grass (10.3%) (Table 8). Mulato II accumulated the highest CP content while MG4 had the lowest. All the Brachiaria cultivars except Xaraes and Piata recorded significant lower NDF than Rhodes grass and consequently were more digestible than Rhodes grass. Increasing the cutting intervals to 12 weeks significantly ($p < 0.05$) reduced the CP and OMD concentrations (Figure 5). No significant differences on CP and OMD were observed when the harvesting was conducted at either 6 or 8 weeks intervals (Figure 5).

**Table 8** Crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and *in-vitro* organic matter digestibility (OMD) concentrations of grass cultivars in Kitale.

| | CP (%) | NDF (%) | ADF % | OMD% |
|---|---|---|---|---|
| Basilisk | 14 | 60.7 | 38.5 | 64.6 |
| MG4 | 12.1 | 61.1 | 38.4 | 64.1 |
| Marandu | 13.7 | 56.7 | 38.0 | 66.4 |
| Mulato II | 16.2 | 53.4 | 40.9 | 65.1 |
| Piata | 13.5 | 62.1 | 36.8 | 66.7 |
| Xaraes | 12.9 | 61.8 | 38.7 | 61.6 |
| Rhodes grass | 10.3 | 66.9 | 41.9 | 57.5 |
| Mean | 13.2 | 60.4 | 39.04 | 63.71 |
| SED variety | 1.58 | 3.95 | 0.94 | 1.67 |

SED- standard error of difference

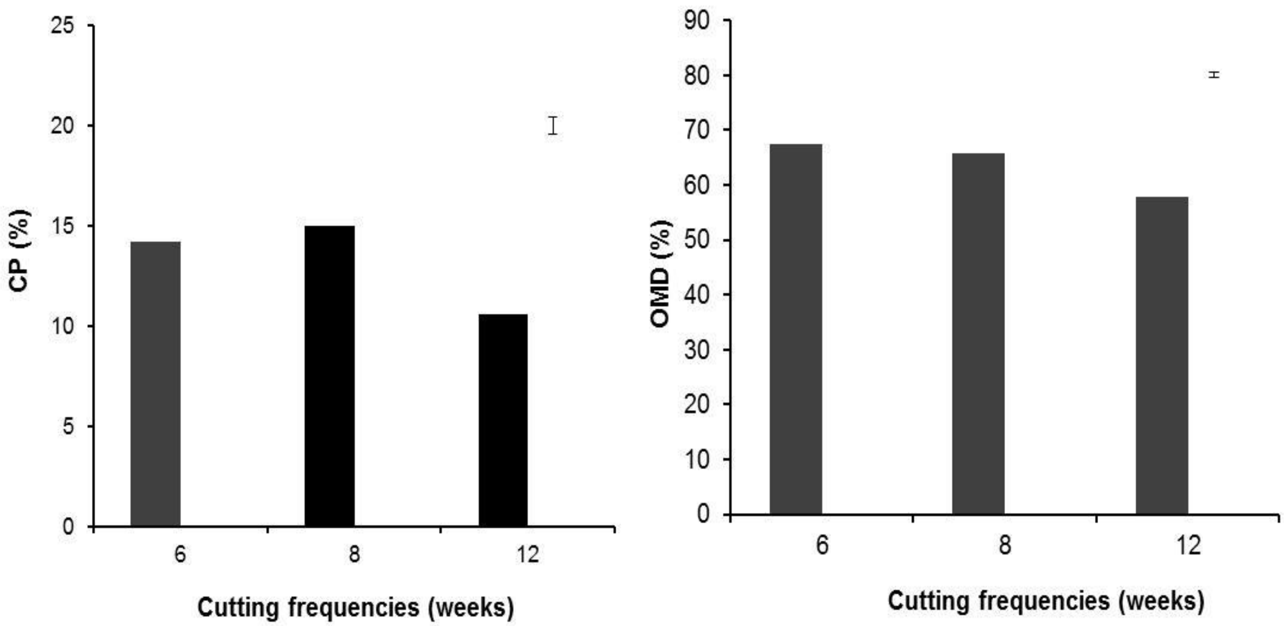


**Figure 5** Effects of cutting frequency on crude protein (%) and *in-vitro* organic matter digestibility (OMD) in Kitale.

**Discussions**

On the basis of DMY production, the most promising Brachiaria cultivars are Basilisk, MG4, Piata and Xaraes. Although Mulato II gave lower DMY in Eldoret, in Kitale and Alupe it accumulated higher DMY particularly during the dry season. In addition, the highest CP and lowest NDF concentrations were recorded in Mulato II in Kitale. Reduced total herbage accumulation in Mulato II in Eldoret was possibly due to poor establishment. Visual observation showed that Mulato II had higher spider mites infestation compared with the other Brachiaria cultivars. Basilisk, MG4, and Llanero had good cover (>50%) remained green and healthy an indication of their increased level of drought tolerance and ability to scavenge and utilize soil nitrogen efficiently. Nitrogen increases moisture use efficiency due to increased roots development (Tedonkeng Pamo and Pieper, 1995) which could be the reason why they showed outstanding DMY during dry season. Nguku *et al.* (2016) reported that while Basilisk has good ground cover and spreading habit, MG4 has erect growth habit whereas Llanero has a decumbent habit and form dense ground cover.

Total production over the year showed that most Brachiaria cultivars particularly in Eldoret and Alupe out yielded Rhodes grass which was an indication that the former is a good alternative to Rhodes grass in western Kenya. Rhodes grass was however well adapted to Kitale possibly due to the humid climate throughout the year. Most Brachiaria cultivars in Kitale and Eldoret gave total DMY within the range reported by Hare *et al.*, (2009) in Thailand. Low DMY production in Alupe was attributed to poor distribution of rainfall, low inherent soil fertility (Nitrogen-0.12%, carbon-1.12%, Olsen P-2ppm P) and slow grass recovery after dry spells (Omondi, 2013). Crude protein for all Brachiaria cultivars was well above 7% which is considered critical for livestock production (Milford and Minson, 1966). This study clearly demonstrated that Brachiaria cultivars have higher nutritive value and are more productive relative to Rhodes grass particularly during the dry season.

Effect of cutting frequency on DMY production in this study was variable. Whereas, cutting Brachiaria cultivars at shorter intervals (6 or 8 weeks) during the dry season resulted in significant higher DMY in Eldoret and Alupe, in Kitale prolonging the cutting interval to 12 weeks resulted in greater DMY accumulation. Due to close proximity of Kitale to Mt Elgon, the site receives rains in most parts of the year which ensures continuous growth of grass and this explain why increasing cutting intervals resulted to higher DMY during the dry season. During the wet season, increasing the cutting intervals to 12 weeks gave significant higher DMY irrespective of the sites. Results on effects of harvesting intervals on grass herbage accumulation have been variable in most parts of the world. In Florida, Vendramini *et al* (2014), while working on Mulato II and Cayman found no difference in herbage accumulation between 3 - and 6- week regrowth intervals in the first period but greater accumulation for 3 week than 6 week regrowth interval in the second period. In Thailand, Hare *et al*., (2013) while working with Brachiaria hybrids found that extending cutting intervals to 90 days greatly increased DM production but reduced CP concentrations. He further noted that cutting at 30 day intervals would produce CP levels 3 - 4 percentage point higher than cutting at 45 and 60 days intervals. For many grasses, longer regrowth intervals result in greater herbage accumulation (Interrante *et al*., 2009) and results of this study therefore support conclusion of other authors (Hare *et al*., 2013; Njarui and Wandera, 2004).

**Conclusions**

This study has demonstrated that the most promising Brachiaria cultivars are Basilisk, MG4, Piata and Xaraes across sites while Llanero performed well in Alupe. Generally, Mulato II had higher nutritive value than all Brachiaria cultivars in Kitale. Cutting Brachiaria cultivars at shorter intervals (6 or 8 weeks) during the dry season resulted to significant higher DMY in Eldoret and Alupe. In Kitale, extending cutting interval to 12 weeks gave higher DMY but reduced CP and OMD during the dry season. Cutting at 8 weeks intervals resulted in high proportions of CP and lower NDF. This study clearly demonstrated that Brachiaria cultivars have higher nutritive value and are more productive than Rhodes grass. To maximize production and quality, cutting Brachiaria at 8 weeks intervals is recommended.

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# Effects of nitrogen and phosphorus on shoots and roots biomass of Brachiaria grasses in low fertility soils of North western Kenya

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## Abstract

One of the major constraints to forage production in north western highlands of Kenya, is low soil fertility. The objective of this study was to assess the influence of nitrogen (N) and phosphorus (P) on growth and biomass production of Brachiaria grasses. Seven Brachiaria cultivars: *Brachiaria brizantha* cvs. Marandu, MG4, Piata, Xaraes, *B. decumbens* cv. Basilisk, *B. hybrid* cv. Mulato II and *B. humidicola* cv. Llanero were compared with two commonly grown forages, Rhodes grass and Napier grass cv. Kakamega 1. The treatments were tested in a split plot arrangement in a randomized complete block design with two rates of NP fertilizer, 0 and 40 kg P $ha^{-1}$ applied at planting and 50 kg N $ha^{-1}$ as a seasonal top-dress assigned to the main plot and the grass varieties as subplots. Shoots biomass was determined 14 weeks after seedlings emergence (WAE) and subsequent sampling done after every eight weeks. Roots biomass was assessed from 0-15 and 15 - 30 cm depths at 22, 46 and 80 WAE. During the wet season, the shoots biomass was significantly influenced by NP fertilizer ($p = 0.05$) and cultivars ($p=0.001$), but no significant interaction effects occurred. Fertilized grasses yielded 17% more shoots biomass than the unfertilized grasses. Basilisk, Marandu and Xaraes had the highest biomass yields (8.6 – 11.3 t $ha^{-1}$) and were higher ($p = 0.05$) than Rhodes grass, and also the other Brachiaria cultivars. Roots biomass was higher at the 0 - 15 cm depth at all sampling times and decreased with depth. Brachiaria cultivars had high roots biomass than Napier and Rhodes grass. Fertilizer response to roots biomass was only noted at 22 WAE only. Basilisk and MG4 are the best Brachiaria options for the north western highlands due to their higher shoots and roots biomass yields.

**Key words:** Dry and wet season, fertilizer, soil quality, shoots biomass.

## Introduction

Livestock production is one of the most important agricultural land use systems in the world, with grasslands covering 25% of land surface and contributing to the livelihoods of more than 800 million people (Steinfeld *et al.,* 2006). However, the Kenyan dairy industry is threatened by seasonal forage production of low nutritional quality. Excess forage production is experienced during the rainy season but more often, acute shortages occur in the dry season (Njarui *et al.,* 2016). The abundant natural forages relied upon by farmers is not sufficient to satisfy animal requirements especially in the dry season when biomass production decreases by 25 - 50% and crude protein levels reduce significantly (Penning de Vries and Djiteye, 1982). Soil nutrient depletion and improper management of improved forages exacerbates the problem leading to soil degradation and reduced livestock production.

Forage grass production is influenced by the varietal potential including growth and development processes within the grass and soil nutrient availability. Grasses have a relatively high demand for nutrients. McKenzie (2005) for example reported that most hay grasses in

Alberta removed an average of 14-18 kg nitrogen (N) and 1.8 kg of phosphorus (P) per ton of dry matter. However, the amounts of nutrients removal vary depending on the grass species and the seasonal conditions. High nutrient uptake and removal occur in the wet seasons due to increased nutrient availability in soil solution. Productivity of forages is limited by low soil fertility especially in the low pH and N and P deficient soils that are common in the north western highlands of Kenya. Nitrogen is an important element in grass production due to its role in photosynthesis and influence in the chlorophyll molecule (Oliveira *et al.* 2010). Where available N from soil is not sufficient for forage production, application of fertilizer N may provide immediate responses in forage grasses (Batista *et al.*, 2014). Silveira *et al*. (2015) working with different N rates (0, 60 and 120 kg $ha^{-1}$ per year) on *Paspalum notatum* for a period of three years, observed that the dry matter yields increased linearly with application rates of N. However, responses to P have only been reported in the season of application during grass establishment due to the role of P in roots formation but not in later seasons. Higher nutrient removal is reported in improved grasses than indigenous grass species though some grasses have also been bred for low soil fertility regions (Rao *et al.*, 1993).

Brachiaria grasses though indigenous to East Africa, have been recently introduced in Kenya. The introduction of these grasses into the smallholder farming systems of north western highlands, necessitates, proper soil fertility management and an understanding of its nutritional requirements for pasture management. Studies on the fertilizer needs of Brachiaria grasses in the low fertility soils in the region is therefore necessary in order to increase the production of feeds for higher milk and meat production per unit of area. In the previous studies, Miles and Lapointe, (1992) reported good performance of *B. humidicola* in infertile soils of Carimagua, while *B. ruziensis* performed better in relatively fertile clay loam soils due to its high N demand (Humphreys *et al.*, 1988). The objective the study was to assess the effects of N and P fertilizers on the shoots and roots biomass of seven Brachiaria cultivars in north western highlands of Kenya. We hypothesized that the shoots and roots biomass of Brachiaria grasses is increased by N and P fertilization in nutrient depleted soils, and the response is also dependent on the season.

## Materials and methods

### *Study sites*

The experiment were conducted in the Kenya Agricultural and Livestock Research Organization (KALRO) Kitale and at the University of Eldoret farms, located in Trans Nzoia and Uasin Gishu Counties respectively within the north western highlands of Kenya. The KALRO Kitale site (1° 0′ 6.6′′N and 34° 59′ 10′′E) lies within the Upper Midlands (UM) 4 agro-ecological zone at 1890 m asl. The site has a cool and temperate climate with average annual temperatures ranging between 10 and 27$^0$C and a mean annual precipitation of 1100 mm, making it reliable for rain fed agriculture. The rainfall is unimodal and starts from April to December. The soils are mainly humic Acrisols (FAO, 2008; Jones *et al.*, 2013), which have low fertility, are deficient in N and P, have a weak to moderate sub-angular blocky structure and low organic matter. They are well drained with high moisture storage capacity.

The University of Eldoret site lies between 1°0′6.6′′N and 34°59′10′′E at an elevation of 2073 m asl in the Lower Highland (LH) 3 agro-ecological zone which covers 58% of the county. Eldoret receives a unimodal rainfall between April and November with averages of 900 mm. Since the area has no distinct dry and wet seasons, the dry season in this study was considered as the periods with lower rainfall amounts (October to April). Temperature ranges between 7 and 29$^{0}$C, making the area favorable for fodder and livestock production. The soils are underlain by murram, well drained, shallow to moderately deep and with dark red friable clay of petroplinthite. They are classified as Rhodic Ferralsols and are low in soil pH (<5.5), N and P contents, moisture storage capacity and prone to Al toxicity (Kisinyo, 2011; NAAIAP and KARI, 2014).

*Site characterization*

Soil samples were collected prior to commencement of the trials using a soil auger. Samples were taken using a W- pattern from four depths: 0 – 15 cm, 15 – 30 cm, 30 – 60 cm, and 60 - 100 cm thoroughly mixed and a sub sample was taken from each depth for each block. After air drying, the samples were gently crushed to pass through a 2-mm sieve. Available P (Mehlick III), exchangeable K, Ca, Mg and total N were estimated after wet digestion with $H_2O_2/H_2SO_4$ as described by Okalebo *et al.* (2002). Total $Ca^{2+}$, $Mg^{2+}$, and $K^+$ were determined by atomic absorption spectrometry and P measured as described by Murphy and Riley (1962). Soil pH was measured in water (soil: water ratio of 1: 2.5) using a pH meter with a glass and reference calomel electrode (Model pH 330 SET-1, 82362). Soils were vertically sampled using stainless steel rings (diameter 10 cm) for different depths; resulting in undisturbed soil samples for bulk density determination as described by Blake and Hartge (1986). The samples were dried at 65ºC to a constant weight to allow soil bulk density determination. All determinations were made in triplicate and expressed on a dry weight basis.

*Soil chemical characteristics*

The soils were deeper in Kitale (>100 cm) than Eldoret (up to 50cm). Soil depth is an indicator of volume of water storage and extent of root growth expected in the soil especially for deep rooted forage grasses (Bengough *et al.* 2016). Low soil depth coupled with low water storage capacity of Ferralsols (Jones *et al.,* 2013) could hinder growth of grasses with extensive deep roots systems in Eldoret. The bulk density ranged from 1.34 to 1.49 g cm$^{-3}$ and between 1.29 to 1.35 g cm$^{-3}$ in Kitale and Eldoret, respectively. The bulk density were within the normal range (<1.5 g/cm$^{3}$) that doesn't restrict root growth in soil as proposed by Hunt and Gilkes (1992). Soils were generally medium acidic in both sites, but slightly more acidic in the 30 - 60 cm depth in Kitale (Table 1). The soils were mainly deficient in N, available P and Zn with nutrient levels decreasing with depth in both sites. The soil are generally low in organic C ranging from 0.2 - 0.3% in both sites. However, K, Ca, Mg and Mn were adequate in both sites.

**Table 1** Initial soil physical and chemical properties at the experimental sites, Kitale and Eldoret

| | Kitale | | | Eldoret | |
|---|---|---|---|---|---|
| Parameters | 0 - 30 cm | 30 - 60cm | 60 - 100cm | 0 - 30cm | 30 - 50cm |
| Soil pH | 5.76 | 5.50 | 5.66 | 5.86 | 5.77 |
| Bulk density ($g/cm^3$) | 1.49 | 1.46 | 1.34 | 1.29 | 1.35 |
| Total N (%) | 0.03 | 0.02 | 0.02 | 0.03 | 0.02 |
| Organic C (%) | 0.3 | 0.2 | 0.2 | 0.3 | 0.2 |
| Available P (ppm) | 18 | 6 | 5 | 15 | 10 |
| Potassium (me %) | 1.17 | 0.74 | 0.51 | 2.01 | 1.56 |
| Calcium (me %) | 4.5 | 4.3 | 2.2 | 6.6 | 5.5 |
| Magnesium (me %) | 1.2 | 1.04 | 1.2 | 1.22 | 1.74 |
| Manganese (me %) | 0.29 | 0.16 | 0.20 | 0.38 | 0.36 |
| Zinc (ppm) | 1.79 | 1.56 | 1.03 | 4.92 | 2.41 |

*Experimental design and management*

The treatments consisted of seven Brachiaria grass cultivars *Brachiaria decumbens* cv. Basilisk, *B. brizantha* cvs. Marandu, MG4, Piata, Xaraes, *B. hybrid* cv. Mulato II and *B. humidicola* cv. Llanero and two commonly grown grasses (Rhodes grass cv. KAT R3 and Napier grass cv. KK1). The design was a randomized complete block in a split plot arrangements with or without fertilizer (40 kg P $ha^{-1}$ and 50 kg N $ha^{-1}$) being the main plot and the grasses as subplots and replicated four times. Phosphorus was applied at planting as triple super phosphate (TSP 46% $P_20_5$) while N was top dressed with calcium ammonium nitrate (CAN 26% N) each season. The bare plots were kept weed free throughout the experimental period. These treatments were tested in subplots of 4 m by 4 m.

The seeds were drilled in a well prepared land at 5 kg $ha^{-1}$ for Brachiaria and 10 kg $ha^{-1}$ for Rhodes grass in 2 cm deep furrows and an inter-row spacing of 0.5 m. Root splits of Napier grass were planted at 1 m x 0.5 m spacing. Minimum tillage was practiced throughout the growth period to encourage maximum root growth. After full establishment phase (14 weeks after seedling emergence- WAE) a standardization cut was made to stimulate uniform plant growth. Subsequent harvests were carried out after every 8 weeks. During the harvest, the grass was cut at 5 cm above the ground within inner 4 $m^2$ using sickles. The fresh herbage was weighed, and a subsample taken, and then dried at 60°C to constant weight for determination of shoots biomass. The number of tillers from four (4) randomly selected plants within a 1 m x 1 m fixed quadrat frame was counted. Plot cover was determined by counting the number of fully covered subdivisions in a 1 $m^2$ quadrat (with 25 squares of 0.2 m x 0.2 m each) as described by (Njarui and Wandera, 2004) while in Napier grass, the plot cover was determined according method described by Sarrantonio (1991). Plant height and spread were measured in four (4) randomly chosen plants within a 1 $m^2$ quadrat. Spread was considered as the width of the widest point while height was taken from the base to the highest flag tip excluding inflorescence.

At harvest (22 WAE), subsamples were taken for determination of shoots biomass and nutritive value. After oven drying (60 ºC for 48 hours) and milling, samples were analyzed for digestibility according to the procedure of Goering and Van Soest (1970). Crude protein was determined using micro-Kjeldahl according to the method of the Association of Official Analytical Chemist (AOAC, 2000).

*Roots biomass assessment*

Roots biomass assessment was conducted at 22, 46 and 80 WAE, representing one complete season in the area. The roots were sampled using the soil-core method (Bohm, 1979). Four soil cores were randomly sampled using a stainless steel auger at 0 - 15 cm and 15 - 30cm depths from the intra- and inter-row spacing in each plot. To prevent edge effects, the samples were taken at least 1 m from the edges. The samples from each depth were thoroughly mixed, weighed to determine the total bulk soil weight and a sub sample was collected for moisture correction. The roots contained in the samples were recovered under a tap of running water at low pressure using 2.8 cm and 2 cm mesh sieves (Bohm, 1979). Samples were dried in an air-forced oven at 65º C to constant weight and then weighed for determination of dry root weight. The roots biomass was calculated as a factor of the bulk density for each depth.

**Statistical analysis**

Analysis of variance was undertaken to determine the effects of fertilizer N and P on the shoots and roots biomass of Brachiaria grasses using Statistix 10 package (Statistix, 2003). Means were separated using the Tukey's HD test. Where ANOVA was significant ($P \leq 0.05$), Pearson correlation was performed to assess relationships between the shoots and roots biomass and growth parameters including tiller numbers, plant height, spread and cover.

**Results and Discussion**

*Shoots biomass*

During the dry season (October – April), a significant site ($P<0.001$) and cultivar effect ($P<0.001$) was noted. The grasses produced higher yields in Kitale (8.9 t ha$^{-1}$) than in Eldoret (4.2 t ha$^{-1}$) mainly due to differences in moisture. The soils in Kitale are mainly Acrisols which have a higher soil water retention due to high accumulation of clay in the lower horizons compared with the Ferralsols in Eldoret. Ferralsols have low water retention capacity, that may lead to stress during dry spells (Jones *et al.* 2013). Regardless of the sites, fertilizer NP treatments had higher biomass yields (6.8 t ha$^{-1}$) compared to 5.8 t ha$^{-1}$ in the unfertilized grasses.

**Kitale**

The shoots biomass were significantly influenced by the grass cultivars ($P$ = 0.001) but not by fertilizer NP ($P$ = 0.26) and no significant interaction between cultivars and fertilizer NP ($P$ = 0.57) were noted during the dry season in Kitale. The lack of response to fertilizer NP during the dry season can be attributed to low nutrient uptake due to low moisture. Among the Brachiaria grasses, Basilisk, MG4 and Marandu accumulated the highest shoots biomass which ranged from 8.9 to 11.2 t ha$^{-1}$ and was significantly higher (*P* = 0.05) than the commonly grown Rhodes grass while cvs. Llanero and Mulato II had the lowest shoots biomass (Table 2). However, Napier grass produced between 56 to 106% higher biomass compared with the highest yielding Brachiaria cultivars (Basilisk and MG4) during the dry season. In the wet season, Xaraes and MG4 had the highest biomass yields (16 t ha$^{-1}$) followed by Basilisk > Mulato II > Piata> and Marandu (Table 2). All the Brachiaria cultivars except Llanero produced significantly higher yields than Rhodes grass, while Napier grass out yielded all the grass varieties in the wet season.

**Table 2** Shoots biomass of Brachiaria cultivars, Rhodes grass and Napier grass during the dry and wet seasons in Kitale.

| Grass cultivars | Dry season | Wet season |
|---|---|---|
| *B. decumbens* cv. Basilisk | 11.3b | 14.4b |
| *B. humidicola cv.* Llanero | 3.8d | 8d |
| *B. brizantha* cv. Marandu | 8.9c | 12.4c |
| *B. brizantha* cv. MG4 | 10.4b | 15.6b |
| *B. hybrid* cv. Mulato II | 4.6d | 14.1c |
| *Pennisetum purpureum* cv KK1 | 17.8a | 24.5a |
| *B. brizantha* cv. Piata | 8.1c | 13.6c |
| Rhodes grass | 6.6cd | 11.2d |
| *B. brizantha* cv. Xaraes | 8.6c | 15.8b |
| Mean | 8.9 | 14.42 |
| SED | 1.5 | 1.82 |
| $P_{(CV)}$ | *** | *** |
| $P_{(NP)}$ | NS | * |
| $P_{(CV*NP)}$ | NS | NS |

Where- SED- Standard error of difference at p≤ 0.05; NP- fertilizer applied at 40 kg P ha$^{-1}$ and 50 kg N ha$^{-1}$ per season; CV – cultivar, NS- Not significant at p≤ 0.05. Means followed by the same letter for a parameter are not significantly different at P≤0.05. Mean separation was done by Tukeys HSD test.

During the wet season, all the treatments where N and P was applied produced significantly higher shoots biomass (*P*=0.05) than those with no fertilizer application (Figure 1). Generally, application of fertilizer NP produced 17% higher shoots biomass than the unfertilized treatments. Among the Brachiaria grasses, MG4 and Xaraes had the highest responses to fertilizer NP with 44.7 and 34.8% increase in yields due to fertilizer addition respectively. These cultivars developed chlorosis in treatments without NP fertilizer throughout the season, while the fertilized treatments showed N deficiencies two months after N application particularly during the high rainfall periods. This indicates that cvs. MG4 and Xaraes had higher N demand than the other cultivars which may not have been satisfied by the rate applied. The cvs. Mulato II, Basilisk and Marandu did not respond to application of NP, and were ever green throughout the season. In grasses, N has a major role in chlorophyll formation and increased vegetative growth, while P is important in roots development. The site had low initial soil N (Table 1); therefore, the grasses with little or no response to NP application could be more efficient in N utilization thereby requiring low N application. Xaraes and MG4 and require N and P fertilization in NP deficient sites to achieve increased production.

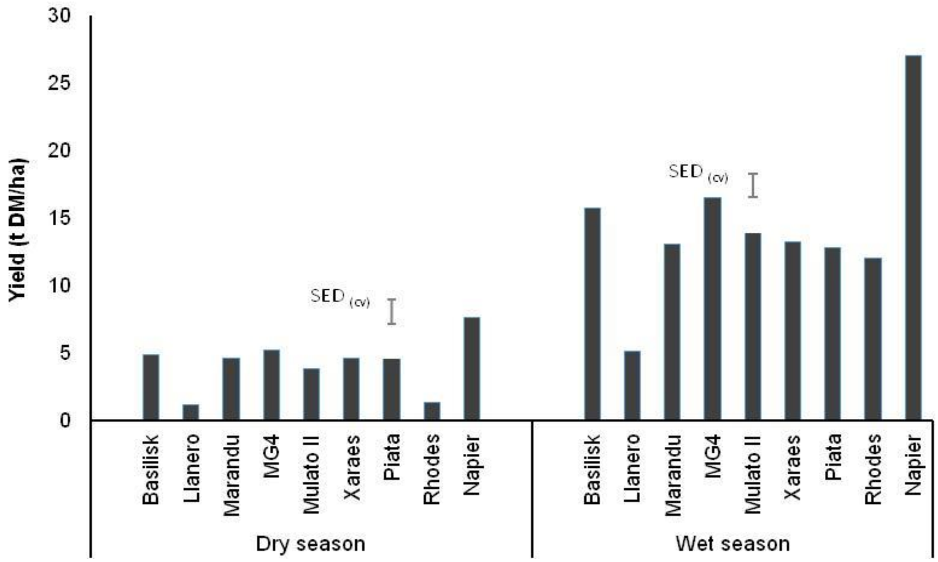


**Figure 1** Influence of fertilizer NP on shoots biomass of Brachiaria grasses during the dry and wet seasons in Kitale. Where: cv- cultivar; SED- standard error of difference at P<0.05

*Nutritive quality of grass cultivars in Kitale*

Generally, the Brachiaria varieties had significantly higher crude protein than the control grasses (Table 3). Mulato II accumulated the highest amounts of crude protein, followed by Basilisk, Piata and Marandu. Cultivars Llanero and MG4 had the lowest crude protein and were comparable to Napier and Rhodes grass (Table 3). Although Napier grass yield was high in both seasons, its nutritional value was lower than that of the Brachiaria grasses. However, the nutritional values were higher than those reported elsewhere by (Paulino *et al*., 2011) probably due to the geographical location and environmental conditions for the site. Crude protein is higher, in fodder grown in high rainfall areas while structural carbohydrates (NDF) in the grasses is lower (Peterson *et al*., 1988).

**Eldoret**

In Eldoret no significant fertilizer ($P \leq 0.05$) effect on shoots biomass was noted in the dry and wet seasons despite the low N and available P content in those soils (Table 1). However, the biomass yields were significantly ($P = 0.001$) influenced by the grass cultivars in the dry season. All the Brachiaria cultivars except cv. Llanero produced similar yields that were significantly ($P = 0.05$) higher than those of Rhodes grass (Figure 2). Napier grass out-yielded all the grasses and accumulated between 2.4 to 6.5 t $ha^{-1}$ more shoots biomass than the lowest and highest yielding Brachiaria grasses. During the wet season, the biomass yields were influenced by the grass cultivars, but no interaction between the grass cultivars and fertilizer NP occurred. The cvs. MG4 and Basilisk accumulated the highest shoots biomass (14 t $ha^{-1}$), which was significantly

(P=0.05) higher than that of other Brachiaria cultivars and Rhodes grass. However, cvs. Marandu, Piata, Xaraes and cv. Mulato II yielded shoots biomass that was similar to that of Rhodes grass, but higher than cv. Llanero (Figure 2). Napier grass out-yielded all the grasses during this season. The high biomass yield accumulated by cvs. MG4 and Basilisk in the dry and wet seasons in the Eldoret site suggest that these are the best bet Brachiaria cultivars.

**Table 3** Crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) (% of DM) of Brachiaria grasses at 22 weeks after emergence in Kitale

| Cultivar | CP | NDF | ADF |
|---|---|---|---|
| *B. decumbens* cv. Basilisk | 16.5ab | 59.8d | 37.6c |
| *B. humidicola cv.* Llanero | 13.6c | 66.9a | 41.9a |
| *B. brizantha* cv. Marandu | 15.1ab | 59.9d | 39.6ab |
| *B. brizantha* cv. MG4 | 12.6c | 59.4d | 36.8c |
| *B. hybrid* cv. Mulato II | 17.3a | 59.1d | 40.6ab |
| Napier grass | 13.1c | 64.6b | 42.9a |
| *B. brizantha* cv. Piata | 16.1ab | 61.4c | 37c |
| Rhodes grass | 14ab | 62.7bc | 39.3ab |
| *B. brizantha* cv. Xaraes | 14.91 | 61.78 | 39.5 |
| SED | 0.88 | 0.98 | 1.06 |
| $P_{(CV)}$ | *** | *** | *** |

Where- SED- Standard error of difference at p≤ 0.05; CV- cultivar; Means followed by the same letter for parameters are not significantly different at *P≤0.05.*

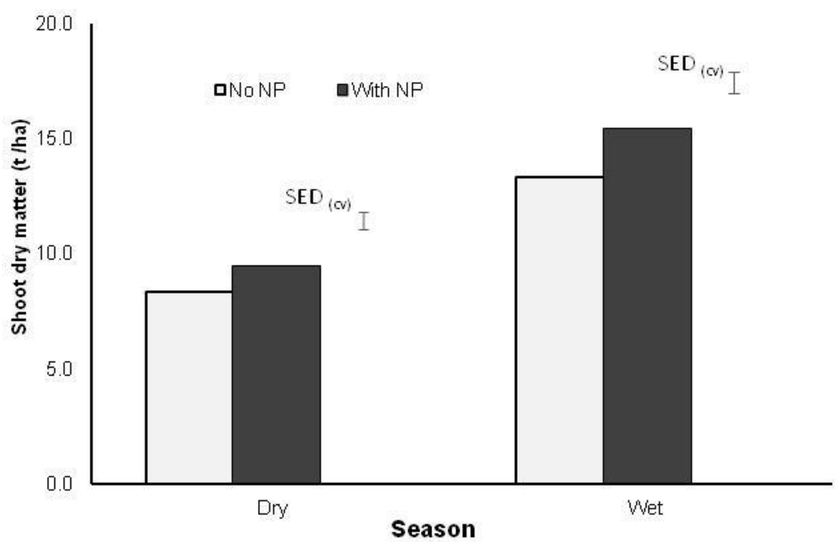


**Figure 2** Shoots biomass yields of Brachiaria cultivars, Rhodes grass and Napier grass during the dry and wet season in Eldoret

*Effects of cultivar and fertilizer addition on roots biomass*

**Kitale**

Roots biomass is important in maintaining soil fertility due to its contribution to C and N sink and as a source of exudates for microbial growth resulting to increased microbial activity within the rhizosphere. Application of fertilizer NP had no effect on roots biomass in all sampling periods. The lack of fertilizer response in roots biomass is partly attributed to low NP rates (40 kg P $ha^{-1}$ and 100 kg N $ha^{-1}$) that may not have been responsive in these P deficient soils (Table 1). Other studies on effects of fertilization on roots biomass have noted similar results. Kibet *et al.* (2016) for example found no responses to fertilizer N by switch grass fertilized for 4 years, while Jung *et al.* (2011) reported increased above ground biomass due to N application but no changes in roots biomass of switch grass. In the current study, accumulation of roots biomass differed across the two depths sampled with 70% of roots biomass located in the 0-15 cm depth. Roots biomass increased with age but reduced with depth. The highest roots biomass was recorded at 80 WAE (Table 4).

At 22 WAE, Basilisk, Piata and Xaraes had comparable roots biomass in the 0-15 cm depth, which was higher than that of Rhodes and Napier grass while cvs. Llanero and Mulato II accumulated the lowest roots biomass. The roots system of Basilisk consists of finer and longer roots than some other Brachiaria species providing superior uptake of P and N from the soil. Most of the grasses had very low biomass at the subsoil level (15 - 30cm) at 22 WAE. Piata had the highest roots biomass at the 15 - 30 cm depth which was between 80 – 200% higher than the other grasses. Less abundant roots biomass accumulation was noted in Mulato II, Llanero, Napier and Rhodes grass at the lower depth (Table 4).

At 80 WAE, no varietal or fertilizer differences were noted on roots biomass at both the top and lower level but generally, the grasses had higher roots biomass at the 0 - 15 cm depth. Roots biomass was 10 times higher in the 15 - 30 cm depth from soils sampled 80 WAE compared to those sampled 22 WAE, showing that as the growth period increased, more roots were distributed in the deeper horizons. By this period, the varieties may have attained near maximum lateral growth and utilized most of the soil area available for growth thereby investing more roots growth at the lower soil depths. This is important for C sequestration, since most of the C stored in the roots at the deeper horizons contribute to C sink and is better protected from degradation. At the 80 WAE, the Brachiaria grasses had higher roots biomass at the lower horizons than the controls, Napier and Rhodes grass, making Brachiaria a better alternative for soil fertility restoration.

Roots biomass had a significant site x cultivar interaction ($P<0.001$) at the 22 WAE period in both depths. MG4 had the highest roots biomass in Eldoret (1445 kg $ha^{-1}$) while Xaraes, Piata and Basilisk had higher roots biomass than other grasses in Kitale at the 0-15 m depth. The grasses differed in roots spread beyond the 15 m depth in both sites. At the 15- 30 m depth, roots biomass ranged from 113 to 438 kg $ha^{-1}$ in Kitale and 144 to 460 kg $ha^{-1}$ in Eldoret. A significant varietal effect ($P<0.001$) was noted in roots biomass production in Eldoret. In general, all the Brachiaria cultivars had higher roots biomass than the control grass (Napier grass). At 22 WAE, Rhodes grass had not yet fully established due to slow germination and growth in this site, sampling was not done. The cv. MG4 accumulated the highest roots biomass (1445 kg $ha^{-1}$) while Napier grass had the lowest roots biomass (345 kg $ha^{-1}$) in this site (Figure 3). As was observed in the Kitale site, roots biomass in all cultivars decreased with sampling depth, and was higher in the 0 - 15 m and lowest at the sub soil (15 - 30 m). Beyond the 15 m depth, Xaraes accumulated the highest roots biomass while Mulato II and Basilisk had the lowest accumulation of roots biomass at this depth.

**Table 4** Roots Biomass (kg $ha^{-1}$) of Brachiaria grasses, Napier grass and Rhodes grass at two depths and three sampling periods in Kitale

| Grass cultivars | 0-15cm | | | 15-30 cm | | |
|---|---|---|---|---|---|---|
| | 22WAE | 46WAE | 80WAE | 22WAE | 46WAE | 80WAE |
| *B. decumbens* cv. Basilisk | 1192a | 2951b | 8754a | 231b | 806a | 2739a |
| *B. humidicola* cv. Llanero | 306b | 2225bc | 9030a | 113c | 597a | 3335a |
| *B. brizantha* cv. Marandu | 668b | 2330b | 8483a | 264b | 853a | 1517a |
| *B. brizantha* cv. MG4 | 710b | 2567b | 7318a | 215bc | 848a | 2649a |
| *B. hybrid* cv. Mulato II | 579b | 2367b | 9175a | 113c | 1059a | 1929a |
| *B. brizantha* cv. Xaraes | 1338a | 2570b | 8390a | 247b | 670a | 2454a |
| *B. brizantha* cv. Piata | 1324a | 3150a | 8964a | 438a | 839a | 3504a |
| Rhodes grass | 614b | 2634b | 4332a | 140c | 658a | 1422a |
| Napier grass | 466b | 2876b | 6986a | 195c | 804a | 1491a |
| Mean | 772.4 | 2499.5 | 7791 | 208.1 | 764.2 | 2408.9 |
| SED (cultivar) | 232.5 | 449.1 | NS | 68.1 | NS | NS |

WAE- weeks after emergence; cv – cultivar, NS- not significant at *P≤0.05.* Means followed by the same letter for a parameter are not significantly different at *P≤0.05.* Mean separation was done by Tukeys HSD test.

Increased roots biomass is essential in plant productivity because in grasses, high roots biomass improves nutrient and water uptake (Bowman *et al.,* 1998) particularly during periods of water scarcity. Xaraes, MG4, Piata and Basilisk had high roots biomass in both sites and accumulated higher shoots biomass than the other grasses especially during the dry season. Biomass investment in shoots and roots differed between Napier grass and the Brachiaria cultivars. Napier grass invested up to 20 times more biomass in shoots than roots in Kitale and nearly 10 times in Eldoret. The lower investment in Kitale would be due to sufficient moisture which encouraged higher shoots growth at the expense of roots. In Eldoret, soil depth and moisture was limiting leading to higher roots biomass to scavenge for water and nutrients. The Brachiaria cultivars had comparatively lower investment in shoots: roots biomass, which ranged between 1.2 to 2.1 and 2.5 to 5.0 in Eldoret and Kitale, respectively.

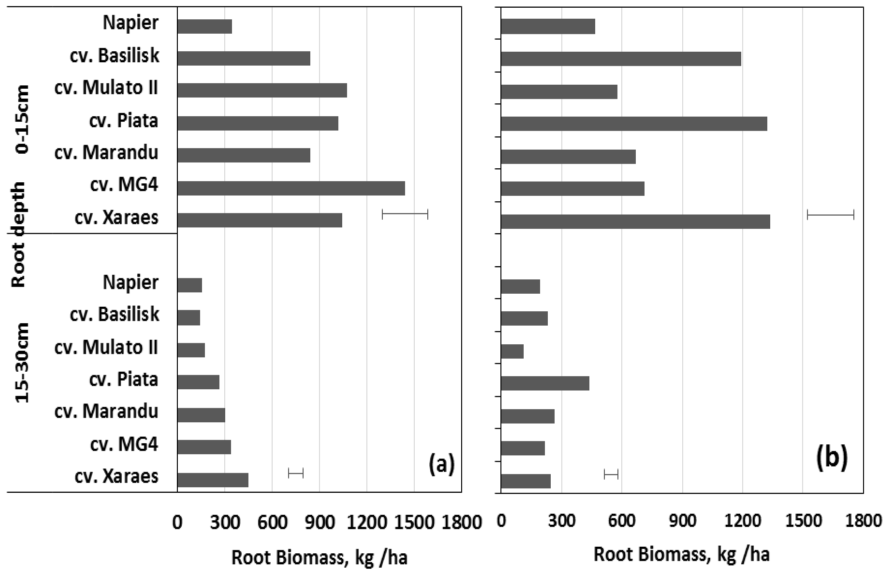


**Figure 3** Roots Biomass (kg ha$^{-1}$) of Brachiaria grasses and Napier grass at different sampling depths in Eldoret (a) and Kitale (b). Bars resprsent LSD P<0.05.

*Relationship between shoots biomass yields and growth parameters*

In Kitale, shoots biomass was strongly and positively correlated with plot cover and plant spread (P=0.001) and was also positively but weakly correlated with plant height (Table 5). Cultivars that had wider spread and high plot cover such as Basilisk and MG4 accumulated higher biomass during the dry season. The number of tillers and roots biomass did not influence the shoots biomass in the dry season.

The relationship between grass growth parameters and shoots yield in Eldoret are shown in Table 6. It was observed that during the dry season, shoots biomass was dependent on plant spread, height and cover but had no relationship with plant height, tiller density or roots biomass. A significant linear relationship ($R^2$ = 0.491; P = 0.001) was observed between plant spread and shoots biomass. Plot cover plays a major role in soil and water conservation. In this study, Basilisk, MG4, Xaraes and Piata were more decumbent, with high spread and plot over (60%) thereby accumulating higher biomass in the dry season. However, the erect grasses, Rhodes grass and Mulato II had low plot over (45%), and plant spread (24 m), which contributed to lower shoots biomass during the dry season. Mganga (2009) reported similar results, with C*enchrus ciliaris* producing higher biomass among four local grasses due to increased horizontal spread. During the wet season, grass spread, cover and height were the most important growth parameters influencing shoots biomass, with spread and cover having the highest significant linear relationship with shoots biomass in Eldoret (Table 6). Therefore, the grasses that had more spread and higher plot cover accumulated more biomass including MG4 and Basilisk.

**Table 5** Pearson correlations between grass growth parameters and shoots biomass yield during the dry and wet season in Kitale

| | | Shoots biomass | Tillers | Height | Spread | Plot cover | Roots biomass |
|---|---|---|---|---|---|---|---|
| Dry season | Shoots biomass | 1 | | | | | |
| | Tillers | -0.289 | 1 | | | | |
| | Height | 0.435*** | -0.6789*** | 1 | | | |
| | Spread | 0.605*** | -0.118 | 0.452*** | 1 | | |
| | Plot cover | 0.613*** | -0.165 | 0.0956 | 0.417*** | 1 | |
| | Roots biomass | -0.027 | 0.096 | -0.271* | -0.312 | 0.002 | 1 |
| Wet season | Shoots biomass | 1 | | | | | |
| | Tillers | -0.113 | 1 | | | | |
| | Height | 0.441*** | -0.725*** | 1 | | | |
| | Spread | 0.245* | 0.676 | -0.525*** | 1 | | |
| | Plot cover | 0.501*** | -0.034 | 0.325** | 0.216 | 1 | |
| | Roots biomass | -0.019 | -0.098 | 0.626* | -0.067 | 0.129 | 1 |

Where * - P≤0.05; ** - P≤0.01; ***- P≤0.001

**Table 6** Pearson correlation coefficients showing relationship between grass growth parameters and shoots biomass yield during the dry and wet season in Eldoret

| | | Shot biomass | Tillers | Height | Spread | Plot cover | Roots biomass |
|---|---|---|---|---|---|---|---|
| Dry season | Shot biomass | 1 | | | | | |
| | Tillers | 0.792 | 1 | | | | |
| | Height | 0.191 | 0.474*** | 1 | | | |
| | Spread | 0.491*** | 0.577*** | 0.485*** | 1 | | |
| | Plot cover | 0.564*** | 0.658*** | 0.385** | 0.285* | 1 | |
| | Roots biomass | 0.385 | 0.264 | 0.069 | 0.084 | 0.195 | 1 |
| Wet season | Shot biomass | 1 | | | | | |
| | Tillers | 0.067 | 1 | | | | |
| | Height | 0.488*** | -0.470*** | 1 | | | |
| | Spread | 0.700*** | 0.191 | 0.115 | 1 | | |
| | Plot over | 0.849*** | -0.135 | 0.656*** | 0.608*** | 1 | |
| | Roots biomass | 0.385 | 0.175 | 0.156 | 0.098 | 0.098 | 1 |

Where * - P≤0.05; ** - P≤0.01; ***- P≤0.001

## Conclusions

In general, results from this study show that shoots biomass varied considerably depending on the cultivar and the site. Fertilizer NP response was minimal despite the low soil N and P in both sites. The shoots biomass was mainly influenced by the spread and plot cover attributes both in the dry and wet seasons. Brachiaria cultivars out-yielded Rhodes grass, the commonly grown grass in the region and accumulated high roots biomass in early establishment phase.

Basilisk and MG4 and may be considered as the best bet cultivars for the north western highlands region due to their superior biomass yields.

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# Theme 2

# Participatory evaluation of Brachiaria grasses

# Participatory evaluation of Brachiaria grass cultivars for livestock production in the coastal lowlands of Kenya

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## Abstract

Despite past efforts to introduce cultivated forages in coastal lowlands Kenya, their contribution to livestock feeding is less than 10%. The low adoption of forage cultivation is likely to be due to limited involvement of farmers during the early stages of technology development. Participatory variety selection helps the farmers to select desired characteristics, as scientists obtain feedback from potential end users early in the technology development process. A total of 115 farmers drawn from different zones within the coastal lowlands participated in evaluation and selection of Brachiaria grasses at Mtwapa. Eight Brachiaria grass cultivars: *B. brizantha* cv. Marandu, Xaraes, Piata and MG4, *B. decumbens* cv. Basilisk, *Brachiaria hybrid* cv. Mulato II, *B. humidicola* cv. Humidicola and Llanero along with two controls, Rhodes grass cv. ex-Tozi and Napier grass were evaluated. The farmers were facilitated to hold focused group discussions sessions during which they developed their own criteria for grass evaluation. The farmers listed sixteen criteria which were then prioritized using pair-wise ranking technique. The seven priority criteria identified were: drought tolerance, fast re-growth ability after cutting, hairlessness, numerous tillers that are source of planting materials, nutritious, highly palatable and high yielding. Farmers used the Likert scale of 1 to 5 (1=very poor, 5= very good) to rate each grass cultivar based on the seven criteria. With the exception of cv. Humidicola that did not establish well all the Brachiaria grasses scored better (score >2.4) than *C. gayana* cv. ex-Tozi (score 2.1). The cv. Mulato II (score =3.3) and Xaraes (score=3.2) was significantly ($P < 0.05$) higher than Napier grass (score =3.0) and Marandu (score =3.0). It was concluded that the evaluated Brachiaria cultivars would meet the farmers' criterion for introduction in the mixed smallholder farms in coastal lowlands. On-farm testing of the grasses and evaluation with livestock was recommended.

**Keywords:** Brachiaria, coastal lowlands, cultivars, farmer, participatory evaluation.

## Introduction

Past efforts to introduce cultivated forages to farmers in coastal lowlands Kenya were reported in the forage adoption study by Mureithi *et al.* (1998). Cultivated fodder, mainly Napier grass (*Pennisetum purpureum*) was first introduced in coastal lowlands by the then Ministry of Agriculture in 1960. In 1978-79, the Ministry of Lands and settlement did further promotion of Napier grass mainly for soil conservation. From 1980 to 1992, Kenya Agricultural Research Institute (now Kenya Agricultural Research Organization, KALRO), in collaboration with the National Dairy Development Project (NDDP) and International Livestock Research Institute (ILRI) systematically promoted forage cultivation for improved dairy in the region. The list of forages promoted expanded to include Panicums, herbaceous and tree forage legumes in addition to Napier grass (Njunie *et al*., 1995; Mwatate *et al*., 1998). Forage technologies' adoption was high immediately after promotion, but some farmers abandoned them after a few years

(Nicholson *et al*., 1999). For example, the number of farms with pure stands of Napier grass and the amount planted per cow declined by nearly 50% from 1988 to 1993. In a recent study by Njarui *et al*. (2016b), natural pastures was the most popular feed in coastal lowlands, with over 98% of farmers using them to feed livestock. Utilization of cultivated forages for the region was low; with Napier grass contributing on average 10% of the feeds in most months. The low contribution of improved forages to dairy cattle feeding was partly because farmers gave less priority in the allocation of resources to forage cultivation (Mureithi *et al*., 1998). For example, Napier grass was planted in the least fertile part of the farm land and after maize. Napier grass plots were not weeded in time and most farmers were not able to return slurry to the Napier grass as recommended. The low priority farmers gave to the cultivated forages implies that they were more interested in crops. Past records indicate that there was little or limited involvement of farmers as co-researchers during forage technology development.

In a programme to improve livestock production in East Africa, Brachiaria grass genotypes selected and improved in Latin America were re-introduced to coastal lowlands Kenya. Brachiaria species adapt to diverse habitats ranging from shaded to open areas and desert to swampy areas (Miles *et al*., 1996). Consequently, the grasses have great potential in the intensification of livestock production systems as sown forages in coastal Kenya. The programme sought to involve the farmers as co-researchers from the initial stages of technology development through participatory evaluation so that their priorities and views can be considered in selecting suitable forages for integration in their farming system. Participatory variety selection helps the farmers to select desired characteristics, as scientists obtain feedback from potential end users early in the technology development process.

**Materials and methods**

*Site description*

The grass cultivars for farmer evaluation were established at KALRO-Mtwapa, located 3°36'S, 39°44'E, 15 m asl. The site is in the coconut-cassava agro-ecological zone (Jaetzold *et al*., 2006). There are two distinct rainfall patterns in the area, a long rain season from April to June and a short rain season from October to December. The soils are well drained, deep, low in available nutrients, and have low to moderate moisture storage capacity. The topsoil texture is sandy loam to sandy clay loam with low organic matter content. The mean temperature ranges from a minimum of 24 - 27°C in May-July to a maximum of 30 - 32°C in January to April.

*Brachiaria grasses*

Eight Brachiaria varieties: *B. brizantha;* cvs. Marandu, Xaraes, Piata and MG4, *B. decumbens* cv. Basilisk, *B. hybrid* Mulato II, *B. humidicola* cv. Humidicola and Llanero and two controls, Napier grass cv. Bana and Rhodes grass cv. ex-Tozi were established during the short rain season in November 2013.

*Development of selection criteria*

The farmers were facilitated to develop criteria to be used when selecting grasses for planting on their farms. A multistage stratified sampling technique was used for selection of the farmers. Diverse sites where smallholder farmers practiced mixed-farming were identified within Kilifi County and visited by researchers for information gathering. Sites visited were Kaloleni and Chonyi (CL3), Gotani and Msabaha (CL4) and Tsangatsini (CL5). In each site, groups of 20 to 30 farmers were involved in focused group discussions (FGD). During the FGD, farmers were requested to suggest the things they considered important when selecting grasses to establish in their farms. The suggested criteria were clearly recorded by the research scientist and pair-wise ranking carried out as suggested by the farmers.

*Selection of Brachiaria cultivars by farmers*

Smallholder farmers were invited to KALRO Mtwapa centre, twice for participatory evaluation and selection of Brachiaria cultivars: in April, 2014 at the vegetative grass-growth stage and at maturity in July, 2014. Farmers were drawn from coastal lowlands (CL) agro-ecological zones 3, CL4 and CL5 (Table 1). Between 112-115 farmers participated in the evaluation (Table 1). There was equal mean representation of men and women (1:1) in the grass evaluation exercise.

**Table 1** Number of farmers involved in participatory evaluation of Brachiaria disaggregated by gender

| Agro-ecological zones | Number of participating farmers | | | | | |
|---|---|---|---|---|---|---|
| | April 2014 | | | July 2014 | | |
| | Males | Females | Total | Males | Females | Total |
| Coastal Lowlands 3 | 19 | 36 | 55 | 22 | 31 | 53 |
| Coastal Lowlands 4 | 29 | 17 | 46 | 28 | 15 | 43 |
| Coastal Lowlands 5 | 7 | 7 | 14 | 9 | 7 | 16 |
| Total | 55 | 60 | 115 | 59 | 53 | 112 |

*Grass evaluation*

At the field site, each farmer was provided with an evaluation score data sheet. The farmers were trained on how to use the Likert scale of 1 - 5 in the evaluation of each grass cultivar and record the data for each grass in the data sheet. They were then guided through a specific route within the experimental field, where they made their observations on each plot (grass cultivar) and recorded the information in the score sheet.

**Data analysis and presentation**

Data was entered in MS excel spreadsheet and analysed using the general linear model of Statistical Analysis System (SAS, 2010). The mean scores for each evaluation criteria were separated using least significant differences (LSD) at $P<0.05$. The results are presented using descriptive statistics, charts and tables.

## Results

*Farmers' selection criteria*

Farmers identified 16 criteria they consider when selecting suitable forages for their livestock. These criteria were ranked based on their importance and are summarized in Table 2. Forages that are less hairy (LH), high yielding (HY), easy to establish and spread due to availability of planting materials (PM), with drought tolerance characteristics (DT), highly palatable when fed fresh (green) or at maturity (P) and with high re-growth ability after cutting (RA) would be most preferred by farmers. Criteria that were difficult to quantify or qualify were ranked lowest. High nutritive value of the foliage (NV), self-regeneration ability of the plants (SR), milk quality (less watery milk probably due to high butter fat in milk) (QM), palatability of dry grass (standing hay) and easily harvested due to firm plant anchorage in the soil (GA) were thus lowly ranked.

*Grass evaluation*

Results of the farmer evaluation are summarized in Table 3. Humidicola failed to establish and therefore it was not included in the evaluation. All the Brachiaria grasses scored better (score >2.4) than Rhodes grass cv. ex-Tozi (score 2.1). Mulato II (score =3.3), Xaraes (score=3.2) had a higher score than Napier grass (score =3.0) while Marandu had similar score to Napier grass. Results show that both male and female farmers did not differ in their ranking of most the grasses (Table 4). The only exception was Xaraes which was ranked higher than Napier grass by men.

## Discussion

*Farmers' selection criteria*

The farmers in coastal Kenya sought after grasses that would be less hairy, produce large material for planting and productive even under low rainfall conditions. In cut-and-carry feeding systems, farmers preferred grass that does not irritate the skin when cutting and carrying to the feeding pens. Generally less hairy grasses are also more palatable. Farmers were conscious of the need to have plenty of planting materials for ease of establishment of forage crops. Consequently, grasses with many tillers would imply increase availability of planting materials as root splits or cuttings (Ramadhan *et al*., 2015). In a past survey, farmers identified lack of planting materials as a major constraint to fodder acreage expansion in the region (Ramadhan *et al*., 2008). Farmers' placement of "drought tolerance' as among the most important criteria may be attributed to poor survival rates of previous cultivated grasses, especially Napier grass during prolonged drought periods. A survey in the region by Ramadhan *et al*. (2008) reported drought as the main factor affecting survival of Napier grass in coastal lowlands. Growth vigour, quick re-growth after cutting and yield criteria can be associated with increased fodder production per unit area. Inadequate feed was identified as a constraint to livestock production in the region (Reynolds *et al*., 1993; Njarui *et al*., 2016b). High palatability, soft and large leaves for ease of forage ingestion are desirable for increased feed intake. Milk yield and /or weight gain are closely related to feed intake (Orodho, 2006). Though not highly ranked, shade tolerance of the grass is important for coastal lowlands farming systems. The cropping system in coastal Kenya is dominated by tree crops, mainly fruit trees, coconut and cashew nuts (Jaetzold *et al*., 2006). Pasture development in this region is integrated with tree crops (Orodho, 1997). Farmers who participated in the discussions clearly identify areas under tree crops as a likely niche for planting grasses.

*Grass evaluation*

The good performance of three Brachiaria grasses is of significance, considering that Napier grass is the recommended fodder grass for dairy production in the region (Mureithi *et al*., 1998; Nicholson *et al*., 1999). The better performing Brachiaria grasses could replace Napier grass in smallholder dairying systems. Furthermore, production of Napier grass especially in the highlands is threatened by Napier grass stunt disease which causes total loss of the crop in severe cases (Khan *et al*., 2014). Farmers have complained of poor survival of Napier grass on-farm resulting in low adoption of cultivated fodder in the region (Mureithi *et al*., 1998; Njarui *et al.,* 2016a). The Brachiaria grasses offer alternative cultivated fodder to Napier grass and ex-Tozi in the region.

**Conclusions**

- Farmers indicated that they would prefer grasses that are easy to establish, productive, can tolerate drought, palatable and with high nutritive value.
- The Brachiaria cultivars that established well were all superior to Rhodes grass cv. ex-Tozi.
- *Brachiaria hybrid* cv. Mulato II and cvs. Xaraes were ranked higher than Napier grass.

**Recommendation**

There is need for further evaluation of the best ranked Brachiaria cultivars to determine their nutritive value for milk and meat production on station and under farmer participation on farm.

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**Table 2** Matrix on pairwise ranking of selection criteria of suitable forages by farmers

| Key attributes considered | DT | NV | MY | P | HY | VG | RA | LL | ST | SR | HM | SF | LH | GA | PM | QM | Total score | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Drought tolerance (DT) | | DT | MY | DT | HY | DT | RA | DT | DT | DT | DT | SF | LH | DT | DT | DT | 10 | 3 |
| Nutritive value (NV) | | | MY | P | HY | VG | RA | LL | ST | NV | HM | NV | LH | GA | PM | QM | 2 | 16 |
| Milk production potential (MY) | | | | P | MY | VG | MY | LL | MY | MY | HM | MY | LH | MY | MY | QM | 9 | 5 |
| Palatability (P) | | | | | P | VG | RA | P | P | P | P | SF | P | P | PM | QM | 9 | 6 |
| High yield (HY) | | | | | | HY | RA | HY | HY | HY | HM | SF | HY | HY | PM | HY | 9 | 7 |
| Vigorous growth (VG) | | | | | | | RA | VG | VG | SR | HM | VG | LH | VG | PM | VG | 8 | 8 |
| Re-growth ability after cutting (RA) | | | | | | | | LL | RA | RA | HM | RA | LH | RA | PM | RA | 10 | 4 |
| Large leaves for easy ingestion (LL) | | | | | | | | | ST | LL | LL | SF | LH | GA | PM | HM | 5 | 13 |
| Shade tolerant (ST) | | | | | | | | | | ST | ST | ST | LH | GA | PM | ST | 5 | 14 |
| Self-regeneration (SR) | | | | | | | | | | | SR | SF | LH | GA | SR | SR | 4 | 15 |
| High moisture content (HM) | | | | | | | | | | | | SF | HM | GA | PM | QM | 7 | 9 |
| Soft forage (SF) | | | | | | | | | | | | | LH | GA | PM | SF | 7 | 10 |
| Less hairy (LH) | | | | | | | | | | | | | | LH | PM | LH | 11 | 1 |
| Firm anchorage of plants in soil (GA) | | | | | | | | | | | | | | | PM | QM | 6 | 11 |
| Planting materials production (PM) | | | | | | | | | | | | | | | | QM | 11 | 2 |
| Quality milk (not watery) (QM) | | | | | | | | | | | | | | | | | 6 | 12 |

**Table 3** Farmers' scores for grass cultivars across all selection criteria for the two evaluations at KALRO Mtwapa in coastal lowlands Kenya

| Grass cultivar | Drought tolerance | Hairless | Soft leaves | Easy to cut | Tiller ability | Greenness | Large leaves | Mean score |
|---|---|---|---|---|---|---|---|---|
| Mulato II | 3.4 | 2.8 | 3.2 | 3.3 | 3.5 | 3.4 | 2.9 | 3.3 |
| Xaraes | 3.3 | 2.7 | 2.9 | 3.0 | 3.2 | 3.3 | 3.1 | 3.2 |
| Marandu | 3.1 | 2.8 | 2.9 | 3.0 | 3.3 | 3.0 | 2.9 | 3.0 |
| Napier grass | 3.1 | 2.8 | 2.8 | 3.1 | 3.0 | 3.0 | 3.4 | 3.0 |
| Piata | 3.1 | 2.7 | 2.6 | 2.9 | 3.0 | 3.0 | 2.7 | 2.8 |
| MG4 | 2.7 | 2.5 | 2.6 | 2.6 | 2.9 | 2.5 | 1.9 | 2.6 |
| Basilisk | 2.4 | 2.5 | 2.5 | 2.5 | 2.8 | 2.6 | 2.2 | 2.5 |
| Llanero | 3.0 | 2.6 | 2.5 | 2.3 | 3.1 | 3.0 | 1.9 | 2.4 |
| Rhodes grass | 2.0 | 2.3 | 2.1 | 2.4 | 2.3 | 1.9 | 1.5 | 2.1 |
| Humidicola† | - | - | - | - | - | - | - | - |
| LSD ($P<0.05$) | 0.16 | 0.17 | 0.16 | 0.19 | 0.16 | 0.15 | 0.15 | 0.14 |

†Cultivar established poorly and was not evaluated

**Table 4** Gender categorized farmers' scores for grass cultivars across all selection criteria for the two evaluations at KALRO Mtwapa in coastal lowlands Kenya

| Grass cultivar | Mean score across all criteria | |
|---|---|---|
| | Female | Male |
| Mulato II | 3.3 | 3.2 |
| Xaraes | 3.0 | 3.1 |
| Marandu | 3.0 | 3.0 |
| Napier grass | 3.1 | 2.9 |
| Piata | 2.8 | 2.9 |
| Llanero | 2.6 | 2.6 |
| MG4 | 2.6 | 2.6 |
| Basilisk | 2.5 | 2.6 |
| Rhodes grass | 2.0 | 2.1 |
| LSD ($p<0.05$) | 0.16 | 0.15 |

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# Participatory evaluation and selection of suitable Brachiaria grass cultivars for production in the semi-arid eastern Kenya

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**Abstract**

Low adoption of agricultural technologies has been attributed to insufficient attention to farmers' priorities and perceptions while developing and promoting technologies. A Participatory Variety Selection (PVS) involving 84-89 farmers was carried out at Katumani to evaluate and select adapted and productive Brachiaria grasses for the semi-arid environment. The Brachiaria cultivars evaluated were; *Brachiaria brizantha* cvs. Marandu, Xaraes, Piatã, MG4, *Brachiaria decumbens* cv. Basilisk, *Brachiaria humidicola* cv. Llanero, *Brachiaria hybrid* cv. Mulato II and Rhodes grass as a control. The criteria used for selection were identified through focus group discussions by farmers from Upper Midlands 3 and Lower Midlands 4 agro-ecological zones in Machakos and Makueni Counties. Nine attributes; drought tolerance, soil erosion control, plant height at harvest, growth habit, colour of leaves, disease and pest tolerance, suitability for grazing, and suitability for cut-and-carry were used for selection criteria. For each criterion, farmers' scores on individual grass cultivars were recorded using a Likert scale of 1 to 4 with higher scores indicating high cultivar preference. There were no significant ($P>0.05$) differences in the scores between female and male farmers. Drought tolerance and colour had the highest mean score (>3.0), implying that they were important for consideration when selecting forages. Based on the overall criteria, MG4, Basilisk, Mulato II, and Xaraes were found to be most suitable and this concurred with results from agronomic evaluation where they produced the highest dry matter yield.

**Key words:** Drought tolerance, focus group discussion, Likert scale, pairwise ranking, variety selection

## Introduction

There is limited adoption of improved forages among smallholder farmers in Kenya. One of the major factors limiting adoption is insufficient attention to farmers' priorities and perceptions while developing and promoting agricultural technologies (Ashby and Sperling 1995; Chambers *et al*., 1989). For example, forage research and development in many countries including Kenya in the past often involved a process where researchers evaluated forages and selected those which had higher yield potential without farmer participation. The selected forages were then given to extension agents to be passed on to farmers (Gabunada *et al*., 1997). Unfortunately, technologies selected through this approach often failed to fulfill their potential from research evaluations and this resulted in low adoption. Since 1990s, participatory approaches have become a driving force for agricultural research and rural development.

One of the recently adopted approaches is the Participatory Varietal Selection (PVS) that has been found to be very effective in addressing the problem of inadequate adoption of new varieties in many crops in different countries of the world (Islam *et al*, 2008). The approach involves the selection by farmers on their own fields of finished or near-finished products from plant breeding programmes (Paris *et al*., 2011). Successful application of PVS in Sub-Saharan Africa include bean crop improvement in Rwanda, Tanzania, and Malawi, the adaptation of the New Rice For Africa (NERICA) varieties (Walker, 2006), the development of maize varieties for drought tolerance, low nitrogen and pest tolerance (Hugo and Siambi, 2002) and identification of Napier grass (*Pennisetum purpureum*) cultivars with farmer preferred traits in northern Tanzania (Sikumba *et al*., 2015). The first step in participatory varietal selection (PVS) is identifying the needs of farmers by discovering what crops they grow, and what traits they consider important when selecting varieties they grow within their agro-ecological and socio-cultural environment (Witcombe, 1996; Paris *et al*., 2011). Further, participatory varietal selection is a means for social scientists to identify the varieties that most men and women farmers prefer, including the reasons for their preference and constraints to adoption.

Analysis of participatory of CIMMITY research projects by Lilja and Bellon (2006) reported that some outcomes associated with participatory research include farmers' access to seed and faster adoption, awareness of new varieties and, provision of varieties with valued traits to farmers. Participatory evaluation and selection has been recommended as a key for improving of forages in Kenya (Mwangi and Wambugu, 2003). The objective of the study was to identify the selection criteria used by farmers to select forages and select suitable Brachiaria grass cultivars for integration in production system within semiarid environment of eastern Kenya.

## Materials and methods

### *Brachiaria grass cultivars*

The Brachiaria grass cultivars were established at Kenya Agricultural and Livestock Research Organization (KALRO), Katumani during the short rains season of 2013. The site (37°28′0′′E, 1°58′0′′S) is located at 75 km south-east of Nairobi at an elevation of 1580 m asl. The mean annual rainfall is 717 mm, bimodal pattern and the dominant soils are chromic luvisols, which are low in organic C, highly deficient in N and P and to some extent Zinc (NAAIAP, 2014). The seven cultivars used in the evaluation were *Brachiaria brizantha* cvs. Marandu, Xaraes, Piatã, MG4, *Brachiaria decumbens* cv. Basilisk, *Brachiaria humidicola* cv. Llanero and *Brachiaria hybrid* cv. Mulato II. These were compared with Rhodes grass (*Chloris gayana*), a locally cultivated grass.

### *Development of selection criteria*

The criteria used by farmers in the selection of grass cultivars were identified through focus group discussions (FGDs) following the procedure described by Krueger (2002). Groups of dairy farmers were purposefully selected from Upper Midlands 3 (UM3) and Lower Midlands 4 (LM4) agro-ecological zones in Machakos and Makueni Counties respectively. The farmers were randomly selected from a list of farmers who had participated in the baseline survey conducted earlier to identify niches for establishing Brachiaria grasses in the study area (Njarui *et al*., 2016b). With guidance of researchers and extension workers, farmers listed criteria they consider important for selection of forages for livestock. A pairwise ranking matrix was used to determine the most important criteria which were then applied in the evaluation and selection.

*Farmers' evaluation and selection*

Between 84 - 89 farmers (Table 1) participated in evaluation and selection of Brachiaria grasses at Katumani at three separate occasions; March 2014 (wet season), June 2014 (end of wet season) and Oct 2014 (peak of dry season). Attributes that could not be directly observed in the field such as high milk yield, nutritionally balanced, palatability of forages etc. were dropped. The final criteria included drought tolerance, disease tolerance, soil erosion control, pest resistance, height at harvest and uses (suitable for grazing and for cut-and-carry). Green colour of the leaves was also included as a selection criterion. For each criterion, farmers' opinions on individual grass cultivars were recorded in an evaluation form using a Likert scale of 1 to 4 where, 1 =poor, 2=fair, 3=good and 4=very good.

**Table 1** Number of farmers involved in Participatory Variety Selection of Brachiaria grasses

| Agro-ecological zones | Number of participating farmers | | | | | |
|---|---|---|---|---|---|---|
| | March 2014 | | June 2014 | | October 2014 | |
| | Males | Females | Males | Females | Males | Females |
| Upper Midland 3 | 32 | 15 | 38 | 12 | 32 | 17 |
| Lower Midlands | 27 | 15 | 19 | 15 | 29 | 9 |
| Total | 59 | 30 | 57 | 27 | 61 | 26 |

*Data analysis*

For each evaluation, the scores from each farmer were entered in Microsoft Excel spreadsheet and a mean score for each criterion calculated for each grass cultivar by gender. The mean scores for each criterion were subjected to Analysis of Variance (ANOVA) and where significant differences occurred, means were separated by the least significant difference (LSD) test using the statistical software Genstat 15 for windows (VSN Int., 2013). To determine the best grass cultivar the scores for each farmer on each criterion were averaged over the three evaluations and the mean scores subjected to Analysis of Variance (ANOVA).

**Results and Discussion**

*Selection criteria*

Farmers identified 14 criteria that are important when selecting forages for livestock production (Table 14). Forages that give high milk yield when fed to livestock were ranked first followed by nutritionally balanced forages and palatability. Forages that are nutritionally balanced and highly palatable influencing milk production in livestock hence they were highly ranked. Drought is prevalent in the semi-arid region and therefore drought tolerant is an important criterion and was ranked fourth. Besides attribute related to livestock, farmers also considered other benefits of forages such as erosion control. In the semi-arid crops and livestock are closely integrated in the farming system and forages are planted along the terrace banks for control of erosion and also provide feed for livestock. Surprising high yield was ranked last because farmers prefer forages that have stable yield even during the dry season.

*Evaluation and selection of grass cultivars*

The order of ranking of the grass cultivars was similar and not significantly different between farmers from UM3 and LM4 in the three evaluations and therefore analysis of the combined results are presented. The average scores for the three evaluations are shown Table 3. There were significant differences ($P<0.05$) among the grass cultivars on all the 9 selection criteria. The cv. MG4 had the highest score in 8 out of the 9 selection criteria and consequently had the highest mean score (3.08). The cvs. Basilisk, Mulato II and Xaraes were ranked second, third and fourth best cultivars respectively. Drought resistance and colour had the highest mean score (>3.0) (Table 3), implying that they were important for consideration when selecting forages. Deep green colour is associated with high nutritional quality, with Mulato II, MG4 and Basilisk being highly ranked for this attributes. Results from chemical analysis showed these grasses had high crude protein, 9.-13% at 6 week growth and 8 -11% at 8 weeks growth (Njarui *et al*., 2016a). The ranking of the Brachiaria cultivars largely concurred with results from agronomic evaluation reported by Njarui *et al.* (2016a) where cvs. MG4, Xaraes, Piatã and Basilisk recorded highest dry matter. On-farm study on adaptability of improved Brachiaria grasses in low rainfall and Aluminum toxicity prone areas of Rwanda (Mutimura and Everson, 2012) showed that although cv. Mulato II was not the most productive grass; farmers preferred it due to its adaptability to low rainfall and acidic soil stress, and its production of green forage year round. Similar results were reported from trials in D.R. Congo (Katunga *et al*., 2014) where forage trees and shrub legumes chosen by farmers were, in general, the same as those from the agronomic evaluation which emphasis the importance of involving farmers during the evaluation. There were no significant differences ($P > 0.05$) between female and male scores for the grass cultivars (Figure 1). However, scores for female farmers were generally higher than those for male farmers.

**Conclusions**

Based on the criteria developed, the cultivars selected by farmers were found to be more productive and nutritious through the agronomic evaluation. Among the Brachiaria cultivars evaluated the cvs. MG4, Basilisk, Mulato II and Xaraes were ranked highly and could further be evaluated and up-scaled in other regions with similar environment. Participatory evaluation incorporates farmers view and preferred plant attributes and is likely to enhance adoption.

**Acknowledgements**

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**Table 2** Farmers' key criteria in selection of grasses for forage production

| Key attributes considered | HY | P | HM | DT | SEC | PR | DL | NB | EE | EM | LP | GH | U | HH | Total Score | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbage yield (HY) | | P | HM | DT | SEC | PR | DL | NB | EE | EM | GP | GH | U | HH | 0 | 14 |
| Palatability (P) | | | HM | P | P | P | P | NB | P | P | P | P | P | P | 11 | 3 |
| High milk yield (HM) | | | | HM | HM | HM | HM | HM | HM | HM | HM | HM | HM | HM | 13 | 1 |
| Drought tolerant (DT) | | | | | DT | DT | DT | NB | DT | DT | DT | DT | DT | DT | 10 | 4 |
| Soil erosion control (SEC) | | | | | | SEC | SEC | NB | SEC | SEC | SEC | SEC | U | HH | 7 | 6 |
| Pest resistant (PR) | | | | | | | DL | NB | PR | PR | PR | PR | PR | PR | 7 | 7 |
| Disease tolerant (DL) | | | | | | | | NB | DL | DL | DL | DL | DL | DL | 8 | 5 |
| Nutritionally balanced (NB) | | | | | | | | | NB | NB | NB | NB | NB | NB | 12 | 2 |
| Easy to establish (EE) | | | | | | | | | | EM | GP | GH | U | HH | 1 | 13 |
| Easy to manage (EM) | | | | | | | | | | | GP | H | U | HH | 2 | 12 |
| Good persistence (GP) | | | | | | | | | | | | GP | GP | HH | 5 | 9 |
| Growth habit (GH) | | | | | | | | | | | | | GH | HH | 4 | 11 |
| Uses (U) | | | | | | | | | | | | | | U | 5 | 10 |
| Height at harvesting (HH) | | | | | | | | | | | | | | | 6 | 8 |

Uses: 1=Suitable for grazing 2=Suitable for cut-and-carry

**Table 3** Farmers' scores for grass cultivars averaged over three evaluations and the two counties

| Grass cultivar | Selection criteria: Drought tolerance | Soil erosion control | Height at harvest | Growth habit | Colour | Disease tolerant | Pest tolerant | Suitable for grazing | Suitable for cut-and-carry | Mean score | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Marandu | 2.47 | 2.48 | 1.76 | 1.94 | 2.46 | 1.86 | 1.72 | 2.23 | 1.91 | 2.09 | 6 |
| MG4 | 3.18 | 2.90 | 3.17 | 2.95 | 3.17 | 3.45 | 2.94 | 3.01 | 2.93 | 3.08 | 1 |
| Piata | 2.68 | 2.37 | 2.57 | 2.44 | 2.72 | 1.83 | 2.05 | 2.50 | 2.44 | 2.40 | 5 |
| Xaraes | 2.65 | 2.19 | 2.27 | 2.33 | 2.88 | 3.00 | 2.28 | 2.47 | 2.23 | 2.48 | 4 |
| Basilisk | 3.04 | 2.88 | 2.70 | 2.61 | 3.09 | 3.30 | 2.72 | 2.77 | 2.75 | 2.87 | 2 |
| Llanero | 2.33 | 2.81 | 1.23 | 1.52 | 1.93 | 2.46 | 2.43 | 1.97 | 1.45 | 2.01 | 7 |
| Mulato II | 2.97 | 2.66 | 1.81 | 2.11 | 3.08 | 3.40 | 2.60 | 2.65 | 2.04 | 2.59 | 3 |
| Rhodes grass | 2.79 | 2.75 | 2.99 | 2.72 | 2.66 | 2.25 | 2.45 | 2.66 | 3.08 | 2.71 | |
| Mean | 2.76 | 2.63 | 2.31 | 2.33 | 2.75 | 2.69 | 2.40 | 2.53 | 2.35 | 2.53 | |
| LSD (P<0.05) | 0.25 | 0.25 | 0.26 | 0.27 | 0.29 | 0.35 | 0.31 | 0.30 | 0.26 | | |

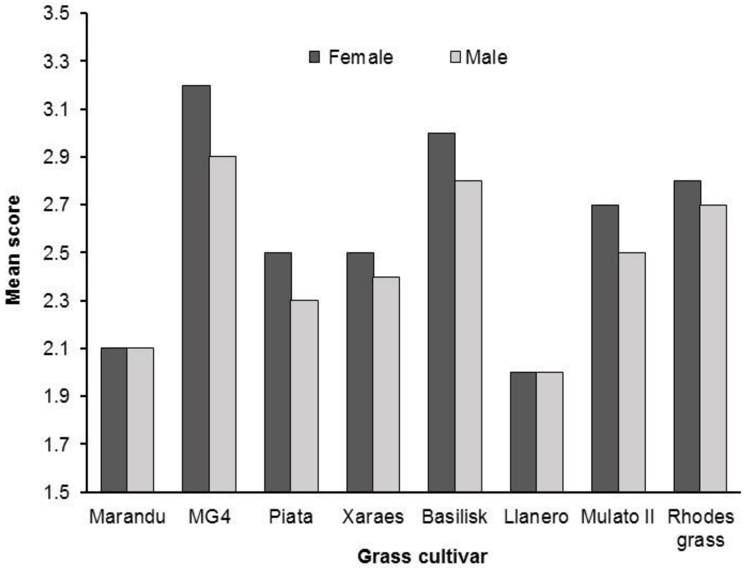


**Figure 1** Farmers' criteria mean scores for grass cultivars for the three evaluations by gender

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# Participatory evaluation and selection of Brachiaria grass cultivars in the cool central highlands of Kenya

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**Abstract**

Inadequate feed resource is a major constraint to the performance of the livestock sub-sector in Kenya. A farmer participatory study involving 60 participants was conducted to evaluate and select suitable Brachiaria grasses for adaptation and up-scaling in the cool highlands of central Kenya. Farmers used their own criteria to evaluate and select eight Brachiaria cultivars established in an on-station experiment at KALRO Ol Joro Orok. The cultivars consisted of *Brachiaria decumbens* cv. Basilisk, *B. humidicola* cv. Llanero, *B. brizantha* cvs Marandu, MG4, Piatã, Xaraes and *B. hybrid* cv. Mulato II They were compared with two commonly cultivated local grasses; Rhodes grass (*Chloris gayana* cv. Boma) and Napier grass (*Pennisetum purpureum* cv. Kakamega 1). The five most important criteria listed by farmers were; ground cover, hairiness, plant height at harvest, resistance to pests and disease and the amount of forage. The 5 criteria used by the farmers to evaluate the grasses were fixed in an evaluation sheet using a Likert scale of 1 to 5 (1= very poor to 5= very good). The scores for different Brachiaria cultivars were Piata (3.47), MG4 (3.63), Basilisk (3.50), Marandu (3.08), Llanero (2.51), Humidicola (2.44), Mulato II (2.50) and Xaraes (3.28). However, Brachiaria grasses had lower ranking than Napier grass (4.09) and Rhodes grass (4.28). Farmers selected MG4, Piata, Basilisk and Xaraes as the most promising cultivars for up scaling.

**Key words:** Brachiaria grasses, Likert scale; pairwise ranking

## Introduction

Feed is a major component of the livestock production systems in Kenya accounting for 60-80% of the total production cost in the intensive systems. One of the major source of feed for livestock in central highland of Kenya is natural pasture, which is low in quantity and quality and consequently livestock productivity is low (Njarui *et al.*, 2016). Napier and Rhodes grass are the major cultivated forage grasses in Kenya due to their relatively wide ecological adaptability, relatively high herbage yield and ease of propagation and management. In the intensive market oriented smallholder livestock production systems, Napier grass constitutes between 40 - 80% of forages used by smallholder dairy farmers in Kenya. Although there have been efforts to evaluate new cultivars of Napier grass (Nyambati *et al*., 2010) there has been limited research on other grasses to increase the genetic base of fodders for production in mixed crop-livestock smallholder farms. In addition, the emerging lethal diseases such as Napier smut (Farrel *et al*., 2001) and Napier stunt (Jones *et al*., 2004) threaten the production of Napier grass for livestock feeding.

Brachiaria grass, an indigenous grass in eastern, central and southern Africa (Ndikumana and de Leeuw, 1996) has been widely adapted as livestock forage in South America and East Asia. Besides its use as livestock feed, Brachiaria is known to contribute significantly to carbon sequestration, ecological restoration and soil erosion control hence it plays an important role in reducing greenhouse gases and nutrient losses from soils. Although Brachiaria grasses, have revolutionized the livestock industry as the most adaptable and high yielding grass in south and central America (Miles *et al.*, 2004), their potential in its native land Africa remains largely unexploited. Limited research has been conducted to evaluate the agronomic performance of improved Brachiaria grasses under different agro ecological conditions in Kenya. Furthermore, farmers' participatory approaches in forage technology development have been ignored and this has contributed to low adoption of technologies by the farmers (Nicholson *et al.*, 1999). In a programme to improve livestock production in East Africa, improved Brachiaria grass genotypes selected in Latin America were introduced to the cool highlands of central Kenya. However, these cultivars are new and unknown to the farmers and their performance and response to biophysical conditions in the Central highlands of Kenya are not known. It was therefore imperative to incorporate farmers' views and priorities in the initial development and introduction of Brachiaria cultivars before up scaling the cultivars in the farming systems. The objective of the study was to evaluate and select promising Brachiaria grasses in the cool highlands of Central Kenya using a farmer participatory approach.

## Materials and methods

### *Study site*

The study was conducted at KALRO Ol Joro Orok from November 2013 to May 2014. The site is located at 0˚03'S, 36˚06'E, and lies 2393 m asl in Upper highlands 2-3 (UH2-3) in Nyandarua County (Jaetzold *et al.*, 2006). Average annual rain-fall is 950 mm with weak bi-modal distribution and temperature ranging between 8 and 22°C. The dominant soils at the site are classified as Verto-luvic and Chromo-luvic Phaeozems (Sombroek *et al.*, 1982).

### *Establishment of Brachiaria grasses*

Eight Brachiaria grasses: *Brachiaria brizantha* cvs. Marandu, Xaraes, Piatã, MG4, *Brachiaria decumbens* cv. Basilisk, *B. humidicola*, cvs. Humidicola and Llanero and *B. hybrid* Mulato II were evaluated. Napier grass *(Pennisetum purpureum)* cv. KARI-Kakamega 1 and Rhodes grass (*Chloris gayana* cv. Boma) were included in the evaluation as control. The plots were established in November 2013 and triple super-phosphate (TSP, 46% $P_2O_5$) fertilizer was applied at a rate of 200 kg $ha^1$ in the rows prior to sowing the seeds. The plots were kept weed free by hand weeding. The grasses were allowed to establish for 20 weeks before farmers were facilitated to perform a participatory evaluation and selection of the most promising cultivars.

### *Development of evaluation and selection criteria*

Twenty dairy farmers from three sub counties of Mirangine (UH2 and UH3), Nyandarua central (UH 3 and LH 1) and Nyandarua west (UH 2 and UH 3) participated in a one day workshop in April 2014 and developed farmer based criteria for evaluating the Brachiaria grasses. The farmers listed 12 criteria for rating the grasses. The criteria included the following Forage biomass (FB), Hairiness (H), Ground cover(GC), Plant height (PH), Soil and user friendly (SUF), Easy to store (ES), smell (S), Colour (C), Pest and disease resistant (PDR), withstand water logging (WL), Frost tolerant (FT) and Drought tolerant (DT). The twelve (12) criteria developed by farmers were further ranked and reduced to five (5) in order of importance using pairwise ranking. The five most important criteria were Forage biomass, Plant height, Ground cover, Hairiness and Pest and disease resistance.

*Participatory evaluation and selection of Brachiaria grass*

60 (39 male and 21 female) farmers drawn from the sub counties of Mirangine -19 (10 male and 9 female), Nyandarua central - 21 (14 male and 7 Female) and Nyandarua west-20 (15 male and 5 female) representing UH2, UH3, and LH 1 participated in a workshop in April, 2014 to evaluate the Brachiaria grasses. Farmers were trained on how to evaluate and fill the scores on the evaluation data sheet prior to the evaluation exercise. After the training, each farmer individually evaluated and rated the grass cultivars using a Likert scale of 1 to 5 (1=very poor to 5= very good) to rate each grass cultivar. Farmers individually recorded their ratings on each grass cultivar in the evaluation sheet.

**Statistical analysis**

The data were analyzed using PROC MIXED of SAS (SAS Institute Inc. 2001) with grass cultivar as fixed effects. Means were separated using Fisher's protected least significant difference (LSD) at $P<0.05$. The means from farmer ranking were subjected to Pearson's linear correlation procedure to see the correlation significance. The results are presented using descriptive statistics.

**Results and Discussion**

*Farmers' selection criteria*

The results of farmers' most preferred criteria are summarized in table 1. Farmers evaluated the grasses using the following five (5) criteria forage biomass, hairiness, plant height at harvest, ground cover and resistance to pests and disease (Table 1). The forages which produced the most biomass and were less hairy were most preferred. Likewise, the tall forages with good ground cover were preferred also preferred by the farmers. Last but not least, farmers preferred grasses which would resist diseases and pests. The farmers' considerations implied that plant height and ground cover are among the important factors in determining forage yield. The results agreed with the findings of Tessema *et al.* (2003) who reported that increasing foliage height had a direct relationship with increased foliage biomass yield.

*Farmers' evaluation of Grasses*

The results of farmers' evaluation are summarized in Table 2. MG4 and Piata were the most preferred Brachiaria grasses. Brachiaria brizantha cv. MG4 was higher in forage biomass ($P<0.05$) compared to the other Brachiaria grasses. The most preferred Brachiaria grasses by the farmers were Piatã, MG4, Basilisk and Xaraes. However, the ranking of Brachiaria grasses were lower compared to both Napier and Rhodes grass controls. Basilisk was slightly lower than MG4 but was significantly higher than the other Brachiaria grasses. In a related study, Cook *et al.* (2005) reported decumbent growth habit in Basilisk which makes it to form a dense plant spread and cover which may have led to its high rating by the farmers. Specifically, according to the farmers evaluation, MG4 had a significantly higher ($P<0.05$)

rating for ground cover compared to Basilisk but both were rated lower than Napier and Rhodes grass controls. Piatã and Basilisk had a similar rating. Llanero, Humidicola and Brachiaria hybrid Mulato II had the lowest rating (Table 2). MG4, Piatã and Xaraes and Basilisk were rated as having less hair compared with the other Brachiaria grasses but was rated (>0.05) lower than Rhodes grass control. Mulato II had the least rating in terms of hairiness followed by Napier grass control (Table 1). MG4 had the best rating for plant height and was significantly ($P<0.05$) taller than the other Brachiaria grasses. It was followed by Basilisk and Piatã in plant height. The two control grasses of Napier and Rhodes grass were similar in rating but were significantly rated higher ($P<0.05$) than Brachiaria grasses (Table 2). There was no significant difference between the Brachiaria grasses rating for pest and disease resistance. This could mean that farmers were not able to identify physical pests on the grasses. Llanero and Humidicola had the lowest rating in terms of forage biomass Napier control had the best rating followed by Rhodes grass (Table 2). There was a significant positive correlation between height (0.723, $P<0.0001$) and forage yield, suggesting that farmers rank estimate using height had a direct linear relationship to forage yield. Similar relationship was also observed between cover and forage yield (Table 4).

The correlations between forage biomass and plant height and cover are in agreement with those reported by Munyasi *et al*. (2015), implying that height and cover could be used to assess biomass yield. Study by Skerman and Riveros (1990) also confirmed that pasture species which grow fast and tall are more efficient in resource use of and therefore more competitive and productive. In this study, the farmers indicated their preference for grasses that were tall, with high forage yield, resistance to pest and diseases, less hair and good ground cover. They selected MG4, Xaraes and Piata, and Basilisk as the most promising. However, they still preferred both Napier and Rhodes grass over the Brachiaria cultivars. Rhodes grass was however ranked highly and therefore the most preferred grass.

**Conclusions**

The farmers indicated their preference for grasses that were tall, with high forage yield, resistance to pest and diseases, less hair and good ground cover. They selected *Brachiaria brizantha* cvs MG4, Xaraes and Piata, and *Brachiaria decumbens* cv. Basilisk as the most promising. However, they still preferred both Napier and Rhodes grass over the Brachiaria cultivars. Rhodes grass was however ranked highly and therefore the most preferred grass.

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**Table 1** Pairwise ranking matrix of farmers' selection criteria for Brachiaria grass cultivars

| **Attribute** | **FB** | **H** | **GC** | **PH** | **SUF** | **ES** | **SM** | **C** | **PDR** | **WL** | **FT** | **DT** | **Total score** | **Rank** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Forage biomass (FB) | | FB | FB | FB | SUF | FB | FB | FB | FB | FB | FB | FB | 10 | 1 |
| Hairiness (H) | | | H | H | H | H | H | H | H | LH | H | H | 9 | 2 |
| Ground cover (GC) | | | | GC | SUF | GC | GC | C | GC | GC | FT | GC | 6 | 5 |
| Plant height (PH) | | | | | PH | PH | PH | PH | PH | PH | PH | PH | 8 | 3 |
| Soil &and user friendly (SUF) | | | | | | ES | SUF | SUF | PDR | WL | FT | SUF | 5 | 6 |
| Easy storage (ES) | | | | | | | SM | C | PDR | ES | ES | ES | 4 | 10 |
| Smell (SM) | | | | | | | | SM | PDR | WL | FT | SM | 3 | 11 |
| Colour (C) | | | | | | | | | PDR | C | FT | C | 4 | 8 |
| Pest & and Disease resistance (PDR) | | | | | | | | | | WL | PDR | PDR | 6 | 4 |
| Withstand water logging (WL) | | | | | | | | | | | WL | DT | 4 | 9 |
| Frost Tolerant (FT) | | | | | | | | | | | | DT | 4 | 7 |
| Drought tolerant (DT) | | | | | | | | | | | | | 2 | 12 |

**Table 2** Farmer's mean scores for all selection criteria for the evaluated grass cultivars

| Grass cultivars | Cover | Hairiness | Height | Pests | Forage | Mean scores | Rank |
|---|---|---|---|---|---|---|---|
| Rhodes grass | 4.49$^{a}$ | 4.12$^{a}$ | 4.25$^{a}$ | 4.29$^{a}$ | 4.25$^{b}$ | 4.28$^{a}$ | 1 |
| Napier grass | 3.90$^{b}$ | 2.94$^{cd}$ | 4.51$^{a}$ | 4.37$^{a}$ | 4.73$^{a}$ | 4.09$^{b}$ | 2 |
| MG4 | 3.69$^{bc}$ | 3.92$^{ab}$ | 3.19$^{b}$ | 4.06$^{abc}$ | 3.27$^{c}$ | 3.63$^{c}$ | 3 |
| Basilisk | 3.55$^{bcd}$ | 3.69$^{ab}$ | 3.06$^{bc}$ | 4.18$^{abc}$ | 3.02$^{cd}$ | 3.50$^{d}$ | 4 |
| Piatã | 3.45$^{bcd}$ | 3.98$^{ab}$ | 2.78$^{bcd}$ | 4.23$^{ab}$ | 2.92$^{cde}$ | 3.47$^{d}$ | 5 |
| Marandu | 3.24$^{cd}$ | 3.45$^{bc}$ | 2.43$^{de}$ | 3.74$^{bc}$ | 2.51$^{e}$ | 3.08$^{f}$ | 6 |
| Xaraes | 3.12$^{d}$ | 3.86$^{ab}$ | 2.71$^{cd}$ | 3.96$^{abc}$ | 2.74$^{de}$ | 3.28$^{e}$ | 7 |
| Llanero | 2.12$^{e}$ | 3.45$^{bc}$ | 1.73$^{fg}$ | 3.65$^{c}$ | 1.63$^{g}$ | 2.51$^{g}$ | 8 |
| Mulato II | 1.86$^{e}$ | 2.76$^{d}$ | 2.06$^{ef}$ | 3.73$^{c}$ | 2.08$^{f}$ | 2.50$^{g}$ | 9 |
| Humidicola | 1.90$^{e}$ | 3.49$^{bc}$ | 1.55$^{g}$ | 3.65$^{c}$ | 1.61$^{g}$ | 2.44$^{h}$ | 10 |

Means with the same superscripts within columns are not significantly different at $P<0.05$

**Table 3** Scores of grass cultivars as categorized by gender

| | Mean score | |
|---|---|---|
| Grass cultivar | Male | Female |
| Rhodes grass | 4.28 | 4.26 |
| Napier grass | 4.1 | 4.07 |
| MG4 | 3.59 | 3.6 |
| Basilisk | 3.48 | 3.49 |
| Piatã | 3.47 | 3.46 |
| Marandu | 2.99 | 3.08 |
| Xaraes | 3.26 | 3.28 |
| Llanero | 2.51 | 2.50 |
| Mulato II | 2.49 | 2.50 |
| Humidicola | 2.42 | 2.44 |

**Table 4** The relationship between performance indicators for evaluating Brachiaria grasses

| Variable | Cover | hairiness | height | pest |
|---|---|---|---|---|
| Hairiness | 0.19501** | | | |
| Height | 0.53307** | 0.04683* | | |
| Pest | 0.22689** | 0.42034** | 0.09571* | |
| Forage | 0.56248** | 0.07555* | 0.72302** | 0.19522** |

*Correlation significant p<0.05; **Correlation significant p<0.01

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# Participatory evaluation and selection of Brachiaria grass cultivars for North western highlands of Kenya

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KALRO - Kitale

**Abstract**

Feed scarcity contributes to sub-optimal livestock production thereby limiting its contribution to economic development and agrarian livelihoods in Kenya. Promotion of improved climate-smart Brachiaria grasses has potential to not only mitigate the effects of climate change but also enhance resilience of smallholder farmers. Attaining greater benefits for smallholder farmers requires consideration of their own expectations and preferences in targeting suitable Brachiaria cultivars with high probability for uptake. It is not clear however which criteria farmers consider in selecting the best Brachiaria grass for integration in their farming systems. This study aimed to consider farmers' criteria in the selection of Brachiaria cultivars for North western highlands of Kenya. A sample of 96 farmers was randomly drawn from project participants. Participants identified the selection criteria disaggregated by gender in focused group discussions. Using a pairwise ranking method the criteria were harmonized and prioritized. Five key criteria: height at harvest, amount of forage, early maturity, pest and disease tolerance and hairiness, were designed in a simple evaluation tool. Scores were awarded against each criteria for every Brachiaria cultivar and the local fodder checks based on a Likert scale of 1-4 (where 1= poor and 4= very good). Data were analysed using mean scores and chi-square statistics. Results showed that the most preferred Brachiaria cultivars were cvs. MG4, Basilisk, Piata and Xaraes, which had significantly ($P \leq 0.05$) higher mean scores than Rhodes grasses. Our findings are relevant for targeted promotion of these farmer-preferred Brachiaria cultivars with better prospects of acceptance by the farming community in the study area. Their successful adoption can ultimately boost feed availability and rise livestock productivity while contributing to environmental and livelihood resilience in Kenya.

**Key words:** Criteria development; farmer assessment; pairwise ranking

## Introduction

Sustainable intensification of livestock production driven by the adoption of improved fodder production technologies has potential to not only mitigate the effects of climate change but also enhance the resilience and livelihoods of smallholder farmers in Africa (FAO, 2010; Rioux *et al*., 2016). Recent studies show that improved Brachiaria grasses offer opportunities to address the challenge of feed scarcity, improve livestock production and livelihoods in African farming systems (Maass *et al*., 2015; Mutimura and Everson, 2012; Pizarro *et al*., 2013). For instance Ghimire *et al*. (2015) found considerable improvement in on-farm feed availability, body weight gain of cattle and milk yield increases owing to the recent re-introduction of Brachiaria grasses in Rwanda and Kenya. There is need therefore to promote suitable Brachiaria grasses for integration into smallholder farming systems to enhance uptake for livestock production in the East African region.

Successful integration of the new Brachiaria cultivars to generate greater benefits for smallholder farmers would require precise fitting of the correct cultivars into different farming systems of Kenya (Maass *et al*., 2015). Farmer-centered research and development is recognised as an effective approach that enhances ownership, decision-making, uptake and scaling-out of improved technologies (KAPP and IIRR, 2015; Wanyama *et al.*, 2003). Matching the promising cultivars to suitable socio-ecological contexts should carefully consider farmers' expectations driven by their needs and preferences as they are likely to influence adoption behaviour (Misiko *et al*., 2008; Wanyama *et al*., 2003). Yet it remains unclear what key attributes farmers expect the best Brachiaria grass to possess and which criteria they are likely to consider in selecting the most suitable cultivar for integration in the farming systems of North western highlands of Kenya.

Participatory variety selection (PVS) is recognized as an effective and efficient participatory approach involving farmers as co-researchers to incorporate their input in selecting socially acceptable technologies that match well with their needs and environment (Horne and Stür, 1999; Nkongolo *et al*., 2008; Singh *et al*., 2014). It is an approach that facilitates the potential end-users to provide information on performance and acceptability of varieties, which then provides feedback to researchers to consider in their final recommendation of promising cultivars (Witcombe *et al*., 1996). This is essential because being conversant with farmers' preferences can enhance adoption prospects for a variety (Paris *et al*., 2011). The interactive learning process makes farmers more appreciative of the technology, enhances trust and builds ownership within the farming community thereby accelerating its wider dissemination and scaling-out.

Despite its proven importance in successful varietal selection and wider uptake, PVS has mostly been applied in cereals and legumes such as maize, rice, wheat, sorghums, barley and beans (e.g. Ceccarelli *et al*., 2007; Misiko *et al*., 2008; Mitchell *et al*., 2014; Nkongolo *et al*., 2008; Nyende and Delve, 2004; Pandit *et al*., 2007; Paris *et al*., 2011) and hardly in forages like Brachiaria grasses (e.g. Mutimura and Everson, 2012; Phengsavanh *et al*., 2004). In Kenya, farmers have rarely been involved as co-researchers in selecting promising Brachiaria grass cultivars aligned with their needs and suitable for their farming environments. The current study therefore is an effort to fill this knowledge gap so as to accelerate the uptake and impact of promising Brachiaria grasses on livestock productivity and agrarian livelihoods in the country.

The objective of the study was to consider farmers' criteria in the selection of acceptable Brachiaria grass cultivars for integration into the farming system of North western highlands of Kenya. Specifically, we examine farmers' development of choice criteria with special emphasis on gender preferences. This study documents participatory harmonization and ranking of priority criteria and test them in the actual farmer selection of the best Brachiaria grass cultivars.

## Materials and methods

*Selection of farmers*

A sampling frame of farmers who were earlier interviewed during a baseline survey on delineation of niches for integration of Brachiaria grasses into the farming systems of North western Kenya was relied upon (Mutoko *et al*., 2015). Following Alreck and Settle (1985) methodology, the main sample of 215 households was drawn using stratified random sampling technique, from Upper Highlands (UH) 3, Midland Upper Midlands (UM) 4 and Lower Highlands (LH) 3 agro-ecological zones located across counties of Trans Nzoia, Uasin Gishu and Elgeyo Marakwet. A proportionate sub-sample of 100 farmers was then randomly drawn for criteria development and participatory Brachiaria cultivars evaluation as shown in Table 1. About 69% were male and the rest female farmers. As suggested by Misiko (2013), we deliberately sampled the same farmers who participated in the baseline survey with a view to enhance consistency and eliminate the effect of 'impulsive buying' during Brachiaria cultivars selection. The selected farmers were invited to participate in criteria identification, prioritization and evaluation of Brachiaria cultivars at Kenya Agricultural and Livestock Research Organization (KALRO) Kitale. The site is located at longitude 1° 0′ 6.6′′N and latitude 34° 59′ 10′′E, at 1890 m above sea level. The site lies in UM4 agro-ecological zones with temperatures ranging between 10 °C and 27 °C and receives bimodal rainfall of 1000 - 1200 mm per year. Soils are mainly humic Acrisols, which are deep and well-drained (Jaetzold *et al*., 2006).

**Table 1** Distribution of the sample of households by agro-ecological zone in North western highlands of Kenya

| Agro-ecological zone | Main sample | Sub-sample |
|---|---|---|
| Upper Highlands 3 (UH3) | 17 | 12 |
| Upper Midlands 4 (UM4) | 108 | 48 |
| Lower Highlands 3 (LH3) | 90 | 40 |
| Total | 215 | 100 |

*Participatory criteria development*

Participating farmers convened at KALRO Kitale in October 2014, where they were briefed on the need to develop the criteria they would use in evaluating and selecting the most suitable Brachiaria grass cultivars. To avoid gender bias and possible dominance in the process, participants were disaggregated by gender into three groups: men, women and youth. With the guidance of researchers and extension officers, each group listed and ranked 10 key criteria that they consider important while selecting suitable forages for their livestock. The identified criteria were presented in a plenary by the leader of each group.

During the plenary, focused discussions were conducted to harmonize the identified criteria. These criteria were then ranked in order of priority using a pairwise ranking matrix. Each listed criterion was compared against the others in the set and farmers picked the preferred one for every pair. Entries of criteria were counted in the entire matrix and the criteria having highest score ranked first. Where criteria tied, farmers were requested to break the tie by comparing each pair and selecting the priority criterion between them.

*Evaluation of Brachiaria cultivars*

The current empirical study was conducted on a researcher-managed trial under controlled, uniform conditions. The study therefore minimized the effect of extraneous factors on farmer preferences and availed reliable information. Output from the participatory criteria development process informed the design of a simple evaluation tool with solely those criteria that farmers could observe and appraise in the experimental fields. The final set of five criteria included height at harvest, amount of forage, pest and disease tolerance, hairiness and early maturity. A sample of 96 farmers participated in the evaluation of seven Brachiaria cultivars (B. *brizantha* cvs. MG4, Marandu, Xaraes, Piata, B. *decumbens* cv. Basilisk, B. *humidicola* cv. Llanero and B. *hybrid* cv. Mulato II) and two local fodder checks (Napier and Rhodes grass) earlier established in June 2014 at KALRO, Kitale. Details on the forage management practices are provided by Ndung'u- Magiroi *et al*. (2016). Guided farmer evaluations were conducted in December 2014 and March 2015 on the on-station experiment. Scores were recorded against each criteria for each cultivar using a Likert scale of 1-4, where 1=poor, 2=fair, 3=good and 4=very good.

**Data analysis**

Mean scores and standard errors were computed by county, agro-ecological zone and gender of the farmers. Chi-square ($\chi^2$) test was applied to determine significant mean score differences between cultivars based on the key selection criteria. Mean scores were then used to determine the best farmer-acceptable Brachiaria grass cultivars. Data processing and analysis was done in SPSS version 20.

**Results and Discussion**

*Farmers' selection criteria*

Results from participatory criteria identification showed that farmers largely desired forages that increase milk production, are persistent and improves soil fertility (Table 2). Other attributes were palatability, high herbage yield and tolerance to drought, weeds, pests and diseases. Farmers' prioritized preferences indicate their expectation for forages that have multiple benefits to meet their needs and are tolerant to biotic and abiotic stresses as highlighted by Kidake *et al*. (2016). Identification of these preferences by farmers at the variety evaluation stage is essential for wider adoption of Brachiaria grasses. This is because consideration of farmer criteria would essentially ensure that recommended Brachiaria grass cultivars meet farmers' requirements and their opportunities, thus they have better prospects to adapt as earlier reported by Nkongolo *et al.* (2008) on sorghums in Malawi, Misiko *et al.* (2008) on soya beans in Kenya and Phengsavanh *et al.* (2004) on improved forages in Lao PDR.

Results in Table 3 show differences in mean score among the seven Brachiaria cultivars and two local fodder checks based on key selection criteria. Basilisk, MG4 and Piata had significantly higher ($p \leq 0.05$) mean scores than Napier and Rhodes grass on pest and disease tolerance, forage amount and hairiness. There was however no significant; differences on maturity between the three most preferred Brachiaria grasses, cvs. MG4, Basilisk and Piata and Napier grass.

**Table 2** Pairwise ranked criteria for selection of forages in North western highlands of Kenya

| Criteria of importance | Total scores | Rank |
|---|---|---|
| High milk production | 12 | 1 |
| Persistence in the field | 10 | 2 |
| Improves soil fertility | 10 | 3 |
| Palatability | 9 | 4 |
| High yielding | 8 | 5 |
| Resistant to weeds, pest and diseases | 7 | 6 |
| Drought tolerant | 6 | 7 |
| Adaptable to all soil types | 5 | 8 |
| Easy to cut-not hairy | 3 | 9 |
| Both for cut-and-carry and grazing | 3 | 10 |
| Intercropped with other crops | 3 | 11 |
| Resistant to water logging | 2 | 12 |
| Fast maturity | 1 | 13 |

**Table 3** Mean score differences across Brachiaria cultivars and local checks evaluated for North western highlands of Kenya

| Grass cultivars | Criteria | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pest and disease tolerant | Early maturity | Height at harvest | Forage amount | Hairiness | Mean score | Rank |
| MG4 | 3.3a | 3.0a | 3.0ab | 3.3a | 2.7abc | 3.06 | 1 |
| Basilisk | 3.0ab | 2.9a | 2.9ab | 2.9abc | 2.9a | 2.92 | 2 |
| Piata | 3.1ab | 2.9a | 2.7ab | 3.0ab | 2.8ab | 2.90 | 3 |
| Xaraes | 2.9abc | 2.4b | 2.1b | 2.7b | 2.7abc | 2.56 | 4 |
| Marandu | 2.7b | 2.3b | 2.0b | 2.5bc | 2.6abc | 2.42 | 5 |
| Mulato II | 2.0d | 1.5c | 1.4c | 1.6d | 2.2bc | 1.74 | 6 |
| Llanero | 2.1c | 1.5c | 1.3c | 1.3d | 2.3b | 1.70 | 7 |
| Napier grass | 2.7b | 3.3a | 3.5a | 2.8b | 2.7abc | 3.00 | |
| Rhodes grass | 2.5bc | 2.1b | 2.2b | 2.3c | 2.6abc | 2.34 | |

Note: Mean scores in the same column not having similar letter are significantly different at $P < 0.05$. Tests assume equal variances adjusted using the Bonferroni correction.

Napier grass had highest score (3.5) on height while Llanero has the lowest. This difference is attributed to growth habit. Llanero has a prostrate growth habit and spread by stolon while Napier is erect in it growth habit. Rhodes grass had significantly lower ($p \leq 0.05$) mean score than MG4, Basilisk and Piata across all the selection criteria except hairiness, which was comparable with MG4 (Table 3). The mean scores for Rhodes grass were only significantly higher ($p \leq 0.05$) than Mulato II and Llanero across all criteria. The mean scores for Marandu and Xaraes did not differ significantly ($p > 0.05$) from Rhodes grass in most of the selection criteria except forage amount and tolerance to pests and disease (Table 3). This suggests that farmers would be indifferent between cultivating either Marandu, Xaraes or Rhodes grass for livestock feeding.

*Prioritized Brachiaria grass cultivars*

Results presented in Table 4 show the mean scores disaggregated by county for each Brachiaria cultivar and the local fodder checks. Farmers from Trans Nzoia and Uasin Gishu counties reported comparable preferences although the former recorded relatively high mean scores. This is because the two counties have similar predominant agro-ecological zones, UM4 and LH3 (Jaetzold *et al*., 2009), thereby farmers from these counties face comparable biophysical conditions for adoption and adaptation of the preferred Brachiaria grasses (Paris *et al*., 2011). The most preferred Brachiaria cultivars by farmers from Trans Nzoia and Uasin Gishu counties were *B. brizantha* cv. MG4, *B. decumbens* cv. Basilisk, *B. brizantha* cv. Piata and *B. brizantha* cv. Xaraes, in that order.

**Table 4** Participatory Brachiaria grasses selection by county in North western highlands of Kenya

| Brachiaria cultivar | Mean score by county | | | All sample (n=96) | | |
|---|---|---|---|---|---|---|
| | Trans Nzoia (n=44) | Uasin Gishu (n=39) | Elgeyo Marakwet (n=13) | Mean | S.E | Overall Rank |
| MG4 | 3.2 | 2.9 | 2.3 | 3.1 | 0.07 | 1 |
| Basilisk | 3.1 | 2.8 | 2.7 | 2.9 | 0.06 | 2 |
| Piata | 3.0 | 2.8 | 2.6 | 2.9 | 0.08 | 3 |
| Xaraes | 2.6 | 2.5 | 2.3 | 2.6 | 0.06 | 4 |
| Marandu | 2.6 | 2.4 | 2.3 | 2.5 | 0.09 | 5 |
| Mulato II | 1.7 | 1.7 | 1.9 | 1.7 | 0.07 | 6 |
| Llanero | 1.7 | 1.8 | 1.8 | 1.7 | 0.06 | 7 |
| Napier grass | 3.1 | 2.9 | 3.3 | 3.0 | 0.07 | |
| Rhodes grass | 2.4 | 2.3 | 2.1 | 2.3 | 0.07 | |

Conversely, Basilisk and Piata were highly rated by farmers from Elgeyo Marakwet County, with cvs. MG4, Xaraes and Marandu tying up at third position. This significant difference ($p < 0.05$) in the selection of cultivars could be attributed to the distinctive agro-ecological zone UH3, which prevails mainly in Elgeyo Marakwet County (Jaetzold *et al*., 2009). The result clearly indicates that farmers consider multiple criteria when selecting suitable forages for their livestock. This finding is consistent with Mutimura and Everson (2012) who found differences in farmer preferences for Brachiaria grasses between Nyamagabe and Bugesera districts in Rwanda. As recognized by Pandit *et al*. (2007) on PVS and scaling-out of wheat, farmers' context-specific needs coupled with adaptation potential of varieties to local biophysical conditions influence their choices of promising crop varieties.

There was however no significant difference (p>0.05) across the counties for the least preferred Brachiaria grass cvs. Llanero and Mulato II, indicating that these cultivars do not meet farmers' expectations on performance in terms production attributes. These Brachiaria cultivars are therefore the least adaptable thus are unlikely to be adopted in the North western highlands of Kenya. This result contradicts Mutimura and Everson (2012) who found that cv. Mulato II was the most preferred Brachiaria cultivar by farmers from Nyamagabe and Bugesera districts in Rwanda, thereby signifying the importance of agro-ecological adaptation and context-specific farmer needs in the selection of acceptable Brachiaria grasses. Comparison with the local checks revealed that cv. MG4 had significantly higher ($p \leq 0.05$) mean score than Napier grass cv. KK1, whereas cvs. Basilisk and Piata were not significantly different from Napier grass cv. KK1 (Table 4). The overall mean score of 2.3 for Rhodes grass was significantly lower ($p \leq 0.05$) than the best four Brachiaria cultivars. Agro-ecological zonation had significant influence on farmer selection of Brachiaria cv. MG4 alone (Table 5). Which was rated cv. MG4 significantly lower ($p \leq 0.05$) by farmers in UH3 than their counterparts from UM4 and LH3.

**Table 5** Participatory Brachiaria grasses selection by agro-ecological zones in North western highlands of Kenya

| Brachiaria cultivar | Mean score by AEZ | | | All sample (n=96) | |
|---|---|---|---|---|---|
| | UM4 (n=43) | LH3 (n=41) | UH3 (n=12) | Mean | Overall rank |
| MG4 | $3.2_a$ | $3.0_{a,b}$ | $2.5_b$ | 3.1 | 1 |
| Basilisk | $3.1_a$ | $2.8_a$ | $2.6_a$ | 2.9 | 2 |
| Piata | $2.9_a$ | $2.9_a$ | $2.5_a$ | 2.9 | 3 |
| Xaraes | $2.6_a$ | $2.6_a$ | $2.3_a$ | 2.6 | 4 |
| Marandu | $2.4_a$ | $2.6_a$ | $2.5_a$ | 2.5 | 5 |
| Mulato II | $1.7_a$ | $1.7_a$ | $2.2_a$ | 1.7 | 6 |
| Llanero | $1.7_a$ | $1.7_a$ | $1.9_a$ | 1.7 | 7 |
| Napier grass | $3.0_a$ | $3.0_a$ | $3.0_a$ | 3.0 | |
| Rhodes grass | $2.3_a$ | $2.4_a$ | $2.3_a$ | 2.3 | |

Note: Mean scores in the same row not sharing the same subscript are significantly different at p< 0.05.Tests assume equal variances adjusted using the Bonferroni correction.

Although farmers from UH3 recorded lower scores for all Brachiaria cultivars except cvs. Mulato II and Llanero, the average scores did not significantly differ across the major AEZs in North western highlands of Kenya. This finding indicates good prospects for integration of the farmer-preferred Brachiaria grasses into the existing fodder production systems of North western highlands of Kenya. Disaggregation of data by gender showed similar preference trend between male and female farmers (Table 6). This result supports a recent study in Kenya, which did not establish significant gender disparity on farmers' preference of promising integrated soil and water management technologies in Northwestern Kenya (Esilaba *et al*., 2015). Across all Brachiaria cultivars however, female farmers recorded relatively higher scores than their male counterparts although it was not significant.

Irrespective of gender of the farmers, MG4, Basilisk, Piata and Xaraes were the most preferred. This finding clearly points to universal farmer-preference of these cultivars and implies that they have good prospects for uptake when availed to both genders of livestock farmers in the study area. Comparison with Napier and Rhodes grasses showed a similar trend as reported for inter-county results and revealed no gender disparity.

**Table 6** Participatory Brachiaria selection by gender of farmers in North western highlands of Kenya

| Brachiaria cultivar | Mean score by gender | | | | All sample (n=96) | | Overall Rank |
|---|---|---|---|---|---|---|---|
| | Male (n=66) | | Female (n=30) | | | | |
| | Mean | S.E | Mean | S.E | Mean | S.E | |
| MG4 | 3.0 | 0.08 | 3.2 | 0.15 | 3.1 | 0.07 | 1 |
| Basilisk | 2.9 | 0.07 | 3.0 | 0.11 | 2.9 | 0.06 | 2 |
| Piata | 2.8 | 0.10 | 3.0 | 0.10 | 2.9 | 0.08 | 3 |
| Xaraes | 2.5 | 0.06 | 2.7 | 0.14 | 2.6 | 0.06 | 4 |
| Marandu | 2.4 | 0.06 | 2.7 | 0.27 | 2.5 | 0.09 | 5 |
| Mulato II | 1.7 | 0.09 | 1.8 | 0.11 | 1.7 | 0.07 | 6 |
| Llanero | 1.7 | 0.07 | 1.7 | 0.13 | 1.7 | 0.06 | 7 |
| Napier grass | 3.0 | 0.08 | 3.0 | 0.12 | 3.0 | 0.07 | |
| Rhodes grass | 2.4 | 0.09 | 2.3 | 0.12 | 2.3 | 0.07 | |

*Implications of farmer preferences for on-farm promotion of Brachiaria grasses*

The study revealed that farmers in north western highlands of Kenya preferred MG4, Basilisk, Piata, Xaraes and Marandu. When the identified farmers' perceptions guide the promotion of these farmer-acceptable Brachiaria cultivars, wide adoption will enhance their resilience to the effects of climate change, improve livestock productivity and household food security as established by Rioux *et al*. (2016). Studies on accelerating uptake of improved rice varieties in Lao PDR (Mitchell *et al*., 2014), sorghum in Malawi (Nkongolo *et al*., 2008), wheat in Bangladesh (Pandit *et al*., 2007) and legume cover crops in Uganda (Nyende and Delve, 2004) provides scientific evidence that incorporating farmers' preference information to complement scientists' data ensures only the best cultivars possessing attributes preferred by most farmers are progressed for on-farm adoption and scaling-out. This is because active farmer participation in the selection of farmer-acceptable Brachiaria cultivars will enhance their adoption and adaptation within the local farming contexts, as found by Wanyama *et al.* (2003). Our findings therefore are relevant for targeted promotion of four better performing and most suitable Brachiaria cultivars for successful uptake in the farming system of North western highlands of Kenya. Results from this study have potential to boost farmers' ownership and decision-making on suitable Brachiaria cultivars to ultimately catalyse their diffusion to bring more benefits to many farming households in North western highlands of Kenya (e.g. IIRR, 2000; Paris *et al*., 2011)

## Conclusions

Recognizing farmers' expectations and being responsive to their preferences for a new technology or improved intervention is central to its successful promotion and acceptance in the farming community. Using farmers own identified criteria on forage amount, height at harvest, maturity period, hairiness and tolerance to pests and diseases, MG4, Basilisk, Piata, Xaraes and Marandu were the most preferred cultivars in western Kenya. Based on the findings from this study, we recommend for targeted promotion of these most farmer-preferred Brachiaria cultivars for successful uptake in the region.

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# Theme 3

# Brachiaria Grasses, Microbes and Soil Health

# Fungal endophyte communities of Brachiaria grass (*Brachiaria* spp.) in Kenya

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**Abstract**

Endophytes are microbes that live inside host plants without causing disease but form beneficial associations with the plants. The objective of this study was to explore endophytic fungal communities inhabiting the aerial and roots tissues of Brachiaria grasses grown in natural environment. Fungal endophytes were isolated from surface disinfected, asymptomatic foliage, seeds and roots tissues of Brachiaria, and identified to the lowest possible taxonomic unit using ITS rDNA sequence analysis. A total of 354 fungal isolates (consisting 94 from aerial tissue and 260 from roots tissue) were identified to 45 and 44 taxa from aerial parts and roots, respectively. The fungal community was estimated for different diversity indices using EstimateS and communities from aerial parts and roots were compared. The aerial fungal community had significantly ($P<0.05$) higher species diversity ($H'= 5.17$) than the below ground community ($H'= 4.06$) and only 5 species were shared between aerial and below ground parts. These included members of *Aspergillus, Cladosporium, Fusarium, Periconia* and *Gaeumannomyces* with members of the genus *Cladosporium* (13.8%) being the most frequently detected in the aerial tissues whereas the genus *Fusarium* (59.2%) dominated the roots tissues. Among the beneficial endophytes isolated were members of *Acremonium*, *Sarocladium*, *Trichoderma*, *Metarhizium*, *Lecanicillium* and *Paecilomyces*. This study showed that Brachiaria grasses harbour a wide range of fungi with diverse relationships with host ranging from pathogenic to mutualistic.

**Keywords:** Acremonium, diversity indices, endophytes, phylogeny, species diversity

## Introduction

The genus Brachiaria from the tribe Panicaeae consists of about 100 documented species distributed across tropical and sub-tropical regions of both eastern and western hemisphere (Renvoize *et al.*, 1996). Most Brachiaria species are of African origin, and Africa is the center of diversity for the genus Brachiaria (Parsons, 1972). Seven perennial Brachiaria species of African origin (*B. arrecta*, *B. brizantha*, *B. decumbens*, *B. dictyoneura*, *B. humidicola*, *B. mutica* and *B. ruziziensis*) are grown as forage, particularly in humid tropical regions of South America, Asia, South pacific and Australia (Argel and Keller-Grein, 1996; Pizarro *et al.*, 1996; Stur *et al.*, 1996). The Brachiaria are most probably widely grown forage grass species in the tropics with estimated acreage of 99 million hectares in Brazil (Jank *et al.,* 2014). The widespread adoption of Brachiaria is attributed to several desirable agronomic traits such as tolerance to drought, shade and flooding; adaptation to low fertility and acid soils; high biomass production potentials; ability to sequester carbon into soils; increase nitrogen use efficiency and minimize greenhouse gas emission (Fisher *et al*., 1994; Fisher and Kerridge, 1996; Rao *et al.,* 1996; Subbarao *et al.*, 2009).

Brachiaria are highly palatable and nutritious forages thus increase livestock productivity. Moreover, Brachiaria plays important roles in ecological restoration and soil erosion control.

Most, if not all, plants in natural ecosystems are symbiotic with mycorrhizal fungi and/or fungal endophytes (Petrini, 1986). Fungal symbionts can have influence on ecology, fitness and evolution of host plants (Brundrett, 2006), shaping plant communities (Clay and Holah, 1999) and influencing community structure and diversity of microbial community (Omacini *et al.*, 2001). Enhance water and nutrients uptake, greater stress tolerance, protection from pests and diseases, increased yields are among benefits from endophytes to plants (Clay and Schardl, 2002). Therefore, fungal endophytes have important roles in the adaptation, healthy and overall performance of host plants (Clay *et al.*, 1989; Schardl, 1996; Cheplick *et al.*, 2000; Clay and Schardl, 2002; Ghimire and Craven, 2011). However, some fungal endophytes are detrimental to livestock e. g. *Neotyphodium* x *coenophialum* and *N. lolii* cause fescue toxicosis in cattle ( Schmidt *et al.*, 1982) and ryegrass staggers in sheep (Fletcher and Harvey, 1981) , respectively. Therefore, the relative importance of these endophytes in agriculture and natural ecosystems depend partly on their abilities to produce different types of alkaloids.

Despite being native to Africa, it is not widely cultivated in the region. Its importance has been recently realized to support the growing livestock industries in Africa, and many institutions are currently involved in Brachiaria research and development in the region. Brachiaria are known to harbor an endophytic fungus, *Acremonium implicatum* with anti-mycotic properties against *Drechslera* sp., a causal agent of leaf spot disease (Dongyi and Kelemu, 2004). Despite importance of microbes on host adaptation and overall performance, current understanding of endophytic microbes of Brachiaria is very limited. Therefore, the objective of this study was to isolate fungal endophytes of Brachiaria grass from above and below ground tissues, and identify candidate fungi for re-introduction into Brachiaria cultivars for enhanced adaptation to drought and low fertility acid soils.

## Materials and methods

### *Sample composition and Sampling*

Study materials were composed of both below and above ground parts of Brachiaria grass grown in wild at the International Livestock Research Institute (ILRI), Nairobi, Kenya; and foliar samples from a two year old stand of *Brachiaria hybrid* cv. Mulato II at Kenya Agricultural and Livestock Research Organization (KALRO), Katumani, Kenya; and seeds of 15 Brachiaria accessions of six African species (*Brachiaria brizantha, B. decumbens, B. humidicola, B. jubata, B. nigropedata* and *B. ruziziensis*) from the Genetic Resource Program of International Centre for Tropical Agriculture (CIAT), Colombia.

Thirty whole plant samples of Brachiaria ecotypes consisting multiple tillers and 2 to 3 inflorescences were collected from ILRI Farm (1°16'S and 36°43'E), Nairobi whereas five plant samples of Mulato II were collected from KALRO-Katumani (1°35'S and 37°14'E) during July and August, 2013. The ILRI Farm is located at the altitude of 1795m ASL with annual rainfall of

900 mm, and mean minimum and maximum temperatures of 10°C and 24°C, respectively. The KALRO-Katumani is located at 1600 m asl with annual rainfall of 717 mm and annual mean minimum and maximum temperatures of 13.7°C and 24.7°C, respectively. Both sites have two seasons (wet and dry), and the differences between wet and dry seasons are minimal.

*Plant sample processing*

The leaf, pseudo-stem and root samples were rinsed thoroughly in tap water to remove soil. Samples were cut into 3-4 cm pieces and surface disinfected in 70% ethanol for 60 sec followed by further disinfection in Tween-80 amended 1.2% sodium hypochlorite for 10 min for leaves and 20 min for stems and roots. Surface disinfected tissues were rinsed three times in sterile water, blot dried, cut into small pieces (1-1.5 cm) and plated on Potato Dextrose Agar (PDA) plates amended with antibiotics cocktail (Ghimire *et al.*, 2011) Seed samples were processed as following the procedure of (Charlton *et al.*, 2014). Plates were incubated in the dark at 24°C for up to two months and examined periodically for emerging fungal colonies. Emerging fungi were harvested regularly and purified through minimum of three rounds of subcultures and fungal cultures from repeated subcultures were used for DNA extraction and long-term storage.

*DNA Isolation*

Fungal material for DNA extraction was harvested from 1 to 2 weeks old culture grown on PDA by scrapping edge of fungi colony (5-7 $mm^2$). The fungal material was suspended to 100 µl of PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA) in the micro-centrifuge tube, vortexed for 30 sec. and incubated into boiling water for 10 min. Subsequently, the tube was allowed to cool at room temperature for 2 min, centrifuged at 12,000 rpm for 2 min. and 50 µl supernatant was transferred to a new tube. The supernatant was diluted ten times for subsequent PCR reactions or stored at 4°C and -20°C as necessary.

*Polymerase chain reaction, sequencing and database search*

The Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) are highly variable thus are extremely useful in distinguishing fungal species by PCR analysis (Martin and Rygiewicz, 2005). Fungal specific primer pairs ITS1F and ITS4 that amplify 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and partial 28S rRNA gene (partial) were used in this study, and sequences generated using these universal primers are highly represented in the NCBI nucleotide databases. The PCR primers were used to sequence the purified PCR products as described previously (Puckette *et al.*, 2009). Nucleotide sequences were trimmed, assembled and aligned using CLC Main Workbench 6.8.4 (http://www.clcbio.com). Manual editing was done on the sequences to ensure accuracy in the assembly. Sequences were then blasted on the NCBI server against the non-redundant database to assign taxonomy. Phylogenetic analysis of the sequences was performed using the maximum likelihood method based on the Tamura 3-parameter model in MEGA version 6 (Tamura *et al.*, 2013). The sequences of representative fungal taxa including variants of the same species were submitted to the NCBI and are published with accession numbers KU574663 to KU574721 and KU680347 to KU680417.

**Data Analysis**

The observed isolation frequencies for fungal taxa isolated from above ground (foliage and seeds) and below ground (roots) tissues of Brachiaria grasses were documented. The relatedness of each taxon to at different taxonomic levels (species, genus, family, class and phylum) was established and associated frequencies were calculated. The fungal diversity analysis was done using an online biodiversity calculator and EstimateS version 9.1.0 software (Colwell, 2013).

**Results and Discussion**

The results presented in this study are based on the 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and partial 28S rRNA gene (partial) sequences of 354 fungal isolates originated from surface sterilized asymptomatic above ground foliar and seeds tissues and below ground root tissues of *Brachiaria* grass. The 94 above ground (shoots and seeds) and 260 below ground isolates from roots belong to 45 and 44 taxa, respectively from phyla Ascomycota and Basidiomycota with Ascomycota constituting 98.8% of the whole fungal community. Grouping of the 94 aerial fungal isolates into different taxonomic levels revealed them into two phyla, six classes, 13 orders, 18 families, 31 genera and 45 distinct taxa. The 260 isolates from roots belonged to two phyla, five classes, 10 orders, 12 families, 20 genera and 44 distinct taxa. There were also 12 isolates with unknown identity (Figure 1 and Figure 2). *Cladosporium, Microspaeropsis, Acremonium, Fusarium* and *Alternaria* were the most frequently detected genera in the above ground community whereas *Fusarium, Gibberella, Gaeumannomyces* and *Magnaporthe* were the most frequently detected genera in the below ground community. To our knowledge this is the first study that documents the fungal community diversity in the symptomless aerial and below ground tissues of Brachiaria grass from Kenya and Sub-Saharan Africa.

The phylogenetic relationships among the fungal isolates from above and below ground populations have been illustrated separately using 45 and 44 representative taxa, respectively. The phylogenetic tree for above ground taxa showed 9 major clusters (Figure 3). In addition, the relationships among diverse species identified including *Acremonium* isolates and the isolates of the genus *Sarocladium*, which was extended to include seven species that were formerly placed in *Acremonium* (Summerbell *et al.,* 2011) has been shown in the above ground phylogenetic tree. The below ground phylogenetic tree (Figure 4) on the other hand, shows 6 clusters with a major clade of *Fusarium* isolates that sub-clusters into two groups comprising 59.2% of the below ground fungal community isolated.

The Shannon Index value calculated (H'=5.17) for the above ground population and that of the below ground population, (H'= 4.06) showed that the Brachiaria fungal community is rich in the species diversity (Table 1a) supported by a high Equitability Index (EI) in the above ground community. A high EI value denotes an even species distribution that tends to 1 whereas unequal distribution tends to zero. Simpson's Index (SI) which focus on the most abundant species without considering the richness of that population showed the above ground population had higher species diversity denoted by a low index than in the below ground community since as the abundance index increases, the diversity is known to decrease implying they are inversely correlated (Magurran, 2004). Other indices like Menhinick Index (MI) and Margalef Richness Index (MR) which compensate for sampling effects by dividing the species recorded in each population by the number of individuals in that population (Magurran, 2004) further supported the results as indicated by low indices which depicted a much richer above ground community than in the below ground community.

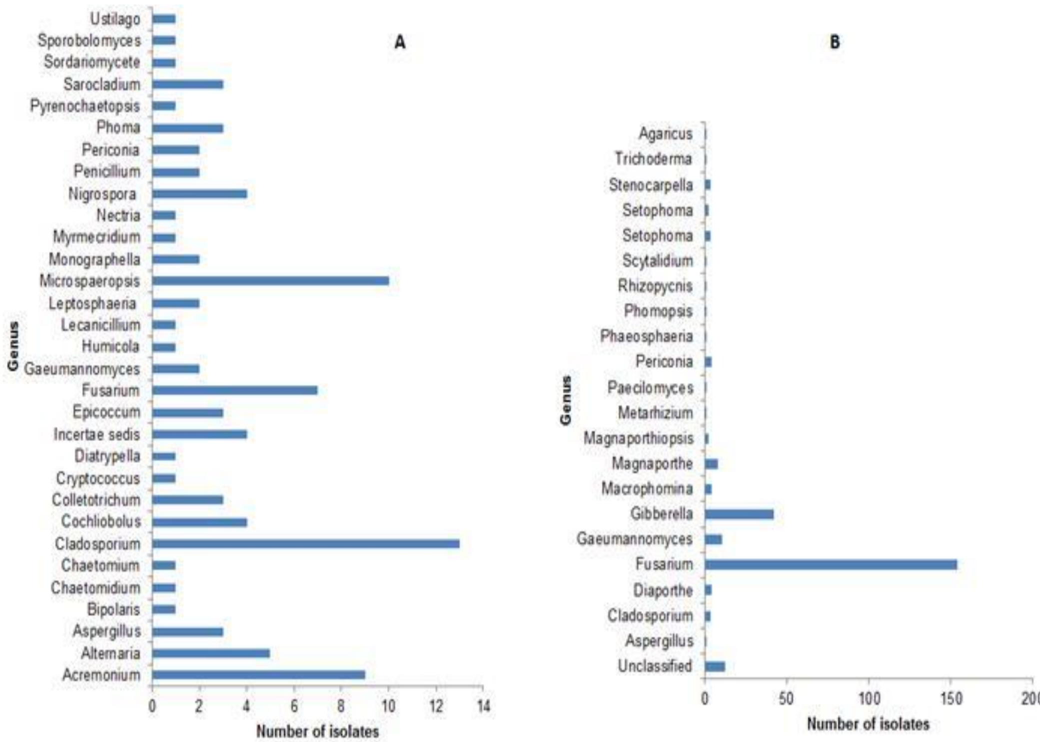


**Figure 1** Fungal genera associated with tissue of Brachiaria grass; (a) Above ground fungal endophyte community (b) Below ground fungal endophyte community

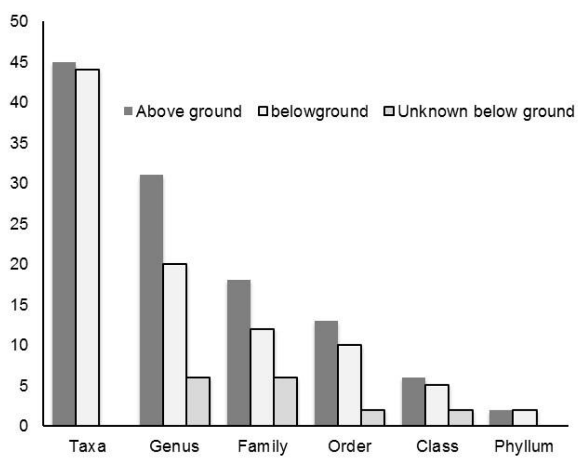


**Figure 2** Above versus below ground groupings of Brachiaria fungal endophytes at different taxonomic levels

**Table 1** Comparision of different diversity (a) and similarity (b) estimates from above and below ground fungal communities.

| (a) | Taxa | SI | SH | DI | MI | EI | GC | BGI | MRI |
|---|---|---|---|---|---|---|---|---|---|
| *Above- ground* | 45 | 0.022 | 5.17 | 0.977 | 5.095 | 0.94 | 20.6 | 0.80 | 10.1 |
| *Below- ground* | 44 | 0.101 | 4.06 | 0.899 | 2.846 | 0.74 | 10.6 | 0.38 | 7.85 |

SI, Simpsons Index; SH, Shannon Index; DI, Dominance Index; MI, Menhinick Index; EI, Equitability Index; GI, Gini Coefficient; BGI, Buzas and Gibson's Index; MRI, Margalef Richness Index

| *(b) Above vs. Below ground* | SS | MH | BC | J | SC |
|---|---|---|---|---|---|
| | 5 | 0.049 | 0.061 | 0.059 | 0.112 |

SS, Species shared; MH, Morisita-Horn similarity Index; BC, Brays-Curtis similarity index; J, Jaccard Classic similarity index; SC, Sorensen Classic similarity index

Comparison between the two communities from the similarity indices showed quite dissimilar species between them. In a similarity index, a value of 1 means the two populations compared share all their species whereas a value of 0 means they share none. In this case, both Jaccard (J) and Sorenson's Classic similarity (SC) indices as well as Bray-Curtis similarity (BC) index which calculate similarity based on presence/absence or abundance data while Morisita- Horn index (MH) which is not influenced by species richness and sample size (Wolda, 1981) revealed very low indices (Table 1b**)** thus showing quite dissimilar species existing between the two populations. The results revealed only five shared species among them. These included members from the genera, *Aspergillus, Cladosporium, Fusarium, Periconia* and *Gaeumannomyces*.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. Evolutionary analyses were conducted in MEGA6 unequal distribution between the two populations. The genus *Cladosporium* (13.8%) was the most frequently detected in the above ground population whereas the genus *Fusarium* (59.2%) dominated the below ground population.

Most of the taxa detected in the above ground community included *Cladosporium, Acremonium, Alternaria* and *Epicoccum* which have also been found dominant as non-systemic endophytes in most temperate grasses and also at their senescence stages of the above ground plant parts existing as latent saprophytes (Sanchez Marquez *et al*., 2012) but are not host specific. For example, endophytic mycobiota isolation from a perennial grass, *Dactylis glomerata* from both aerial and below ground communities, revealed 91 different species from 3 phyla, Ascomycota, Basidiomycota and Zygomycota representing 63 genera with ascomycetes dominating represented by the genera *Penicillium, Cladosporium, Acremonium, Phaeosphaeria, Fusarium, Epicoccum* and some members of *Epichloë* (Sánchez Márquez, Bills, and Zabalgogeazcoa 2007). In addition, the fungal diversity of two coastal grasses, *Ammophila arenaria* and *Elymus farctus* from leaves and rhizomes identified 103 different species classified into 2 phyla, Ascomycota and Basidiomycota among which represented 62 genera with some of the most abundant being generalists such as *Alternaria, Acremonium, Cladosporium* and *Epicoccum* (Sánchez Márquez, Bills, and Zabalgogeazcoa 2008). In the below ground community of the Brachiaria grass, the most detected genera comprised *Fusarium* followed by few species of *Gibberella, Gaeumannomyces* and *Magnaporthe* and other 16 genera represented by singleton species.

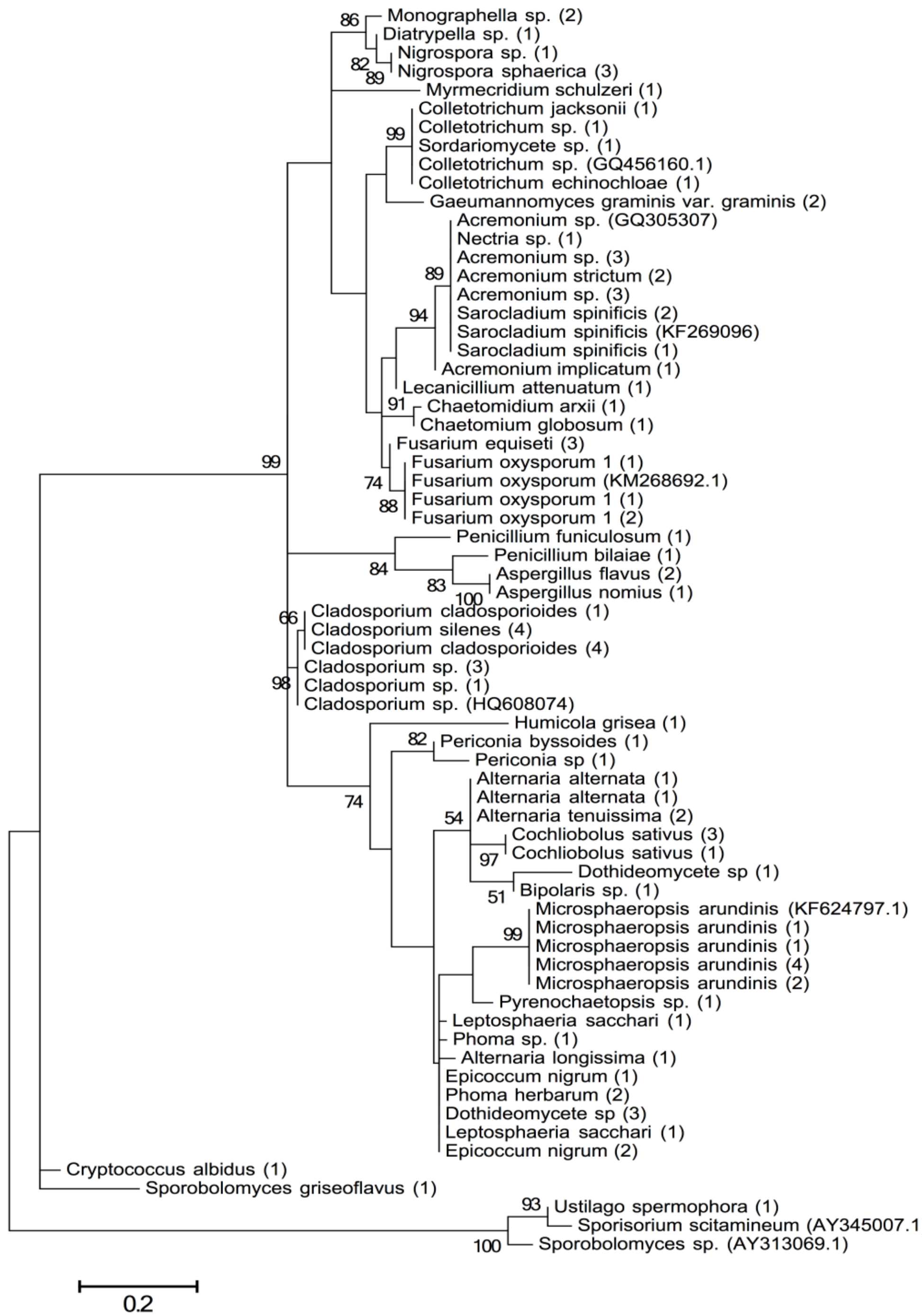


**Figure 3** Phylogenetic analysis of 45 above ground fungal taxa. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. Evolutionary analyses were conducted in MEGA6.

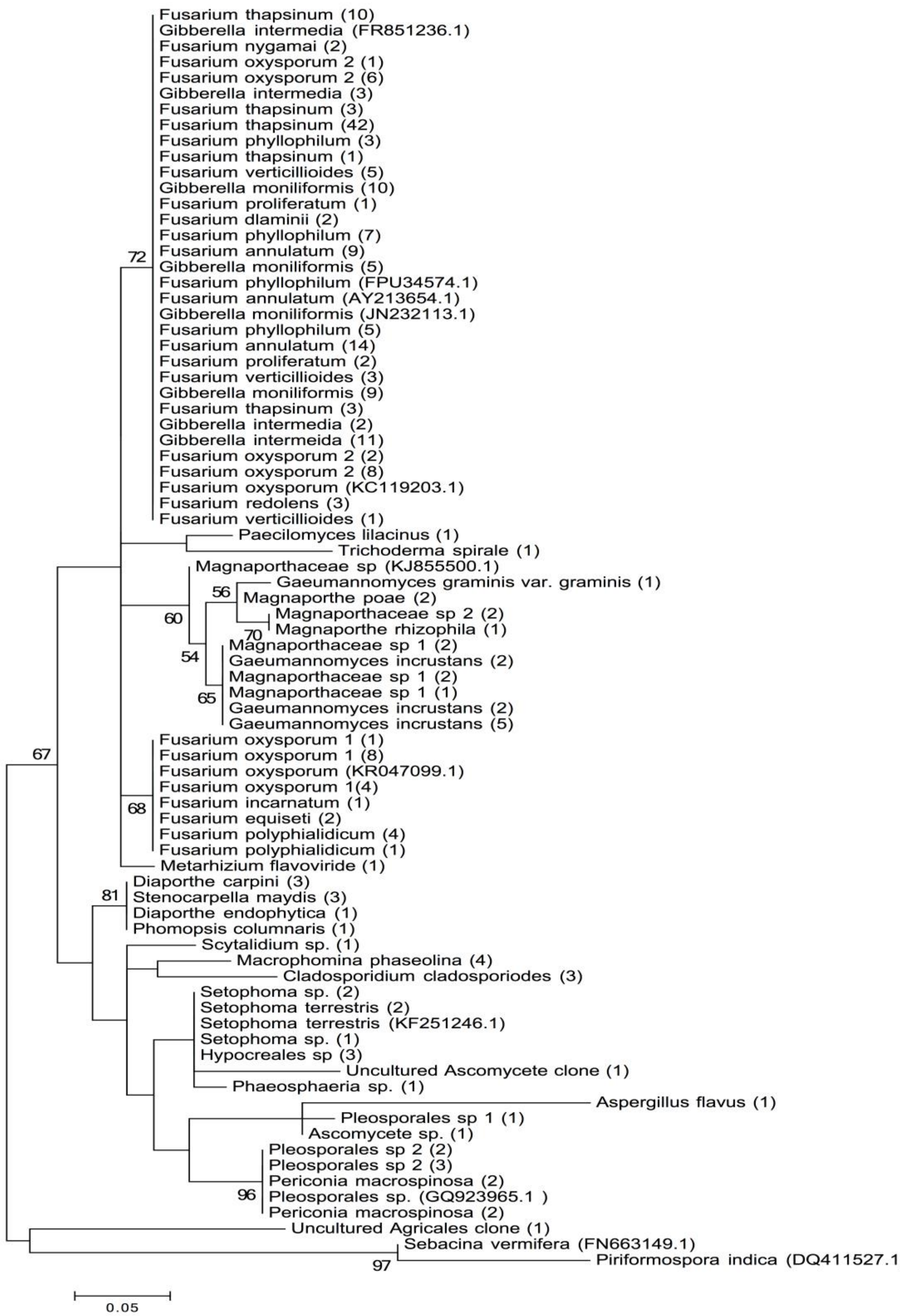


**Figure 4** Phylogenetic analysis of 44 below ground fungal taxa.

While comparing the Brachiaria fungal community with other tropical grasses like the switchgrass (*Panicum virgatum* L.), the most frequently detected genera from both aerial and root tissues were members of *Fusarium, Alternaria, Gibberella* and *Periconia* (Ghimire *et al.*, 2011) which were also isolated in this study. In cereal crops such as rice (*Oryza sativa* L.), the most frequently detected genera of fungal isolates were members of *Cladosporium, Fusarium and Penicillium* (Naik, Shashikala, and Krishnamurthy, 2009). Members of *Penicillium* as well as *Aspergillus,* and *Trichoderma* species are among the filamentous fungi isolated from different rhizosphere soils which have been tested to be associated with solubilization of insoluble phosphorus important for promotion of growth in plants (Saber *et al.*, 2009; Yasser *et al.*, 2014). In addition, (Danielsen and Jensen 1999) isolated endophytic fungi from aerial parts of 4 Costa Rican $C_4$ grasses and local maize variety to screen for antagonism against the mycotoxins producer, *Fusarium verticillioides*. They found the most dominant genera were *Fusarium* and *Nigrospora* whereas *Cladosporium, Penicillium, Phoma* and *Trichoderma* were infrequently detected but were able to reduce necrosis of *P. verticillioides* infection on maize to some extent hence proved as potential biocontrol agents.

Members of the genera *Acremonium, Lecanicillium, Fusarium* and *Nectria* spp. were detected in the aerial tissues of these Brachiaria grasses whereas *Gibberella, Metarhizium, Trichoderma* and several *Fusarium* isolates were detected in the root tissues belonging to the Order Hypocreales often reported as beneficial endophytes in grasses (Dongyi and Kelemu 2004; Ghimire *et al.*, 2011; Sanchez Marquez *et al.*, 2012). It is interesting to note that we did not detect any members of the family Clavicipaceae in our isolation, which accommodate the *Epichloë* species that also belong to the order Hypocreales (Summerbell *et al.*, 2011). These have been detected in some grasses (Sánchez Márquez, Bills, and Zabalgogeazcoa 2007) and have been associated with livestock toxicosis (Fletcher and Harvey 1981;Schmidt *et al.*, 1982).

Members of genera *Acremonium* and *Sarocladium* were among a group of endophytes isolated from Brachiaria grasses in the above ground population. Both genera share similar morphological features but phylogenetic analysis has revealed genetic differences between them (Summerbell *et al.*, 2011; Giraldo *et al.*, 2015). Moreover, *Sarocladium* accommodate seven species (*A. strictum, A. kiliense, A. zeae, A. basillisporum, A. bactrocepharum, A. ochraccum* and *A. glaucum*) that were formerly placed under *Acremonium* (Summerbell *et al.*, 2011). Some *Acremonium/Sarocladium* establishes endophytic association with plant and provides fitness advantages to the host. For example, *Acremonium implicatum* is an endophyte of *Brachiaria* spp. That is transmitted through seeds and provides protection against fungal pathogen *Drechslera* spp. the causal agent of leaf spot disease (Kelemu and Takayama, 1998; Dongyi and Kelemu, 2004). Similarly, *Acremonium zeae* has been considered as a protective endophyte of maize and displays antifungal activity against kernel rotting and mycotoxin producing fungi *Aspergillus flavus* and *Fusarium verticillioides*, and interferes with *A. flavus* infection and aflatoxin contamination of pre-harvest maize kernels (Wicklow *et al.*, 2005). Our study demonstrated a substantial association of *Acremonium* spp. in the native Brachiaria populations. *A. implicatum*, a common endophyte of Brachiaria species in South America was not detected at all in Kenyan Brachiaria populations, and the only one *A. implicatum* isolated in this study was from the foliage tissue of second year crop of hybrid Mulato grown from the seeds imported from South America. *Sarocladium spinificis* that shows a close relationship to *Acremonium* species detected in this study was recently reported as endophyte of coastal grass *Spinifex littoreus* in Taiwan (Yeh and Kirschner, 2014). Endophytic fungi isolated from Brachiaria grasses were non-systemic, generalist grass endophytes as reported from several temperate and tropical grasses *(Sanchez Marquez et al.*, 2012).

Examples of beneficial endophytes isolated as single isolates were members of the genera *Metarhizium*, *Lecanicillium Paecilomyces, Penicillium* and *Trichoderma* that are useful in agricultural and industrial applications. *Metarhizium anisopliae (M. flavoviride)*, *Paecilomyces lilacinus,* are well-known entomopathogenic fungi that are environmentally friendly and used as biocontrol agents against insect pests and against other plant pathogens on the root rhizosphere of plants in integrated pest management strategies (Peveling and Demba, 1997; Kiewnick and Sikora, 2006; Anastasiadis *et al.,* 2008). *Trichoderma* spp. have also been used as biological control agents together with other microbes in defense against plant disease causing pathogens using different mechanisms. For example, *T. virens* and *T. harzianum* work against pathogenic *Pythium* species and *Rhizoctonia solani* and also promote overall plant biomass growth and resistance to biotic and abiotic stresses that affect general plant health (Howell, 2003). *Penicillium* spp. are ubiquitous fungi known, that are not only pathogenic but are also beneficial. They are not only mycotoxins producers, but also produce other secondary metabolites associated with antagonism against plant pathogens e. g. *P. chrysogenum* isolated from rice has been tested in dual cultures and shown to be antagonistic against other plant pathogens such as *Rhizoctonia solani, Alternaria alternata, Phoma sorghina and Macrophomina phaseolina* (Naik *et al.*, 2009).

In conclusion, Brachiaria fungal community was complex harbouring a wide range of fungi that potentially have varied relationships with the host. In an agricultural perspective, some are applicable as biological control agents, plant growth promoters, promote antagonism against plant pathogens as causal agents of diseases or enhancing plant resilience against drought and other stresses. Most endophytes detected in Brachiaria were generalist as observed in other $C_4$ grasses with no *Epichloë* detected in this study. The rich diversity of endophytic fungi in the native Brachiaria grasses exhibited in this study is very important in improving their productivity and building resilience for sustainable forage availability in Kenya and Sub-Saharan Africa countries. Therefore, this being the very first study on fungal endophytes existing in these grasses in their native environment, this core collection of endophytes would be very important for identifying the most beneficial fungi for re-introduction into the grasses for their adaptation to abiotic and biotics stresses as we adopt them back to their native environments in Africa.

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# Bacterial community associated with *Brachiaria* spp. in Kenya

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**Abstract**

Endophytic and plant associated bacteria were isolated from plants and rhizosphere soils of naturally grown Brachiaria grasses at International Livestock Research Institute in Nairobi, Kenya. Eighty four bacterial strains were isolated from aerial tissues, root tissues and rhizosphere soils on nutrient agar and 869 media. All bacterial strains were identified to the lowest possible taxonomic unit using 16S rDNA universal primers, and were characterized for phosphate solubilization; production of IAA, siderophore and hydrogen cyanide; AAC deaminase activity; antifungal properties; and plant growth promotion activities. The 16S rDNA based identification revealed the 84 bacterial strains into three phyla, five classes, eight orders, twelve families and sixteen genera. The most frequent genera were *Pseudomonas* (23%), *Pantoea* (17%), *Acinetobacter* (9%) and *Enterobacter* (8%). The functional characterization of these strains showed that 41 (48.8%) of 84 bacterial strains had at least three plant beneficial properties. Inoculation of maize seedlings with *Acinetobacter* spp., *Microbacterium* spp., *Pectobacterium* spp., *Pseudomonas* spp., and *Bacillus* spp. showed positive effect on seedling biomass. The ability of Brachiaria grasses to host genetically diverse bacteria, many of them with multiple benefit to plants might have contributed to the adaptation of Brachiaria to drought and low fertility soils.

**Key words:** ACC deaminase, anti-fungal activity, auxins, hydrogen cyanide, phosphate solubilization, siderophore production

## Introduction

Brachiaria grass is an important constituent of Savannah grassland ecosystem that supports millions of African herbivores for thousands of years (Kelemu *et al*., 2011). They consist of about 100 documented species and several of them are used as cultivated forage across the tropics. Brachiaria is the most extensively grown tropical forage in Latin America, Asia, South Pacific and Australia with an estimated acreage of 99 million hectares in Brazil alone (Jank *et al*., 2014). Recently, there has been considerable interest in Brachiaria grass in its native home of Africa, and several initiatives are on-going to promote Brachiaria as an additional forage option for sustaining the emerging livestock industry in the region; especially for dry season feeding (Maass *et al*., 2015). Brachiaria grasses has several desirable traits that include adaptation to marginal soils; water stresses and shade tolerance; high biomass production potential; ability to sequester carbon; increase nitrogen use efficiency through biological nitrification inhibition (BNI) and subsequently reducing greenhouse gas emissions and ground water pollution (Rao *et al*., 1996; Subbarao *et al*., 2009). The grass is highly palatable and nutritious forages thus increase livestock productivity. Moreover, Brachiaria is an important ecological agent with significant roles in soil reclamation and erosion control. Despite the plethora of desirable attributes and high annual biomass production potential of 30 t/ha, the on-farm productivity of Brachiaria in Africa is quite low.

The global demand for livestock product is projected to increase by 70 percent in 2050 due to growing population; rising affluence and urbanization (www.fao.org/livestock-environment/en). Forages are the main component of livestock feeds accounting for 60 to 80 percent of livestock production costs (Ademosun, 1976). The economic production of forages can be attained through minimizing the production costs and by closing the yield gaps. As for many cultivated crops there is substantial yield gap on forages since forage yields are highly responsive to expensive inputs such as water, fertilizers and other agro-chemicals (Lobell *et al*., 2013). There is need, therefore, for concerted multidisciplinary effort to increase the livestock productivity through the development of low input forage production systems that minimize the use of resources without compromising forage productivity.

Endophytic and plant growth promoting rhizobacteria (PGPR) are known to provide several fitness benefits to plant hosts. These benefits include nitrogen fixation (Bahulikar *et al*., 2014; James 2000), the production of auxins, cytokinins and gibberellins (García de Salamone *et al*., 2001; Gutierrez-Manero *et al*., 2001; Taghavi *et al*., 2009), suppression of the ethylene production by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Taghavi *et al*., 2009; Zhang *et al*., 2011), alteration of sugar sensing mechanisms in plants (Taghavi *et al*., 2009), solubilization of mineral phosphorous to a form that is readily available to plants (Turan *et al*., 2012), and synthesis of siderophores (Rungin *et al*., 2012) and other low molecular mass compounds or enzymes that can modulate plant growth and development (Lamber and Joos 1989). Endophytic and PGPR also benefit host plants by preventing or suppressing plant pathogens by competing for niche and nutrients, by antibiosis, through the production of hydrolytic enzymes and through induced systemic resistance (Mendes *et al*., 2011). A model system involving plant bacterial association (poplar host and endophytic bacteria *Enterobacter* sp.) has been well recognized for variety of fitness enhancement on poplar and other plant species (Taghavi *et al*., 2010). Endophytic and PGPR seem to exist in most, if not all, higher plant species (Wu *et al*., 2012). The utilization of endophytes and PGPR bacteria is, therefore, a feasible strategy for enhancing the productivity of wide range of plant species but this is severely constrained due to limited understanding of these microbes in different hosts. The objective of the study was to enumerate cultivable bacterial endophytes and PGPR of important tropical forage *Brachiaria* spp. and to characterize them for functional roles in plants for potential applications in the commercial cultivation of Brachiaria grasses in sub Saharan Africa.

## Materials and methods

### *Sample collection*

Endophytic and plant-associated bacteria were isolated from leaves, roots and rhizoplane soils collected from thirty apparently healthy looking Brachiaria plants grown in wild at the farm of the International Livestock Research Institute (ILRI) in Nairobi, Kenya.  These plants are maintained in experimental plot at ILRI Campus after collecting samples for bacterial isolations.

**Isolation of endophytic and rhizoplane bacteria**

*Surface disinfection*

The Brachiaria leaves and roots samples were processed and surface disinfected in 70% ethanol and 1.2% sodium hypochlorite (NaOCl) as described by Taghavi *et al.*, (2009) with slight modification. After surface-sterilization, the samples were rinsed three times in sterile distilled water and blot dried in sterile paper towels.

*Bacteria isolation*

One gram of finely chopped surface-disinfected plant samples was macerated in 9 ml of 10 mM magnesium sulfate ($MgSO_4$) solution using a sterile mortar and pestle, and the suspension was diluted serially. For bacterial isolation from rhizoplane soils, one gram of soil was added with 9 ml of 10 mM $MgSO_4$, mixed vigorously for 5 min, allowed to settle for 5 min and the supernatant was collected for serial dilution. The dilutions were plated (100µl/ 90mm petri plate onto nutrient agar and 869 media), incubated at 28°C for 1-3 days and emergent colonies were purified through a series of three subcultures.

*DNA extraction, 16S rRNA gene amplification and sequencing*

Bacterial genomic DNA was extracted using PrepMan® reagent (Applied Biosystems) according to the manufacturer's instructions.16S rRNA gene was amplified using 16S primer pairs 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Frank et al., 2008). The PCR was performed using AccuPower® PCR PreMix (Bioneer) in 25 µl reactions under cycling conditions consisting of an initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 57°C for 45 sec and extension at 72°C for 45 sec; and a final extension at 72°C for 10 min. Amplification products were resolved on 1% agarose gels, purified using a QIAquick PCR Purification Kit (Qiagen) and sequenced on the ABI 48-capillary 3730 DNA Analyzer (Applied Biosystems).

*Bio-informatics analysis*

Sequence analysis was performed using the CLC Genomics Workbench 7.0.3 (http://www.clcbio.com) and molecular identities of the bacterial strains obtained using the SeqMatch tool on the Ribosomal Database (Cole *et al.*, 2014). BLAST analysis was also performed on the NCBI database and results from the two databases compared. Phylogenetic trees were generated using the tree builder tool on the RDP database (Cole *et al.*, 2014) as well as MEGA-6 (Tamura *et al.*, 2013).

*Biochemical characterization of bacterial strains*

Bacterial strains isolated from Brachiaria leaf and roots and rhizoplane soils were tested for several attributes beneficial for plant growth and development.

*Indole-3-acetic acid (IAA) production*

Bacterial strains were grown in 1/10$^{th}$ strength 869 broth supplemented with 0.5 g per litre of L-tryptophan at 28°C for 5 days at 150 rpm in dark. After growth the number of bacterial cells was estimated using a spectrophotometer at 600 nm. To detect and quantify IAA production, 1 volume of clarified culture supernatant added with two volumes of Salkowski reagent (Mayer, 1958) and incubated for 35 min at room temperature. Development of a pink color (indication of IAA production) was quantified by measuring absorbance at 535 nm.

*Siderophore production*

Bacterial siderophoregenesis was determined using Chrome-Azurol (CAS) media as described previously (Vellore, 2001). Briefly, bacterial strains were grown overnight at 28°C on a shaker in two variants of Modified Fiss Minimal Media (5.03 g/L $KH_2PO_4$, 5.03 g/L L-asparagine, 5.0 g/L glucose, 40 mg/L $MgSO_4$, 100 µg/L $MnSO_4$, and 500 µg/L $ZnCl_2$). Iron-restricted modified Fiss Minimal Medium and high iron Modified Fiss Minimal Medium were prepared by adding 139 µg/L $FeSO_4$ (5 µM) and 5.56 mg/L $FeSO_4$ (20 µM) to the final media respectively. Siderophore production was examined by loading 60 µl of clarified culture supernatant into wells made with a cork borer in the CAS media. Plates were incubated at 28°C for 3-5 days. The development of yellow or orange halos around the inoculated wells is indicative of siderophore production.

*Phosphate solubilization*

Bacterial ability to solubilize phosphate was determined by spotting 10 µl of fresh bacterial cells onto NBRIP media (Mehta and Nautiyal, 2001). Inoculated plates were incubated at 28°C for 2-3 days. Formation of clear halos around the colony is indicative of phosphate solubilization. The size of halo produced by each test strain was used as a measure of the strength of the phosphate solubilizing ability of each strain.

*Hydrogen cyanide (HCN) production*

Qualitative HCN detection was performed using Lorck's alkaline picrate assay (Lorck, 1948). Test strains were cultured in media supplemented with the amino acid glycine. HCN production was detected by placing Whatman No.1 paper discs soaked in alkaline picrate (0.5% picric acid in 2% sodium carbonate) solution a few millimeters above the surface of inoculated media in each well and incubation at 28°C. Change in the color of filter paper from yellow to light brown, brown and reddish-brown was indicative of HCN production.

*Aminocyclopropane-1-carboxylic acid (ACC) deaminase production*

Bacterial strains were tested for ACC deaminase production as described previously (Ali *et al.*, 2014). Strains were cultured in Dworkin and Fosters ACC Minimal Salts supplemented with AAC as a sole source of nitrogen. Media without ACC and with a nitrogen source (2 g/L of

$(NH4)_2SO4)$ were used as controls. The ability of a strain to grow in media with ACC was indicative of ACC deaminase production.

*Antifungal activities*

Bacterial strains were tested for the ability to inhibit the growth of seven plant pathogenic fungal isolates: *Aspergillus flavus* isolate F06**,** *A. flavus* isolate F23, *Fusarium equiseti* isolate F05, *Mangnaporthe grisea* isolate MG01, *Nigrospora oryzae* isolate F25, *N. sphaerica* isolate F10 and *Phoma herbarum* isolates F20. Fungal inoculum was prepared by excising 25 $mm^2$ PDA block from a fresh fungal colony followed by grinding in 300 µl sterile water. A 100 µl of finely ground mycelial suspension was added to 100 ml PDA at 55°C, thoroughly mixed, poured onto 90 mm Petri-dish and allowed to set at room temperature. Thereafter, 10 µl of fresh bacterial cells of each bacterial test strain was spotted onto the plates and incubated at 28 ˚C for 7-10 days to allow the fungi and bacteria to grow together. Inhibition of fungal growth around the bacterial colonies is indicative of antifungal activity of test strain.

*Screening for plant growth promotion*

Thirty of 84 bacterial strains with varied functional properties and water inoculated control were tested in a greenhouse for biomass production using a maize seedling system. Three maize seeds of variety H614 (KALRO/KSCO) were planted in pots filled with heat-sterilized virgin forest soil and each seedling inoculated with 2 ml of bacterial suspension ($10^8$ bacterial cells per ml) while control plants were inoculated with 2 ml of sterile water. Each treatment was done in two replications, and the experiment was repeated twice. Inoculated plants were maintained in a greenhouse (daily mean minimum temperature of 20.35°C, mean maximum temperature of 23.41°C and mean relative humidity of 51.32 to 66.9% and 12 hours day length) for three weeks. Plants were harvested, dried at 60°C for 72 hours and weighed for biomass. Roots and shoots biomass data was recorded and the effect of inoculation on maize seedling biomass determined by mean separation using standard errors of means.

## Results

*Bacterial isolation and molecular identifications*

A total of 84 bacterial strains were successfully isolated from Brachiaria leaf, roots and rhizoplane soils. Bacterial strains from leaf, roots and rhizoplane soil constituted 32%, 31% and 37% of the total population, respectively. The 16S rDNA sequences were generated for all test strains and homology search in RDP and NCBI databases revealed three phyla, five classes, eight orders, twelve families and fifteen genera (Table 1). The sequences of 50 representative bacterial are available in NCBI Genbank ® Database with accession number KU725918 to KU725967.

**Table 1** Grouping of endophytic and plant growth-promoting rhizobacteria (PGPR) strains of Brachiaria grasses at different taxonomic levels

| PHYLA (3) | CLASSES (5) | ORDERS (8) | FAMILIES (12) | GENERA (16) |
|---|---|---|---|---|
| Proteobacteria (70) | Alphaproteobacteria (5) | Burkholderiales (4) | Burkholderiaceae (1) | Acinetobacter (9) |
| | Betaproteobacteria (4) | Enterobacteriales (26) | Comamonadaceae (2) | Burkholderia (1) |
| | Gammaproteobacteria (61) | Pseudomonadales (32) | Enterobacteriaceae (26) | Enterobacter (8) |
| | | Rhizobiales (2) | Moraxellaceae (9) | Herbaspirillum (1) |
| | | Sphingomonadales (3) | Oxalobacteraceae (1) | Pantoea (17) |
| | | Xanthomonadales (3) | Pseudomonadaceae (23) | Pectobacterium (1) |
| | | | Rhizobiaceae (2) | Pseudomonas (23) |
| | | | Sphingomonadaceae (3) | Rhizobium (2) |
| | | | Xanthomonadaceae (3) | Shingomonas (3) |
| | | | | Stenotrophomonas (2) |
| | | | | Variovorax (2) |
| | | | | Xanthomonas (1) |
| Actinobacteria (12) | Actinobacteria (12) | Actinomycetales (12) | Micrococcaceae (2) | Arthrobacter (2) |
| | | | Microbacteriaceae (10) | Curtobacterium (4) |
| | | | | Microbacterium (6) |
| Firmicutes (2) | Bacilli (2) | Bacillales (2) | Bacillaceae (2) | Bacillus (2) |

Note: Value in the parenthesis is the number of strains belonging to the corresponding taxon.

The frequency of isolation and distribution of the sixteen identified bacterial genera are as presented in Figures 1 & 2. The three most frequently isolated genera were *Pseudomonas*, *Pantoea*, and *Acinetobacter*, representing 23%, 17% and 9% of the population, respectively. *Pseudomonas* and *Pantoea* were isolated from all the three sources while Acinetobacter was isolated from roots and rhizoplane soil only. Similarly, *Bacillus*, *Microbacterium*, *Stenotrophomonas* and *Enterobacter* had two different origins. Five genera (*Arthrobacter*, *Burkholderia*, *Pectobacterium*, *Rhizobium*, *Variovorax* and *Xanthomonas*) were isolated exclusively from roots samples whereas another three genera (*Curtobacterium*, *Herbaspirillium* and *Shingomonas*) were isolated exclusively from leaf samples (Figure 2).

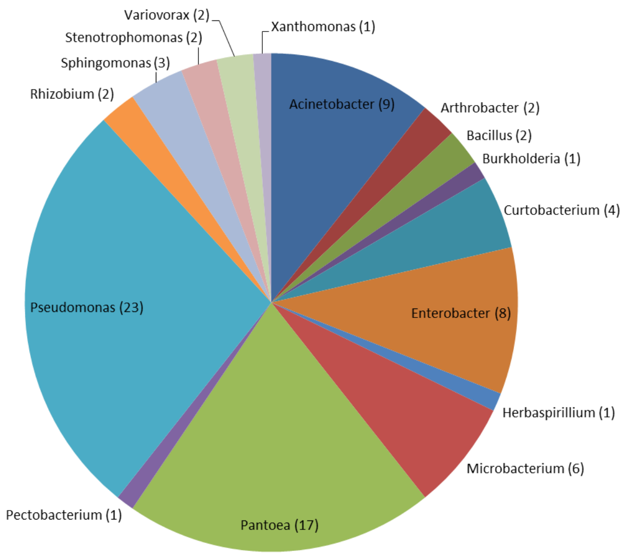


**Figure 1** Genus level taxonomic classification of bacterial strains isolated from the roots, leaves and rhizoplane soils of naturally growing *Brachiaria*

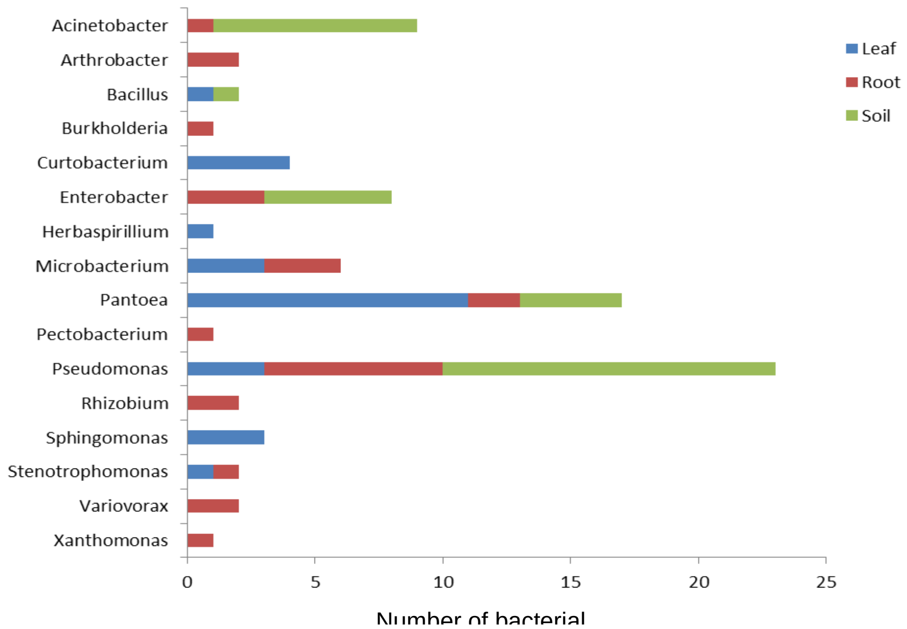


**Figure 2** Distribution of bacterial genera isolated from three sources (root, leaf and rhizoplane soil) of naturally growing *Brachiaria* spp.

*Phylogenetic relationships among bacterial isolates*

The 16S rRNA sequence-based neighbor-joining cladogram revealed that test bacterial strains form seven major clades with a strong bootstrap support (Figure 3). The grouping of the bacterial strains in clades was not based on the origin of the strains.

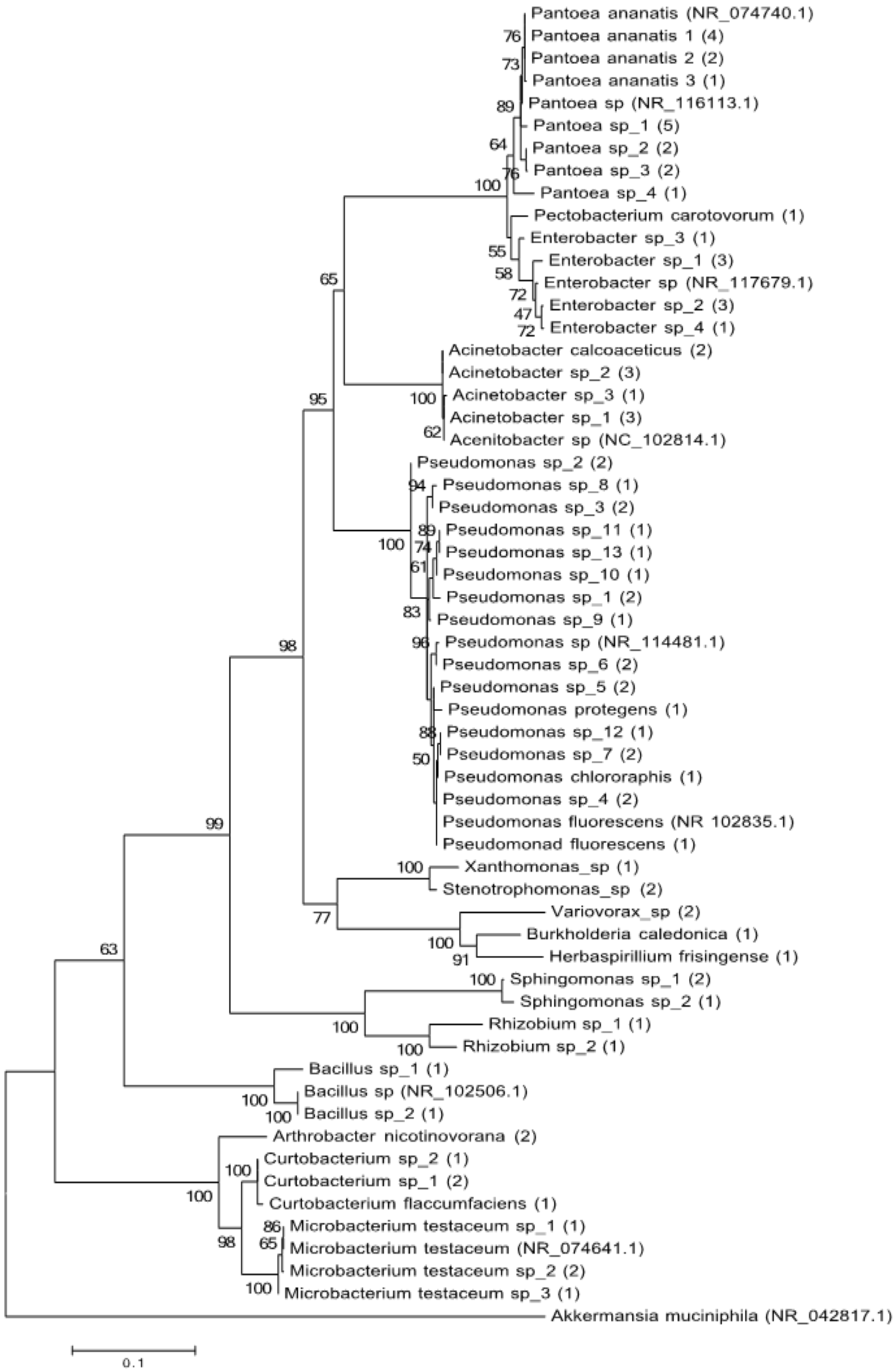


**Figure 3** Molecular phylogenetic analysis of 50 representative bacterial taxa inferred using the Maximum Likelihood method based on the Tamura 3-parameter model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 1051 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

*Biochemical characterization of bacteria*

All 84 bacterial strains from leaf, roots and rhizoplane soils were tested for six biochemical characteristics: IAA production, siderophores production, phosphate solubilization, hydrogen cyanide production, ACC deaminase activity and antifungal activities (Figure 4). A total of 49 (58%) of the 84 bacterial strains were positive for IAA production (Figure. 4A). Majority of these positive strains were isolated from leaf (43%) and roots (40.8%). On the other hand the majority of the strains that tested negative for IAA production were from rhizoplane soils (65.7%). Fifty seven (51%) of the test strains were positive for siderophore production and these positive strains represented all three sources. The majority of the strains that tested positive for siderophoregenesis belonged to the genus *Pseudomonas* and was isolated from rhizoplane soil. Most of the strains that were negative for siderophoregenesis were from roots and leaf (Figure 4B). Twenty three (27%) of the test strains were able to grow in media supplemented with ACC as the sole carbon source confirming their ability to produce ACC deaminase (Figure 4C). The majority of these positive strains were Gram negative bacteria belonging to the genera *Pseudomonas*, *Pantoea* and *Enterobacter*. These strains tested positive for other plant growth-promoting properties.

Forty seven (56%) of the 84 bacterial strains tested positive for the ability to solubilize phosphates (Figure 4D). This characteristic was evenly distributed among the bacterial strains isolated from the three different sources. Twenty two (26%) of the tests bacterial strains were positive for cyanogenesis with both the negative and positive strains distributed across the three sample sources (Figure 4E). The strains that tested strongly positive for cyanogenesis were mainly from the genus *Pseudomonas*, and to a lesser extent from the genus *Microbacterium* and *Pantoea*.

*Antifungal activity*

Some bacterial strains were detected with antifungal activities against *A. flavus* isolate F006**,** *A. flavus* isolate F023, *M. grisea* isolate MG001, *N. oryzae* isolate F025 and *N. sphaerica* isolate F010. A total of 15, 12, 4, 2 and 1 strains showed antifungal activities to *N. oryzae* isolate F025, *N. sphaerica* isolate F010, *A. flavus* isolate F006**,** *A. flavus* isolate F023 and *M. grisea* isolate MG001, respectively. Some strains e. g. *Pseudomonas s*pp. strain CSBB-072 and *Pectobacterium carotovorum* strain CSBB-046 showed antifungal activities against four and three pathogens, respectively (Figure. 4F-L). Majority (79%) of bacterial strains with antifungal activity were from rhizoplane soils and Brachiaria roots.

Evaluations of the 84 bacterial strains isolated from Brachiaria grasses and rhizoplane soils for six plant growth promotions (PGP) properties showed that they possessed up to five properties beneficial for plant growth and development (Figure 5). Almost half (48.8%) of the strains had three or more PGP properties whereas only two strains were detected with no PGP activity.

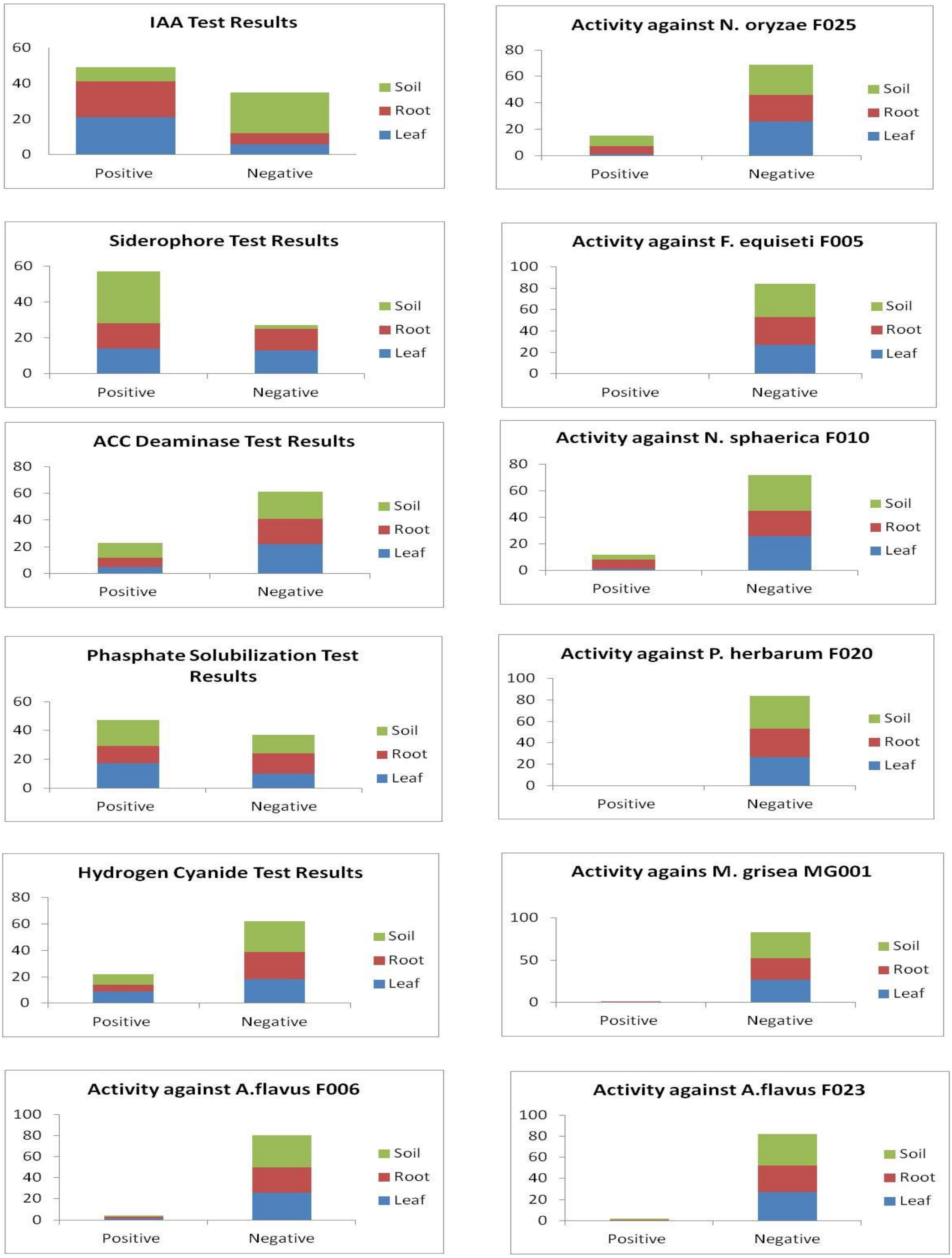


**Figure 4** Biochemical characterizations of endophytic and plant-associated bacteria of Brachiaria grasses. Isolated strains were tested for various plant growth promoting attributes including auxin(IAA) production (A), siderophogenesis (B), ACC-deaminase production (C), phosphate solubilization (D), cyanogenesis (E) and antifungal activity (F-l).

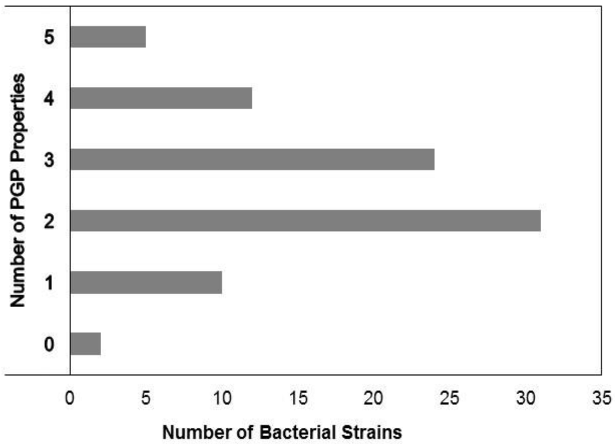


**Figure 5** Screening endophytic and plant-associated bacteria of Brachiaria grasses for plant growth promotion properties. Isolated strains were tested for six plant growth promoting attributes (IAA production, siderophogenesis, ACC-deaminase production, phosphate solubilization, cyanogenesis and antifungal activity against seven known fungal pathogens of plants.

*Plant growth promotion*

The effect of inoculating 30 test strains on the total biomass of maize seedlings was evaluated in two separate experiments. In these two experiments eight test strains were found to consistently produce a positive increase of up to 39% in seedling biomass compared to the un-inoculated control seedlings (Figure 6).

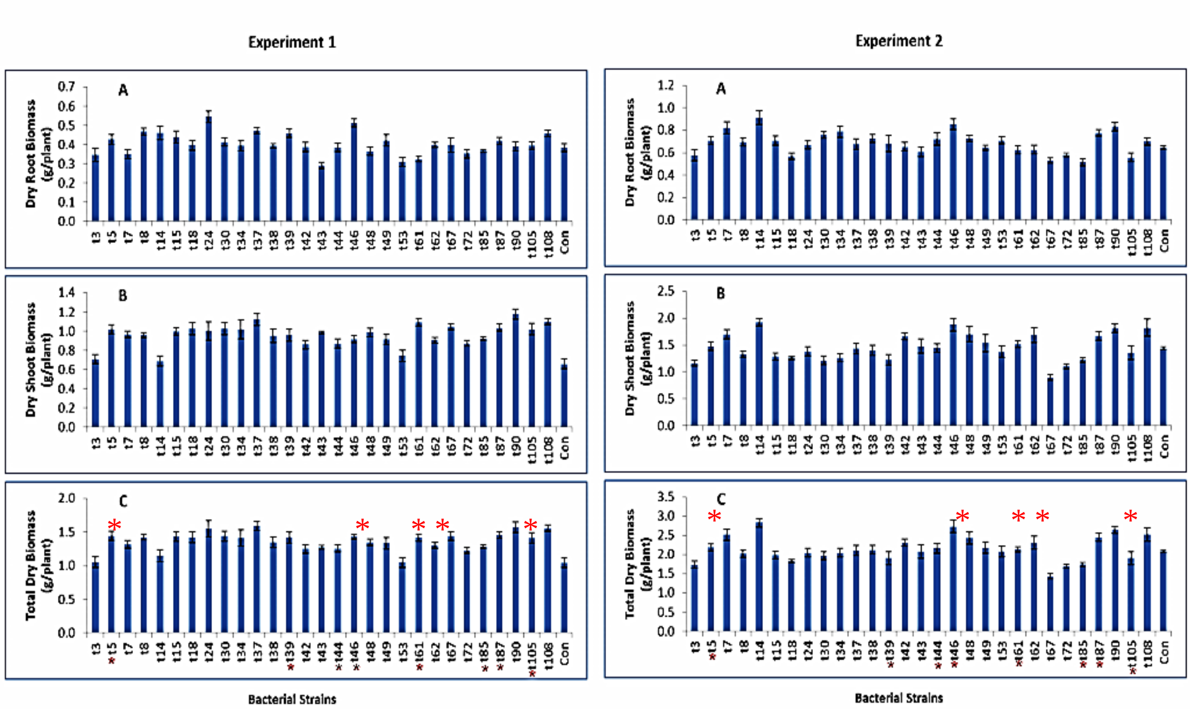


**Figure 6** Effect of bacterial inoculation on roots (A), shoots (B) and total biomass (C) of maize seedlings. Experiment was conducted twice in two replications. Eight bacterial strains (CSB_B007, CSB_B042, CSB_B046, CSB_B048, CSB_B062, CSB_B087, CSB_B090 and CSB_B108) consistently showed a positive increase in the biomass of inoculated plants compared to un-inoculated controls.

The bacterial communities of Brachiaria grasses were composed of many closely related strains, the majority (73%) of which belongs to the phylum *Gammaproteobacteria*; a result similar to that reported for endophytic bacteria from poplar and willow, rhizoplane bacteria from tree peony and rhizospheric bacteria of maize (García-Salamanca *et al.*, 2006; Han *et al.*, 2011; Taghavi *et al.*, 2009). *Gammaproteobacteria* respond chemotactically to roots exudates and are very efficient in utilizing plant exudate products (García-Salamanca *et al.*, 2012) therefore they are abundant in rhizoplane soils, roots and leaf samples. Members of the genera *Pantoea*, *Pseudomonas* and *Acinetobacter* constituted 23%, 17% and 9% of the microbial populations and were consistently isolated from rhizoplane soils, roots and leaves respectively. This is in line with the bacterial colonization of plants via roots as a form of continuum that starts from the rhizosphere to internal root tissues, to plant vascular systems and eventual colonization of above ground plant tissues (Kloepper *et al.*, 1999).

Analysis of the 16S rRNA sequences of 84 bacterial strains detected 50 OTUs belonging to 16 genera at variable frequencies that ranged from one to twenty three; an observation comparable to similar studies for poplar and willow (Taghavi *et. al.*, 2009). The number of bacterial genera isolated from rhizoplane soil, roots and leaf of Brachiaria were 5, 12 and 5, respectively. Bacteria from roots were more diverse than those from leaf whereas rhizoplane soils had the least diversity at genus level. A low diversity in rhizoplane soil bacteria might have been attributed to the high affinity between selected bacterial species and roots exudates resulting in these bacteria out-competing the rest. Phylogenetic analysis of the strains revealed seven distinct clades; with all but one clade dominated by members of the phylum Proteobacteria. This is in agreement with the fact that Proteobacteria are morphologically, physiologically and ecologically extremely diverse accounting for over 45% of all cultured bacteria (Kersters *et al.*, 2006).

Endophytes and rhizobacteria are part of the natural microflora of healthy plants and may be considered to be important contributors to plant growth and biological control of pathogens and weeds (Hallmann *et al.*, 1997). Fifty eight percent of the bacterial strains isolated in this study were able to produce the auxin IAA. Production of IAA has been reported for many bacteria and it is assumed that over 80% of the bacteria isolated from the rhizosphere are capable of synthesizing IAA (Khalid *et al.*, 2004). Auxin plays a major role in the regulation of various plant physiological processes such as cell division and enlargement, cell differentiation and cellular response to physical factors like light and gravity (Meuwly and Pilet, 1991). The level of IAA in a plant has an effect on primary root length and formation of adventitious and lateral roots and this consequently influences water and nutrient uptake. A number of plant-associated bacteria have the ability to produce IAA and contribute to plant growth promotion by altering the plant auxin pool (Bharucha *et al.*, 2013). More than half (51%) of the isolated bacterial strains in our study were positive for siderophore production. Siderophores are low molecular weight iron-chelating agents secreted by bacteria in iron-limiting conditions to help them scavenge for iron from the environment (Neilands and Nakamura, 1991). The production of siderophores by plant-associated microorganisms stimulates plant growth by depriving the plant pathogens of iron which inhibits the growth of such pathogens (Costa and Loper, 1994).

About 56% of test bacterial strains were able solubilize phosphorous; an important plant mineral nutrient that often occurs in abundance in soils but whose availability to plants is limited by the fact that it occurs mainly in the form of insoluble complexes that cannot be taken up by plants (Goldstein, 1986). Phosphate solubilizing microorganisms convert inorganic and organic phosphate complexes into bioavailable forms that can easily be taken up by plants therefore promote plant growth (Hilda and Fraga 1999). The use of such microbes can, therefore, be a sustainable approach for managing phosphorus deficiency in agricultural soils. Similarly, 26% test bacterial strains were able to produce hydrogen cyanide; an attribute that has been demonstrated as one of the mechanisms for biological control of weeds, nematodes and microbial pathogens (Kremer and Souissi, 2001). In bacteria, cyanogenesis has been reported mainly in the genus *Pseudomonas* (Ryall *et al.*, 2009), a few Bacilli (Grover *et al.*, 2009) and members of the *Burkholderia cepacia* complex (Ryall *et al.*, 2008).

Twenty seven percent of the isolated bacterial strains were found to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase. The hormone ethylene plays a role in various physiological processes in plants, including the breaking of seed dormancy, but sustained high levels in response to biotic and abiotic stress can inhibit root growth and induce senescence (Akhgar *et al.*, 2014). Endophytic bacteria that can produce the enzyme ACC deaminase contribute towards the catabolism of the plant ethylene precursor, ACC, consequently decreasing plant ethylene level and enabling plants to better tolerate biotic and abiotic stresses (Glick *et al.*, 1998, 2007). Microbial ability to produce ACC deaminase has been identified as one of the direct mechanisms of plant growth-promotion and has been linked to drought and salt tolerance in various plant species (Akhgar *et al.*, 2014; Glick, 2005; Sgroy *et al.*, 2009). Some bacterial strains from Brachiaria showed antifungal activity against five plant pathogenic fungi as has been demonstrated in several genera of bacteria (Kerr, 1999). Iron-deprivation through siderophores, cyanogenesis and antibiosis through the secretion of enzymes and volatile compounds have been describe as some of the possible mechanisms through which such bacteria effect antifungal activity (Cornelison *et al.*, 2014; Frey-Klett *et al.*, 2011; Minaeva *et al.*, 2008).

This study shows that the bacterial community associated with Brachiaria is quite diverse and include strains beneficial for plant growth promotion and suppression of plant pathogens. It is worth noting that an impressive majority (98%) of the isolated strains possess traits that are beneficial for the plant, with 49% of the bacterial strains testing positive for at least three traits beneficial to plants. Brachiaria are extensively cultivated tropical forages known for several desirable attributes such as drought tolerance; adaptation to low fertility soils; high nitrogen use efficiency, less input demand, high biomass production; carbon sequestration and resistance to several pests and diseases. It is fair to assume that some of the PGP attributes identified in the endophytic and rhizoplane bacteria of Brachiaria may have played a role, individually or in combination, in increasing the total biomass of test seedlings inoculated with some of the test strains. What makes Brachiaria so successful even under apparently harsh environmental and low input conditions has been a common cause of speculation among scientists and therefore our current findings on bacterial community composition, and the traits these microbes hold, will provide some evidence base on the role of these microbes for the adaptation and survival of the Brachiaria in such challenging environments.

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# Effects of Brachiaria grass cultivars on soil microbial biomass carbon, nitrogen and phosphorus in soils of the semi arid eastern Kenya

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**Abstract**

A study was conducted to investigate the changes in microbial biomass carbon (C), nitrogen (N) and phosphorus (P) following cultivation of Brachiaria grasses in semi-arid region of Kenya. The Brachiaria grass cultivars included *Brachiaria decumbens* cv. Basilisk, *B. brizantha* cvs Marandu, MG4, Piatã and Xaraes, *B. humidicola* cv. Llanero and *B.* hybrid cv. Mulato II which were compared with two locally cultivated forage grasses (*Chloris gayana cv.* KAT R3 and *Pennisetum purpureum cv.* Kakamega 1) and a bare plot (negative check). The grass treatments were evaluated with fertilizers application (40 kg P applied at sowing and 50 kg N $ha^{-1}$ in each wet season) and with no fertilizer applications. Microbial biomass C, N and P were determined on field moist rhizosphere soil (18-23% by weight) from a depth of 10 cm using the chloroform fumigation-extraction technique. Microbial biomass was significantly ($p < 0.01$) influenced by grass cultivars and N and P fertilizers. Generally, microbial biomass N was higher in plots with grasses than in the bare plots. A significant enrichment of organic matter was noted in the microbial biomass when Brachiaria grasses are grown with N and P fertilizers. Among Brachiaria cultivars, the highest microbial biomass C was recorded in plots with cv. Mulato II and the lowest from the plots with cv. MG4. Brachiaria grasses with fertilizers application accumulated the highest microbial C and N compared to grasses without fertilizers, but no interaction was observed between fertilizer and grass cultivars. The cv. Marandu had the highest microbial biomass N (21.2 mg N $kg^{-1}$) in fertilizer treatments whereas cv. Mulato II hybrid had the highest microbial N (14.6 mg N $kg^{-1}$) in no fertilizer treatments.

**Keywords**: Brachiaria; Carbon sequestration; *Chloris gayana;* microbial biomass; *Pennisetum purpureum*

## Introduction

Soils in the semi-arid regions of Kenya are often low in organic matter content (< 1%) and deficient in plant-available nutrients, especially nitrogen (N) and phosphorus (P). Any practice that increases the production of biomass carbon (C) via photosynthesis and slows down the return of C to the atmosphere increases C reserves in the soils through Carbon sequestration process. This would improve the soil quality and productivity (Smith *et al*., 2007) and make soils more resilient to climate change. One way to achieve this is to increase the amount of biomass C in soil where it is less susceptible to loss. This way the soil becomes a C 'sink' by absorbing atmospheric $CO_2$ from circulation and locking it in organic C from in the soil. Brachiaria grasses are endaphytic and have a greater ability sequester and accumulate large amounts of SOC through their large and extensive roots biomass and survive in dry areas of low soil fertility (Clapperton *et al*., Fisher *et al*., 2007; Peters *et al*., 2012). This makes Brachiaria grasses a better option for livestock feed production and soil improvement.

Soil organic matter is an important component of soil quality and productivity. However its measurement alone does not adequately reflect the short term changes in soil quality and nutrient status. Measurements of biologically active fractions of organic matter, such as microbial biomass C (MBC), N (MBN) and P (MBP) reflect changes in soil quality due to more recent (1-5yr) management and cultural practices (Hargreaves *et al.,* 2003). These measurements are based on rapidly changing capacity of both C and N forms in the soils. Microbial biomass is part of the active pool of soil organic matter that plays focal roles in decomposition of organic matters, nutrient cycling and biophysical manipulation of soil structure. It is considered to be a labile reservoir of potentially plant- available nutrients, since it acts as a source and sink of plant nutrients (Brookes et al., 1984). Microbial processes are driven by the availability of decomposable organic C, which highlights the importance of sustaining and improving soil organic matter concentrations if large populations of microbes are to be active in the soil. Roots exudates are a major source of substrate for soil microorganisms. These compounds can be utilized by microorganisms immediately, increasing significantly the diversity, number and activity of microorganisms in the rhizosphere. It has been accounted that nearly 5 to 21% of all photosynthetically fixed C is transferred to the rhizosphere through roots exudates, which range from 20 to 50% of plant biomass (Jones *et al*., 2009).

Organic amendments such as manure, plant residues and roots exudates are a major source of organic substrate in the soil. During the process of biomass turnover, the nutrients may be released slowly and taken up by the crop more efficiently (Brookes et al, 1984; Parham *et al.,* 2003; Gichangi *et al.,* 2010). These nutrients can be utilized by microorganisms immediately, increasing significantly the diversity, number and activity of microorganisms in the rhizosphere. This makes microbial biomass a fundamental component of nutrient cycling in agro-ecosystems and critical in determining soil quality (Belay *et al.,* 2002). Soil microbial biomass is an important early indicator of long term changes in soil (Saffigna *et al*., 1989; Romaniuk *et al*., 2011; Lauer *et al*., 2011) and therefore can be used to determine the level of degradation or improvement of the soil following changes in management and cultural practices (Brookes 1995; Sparling, 1997). We tested the hypothesis that, cultivation of climate smart Brachiaria grasses improves soil quality through increased organic matter resulting from the large roots biomass. The objective of this study was therefore to quantify the amount of soil microbial biomass C, N and P as indicators of improved soil quality resulting from 2-year cultivation of Brachiaria grasses.

**Materials and methods**

*Description of the study site*

The experiment was established in November during the short rains of 2013 at the Kenya Agricultural and Livestock Research Organization (KALRO) Katumani. The site is located (37°28′0′′E, 1°58′0′′S) 75 km southeast of Nairobi at an elevation of 1580 m above sea level. It receives mean annual rainfall of 717 mm in bimodal pattern with the long rains (LR) occurring from March to May and the short rains (SR) from October to December with peaks in April and November, respectively and a mean temperature of 19.6˚C. The dominant soils are chromic Luvisols, which are low in organic C and highly deficient in N and P and to some extent Zinc (NAAIAP, 2014).

*Site characterization*

Soil samples were collected in November 2013 before establishing the experiment at depths of 0–15 cm, 15–30 cm, 30–60 cm, and 60-100 cm using a bucket auger for initial analysis of the soils at the testing site. Plant litter on the soil surface was removed before collecting the soil samples. A composite soil sample, consisting of 12 cores, was collected in a grid pattern from within the 25 × 10 m blocks. Samples from each block were air-dried, visible plant roots removed, and the samples gently crushed to pass through a 2 mm sieve. The fractions sample <2 mm were used for subsequent chemical and physical analyses. Total soil N, available P (Mehlick III), exchangeable K, Ca, and Mg were estimated following standard methods as described by Okalebo *et al.*, (2002). Cations $Ca^{2+}$, $Mg^{2+}$, and $K^{+}$ were determined by atomic absorption spectrometry and soil P was measured as described by Murphy and Riley (1962).

Soil texture was determined by the hydrometer method. Soil pH was measured in water (soil: water ratio of 1: 2.5) using a pH meter and reference calomel electrode (Model pH 330 SET-1, 82362) after the suspensions were shaken for 30 minutes and allowed to stand for 1 hour. Organic carbon, was determined by the modified Walkley and Black procedure (Nelson and Sommers 1982. Cation exchange capacity (CEC) was based on the sum of exchangeable Ca, Mg, K, H and Al after extraction with ammonium acetate. Soil bulk density was determined according to Blake and Hartge (1986). Soils were vertically sampled using stainless steel rings (diameter 10 cm) at soil depths of: 0–15 cm, 15–30 cm, 30–60 cm, and 60-100 cm, resulting in undisturbed soil samples for bulk density determination. Soil samples were dried at 65ºC to a constant weight to allow soil bulk density calculation. All determinations were made in triplicate and expressed on a dry weight basis.

Tables 1 and 2 below show the initial main soil characteristics of the experimental site. Soil pH was moderately acidic in all the depths (Table 1) and organic C content was low and decreased with depth. Similarly, N, P and Zn were low. Calcium, K and Fe levels in the soil were adequate (Table 1).

**Table 1** Initial soil chemical characteristics

| Properties | Sampling depth (cm) | | | |
|---|---|---|---|---|
| | 0-15 | 15-30 | 30-60 | 60-100 |
| Soil pH (water) | 5.88 | 5.76 | 5.81 | 6.10 |
| Total nitrogen % | 0.12 | 0.12 | 0.07 | 0.05 |
| Organic carbon % | 1.16 | 1.15 | 0.65 | 0.49 |
| Phosphorus ppm | 10 | 12 | 10 | 15 |
| Potassium me% | 0.29 | 1.01 | 0.52 | 0.32 |
| Calcium me% | 3.1 | 3.4 | 2.2 | 2.4 |
| Magnesium me% | 5.72 | 5.99 | 5.96 | 6.31 |
| Iron ppm | 17.0 | 17.4 | 18.8 | 18.3 |
| Zinc ppm | 1.78 | 1.44 | 0.97 | 0.64 |

Physical analysis of soil samples from the test site indicated that the soils were sandy clay loam in the 0 -30cm depth and clay in the lower depths (Table 2). Cation exchange capacity ranged from 20.2 to 27.8 me%, increasing with depth. This was expected as the clay content also increased with depth resulting to increased number of exchange sites (Table 2). Bulk density ranged from 1.32 to 1.45 g $cm^{-3}$ and was greater than the ideal range of 1.1-1.3 g $cm^{-3}$ for non-restricted plant root growth. Soil bulk density exceeding 1.46 g $cm^{-3}$ for such soils would restrict root growth and could negatively interfere with soil aeration through reduced air-filled pore space (Landon, 1991).

**Table 2** Initial soil physical characteristics

| Properties | Sampling depth (cm) | | | |
|---|---|---|---|---|
| | 0-15 | 15-30 | 30-60 | 60-100 |
| Bulk density g/$cm^3$ | 1.32 | 1.35 | 1.41 | 1.45 |
| Sand % | 50.7 | 48.7 | 44.0 | 40.0 |
| Silt % | 6.0 | 8.0 | 5.3 | 7.3 |
| Clay % | 43.3 | 43.3 | 50.7 | 52.7 |
| Cation exchange capacity me% | 20.2 | 21.3 | 26.9 | 27.8 |
| Base saturation % | 92.4 | 85.7 | 78.9 | 64.2 |
| Exchangeable Sodium Potential (ESP) | 0.9 | 0.7 | 0.6 | 0.7 |
| Texture Class | Sandy clay | Sandy clay | Clay | Clay |

*Treatments and experimental design*

The treatments consisted of seven Brachiaria grasses: *Brachiaria decumbens* cv. Basilisk, *B. humidicola* cv. Llanero, *B. brizantha* cvs Marandu, MG4, Piatã, Xaraes and *B. hybrid* cv. Mulato II, two commonly cultivated local grasses [(*Chloris gayana* cv. KAT R3 and *Pennisetum purpureum cv*. Kakamega 1 (KK1) as local checks)] and a bare plot (as negative control). These treatments were evaluated in the plots with fertilizer (40 kg P $ha^{-1}$ applied at sowing and 50 kg N top-dressed in each wet season) and without fertilizer application. The treatments were laid out in a randomized complete block design in a split plot arrangement (fertilizer treatments as main plots and the grass treatments as sub plots) in three replications. The grasses were sown in November 2013 during the short rains. All the plots were kept free of weeds throughout the experimental period by hand weeding. The grasses were first harvested 16 weeks after establishment and later, on an 8-week interval harvestings during the wet seasons.

*Above-ground and roots biomass determination*

Data for aboveground plant biomass was collected eight times on an 8 weeks interval after plants were well established. The establishment period was considered as 16 weeks after seedling emergence. Harvesting of plant shoots was conducted from 2 m x 2 m net plots at a cutting height of 5 cm above ground. Samples of fresh shoots biomass were recorded, and approximately 500g subsamples were dried at 65°C to constant weight in forced-air drier for determination of dry matter. Roots were sampled using the soil-core method (Bolinder, *et al*., 2002). In each plot, four soil cores were randomly taken with a 5 cm diameter stainless steel auger to a depth of 0–15 and 15–30 cm from the inter-row and intra-row positions and composited into one sample per plot for each depth. The sampling was carried out at least 1m apart from the edge of the plot to avoid edge effects. Sampling was conducted at 24 and 48 weeks of plants establishment. The roots from each soil layer were washed separately by hand with a 2.8 mm and a 2 mm soil sieve under running tap water. Root samples integrating both living and dead roots were then dried at 65°C to constant weight and roots dry weights were recorded.

*Soil microbial biomass C, N, P*

Soil samples for microbial biomass carbon, nitrogen and phosphorus analysis were collected in November 2015 twenty four months after the grasses had established. Four soil samples were carefully collected from a depth of 0-10 cm using an auger in each pasture plot and from the bare plot controls. In this study, only the top 10 cm soil was sampled which was assumed to contain the highest biological activity and most likely exhibit short-term changes in response to Brachiaria grasses cultivation. Soils from the four sampling positions of a plot were pooled to one sample and used in the subsequent analysis as described below.

Microbial biomass C, N and P were determined on field moist soil (18-23% by weight) by the chloroform fumigation-extraction technique as described in Brookes *et al*. (1984, 1985) and Vance *et al*. (1987). Briefly, 10 g dry weight equivalent of soil was fumigated with ethanol-free chloroform in a glass desiccator; and another 10g was incubated without fumigation at the same moisture content, time period and temperature for 24 h at 25°C. Both sets were extracted with 0.5 M $K_2SO_4$ (for C and N) or 0.5 M $NaHCO_3$ (for P). Soil microbial biomass element content was calculated as the difference between the fumigated and un-fumigated samples and corrected for incomplete recovery using conversion factors of 0.45 for C (Wu *et al*., 1990), 0.45 for N (Jenkinson *et al*., 2004) and 0.40 for P (Hedley *et al*., 1982). All determinations were made in triplicate and expressed on a dry weight basis.

**Statistical analysis**

The concentrations of the microbial biomass C, N and P were compared by 2-way analysis of variance (ANOVA) using GenStat statistical software (GenStat Release 4.24DE, 2005). This was evaluated by running a full model (20 treatments, 19 df), which was further split into a fertilizer effect (1 df), cultivar effect (9 df), fertilizer*cultivar effect (9 df). Differences at $p \leq 0.05$ were considered significant and means separation was done using Fischer's protected least significant difference (LSD). Regression analyses and Pearson correlation coefficient (r) were used to find models best describing the relationships between soil microbial biomass and other soil and plant properties.

**Results and Discussion**

*Microbial biomass carbon*

Although it is only a small part of soil organic C, the soil microbial biomass is regarded as one of the most sensitive indicators of ecosystem function. The effects of the different Brachiaria cultivars and fertilizer application on microbial biomass were significant ($p \leq 0.05$). Microbial biomass C was significantly higher ($p < 0.01$) in grass vegetated soils compared to bare plots (Table 3).

The MBC ranged from 23.9 to 200.5 mg C $kg^{-1}$ of soil and 12.9 to 107.9 mg C $kg^{-1}$ of soil in the fertilized and non-fertilized treatments, respectively. The highest MBC in cultivated Brachiaria soils was recorded under Mulato II hybrid and lowest under MG4 (Table 3). The effects of the different treatments on MBC followed the order Mulato II hybrid > Basilisk > Marandu > Xaraes > Llanero > KK1 > Piatã > MG4 > KAT R3 > bare for plots that received N and P fertilizer. Microbial biomass C increased in the grass plots probably due to rhizo-deposition (Benizri *et al*., 2007). Rhizo-deposition may occur by roots exudation and root cell sloughing (Rasse *et al*., 2005). These compounds represent a source of labile C in soil, which is rapidly consumed by micro-organisms (Jones *et al*., 2009), thereby stimulating microbial biomass production (Benizri *et al*., 2007). Similarly, MBC had a higher range of values in fertilized plots. This small component of the soil organic matter has been shown to be more responsive to cultural treatments than is total soil organic matter (Jenkinson *et al*., 2004).

**Table 3** Effects of cultivar and fertilizer N and P on microbial biomass C

| Grass type | Microbial biomass C (mg C $kg^{-1}$ soil) | |
|---|---|---|
| | No-Fertilizer NP | Fertilizer NP |
| *B. decumbens* cv. Basilisk | 91.3 | 199.3 |
| *B. humidicola* cv. Llanero | 65.3 | 179.2 |
| *B. brizantha* cv. Marandu | 97.6 | 198.0 |
| *B. brizantha* cv. MG4 | 47.5 | 125.4 |
| *B. brizantha* cv. Piatã | 61.3 | 150.3 |
| *B. brizantha* cv. Xaraes | 79.1 | 189.5 |
| *B. hybrid* cv. Mulato II | 107.9 | 200.5 |
| *Pennisetum purpureum cv* KK1 | 77.3 | 177.5 |
| *Chloris gayana* cv. KAT R3 | 37.5 | 101.0 |
| Control bare plot | 12.9 | 23.6 |
| LSD (interaction) | 33.9 | |

LSD= Fischer's protected least significant difference

*Microbial biomass nitrogen*

There was a highly significant interaction effect ($p < 0.01$) of grass cultivars by fertilizer application on MBN (Table 4). Among the N and P fertilized treatments, the soils under cv. Marandu had the highest MBN (21.2 mg N $kg^{-1}$ soil) whereas cv. Mulato II hybrid had the highest MBN (14.6 mg N $kg^{-1}$ soil) in non-fertilized treatments (Table 4). However, the amounts of MBN of soils under cv. Marandu in the N and P treatments were statistically similar to those recorded from soils under cultivars Mulato II, Basilisk, KK1, Xaraes, Llanero, and Piatã (Table 4). The bare plot treatment had the least amount of microbial N (1.5 mg N $kg^{-1}$ soil) and (1.1 mg N $kg^{-1}$ soil) in the N and P fertilized and un-fertilized treatments respectively. This indicates that the cultivated grasses had greater contribution to the amounts of MBN recorded. The increases in MBN in the grasses plots due to N addition may be attributed to increased N availability to soil microorganisms. Gama Rodrigues *et al.* (2005) reported that only 40 to 60% fertilizer N is absorbed by plants, while 20-50% of the applied N is incorporated into the soil as organic N which contributes to the microbial biomass.

Studies on the effects of fertilizer application on soil microbial biomass remain equivocal. For instance, Zhang *et al*. (2005) measured significant increase of soil microbial biomass after two year of N fertilization in deteriorated grassland in China. However, Sarathchandra *et al*. (2001) reported significant decrease of soil microbial biomass in a perennial pasture of New Zealand due N fertilization. Meanwhile, Johnson *et al*. (2005) found that no effect of N applications on soil microbial biomass in upland grassland in Scotland. The mechanisms behind the variations may depend on other soil features, such as soil moisture, soil organic matter, total N, pH, the rate of N addition etc. (Compton *et al*., 2004; Heinze *et al*., 2010), but the specific drivers are still not completely identified (Zhang and Zak, 1998).

As an important component in regulating below ground ecological processes, the soil microbial populations are facilitators of nutrient availability, particularly soil N availability (Coleman and Crossley, 1996). Thus, any changes in the availability of soil N may in turn, affect the soil microbial community, and hence obviates their role in the turnover of soil organic matter. Additionally, changes in soil microbial function and community composition may trigger a series of responses, such as impacting litter and organic matter decomposition rates (Carreiro *et al*., 2000), humus formation (Magill and Aber, 1998), nutrient transformation and cycling (Fisk and Fahey, 2001), and then alter the interaction between soil microbes and plant communities.

**Table 4** Effects of cultivar and fertilizer N and P on microbial biomass N

| Grass type | Microbial biomass N (mg N $kg^{-1}$ soil) | |
|---|---|---|
| | No-Fertilizer NP | Fertilizer NP |
| *B. decumbens* cv. Basilisk | 12.2 | 19.9 |
| *B. humidicola* cv. Llanero | 9.2 | 17.9 |
| *B. brizantha* cv. Marandu | 13.7 | 21.2 |
| *B. brizantha* cv. MG4 | 4.3 | 16.6 |
| *B. brizantha* cv. Piatã | 5.6 | 17.9 |
| *B. brizantha* cv. Xaraes | 7.7 | 18.7 |
| *B. hybrid* cv. Mulato II | 14.6 | 20.0 |
| *Pennisetum purpureum cv* KK1 | 11.6 | 19.7 |
| *Chloris gayana* cv. KAT R3 | 3.6 | 15.4 |
| Control bare plot | 1.1 | 1.5 |
| LSD (interaction) | 3.9 | |

LSD= Fischer's protected least significant difference

*Microbial biomass phosphorus*

Microbial biomass P was significantly higher ($p < 0.01$) in treatments that received N and P fertilizer and the amounts were even much higher in soils under grasses than in bare plots (Table 5). The values of P in the microbial biomass were larger in the presence of Brachiaria grasses with significant ($p < 0.01$) interaction effect with N and P addition. Among the Brachiaria grasses cvs. Piatã and MG4 had the lowest MBP in treatments without N and P application but in fertilizer treatments the MBP levels in these soils increased by 60.9% and 69.9%, respectively. Brachiaria cultivar MG4 had the highest response to fertilizer with an increase of 69.9% in fertilized soils compared to no fertilizer treatments. Basilisk, Marandu and Mulato II cultivars contributed to the highest MBP (4.4 – 5.4 mg P $kg^{-1}$soil) in no fertilizer treatments and (10.4 – 12.0 mg P $kg^{-1}$soil) under fertilizer treatments (Table 5).

Since microbial biomass acts as an important source of P in soils, cultivation of Basilisk, Marandu and Mulato II could contribute to increased available P levels in these P deficient soils of eastern Kenya (Table 1). The Brachiaria cultivars gave higher MBP than the commonly grown *Chloris gayana* cv. KAT R3 making them a good alternative for soil amelioration and animal feeds in the drylands of Kenya. However, the MBP in Brachiaria grasses were comparable to the widely grown Napier grass (cv. KK1). During growth, Brachiaria grasses may also have encouraged higher microbial populations within their rhizosphere, which contributed to the increased MBP. Phosphate immobilization by microorganisms is an important sink, which contributes to microbial P pool. Addition of N and P fertilizers doubled the MBP in all the grass cultivars.

**Table 5** Effects of cultivar and fertilizer N and P on microbial biomass phosphorus

| Grass type | Microbial biomass P ($mg\ P\ kg^{-1}$ soil) | |
|---|---|---|
| | No-Fertilizer NP | Fertilizer NP |
| *B. decumbens* cv. Basilisk | 4.4 | 10.4 |
| *B. humidicola* cv. Llanero | 3.7 | 10.5 |
| *B. brizantha* cv. Marandu | 5.1 | 11.7 |
| *B. brizantha* cv. MG4 | 2.3 | 7.7 |
| *B. brizantha* cv. Piatã | 3.5 | 8.9 |
| *B. brizantha* cv. Xaraes | 4.4 | 11.9 |
| *B. hybrid* cv. Mulato II | 5.4 | 12.0 |
| *Pennisetum purpureum cv* KK1 | 5.0 | 11.2 |
| *Chloris gayana* cv. KAT R3 | 2.0 | 6.0 |
| Control bare plot | 1.1 | 2.0 |
| LSD (interaction) | 1.8 | |

LSD= Fischer's protected least significant difference

The P concentration in the microbial biomass in this study falls within the range reported earlier (Brookes *et al*., 1984; Singh, 2007). It has been reported that incorporation of P into the soil microbial biomass is a mechanism that significantly increases the availability of P to plants and forms a significant pool of plant nutrients (Nziguheba *et al*., 1998). This pool play a key role in P dynamics in soils by immobilizing inorganic P which is later mineralized (Parham *et al*., 2003; Gichangi *et al*., 2009, Gichangi *et al*., 2010). For example, Nziguheba *et al.* (1998) reported increased soil MBP and decreased P sorption following incorporation of wild sunflower (*Tithonia diversifolia*) as green manure in an acid soil in western Kenya.

A high microbial biomass may indicate greater accumulations of C, N and P in the organic pool, and could represent either a sink or a source of plant-available nutrients, depending on the soil management. The higher C, N and P in the soil microbial biomass under Brachiaria grasses in this study may be due to a higher capacity of nutrient immobilization by the microbes from the decomposing litter fall and roots residues in addition to the roots exudates released which serves as substrate for microbial growth in the soil. Roots exudates are a major source of substrate for soil microorganisms. These compounds can be utilized by microorganisms immediately, increasing significantly the diversity, number and activity of microorganisms in the rhizosphere. It has been accounted that nearly 5 to 21% of all photosynthetically fixed C is transferred to the rhizosphere through roots exudates, which range from 20 to 50% of plant biomass (Jones *et al*., 2009). Due to its highly dynamic character, the microbial biomass responds more rapidly to soil fertility than the physical/chemical properties, which change relatively slowly (Sparling, 1997) and this might explain measurable changes in microbial biomass in the N and P fertilized plots in this study. The amounts of roots in the plots with fertilizer were significantly higher than no fertilize plots (Figure 1). Increasing roots biomass influences soil organic matter: i) directly by increasing organic inputs to soil and ii) indirectly by influencing the location of roots and production of roots exudates that may stimulate mineralization (Jones *et al*., 2009). Roots exudates and other by-products are also more readily absorbed and protected by soil aggregates and where concentrated are more likely to persist in the particulate organic matter and humus fractions than shoots-derived soil organic C (Clapperton *et al*., 2003; Walker *et al*., 2003; Zhang *et al*., 2005).

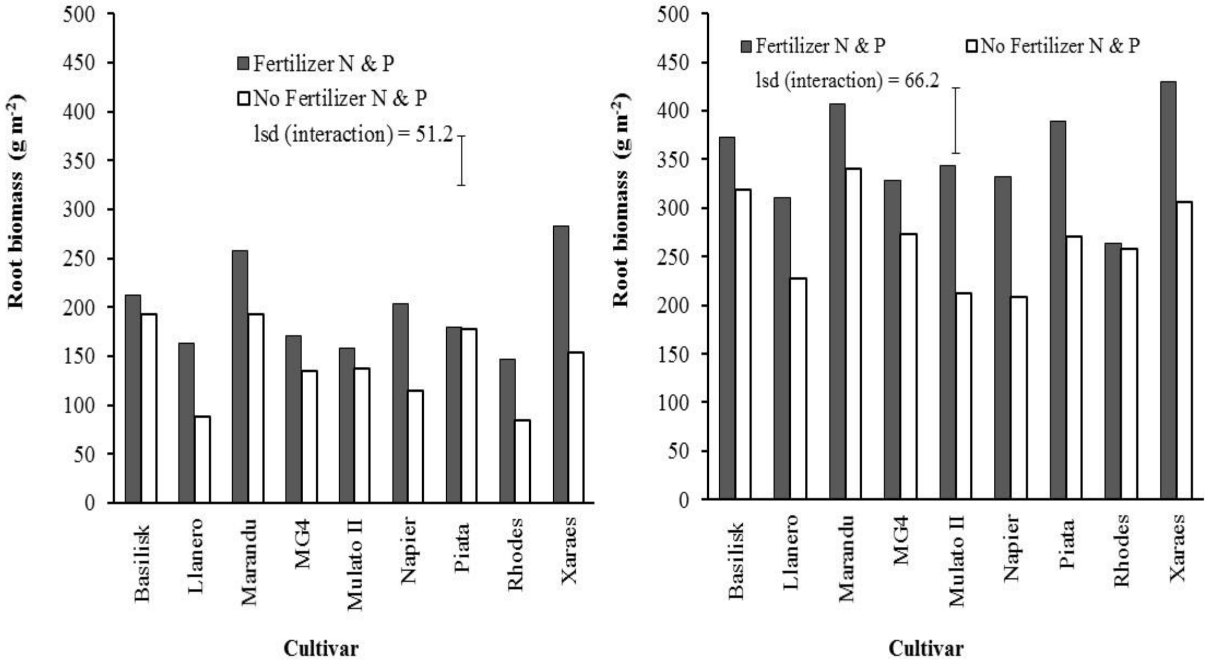


**Figure 1** Effects of cultivar and fertilizer NP on roots biomass (0-15cm) a) 24 weeks and b) 48 weeks after plants had established

*Relationships between microbial biomass carbon, nitrogen and phosphorus*

There were a number of significant correlations between microbial biomass and soil and plant properties (Figure 2; Table 6). Microbial biomass N and P showed a significant positive correlation with microbial biomass C (Figure 2). Multiple regression across all treatments showed that there were high coefficients of determination between MBC and MBN ($r^2 = 0.83$, $p < 0.01$, Figure 2a), MBC and MBP ($r^2 = 0.85$, $p < 0.01$, Figure 2b) as well between MBN and MBP ($r^2 = 0.82$, $p < 0.01$, Figure 2c). This results show evidence that soil C, N and P cycles are intimately related through the processes of mineralization and immobilization, suggesting a strong relationship that may exist between soil N and P transformations and soil C.

Microbial biomass was significantly and positively correlated to soil organic carbon ($r = 0.3139$, $p < 0.05$); ($r = 0.4596$, $p = 0.01$) and ($r = 0.2583$, $p = 0.05$) for MBC, MBN and MBP, respectively and total N ($r = 0.356$, $p < 0.05$); ($r = 0.5029$, $p = 0.01$) and ($r = 0.3521$, $p = 0.05$) for MBC, MBN and MBP, respectively (Table 6). This indicates that microbial biomass is highly influenced by the concentration of soil nutrients. Positive relationship between microbial biomass C, N and P and soil organic C and total N in grassland has been reported elsewhere by Moore *et al*. (2000). Similarly, microbial biomass correlated, significantly and positively, to roots biomass measured at various stages of growth 24 and 48 weeks after plants had established (Table 6). However a stronger relationship was recorded for roots biomass measured 24$^{th}$ week (Table 6). Roots are major C source in soil and can also stimulate SOM mineralization (Jones *et al*., 2009). The capacity to generate roots, in part explains why perennials and pastures are sometimes associated with increasing soil organic matter compared to annuals. In conclusion, soil microbial biomass C, N and P in this study varied in the different grass types and was higher in the N and P fertilizer treatments and were therefore useful parameters to elucidate the changes of organic matter in the different grass type's soils. The results showed a significant enrichment of the microbial biomass component of organic matter due to the cultivation of the grasses and which was further enhanced by applications of N and P fertilizer.

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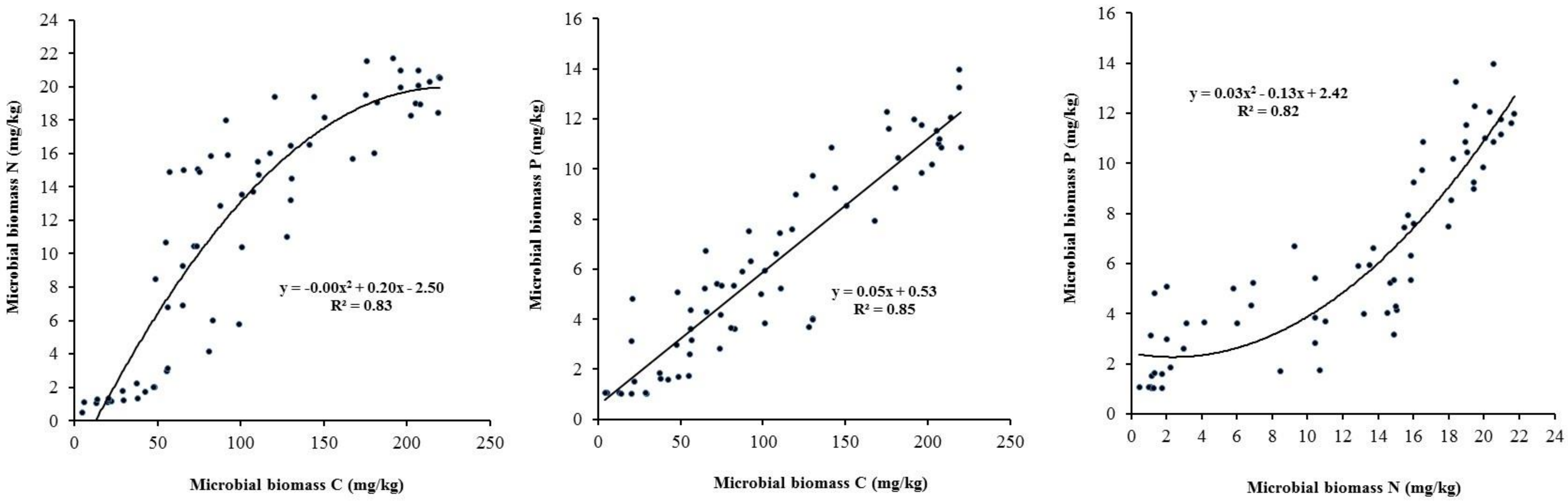


**Figure 2** Relationships between a) MBC with MBN and b) MBC with MBP c) MBN with MBP

**Table 6** Relationships between microbial biomass carbon, nitrogen and phosphorus with soil and plant properties

| Properties | MBC | MBN | MBP | NH4-N | NO3-N | Roots biomass-Week 24 | Roots biomass-Week 48 | Total C | Total N | Above ground biomass | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MBC | 1.0000 | | | | | | | | | | |
| MBN | 0.8759** | 1.0000 | | | | | | | | | |
| MBP | 0.9210** | 0.8633** | 1.0000 | | | | | | | | |
| NH4-N | 0.1047 | 0.0998 | 0.1711 | 1.0000 | | | | | | | |
| NO3-N | 0.1121 | 0.0848 | 0.1750 | 0.9202** | 1.0000 | | | | | | |
| Roots biomass-Week 24 | 0.7172** | 0.6904** | 0.6905** | -0.1467 | -0.2096 | 1.0000 | | | | | |
| Roots biomass-Week 48 | 0.6225** | 0.6290** | 0.6445** | -0.0335 | -0.1143 | 0.8210** | 1.0000 | | | | |
| Total C | 0.3139* | 0.4596** | 0.2583* | -0.1984 | -0.1747 | 0.4527** | 0.4059** | 1.0000 | | | |
| Total N | 0.3568* | 0.5029** | 0.3521* | 0.0219 | 0.0480 | 0.4502** | 0.4734** | 0.8403** | 1.0000 | | |
| Above ground biomass | 0.6363** | 0.6502** | 0.6366** | 0.0830 | 0.0227 | 0.6802** | 0.6992** | 0.4287** | 0.4777** | 1.0000 | |
| pH | 0.1561 | 0.1701 | 0.0576 | -0.5637** | -0.6536** | 0.3076* | 0.2310 | 0.2568* | 0.1101 | 0.3206* | 1.0000 |

** $p < 0.01$ and *$p < 0.05$, MBC-microbial biomass carbon, MBN-microbial biomass nitrogen, MBP-microbial biomass phosphorus

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# Soil microbial biomass and changes in soil properties in cultivated Brachiaria grass in North western Kenya

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KALRO – Kitale

**Abstract**

Microbial biomass is part of the active pool of soil organic matter which determines decomposition of organic matter, nutrient release and soil structure formation. Farming practices that enhance high accumulation of microbial biomass play an important role in increasing soil organic matter. The study was conducted at Kitale in the north western highlands to assess the effects of Brachiaria grasses on soil pH, available P and microbial biomass in a low fertility soil. Seven Brachiaria cultivars: *Brachiaria brizantha* cvs. Marandu, MG4, Piata, Xaraes, *B. decumbens* cv. Basilisk, *B. hybrid* cv. Mulato II and *B. humidicola* cv. Llanero were compared with two popularly grown forages, Rhodes grass (*Chloris gayana*)and Napier grass (*Pennisetum purpureum* cv KK). The treatments were tested in a split plot arrangement with two rates of fertilizer N and P, 0 and 40 kg P ha$^{-1}$ applied at planting and 50 kg N ha$^{-1}$ as a seasonal top-dressing assigned to the main plots and the grass cultivars as subplots. After 24 months, moist rhizosphere soil was sampled at 0 - 10cm depth for determination of microbial biomass. No significant fertilizer NP effects ($P > 0.05$) were noted in either the ammonium N, nitrate N, Microbial biomass C (MBC) or N (MBN). The Brachiaria cultivars did not significantly ($P > 0.05$) influence the microbial biomass. Significant ($P = 0.001$) changes in soil properties including ammonium N, nitrate N and soil pH due to grass cultivars were observed. Although bare plot and Napier grass accumulated higher pools of ammonium and nitrate N, the MBC and MBN it was low. Soil pH was also low, indicating increased nitrification. Ammonium N was the most dominant form of inorganic N in Llanero and Piata with lower nitrate N due to either increased plant uptake or reduced nitrification rates. However, further studies are required to ascertain the role of the two grasses as potential biological nitrogen inhibitors.

**Keywords**: Ammonium N, microbial biomass N, nitrate, soil organic matter, soil quality

## Introduction

Microbial biomass is part of the active pool of soil organic matter which determines the part of labile organic matter that can easily be decomposed leading to nutrient mineralization. Soil microbial biomass comprises only 1 to 3% of the total organic carbon (C) in the soil but is a labile fraction of the soil organic matter (Jenkinson and Ladd, 1981). Changes in organic matter input or rate of decomposition are more readily determined from soil microbial biomass than the total organic matter (Powlson *et al.*, 1987).The flow of C, nitrogen (N) and phosphorus (P) through the microbial biomass is useful in assessing soil quality in farming systems (Sparling *et al.*, 1991; Tangjang *et al.*, 2010). Accumulation of soil microbial biomass (SMB) is affected by management systems employed in the soil. Human activities including tillage, conversion of agricultural land to forage grassland and fertilizer inputs can lead to alteration of biogeochemical cycles and nutrient enrichment or depletion especially N and P (Burger and Jackson, 2003). Assessment of soil microbial biomass gives an indication of management induced changes brought about by these activities.

Generally, SMB is higher in grass systems than in open cultivated cropland because of the high turnover in pasture systems. However, while investigating the effects of different grass species in four separate soil types, Groffman *et a.l* (1996) found significant impact of soil type but small and infrequent differences in SMB and microbial activity due to grass species. Application of N and P fertilizers to grasslands can influence the root growth, shoots dry matter accumulations and therefore lead to increased microbial biomass especially in low organic matter sandy soils (Gichangi *et al.* 2016; Li *et al.* 2014). However, other authors have reported no differences in microbial C due to N and P fertilization (Li *et al.* 2010). Humid regions of Kenya are threatened by declining soil fertility and low soil organic matter due to continuous cropping and tillage. The introduction of high quality grass species with increased roots biomass may improve the microbial biomass and restore fertility of degraded lands. The study was conducted in the north western highlands to assess the effects of Brachiaria grasses on soil pH, available P and microbial biomass in a low fertility soil.

**Materials and methods**

*Study site*

The experiments were conducted at the Kenya Agricultural and Livestock Research Organization (KALRO), Kitale farm, located in Trans Nzoia County within the north western highlands of Kenya. The site (1° 0′ 6.6′′N and 34° 59′ 10′′E) lies within the Upper Midlands (UM4) agro-ecological zone at an elevation of 1890 m asl. The site has a cool and temperate climate with mean annual temperatures between $10^0$ C and $27^0$ C. The mean annual precipitation is 1100 mm, is unimodal and normally starts in April and continues through to October/November with peak in May and August. The soils in the study site are mainly humic Acrisols (FAO, 2006; Jones *et al.,* 2013). Acrisols have low fertility, are mainly N and P deficient, with a weak to moderate sub-angular blocky structure, low organic matter (NAAIAP and KARI, 2014) and are well drained with high moisture storage capacity.

*Soil chemical characteristics*

Soil samples to assess initial soil physical and chemical properties were collected prior to commencement of the trials using a soil auger. Samples were taken using a w- pattern from four depths: 0 – 15 cm, 15 – 30 cm, 30 – 60 cm, and 60 - 100 cm thoroughly mixed and a sub sample taken from each depth for each block. After air drying, the samples were gently crushed to pass through a 2-mm sieve. Available P (Mehlick III), exchangeable K, Ca, Mg and total N were estimated after wet digestion with $H_2O_2/H_2SO_4$ as described by Okalebo *et al.* (2002)**.** Total $Ca^{2+}$, $Mg^{2+}$, and $K^+$ were determined by atomic absorption spectrometry and P measured as described by Murphy and Riley (1962). Soil pH was measured in water (soil: water ratio of 1: 2.5) using a pH meter with a glass and reference calomel electrode (Model pH 330 SET-1, 82362). Soils were vertically sampled using stainless steel rings (diameter 10 cm) for different depths; resulting in undisturbed soil samples for bulk density determination as described by Blake and Hartge (1986). The samples were dried to 65ºC to a constant weight to allow soil bulk density calculations. All determinations were made in triplicate and expressed on a dry weight basis.

The bulk density ranged from 1.34 to 1.49 g/cm$^3$ in Kitale, which was within the normal range (<1.5 g/cm$^3$) that doesn't restrict root growth in soil as proposed by Hunt and Gilkes (1992). Soil pH was generally medium acidic, but slightly more acidic in the 30 - 60 cm depth (Table 1). Deficiencies of N, available P and Zn were observed with nutrient levels decreasing with depth. Soil organic C ranged from 0.2-0.3% and these levels are generally considered low (Okalebo *et* *al.* 2002). Soil organic C is an indicator of soil quality, due to its contribution to microbial activity, soil structure and promotion of water storage and drainage. However, K, Ca, Mg and Mn were adequate.

**Table 1** Initial soil physical and chemical properties at varying depths in Kitale

| Parameters | 0-30cm | 30-60cm | 60-100cm |
|---|---|---|---|
| Soil pH | 5.76 | 5.50 | 5.66 |
| Bulk density (g/cm$^3$) | 1.49 | 1.46 | 1.34 |
| Total N (%) | 0.03 | 0.02 | 0.02 |
| Organic C (%) | 0.3 | 0.2 | 0.2 |
| Available P (ppm) | 18 | 6 | 5 |
| Potassium (me %) | 1.17 | 0.74 | 0.51 |
| Calcium (me %) | 4.5 | 4.3 | 2.2 |
| Magnesium (me %) | 1.2 | 1.04 | 1.2 |
| Manganese (me %) | 0.29 | 0.16 | 0.20 |
| Zinc (ppm) | 1.79 | 1.56 | 1.03 |

*Experimental design and management*

The treatments consisted of seven Brachiaria grass cultivars: *Brachiaria decumbens* cv. Basilisk, *B. brizantha* cvs. Marandu, MG4, Piata, Xaraes, *B. hybrid* cv. Mulato II *and B. humidicola* cv. Llanero and two commonly grown grasses, (Rhodes grass, C*hloris gayana* cv. KAT R3 and Napier grass, *Pennisetum purpureum* cv. Kakamega 1) as positive controls and a bare plot as negative control. These treatments were arranged in a split plot and randomized in complete block design with either fertilizer application (40 kg P ha$^{-1}$ and 50 kg N ha$^{-1}$ per season) or no fertilizer in the main plot and the grasses as subplots and replicated four times. Phosphorus was applied at planting as Triple Super Phosphate (46% $P_20_5$) while N was top dressed with Calcium Ammonium Nitrate (CAN 26% N) each season. The bare plots were kept weed free throughout the experimental period by hand weeding. These treatments were tested in subplots of 4 m by 4 m. Seeds were drilled by hand in about 2 cm deep furrows at a rate of 5 kg ha$^{-1}$ for Brachiaria and 10 kg ha$^{-1}$ for Rhodes grass in a well prepared land. The inter-row spacing was 0.5 m. Splits of Napier grass was planted at 1 m x 0.5 m spacing. Minimum tillage was practiced throughout the growth period to minimize root damage. After establishment period (14 weeks after seedling emergence - WAE) the grasses were harvested at a cutting height of 5 cm above the ground in an effective area of 4 m$^2$ using sickles and removed from the plot to stimulate uniform growth. Subsequent harvests were made every eight weeks, to determine shoots biomass yields as this was recommended management practice for the grass (Kifuko *et al.*, 2016, this proceedings).

*Assessment of roots biomass*

Roots biomass assessment was carried out after 24 months. Roots were sampled using the soil-core method (Bohm, 1979). Four soil cores were randomly taken using a stainless steel auger at 0 - 15 cm and 15 – 30 cm depths from the intra- and inter-row spacing in each plot. To prevent edge effects, the samples were taken at least 1 m from the edges. The samples from each depth were thoroughly mixed, weighed to determine the total bulk soil weight and a sub sample was taken for moisture correction. The roots contained in the samples were recovered under a tap of running water at low pressure using 2.8 cm and 2 cm mesh sieves (Bohm, 1979). Samples were dried in an air-forced oven at 65º C to constant weight and then weighed for determination of dry roots weight. The roots biomass was calculated as a factor of the bulk density for each depth.

*Soil sampling for microbial biomass and ammonium and nitrate N determination*

Soil samples were collected from 0 – 10 cm in each plot at the intra-row and inter-row positions to target the rhizosphere soil under Brachiaria grasses two years after the grasses had established. The sub samples from each plot were thoroughly mixed, large plant material removed and samples packaged in sterile polythene bags and packaged in ice boxes for transportation to the laboratory. In the laboratory, the samples were stored at 4$^{o}$C until analysis of the microbial properties. The microbial biomass C (MBC) was determined by a fumigation extraction method according to Vance *et al*. (1987). The microbial biomass N (MBN) was determined using Brookes *et al*. (1985), while ammonium and nitrate N were determined as highlighted by Anderson and Ingram (1993). All determinations were conducted in triplicate and expressed on dry weight basis.

**Statistical Analysis**

Analysis of variance was undertaken to determine the effect of fertilizer N and P on microbial biomass, soil pH and available P using Statistix 10 package (Statistix, 2003). Means were separated using the Tukey's HD test. Where ANOVA was significant ($P \leq 0.05$), Pearson correlation was performed to assess relationships between MBN, MBC, shoots and roots biomass.

**Results and Discussion**

The effect of Brachiaria grasses on MBC and MBN is shown in Table 2. No significant fertilizer NP differences ($P > 0.05$) were noted in either the MBC or MBN. The amount of MBC accumulated in this site was lower (79 - 128 mg N kg$^{-1}$ soil) than the ranges of 121 - 200 mg N kg$^{-1}$ soil reported elsewhere by Gichangi *et al.* (2016). Due to the low initial N and P in this site (Table 1), the nutrient rates applied were probably not sufficient to improve N availability. Organic C was also low and probably recalcitrant hence, with removal of shoots biomass, accumulation of C in the roots through roots exudates did not contribute to sufficient C for microbial activities. Soils without sufficient labile C may hinder microbial biomass as labile C provides a readily available energy source for microbial decomposition (Hoyle and Murphy, 2006). Nonetheless, Llanero, Mulato II, Marandu gave higher MBC and MBN while Basilisk and Rhodes grass had lower microbial biomass.

$NH_4$ -N and $NO_3$ -N was significantly ($P=0.034$) influenced by the grass cultivars. The pools of $NH_4^+$N and $NO_3^-$N were higher in bare plot and Napier grass which also accumulated lower MBC and MBN. The high accumulation of $NO_3^-$N in the bare plot is due to lack of plant uptake and increased nitrification. $NH_4^+$N was the most dominant form of inorganic N in Llanero, Marandu and Piata. These cultivars also had lower $NO_3^-$N, MBC and MBN an indication of either low nitrification rates or a high rate of $NO_3^-$N uptake by plants. Nitrification is a biological process that converts $NH_4$ -N to $NO_3$ -N creating acidification in the process as noted in the bare, Mulato II, Napier and Rhodes grass plots (Table 2).

The significant relationship between MBC and both $NH_4^+N$ and $NO_3^-N$ showed that $NH_4^+N$ and $NO_3^-N$ decreased with microbial biomass (Table 3). These results are contrary to findings reported by Burger *et al.* (2003) who observed positive relationship between MBC and $NH_4^+N$, and attributed it to faster decomposition and mineralization of N. In the present study, grasses with higher MBC accumulated lower $NH_4^+N$ and $NO_3^-N$ since most of the N may have been immobilized. Llanero, Marandu and Piata accumulated lower $NO_3$-N compared to $NH_4$-N with higher $NH_4^+N$: $NO_3^-N$ ratio and increased soil pH (6.05 – 6.12) an indication of lower nitrification. Similar results were reported by Fernandes *et al*. (2011) who showed increased $NO_3^-N$ and lower or similar levels of $NH_4^+N$ in *B. decumbens* and *B. ruziziensis*.

**Table 2** Influence of Brachiaria grasses on microbial biomass C and N and soil chemical properties in Kitale

| | mg kg$^{-1}$ soil | | | | $NH_4^+N:NO_3^-$ | | Available P |
|---|---|---|---|---|---|---|---|
| Treatments | MBC | MBN | $NH_4^+N$ | $NO_3^-N$ | N Ratio | Soil pH | Mg kg$^{-1}$ soil |
| Bare plot | 79 | 4.9 | 13.4 | 13.9 | 1:1 | 5.20 | 15.0 |
| Basilisk | 108 | 8.6 | 8.1 | 6.6 | 1:1 | 5.85 | 16.7 |
| Llanero | 107 | 6.0 | 12.1 | 4.7 | 3:1 | 6.01 | 16.7 |
| Marandu | 113 | 7.9 | 8.9 | 4.2 | 2:1 | 6.00 | 17.5 |
| MG4 | 128 | 9.2 | 9.6 | 7.6 | 1:1 | 5.91 | 15.8 |
| Mulato II | 112 | 7.7 | 6.9 | 5.4 | 1:1 | 5.73 | 16.7 |
| Piata | 104 | 7.9 | 9.6 | 4.9 | 2:1 | 6.07 | 15.0 |
| Xaraes | 124 | 7.5 | 7.3 | 4.9 | 1:1 | 5.92 | 13.8 |
| Rhodes grass | 99 | 7.1 | 9.5 | 7.5 | 1:1 | 5.78 | 14.2 |
| Napier grass | 99 | 7.1 | 11.2 | 8.4 | 1:1 | 5.74 | 18.3 |
| Mean | 111 | 7.5 | 9.70 | 6.98 | | 5.89 | 16.25 |
| $SED_{cv}$ (P=0.05) | ns | ns | 1.85 | 1.85 | | 0.08 | ns |

Where cv- cultivar; ns- not significant at P≤ 0.05; SED- standard error of deviation; MBC- Microbial biomass C; MBN- microbial biomass N;

The current study shows possibility that the grasses with lower $NH_4$-N: $NO_3$-N do not inhibit the nitrification in the rhizosphere which may contribute to higher N losses through leaching and denitrification. In this study, Llanero was the most effective in improving soil pH probably from suppression of nitrification, confirming the results by Ishikawa *et al*. (2003). To minimize nitrification and reduce N loss in agricultural or pasture areas, it is necessary to maintain soil N in the form of $NH_4^+N$ for as long as possible to synchronize N fertilizer supply and the plant demand (Ishikawa *et al.*, 2003).

Available P was not significantly (P > 0.05) correlated with any microbial biomass indicators or soil properties. Application of N and P fertilizers increased the levels of available P (18.94 mg P kg$^{-1}$ soil) above the non-fertilized treatments (13.56 mg P kg$^{-1}$ soil). Phosphate fertilizers were only added at planting, but seasonal application of ammoniacal-N fertilizers such as CAN improved P availability to plants and thereby increasing crop growth.

**Table 3** Pearson correlation between microbial biomass and roots biomass in Kitale

| | amm | nit | pH | P | MBC | MBN | RB22 | RB46 | RB80 | SDM |
|---|---|---|---|---|---|---|---|---|---|---|
| nit | 0.65* | 1 | | | | | | | | |
| pH | -0.44 | -0.84*** | 1 | | | | | | | |
| P | 0.135 | 0.02 | 0.16 | 1 | | | | | | |
| MBC | -0.68* | -0.69* | 0.66* | -0.07 | 1 | | | | | |
| MBN | -0.79** | -0.52 | 0.60* | -0.03 | 0.83** | 1 | | | | |
| RB22 | -0.44 | -0.27 | 0.06 | -0.51 | 0.15 | 0.40 | 1 | | | |
| RB46 | -0.20 | -0.37 | 0.14 | -0.36 | 0.30 | 0.12 | 0.53 | 1 | | |
| RB80 | -0.11 | -0.45 | 0.02 | -0.31 | 0.17 | -0.09 | 0.51 | 0.75* | 1 | |
| SDM | -0.25 | -0.19 | 0.01 | -0.15 | 0.42 | 0.33 | 0.47 | 0.79** | 0.54 | 1 |

Where * - P≤0.05; amm- Ammonium N; pH- Soil pH; nit – Nitrate N; P – Available P; MBC- Microbial biomass C; MBN- Microbial biomass N; RB22,46,80- Roots biomass at 22, 46 and 80 WAE; SDM- Shoots dry matter

## Conclusions

There were no varietal differences in MBC, MBN since the nutrients applied were not sufficient to improve N availability in these low fertility soils. Piata and Llanero had high NH4-N:NO3-N ratio and may have lowered the nitrification processes. However, further studies are required to ascertain the role of the two grasses as potential biological nitrogen inhibitors.

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# Effects of Brachiaria grass cultivars on soil aggregation and stability in the semi-arid eastern Kenya

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## Abstract

Soil aggregation is among the key short term indicators of soil quality attributed to changes in land management. A study was conducted to investigate the changes in the size distribution and stability of soil aggregates in a structurally unstable sandy loam soil following cultivation of Brachiaria grass in semi-arid region of Kenya. The Brachiaria grass cultivars included Brachiaria decumbens cv. Basilisk, B. brizantha cvs. Marandu, MG4, Piatã and Xaraes, B. humidicola cv. Llanero and B. hybrid cv. Mulato II which were compared with two locally cultivated forage grasses (Chloris gayana cv. KAT R3 and *Pennisetum purpureum* cv. Kakamega 1) and a bare plot (negative check). The grass treatments were evaluated with fertilizers application (40 kg P applied at sowing and 50 kg N $ha^{-1}$ in each wet season) and with no fertilizer applications. Aggregate size fractions (>2000, 250–2000, 53–250 and 53 μm) were isolated using the wet sieving method. Aggregation based on the proportion of small macro-aggregates (250–2000 μm) increased in soils cultivated with all grass types compared to the bare plots control and was greatest in soils under B. hybrid cv. Mulato II. Aggregate stability in terms of mean weight diameter (MWD) differed among the grasses and was highest in soils under cv. Mulato II and cv. Marandu with mean weight diameters of 4.49 and 4.31 mm, respectively. Changes in small macro-aggregates fraction was positively correlated with particulate organic matter (POM) ($r=0.9104$, $p = 0.001$), microbial biomass carbon (MBC) ($r=0.5474$, $p = 0.01$), soil organic carbon (SOC) ($r=0.3654$, $p = 0.05$) and roots biomass ($r=0.4977$, $p = 0.01$). This indicated that the binding agents were important in the aggregation of soils cultivated with Brachiaria grasses.

**Keywords:** Macro-aggregates; micro-aggregates; microbial biomass; particulate organic matter; soil organic carbon

### Introduction

Soil aggregation is among the key short term indicators of soil quality associated with changes in land management. The aggregate stability of soils improve under certain crops, notably grasses and these improvements are frequently associated with increases in soil organic carbon (SOC) levels caused by plant residues (Lal *et al*., 2003; Marquez *et al* 2004; Denef *et al*., 2007). Low SOC, weak soil structural stability and degradation are common attributes of most semi-arid soils of eastern Kenya (Gicheru *et al*., 2004). Agricultural practices that could improve SOC, coupled with increase in soil surface cover, would significantly increase soil aggregate stability and reduce the soil degradation. Soil aggregate stability, defined as the ability of the aggregates to remain intact when subject to a given stress, is an important soil property that affects the movement and storage of water, aeration, erosion, biological activity and plant growth (Spohn and Giani, 2011; Pohl *et al*., 2012). There exist complex interactions between SOC storage and aggregate stability. Soil organic carbon, can encapsulate within stable aggregates thereby offering protection against microbial processes and enzymatic reaction (Lal *et al*., 2003; Holeplass *et al*., 2004).

The size of aggregates and aggregation state can be influenced by different agricultural activities that alter the content of SOC and the biological activity of the soil (Mills and Fey, 2003; Wick *et al*., 2009; Fonte *et al*., 2014). The article by Bronick and Lal (2005) provides overview of chemical compounds that are thought to be involved in the formation of soil aggregates, a list of factors that determine extent of this aggregation, and a description of the influence of soil structure on a wide range of soil processes. Over short periods, the stability of soil aggregates is modified under the influence of different cropping practices, probably being more related to changes in the organic constituents than to the actual total organic matter content (Reid and Goss, 1980; Milne and Haynes, 2004). Reid and Goss (1980) for example, demonstrated that after only 4 weeks growth the living roots of perennial rye grass (*Lolium perenne*) increased the aggregate stability of a sandy loam as measured by turbidimetric and wet sieving analyses which was most strongly associated with the larger aggregates. This effect was probably caused by organic substances released from the roots which either stabilized the aggregates directly or indirectly after microbial colonization (Leifeld *et al*., 2005; Franchini *et al*., 2007). However, over long periods of time, the stability of soil aggregates diminishes as the SOC content declines as a result of it being used as an energy source by the microorganisms in the soil (Mills and Fey, 2003). Loss of SOC will therefore reduce soil fertility, degrade soil structure and water holding capacity and ultimately, leads to land degradation. Grasses, present the greatest effect on the aggregation and aggregate stability due to their extensive roots system (Harris *et al*., 1996). Brachiaria grasses are endaphytic and have a greater ability sequester and accumulate large amounts of SOC through their large and extensive roots biomass and survive in dry areas of low soil fertility (Fisher *et al*., 2007; Peters *et al.,* 2012). This makes Brachiaria grasses a better option for livestock feed production and soil improvement.

The resistance of soil aggregates to breakdown from physical forces is a measure of coherence or strength of cementation between or within soil aggregates. Aggregate size is important in determining the dimensions of pore space in cultivated soils. The size of the pores in turn affects the movement and distribution of water and aeration that are major factors affecting plant growth. Soil organic carbon increases aggregate water repellence therefore minimising their disruption and breakdown when wetted through mechanical manipulation such as tillage (Chenu *et al*., 2000). Soil aggregation can be determined by mean weight diameter (MWD), geometric mean weight diameter (GMD) and aggregate stability (AS, %) index, which are obtained by fractioning the soil material into aggregate classes by wet sieving (Kemper and Rosenau, 1986).

Disruption of soil structure is common in semi-arid zones of eastern Kenya, due to the inherent soil type that has weak structure, overgrazing, compaction, and poor land management, which have negative consequences on SOC storage and degradation of the soil structure. There is therefore a need to examine the potential effects of introduced Brachiaria grasses on aggregation and stability of aggregates in these fragile soils. The objective of this study was therefore, to investigate the short-term (2-years) changes in aggregate size distribution and the stability of soil aggregates following cultivation of Brachiaria grasses. We examined linkages between SOC, particulate organic matter (POM), microbial biomass carbon (MBC), and roots biomass with aggregation by comparing Brachiaria cultivated soils verses commonly grown Napier and Rhodes fodders and not cultivated weed free soils. We tested the hypothesis that, cultivation of Brachiaria grasses improves soil aggregation through increased SOC and aggregate associated C resulting from large roots biomass.

## Materials and methods

*Description of the study site*

The experiment was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO), Katumani farm between November 2013 and November 2015. The site is located (37°28′0′′E, 1°58′0′′S) 75 km south-east of Nairobi at an elevation of 1580 m above sea level. It receives mean annual rainfall of 717 mm in bimodal pattern with the long rains (LR) occurring from March to May and the short rains (SR) from October to December with peaks in April and November, respectively. The mean temperature is 19.6˚C. The dominant soils are chromic luvisols, which are low in organic C, highly deficient in N and P and to some extent Zinc (NAAIAP, 2014) and generally have poor structure.

*Site characterization*

Composite soil samples from 12 sampling positions within a plot were collected in November 2013 before establishing the experiment at depths of 0–15 cm, 15–30 cm, 30–60 cm, and 60-100 cm using a bucket auger for initial characterization of the soils. Plant litter on the soil surface was removed before collecting the soil samples. Samples were air-dried, visible roots removed, and the samples gently crushed to pass through a 2-mm sieve. The sample was used for subsequent chemical and physical analyses. Total soil N, available P (Mehlick III), exchangeable K, Ca, and Mg were estimated following standard methods as described by Okalebo *et al.* (2002)**.** Cations $Ca^{2+}$, $Mg^{2+}$, and $K^+$ were determined by atomic absorption spectrometry and soil P was measured as described by Murphy and Riley (1962).

Soil texture was determined by the hydrometer method. Soil pH was measured in water (soil: water ratio of 1: 2.5 w/w) using a pH meter and reference calomel electrode (Model pH 330 SET-1, 82362) after the suspensions were shaken for 30 minutes and allowed to stand for 1 hour. Organic carbon was determined by the modified Walkley and Black procedure (Nelson and Sommers 1982). Cation exchange capacity (CEC) was based on the sum of exchangeable $Ca^{2+}$, $Mg^{2+}$, $K^+$, $H^+$ and $Al^{3+}$ after extraction with ammonium acetate. Soil bulk density was determined according to Blake and Hartge (1986). Soils were vertically sampled using stainless steel rings with 10 cm diameter at depths of 0–15 cm, 15–30 cm, 30–60 cm, and 60-100 cm, resulting in undisturbed soil samples for bulk density determination. Soil samples were dried at 65º C to a constant weight. All determinations were made in triplicate and expressed on a dry weight basis.

Soil characteristics of the experimental site are shown in Tables 1 and 2. Soil pH was moderately acidic in all the depths (Table 1) and soil organic C content was low and decreased with increasing depth. Similarly N, P and Zn were low. Physical analysis of soil samples from the test site indicated that the soils were sandy clay loam in the 0–30 cm depth and clay in the lower depths (Table 2). Cation exchange capacity ranged from 20.2 to 27.8 me%, and increased with depth. This is expected as the clay content also increased with depth resulting to increased number of exchange sites (Table 2). Bulk density ranged from 1.32 to 1.45 g $cm^{-3}$ and was greater than the ideal range of 1.1-1.3 g $cm^{-3}$ for non-restricted roots growth. Soil bulk density exceeding 1.46 g $cm^{-3}$ for such soils would restrict root growth and negatively interfere with soil aeration through reduced air-filled pore space (Landon, 1991).

**Table 1** Initial soil chemical characteristics

| Properties | Sampling depth (cm) | | | |
|---|---|---|---|---|
| | 0-15 | 15-30 | 30-60 | 60-100 |
| Soil pH (in water) | 5.88 | 5.76 | 5.81 | 6.10 |
| Total Nitrogen (%) | 0.12 | 0.12 | 0.07 | 0.05 |
| Organic Carbon (%) | 1.16 | 1.15 | 0.65 | 0.49 |
| Phosphorus (ppm) | 10 | 12 | 10 | 15 |
| Potassium (me %) | 0.29 | 1.01 | 0.52 | 0.32 |
| Calcium (me %) | 3.1 | 3.4 | 2.2 | 2.4 |
| Zinc (ppm) | 1.78 | 1.44 | 0.97 | 0.64 |

**Table 2** Initial soil physical characteristics

| Properties | Sampling depth (cm) | | | |
|---|---|---|---|---|
| | 0-15 | 15-30 | 30-60 | 60-100 |
| Bulk density ($g/cm^3$) | 1.32 | 1.35 | 1.41 | 1.45 |
| Sand (%) | 50.7 | 48.7 | 44.0 | 40.0 |
| Silt (%) | 6.0 | 8.0 | 5.3 | 7.3 |
| Clay (%) | 43.3 | 43.3 | 50.7 | 52.7 |
| Cation exchange capacity (me %) | 20.2 | 21.3 | 26.9 | 27.8 |
| Base saturation (%) | 92.4 | 85.7 | 78.9 | 64.2 |
| Texture Class | Sandy clay | Sandy clay | Clay | Clay |

*Treatments and experimental design*

The treatments consisted of seven Brachiaria grass cultivars *Brachiaria decumbens* cv. Basilisk, *B. humidicola* cv. Llanero, *B. brizantha* cvs. Marandu, MG4, Piatã, Xaraes and *B. hybrid* cv. Mulato II), two commonly cultivated local grasses [(*Chloris gayana* cv. KAT R3 and *Pennisetum purpureum cv*. Kakamega 1 (KK1) as local checks)] and a bare plot (as negative control). These treatments were evaluated in the plots with fertilizer (40 kg P $ha^{-1}$ applied at sowing and 50 kg N top-dressed in each wet season) and without fertilizer application. The treatments were laid out in a randomized complete block design in a split plot arrangement (fertilizer treatments as main plots and the grass treatments as sub plots) in three replications. The grasses were sown in November 2013 during the short rains. All the plots including the bare plots were kept weed free throughout the experimental period by hand weeding. The grasses were first harvested 16 weeks after establishment and later, harvestings were done eight times on an 8 weeks interval during the 5 wet seasons.

*Roots biomass determination*

Roots were sampled using the soil-core method (Bolinder, *et al*., 2002). In each plot, four soil cores were randomly taken to a depth of 0–15 and 15–30 cm, two each from the inter-row and intra-row spacing and composited into one sample per plot for each depth. The sampling was carried out using a 5 cm diameter stainless steel auger at least 1m apart from the edge of the plot to avoid edge effects. Sampling was conducted at 24 and 48 weeks after establishment, high roots accumulation was expected at these sampling periods. The roots from each soil layer were washed separately by hand with a 2.8 mm and a 2 mm soil sieve under running tap water. Root samples integrating both living and dead roots were then dried at 65°C to constant weight and roots dry weights were recorded.

*Soil sampling and determination water-stable aggregate distribution and microbial biomass carbon*

Soil samples for aggregate, POM and microbial biomass carbon analysis were collected in November 2015 twenty four months after the grasses had established. Four soil samples were carefully collected from a depth of 0-10 cm using a spade, so as to minimize aggregates disruption in each pasture plot and from the bare plot controls. In this study, only the top 10 cm soil was sampled which was assumed to contain the highest biological activity and most likely exhibit short-term changes in response to Brachiaria grass cultivation. Soils from the four sampling positions of a plot were pooled to one sample. The soils were then dried at room temperature ($21^0$ C) and sieved by gently breaking soil clods along natural planes of weakness, so that they passed through an 8 mm sieve. Soil sub-samples of approximately 400g were taken using the quartering method for further processing and analysis at International Center for Tropical Agriculture (CIAT, Nairobi) as described below.

*Water-stable aggregate distribution and particulate organic matter*

Four aggregates-size fractions were isolated using triplicate 80 g of air-dry 8 mm sieved soil by the wet sieving method as described by Six *et al*. (1998), and each fraction was named as large macro-aggregates (> 2000 μm), small macro-aggregates (250–2000 μm), micro-aggregates (53–250 μm), and silt + clay fraction (<53 μm). The soil subsamples were spread evenly onto a 2000-μm sieve and slaked for 5 min in distilled water. The soil was then sieved for 2 min by oscillating the sieve 50 times up and down (approximately 3-cm amplitude). Large macro-aggregates retained on the 2000 μm sieve mesh were backwashed into pre-weighed pans for drying. Large (>2000 μm) floating litter was removed, while soil passing through the 2000 μm sieve was transferred to a 250 μm sieve and the process was repeated to obtain the small macro-aggregates fraction (250–2000 μm). The sieving process was repeated once more using a 53 μm sieve to separate micro-aggregates (53–250 μm) from the silt and clay fraction (<53 μm). All pans and soil solutions were placed in an oven at 65°C until dry and weighed in order to determine the mass of each aggregate size class. Aggregate fractions > 53 μm were corrected for sand prior to calculation of the proportional weight of aggregates and mean weight diameter (MWD) was determined. However, large macro aggregate > 2000 μm were not used in computing or calculating MWD, because the proportion of aggregates > 2000 μm recovered after the wet sieving were too small in weight to be corrected for sand (sand free fraction). The proportional weight of sand free aggregates (aggregate size distribution) was calculated as follows:

$$\frac{\text{Weight of fractioned aggregate - \% sand content}}{\text{Weight of bulk soil - \% sand content}} \quad \text{.................................. Equation1}$$

The MWD was then calculated as an index of aggregate stability using the formula of Kemper and Rosenau (1986) as follows:

$$MWD = \frac{\sum w_i x_i}{100} \quad \text{.......................................................................................... Equation 2}$$

Where $x$ (μm) is the average diameter of the openings of two consecutive sieves, and $w$ the weight ratio of aggregates remained on the $i^{th}$ sieve.

Particulate organic matter was separated from water-stable aggregate fractions by floatation and decanting after mechanical dispersion of the soil by agitation in water with glass beads. The collected organic size fraction was oven dried at 65 °C for 24 h and their weight determined. The soil POM was expressed in g $kg^{-1}$ after adjusting for soil moisture using the weight loss of sub-samples oven dried at 105 °C to a constant weight.

*Soil microbial biomass C*

Microbial biomass C was determined on field moist soil (18-23% by weight) by the chloroform fumigation-extraction technique as described in Vance *et al*. (1987) on soils sampled in November 2015 as described above. Briefly, 10 g dry weight equivalent of soil was fumigated with ethanol-free chloroform in a glass desiccator; and another 10 g was incubated without fumigation at the same moisture content, time period and temperature for 24 h at 25°C. Both sets were extracted with 0.5 M $K_2SO_4$ for microbial biomass C determination. Soil microbial biomass element content was calculated as the difference between the fumigated and un-fumigated samples and corrected for incomplete recovery using conversion factors of 0.45 for C (Vance *et al*., 1987). All determinations were made in triplicate and expressed on a dry weight basis.

**Statistical analysis**

Treatment effects on soil aggregate stability and POM were tested using an analysis of variance (ANOVA) as a split-plot with fertilizer NP as the main factor and grass type as the sub-plot factor using GENSTAT statistical software (GENSTAT Release 4.24DE, 2005). This was evaluated by running a full model (20 treatments, 19 df) which was further split into a fertilizer effect (1 df), cultivar effect (9 df), fertilizer*cultivar effect (9 df). Differences at $p \leq 0.05$ were considered significant and means separation was done using Fischer's protected Least Significant Difference test (LSD). Regression analyses and Pearson correlation coefficient (r) were used to find models best describing the relationships between soil aggregate stability and other soil and plant properties.

**Results and Discussion**

*Soil aggregate size fractions and stability*

The growth of perennial grasses enhance aggregate formation due to the production of large quantities of polysaccharide and phenolic binding agents by the large microbial biomass in the pasture rhizosphere (Milne and Haynes, 2004). Additionally, the fine grass roots and associated fungal hyphae physically enmesh fine soil particles into aggregates (Milne and Haynes, 2004). The results of aggregate size distribution and stability determinations are presented in Table 3. The effects of grass types on the proportion of aggregate size fractions 250-2000, 53-250 and <53µm were significantly ($p<0.01$) different. The small macro-aggregates (250-2000 µm) comprised the largest proportion, which accounted for 34.1 – 64.2% of the total soil dry weight, and the fraction of micro-aggregates (53-250 µm) was the second largest, being 28.5–48.2% of whole soil dry weight. The large macro-aggregates (>2000 µm) and silt + clay (<53µm) fractions were the least components. The silt + clay fractions accounted for only 8.9-17.6% of whole soil dry weight (Table 3). In contrast, there were no significant differences ($p \geq 0.05$) in the distributions of water stable aggregates with aggregates sizes >2000 µm between the grass types and accounted for less than 1% of the bulk soil. Fertilizer NP addition did not influence the aggregate size distribution. More than 99.6% of whole soil dry weight was recovered after wet-sieving, indicating that losses during the fractionation process were negligible.

The effects of grass types on water stable aggregates (Table 3) revealed that soils under *B. hybrid* cv. Mulato II and *B. brizantha* cv. Marandu, significantly increased small macro aggregates (250-2000 μm) fraction. However, *B. brizantha* cv. MG4 and the control bare plots, *Chloris gayana* cv. KAT R3 had the lowest levels of small macro-aggregates (250-2000 μm). The least proportion of silt and clay aggregates (<0.053mm) was recorded in soil planted to *B. brizantha* cv. Xaraes and *B. hybrid* cv. Mulato II. This could have resulted from the effect of soil cementing agents binding primary particles to micro-aggregates and macro-aggregates. Macro-aggregates (diameter >250 mm) are considered as a secondary soil structure associated with pores, microbial habitat, and physical protection of organic matter (Christensen, 2001). In addition significant differences were observed in aggregate stability expressed by MWD among the grass species and were much higher in *B. hybrid* cv. Mulato II, *B. brizantha* cv. Marandu and *B. decumbens* cv. Basilisk (Table 3). Similarly as in the proportion of aggregate fractions, the effects of fertilizer application on MWD were not significantly different.

High macro-aggregates proportion favoured soil aggregate stability as indicated by high MWD values which might have resulted from increased soil cementing by organic compounds (Bronick and Lal, 2005). The soil cementing agents bind micro-aggregates and primary particles to macro-aggregates, and minimize microbial decomposition by promoting physical protection through sorption to clay minerals and encapsulation within soil aggregates (Mikha and Rice, 2004). The increase in macro-aggregates and the decrease in fine size aggregates in the 0–10 cm layer as a result of grass treatments accelerated the integration of fine particles into the coarse elements. The results generally agree with the finding of Sommerfeldt and Chang (1985), that macro-aggregates were increased while micro-aggregates were decreased due to increased organic matter. Macro-aggregates are good predictors of potential C responses to pasture establishment because of their importance in protecting recently deposited, labile, organic matter (Dungait *et al*., 2012). Tisdall and Oades (1982), reported that the water stable micro-aggregates (<250μm) are insensitive to cropping and management, whereas, macro-aggregates are found to be dependent on soil management. According to the conceptual model of Six *et al*. (2002), recent inputs of organic matter induces macro-aggregates formation, while the decomposition of SOC within these macro-aggregates leads to the formation of stable micro-aggregates and organo-mineral complexes. Consequently, macro-aggregates formation leads to longer mean residence time of SOC in soil over time through the formation of smaller, more stable soil fractions with increasingly intimate associations between organic matter and mineral surfaces (Gale *et al*., 2000). In this study, small macro-aggregates were the most prominent aggregates fraction in the 0-10 cm soil layer in the grass treatments whereas 53-250 μm aggregates fraction was most prominent the bare plot soils. This indicates increased aggregation in the grass treatments implying that these poorly structured soils should not be left bare due to risks of erosion and surface sealing/crusting due to their high silt content.

*Particulate organic matter and microbial biomass carbon*

Both POM and MBC are labile non-humic fraction of organic matter and constitute important pools of nutrients in the soil. Particulate organic matter defined as organic matter that is intermediate in the decay continuum between fresh litter and humified organic matter has high sensitivity to management than total soil organic carbon (Grandy and Robertson, 2006; Todd *et al*., 2015). The POM fraction hosts a large concentration of microorganisms because it provides a substrate for their activities (Zhang *et al*., 2014). The POM and MBC are therefore important in maintaining soil structure in that the microorganisms associated with them in the decomposition process exude mucilaginous carbohydrate material which acts as a glue and helps cement soil aggregates together. For example, MBC has been shown to be positively correlated with aggregate stability, indicating the important role of MBC in aggregation (Milne and Haynes, 2004). The analysis of light organic fractions separated from wet sieved aggregates showed that POM differed between the grass types (Table 3). The POM concentration in 0-10 cm depth ranged from the minimum of 0.16 g $kg^{-1}$ in the bare soil plots to the maximum of 0.93 g $kg^{-1}$ in soil under *B. hybrid* cv. Mulato II (Table 3). The gains in POM within macro-aggregates shown here concur with the results of others that suggest macro-aggregates may be good predictors of potential C responses to changes in agro-ecosystems management (Grandy and Robertson, 2006; Todd *et al*., 2015). This also supports the findings of Six *et al.* (2002) who reported that soil aggregation was enhanced as soil organic matter increased, due to increased production of organic matter derived binding agents resulting from the activity of microbes on deposited residues in soils. While we found that total SOC did not vary among Brachiaria grasses over the short duration of the study (Table 3), changes in below ground C cycling were apparent through aggregates formation. Aggregation increased over the two years of this study in all grass types compared to the bare control treatment, with *B. hybrid* cv. Mulato II and *B. brizantha* cv. Marandu showing the largest proportion of small macro-aggregates and mean weight diameter (Table 3).

*Roots biomass*

The effect of grass types on roots biomass was significantly different with higher roots biomass recorded in treatments with cvs. Xaraes, Marandu, Piatã and cv. Basilisk (Figure 1). The amounts of roots recorded in the fertilized plots in this study were also significantly higher than where fertilizers N and P were not added for cv. Llanero, cvs. Piatã, Xaraes and *B. hybrid* cv. Mulato II) and *Pennisetum purpureum cv*. Kakamega 1 (Figure 1). Generally, the composition of POM consists mainly of roots fragments (Cambardella and Elliot, 1992) and therefore this affirms that significant differences in the levels of POM observed in this study were due to differences in the amounts of roots biomass among grass types. Reid and Goss (1980) for example demonstrated that after only 4 weeks growth the living roots of perennial rye grass (*Lolium perenne*) increased the aggregate stability of a sandy loam soil. Plant roots can increase aggregation by enmeshing small particles into stable macro-aggregates; by supplying organic substrates such as sloughed cells and mucilage and by influencing soil moisture content (Grandy and Robertson, 2006). According to Broersma *et al*. (1997), crops affect soil structure differently because of diverse rooting habits, type of organic matter and the rhizosphere processes. Increasing roots biomass influences soil organic matter: i) directly by increasing organic inputs to soil and ii) indirectly by influencing the production of root exudates that may stimulate mineralization (Jones *et al*., 2009). Roots exudates and other by-products are also more readily absorbed and protected by soil aggregates and where concentrated are more likely to persist in the POM and humus fractions than shoots-derived SOC (Clapperton *et al*., 2003; Walker *et al*., 2003; Hurisso, *et al*., 2013; Zhang *et al*., 2014). Depending on the roots turnover rates the amount of C stored in the soil can be determined from the roots biomass, plant residue and SOC. Commonly, roots mass and plant residue in the soil form between 3,400 (annual crop) and 17,000 (perennial grasses) kg $ha^{-1}$ $year^{-1}$ of the soil biomass (Harwood *et al.*, 1998).

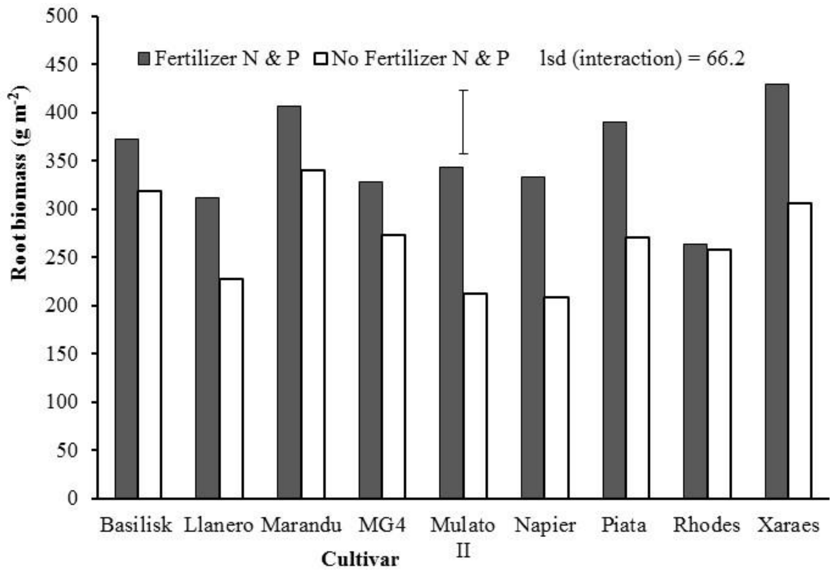


**Figure 1** Effects of cultivar and fertilizer N and P on roots biomass (0-15 cm depth) 1 year after the grasses were established

**Table 3** Effects of grass types on soil aggregation, MWD, POM, SOC and MBC

| Grass type | Proportion of aggregate size fraction | | | | MWD (mm) | POM ($gkg^{-1}$) | SOC ($gkg^{-1}$) | MBC ($\mu gg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | >2000 μm | 250-2000 μm | 53-250 μm | <53μm | | | | |
| Basilisk | 0.002 | 0.569 | 0.310 | 0.103 | 4.08 | 0.65 | 13.97 | 145.3 |
| Llanero | 0.002 | 0.488 | 0.416 | 0.101 | 3.73 | 0.46 | 13.82 | 122.3 |
| Marandu | 0.003 | 0.604 | 0.319 | 0.099 | 4.31 | 0.77 | 14.13 | 147.8 |
| MG4 | 0.001 | 0.432 | 0.441 | 0.120 | 3.43 | 0.37 | 13.87 | 86.5 |
| Xaraes | 0.002 | 0.516 | 0.354 | 0.089 | 3.81 | 0.66 | 14.24 | 134.3 |
| Piatã | 0.001 | 0.461 | 0.419 | 0.126 | 3.58 | 0.38 | 13.86 | 105.8 |
| Mulato II | 0.001 | 0.642 | 0.285 | 0.090 | 4.49 | 0.93 | 13.99 | 154.2 |
| Napier grass | 0.002 | 0.519 | 0.378 | 0.104 | 3.87 | 0.51 | 13.97 | 127.4 |
| Rhodes grass | 0.002 | 0.422 | 0.440 | 0.140 | 3.38 | 0.35 | 14.07 | 69.3 |
| Bare plot | 0.002 | 0.341 | 0.482 | 0.176 | 2.95 | 0.16 | 12.09 | 18.3 |
| Lsd ($p \leq 0.05$) | NS | 0.046 | 0.038 | 0.014 | 0.18 | 0.08 | 0.96 | 24.0 |
| CV (%) | - | 4.9 | 6.2 | 10.4 | 4.1 | 12.3 | 6.0 | 19.6 |

MWD= Mean weight diameter; POM= Particulate organic matter, SOC = Soil organic carbon; MBC= Microbial biomass carbon, Lsd= Fischer's protected least significant difference; NS= Not significant, Cv = coefficient of variation

*Relationships between soil aggregation and stability with binding agents*

Particulate organic matter and MBC all act as important binding agents for aggregation (Six *et al.*, 2004; Bronick and Lal, 2005). Previous studies have reported that soil aggregate stability was strongly correlated with POM and MBC in different soils (Six *et al.*, 2002). Regression of the proportional weights of the 250-2000 μm aggregates fraction and MWD with POM showed that POM explained 79.4% and 81.7% of the variations of the aggregates fraction and MWD, respectively (Figure 2). Similar results were also reported by Ashagrie *et al.* (2007) and Spohn and Giani (2011), who suggested that POM contribute to soil aggregation as it acts as nucleation sites for the formation of macro-aggregates .

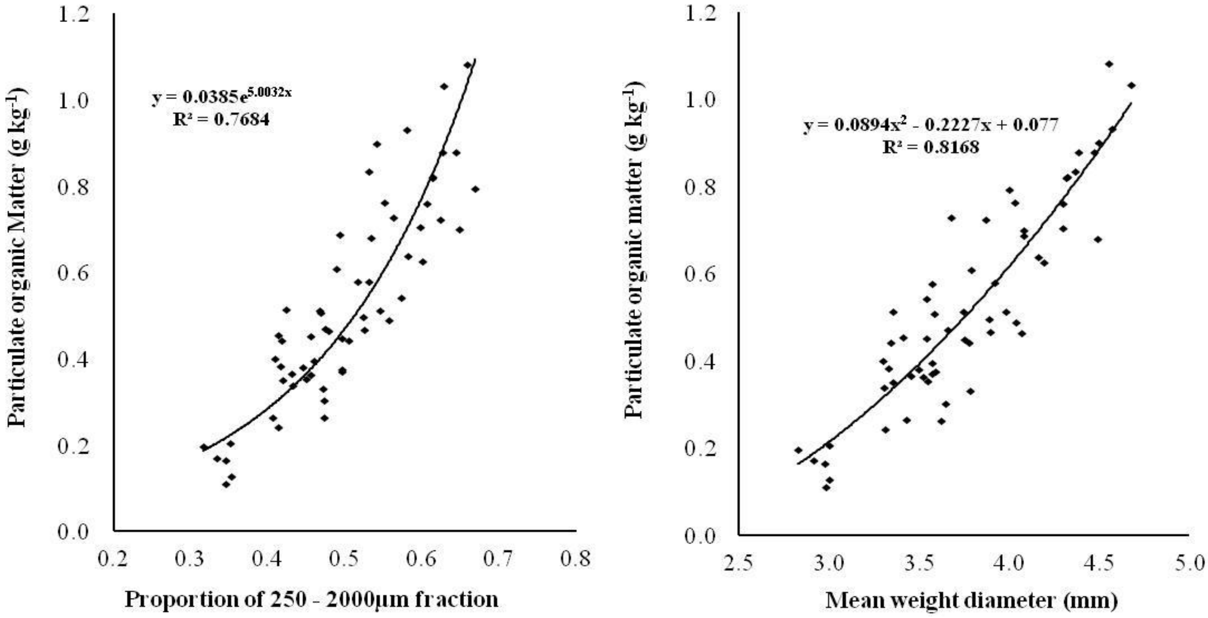


**Figure 2** Relationships between particulate organic matter (POM) with a) proportional weights of 250-2000 μm aggregates fraction and b) mean weight diameter (MWD)

The proportional weights of the 250-2000 μm aggregates fraction and MWD in this study was found to be positively but weakly correlated with MBC (Figure 3) which however indicated that soil aggregation and stability increased with increasing levels of MBC in the bulk soil. Overall, POM made the greatest direct contributions to aggregate stability (Figure 2), suggesting that greater POM in Brachiaria cultivated soils enhanced aggregate stability and by extension improved soil structure was comparable to soils under *Pennisetum purpureum cv.* KK1 a commonly cultivated fodder in the region. Gartzia-Bengoetxea *et al*. (2009) also found a strong relationship between MWD and POM. Other previous studies using other sources of SOC have reported higher aggregate MWD with increased organic C in soils (Gulde *et al*., 2008; Min *et al*., 2003; Whalen *et al*., 2003). For example, Wortmann and Shapiro (2008) observed higher macro-aggregates formation with composted manure application than unamended control. Similarly, Min *et al*. (2003) observed that livestock manure added at 32.7 Mg C $ha^{-1}$ resulted in 30% higher aggregate stability than an unamended control.

Small macro-aggregates fraction were found to be positively correlated with POM (r=0.9104, p=0.001), MBC (r=0.5474, p=0.01), and SOC (r=0.3654, p=0.05) and roots biomass (r=0.4977, p=0.01) but other fractions (>2000, 53-250 and <53 μm) were negatively correlated with the binding agents (Table 4). This agrees with other studies that have reported strong correlation of soil aggregate stability with POM in different soils (Six *et al*., 2002; Franchini *et al*., 2007) and due to the sensitivity of this parameter, POM has been used in previous studies as an indicator of changes caused by soil and crop management (Leifeld, *et al*., 2005; Franchini, *et al*., 2007). Recent work elsewhere has also shown strong positive links between roots biomass and aggregation suggesting that changes in roots biomass alters the structure of soil food webs, changing below ground C cycling and the mean residence time of different SOC pools (Reid *et al*., 2012). It is generally understood that formation of larger aggregates is enhanced by fine roots and fungal hyphae, while micro-aggregates are stabilized by long-chained organic compounds (e.g., polysaccharides) and fungal hyphae. In our study, correlations of roots biomass with MWD and the 250-2000 µm size fraction were significant and positive (Table 4) which supports the hypothesis that roots act as temporary binding agents which aid in stabilizing larger aggregates (Tisdall and Oades 1982).

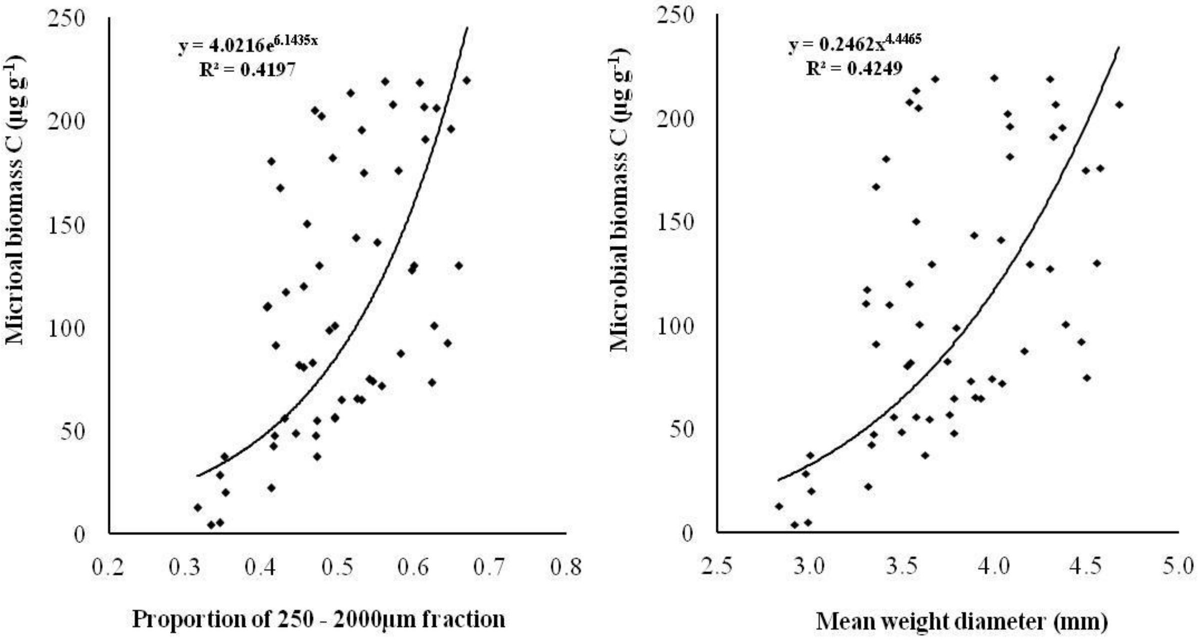


**Figure 3** Relationships between microbial biomass carbon (MBC) with a) proportional weights of 250-2000 µm aggregates fraction and b) mean weight diameter (MWD)

Larger size fractions (>2000 µm), micro-aggregates (53-250 µm) and the silt+ clay (<53 µm) fractions were negatively correlated with roots biomass. According to hierarchical theory of soil aggregation, binding of micro-aggregates into macro-aggregates occurs through the entanglement by roots and fungal hyphae, particularly vascular arbuscular mycorrhiza (VAM) hyphae (Tisdall and Oades 1982; Bearden, 2001). The production of mucigel, rhizo-deposition, increases of poly-cations in the rhizosphere, and soil water extraction by plant roots have been implicated in the formation of soil aggregates (Perfect *et al*., 1990). Roots and hyphal growth stimulate microbial activity and simultaneously promote the formation of macro-aggregates (Denef, *et al*., 2007). Aggregates up to <1000 µm are predominantly assembled by fungal hyphae, mechanically through entanglement of soil particles and chemically with glue-like metabolites (Bearden 2001). Pohl *et al.* (2009) found a positive and significant correlation between roots length density and soil aggregate stability. Similarly, Reid *et al.* (2012) have reported strong positive links between roots biomass and the abundance of nematodes and several taxa of mesofauna, suggesting that changes in roots biomass alters the structure of soil food webs, changing belowground C cycling and the mean residence time of different SOC pools.

The direct influence of roots as the primary C source to soil and particularly to POM is reflected in the significance influence (50.3% relative importance) of roots biomass to changes in POM (Table 4). Likewise, roots biomass was positively correlated to changes in both MBC (68.3% relative importance) and small micro-aggregates (49.8% relative importance). The greater below ground roots biomass of the Brachiaria grasses likely increased microbial activity, stabilizing aggregates through increases in microbially-derived soil binding agents leading to increases in physically protected POM (O'Brien and Jastrow, 2013; Zhang *et al*., 2013; Zhang *et al*., 2014). In other studies on soil aggregate stability in agricultural systems, Milne and Haynes (2004), Pohl et al. (2012), Hurisso et al. (2013 and O'Brien and Jastrow (2013) found highest percentages of large aggregates in systems with permanent pasture. The authors showed that the activity of the grass roots system, coupled with the absence of soil disturbance, effectively contribute to the formation of stable macro-aggregates. The authors also reported the importance of relations between the MWD and the organic C pools, confirming the statement of Christensen (2001) that, aside from the interactions between minerals, the interaction of MWD with SOC strongly affects the size of water stable aggregates. Soils with higher water stable aggregates are likely to have greater resistance to soil degradation and erosion.

**Conclusions**

Aggregate stability in terms of MWD differed among the Brachiaria grasses and was highest in soils under *Mulato II* hybrid and lowest under cv. MG4. This was attributed to the presence of higher POM and MBC in *Mulato II* hybrid cultivated soils. While we found that SOC did not vary among Brachiaria grasses over the short duration of the study, changes in below ground C cycling were apparent through effect on aggregate formation and higher POM and MBC in Brachiaria cultivated soils. By significantly improving soil aggregation and associated C content, the potential of Brachiaria grasses for enhancing C storage was noted.

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**Table 4** Relationships between soil aggregate fractions and MWD with other soil and plant properties

| Properties | >2000 μm | 250-2000 μm | 53-250 μm | <53 μm | MWD mm | POM $gkg^{-1}$ | MBC $\mu gg^{-1}$ | SOC $gkg^{-1}$ | Total N $gkg^{-1}$ | Roots biomass $gm^{-2}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| >2000 μm | - | | | | | | | | | |
| 250-2000 μm | 0.0069 | - | | | | | | | | |
| 53-250 μm | -0.0030 | -0.8942*** | - | | | | | | | |
| <53 μm | 0.0238 | -0.7735*** | 0.6688** | - | | | | | | |
| MWD mm | 0.0085 | 0.9950*** | -0.8457*** | -0.7618*** | | | | | | |
| POM $gkg^{-1}$ | -0.0167 | 0.9104*** | -0.8355** | -0.7636*** | 0.8991*** | - | | | | |
| MBC $\mu gg^{-1}$ | -0.1419 | 0.5474** | -0.4917** | -0.6415** | 0.5371** | 0.6469** | - | | | |
| SOC $gkg^{-1}$ | 0.1243 | 0.3654* | -0.3229* | -0.4907** | 0.3577* | 0.3357* | 0.2774* | - | | |
| Total N $gkg^{-1}$ | 0.1527 | 0.2861* | -0.2807* | -0.4207* | 0.2727* | 0.2922* | 0.3351* | 0.8670*** | - | |
| Roots biomass $gm^{-2}$ | -0.0754 | 0.4977** | -0.4553* | -0.6590** | 0.4841** | 0.5034** | 0.6833** | 0.4654* | 0.5017** | - |

*** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$, MWD-Mean weight diameter, POM-Particulate organic matter, MBC-microbial biomass carbon, SOC-Soil organic carbon

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# Plant shoots and roots biomass of Brachiaria grass cultivars sown in the semi-arid eastern Kenya and the effects on soil carbon

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KALRO - Katumani

**Abstract**

Grassland management practices that increase carbon uptake by increasing productivity or reducing carbon losses can lead to net accumulation of carbon in grassland soils. A study was conducted to quantify the amounts of shoots and roots biomass of cultivated Brachiaria grass cultivars on two sites Ithookwe and Katumani in semi-arid tropical Kenya. The grass cultivars were *Brachiaria decumbens* cv. Basilisk, *B. brizantha* cvs. Marandu, MG4, Piatã and Xaraes, *B. humidicola* cv. Llanero and *B.* hybrid cv. Mulato II. These were compared with two locally cultivated grasses (*Chloris gayana* cv. KAT R3 and *Pennisetum purpureum cv.* Kakamega 1). The grass treatments were evaluated with fertilizer application (40 kg P applied at sowing and 50 kg N ha$^{-1}$ in each wet season) and with no fertilizer application. Shoots biomass of the Brachiaria cultivars ranged from 3.0 to 11.3 t ha$^{-1}$ and 5.5 to 8.3 t ha$^{-1}$ at Ithookwe and Katumani sites, respectively in year 1. The highest shoots biomass was recorded from cv. Piata and cv. MG4 at Ithookwe and Katumani, respectively. Similar trends were recorded in year 2 of growth though the shoots biomass was lower at Katumani. However the yields were significantly lower than those recorded from control Napier grass in both years. The cv. Marandu, Xaraes, Basilisk and Piata had higher roots biomass than the control (Napier and Rhodes grass) indicating greater potential for the Brachiaria grasses to sequester more carbon in the soil. The results of this study indicate that introduction of Brachiaria grasses in the semi-arid tropics of Kenya and in other similar environments can help increase soil carbon stocks that would aid in offsetting the adverse effects of climate change and have greater economic returns.

**Key words**: Biomass; productivity; Brachiaria; soil carbon; climate change.

## Introduction

A large part of the world's grasslands is under pressure to produce more livestock by grazing more intensively, particularly in Africa's rangelands, which are vulnerable to climate change and are expected to supply most of the beef and milk requirements in Africa (Reid *et al*., 2004). Previous research has documented that improved pasture management can lead to greater forage production, more efficient use of land resources and rehabilitation of degraded lands (Oldeman, 1994). Implementing grassland management practices that increase carbon uptake by increasing productivity or reducing carbon losses can lead to net accumulation of carbon in grassland soils sequestering atmospheric carbon dioxide (Lal, 2009).

Soil organic matter (SOM) a key regulator of ecosystem processes plays an important role in soil fertility and is critical factor for the reduction of soil erosion through aggregate stabilization (Gichangi, *et al*., 2016). In addition, the preservation of SOM in soil mitigates greenhouse gas emissions ($CO_2$) into the atmosphere (Cole *et al*., 1996; Pan *et al*., 2006). Atmospheric carbon can be sequestered in long-lived carbon pools of plant biomass both above and below ground or recalcitrant organic carbon in soils. Apart from offsetting $CO_2$ emissions and global warming, sequestration of carbon in soils also helps to improve soil quality by improving many physical, chemical and biological properties of soils such as infiltration rate, aeration, bulk density, nutrient availability, cation exchange capacity, buffer capacity, etc. Practices that sequester carbon in grasslands also tend to enhance resilience, and are thus likely to enhance longer-term adaptation to changing climates more especially in the semi-arid environments where soils are inherently low in organic carbon content. Therefore, practices that sequester carbon should be promoted to provide near-term dividends in greater forage production for enhanced producer income and better environmental protection.

Perennial grasses hold promise for increasing belowground C storage, sequestering C in extensive roots structures (Norby and Jackson, 2000; Fornara and Tilman 2008; Dohleman *et al*., 2012) and accumulating SOC at rates averaging 40–100 g C $m^{-2}$ $y^{-1}$ (Chevallier *et al*., 2000; Anderson-Teixeira *et al*., 2009). Indeed, some pastures have higher soil C stocks than forests (Cole *et al*., 1996; Neill *et al*., 1996). Scharpenseel and Becker-Heidmann (1997) for example reported that the mean residence time of C derived from pasture was longer than for C derived from forest in an Australian vertisols. Roots are the major C source in soil (Matamala 2008; Frank *et al*., 2004 Fornara and Tilman 2008), and can also stimulate SOM mineralization (Ladd *et al*., 1994). The C sequestration rates vary widely for tropical zones (0.03 to 1.7 Mg $ha^{-1}$ $yr^{-1}$) and could be increased knowing the potential of biomass production of those agroecozones (Bayer *et al*., 2006; Corbeels *et al*., 2006; Cerri *et al*., 2007).

Brachiaria grasses are endaphytic and have a great ability sequester and accumulate large amounts of SOC through their large and extensive shoots and roots biomass and survive in dry areas of low soil fertility (Clapperton *et al*., Fisher *et al*., 2007; Peters *et al*., 2012). This makes Brachiaria grasses a better option for livestock feed production and soil improvement. The grass is widely planted in the tropics of South America to sustain livestock production (Miles *et al*., 2004). About 55% of the total area of pastures is composed by grasses of the genus *Brachiaria.* Brazil for example, has around 172 million hectares of grasslands that support a cattle herd of approximately 170 million heads. The objective of this study was therefore to quantify the amounts of plant shoots and roots biomass resulting from 2 years cultivation of Brachiaria grasses in the semi-arid region of Kenya. We tested the hypothesis that Brachiaria grasses have higher shoots biomass and allocate more C to roots resulting in greater belowground biomass particularly when N and P fertilizers are applied compared to commonly cultivate local grasses.

**Materials and methods**

*Description of the study sites*

The experiments were established in November during the short rains of 2013 at the Kenya Agricultural and Livestock Research Organization (KALRO) centres at Ithookwe (38°02′E, 1°37′S) and Katumani (37°28′E, 1°58′S). The elevations for Ithookwe and Katumani are 1150 m and 1600 m ASL, respectively. The sites have a long term mean annual rainfall of 1010 and 717 mm, respectively in bimodal pattern with the long rains (LR) occurring from March to May and the short rains (SR) from October to December with peaks in April and November, respectively. Mean temperatures are 22.5 and 19.6˚C for Ithookwe and Katumani, respectively. The dominant soils in both sites are chromic Luvisols, which are low in organic C and highly deficient in N and P and generally have poor structure (NAAIAP, 2014).

*Site characterization*

Soil samples were collected in November 2013 before establishing the experiment at depths of 0–15 cm, 15–30 cm, 30–60 cm, and 60-100 cm using an auger for analysis at the testing sites. Plant litter on the soil surface was removed before collecting the soil samples. A composite soil sample, consisting of 12 cores, was collected in a grid pattern from within the 25 × 10 m blocks. Samples from each block were air-dried, visible plant roots removed, and the samples gently crushed to pass through a 2-mm sieve. The fractions sample <2 mm were used for subsequent chemical and physical analyses. Total soil N, available P (Mehlick III), exchangeable K, Ca, and Mg were estimated following standard methods as described by Okalebo *et al*. (2002)**.** Cations $Ca^{2+}$, $Mg^{2+}$, and $K^{+}$ were determined by atomic absorption spectrometry and soil P was measured as described by Murphy and Riley (1962).

Soil texture was determined by the hydrometer method. Soil pH was measured in water (soil: water ratio of 1: 2.5) using a pH meter and reference calomel electrode (Model pH 330 SET-1, 82362) after the suspensions were shaken for 30 minutes and allowed to stand for 1 hour. Organic carbon was determined by the modified Walkley and Black procedure (Nelson and Sommers 1982). Cation exchange capacity (CEC) was based on the sum of exchangeable Ca, Mg, K, H + Al after extraction with ammonium acetate. Soil bulk density was determined according to Blake and Hartge (1986). Soils were vertically sampled using stainless steel rings (diameter 10 cm) at soil depths of: 0–15 cm, 15–30 cm, 30–60 cm, and 60-100 cm, resulting in undisturbed soil samples for bulk density determination. Soil samples were dried at 65ºC to a constant weight to allow soil bulk density calculation. All determinations were made in triplicate and expressed on a dry weight basis.

The soil characteristics are shown in Table 1. Mean surface (0-15cm) soil pH was moderately acidic and was 5.8 and 5.3 in Katumani and Ithookwe, respectively. The soil pH in Ithookwe was slightly below the range (pH 5.5–7.0) for good nutrient availability without toxicity problems (Landon, 1984). Organic carbon content was low in both sites and varied from 0.49 – 1.16% with depth for the Katumani and 0.52 – 0.83% for the Ithookwe (Table 1). Similarly nitrogen, phosphorus and zinc were low in both sites. Potassium levels were adequate at Katumani site but were generally low in soil samples collected from Ithookwe. According to Foster (1971), critical values for soil pH, organic matter, total N, P and K are 5.5, 3.0%, 0.18%, 5 mg $kg^{-1}$ and 13.3 $cmolkg^{-1}$ respectively. Calcium and iron levels were adequate in both sites (Table 1).

Results of the physical soil analysis of samples collected from the test sites at 0-15 cm indicated that the soils were sandy clay loam in Ithookwe and sandy clay in Katumani (Table 2). Cation exchange capacity ranged from 16.1 to 18.6me% and 20.2 to 27.8me% in Ithookwe and

Katumani, respectively and tended to increase with depth. This would be expected as the clay content also increased with depth resulting to increased number of exchange sites. Bulk density ranged from 1.32 to 1.49 g/cm$^3$ and for both sites was greater than the ideal range of 1.1 - 1.3 g cm$^{-3}$ for non-restricted plant roots growth (Landon, 1991). According to Landon (1991), soil bulk density exceeding 1.3 g cm$^{-3}$ for clay soils could negatively interfere with soil aeration through reduced air-filled pore space. Silt and clay comprised over 50 % of the soil in both sites (Table 2).

**Table 1** Initial soil chemical properties at the experimental sites, Ithookwe and Katumani

| Soil properties | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ithookwe | | | | Katumani | | | |
| Sampling depth (cm) | 0-15 | 15-30 | 30-60 | 60-100 | 0-15 | 15-30 | 30-60 | 60-100 |
| Soil pH | 5.36 | 5.34 | 5.39 | 5.79 | 5.88 | 5.76 | 5.81 | 6.10 |
| Total Nitrogen % | 0.08 | 0.08 | 0.08 | 0.05 | 0.12 | 0.12 | 0.07 | 0.05 |
| Org. Carbon % | 0.83 | 0.78 | 0.77 | 0.52 | 1.16 | 1.15 | 0.65 | 0.49 |
| Phosphorus ppm | 13 | 10 | 15 | 12 | 10 | 12 | 10 | 15 |
| Potassium me% | 0.25 | 0.14 | 0.11 | 0.61 | 0.29 | 1.01 | 0.52 | 0.32 |
| Calcium me% | 1.9 | 1.7 | 1.8 | 1.3 | 3.1 | 3.4 | 2.2 | 2.4 |
| Magnesium me% | 3.62 | 4.11 | 3.75 | 3.59 | 5.72 | 5.99 | 5.96 | 6.31 |
| Iron ppm | 10.3 | 14.3 | 14.6 | 11.3 | 17.0 | 17.4 | 18.8 | 18.3 |
| Zinc ppm | 1.09 | 1.29 | 1.28 | 1.01 | 1.78 | 1.44 | 0.97 | 0.64 |

**Table 2** Initial soil physical properties at the experimental sites, Ithookwe and Katumani

| Soil properties | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ithookwe | | | | Katumani | | | |
| Sampling depth (cm) | 0-15 | 15-30 | 30-60 | 60-100 | 0-15 | 15-30 | 30-60 | 60-100 |
| Bulk density (g/cm$^3$)….. | 1.59 | 1.48 | 1.49 | 1.39 | 1.32 | 1.41 | 1.35 | 1.45 |
| Sand % | 56.7 | 49.3 | 49.3 | 50.0 | 50.7 | 48.7 | 44.0 | 40.0 |
| Silt % | 12.7 | 8.7 | 8.0 | 18.0 | 6.0 | 8.0 | 5.3 | 7.3 |
| Clay % | 30.7 | 42.0 | 42.7 | 32.0 | 43.3 | 43.3 | 50.7 | 52.7 |
| Cat. Exch. Cap. me% | 16.1 | 14.2 | 18.6 | 11.9 | 20.2 | 21.3 | 20.9 | 27.8 |
| Base saturation % | 33.3 | 50.2 | 27.6 | 58.1 | 92.4 | 85.7 | 78.9 | 64.2 |

*Treatments and experimental design*

The treatments consisted of seven Brachiaria grass cultivars; *Brachiaria decumbens* cv. Basilisk, *B. humidicola* cv. Llanero, *B. brizantha* cvs. Marandu, MG4, Piatã, Xaraes and *B. hybrid* cv. Mulato II., Two commonly cultivated local grasses, [(*Chloris gayana* cv. KAT R3 and *Pennisetum purpureum cv*. Kakamega 1 (KK1) were included as control. These treatments were evaluated in the plots with fertilizer (40 kg P ha$^{-1}$ applied at sowing and 50 kg N top-dressed in each wet season) and without fertilizer application. The treatments were laid out in a randomized complete block design in a split plot arrangement (fertilizer treatments as main plots and the grass cultivars as sub plots) in three replications. The grasses were sown in November 2013 during the short rains. All the plots were kept weed free throughout the experimental period by hand weeding. The grasses were first harvested 16 weeks after establishment and later, harvesting was done on an 8 weeks interval during the wet seasons.

*Shoots and roots biomass determination*

Data for shoots biomass was collected eight times on an 8 weeks interval after plants were well established. The establishment period was considered as 16 weeks after seedling emergence. Harvesting of plant shoots was carried out from 2 m x 2 m net plots at a cutting height of 5 cm above ground. Samples of fresh shoots biomass were recorded, and approximately 500g subsamples were dried at 65°C to constant weight in forced-air drier for determination of dry matter. Roots were sampled using the soil-core method (Bohm, 1979). In each plot, four soil cores were randomly taken with a 6.5 cm diameter stainless steel auger to a depth of 0–15 and 15–30 cm from the inter-row and intra-row positions and composited into one sample per plot for each depth. The sampling was carried out at least 1m apart from the edge of the plot to avoid edge effects. Sampling was conducted at 24 and 48 weeks of plants establishment. The roots from each soil layer were washed separately by hand with a 2.8 mm and a 2 mm soil sieve under running tap water. Root samples integrating both living and dead roots were then dried at 65°C to constant weight and roots dry weights were recorded. Total N and P in the plant samples were measured after digestion in a 1.2:1 $H_2SO_4$: $H_2O_2$ mixture at 360°C after which total N and P were measured colorimetrically (Anderson and Ingram, 1993).

Microbial biomass C was determined on field moist soil (18-23% by weight) taken from a depth 10 cm in the rhizosphere by the chloroform fumigation-extraction technique as described in Brookes *et al*. (1984, 1985) and Gichangi *et al.* (2016). Briefly, 10 g dry weight equivalent of soil was fumigated with ethanol-free chloroform in a glass desiccator; and another 10g was incubated without fumigation at the same moisture content, time period and temperature for 24 h at 25°C. Both sets were extracted with 0.5 M $K_2SO_4$ and C in the extracts determined using the standard method as described by Okalebo *et al*. (2002). The Soil microbial biomass carbon was then calculated as the difference between the fumigated and un-fumigated samples and corrected for incomplete recovery using a conversion factor of 0.45 for C (Wu *et al*., 1990). All determinations were made in triplicate and expressed on a dry weight basis.

**Statistical analysis**

Treatment effects on shoots and roots biomass were tested using the analysis of variance (ANOVA) as a split-plot with fertilizers N and P as the main factor and grass type as the sub-plot factor using GENSTAT statistical software (GENSTAT Release 4.24DE, 2005). Differences at $p \leq 0.05$ were considered significant and means separation was done using Fischer's protected test (LSD). Regression analyses and Pearson correlation coefficient (r) were used to find models best describing the relationships between shoots and roots biomass with other soil and plant properties.

**Results and Discussion**

*Shoots biomass and N and P uptake*

The shoots biomass differed significantly ($p \leq 0.05$) among grass cultivars and across experimental sites. The shoots biomass of the Brachiaria cultivars ranged from 3.0 to 11.3 t $ha^{-1}$ and 5.5 to 8.3 t $ha^{-1}$ at Ithookwe and Katumani, respectively in year 1 with the highest shoots biomass recorded from cv. Piata at Ithookwe and cv. MG4 at the Katumani (Figure 1a). Similar trends were recorded in year 2 growth, but the shoots biomass was much lower at Katumani (Figure 1b) than in year 1 growth (Figure 1a). However the yields were significantly lower than those recorded from locally cultivated Napier grass (*Pennisetum purpureum cv*. Kakamega 1) for both periods. Generally, higher shoots biomass was recorded from the Ithookwe site than that recorded from the Katumani site (Figure 1) indicating that the Brachiaria cultivars are more suited to that site. The site has a higher annual rainfall with a long term mean of 1010 mm compared to 717 mm received at Katumani. There was a strong positive relationship between shoots biomass recorded with N and P uptake (Figure 2). Higher shoots biomass resulted to higher N and P uptake indicating better utilization of the fertilizer applied. Bonfim and Monteiro (2006) and Batista and Monteiro (2008) have previously reported that the combined application of nitrogen with phosphorus was more effective in maximizing the leaf area and the production of higher dry matter of grasses.

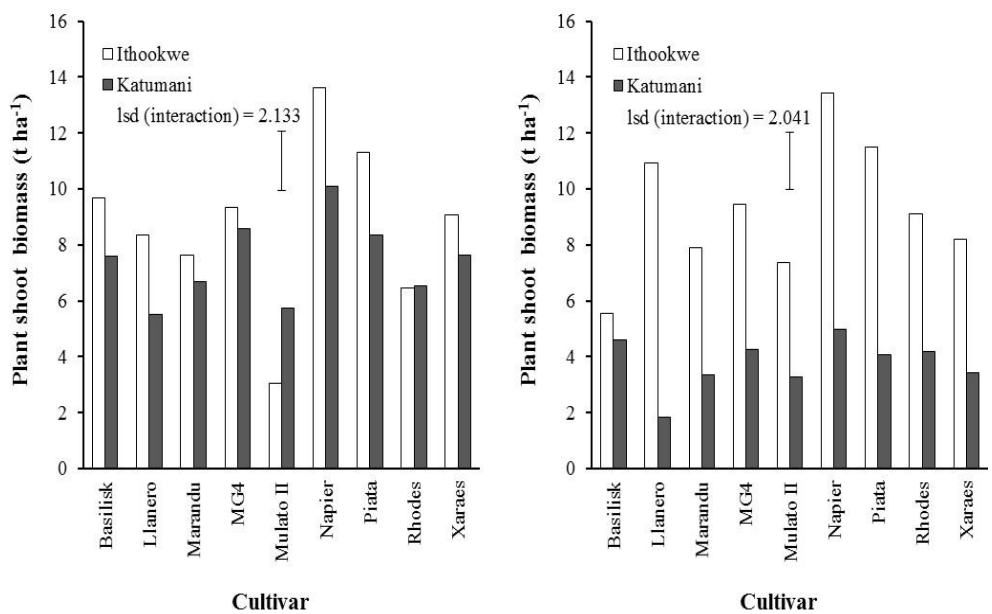


**Figure 1** Effects of grass type and site on total annual shoots biomass a) Year 1 and b) Year 2

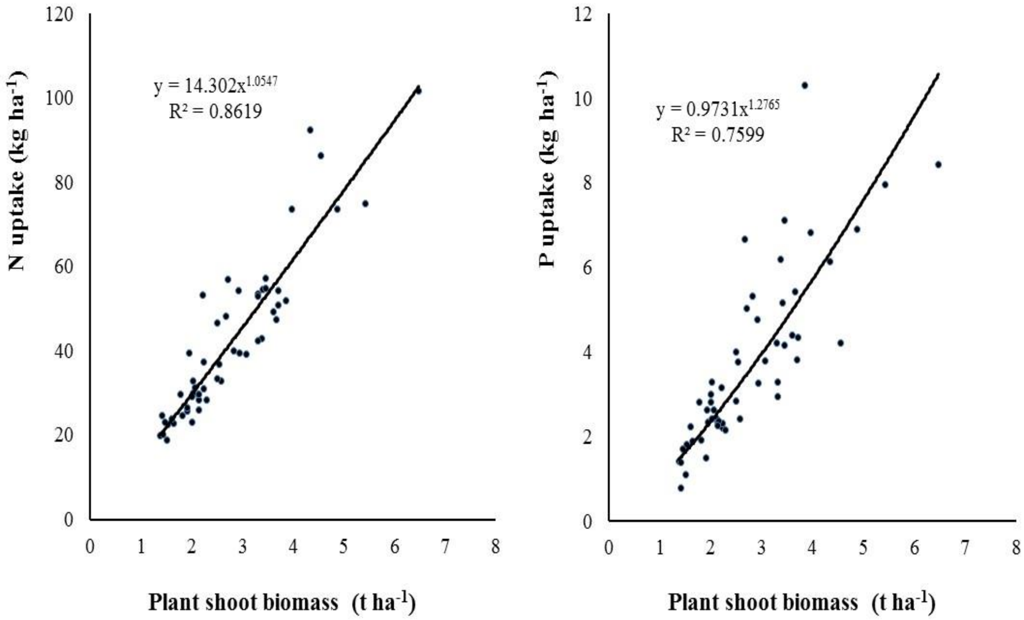


**Figure 2** Relationships between shoots biomass with a) N and b) P uptake of Brachiaria grasses

*Roots biomass*

Brachiaria cultivars; Marandu, Xaraes, Basilisk and Piata had higher roots biomass than the local checks (Napier and Rhodes grass) indicating greater potential for the Brachiaria grasses to sequester more carbon in the soil. A vigorous roots system increases plant growth rate, tolerance to water deficit, and ability to compete for soil nutrients and consequently, leads to an increase in pasture productivity. These results are in agreement with observations made by Peters *et al.,* 2012 that Brachiaria grasses have greater ability to sequester and accumulate large amounts of organic carbon through their large roots biomass. Generally roots biomass was significantly higher from samples collected from Ithookwe than those obtained from Katumani (Figure 3). Grass type by sampling depth interaction was highly significant ($P<0.001$) for roots biomass with the highest roots concentration recorded in the upper (0 - 15 cm) soil layer regardless of the grass type and sampling period (Figure 4). There was approximately 79% of dry roots matter in the 0 - 15 cm soil layer and as expected, roots biomass, increased with age (Figure 4b). Among the Brachiaria cultivars, cv. Mulato II hybrid had the lowest amount of roots; 242.3 g $m^{-2}$ and 409.9 g $m^{-2}$ in the 0-15 cm depth 24 and 48 weeks after plants had established, respectively. The trend was similar in the 15 – 30 cm depth (Figure 4).

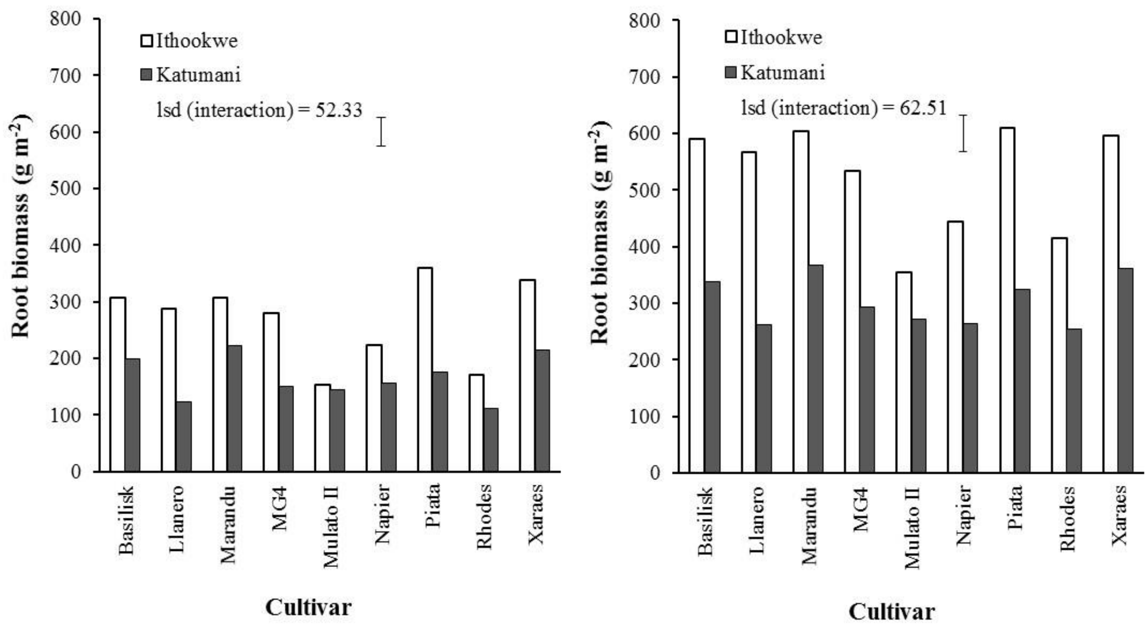


**Figure 3** Effects of grass type and site on roots biomass a) 24 weeks and b) 48 weeks after grasses had established

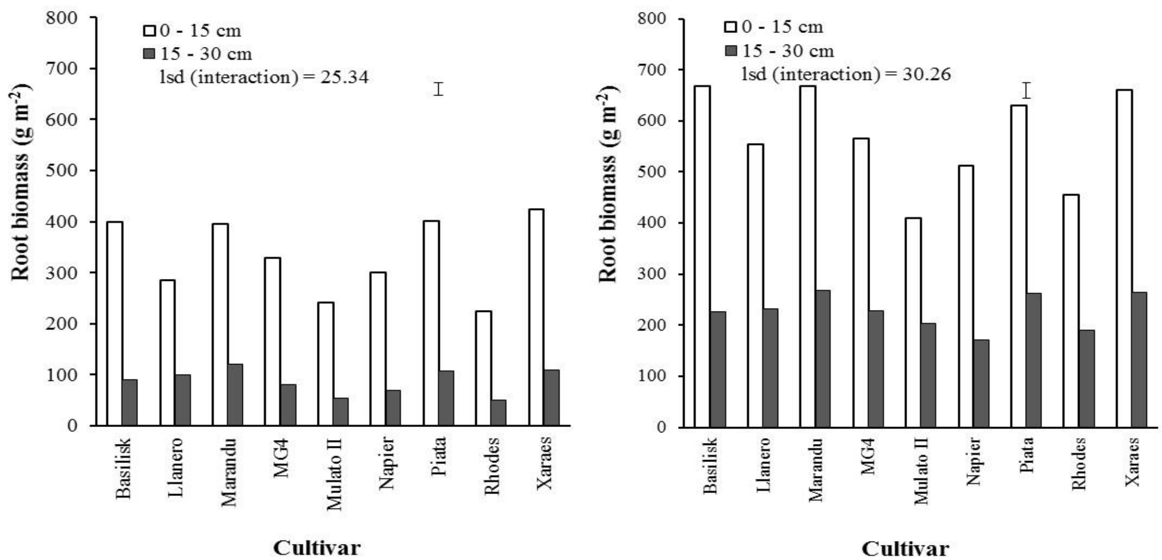


**Figure 4** Effects of grass type and sampling depth on roots biomass across 2 sites (Ithookwe and Katumani) a) 24 weeks and b) 48 weeks after grasses had established

Many management techniques intended to increase livestock forage production have the potential to augment soil carbon stocks, thus sequestering atmospheric carbon in soils. Grassland management that enhance production (through sowing improved species, irrigation or fertilization), minimizing the negative impacts of grazing or rehabilitating degraded lands can each lead to carbon sequestration (Follett, *et al*., 2001; Conant *et al*., 2001). In this study fertilizer addition significantly ($p<0.001$) increased roots dry matter of all grass types except cvs. Basilisk, MG4 and Mulato II 24 weeks after plants had established (Figure 5a). However, the fertilizer effects were significant in all Brachiaria cultivars at 48 weeks (Figure 5b). The cultivar x fertilizer treatments interaction was also significant for roots biomass. This confirmed that low nutrient availability, especially phosphorus (P) and nitrogen (N) supply are a major limitation to forage production in infertile soils of the region. Our results are in agreement to those of Conant *et al*. (2001) who reported that intensively managed and fertilized grassland had higher roots biomass than less managed grasslands. As forage production increases with fertilizer application, an ancillary benefit may lie in increased sequestration of atmospheric carbon. Indeed, Gifford *et al*. (1992) noted that improved pasture management is an important consideration when computing a national carbon budget.

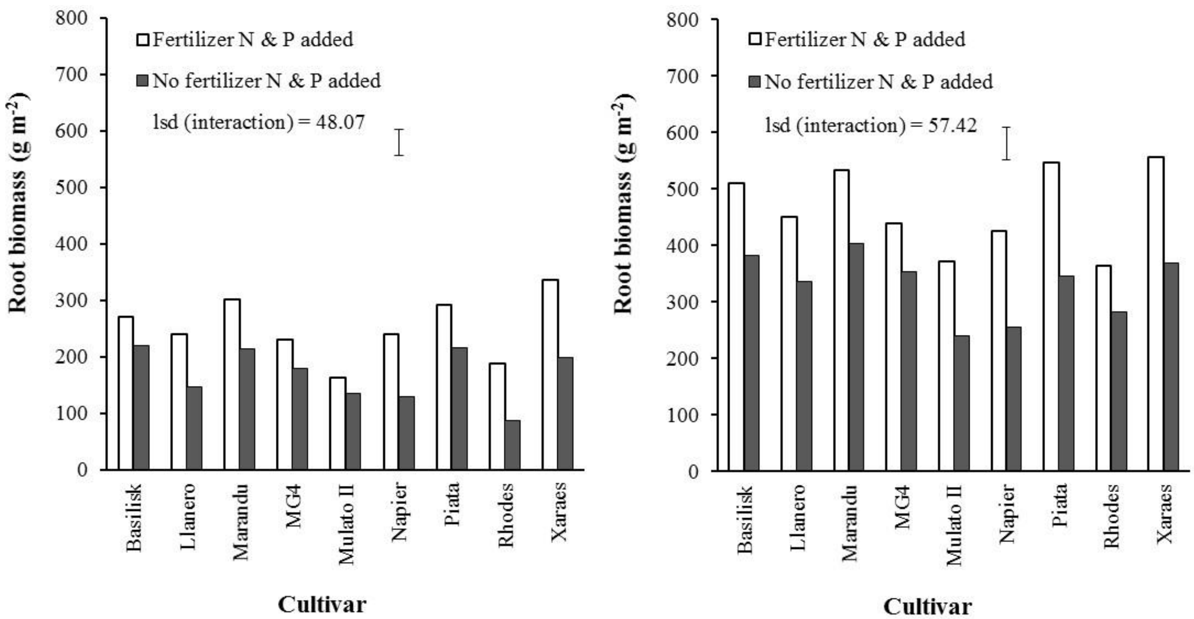


**Figure 5** Effects of grass type and fertilizer application on roots biomass across 2 sites (Ithookwe and Katumani) a) 24 and b) 48 weeks after grasses had established

The synthesis by Smith *et al.* (2008) suggests that improvement of soil fertility could lead to C sequestration of between 0.42 and 0.76 t C $ha^{-1}$ $yr^{-1}$ depending on the region. Similar observations were also made by Follett *et al*. (2001) who reported that grassland management to enhance production through sowing improved species, irrigation and fertilization can each lead to carbon sequestration. The increased carbon allocation to the roots by the Brachiaria grasses resulted in net belowground sequestration of carbon as indicated by the positive correlation between roots biomass with microbial biomass carbon (MBC) and soil organic carbon (SOC) (Table 3) even though the changes in SOC were very small and not significant among the Brachiaria grasses.

**Table 3** Relationships between roots biomass and soil and plant properties

| Properties | MBC | Roots biomass Week 24 | Roots biomass Week 48 | SOC | Shoots biomass |
|---|---|---|---|---|---|
| MBC | 1.0000 | | | | |
| Roots biomass-Week 24 | 0.7172** | 1.0000 | | | |
| Roots biomass-Week 48 | 0.6225** | 0.8210** | 1.0000 | | |
| SOC | 0.3139* | 0.4527** | 0.4059** | 1.0000 | |
| Shoots biomass | 0.6363** | 0.6802** | 0.6992** | 0.4287** | 1.0000 |

** $p < 0.01$ and *$p < 0.05$, MBC-microbial biomass carbon, SOC – Soil organic carbon

## Conclusions

The results of this study indicate that the introduction of Brachiaria grasses in the semi-arid tropics of Kenya and in other similar environments would increase soil carbon stocks through their higher shoots and roots biomass that can aid in offsetting the adverse effect of climate change and have greater economic returns.


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# Theme 4

# Brachiaria grass and livestock production

# The effects of Brachiaria grass cultivars on lactation performance of dairy cattle in Kenya

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**Abstract**

Brachiaria grass is a high quality tropical grass native to East and Central Africa. It is widely grown for grazing, hay and silage making in South America, Australia and East Asia. An on-farm feeding trial was carried out in Kangundo sub County in Machakos County in mid-altitude eastern region of Kenya to evaluate the effects of feeding Brachiaria cultivars on milk yield. Twelve farmers fed cows on either *Brachiaria brizantha* cvs. Piata, Xaraes and MG4 or *B. decumbens* cv. Basilisk. A total of 12 dairy cows in mid lactation (between 3 and 6 months into lactation) were used. Milk yield increased by 15-40% in cows fed Brachiaria grasses compared to local feeds which were varied mixtures of Napier grass, maize stover and natural pastures. A second study was conducted at KALRO Mtwapa in coastal lowlands to evaluate the effect of Mulato II, Piata and Xaraes on milk production. Sixteen (16) Jersey cows in mid to late lactation were grouped into four and fed on either Mulato II, Piata, Xaraes or Bana as the control. There were no differences ($P > 0.05$) between the daily grass dry matter intake which ranged from 5.7 to 6.4 kg/cow. Daily milk yield was similar between cows fed Bana (4.6 kg/cow) and Piata (4.7 kg/cow). However, cows fed on either Mulato II or Xaraes produced less ($P<0.05$) milk (4.4 and 3.6 kg/cow respectively) than cows fed Bana (4.6 kg/cow). The study concluded that; compared to local feeds in Kangundo sub County, Brachiaria increased milk by 15-40%. It also has potential to compliment Napier grass in dairy feeding in coastal Kenya. Farmers should be encouraged to grow more Brachiaria for increased milk production.

**Key words:** Jersey cows; milk yield; Mulato II; Napier grass, Piata; Xaraes;

## Introduction

A major biotic constraint to dairy production in Kenya is inadequate and low quality feed resources to meet year-round nutrient requirements for lactating cows (Reynolds *et al*., 1993). Natural pastures are the main feed resource mainly under free-grazing system in coastal lowlands (Muinga *et al*., 1998; Njarui *et al*., 2016). Milk production for local and exotic/crossbred cattle is low, ranging from 1.0 to 6.4 kg day$^{-1}$ respectively (Ramadhan *et al*., 2008). Over several decades, Napier grass (*Pennisetum purpureum* Schum.), was promoted for improving fodder availability (Mureithi *et al*., 1998). The grass which is one of the highest yielding tropical grasses, is versatile and grows under a wide range of conditions and systems. It is a valuable forage notably in cut-and-carry systems (FAO, 2015). Increased milk production has been recorded where lactating dairy cattle were fed Napier grass cv. Bana supplemented with forage legumes (Muinga *et al*., 1992; Juma *et al*., 2006). Despite past efforts to promote fodder technologies for dairy feeding in Kenya, Napier grass contributes about 10% of feed in coastal Kenya, 35-45% in North western highlands and about 50% in the central highlands (Njarui *et al*., 2016). In some parts of Kenya, Napier grass is challenged by the stunting disease and head smut (Kabirizi *et.*

*al*., 2016) which calls for alternative grasses. Brachiaria species are being re-introduced to the region as potential alternatives to Napier grass (KALRO-BecA-ILRI Hub project 2012).

Brachiaria species are native to eastern and central Africa and are extensively grown as livestock forage in South America and East Asia (FAO, 2015). Important species include *B. ruziziensis*, *B. decumbens*, *B. brizantha* and *B. humidicola*. The last two have been hybridized to form a series of cultivars known as Mulato (FAO, 2015). Compared to Napier grass, *B. hybrid* cv. Mulato II is tolerant to drought and can withstand heavy grazing (The Organic Farmer, 2015). The annual dry matter yield ranges from 8 to 20 t/ha depending on moisture and nutrients (FAO, 2015). *Brachiaria brizantha* yield in Tanzania increased from 6 to 26.5 t/ha on nitrogen (896 kg/ha/year) application (Urio *et al*., 1988). Brachiaria grasses are among the most nutritious forages in the humid tropics. For example, *B. brizantha* contains about 10% (range: 5 to 16%) crude protein (CP) in dry matter, 66% neutral detergent fibre (NDF) and 58% *in vivo* organic matter digestibility (Heuzé *at al.,* 2016). In Cameroon during the dry season, a decrease in CP from 15.6 to 5.4% and an increase in NDF and acid detergent fibre (ADF) from 34.2 to 48.6% to 70.5 to 76% respectively for B. *ruziziensis*, were recorded compared to the wet season (Pamo *et al*., 2007).

Hardly any animal performance studies involving Brachiaria have been reported within the East African region. The current studies were therefore designed to evaluate Brachiaria grasses as basal feed for lactating cows, on-farm in mid-altitude eastern region and under controlled conditions on-station in coastal lowlands of Kenya. The potential of using Brachiaria grasses in dairy feeding as alternatives to Napier grass and other locally available feeds is reported.

## Materials and methods

### On farm study

The study was carried out in Kangundo sub-County in mid-altitude eastern region of Kenya. Farmers in this sub-County (Machakos County) were introduced to Brachiaria in an earlier study to evaluate the performance of various cultivars. Typically the farmers have smallholdings where they practice mixed crop-livestock farming. They keep different species of livestock and dairy farming is an important livelihood strategy. About 69% of the farmers keep dairy cattle and the average herd size is 3.1±2.1 animals (Njarui *et al*., 2016).

A meeting was held on 5 May 2015 where 60 farmers attended. They were given an overview of livestock feeding which included the different types of feeds which can be used as sources of basal diet, protein and energy. The session was interactive and farmers shared information freely. The lactation curve was highlighted to show early lactation where milk increased to a peak at about 2-3 months after calving (Figure 1).

Cows after peak lactation when milk yield decreases, were selected for the feeding trial because an increase in milk yield during this period could be attributed to feed quality. Eighteen (18) farmers who indicated that they had planted more than 0.1 ha of any Brachiaria cultivar were included in the study. Out of the 18 farmers who registered for the feeding trial, only data from

12 farms were analysed. The other farmers did not have adequate amount of Brachiaria forage to cover the feeding period as indicated in the meeting. Farmers used four Brachiaria grass cultivars in the trial depending on what they had. These included: cultivars Piata, Xaraes, MG4 and Basilisk.

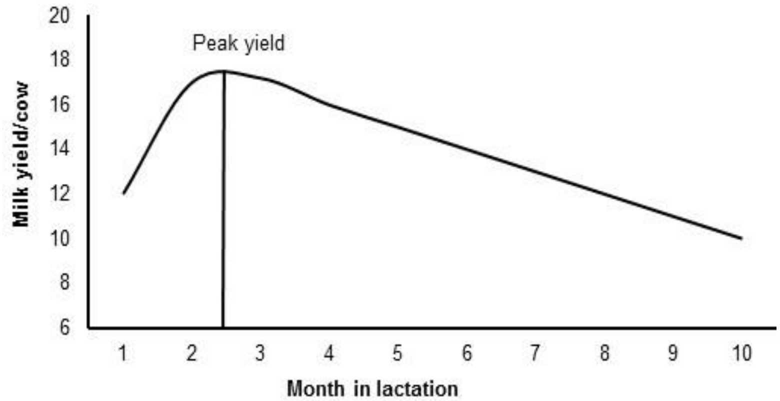


**Figure 1:** Typical lactation curve

Both the farmers, researchers and extension staff jointly participated in data collection. At the commencement of the study, milk yield of cows fed on local feeds was recorded for three consecutive days. This was followed by feeding the cows on Brachiaria grass for seven days. Milk yield for the last three days was compared with the initial three days milk yield for the period when cows were fed on locally available feeds. A total of 12 lactating dairy cows in mid lactation (3 and 6 months) were used for milk yield analysis.

**Data analysis**

The mean milk yield per farm was calculated for the first three (3) days when the cows were fed on local feeds and for the last three (3) days when cows were fed on Brachiaria grass. The means were subjected to Analysis of Variance (ANOVA) using the statistical software Genstat 15 (VSN International, 2013) using the following model:

$Y_{ij}=\mu + T_i + F_j + \varepsilon_{ij},$

Where:

$Y_{ij}$=Observed milk yield for $i^{th}$ feed in $j^{th}$ farm
$\mu$=Overall mean
$T_i$=Effect of $i^{th}$ feed (i=1, 2 for local feed or Brachiaria grass)
$F_j$=Effect of $j^{th}$ farm (replicate), (j=1 to 12)
$\varepsilon_{ij}$=Random error

**On-station study**

The study was carried out at KALRO Mtwapa, at an altitude of 15 m above sea level, latitude $3^o56'$S, longitude $39^o44'$E, in the coastal lowlands agro-ecological zone 3 (CL3). The site is characterized by light sandy soils and a mean annual rainfall of 1200 mm. The relative humidity ranges from 65 - 95% and the mean annual temperatures range from 24 to 29°C (Jaetzold *et al*., 2006). Napier grass established in gliricidia (*Gliricidia sepium*) alleys and pure plots of Brachiaria grasses (*B. hybrid* cv. Mulato II, *B. brizantha* cvs. Piata, Xaraes) were established in November 2014 at the recommended spacing of 50 cm between the rows. Normal cultural practices were carried out to optimize yield. The grasses were harvested daily and chopped with a motorized chaff cutter. Gliricidia was harvested and wilted a day before feeding the leaves and stems less than 5 mm diameter were fed to the cows.

Sixteen lactating (16) Jersey cows with pre-experiment milk yield ranging from 4 to 5 kg/day and weighing 257±38 kg were used in the experiment. The cows were divided into four groups balanced for milk yield and live weight at the start of the experiment. The groups were allocated to four treatments (cvs. Mulato II, Piata, Xaraes and Napier grass cv. Bana), in a completely randomized design. Each cow was fed the recommended supplement of 8 kg fresh gliricidia and 3 kg maize bran in two equal amounts daily at milking. They were also allowed 60 g of a dairy mineral mix per cow daily and clean cool water was provided *ad libitum*. Refusals were removed and weighed every morning after which fresh feed was added. Data was collected on feed intake and milk yield. The cows were housed in individual feeding stalls and allowed a three weeks acclimatization period on the treatment diets. Data collection commenced on 7$^{th}$ September, 2015 for 10 weeks. Composite samples of each feed (maize bran, gliricidia, grasses) taken at three stages (onset, mid and end of the experiment) were analysed.

*Chemical analysis*

Feed samples were analysed for Ash, Nitrogen, Phosphorous (P) and Calcium (Ca) The total P and Ca were measured according to methods described by Okalebo *et al*. (2002). Ash was determined by heating the samples at 600$^{o}$C for 2 hours in a muffle furnace. Crude protein (CP) was estimated from Nitrogen x 6.5. Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Acid detergent lignin (ADL) and dry matter digestibility (DMD) and organic matter (OMD) digestibility were determined through the method of Goering and Van Soest (1970). The analyses were carried out at KALRO Muguga (Food Crops Research Institute).

**Data analysis**

Analysis of variance (ANOVA) using the General Linear Model was carried out using the following model:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij},$$

Where:
$Y_{ij}$ =The $_j$th observation on the $_i$th treatment;
$\mu$ = Overall mean;
$G_i$= the effect of the $_i$th grass treatment;
$\varepsilon_{ij}$= Random error.

Means were separated using the least significant difference (LSD) at P=0.05 (SAS, 2003)

## Results and Discussion

### On farm study

Two distinct groups of lactating dairy cows were observed, the relatively high and low yielding (Figure 2). On average milk production increased from 4 to 4.6 litres/cow per day for low yielding animals, representing a 15% increase and 9 to12.6 litres/cow per day for the relatively higher yielding dairy cattle representing a 40% increase. This is an indication that Brachiaria is superior to the locally available feeds used by the farmers in the area during the study period. Therefore farmers should be encouraged to increase the acreage under Brachiaria which has potential to increase milk production.

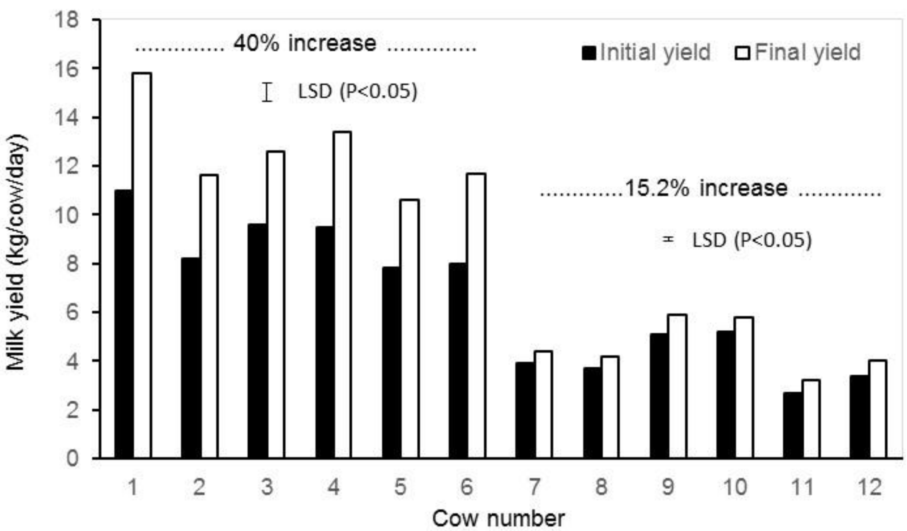


**Figure 2** Milk yield from cows fed local feeds or Brachiaria in Kangundo sub County

### On station study

*Feed composition*

Gliricidia had the highest CP (24.4%) and lowest NDF (34.9%) which was different ($P<0.05$) from the grasses. Its dry matter and organic matter digestibility was however similar ($P>0.05$) to that of Napier grass, Mulato II and Piata and higher ($P<0.05$) than Xaraes (Table 1). Xaraes had the lowest CP and digestibility and the highest NDF but these values were similar ($P>0.05$) to those recorded for Napier grass, Mulato II and Piata. The grasses had similar ADF, ADL, Ca and P. The CP and OMD values of the Brachiaria cultivars in this study were lower than those reported by Nguku (2015) in Katumani. However, the CP and OMD values had a similar trend to those reported in a different study at Mtwapa by Ondiko *et al.* (2016). Their CP was 6.9, 5.8, 5.4 and 4.9% for Napier grass, Mulato II, Piata and Xaraes which was similar to values reported in the current study (Table 1).

**Table 1** Chemical composition and digestibility of the feeds

| | Composition on DM basis (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Feed | CP | NDF | Ash | DMD | OMD | ADF | ADL | Ca | P |
| Maize bran | 12.6 | 58.0 | 8.5 | 66.8 | 61.8 | 21.4 | 2.6 | 0.9 | 0.03 |
| *Gliricidia sepium* | 24.4 | 34.9 | 7.7 | 57.5 | 51.5 | 34.4 | 17.3 | 0.3 | 1.0 |
| *P. purpureum* cv. Bana | 6.8 | 67.3 | 7.9 | 48.6 | 43.3 | 50.1 | 14.0 | 0.1 | 0.3 |
| *B. hybrid* cv. Mulato II | 5.5 | 66.2 | 6.1 | 46.6 | 42.2 | 50.6 | 8.9 | 0.2 | 0.3 |
| *B. brizantha* cv. Piata | 5.3 | 68.9 | 5.0 | 43.2 | 40.0 | 52.5 | 13.6 | 0.1 | 0.2 |
| *B. brizantha* cv. Xaraes | 5.0 | 70.7 | 5.4 | 35.2 | 32.9 | 52.3 | 9.4 | 0.1 | 0.2 |
| LSD (P<0.05) | 3.67 | 8.10 | 2.36 | 16.07 | 14.28 | 10.45 | 9.52 | 0.08 | 0.368 |

*Feed intake and milk yield*

The cows ate all the supplement (8 kg fresh gliricidia and 3 kg maize bran) equivalent to 2.2 kg DM gliricidia and 2.7 kg DM maize bran. The daily basal grass diet DM intake was similar (P<0.05) for cows fed on Bana grass (5.7 kg) and those fed Piata (5.8 kg) and was different (P<0.05) from that of cows fed Mulato II (6.3 kg) or Xaraes (6.4 kg) (Table 2). Mean daily milk production for all the cows was low (4.3 kg $cow^{-1}$) probably as a result of the late stage of lactation at the start of the experiment. Cows fed Piata (4.7kg) and Bana (4.6kg) had the highest milk yield which was not different (P > 0.05). However cows fed on Mulato II (4.4kg) and Xaraes (3.6kg) produced less (P<0.05) milk daily compared to those fed on Piata and Napier grass (Table 2).

**Table 2** Daily basal grass diet intake (kg DM) and milk yield (kg) per cow

| Treatment | Grass DM intake (kg) | Total DOM intake | Milk yield (kg) |
|---|---|---|---|
| *B. brizantha* cv. Piata | 5.8 | 5.3 | 4.7 |
| *P. purpureum* cv. Bana | 5.7 | 5.5 | 4.6 |
| *B. hybrid* cv. Mulato II | 6.3 | 5.1 | 4.4 |
| *B. brizantha* cv. Xaraes | 6.4 | 4.9 | 3.6 |
| LSD (P<0.05) | 0.29 | - | 0.14 |

Total Digestible Organic Matter (DOM) intake = sum of (DM intake*Digestibility) for grass (grass DM intake*DOM), gliricidia (2.2 kg DM*DOM) and maize bran (2.7 kg DM*DOM)

Cows fed on Xaraes had the highest DM intake and the lowest milk yield. The DM intake was equivalent to 2.5, 2.7, 2.3 and 2.1 kg digestible organic matter for cows fed on Bana, Mulato II, Piata and Xaraes respectively. Table 2 shows the calculated total DOM intake which was positively related to milk yield; thus Xaraes had the lowest DOM intake and lowest milk yield. It was noted from Figure 3 that, although the groups were balanced for milk yield at the start of the experiment, cows fed on Xaraes had the lowest milk yield (4.2 kg) by the start of the experimental period (at 3 weeks) compared to the other treatments (Piata 5.0, Bana 4.6, and Mulato 4.8 kg). From the current study, the crude protein, NDF and digestibility (except for Xaraes) of the Brachiaria cultivars were similar to that of Bana. Milk yield from cows fed Piata was comparable to that of cows fed Bana. Brachiaria therefore has the potential to replace Bana in dairy feeding especially in areas where it is threatened by head smut and stunting disease. Compared to the local feeds in Kangundo sub County, Brachiaria increased milk by 15-40%.

Therefore farmers should be encouraged to grow more Brachiaria for increased milk production. There is however need to repeat the on station experiment to verify the results with more Brachiaria cultivars under controlled conditions where grasses are harvested at a similar stage of growth.

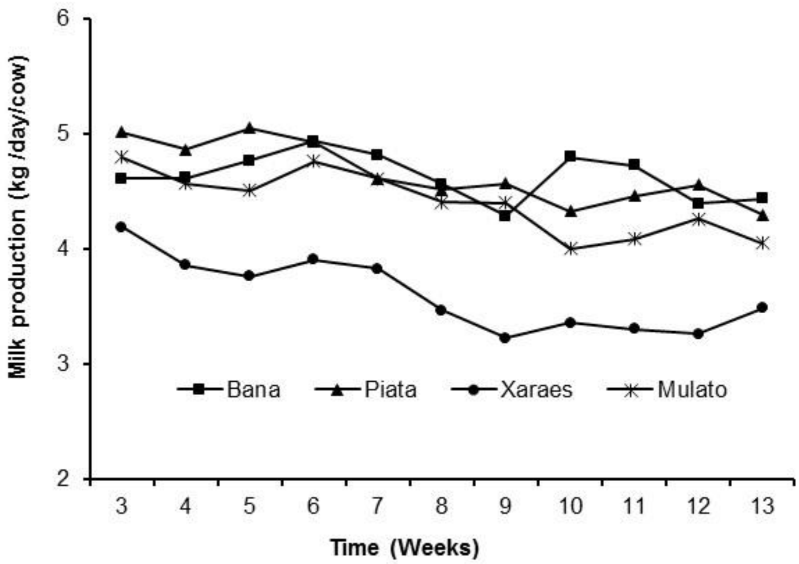


**Figure 3** Milk productions from Jersey cows fed different Brachiaria grasses and Bana in coastal lowlands, Kenya


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# Change in growth of Galla goats fed selected Brachiaria grass cultivars in the coastal lowlands of Kenya

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**Abstract**

Inadequate quality and quantity of feeds is the major constraint to livestock production in the arid and semi-arid regions of Kenya. A study was conducted to determine the chemical composition and dry matter digestibility of three Brachiaria grass cultivars namely; *Brachiaria brizantha* cvs. Piata and MG4, and *B. hybrid* cv. Mulato II and their effects on live weight changes of Galla goats in the coastal lowlands of Kenya. They were compared with the commonly grown grass, Rhodes grass. Sixteen Galla goat bucklings ranging from 10-24 kg were randomly allocated to four dietary treatments with four animals per treatment. Each goat was supplemented with 100 g/day of maize germ. Mineral licks and water were provided *ad libitum*. Piata and MG4 had higher ($P < 0.05$) crude protein (12.6 and 12.1%, respectively) than Mulato II (3.0%) and Rhodes grass (6.7%). The cvs. Piata and MG4 were also more digestible than Mulato II and Rhodes grass. There was no difference ($P>0.05$) in grass dry matter intake among the goats and the daily intake ranged from 513 to 661 g/goat. Average daily live weight gain was higher ($P < 0.05$) for goats fed on Piata (45.2 g/day) and MG4 (41.3 g/day) than those fed on Mulato II (2.0 g/day) and Rhodes grass (9.6 g/day). Likewise goats fed on Piata (3.8 kg) and MG4 (3.5 kg) had the highest total weight gain compared with bucklings fed on Mulato II (0.2 kg) and Rhodes grass (0.8 kg). Based on body weight gain and nutritive value, Piata and MG4 showed the greatest potential to address feed quality constraint in livestock production.

**Key words**: Chemical composition; crude protein; dry matter digestibility; livestock production; live weight gains.

## Introduction

Arid and semi-arid lands (ASALs) cover 80% of Kenya's landmass (Mganga *et al*., 2010). These areas are characterized by low rainfall, high temperatures, poor quality feed resources, and high incidences of livestock diseases (Kahi *et al.,* 2006). The ASALs support 60% of the livestock population and the largest proportion of wildlife (Ngugi and Nyariki, 2003). According to Mbogoh and Shaabani (1999) agro-pastoralism and pastoralism are the main economic activities in ASALs from which majority of the people attain their livelihoods. This is mostly based on cattle (the small East African zebu – SEAZ and Boran), goats, sheep and camels, and thus constitutes a major source of Kenya's meat (Herlocker, 1999).

According to Njarui *et al.* (2011), the productivity of livestock in Kenya is strongly linked to feed availability. The reason for this is that; feed is the major input factor in livestock production systems and account for between 60 - 70% of the production cost. The authors reported that, the productivity of ruminants is considered low due to inadequate and poor quality feeds. There is a feed resource deficit for about 4 - 6 months in a year across many regions in Kenya particularly during the dry season when there is limited pasture growth. Livestock is considered one of the key assets for rural households in most parts of the world and it is a primary livelihood resource for most rural communities. According to FAO (2012), about 752 million of the world's poor keep livestock mainly to; generate cash income, produce food for subsistence use, manage risks and to build up assets for security purposes. Another limitation of livestock production is that there is lack of suitable fodder crops that can produce green forage throughout the year (Leeuw *et al*., 1992). This situation becomes even worse in the areas that are constrained by low rainfall.

Most small ruminants in the ASALs suffer from nutritional stress (Bruinsma, 2003). Most of the grasses have low crude protein (CP) falling below 7% minimum level that is required for optimum microbial growth (Wambui *et al*., 2006). When this occurs, it prompts supplementation, which is not always possible for resource poor farmers (Gitunu *et al*., 2003). There is a need, therefore, for pasture species that can improve the quality of the natural pastures and significantly increase dry matter production to enhance livestock productivity. One of these pastures has been found to be Brachiaria (Machogu, 2013). Lascano and Euclides (1996) and Brighenti *et al*. (2008) reported that apart from the good adaptability, tolerance and resistance of *Brachiaria* species, the grasses have high forage quality and high dry matter production making them capable of meeting the nutritional requirements of animals especially during the dry season.

Goats are found in many parts of Kenya and are an important source of income to many small-holder farmers. They are preferred to cattle as they can be converted to cash easily. They also provide a higher offtake compared to cattle because of their shorter generation interval and higher prolificacy (Ahuya and Okeyo, 2006). Galla goats also known as Somali or Boran goats are indigenous to the arid and semi-arid regions of northern Kenya and are kept mainly for meat (Ahuya and Akeyo, 2006). The full potential of the ASALs for livestock production can be exploited by expanding the forage resource base by introducing climate smart forage species to boost nutrient quality and quantity hence supplying the nutritive requirements of livestock. Studies on climate smart Brachiaria grass species developed elsewhere have shown that they could be the key to improvement of livestock production and also serve to boost composition and nutritive values of local Brachiaria cultivars. However, there are hardly any studies on goat feeding on Brachiaria grasses. The study was therefore carried out to determine its suitability on goat performance. The objective was to evaluate the growth of Galla goats fed selected Brachiaria grass cultivars.

## Materials and methods

*Site description*

The feeding trial was conducted at the Sheep and Goat Multiplication Centre at Matuga ($4^{o}$ 9'6'S; $39^{o}$ 32'40'E), in Kwale County, Kenya. The Centre is located at 60 m asl in coastal lowlands 3 (CL3) agro-ecological zone, also referred to as the coconut-cassava zone (Jaetzold *et al*., 2006).

The average annual rainfall is 1100 mm while the relative humidity ranges from 70 - 80% and an average temperature from 22 - 30$^{o}$C.

*Management of Animals*

Sixteen Galla goat bucklings aged between 6-12 months and weighing 10-24 kg were selected from Centre herd. They were divided into four groups of four animals which were balanced for age and weight and randomly assigned to four dietary treatments. The goats were kept in well ventilated individual pens. Dry grass was used for bedding. Both the feeding and sleeping areas were disinfected before the goats were brought in. During the adjustment period, animals were dewormed against endo-parasites and sprayed weekly against ecto-parasites. The pens were cleaned every morning and beddings changed weekly.

*Feeds and feeding*

The Brachiaria cultivars used for feeding were *Brachiaria brizantha* cvs. Piata and MG4 and *B. hybrid* cv. Mulato II. Rhodes grass was used as the control. The cvs. Piata, MG4 and Rhodes grass were grown at KALRO-Katumani in the semi-arid region of eastern Kenya while Mulato II was grown at KALRO-Mtwapa in the coastal lowlands. The recommended agronomic practices were followed in order to provide good quality forage for feeding. During the harvesting, the grasses were cut at 5 cm above ground and allowed to dry, baled into hay and transported to Matuga.

During the feeding, all the animals were supplemented with a 100g/day of maize germ that was purchased from a commercial maize miller to last for the whole experiment. The supplement was given before the basal diets were offered at 7.00 hrs. Water and a mineral supplement were provided *ad libitum*. The hay made up of stem and leaves were chopped using a motorized chaff cutter to approximately 5 cm length and mixed thoroughly to prevent selection. The feeds were offered for a 14 days adaptation period and 12 weeks experimental period from mid-April to July 2016. The grass basal diet was offered *ad libitum* by offering feed in the morning and adding during the day to ensure feed availability at all times. Any feed that was not consumed was removed and weighed the following day before fresh feed was added.

*Chemical composition of feeds*

A small amount of herbage was taken from each bale used for feeding and a composite sample of about 2 kg per treatment constituted for analysis. The samples were ground to pass through 1 mm screen. The samples were then analysed in duplicates for chemical composition at the Animal and Nutrition Laboratory at KALRO-Muguga. The CP was determined using the micro-Kjeldahl according to the method of the Association of Official Analytical Chemists (AOAC, 2000). Neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, and digestibility were determined according to the procedure of Goering and Van Soest (1970). Ash was determined by heating the samples at 600$^{o}$C for 2 hours in a muffle furnace. Total P and Ca were determined according to the methods described by Okalebo *et al.* (2002).

*Data collection and calculation*

Data was collected on feed daily feed intake for each goat. Animals were weighed weekly before feeding (fasting weight) using a portable electronic weighing scale. Feed intake was calculated from the difference between the feed offered and refused. Live-weight changes were calculated as the difference between the initial and final weight while the average daily weight gain was obtained by dividing the weight change by number of experimental days (84 days).

**Data analysis**

The nutritive quality composition (DM, CP, OM, Ash, NDF, ADF, ADL, Ca, P) and digestibility of feeds were analysed using the general linear model (GLM) procedures of the Statistical Analysis System (SAS, 2010). Values for feed intake and live weight gain were subjected to analysis of variance (ANOVA) in a completely randomised design using GLM procedures of the Statistical Analysis System (SAS, 2010) based on the following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where $Y_{ij}$ is the $_j$th observation of the $_i$th treatment; μ is overall mean; T*i* is the effect of the feed of the $_i$th grass treatment (1-4) and $e_{ij}$ is the residual error. Means were separated by least significance difference (LSD) (Steel and Torrie, 1981).

**Results**

*Feed quality composition*

There were significance ($P < 0.05$) differences in the CP content, dry matter digestibility, ADF, ADL, ash, Ca and P content among the forages (Table 1). Piata had the highest CP content (12.6% of DM) while Mulato II had lowest ($P<0.05$). Similarly, MG4 and Piata were more digestible than Mulato II and Rhodes grass. All the Brachiaria cultivars had similar amount of Ca but were significantly ($P<0.05$) lower than that of Rhodes grass while P content was not different ($P>0.05$) among all the grasses.

*Feed intake*

The goats ate all (100 g) the maize germ supplements offered. The average feed intake on weekly basis for the entire feeding period is shown in Figure 1. There was no difference ($P > 0.05$) in the basal feed intake in all the weeks among the goats. Generally the average daily feed intake increased over time and ranged from 513 - 661 g/goat.

*Live weight gain*

Live weight increased marginally with goats fed on Piata and MG4 maintaining the highest weights during the entire period (Figure 2). Goats fed on Mulato II lost weight initially and gained from week 9. The average daily weight gain (ADWG) differed significantly ($P<0.05$) with bucklings fed on Piata (45.21g/day) and MG4 (41.28g/day) having the highest daily weight gain while those fed on Mulato II had the lowest. Likewise, the average weight change (AVC) was also highest in goats fed Piata (3.80 kg) and MG4 (3.47 kg) than those feed on Mulato II (0.17 kg) and Rhodes grass (0.81 kg).

**Table 1** Chemical composition (%) and digestibility (%) of feeds used in feeding the goats

| Feeds | CP | NDF | ADF | ADL | Ash | DoMD | DMD | Ca | P |
|---|---|---|---|---|---|---|---|---|---|
| Maize germ | 13.9 | 27.5 | 7.7 | 0.4 | 3.6 | 84.7 | 87.4 | 0.03 | 0.73 |
| Piata | 12.6 | 57.9 | 35.4 | 3.6 | 10.8 | 49.0 | 55.0 | 0.27 | 0.20 |
| MG4 | 12.1 | 57.1 | 36.9 | 4.3 | 10.7 | 48.7 | 55.5 | 0.27 | 0.22 |
| Rhodes | 6.7 | 68.6 | 44.3 | 5.5 | 7.7 | 39.8 | 44.6 | 0.39 | 0.08 |
| Mulato II | 3.0 | 70.7 | 46.9 | 6.3 | 5.0 | 38.2 | 41.4 | 0.27 | 0.19 |
| LSD (P<0.05) | 0.8 | 2.6 | 1.5 | 2.9 | 0.7 | 32.8 | 4.1 | 0.03 | 0.15 |
| CV (%) | 3.0 | 1.6 | 1.6 | 25.6 | 3.5 | 2.3 | 2.6 | 4.1 | 18.9 |

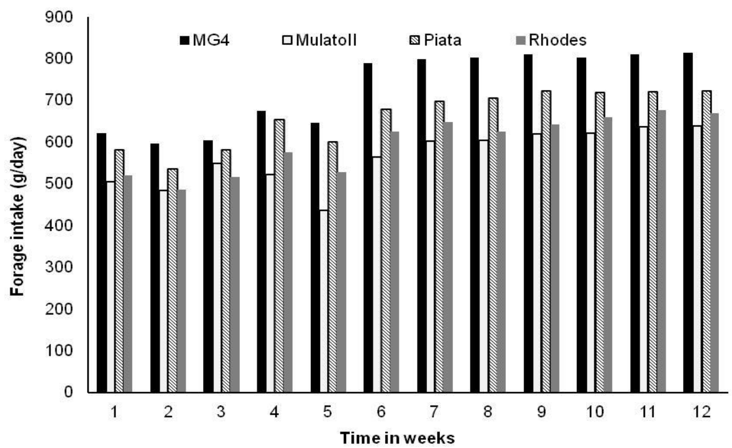


**Figure 1** Average weekly feed intake of goats fed on Brachiaria grass cultivars and Rhodes grass

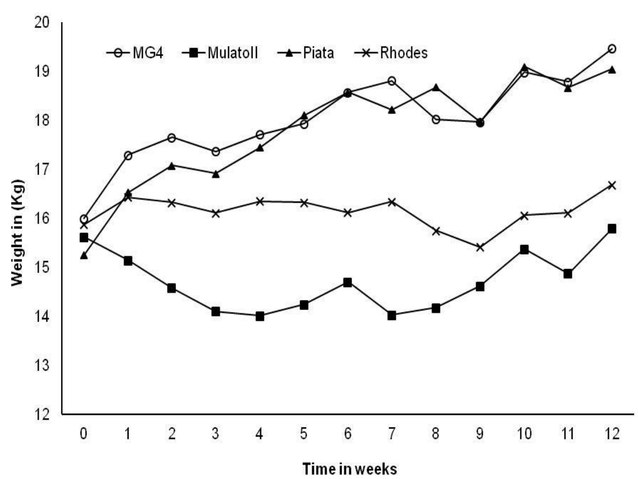


**Figure 2** Average weekly weights of goats fed on Brachiaria grass cultivars and Rhodes grass

**Table 2** Total and average weight gain of Galla goats fed Brachiaria cultivars and Rhodes grass.

| Feeds | IBW (kg) | FBW (kg) | AWC (kg) | ADWG (g/day) |
|---|---|---|---|---|
| MG4 | 16.00 | 19.47 | 3.47 | 41.3 |
| Mulato II | 15.63 | 15.80 | 0.17 | 2.0 |
| Piata | 15.25 | 19.05 | 3.80 | 45.2 |
| Rhodes | 15.87 | 16.68 | 0.81 | 9.6 |
| LSD (P<0.05) | | NS | 1.45 | 17.2 |
| CV (%) | | 31.3 | 43.9 | 43.9 |

IBW=Initial body weight; FBW =Final body weight, AWC=Average weight change; ADWG =Average daily weight gain; NS=Not significant.

## Discussion

*Feed quality composition*

In this study, Piata and MG4 were found to be better sources of protein than Mulato II and Rhodes grass. They contained the minimum CP of 7.5% suggested as necessary for optimum rumen function and production by Van Soest (1994). Afzal and Ullah (2007) reported that crude protein (CP) and digestible dry matter are the most important components of a feed. Crude protein requirement for small ruminant maintenance is 9.6, 11.2 and 11.7% for pregnant ewes, does and kid finishing respectively (NRC, 2007). The CP content of Mulato II content was low compared with that reported by Nguku (2015) of 7-12.8% in a semi-arid region of Kenya, 15% in central Kenya (Nyambati *et al*. 2016) and 12-17% by Vendramini *et al*. (2011) in Florida, USA. The low CP of Mulato II was attributed to poor management of the grass at harvesting and baling. Further Mulato II was grown in the coastal lowlands and generally due to the high temperatures experienced in the region, the growth was fast and accumulated more fibre resulting to low CP and digestibility. On the contrary Piata, MG4 and Rhodes were grown in mid-altitude region where it is cooler resulting in slower growth.

The DMD of Mulato II and Rhodes was lower than that reported by Ondiko *et al*. (2016) in coastal lowlands of Kenya. The digestibility of tropical grasses ranges between 50 and 65%, while that of temperate grasses is slightly higher and ranges between 65 and 80%. Coward-Lord *et al*. (1974) reported that the age of cutting forage crops has an influence on the *o* digestibility, and is a function of the chemical constituents of forages. These results agree with what Njarui *et al*. (2003) who reported that the proportion of potentially digestible components decline as the fibrous content increases.

*Live weight gains*

Bucklings fed on Piata and MG4 gained more weight on daily basis and had the highest total weight gain at the end of experiment. This weight gain was higher than that reported by Njarui *et al*. (2003) for Kenya Dual Purpose goats fed different forage legumes supplements and by Nyako *et al*. (2012) when fed on Pangola grass and supplemented with cotton seed cake. In another study by Wambui *et al*. (2006) on German Alpine crosses supplemented with Tithonia, Calliandra and Sesbania, the goats showed a high average daily weight gain of up to 82.7, 57.3 and 39.3 g/day, respectively. High weight gain for goats fed on Piata and MG4 is attributed to their high CP content, digestibility and low fibres. On the contrary bucklings fed Mulato II had the lowest gain due to low CP content.

## Conclusions

Piata and MG4 contributed to the highest growth of the Galla goats and were superior to Rhodes grass. Thus these grasses could replace Rhodes grass in the coastal lowlands as livestock feeds. Further research should be conducted on Mulato II taking into consideration its management to maintain high quality.

## Acknowledgements

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# Theme 5

# Seed Production and Diseases

# Potential for seed production of Brachiaria grass cultivars in the central highlands of Kenya

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**Abstract**

Brachiaria is a new forage grass in Kenya that has potential for improving livestock productivity but limited seed availability is the major constraints to increasing area under cultivation. A study was carried out to assess seed production potential and understand the phenological and seeding characteristic of different Brachiaria grass cultivars at Embu in the central highlands of Kenya. Nine Brachiaria grass cultivars (*Brachiaria brizantha* cvs. MG4, Piata, Marandu, Xaraes, *B. decumbens* cv. Basilisk, *B. humidicola* cv. Llanero, *B. hybrid* cvs. Mulato II, Cobra and Cayman) were evaluated along with a control; Rhodes grass (*Chloris gayana*). The plots were arranged in a randomized complete block design with four replications. The experiment was established during the long rains season, in April 2015 and monitoring for seed production was carried out for 12 months. The key phenological parameters recorded included; panicle formation, flowering period and seed yield. Time to 50% flowering ranged from 106 to 344 days after seedling emergence with Basilisk taking the shortest period and Xaraes the longest period. Generally, seed production was low in all Brachiaria grass and was significantly ($P < 0.05$) lower than the control, Rhodes grass (307 kg ha$^{-1}$) due to inability of most flowers to form seeds. The cv. Llanero (178 kg ha$^{-1}$) had the highest seed yield while Marandu (23 kg ha$^{-1}$) recorded the lowest yield. There is need to continue monitoring seed production for more seasons to establish the actual potential for seed production.

**Key words**: Brachiaria grass; time to 50% flowering, phenology information; seed yield

## Introduction

The Brachiaria grasses belong to the poacea family, are classified as $C_4$ plants and the life cycle can be either annual or perennial. The genus Brachiaria includes about 100 species which are distributed in the tropical and subtropical regions of both eastern and western hemispheres but mostly in Africa (Renvoize *et al*., 1996). The most common and extensively cultivated Brachiaria species for pastures are; *B. brizantha, B. ruziziensis, B. decumbens* and *B. mutica,* but recently *B. humidicola* and *B. platynota* have also received increased attention (Ndikumana and de Leeuw, 1996). The genus *Brachiaria* originated from Africa but is now widely cultivated in sub-tropical and tropical regions of Australia and South America (Parsons, 1972). An estimated 99 million hectares are planted in Brazil (Jank *et al*., 2014) and about 300,000 hectares in Asia, the South Pacific and Australia (Stur *et al*., 1996). In the Pacific region, over 10,000 hectares of Brachiaria *hybrid* cv. Mulato II pastures were established in Vanuatu since 2007, where it is primarily used for beef cattle grazing (Pizzaro *et al.*, 2013). In USA, nearly 200,000 hectares of Mulato II pastures had been established by 2005 for both dairy and beef cattle grazing (Esteban *et al*., 2013). In Kenya a few cultivars of Brachiaria grass have been introduced to over 4000 farmers (Njarui, per comm.) while in Rwanda Mulato II was introduced to smallholder farmers (Mutimura and Everson, 2012). Feeding Brachiaria grass to dairy cattle showed increased milk production by 15 to 40% in Kenya (Ghimire *et al*., 2015). However, limited seed availability is the major constraints to increasing area under cultivation.

All Brachiaria species can be propagated both vegetatively and from seeds. Vegetative propagation is simple but impracticable except in very small-scale farming (Hopkinson *et al*., 1996) necessitating the need for extensive seed production. Developing an appropriate method for harvesting Brachiaria seeds is important for producing high quality seeds to be commercially available to farmers at a reasonable price. A number of studies have been conducted to determine the most suitable methods for harvesting Brachiaria seeds. The work of Hare *et al*. (2007) found out that high seed yields are obtained from multiple, non-destructive manual harvests, with seed heads tied into living sheaves and the seed knocked daily into seed-net receptacles. Normally in Thailand, seed is ground swept while in Laos it is harvested by knocking the seeds from seedheads (Pizzaro *et al*., 2013). In Mexico and Brazil, all the seeds are ground-swept using machinery (Pizarro *et al*., 2010). The Brachiaria seed industry in Brazil has grown to meet the large internal demand and an expanding export market placing it in competition, in terms of monetary value with major cereal crops (Santos Filho, 1996). Thus, if Brachiaria grasses seeds are made available to smallholder farmers in Kenya, they could boost the forage resource base and propel the livestock industry. Additionally, farmers would benefit through increased incomes from seed trade enterprises and contribute to growth of Kenya economy. The work reported in this paper was conducted to assess the seed production potential and understand the phenological and seeding characteristics of the Brachiaria grasses in central highlands of Kenya.

**Materials and methods**

*Site description*

The study was carried out at the Kenya Agricultural and Livestock Research Organization (KALRO), Embu which is situated 3 km north of Embu town. The centre lies at latitude, $0^0$ 30’S and longitude $37^0$ 27’E, at an elevation of 1492 m asl. The average annual rainfall is 1252 mm, is bimodal with the long rains occurring from mid-March to September and average 650 mm. The short rains occurs from mid-October to February and average 450 mm. The mean annual temperature is $19.5^0$C, with mean maximum and minimum of $25^0$C and $14.1^0$C, respectively. The mean annual potential evaporation is 1422 mm while mean annual evapo-transpiration is 950 mm. The site lies in the transition of Upper Midlands (UM) 2 and UM 3 agro-ecological zones. The soils are mainly humic Nitisols (FAO-UNESCO, 1994) and are derived from basic volcanic rocks. They are deep, highly weathered with friable clay texture and moderate to high inherent fertility (Jaetzold *et al*., 2006).

*Experimental design and treatments*

The experiment was set out in a randomized complete block design with four replications. Plot sizes were 5 m x 4 m with a 1 m path between plots and replications. The seeds were drilled in furrows at about 1.5 cm deep on a well prepared seedbed with an inter-row spacing of 0.5 m, giving 10 rows in each plot. A seeding rate of 5 kg ha$^{-1}$ was used. Triple supper phosphate (TSP, 46% $P_2O_5$) fertilizer was applied during planting at a rate of 40 kg P kg ha$^{-1}$. Calcium ammonium phosphate (CAN, 26% N) was applied at rate of 50 kg N kg ha$^{-1}$ in each season and application commenced from the second wet season. The trial was kept weed free throughout by hand weeding.

**Data collection and analysis**

Phenology data collected include, days to initial and 50% flowering, number of inflorescence in 1 m$^2$, days to initial and to 50% caryopsis hardening and seed yield. Additional information was recorded on plant growth and included plot cover and height at 50% flowering. An area of 2 m x 2 m was marked for determining seed yield. At commencement of maturity, the seeds were harvested by gently shaking the seeds from the inflorescence into a bucket on alternate days. Harvested seeds were dried in bags and weighed. Plants were cut back after all the seeds were harvested for each cultivar, to allow new tillers to develop and form seeds.

The data on growth parameters, phenology and seed yield were entered in an Excel computer spread sheet program and then subjected to an analysis of variance using the SAS (2001) computer software package. The significance of the F-values was determined at the 5% probability levels of significance.

**Results**

*Rainfall*

Rainfall during the experimental period was generally high and evenly distributed. During the first three months after planting the Brachiaria grasses (April-June 2014) the total rainfall received was 650 mm while the short rains 2014 the total amount received was 730 mm (Figure 1.) with peak in April and November, respectively. During the long rains 2015 season the total amount of rains declined to 535 mm.

*Plant growth*

Generally, all the Brachiaria cultivars establishment successfully except Mulato II which had poor seedling emergence. Initial growth was slow but most of the Brachiaria cultivars attained reasonable ground cover within 30 days after seedling emergence and a complete plot cover (100%) at 50% flowering (Table 1). Mulatto II had significantly ($P < 0.05$) lower ground cover (63%) than the other Brachiaria grasses at 50% flowering. Llanero, Marandu, Piata and Xaraes achieved complete (100%) plot cover, the same as Rhodes grass. There were significant ($P < 0.05$) differences on plant height at 50% flowering among the cultivars (Table 1). Piata, Xaraes and Marandu were the tallest while Cayman, Cobra, Mulato II and MG4 had the lowest height.

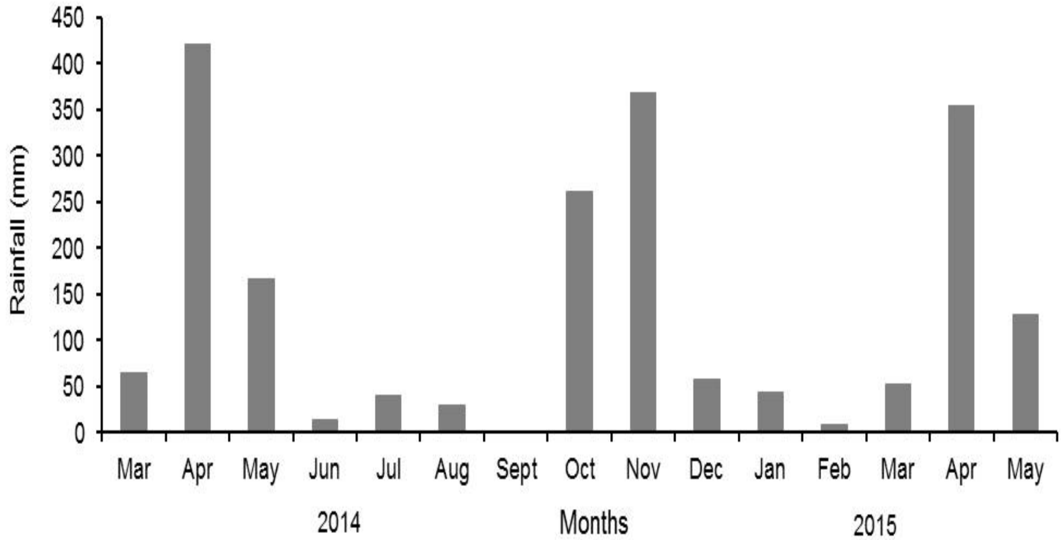


**Figure 1** Total monthly rainfall at Embu during the experimental period (2014–2015)

**Table 1** Plot cover and plant height of Brachiaria cultivars and Rhodes grass at 50% flowering at Embu, central highlands.

| Grass cultivars | Plot cover (%) | Plot cover (%) | Plant height (cm) |
|---|---|---|---|
| Basilisk | 22 | 88 | 108 |
| Cayman | 22.8 | 91.2 | 83 |
| Cobra | 18.8 | 75.2 | 73 |
| Llanero | 25 | 100 | 104 |
| Marandu | 25 | 100 | 219 |
| MG4 | 22.8 | 91.2 | 86 |
| Mulatto II | 15.8 | 63.2 | 64 |
| Piata | 25 | 100 | 231 |
| Xaraes | 25 | 100 | 229 |
| Rhodes grass | 25 | 100 | 74 |
| CV (%) | 12.9 | 12.9 | 11.2 |
| SED | 2.04 | 8.16 | 5.26 |

**Flowering and seed production**

There was large variation in time to flowering among the Brachiaria grasses. All the nine cultivars took longer time to commence flowering and to reach 50% flowering than the control, Rhodes grass. Basilisk, Cayman, Cobra and MG4 took relatively shorter time to commence flowering (91 - 108 days) and to reach 50% flowering (113 - 141 days) while Piata, Marandu, Llanero and Xaraes took the longest time to commence flowering (128 - 206 days). Piata, Marandu, Llanero took the longest time to attain 50% flowering while Xaraes did not reach 50% flowering during the period of the study. The highest number of inflorescence occurred in Cobra (339 flowers/$m^2$) followed by Mulato II and were more than Rhodes grass.

The number of days to seed maturity, denoted by hardening of the caryopsis, ranged from 150 to 362 days (Table 2). Cobra took the shortest period but was not significantly ($P > 0.05$) shorter than Rhodes grass. Cayman and Mulato II followed closely while Xaraes took the longest period. Basilisk and MG4 took similar number of days to reach maturity while Marandu, Piata and Xaraes also had similar number of days to seed maturity. The amount of seed produced for all Brachiaria grass was less than 60 kg/ha except Llanero which produced 178 kg $ha^{-1}$ and was significantly higher ($P < 0.05$) than the other cultivars (Figure 2). The control, Rhodes grass produced more ($P < 0.05$) seeds (307 kg $ha^{-1}$) than all the Brachiaria cultivars.

**Table 2** Flowers and caryopsis development of Brachiaria grass cultivars and Rhodes grass in Embu, central highlands of Kenya.

| Grass cultivars | Days to initial flowering | Days to 50% flowering | Number of inflorescence ($m^{-2}$) | Days to initial caryopsis hardening | Days to 50% caryopsis hardening |
|---|---|---|---|---|---|
| Basilisk | 90 | 106 | 136 | 184 | 194 |
| Cayman | 103 | 116 | 339 | 163 | 191 |
| Cobra | 108 | 141 | 302 | 150 | 204 |
| Llanero | 128 | 272 | 179 | 300 | 308 |
| Marandu | 128 | 321 | - | 330 | 364 |
| MG4 | 91 | 113 | 123 | 184 | 197 |
| Mulato II | 115 | 176 | 317 | 166 | 192 |
| Piata | 131 | 229 | - | 354 | 374 |
| Xaraes | 206 | - | - | 362 | 386 |
| Rhodes | 67 | 105 | 72 | 153* | 187* |
| CV | 10.2 | 6.44 | - | 3.1 | 4.1 |
| SED | 8.5 | 7 | - | 4.6 | 4.89 |

*Seed maturity for Rhodes grass was indicated by brown caryopsis

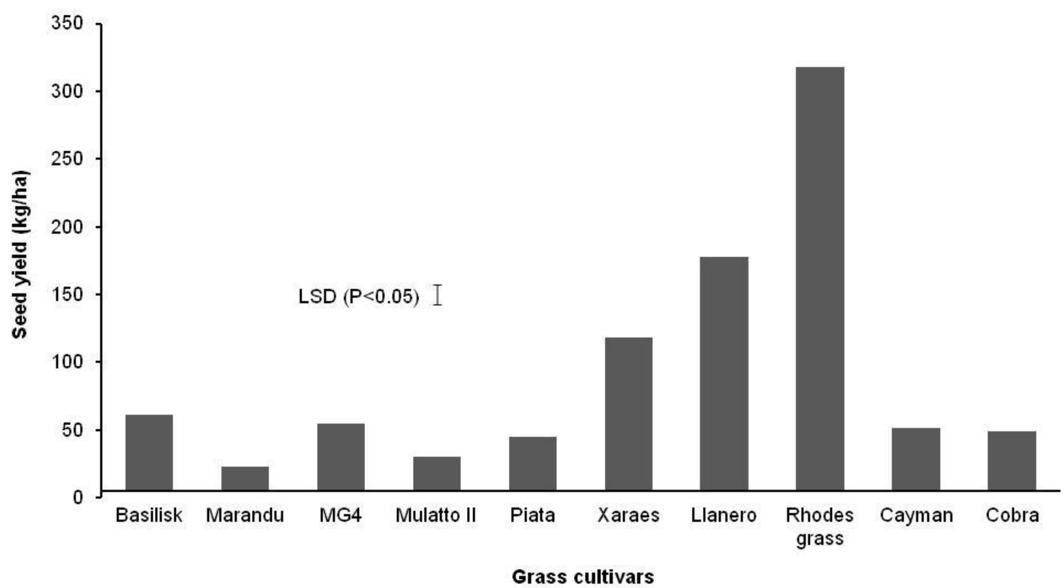


**Figure 2** Seed yield of Brachiaria cultivars and Rhodes grass at Embu, central highlands

## Discussion

All Brachiaria cultivars established successfully and attained good vegetative plot cover within the first month after seedling emergence and complete plot cover at 50% flowering. The height attained by the different Brachiaria cultivars was closely related to the time they took to flower. Those cultivars that took longer to flower were comparatively tall and vice versa for the others. However, the difference in plant height is genetically controlled but the plants also differed in growth habit. Llanero has a prostrate growth habit while the other Brachiaria had erect growth habit.

The variation in the number of days to flowering and to seed maturity (hardening of the caryopsis) was large. Based on day to initiation of flowering and seed maturity, the grasses can be divided in two groups. Basilisk, Cayman, Cobra, Mulato II and MG4 are early maturing while Piata, Marandu, Llanero and Xaraes could be regarded as late maturing. This classification is similar to that reported by Kamidi *et al*. (2016; this proceedings) for some of cultivars grown in north western highland of Kenya. They grouped MG4, Basilisk and Cayman as early maturing together with Piata. In another study at Kitale in western Kenya, *B. ruziziensis* flowered 147 days after seedling emergence while regrowth headed as early as 21 days after cutting (Boonman, 1971). Generally, seeds production was low in all the Brachiaria cultivars and this has also been reported in Thailand (Esteban *et al*., 2013; Hare *et al*., 2007). Esteban *et al*. (2013) reported seeds yield of 150 kg $ha^{-1}$ from Mulato II and Cayman. Earlier work by Hare *et al*. (2007) reported less than 200 kg/ha of seed from Mulato II hybrid in Mexico and attributed the low seed yields to pollen sterility and poor caryopsis maturation. The different quantity of seeds produced by the various Brachiaria cultivars is attributed to the difference in genetic characteristics and environmental conditions. Although most of the grasses flowered profusely, this was not reflected in seed production due to failure to form seeds. Nevertheless, most of the tillers of Marandu did not produce any inflorescence and this contributed to low seed yield. However, it is important to point out that seed harvesting coincided short rains 2014 and long rains 2015 seasons. This had a negative impact on the seed recovery as most of the seeds were washed down by rain water and it was not possible to recover it from the mud. Thus, seed yields realised from this experiment from all the Brachiaria does represent the actual quantities of seed produced.

## Conclusions

Although most of the Brachiaria grasses flowered profusely, there was poor seed formation and consequently seed yield was low. On the basis of seed production, Llanero was the most promising. The experiment was conducted in one year and thus further monitoring would be necessary to ascertain seed production for longer period. In view of the fact reasonable quantity of seeds was not harvest due to wet season, there is need to identify suitable time for planting so that seed maturity does not coincide with rainy season.

**Acknowledgements**

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# The potential of Brachiaria grass cultivars to produce seed in north Western highlands of Kenya

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## Abstract

Brachiaria cultivars recently introduced in the North western highlands of Kenya have high biomass yield and are of superior nutritional quality compared to locally cultivated forages and therefore are highly likely to be adopted by most farmers in the region. There is no commercial Brachiaria seed production in Kenya and imported seeds from South America are expensive. The seed production potential of these grasses in Kenya is still unknown. The objective of the study was to evaluate the seed production potential of six Brachiaria cultivars in North western highlands of Kenya. The cultivars evaluated were, *Brachiaria brizantha* cvs. Xaraes, MG4 and Piata, *B. decumbens* cv. Basilisk and *B. hybrid* cvs. Cayman and Cobra. Data collected included: the number of plants and tillers, plant height, number of inflorescence and seed yield. Results showed that MG4, Piata Basilisk, and Cayman were earlier maturing, reaching 50% flowering within 253 days after seedling emergence (DAE) while the late maturing cultivar, Xaraes achieved 50% flowering in 337 DAE. Significant ($P < 0.05$) differences in the number of inflorescence were noted, with Basilisk, MG4 and Cayman having higher number of inflorescence than Cobra, Piata and Xaraes. A similar trend was observed in seed yield with MG4 and Cayman producing the highest yields; 211 and 192 kg $ha^{-1}$ respectively, while Xaraes had the lowest (18 kg $ha^{-1}$).. Although evidence of flowering and seed formation was observed in all the Brachiaria cultivars, the yields were low due to effect of diseases. There is need to develop an integrated approach to control the diseases in order to enhance seed production.

**Keywords:** Brachiaria, cultivar, diseases, seed production,

## Introduction

Agriculture contributes about 25% of Kenya's gross domestic product (GDP) (KARI, 2009) with the dairy sector contributing 14% of the agricultural GDP (Kiptarus, 2005). Farmers in the north western highlands of Kenya practice mixed farming in which maize and dairy are the main enterprises even though there is a trend to shift to dairy farming because of high production costs and poor prices for maize crop. A major constraint to livestock production in the region is the acute feed shortages that occur during the dry seasons and limited availability of forages of high nutritional quality (Ndung'u-Magiroi *et al*., 2016). The commonly cultivated forage, Rhodes grass (*Chloris gayana*), has limited adaptation. Napier grass, the commonly cultivated fodder by dairy farmers in region is susceptible to smut and stunt threaten its survival and production (Orodho, 2006, Maass *et al*., 2015). With the decreasing land sizes due to subdivisions and the need to produce more feed to sustain the dairy industry in the region, there is need to introduce other grass species that are more productive and with higher nutritional value.

Brachiaria grass, a native of east and central Africa was introduced to Latin America, Southeast Asia and Australia where it has revolutionized grassland farming and animal production (Ndikumana and de Leeuw, 1996). The implementation of the Swedish funded research programme "Climate-smart Brachiaria grasses for improved livestock production in East Africa has led to high publicity of the importance of Brachiaria grasses which has created big interest and high demand for seed among farmers across Kenya (BecA, 2014). The programme aimed at increasing animal productivity through enhanced feed availability using climate smart *Brachiaria* grasses. Brachiaria grass can be established using either seed or vegetative material. Establishment by vegetative material is labour intensive and is more expensive than establishment by seed, which can easily be mechanized (Kandemir and Saygili, 2015, Maass *et al*., 2015). Many Brachiaria cultivars reproduce through apomixis (Araujo *et al.,* 2007) which is seed formation without fertilization (Kandemir and Saygili, 2015). The seeds produce plants that are identical to the mother plants (Hare *et al.*, 2007) thus enabling production of seeds that are true to type.

Seed production potential can be linked to environmental factors (Monteiro *et al*., 2016), and in the humid lowland tropics, especially near the equator grass seed production can be a big challenge (Maass *et al*., 2015; Phaikaew *et al*., 1997). Many species which grow well in such areas often do not produce seed and those that do such as *Brachiaria decumbens* cv Basilisk, the seed yields are normally very low (Hare *et al*., 2015). Hare *et al.,* (2015) observed that altitude and latitude influence flowering and seed setting in *Brachiaria* hybrids. In Brazil and Thailand for example successful Brachiaria seed production is done in latitudes 20°-22°S and at elevations of 700-1000 m above sea level and is in latitudes 19°-20° N at 700-1200 m asl, respectively. Currently there is no commercial Brachiaria seed production in Kenya and the imported seeds from South America are expensive. Therefore, there is need to identify suitable regions where the seed can be produced locally to meet anticipated demand. The objective of the study was to evaluate the seed production potential of Brachiaria cultivars in north western highlands of Kenya.

## Materials and methods

### *Description of the study site*

The study was conducted at Kenya Agricultural and Livestock Research Organization (KALRO) Kitale farm (1° 0′ 6.6′′N and 34° 59′ 10′′E), at 1890 m asl. The mean annual rainfall is 1140 mm and is unimodal occurring from March to November with peaks in May and August and with a distinct dry spell from December to March. The region experiences annual mean minimum and maximum temperatures of 12° C and 25° C, respectively (Jaetzold *et al*., 2012). The dominant soils are humic Acrisols (Jones *et al*., 2013) and are deficient in nitrogen and phosphorus.

*Treatments and experimental design*

Six Brachiaria cultivars cvs Xaraes, MG4, Piata, Basilisk, Cayman and Cobra were evaluated for their seed production potential. The treatments were laid out in a randomized complete block with three replications in plots measuring 4 x 5 m. Triple super phosphate (TSP 46% $P_2O_5$) was applied in all plots at the rate of 200 kg TSP $ha^{-1}$. The seeds were drilled by hand in 2 cm deep furrows separated with an inter-row spacing of 0.5m at a seed rate of 5 kg $ha^{-1}$ and covered with a thin layer of soil. The plots were kept weed free throughout the experimental period by hand weeding. The grasses were irrigated during the dry periods.

*Data collection and analysis*

To assess plant establishment, data was collected on number of plant and tillers and plant height. The phenological data was collected on time to commencement and 50% flowering and number of flowers and seed yield. Seeds were harvested from an area measuring 2 x 2m within the plot by shaking the seeds into labeled khaki bags after every 2 days and sweeping the ground after cutting back to recover fallen seeds. The harvested seeds were weighed and a 1000 seeds weight determined. Germination tests were carried out 2 months after harvesting in Petri-dishes as described by Koech *et al*. (2014) and in soil. Pest and diseases incidence and damage were also monitored using a Likert scale of 0-5 where 0=no pest or diseases incidence/damage and 5=highest incidence /damage.

Treatment effects on seed yield and other plant growth parameters (number of plants/$m^2$, number of tillers/plant, plant height, days to first flowering and 50% flowering, number of inflorescence per plant and per $m^2$, disease scores) were tested using the analysis of variance (ANOVA) using SAS statistical package (SAS, 2003). Differences at $P<0.05$ were considered significant and the means separated using least significant difference (LSD) as described by Steel and Torrie (1986).

**Results and Discussion**

*Seasonal condition*

The rainfall and temperature during the experimental period in 2015/2016 is given in Figure 1. Rainfall was highest in May and June in 2015 (>200 mm) while in 2016 it was highest in April (>250 mm). Mean monthly temperatures ranged from 19.8 - 22.2$^{o}$C and were lower than the optimum range of 25-35$^{o}$C.

*Plant establishment and seed production*

*Brachiaria hybrid* cv. Cobra had significantly higher number of plants ($P < 0.05$) than the other cultivars (Table 1). Piata and Xaraes were more erect and significantly taller ($P <0.01$) than all the other Brachiaria cultivars (Table 1). Taller cultivars are usually prone to lodging that creates moist conditions under the canopy which interferes with seed production and harvesting (Gobius *et al*., 2001). The cultivars Piata and Xaraes had the highest lodging percentages and were highly affected by pathogens confirming earlier observations made by Andrade de *et al.* (1983). The cvs MG4 and Basilisk had the highest number of tillers and were significantly higher ($P < 0.05$) than those of the other cultivars (Table 1). Tropical grasses produce more seed under conditions of closer tiller density which is obtained by closer spacing, as was observed by Boonman (1973) and Andrade de *et al.* (1983). Gobius *et al*. (2001) observed that lower plant population than the recommended resulted in reduced seed yields.

Phenological development differed significantly ($P<0.05$) among the cultivars as expressed by the appearance of first flower and days to 50% flowering (Table 1). Early appearance of first flower was recorded from MG4, Basilisk, Cayman, Cobra and Piata which took 246 days for the first flower to appear. The number of days to 50% flowering followed a similar trend.

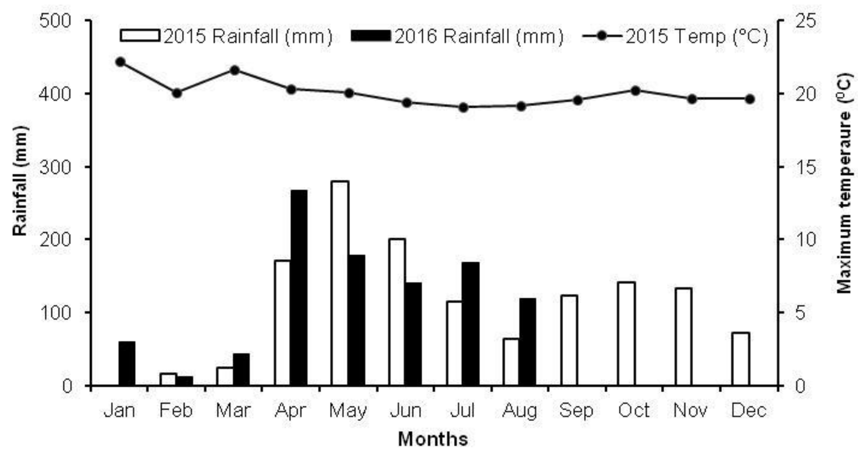


**Figure 1** Monthly rainfall for 2015 and 2016 and maximum temperature for 2015

**Table 1** Components of seed yield and seed yield of Brachiaria grass cultivars at Kitale

| Cultivar | Plants/ m$^2$ | Plant height (cm) | Tillers/ plant | Number of days to flowering | | Number of inflorescence | | 1000 seed weight (g) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | First appearance | 50% flowers | per plant | per m$^2$ | | |
| Basilisk | 9.5 | 119.0 | 96.3 | 93.8 | 246.0 | 9.1 | 86.5 | 3.5 | 96.9 |
| Piata | 8.0 | 205.3 | 52.8 | 144.3 | 252.5 | 3.0 | 24 | 4.0 | 56.3 |
| Cayman | 7.8 | 117.8 | 65.8 | 131.8 | 250.0 | 8.7 | 67.9 | 3.0 | 191.9 |
| Xaraes | 8.3 | 170.8 | 54.5 | 245.7 | 327.0 | 2.0 | 16,6 | 4.0 | 18.7 |
| Cobra | 12.8 | 101.0 | 66.3 | 99.8 | 267.3 | 2.6 | 33.3 | 5.0 | 124.4 |
| MG4 | 7.8 | 126.0 | 126.8 | 94.5 | 243.0 | 9.5 | 74.1 | 4.0 | 211.0 |
| Mean | 9.0 | 139.1 | 77.0 | 139.7 | 264.3 | 5.8 | 50.4 | 3.9 | 116.6 |
| LSD | 3.0 | 27.0 | 51.8 | NS | 11.7 | 3.9 | 11.7 | 0.7 | 113.7 |

The effects of cultivar on seed yield was significant and ranged 18.7 to 211 kg ha$^{-1}$ with cvs MG4 and Xaraes recording the highest and lowest amount of seeds, respectively (Table 1). Seed yield was negatively related with the number of days to 50% flowering though not consistent (Figure 2) indicating that the cultivars that produced flowers much earlier tended to produce more seeds. These results are in agreement with observations made by Boonman (1973) that early maturing cultivars of some tropical grasses produce more seed than the late maturing ones.

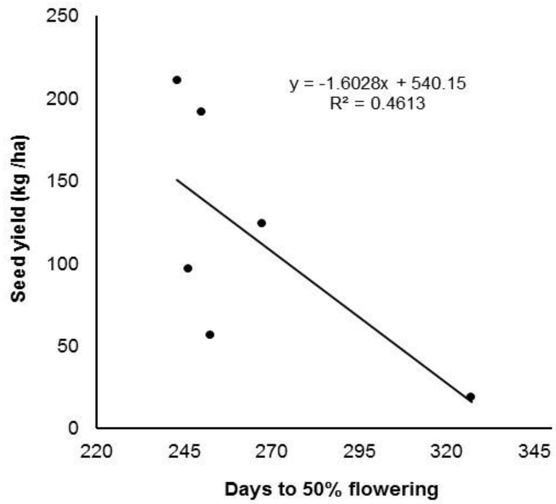


**Figure 2** The relationship between seed yield and days to 50% flowering

There was a positive and significant relationship between seed yield and the number of tillers per plant (Figure 3a) and number of inflorescence per plant (Figure 3b). Cultivars MG4, Basilisk and Cayman, had higher ($P < 0.01$) number of tillers and inflorescence per plant than Piata, Cobra and Xaraes and tended to produce more seed. These results confirm earlier findings by Hare *et al*. (2015) who observed very few inflorescences in Xaraes and corresponding low seed production. Our results contradict the findings by Monteiro *et al*. (2016) who found no direct relationship between the number of vegetative tillers and seed yield in some tropical grasses.

Although the seed yields obtained in this study were low, they are comparable to those reported elsewhere. For example, Gobius *et al*. (200I) reported yields ranging from 81 to 123 kg ha$^{-1}$ for cv. Basilisk compared to 97 kg ha$^{-1}$obtained in this study. Pizarro *et al.* (2013) obtained seed yields of 150 kg ha$^{-1}$ for Cayman which was lower than 192 kg ha$^{-1}$ recorded in this study though the yields are lower than those recommended (600 - 700 kg ha$^{-1}$) for viable commercial production with competitive prices (Hare *et al*., 2015). Phaikaew *et al*. (1997) reported that seed production in the humid lowland tropics near the equator was difficult and this would explain the low yields obtained in this study where the site lies at 1$^{o}$N and 1890 m above sea level. Hare *et al.* (2015) found that in Brazil, successful Brachiaria seed production was possible in latitudes 20$^{o}$-22$^{o}$S at elevations of 700-1000m above sea level, while in Thailand production of seed is done in areas between latitudes 19$^{o}$-22$^{o}$ N and at elevations between 700 to1200 m above sea level. Similarly, Andrade (2001) found latitudes 15$^{o}$-22$^{o}$ S, with annual rainfall not exceeding 1500mm with a distinct dry spell during harvesting to be suitable for Brachiaria seed production. The seeds produced in this study failed to germinate when tested. Several factors may have contributed to the failure of the seed to germinate. Some of the seed may have failed to form caryopsis due to environmental factors as has also been reported by Araujo *et al*. (2007) and Hare *et al.* (2007), abscission that is quite common in Brachiaria (Hare *et al*., 2015). The seeds also might have failed to germinate because of dormancy (Bouathong *et al*., 2011; Koech *et al*., 2014). Generally longer periods are required to break seed dormancy.

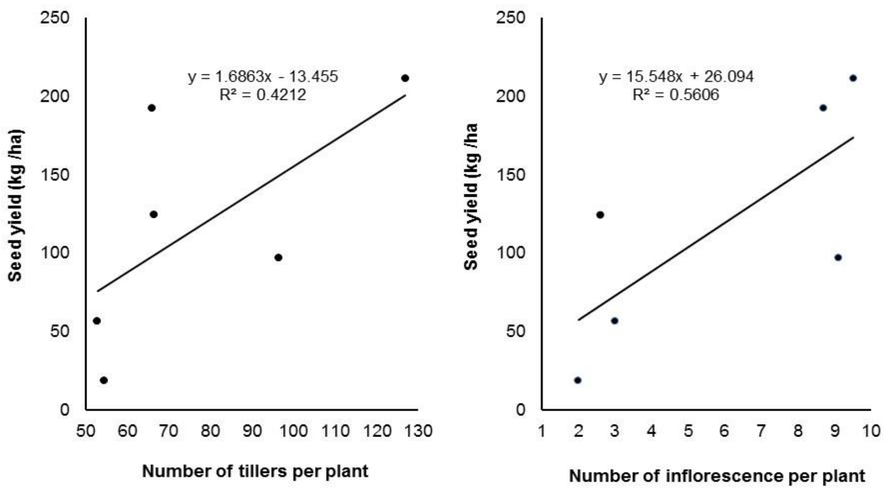


**Figure 3** Relationship between seed yield and number of a) tillers and b) inflorescence per plant

*Diseases damage*

The main prevalent fungal diseases on Brachiaria were rust, smut and ergot (Table 2). Ergot infected the inflorescence after the shedding of pollen. Cultivars Xaraes, Piata and MG4 exhibited significantly ($P<0.05$) lower incidence than the other cultivars, but the extent did not differ significantly ($P<0.05$). Smut (*Ustilaginoidea virens*), a black fungus on the seed heads which gives them a compact appearance, mainly affected the diseased (ergot) seeds. Cobra had the highest incidence and extent of damage by smut compared to the other cultivars. Normally if damage is severe, it leads to death of the affected inflorescences. Leaf rust (*Uromyces setariae-italicae*) is a fungal disease that was noted on the Brachiaria leaf blades, which gives a characteristic deep blue to black spots. The disease incidence was highest during the periods of slowed growth especially towards grass maturity. Cultivars Piata and Cobra had high incidence and damage by rust while Xaraes, Basilisk and Cayman were not attacked implying that they could be resistant to leaf rust. Monteiro *et al.* (2016) has cited diseases and pests as one of the causes of low seed production in Brachiaria grasses.

**Table 2** Incidences and extents of damage by diseases in Brachiaria cultivars

| Grass cultivars | Rust | | Smut | | Ergot | |
|---|---|---|---|---|---|---|
| | Incidence | Extent | Incidence | Extent | Incident | Extent |
| Basilisk | 0.00 | - | 1.63 | 3.00 | 2.25 | 2.75 |
| Piata | 2.72 | 1.40 | 0.38 | 1.28 | 0.26 | 1.50 |
| Cayman | 0.00 | - | 1.14 | 3.26 | 2.03 | 2.50 |
| Xaraes | 0.00 | - | 0.76 | 2.00 | 0.01 | 1.25 |
| Cobra | 1.75 | 1.38 | 3.13 | 3.88 | 1.13 | 1.51 |
| MG4 | 0.01 | - | 0.15 | 1.52 | 0.26 | 1.25 |
| LSD | 0.75 | 0.36 | 2.05 | 2.12 | 1.56 | 1.65 |

## Conclusions

Although evidence of flowering and seed formation was observed in all the Brachiaria cultivars, the yields were low due to effects of diseases. All the cultivars exhibited different levels of susceptibility to rust, smut and ergot diseases that had a negative effect on seed production. There is need for developing an integrated approach to control the diseases


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# Diseases of improved Brachiaria grass cultivars in Kenya

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**Abstract**

Brachiaria is one of the most important tropical forages of African origin. Some Brachiaria species have been improved for quality, biomass production and persistence to biotic and abiotic stresses. The production of Napier grass, the most important forage species in the East Africa has been threatened by stunt and smut diseases. Therefore, effort has been in place to explore the potential of improved Brachiaria grass from South America as an additional forage option. A research program of BecA-ILRI Hub in partnership with KALRO evaluated eight Brachiaria cultivars (Basilisk, Humidicola, Llanero, Marandu, MG4, Mulato II, Piata and Xaraes) at multi-locations in Kenya starting from November 2013. Experimental plots at Nairobi, Ithookwe, Katumani, Msabaha, Mariakani, Mtwapa and Ol Joro Orok were surveyed for the incidence and severity of the diseases. Leaf spots, leaf blights, leaf rust and physiological disorders were observed in four or more sites surveyed while ergot and smut diseases occurred in two sites. Fungi associated with symptomatic tissues were isolated and identified using fungal specific primers. Thirty six fungal taxa were associated with symptomatic tissues of improved Brachiaria cultivars. *Phoma herbarum, Epicoccum nigrum*, *Fusarium equiseti*, *Cladosporium cladosporoides* and *Nigrospora oryzae* were the most frequently detected species. Future studies to confirm the association of pathogen with symptom (Koch's postulate), and the estimation of yield loss and management of diseases of economic importance have been suggested.

**Keywords:** Brachiaria, ergot, incidence, leaf rust, leaf spots, pathogens, severity, smut.

## Introduction

Brachiaria is one of the most important forage grass in the tropics and sub tropics (Keller-Grein *et al*., 1996). Among 100 documented species: *B. brizantha*, *B. decumbens*, *B. humidicola* and *B. ruziziensis* and hybrids (Mulato, Mulato II and Cayman) are of significant importance as they have been improved for quality, quantity and persistence and grown in large acreages across the world (Miles *et al*., 2004; Rao *et al*., 2011). The production of Napier grass, the most important forage grass in Kenya and other East African countries has been threatened by smut and stunt diseases urging the need for alternative forage species to fulfill demand of ever increasing livestock populations in the region. High biomass production potential, highly nutritive herbage, adaptation to low fertility and drought, high nitrogen use efficiencies and other environmental benefits makes Brachiaria grasses an ideal forage option. Therefore, Kenya Agricultural and Livestock Research Organization (KALRO) initiated Brachiaria evaluation in 2011 with the introduction of hybrid Mulato II, and a year later joined a larger initiative of the Biosciences eastern and central Africa - International Livestock Research Institute (BecA - ILRI) Hub to promote Brachiaria grass as an additional forage option. Under this program eight commercial cultivars (*B. brizantha* cv. Marandu, MG4, Piata and Xaraes, *B. decumbens* cv.

Basilisk, *B. hybrid* cv. Mulato II, *B. humidicola* cvs. Humidicola and Llanero) from South America were introduced and evaluated in multiple locations in Kenya for biomass production and adaptation to drought and low soil fertility. As the genetic materials developed in exotic environment were being evaluated in the sites within the centre of diversity of Brachiaria species priority was given to monitor response of these genetic materials to the diseases. As expected some of these cultivars were attacked by diseases.

In the pasture production, diseases are major biotic constraint that reduces herbage and seed yields, lowers nutritive value and palatability of grasslands which eventually impact not only on animal health and productivity but also increase production costs (Pennypacker, 1997). Some Brachiaria diseases reported elsewhere include rust (*Uromyces setariae-italicae*), foliar leaf blight (*Rhizoctonia solani*), bacterial root rot (*Erwinia chrysanthemi pv. zeae*), leaf spot (*Dreschslera sp)* and sugarcane mosaic virus (Kelemu *et al*., 1995; Lenné and Trutmann, 1994). In tropical America, *B. brizantha* is susceptible to rust, foliar leaf blight and bacterial root rot (CIAT, 1992). In *Brachiaria humidicola*, rust can cause yield losses as high as 100% (Lenné, 1990). Therefore, this study was conducted to monitor diseases incidence and severity on improved Brachiaria cultivars planted in multiple locations in Kenya or and identify the causal agent associated with symptomatic Brachiaria plants using molecular technique. The information on the causal agent would be of great value in devising appropriate measures for the effective management of the diseases.

## Materials and methods

### Disease survey and sample collection

Brachiaria experimental plots established at Nairobi (ILRI Campus), Mtwapa, Msabaha, Mariakani, Katumani, Ithookwe, and Ol Joro Orok were surveyed for the disease incidence and severity during May to October 2014. The Brachiaria cultivars included in the study were: *B. brizantha* cvs. Marandu, MG4, Piata and Xaraes, *B. decumbens* cv. Basilisk, *B. hybrid* cv. Mulato II and *B. humidicola* cvs. Humidicola and Llanero. The geographical position, elevation, temperature and rainfall of the study sites are presented in Table 1. Detailed description of climate and soils of the sites are given by Njarui *et al*. (2006), in this proceeding.

Disease symptoms were recorded; photographs were taken along with information on growth stage and crop types (first crop/regrowth). Disease incidence was recorded as percentage of plants with disease symptoms relative to the total number of plants. Disease severity was recorded as percentage of Brachiaria tissue covered by a specific symptom or lesion. The plant parts with disease symptoms were collected in paper envelopes and transported to the BecA-ILRI Hub for causal agent isolation and identification.

The symptomatic plant parts were used for microbial isolation. The leaf with disease symptom was photographed, and cut along with healthy portion into ten pieces (0.4 to 0.6 $mm^2$). The cut pieces were surface disinfected with 1.2% sodium hypochlorite solutions (with Tween-20) for 10 minutes, rinsed three times with sterile water and dried in paper towel. Five leaf pieces were plated on 9 cm petri - dishes containing PDA amended with ampicillin (100 µg/ml), incubated at 25°C and monitored daily for emerging fungal colony. Fungal isolates were sub-cultured three times to obtain pure colonies for subsequent analysis.

**Table 1** Geographical position, elevation, temperature, and rainfall at 7 sites monitored

| Site Description | Mtwapa | Msabaha | Mariakani | Katumani | Ithookwe | Nairobi | Ol Joro Orok |
|---|---|---|---|---|---|---|---|
| Latitude | 3°56′S | 3°16′S | 3°85′S | 1°35′S | 1°37′S | 1°16′S | 0°22′S |
| Longitude | 39°44′E | 40°03′E | 39°46E | 37°14′E | 38°02′E | 36°43S | 36°46′E |
| Altitude (masl) | 15 | 45 | 202 | 1600 | 1160 | 1795 | 2393 |
| Mean temp (°C) | 22 | 32 | 26.1 | 19.6 | 22.5 | 17.7 | 13.0 |
| Annual rainfall (mm) | 1200 | 1000 | 848 | 710 | 1010 | 900 | 950 |

**Microbial isolation**

*DNA extraction*

DNA was extracted using PrepMan kit following manufacturer's directions but with a 100 µl of PrepMan ultra sample preparation reagent instead of 200 µl. The final product was stored at 4 °C until further processing.

*DNA amplification*

DNA from previous step was diluted ten folds in sterile water. The PCR amplification was done using the universal fungal primer sets ITS1F (CTTGGTCATTTAGAGGAAGTA) and ITS4 (TCCTCCGCTTATTGATATGC) (Gardes and Bruns, 1993; White *et al.*, 1990). PCR mix was prepared using 3 µl of diluted genomic DNA as template, 5 µl of PCR master mix, 2 µl of ITS1F, 2 µL of ITS4 and 28 µl of sterile reagent water in a total of volume of 40 µl, and reaction without template DNA was included as negative control. The PCR conditions were 4 min of denaturation at 94 °C, followed by 35 cycles of 94 °C for 45 s, 48°C for 45 s and 72 °C for 45 s, with final extension of 72 °C for 10 min. Three µl PCR product was run in agarose gel to confirm the presence of expected PCR products.

*PCR Product purification, quantification and sequencing*

The PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) following manufacturer's instructions. Purified DNA products were sequenced with PCR primers sets using Sanger Sequencing at BecA-ILRI Hub.

**Data analysis**

Maximum disease index (MDI) was calculated by multiplying score for maximum disease incidence (%) and maximum disease severity (%) for each disease recorded in each Brachiaria cultivar irrespective of type of experiment and survey event. The resulting score was divided by 100 to get MDI. For molecular identification, fungal sequence data were imported into CLC Main Workbench Software v7.5, trimmed and assembled. The sequence identities were determined using BLAST program of NCBI (blast.ncbi.nlm.nih.gov). Criteria used for BLAST search were: e-value below 0.004, the highest identity (%), the highest query coverage and the possibility of a fungus identified by blast search being a plant pathogen as confirmed by literature search. A list of fungal taxa associated with symptomatic Brachiaria plant parts was prepared and the frequency of similar taxa in the population was calculated.

## Results and Discussion

*Monitoring disease incidence and severity*

### Leaf spots

Leaf spots were characterized by black to brown spots on Brachiaria leaves (Figure 1a). Infected leaves had brown to black raised to flat rough irregular spots which in severe cases coalesced and killed the entire leaf. Some spots had dark irregular centers surrounded by a tan to oval margin. Leaf spots were more on upper leaf surface compared with the lower leaf surface. The spots mainly occurred on older senescing leaves in both first and regrowth crops.


**Figure 1** Diseases of improved Brachiaria cultivars in Kenya. (a) Leaf spot, (b) Leaf rust, (c) Leaf blight, (d) False smut, (e) Ergot and (f) physiological leaf disorder (PLD)

Leaf spots occurred in all sites except at Nairobi (Tables 2). At Ithookwe only Llanero was free from the diseases among the seven cultivars evaluated. Basilisk, Humidicola, Marandu, MG4 and Mulato II were infected by leaf spot in Katumani whereas only Llanero was susceptible at Msabaha. Humidicola and Llanero were attacked by leaf spots at Mtwapa and Mariakani. At Ol

Joro Orok, all cultivars except Basilisk had leaf spot. Leaf spots attacked more cultivars at Ithookwe, Ol Joro Orok and Katumani compared with Msabaha, Mariakani and Mtwapa (Table 2). The disease was most damaging at Ithookwe where five of seven cultivars tested had a MDI of more than 21 (Table 2). MG4 was severely damaged by leaf spots at Ithookwe suggesting that MG4 may not be suitable for planting in Ithookwe. The leaf spots MDI was variable across cultivars and test sites. Disease was recorded in the month of July and October (data not shown).

**Leaf rust**

Symptoms of leaf rust consisted of orange or yellowish-orange raised pustules mainly on leaf blades (Figure 1b). As the plant matured, the pustules became brown to black. In severe cases, chlorosis and death of the leaves occurred. The rust disease was recorded in all test sites except at Ol Joro Orok (Table 3). MG4 was affected by leaf rust in five of seven test sites with the highest MDI (60%) at Nairobi. Mulato II was the second most affected cultivars and occurred in four sites: Nairobi, Ithookwe, Katumani and Mtwapa. On the other hand, Humidicola was free from the rust in five test sites. Rust was observed in July and October at Ithookwe and Katumani, in August at Nairobi and in October at Mariakani, Msabaha and Mtwapa. The MDI for leaf rust varied across cultivars, test sites and monitoring months.

**Table 2** Maximum disease index for leaf spot disease in improved Brachiaria cultivars at seven test sites

| Cultivar/location | Nairobi | Ithookwe | Katumani | Msabaha | Mariakani | Mtwapa | Ol Joro Orok |
|---|---|---|---|---|---|---|---|
| Basilisk | 0 | 30 | 0.72 | 0 | 0 | 0 | 0 |
| Humidicola | 0 | NA | 0.1 | NA | 0.12 | 1.1 | 0.08 |
| Llanero | 0 | 0 | 0 | 6.5 | 1 | 0.99 | 1.32 |
| Marandu | 0 | 37.5 | 0.03 | 0 | 0 | 0 | 0.4 |
| MG4 | 0 | 34.83 | 43 | 0 | 0 | 0 | 0.03 |
| Mulato II | 0 | 42.84 | 0.4 | 0 | 0 | 0 | 0.03 |
| Piata | 0 | 21 | 0 | 0 | 0 | 0 | 0.7 |
| Xaraes | 0 | 0.2 | 0 | 0 | 0 | 0 | 0.7 |

NA: The cultivar was not tested in this location

**Table 3** Maximum disease index for leaf rust disease in improved Brachiaria cultivars at different test sites

| Cultivar/location | Nairobi | Ithookwe | Katumani | Msahaha | Mariakani | Mtwapa | Ol Joro Orok |
|---|---|---|---|---|---|---|---|
| Basilisk | 0.05 | 0.6 | 0.48 | 0 | 0 | 0 | 0 |
| Humidicola | 0 | NA | 0 | NA | 0 | 0 | 0 |
| Llanero | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Marandu | 0 | 0 | 0 | 0 | 0.01 | 2.28 | 0 |
| MG4 | 60 | 0.01 | 0.8 | 1.2 | 5.75 | 0 | 0 |
| Mulato II | 0.01 | 1.15 | 3.84 | 0 | 0 | 0.37 | 0 |
| Piata | 0 | 0 | 0.01 | 0 | 0.95 | 0.13 | 0 |
| Xaraes | 0 | 0.01 | 0 | 0 | 0.03 | 0.38 | 0 |

NA: The cultivar was not tested in this location

**Leaf blight**

Leaf blight was characterized by appearance of long, elliptical gray or tan lesions first on the lower leaves. The disease progresses to the upper parts. Severe infection cause premature death and gray appearance that resemble to drought or frost injury (Figure 1c)**.** Leaf blight was recorded in all test sites except at Ol Joro Orok (Table 4). The test cultivars differed in response to the disease, for example, Humidicola was not diseased in all sites whereas Piata encountered disease at four of the seven test sites. Leaf blight was observed at Ithookwe and Katumani in July and October, at Nairobi in August and at Mariakani, Msabaha and Mtwapa in October. In general the MDI was very low for leaf blight with maximum score of 6.5% in Llanero at Msabaha.

**False smut.** The disease was characterized by replacement of individual Brachiaria grains by smut sori (Figure 1d). The disease was recorded at Ithookwe only in Basilisk, Marandu and Mulato II in the month of May and July. The disease was more damaging in May on Basilisk with MDI of 80%. Marandu and Mulato II had indices of 6.93 and 0.23.

**Table 4** Maximum disease index for leaf blight disease in improved Brachiaria cultivars at different test sites

| Cultivar/location | Nairobi | Ithookwe | Katumani | Msahaha | Mariakani | Mtwapa | Ol Joro Orok |
|---|---|---|---|---|---|---|---|
| Basilisk | 0.39 | 0 | 0.9 | 0 | 0 | 0 | 0 |
| Humidicola | 0 | NA | 0 | NA | 0 | 0 | 0 |
| Llanero | 0 | 0 | 0 | 6.5 | 0 | 0.03 | 0 |
| Marandu | 0.22 | 0 | 0.26 | 0 | 0.1 | 0 | 0 |
| MG4 | 0.16 | 0 | 0.08 | 0 | 0.03 | 0 | 0 |
| Mulato II | 0.54 | 0 | 0.56 | 0 | 0 | 0 | 0 |
| Piata | 0.08 | 0.08 | 0.08 | 0 | 0.7 | 0 | 0 |
| Xaraes | 0 | 0.06 | 0 | 0 | 0 | 0 | 0 |

NA: The cultivar was not tested in this location

**Ergot.** Initial symptoms of the disease consisted of a white soft spherical tissue on infected florets which produced a sugary honeydew (Figure 1e) which latter transformed into a hard dry sclerotium. Ergot symptoms were observed and recorded only at Nairobi test site. The disease was recorded during flowering to grain filling stage in August and attacked Piata (70%), Basilisk (40%) Marandu (28%) and MG4 (15%).

**Physiological leaf disorder (PLD).** Physiological leaf disorder (PLD) was first observed on Brachiaria at Mariakani and Msabaha in July. Initial symptoms consisted of purpling, yellowing or bleaching of leaves from the tips. Gradually, the symptoms progressed towards the leaf base and covered the entire leaf or a portion of the leaf (Figure 1f). In some cases, the midrib turned purple. In severe cases, the affected leaves dried up. The symptoms affected young and older leaves of both first crop and regrowth. The PLD occurred in all sites except at Nairobi and Ol joro Orok. The disease attacked all test cultivars at Msabaha, Mariakani and Mtwapa (Table 5). Disease was recorded during the month of July and October at Msabaha, Mariakani and Mtwapa and in October at Katumani.

**Table 5** Maximum disease index for PLD in improved Brachiaria cultivars at different sites

| Cultivar/location | Nairobi | Ithookwe | Katumani | Msabaha | Mariakani | Mtwapa | Ol Joro Orok |
|---|---|---|---|---|---|---|---|
| Basilisk | 0 | 0.6 | 0 | 3.6 | 0.3 | 4.05 | 0 |
| Humidicola | 0 | NA | 0 | NA | 0.32 | 0.23 | 0 |
| Llanero | 0 | 30.2 | 0 | 0.01 | 0.2 | 15 | 0 |
| Marandu | 0 | 0.02 | 0 | 15 | 0.2 | 7.2 | 0 |
| MG4 | 0 | 1.6 | 0.1 | 2.75 | 0.39 | 6.36 | 0 |
| Mulato II | 0 | 0.65 | 0.13 | 16.5 | 0.66 | 5.7 | 0 |
| Piata | 0 | 0 | 0 | 11.7 | 0.3 | 6.72 | 0 |
| Xaraes | 0 | 0.07 | 0 | 12.35 | 0.5 | 5.1 | 0 |

NA: The cultivar was not tested in this location

*Identification of fungi associated with diseases symptoms*

The molecular identification of 240 fungal isolates obtained from symptomatic leaf, stem and seed heads of improved Brachiaria cultivars revealed 36 different pathogenic taxa at variable frequencies (Table 6). The top six plant pathogenic taxa were *Phoma herbarum, Epicoccum nigrum, Fusarium equiseti, Nigrospora oryzae, Clasosporium cladosprioides, Nigrospora sphaerica,* and *Nigrospora sphaerica*.

*Phoma herbaru*m was the most frequently isolated fungus (34.8%) from the Brachiaria tissues with leaf spots symptoms (Figure 1a). *Phoma herbarum* are reported as causal agents of leaf spots disease in pea (Li *et al*., 2011), stem canker on hemp (McPartland *et. al*., 2000) and tip dieback/or stem canker on forest nurseries (James, 1985). *Phoma herbarum* was among 15 *Phoma* species isolated from New Zealand grasses and pastures. Pathogenicity testing of these species showed *P. medicaginis, P. lotivora* and *P. chrysanthemicola* pathogenic on legumes, and other 12 species were weak wound parasites (Johnston, 1981). Besides pathogenic roles, *P. herbarum* has been considered a candidate for biological agent for weed control (Neumann and Boland 2002, Stewart-Wade and Boland 2004, Kalam *et al*., 2014).

*Fusarium equiseti* was second most frequent fungus isolated from Brachiaria leaves. It is a cosmopolitan soil inhabitant (Saemi *et al*., 1999) capable of infecting seeds, roots, tubers, and fruit of several crop plants (Goswani *et al*., 2008). It is a common colonizer of senescent or damaged plant tissue (Leslie *et al*., 2006). Similarly, *Epicoccum nigrum* is a widespread and common saprophyte on dying plant organs but also a parasite on different hosts especially on seeds of cereals (Webster 1983). It produces colored pigments that can be used as antifungal agents (Bamford *et al*., 1961; Brown *et al*., 1987). In Kenya, the fungus was isolated from *coffee*, carnation, bitter lettuce, rice, and sugarcane (Kung'u and Boa, 1997). The fungus was isolated from Lablab bean as a cause agent of leaf spots (Mahadeva kumar *et al*., 2014).

*Nigrospora oryzae* causes ear or cob rot in maize (Shurtleff, 1992) and leaf spot in Rosemary (Moshrefi Zarandi *et al*., 2014), and Dendrobium (Wu *et al*., 2014). *Nigrospora sphaerica* causes Nigrospora blight of turf grasses. Its primary hosts are Kentucky bluegrass, creeping red fescue, perennial rye grass and St. Augustine grass (Smiley *et al*., 2005). Others hosts includes Bermuda and bent grasses (Cho *et al*., 2000).The attack of ergot disease in Brachiaria raises some issues on livestock and human health as the ingestion of ergot cause hallucinations, irrational behavior, convulsions and even death in humans and animals due to the poisonous ergot alkaloids (Tudzynski *et al*, 2001; Eadie, 2003). Besides health concerns, the infection of Brachiaria with ergot significantly reduces the seed quality and seed trades.

**Table 6** List of fungi associated with symptomatic improved Brachiaria cultivars in Kenya

| Fungal taxa | Frequency | Fungal taxa | Frequency |
|---|---|---|---|
| *Alternaria alternata* | 6 (3.0%) | *Fusarium sacchari* | 1 (0.5%) |
| *Alternaria arborescens* | 4 (2.0%) | *Magnoporthe oryzae* | 2 (1.0%) |
| *Alternaria brassicola* | 2 (1.0%) | *Microdochium albescens* | 2 (1%) |
| *Alternaria padwickii* | 1 (0.5%) | *Microdochium bolleyi* | 1 (0.5%) |
| *Alternaria tenuissima* | 2 (1.0%) | *Microdochium nivale* | 3 (1.5%) |
| *Cercospora beticola* | 2 (1.0%) | *Monographella albescens* | 2 (1.0%) |
| *Cercospora capsigena* | 1 (0.5) | *Nigrospora oryzae* | 14 (6.9%) |
| *Choanephora infundibulifera* | 1 (0.5%) | *Nigrospora sphaerica* | 11 (5.4%) |
| *Cladosporium cladosporioides* | 15 (7.5%) | *Olpidium viciae* | 1 (0.5%) |
| *Cladosporium langeroni* | 1 (0.5%) | *Paraphaeosphaeria michotii* | 4 (2.0%) |
| *Cladosporium pengustum* | 1 (0.5%) | *Peyronellaea glomerata* | 2 (1.0%) |
| *Cladosporium tenussimum* | 2 (1.0%) | *Phaesphaeria podocarpi* | 1 (0.5%) |
| *Cochliobolus geniculatus* | 1 (0.5%) | *Phoma glomerata* | 2 (1.0%) |
| *Cochlobolus sativus* | 1 (0.5%) | *Phoma herbarum* | 71 (34.8%) |
| *Epacris micropyla* | 1 (0.5%) | *Phoma medicaginis* | 3 (1.5%) |
| *Epicoccum nigrum* | 14 (6.9%) | *Poistrasia circinans* | 2 (1.0%) |
| *Epicoccum sorghinum* | 4 (2.0%) | *Setosphaeria rostrata* | 4 (2.0%) |
| *Fusarium equiseti* | 17 (8.5%) | *Stagonosporopsis cucurbitacearum* | 1 (0.5%) |

This study with isolation of fungi from symptomatic plants of improved Brachiaria cultivars demonstrated the association of a wide range of fungal species. *Phoma herbarum*, *Fusarium equiseti*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Nigrospora oryzae* and *Nigrospora sphaerica* constituted 70% of the fungal community. The current effort is a beginning of research on Brachiaria diseases and causal agents in Kenya. The future research on establishing relationships between these organisms and disease symptoms on host plant, confirmation of pathogenicity, yield loss assessment, and development of management methods for economically important diseases have been suggested for the effective and timely management of the important Brachiaria diseases in Kenya and neighboring countries. We also suggest further investigation on rust and physiological leaf disorder symptoms highlighted in this study.

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