EVALUATION OF GRASS ECOTYPES FOR POTENTIAL USE IN RESEEDING OF PASTORAL FIELDS IN THE ARID AND SEMI-ARID LANDS OF KENYA

By

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A80/83321/2012

A THESIS SUBMITTED IN FULFILMENT OF REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN GENETICS AND PLANT BREEDING

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

DECLARATION

This is my is my original work and has not been presented for award of any degree at any other University.

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DEDICATION

This thesis is dedicated to my lovely husband Dr. Muge and children Peter, Chantal and Leon for their relentless support and encouragement during the preparation.

ACKNOWLEDGEMENT

I acknowledge the funding support from the East African Agricultural productivity programme (EAAPP) for the scholarship and, Arid and Semi-Arid Lands Agricultural productivity research programme (ASAL-APRP) and National Council for Science and Technology for partially supporting my research work.

I sincerely appreciate the input of my supervisors Prof. Eliud Kahiu Ngugi, Prof. George N. Chemining'wa and Dr. William Ngoyawu Mnene who greatly supported me in the development of this thesis. I would also like to sincerely appreciate posthumously the input of the late Prof. Kiarie Njoroge who guided me through proposal development, research implementation and partly writing of my thesis.

Thanks are also due to the Director General, Kenya Agricultural and Livestock Research Organization and the staff of KALRO Headquarters, Kiboko, Buchuma, Mtwapa and Biotechnology Centre Kabete for logistical support.

Finally to my family, relatives and friends, thanks all for the patience and your prayers. May God almighty bless you all.

ACRONYMS

AEZ	Agro-ecological Zone
AMMI	Additive Main and Multiplicative Interaction
ANOVA	Analysis of variance
ASALs	Arid and Semi-Arid Lands
CBFSB	Community Based Range Forage Seed Bulking
FAO	Food and Agriculture Organization
ICPALD	IGAD Centre for Pastoral Areas and Livestock Development
ILRI	International Livestock Research Institute
KALRO	Kenya Agricultural and Livestock Research Organization
KBK	Kiboko
KLF	Kilifi
MGD	Magadi
PVS	Participatory Variety Selection
SRAP	Sequence Related Amplified Polymorphism
TVT	Taita Taveta
USDA	United States Department of Agriculture

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ABSTRACT

Cenchrus ciliaris L. (African foxtail grass) and *Eragrostis superba* Peyr. (Maasai lovegrass) grass species that are native to the rangelands have been promoted for rehabilitation of degraded areas and improve forage production. A major challenge to successful reseeding of the rangelands has been lack of certified seeds of these species in the formal seed systems except through collections from the wild. The main objective of the study was to contribute to improved livestock production in the ASALs through characterization, evaluation and identification of higher yielding ecotypes of *C. ciliaris* and *E. superba* that can be submitted for certification process.

Germplasm was collected from the wild in four agro-ecological zones (AEZ), represented by Kilifi, Taita Taveta, Makueni and Kajiado Counties for AEZ III, IV, V and VI, respectively. Seeds of eleven ecotypes for *C. ciliaris* and nine for *E. superba* were processed and planted in five rows of four metres long in randomized complete block design with three replicates at KALRO Kiboko Research Centre. Data were collected for stem, leaf and flowering traits while plant samples were analysed for crude protein, crude fibre, ash, percent dry matter, and in-vitro digestibility of dry matter. The levels of correlation and relatedness among the ecotypes was determined. Two clusters of robust and small sized types were formed using plant height, stem thickness, leaf length and leaf width. The small sized ecotypes were clustered as early flowering while the robust types were late flowering indicating presence of early and late maturing ecotypes among the *C. ciliaris* collection. MGD1 ecotype was found to be different from the rest due to clustering as a robust and early flowering type. The recorded correlation between stem and leaf traits and nutritive components, CP and INVDMD, in *C. ciliaris* ecotypes could be used to select for higher yielding plants for the target nutritive values within the ecotypes. Clustering patterns for *E. superba*

ecotypes remained the same using either 16 different morphological traits, selected robustness traits, namely, plant height, stem thickness, leaf length and leaf width, or seed yield traits. KBK1 and KBK2 ecotypes of *E. superba* remained clustered together in one group as robust types against the rest of the seven ecotypes. Effect of the environment of collection including climate and grazingland management may have influenced the plant size and maturity time among the *C. ciliaris* ecotypes. The identified different clusters of *C. ciliaris* ecotypes allows for selection along maturity time such as within the early flowering types, late flowering types and MGD1 as an early maturing and robust type.

Significant genetic differences (<0.01) was recorded among ecotypes of *C. ciliaris* where Kajiado population recorded the highest diversity indices while Kilifi and Narok collections were the most distant populations. High genetic differentiation between populations of *E. superba* was recorded with Fst=0.237, Gst= 0.534, mean Shannon diversity index (I=0.357) and Nei's genetic diversity index (h=0.223) among populations. There was possible exchange of genetic materials between ecotypes of *E. superba* conserved in common gardens.

Evaluation of biomass yield among *C. ciliaris* ecotypes was done in three sites Kiboko, Buchuma and Mtwapa KALRO Centres. Plots were established in three replicates of five rows each measuring four metres long. Dry matter yield data was collected for two seasons and analysed using AMMI stability value (ASV) and Yield stability index (YSI). Mean dry matter yield (DM) of the *C. ciliaris* ecotypes across three sites ranged from 3986 to 11,792 $kgha^{-1}$ where the small sized types had the lowest yield. KBK3 ecotype was ranked the most stable across sites with ASV and the highest yielder with YSI. Ecotype KBK1 was the most suitable for Kiboko and Mtwapa sites and MGD3 for Buchuma.

Evaluation of farmer knowledge and perceptions on ecotypes of *C. ciliaris* grass species was carried out using Focused group Discussions (FGDs) where farmers developed criteria for preferred grass types. The farmers knew of the existence of various ecotypes of *C. ciliaris* and had varied perceived preferences on them. TVT1 and KLF1 ecotypes were selected by over 80 % of the participants due to their perceived tolerance to droughts and heavy grazing. The criteria for selection of ecotypes by farmers varied depending on the type of utilization of the grass. Successful development and promotion of grass varieties should consider the mode of utilization by the target farmer group.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Importance of ASALs in livestock production

The importance of Arid and Semi-Arid Lands (ASALs) has been underscored given the vastness in the area and the greater proportion of land it covers (70%) in the Greater Horn of African countries (ILRI News, 2011). The ASALs are characterized by low, erratic and usually bi-modal rainfall ranging from 200 mm to 1000 mm per annum and contain different associations of natural vegetative cover and soils. Periodic droughts are a common occurrence.

The importance of ASALs is emphasized in the Kenya ASAL Draft Policy document that states that unless the enormous resources of the ASALs are factored into effective national planning and development, the country's hope of ever achieving sustained economic growth remains a dream. Indeed, livestock production is one of the fastest-growing agricultural subsector in Kenya contributing around 45 % of the agricultural GDP (ICPALD, 2013). Beef production in the country is practiced primarily in the ASALs where 70% of livestock population is hosted (Behnke and Muthami, 2011).

Despite the many challenges in the Kenyan ASALs, such as insecurity, resource conflicts and harsh climatic conditions, an estimated 67% of country's red meat comes from these areas (Juma, 2010). Kenya is a meat deficit country with the local beef market considerably relying on cattle imports from Ethiopia, Somalia, Sudan and Tanzania. These countries have developed their ASALs whereas in Kenya the potential is yet to be fully exploited. The ASALs also contribute to

the dairy sector. For instance, the Zebu cattle that are mostly found in the ASALs, contribute 16 % of the total cattle milk production in the country (FAO, 2011).

The ASALs also host about 92% of the land alienated for National Parks and Reserves that is home to 90 percent of the wild game. By extension, the ASALs account for 80% of the country's eco-tourism interests while contributing in excess of Kshs 50 billion annually from the interaction of wildlife and pastoral communities (ALRMP, 2005; Barrow and Mogaka, 2007).

1.2 Problem Statement

Livestock production in the ASALs relies on grazing on natural pastures as the main source of feed. Feed inadequacy in terms of quality and quantity is the major constraint to livestock production in the ASALs (Gitunu et al., 2003, Kibet et al., 2006; Mnene, 2006). Among the contributing factors to challenges in livestock feed availability in ASALs includes increasing effects of droughts, land subdivisions, influx of immigrants and changes in land use systems. The immediate resultant effect of all these factors is continuous overgrazing leading to loss of important native forage species, massive land degradation and increased livestock mortalities even during normal dry seasons.

Land degradation is a major challenge to livestock feed availability and, to a greater extent, the household food security in the ASALs. Land degradation is defined as reduction or loss of the biological or economic productivity of the land (FAO, 2005) with desertification as an extreme form of land degradation. There are several indicators of land degradation with respect to natural pasture. These include net loss of vegetation due to overgrazing, decrease in palatable perennial forage species, species rarity, bush encroachment, weed invasion and increase in annual plants (FAO, 1999; Rayburn, 2000). Therefore, technologies to address land degradation while enhancing

feed availability through identification and promotion of suitable range grass ecotypes would contribute to increased livestock productivity in the ASALs.

Technologies for rehabilitating degraded ASALs have been developed, among them reseeding as an option to reintroduce and increase the cover of the lost forage species (Mnene, 2006; Nyariki et al., 2008; Manyeki et al., 2011). Most of the research done on cultivation of forage grasses in East Africa has been targeted at the high potential areas despite the ASALs regions being endowed with a myriad of grass genetic resources. Researchers from other continents have selected some of these genetic materials and improved them, and are already benefiting commercially from them. For instance, a number of *Brachiaria* species such as *Brachiaria decumbens, B. brizantha and B. humidicola*, which originated from East Africa, are important commonly cultivated forages for the beef industry in South America (Nakamura et al., 2005) and occupy over 80 million hectares (Boddey et al., 2004). In addition, *Brachiaria* cv. Mulato II, which is a widely cultivated grass species in South America, is a hybrid of three locally found *Brachiaria* spp. The variety is currently being evaluated for National Performance Trials (NPT) for potential release in Kenya.

In the past, some of the grass species nominated for reseeding the ASALs of Kenya have been perennials that have developed adaptive mechanisms to ASAL environments and are thus more preferred. Studies indicate that species most likely to be successful in reseeding are native species found on sites similar to those to be re-seeded (Musimba et al., 2004). Planting of a mixture of grass species is increasingly being recommended since no one species can have all the qualities required. For example, no species has sufficiently high nutritive value, tolerance to droughts and can withstand grazing pressures which are found in the ASAL environments. ASALs are expansive and unpredictable in nature and are used for direct grazing by large herds of wildlife and livestock.

Among the many indigenous grass species, *Cenchrus ciliaris* L. (African foxtail) and *Eragrostis superba ([*Peyr] Maasai lovegrass) were identified as preferred species by farmers and commonly found in the ASALs (Mnene, 2006). Despite wide promotion as the preferred grass species for rangeland rehabilitation, there was a challenge of unavailability of quality seeds in the formal market for *C. ciliaris* and *E. superba*. In Kenya, available grass seeds in the market mainly comprise the type that do well in the humid and sub-humid types such as *Setaria sphacelata* and *Chloris gayana.* The only option left for reseeding the ASALs is the collection of seeds from the wild. In the recent past, options to enhance range grass seeds availability have been identified and promoted. For instance, establishment of an irrigation system for seed bulking at KALRO Kiboko research centre and community based range forage seed bulking (CBFSB) in the Southern Rangelands of Kenya by the research centre have been promoted (Kimitei et al., 2010; Manyeki et al., 2011).

The challenge still remained inadequately addressed because the seed production being bulked and promoted was targeted to informal markets. There was need to study and identify ecotypes of potential range grass species that can successfully go through the formal inspection requiring specific descriptors and seed certification process with an objective of supplying quality seeds to Kenyan farmers and others beyond the borders.

1.3 Justification of the study

Reseeding has been noted and recommended to be a viable option to rehabilitate rangelands for improved forage production in ASALs (Mnene, 2006; Nyariki et al., 2008). In the past, the need to improve forage production in the ASALs was lacking since grazinglands were still extensive, communally used with well installed traditional decision making structures controlling the access

and use of forage and water resources responsibly. Earlier efforts to bulk seeds for some of the potential range grasses have been unsuccessful. For instance, bulking of *Cenchrus ciliaris* seeds in Turkana area was abandoned due to low demand since reseeding to improve feed availability was yet to be embraced by ASAL communities (Boonman, 1993).

In recent years there have been changes towards adoption of range reseeding technologies leading to high demands for grass seeds to improve forage productions in the ASAL due to four major factors. First, reducing land sizes and subsequent overgrazing occasioned by continued land subdivisions, influx of other communities from higher potential areas and changes in land use systems such as introduction of crop-production. Secondly, high livestock losses due to climate change effects that have been linked to increased incidence of droughts (Ndikumana et al., 2002). Thirdly, there has been increased sensitization of pastoral and agro-pastoral communities in ASALs on importance of improving forage production (Kimitei et al., 2011). Lastly, with reducing land sizes, farmers have intensified their livestock production by introducing high yielding dairy animals under zero grazing, particularly in urban centers in ASALs leading to increased demand for high yielding forage plants (Mutavi et al., 2016). These trends are likely to intensify in the coming years.

Pasture production in the ASALs is a major priority in the government of Kenya (GOK, 2010; Vision 2030 sessional paper, 2012). Sourcing for the seeds is a major requirement for the success of this effort. Forage seed system has been prioritized in the Kenya Agricultural and Livestock Research Organization (KALRO) strategic plan (KALRO, 2017). Grass species and ecotype characterization is one of the strategies to achieve this purpose.

Some of the unforeseen benefits from improved pastures in the ASALs are changing the attitudes of the communities towards deliberate efforts to improve their pastures. A household survey to assess farmer perceptions on effect of reseeding found that there was an increase in milk yield for both goats and cows by 0.1 and 1.6 litres, respectively (Manyeki et al., 2011). Also, range rehabilitation technologies such as reseeding can be applied as mitigative measures against droughts as highlighted as one of the flagship initiatives in the livestock sector (MoLD, 2010). Grass seed bulking can also be an alternative source of household income through sale of seeds as an economic activity. Formal marketing of the seeds has been impossible since these seeds are being collected from the wild, which calls for the need to study and select ecotypes for potential use in the formal seed systems.

The criteria used to select *C. ciliaris* and *E. superba* grass species for this study included (1) that they are indigenous grasses, commonly found in the ASALs and preferred by farmers (Mnene, 2006); (2) They have been found to have potential for reseeding Kenyan ASALs by various projects (Bogdan and Pratt, 1967; Keya, 1998; Mnene, 2006; Opiyo, 2007; Kimitei, 2010, Mganga, 2010a); (3) They do well in their mixtures (Bogdan and Pratt, 1967; Mganga et al., 2010a). Reseeding with mixtures is recommended for ASALs since no one species would have all the important characteristics as required given the highly unpredictable nature of the fragile ASAL environments

Various studies have been done on *C. ciliaris* and *E. superba* such as on techniques of planting, land preparation, watering regimes and management including production of a training manual on forage seed multiplication (Mnene, 2006; Nyariki et al., 2008; Opiyo, 2007; Ogillo et al., 2011; Mganga et al., 2010a). Mnene (2006) studied technologies for enhancing success in establishment

including watering regimes and Mganga et al., (2010a) studied the potential effect of the two grasses on the environment, particularly the soils. Keya (1998) realized a production potential of 6.6 t ha^{-1} for *C. ciliaris* while determining its productivity and ecophysiology in ASAL environments. In addition, the potential socio-economics effects of reseeding using different techniques have been analysed (Opiyo, 2007; Manyeki et al., 2011; Ogillo et al., 2011).

The various studies done on *C. ciliaris* and *E. superba* grass species have used local, wild collected seeds while several of their ecotypes may exist contributing to differences in recorded study results. The current study was designed to contribute to improved ASAL feed resources through characterization, selection and evaluation of ecotypes of *C. ciliaris and E. superba* for potential herbage and seeding ability and to understand the relative importance and complementary nature of these grasses in the livelihoods of the people in ASALs of Kenya. Both traditional phenotypic and modern molecular approaches for characterizing species were employed in this study.

1.4 Main Objective

To contribute to improved livestock production in the ASALs through evaluation and identification of higher yielding ecotypes of two range grass species, *Cenchrus ciliaris* and *Eragrostis superba*.

1.4.1. Specific Objectives

1. To carry out morphological characterization of ecotypes of *Cenchrus ciliaris* and *Eragrostis superba*
2. To characterize ecotypes of *Cenchrus ciliaris* and *Eragrostis superba* using molecular tools
3. To assess ecotypes of *Cenchrus ciliaris* for dry matter and seed yield
4. To determine farmer knowledge and perceptions on the ecotypes of *Cenchrus ciliaris* grass species through participatory variety selection.

1.4.2. Hypotheses

1. Ecotypes of *C. ciliaris* and *E. superba* are not morphologically distinct
2. Ecotypes of *C. ciliaris* and *E. superba* are not genetically diverse
3. There is no difference among *C. ciliaris* ecotypes in dry matter and seed yield
4. Farmers do not have knowledge on the existence of ecotypes of *C. ciliaris* and have different preferences

CHAPTER TWO

LITERATURE REVIEW

2.1. Importance of grass forage in livestock production

Grasses provide food indirectly to human and directly to grazing animals. Although many of the valuable services of grasses have no clear market value, they are the most important food crops on earth (Gibson, 2009). Grasses provide food to both human and animals thus providing most of the world's milk, meat and wool. The grasses occupy a greater area of the world's land surface than any other plant family covering about 40.5% (52.5 million square kilometers) of the terrestrial area excluding Greenland and Antarctica (Suttie et al., 2005). Grasses have abilities to grow and propagate themselves vegetatively. Domestication of forage grasses is a recent activity which is argued that they are yet to go through the required transition to be classified as domesticated (Brown et al., 2014). Two grass species, *Eragrostis superba* and *Cenchrus ciliaris* were selected for the study due to their high ranking in farmer preference for reseeding (Mganga et al., 2013; Mnene, 2006).

2.2. Description of *Eragrostis superba* Peyr

The taxonomy of the species is as follows: **Kingdom:** *Plantae*, **SubKingdom:** *Tracheobionta*, **SuperDivision:** *Spermatophyta* – **Division:** *Magnoliophyta*, **Class:** *Liliopsida*, **SubClass** *Commelinidae* **Order:** *Poales*, **Family:** *Poaceae* and **Genus:** *Eragrostis* (USDA, 2012). *The* Species was first described in 1860 by Johann Joseph Peyritsch. *Eragrostis superba* is native to Africa where the species occurs naturally in South Africa and northwards through East Africa to Sudan where the grass is grown for hay. The species was introduced into the United States in

1960's where it was released as a cultivar known as palar. The species has a C_4 photosynthetic pathway with a chromosome base number of $x = 10$ (FAO, 2012). The species is cross-pollinated (Busey, 1976).

The species has high tolerance to salinity, alkalinity and droughts and is also used for erosion control. The grass species is adapted to calcareous soil at pH 7.3 (Foy, 1979). The species grows in areas receiving 500 – 875 mm rainfall with an altitude of 0 – 2000 mm above sea level (FAO, 2012). The species is wide spread in the semi-arid areas of East Africa occurring in various vegetation types. The species is deep rooted growing up to 2.2 metres although a larger percentage of the root system occurs at less than 50 cm below the ground which allows for access of moisture during light showers (Opiyo, 2007).

Eragrostis superba is a good seeder with yields varying according to environments and management. Yields of up to 1 ton/ha/season was reported by Bulle et al., (2010) in Marsabit Central Division. Machogu (2013), reported 803.2kg/ha for the species from an irrigated study, which was higher than *Enteropogon macrostachyus, Chloris roxburghiana* and *C. ciliaris* with 542.8, 128.4 and 53.6 kg/ha, respectively. Koech et al., (2014) recorded over 85% depressed seed yield under rain fed conditions compared to irrigation to different field capacities. The seed yields in Koech et al., (2014) were 350, 286, 343 and 39 kg/ha for 80, 50 and 30% field capacity and, rain fed conditions. The high seeding capacity has been associated with high percent flowering tillers, with a longer flowering period that were also recorded in the study (Machogu, 2013).

The species has high ability to spread naturally in the wild that could be due to the high seed yield coupled with good seed viability. Germination capacity of 58% was recorded by Machogu (2013)

and over 70% by Veenendaal and Ernst (1991). Lower germination capacities of 8.6 and 10% were reported by opiyo et al., (2011) and Mganga et al., (2010a). High post-harvest seed dormancy have been reported for the grass species (Mnene, 2006). Mganga et al., (2010a) study used two year old seeds where the long storage period of the seeds could have also affected the viability. Long term viability tests of seeds under storage indicate a gradual increase in germination capacity to a peak point, which is species dependent, then followed by a continued reduction in germination (Mnene, 2006). Mnene (2006) reported 36 weeks of storage as the peak germination point for *E. superba.* Lower germination capacity is reported for the dispersal unit (18%), which is the spikelet, as compared to the naked caryopsis (82%; Veenendaal and Ernst, 1991) implying that the seed coverings contribute to high seed dormancy in *E. superba*.

Eragrostis superba is fairly palatable and readily grazed, although the species gets stemmy and unpalatable near maturity where the nutritive value drops (FAO, 2012). Opiyo et al., (2011) reported reduced leafiness and increased stemminess in the species by end of a 12-weeks study period. Crude protein content of 12% is reported for the species (FAO, 2012). Machogu (2013) recorded 13 % crude protein at week 12 post clipping which was comparable to *Brachiaria* Cv. Mulato II (13.3 %). Machogu (2013) recorded 65.5 % digestible dry matter and 75 % relative feed value for the grass species. Varied dry matter yields have been reported for the species. The species produces high herbage especially during primary Opiyo et al., (2011) reported a range of 1899.5 to 2434.5 kg DM/ha while Machogu (2013) recorded an estimated 1800 kg DM/ha at 12 weeks and about 9000 kg DM/ha at 16 weeks.

So far there is no phenotypic or genetic characterization that has been carried out for *E. superba*.

(a) (b)

Plate 2. 1: *(a) Eragrostis superba; (b) Cenchrus ciliaris at KALRO Buchuma ecotype plots on Dec, 2013*

2.3. Description of *Cenchrus ciliaris* L.

The taxonomy of *C. ciliaris* is as follows: **Kingdom:** *Plantae*, **SubKingdom:** *Tracheobionta*, **SuperDivision:** *Spermatophyta* – **Division:** *Magnoliophyta*, **Class:** *Liliopsida*, **SubClass** *Commelinidae* **Order:** *cyperales*, **Family:** *Poaceae,* **subfamily**: *Panicoideae* **tribe**: *Paniceae* and **Genus:** *Pennisetum*/*Cenchrus*. The species is also known by other synonyms like "Pennisetum ciliare" and "Pennisetum cenchroids" (USDA, 2012). Native to over 20 African countries, Asia and Sicily in Europe (Cook et al., 2005), *C. ciliaris* is widely naturalised in sub-humid and semi-arid tropics and subtropics where the species is consistently tolerant to drought (Humphrey, 1967). Reportedly, *C. ciliaris* is also tolerant to grazing pressure although less tolerant to salinity and waterlogging as well as being more susceptible to diseases e.g. smut in wetter areas of Kenya. Seed yields range between about 150 and 500 kg/ha. When cattle are fed on *C. ciliaris* on fertile soils under good growing conditions, they can gain up to 180-200 kg/hd/yr at 2 ha/beast (Cook et al., 2005).

Various studies on the morphological and physiological characterization of *C. ciliaris* have been done (Pengelly et al., 1992; Hacker and Waite, 2001; Mnif et al., 2005; Jorge et al., 2008), where

great variation between the varieties was noted. Studies have also been conducted to evaluate the species for tolerance to different stress conditions such as salinity (Nadaf et al., 2003). Nadaf et al., (2003) found that mean height, tiller numbers and the green and dry matter yield were significantly reduced with increasing levels of salinity and that the species could not survive up to 10 cuts under high saline conditions as compared to the control. Cultivars have been developed with increasing growth rates and increased ranges of tolerance to different environmental conditions and to diseases. Examples are cultivars 'Laredo' and 'Pecos' that are both blight resistant and Blue', 'Nueces', and 'Llano' that have cold tolerance (Mandy, 2009). Bhatt et al., (2007), realized a triple fold increase in leaf area index resulting in about 25% increase in photosynthesis and almost 50% in stomatal conductance in two different levels of CO_2 whereas Mishra et al., (2010) found that the dry matter yield of *C. ciliaris* is reduced under *Acacia Tortilis* canopy.

Cenchrus ciliaris is wide spread throughout the tropics and subtropics (Figure 2.1) and has been in Northern Australian extension services since 1923 (Humphreys, 1967). The species is widely planted as pasture grass in both Texas and Northern Mexico.

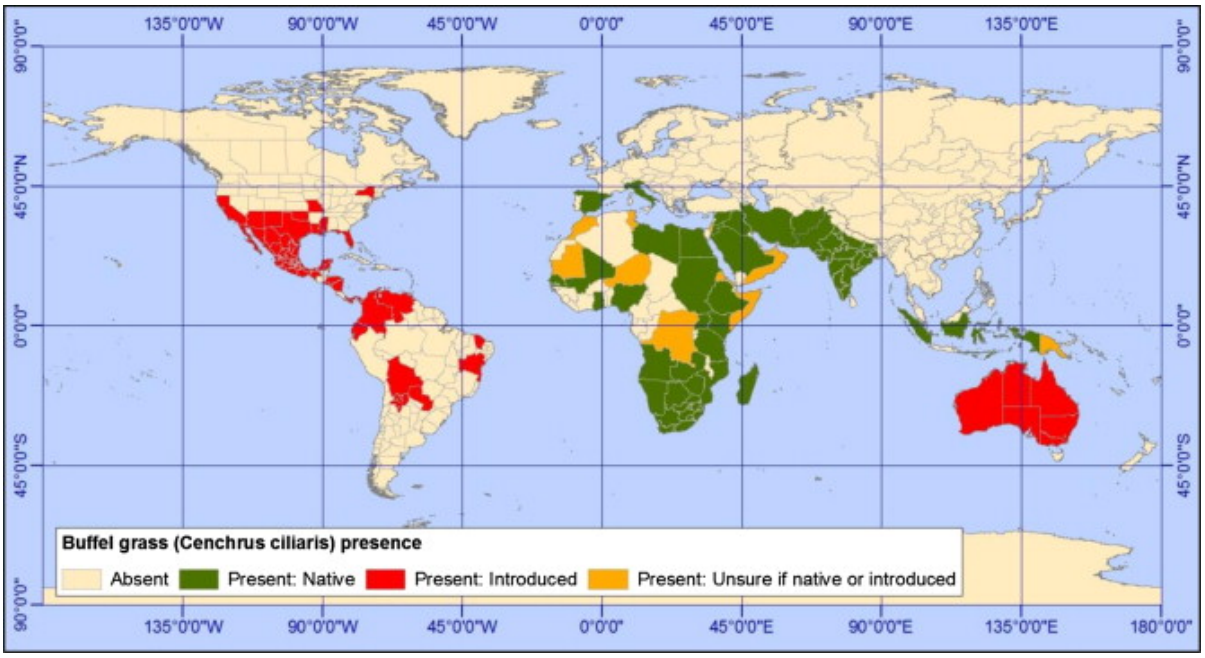

Figure 2. 1: *Distribution of Cenchrus ciliaris in different parts of the world*
Source: Marshall et al., 2012

Although great potential of *Cenchrus ciliaris* has been indicated, the species is associated with one disadvantage. The species is known to dominate weeds and has been declared noxious in some areas (Cook et al., 2005). Introduction of the species in Sonora, Mexico is being blamed for the loss of native desert vegetation due to the complete removal of the native vegetation which is said to have had higher net primary productivity (Franklin et al., 2006). Other than being an invasive species that outcompetes and displaces the native ones, the species modifies the plant communities that it invades through wild fires by providing fuel (Friedel et al., 2006). The aggressiveness exhibited by *C. ciliaris* grass species can be utilized in rehabilitation of degraded rangelands.

In terms of pollination, *C. ciliaris* is protogynous, in that the stigmas are exserted from the floret one or more days prior to anther exsertion (Byron and Bruce, 2001). The stigmas are receptive when they are exserted from the floret, which can be 72 hours prior to anther exsertion. This permits the stigmas to be pollinated by adjacent plants and ensures cross-pollination.

Despite having been the most studied species in the genus *Cenchrus*, the species is yet to be fully domesticated and is still open for exploitation by using naturally occurring variation in the species (Goel et al., (2011).

2.4. Morphological characterization of forage grass

Characterization of forage germplasm is an important requirement in utilization of the genetic resources (Van de Wouw et al., 1999). Appropriate characterization of germplasm provides information that facilitates their utilization for specific purposes and environments (Van de Wouw et al., 2008). Characterization allows for further evaluation and selections of the accessions for improving productivity in targeted livestock production systems. Lack of information on existing accessions results in dissemination of only the most familiar lines locking out the potential utilization of a larger number (Jorge et al., 2008).

There is considerable variation between characters used in morphological characterization even between plants of an accession (Tcacenco and Lance, 1992). Van de Wouw et al., (1999) recommends the use of ten observations per accession with one observation per plant for an acceptable error percentage. In cases of fewer plant establishments, repeated measurements per plant for up to a minimum of six plants per accession could be done to get an acceptable error percentage (Van de Wouw et al., 1999).

Selection of characters used in characterization can be based on factors such as agronomic relevance, expected variation among the accessions and/or ease of recording the attributes especially in case of a large number of accessions (Pengelly et al., 1992; Van de Wouw et al., 2009). Attributes that are influenced by environmental conditions or significantly correlated in a logically way should be avoided (Tcacenco and Lance, 1992). The environment for

characterization of forage germplasm should be based on the species adaptation to allow the targeted characters to be fully expressed by the plants (IBPGR, 1985). For instance, *Cenchrus ciliaris* species should be characterized in semi-arid zones of 600-1000 mm rainfall of 0-180 growing days. The means for the measurements are used in multivariate analysis (Van de Wouw et al., 1999. Multivariate analysis is used to analyse data on multiple traits where methods such as cluster or principal components analysis can be used.

2.4.1 Principal component analysis

Principal component analysis (PCA) is a multivariate analysis tool used to reduce the dimensionality of a data set with large interrelated variables with minimal effect on the variation in the data set (Jolliffe, 2002). The data set is converted into fewer and un-related data sets known as principal components without considerable loss of information (Gallo et al., 2013). The first principal component explains the highest percent variation among the subjects of the study while variability explained by each of the succeeding components keeps reducing. The technique makes data easy to explore and visualize through display of patterns of similarity of the observations and variables in a map. Principal component analysis expounds many aspects of the variance in the samples in a study (Tcacenco and Lance, 1992). The technique identifies variables associated with each principal components allowing for identification

The technique has been used in several forage characterization studies (Tcacenco and Lance, 1992; Van de Wouw et al., 2009; Jorge et al., 2008; Arshad et al., 2007; Kharrat-Souissi et al., 2011; Gallo et al., 2013). In a study to characterize 98 accessions of *Cynodon* grass species using 19 agro-morphological characters, Van de Wouw et al., (2009) was able to identify outliers from the rest of the accessions from PCA1 and PC2 that were explaining a total of 35.1% of the variation

among the accessions. Separation of accessions can be enhanced in subsequent PCA by selecting characters that ranked higher in differentiating accessions in the preceding PCA (Tcacenco and Lance (1992). Tcacenco and Lance (1992) carried out a two level PCA whereby the first analysis involving all 89 morphological attributes explained 57% variation in nine accessions of *Pennisetum purpureum* while the second PCA using few selected characters increased the level of variation explained to 86%. Principal component analysis did not show any groupings in *Cenchrus ciliaris* accessions using 22 agro-morphological characters (Jorge et al., 2008). Gallo et al., (2013) characterized forage populations using nutritive value components using PCA and reported three groups consisting of corn silage, alfalfa hay and the grasses.

2.4.2 Cluster analysis

Cluster analysis is a multivariate method that categorizes a sample of subjects or objects based on data set of selected variables into a number of different groups such that similar subjects are placed in the same group (Cornish, 2007). The technique helps in discovering distinct groupings among accessions during characterization of samples and no predefined classes are required prior to the analysis. One of the limitations of the technique is that there no official guidelines or conventional approaches to identifying or defining formed clusters.

Clustering of accessions is affected by the accuracy of data obtained, which is dependent on the scope and depth of the characterization study. The accuracy of clustering is increased by use of more characters (Rohlf and Wooten 1988). Cluster analysis group accessions with similar characteristics thus allowing for selection and breeding for specific attributes such as drought tolerance, early maturity, among others (Jorge et al., 2008). Clustering facilitates sampling of representative samples from a large number of accessions. Hacker and Waite (2001) used

representative samples from each of the clusters reported in Pengelly et al., (1992) to select for improved spring yield in *C. ciliaris*. Pengelly et al., (1992) had characterized 322 collections of *Cenchrus* species.

2.5. Nutritional value of grasses

The quantity and quality of forage grasses is influenced by a number of factors, mainly hereditary and environmental. These include management regime, plant growth stage and frequency of harvesting, climatic conditions and forage species type (Bumb et al., 2016). Nutritive value of grasses is relative and should always be evaluated with regard to the influencing factors (Boonman, 1993). Although there are several factors to measure forage quality, chemical composition in terms of crude protein, crude fibre and digestibility are commonly used to interpret the quality of forages (Boonman, 1993; Mohajer et al., 2013).

Changes in plant growth stages directly influences the chemical composition of a grass forage (Arzani et al., 2004; Enoh et al., 2005; Bumb et al., 2016). Arzani et al., (2004) reported reduced dry matter digestibility, metabolizable energy and crude protein as growth progressed in various forage species. Enoh et al., (2005) recorded 25% reduction in crude protein levels and 20% increase in fibre with delayed forage harvesting by four weeks which implies decrease in forage quality. Forage quality is directly related to the protein content and inverse to crude fibre (Mohajer et al., 2013). The decrease in forage quality is due to a change in the leaf to stem ratio resulting from an increase in structural carbohydrates especially in the stems as the plant matures (Arzani et al., 2004; De Santis et al., 2004). Differences in nutritive values between species or varieties could be due to the plant's inherent ability to extract nutrients from the soil, previous utilization regimes and proportional variation in important plant parts, such as leaf, stem and flower, at

different growth stages (Arzani et al., 2004; Ashraf et al., 2013). Differences in environmental conditions of study sites including soil fertility results in seasonal and geographical variation in forage quality (Buxton et al., 1995).

The amount of nutrients in the forage determines the quality of livestock production. High nutritive value is regarded when the chemical and physical characters of a grass species supports high animal intake and production (Boonman, 1993). Knowledge of forage quality is necessary in planning and proper utilization of the pastures for optimum livestock performance (Amiri and Shariff, 2012).

2.6. Seasonal variation in grass forage availability

Grass forage availability is influenced by the climatic conditions, plant nutrition and management (Demanet et al., 2015). Grasses are highly seasonal with variability observed between and within growing seasons (Boonman, 1993). Seasonal variation in dry matter has been reported (Demanet et al., 2015; Kalil et al., 2016). Demanet et al., (2015) reported variation in seasonal and annual dry matter yield with a range of 9.8 to 17 ton Dm/ha for the annual yields in a long term study of a pastureland in Chile. Higher forage yields are recorded in newly established pastures which evens out with older establishments by the fourth year due to a steady decline in production over the years (Reheul et al., 2010). The rate of the decline in forage yield between harvest cycles can be minimized by application of nitrogen fertilizer (Onyeonagu and Asiegbu, 2011; Boonman, 1993).

Significant decline in forage availability during the dry season was reported by Kalil et al., (2016). The response to the drying conditions vary among species; for instance, the common guinea grass displays greenness during dry seasons when utilization is minimal and browns off rapidly with grazing (Boonman, 1993).

Forage yield potential of grasses is determined by the length of the vegetative growing season, which is the period between regrowth and flowering date (Jiang et al., 2019). Jiang et al., (2019) reported positive relationship (R^2=0.55) between vegetative growth period and biomass yield in 36 accessions of switch grass.

2.7. Participatory selection of varieties

Participatory selection of varieties (PSV) is a technique where the farmers are used to select finished, near-finished or non-segregating products of plant breeding for introduction into their cropping systems (Joshi and Witcombe, 1998). Use of participatory methods in plant breeding helps in meeting the needs of the market, which is the farmer and the consumer, for the new varieties; leads to higher adoption of the new varieties and aids in identification of important characteristics for selection within a crop (Witcombe et al., 2005). For instance, palatability and biomass yield were identified as important attributes for evaluation of *Brachiaria* grass varieties by farmers in Rwanda (Mutimura and Everson, 2012). The technique improves productivity at farmer level due to enhanced access to information and planting material that help them in selecting suitable and preferred varieties for their own environments (Witcombe, 1999).

Participatory variety selection has three main stages, which are identification of farmers' needs, search for suitable materials to test with farmers and lastly experimentation at farmers' field (Joshi and witcombe, 1998). The level and stage of farmer participation varies widely between research programmes. Participatory variety selection in centralized breeding programmes involves farmers visiting and selecting from trials on-station or by scientists carrying out evaluation, selection and testing of crop varieties in farmers' fields (Ceccarelli, 2009). The farmers participating in the on-station activity should be from similar environments to the research station since PSV is

environment dependent (Ceccarelli, 2009). Witcombe et al., (2005) notes that to enhance the level of participation, the farmer should be able to test a wide diversity of varieties that are preferably non-released or released and non-recommended with farmers' perceptions being used as essential factors during crop evaluation.

Participatory selection approaches have been applied in evaluation and selection among different forage species (Magboo et al., 1998; Aberra et al., 2010; Katunga et al., 2014; Zeleke et al., 2018) with a few using the techniques on developed forage varieties for a given species (Mutimura and Everson, 2012). Although selections are being done at species level in most of forage trials, the studies seem to follow the three phases of PSV as recommended by Joshi and Witcombe (1998). The selection criteria varies depending on farmers' different possible uses of the grasses and herbage yield is a key attribute in forage PSV (Magboo et al., 1998; Mutimura and Everson, 2012; Zeleke et al., 2018). Participatory variety selection assists livestock farmers in selecting forage varieties that are higher yielding than their locally available species (Mutimura and Everson, 2012). The technique also allows for rapid experimentation, adaptation and adoption of new forage varieties (Peters et al., 2001).

2.8. Molecular characterization of grasses

Molecular techniques have been used to assess genotypes at the DNA level and detect minor changes (Griffa et al*.,* 2006). Use of molecular markers in breeding programmes has been increasing over the years. Detection of specific traits in progeny plants have been done successfully thus allowing for development of new varieties within a shorter time. Breeding for resistant traits have been enhanced through use of molecular markers that shorten the time for gene identification. Varied results in terms of genetic diversity have been reported for same plant samples using

different markers. The ability to determine genetic variation among different genotypes is more directly related to the number of polymorphic loci detected with each marker system and not a function of which marker is used (Babu et al., 2009).

2.8.1 Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA (RAPD) is based on the amplification of genomic DNA with single primers of arbitrary nucleotide Sequence (Arif et al., 2010). Some of the limitation of this technique includes non-reproducibility and dominant inheritance. The technique does not allow identifying dominant homozygotes from heterozygotes since majority of the alleles segregate as dominant markers (Semagn et al., 2006). The techniques is less specific since it requires one primer for amplification and the size of the primer is short. Despite development of superior techniques such as AFLP, there has been continued use of RAPD in low-tech labs (Kjolner et al., 2004).

The technique has been used to analyse for genetic diversity in various grass family plants, such as *Chloris roxburghiana* (Mnene et al., 2005), Nappier grass (Babu et al., 2009), elephant grass (de Lima et al*.,* 2011), *Elymus sibiricus* (Ma et al., 2012) and rice, sorghum, maize, barley and wheat (Salem et al., 2007). Mnene et al., (2005) reported 131 polymorphic markers and significant genetic variation among four populations of *C. roxburghiana* grass species. Ma et al., (2012) reported 79% polymorphic markers with RAPD on eight populations of *Elymus sibiricus* grass species. Most studies have used both RAPD and ISSR markers on the same samples giving comparisons of their performances. Babu et al., (2009) used RAPD and ISSR markers on 30 collections of Nappier grass (*Pennisetum purpureum*) and reported significant genetic variation among accessions by each marker and low correlation ($r = 0.33$) between recorded results of the

markers, such as different dendrograms. De Lima et al., (2011) used both RAPD and ISSR markers and recorded 76% polymorphic bands with 6.56 polymorphic bands per primer for ISSR markers against 72% polymorphic bands with 5.11 polymorphic bands per primer for RAPD. De Lima et al., (2011) noted that ISSR and RAPD can provide consistent information for diversity analyses in elephant grass. Szenejko et al., (2016) reported matching polymorphism information content (PIC) for RAPD (0.264) and ISSR (0.270) with varied assay efficiency index (AEI) where ISSR with 37.1 was higher than RAPD (35.3). The assay efficiency index value shows the average number of polymorphic products identified in the presence of a single primer. In conclusion, Szenejko et al., (2016) noted that ISSR was more useful and reliable than RAPD due to the high AEI, better reproducibility and differentiating properties.

2.8.2 Inter Simple Sequence Repeat (ISSR)

This technique involves the use of microsatellite sequences directly in the Polymerase Chain Reaction (PCR) for DNA amplification (Semagn et al., 2006). The technique is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and AFLP to the universality of RAPD (Pradeep, et al., 2002). Inter Simple Sequence Repeats have high reproducibility possibly due to the use of longer primers. The ISSRs are highly polymorphic and broadly spread all over the genome.

The technique has been used successfully to investigate for genotypic diversity in grass species such as, *Cenchrus* species by Gutierrez-Ozuna et al., (2009) and Al-Soqeer (2011), Nappier grass Babu et al., (2009) and elephantgrass (de Lima et al., 2011). Significant variation among grass populations have been reported by Babu et al., (2009) and Adhikari et al., (2015). Babu et al., (2009) reported 89% polymorphic bands (PB) with 10 ISSR primers on 30 accessions of Nappier

grass while Adhikari et al., (2015) reported 87% PB with 12 ISSR markers on *Cymbopogon* species. Using three ISSR primers on 16 *C. ciliaris* populations, Gutierrez-Ozuna et al., (2009), recorded no significant variation and concluded that the dispersal and spread of the populations was not guided by genetic diversity. While working with *C. ciliaris* species, Al-Soqeer (2011) reported low percent polymorphic bands for six ISSR markers at 17.6 and low genetic variability among the five populations in the study. Al-Soqeer (2011) linked the low genetic variability to the apomictic breeding system of the species.

2.8.3 Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) is a polymerase chain reaction based on fingerprinting technology (Vos et al., 1995). The technique is highly reliable, reproducible, does not require any DNA sequence information from organism under study and is information-rich since it can analyze a large number of polymorphic loci simultaneously (Semagn et al*.,* 2006).

This technique has been used successfully to evaluate various grass species for genetic variability, such as Buffelgrass (Griffa et al., 2006; Burson et al., 2015), *Pennisetum purpureum* (Wanjala et al., 2013), *Elymus glaucus* (Hufford et al., 2014) and *Panicum* species (Assaeed et al., 2017). Using three primer combinations that produced 152 bands, Griffa et al., (2006) and was able to characterize 21 genotypes of Buffelgrass and successfully identify 2 hybrids among 15 F1 genotypes. Griffa et al., (2006) identified two asexual lines that were very distinct from the sexual line and recommended them for use in future crossings. While working on the same species, Buffelgrass, Burson et al., (2015) reported considerable genetic variation among pentaploid accessions with genetic separation of cold tolerant and non-cold tolerant genotypes at sub-group levels using AFLP technique. In Wanjala et al., (2013), mean polymorphic loci of 63.4% was

recorded with significant variation within (91%) and among (9%) 281 cultivars of *Pennisetum purpureum.* Hufford et al., (2014) reported 99% polymorphic loci using eight primer combinations on 21 populations of *Elymus glaucus.* Using the technique, Hufford et al., (2014) reports 37.1% variation within population, 55.8% among populations within regions, namely the islands and mainland and, 7.1% between regions.

Despite wide application of AFLP in genetic diversity studies, the technique is a complex and time consuming technique with multiple steps making it difficult to optimize conditions in each step (Li and Quiros, 2001). The technique involves the use of radioactive materials that are expensive and toxic (Mondini et al., 2009).

2.8.4 Sequence Related Amplified polymorphism (SRAP)

Sequence related amplified polymorphism (SRAP) is a novel marker that is based on two-primer amplification with the primers being 17 or 18 nucleotides long (Li and Quiros, 2001). The amplifications are aimed at open reading frames (ORFs). Some of the advantages of the marker include ease of use, reasonable throughput rate, disclosure of numerous co-dominant markers and bands are easily isolated for sequencing (Li and Quiros, 2001).The marker is able to detect polymorphic loci and is more informative than Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence repeat (SSR) markers (Budak, et al., 2004a). The marker was found to be more efficient than ISSR while analyzing genetic diversity of 12 populations of *Hemarthria compressa* grass species in terms of marker index (MI) and higher genetic differentiation resolved (Huang et al., 2012). Huang et al., (2012) reports MI of 5.51 for SRAP against 3.36 for ISSR. While analyzing for diversity among *Ocimum* species, Chen et al., (2013) reported that SRAP marker showed the highest mean value

of polymorphic information content (PIC) of 0.29 and resolving power (Rp) of 30.19 that were much higher than those of RAPD (0.23, 5.13) and ISSR (0.19, 1.39). Zhang et al., (2016) reported lower levels of percent polymorphic loci for SRAP (91.9%) than ISSR (94%) and a strong correlation between SRAP and ISSR (0.8023) while working on *Chrysopogon aciculatus* grass species.

The SRAP marker was developed primarily for *brassica* and has been successfully used on other various crops where good amplification and polymorphism is reported (Li and Quiros, 2001). The technique has been used in analysis for genetic diversity in various grass species such as Buffalograss (Budak et al., 2004a), Orchardgrass (Zeng et al., 2008), *Hemarthria compressa* Huang et al., 2012) and Kentuckey bluegrass (Yuan et al., 2018). The marker is highly polymorphic as shown by Huang et al., (2012), Zeng et al., (2008) and Yuan et al., (2018) who recorded 82.2, 84 and 91.8% polymorphic bands, respectively. A total of 53 accessions of Buffalograss were grouped into four ploidy levels of diploid, tetraploid, pentaploid and hexaploid using 34 primer combinations of SRAP markers (Budak et al., 2004a). Twenty one primer pairs of SRAP gave clear separation of 60 Orchardgrass accessions based on four continents, which were the places of origin (Zeng et al., 2008). Significant genetic diversity among 12 populations of *Hemarthria compressa* was revealed by SRAP with 53.4 and 46.6 % variation recorded between and within populations, respectively (Huang et al., 2012).

2.9. Agro-ecological zones

An Agro-ecological Zone (AEZ) is a land resource mapping unit, defined in terms of climate, landform and soils, and/or land cover, and having a specific range of potentials and constraints for land use (FAO, 1996). The AEZs are zoned based on the combination of landforms, soil and

climatic characteristics. The delineation of the AEZs mainly relies on the climatic and edaphic requirements of crops and on the management systems under which the crops are grown. Some of the climatic factors include temperature and growing period, which is the period of the year when both moisture and temperature conditions are suitable for crop production. Based on the above definition, there are seven main AEZs (Table 2.1; Figure 2.2).

Table 2.1: *Agro-climatic zones, excluding areas above 3000m altitude (Mganga et al., 2010b)*

Zone	R/E_o *(%)	Classification	R (mm)	E_o (mm)
I	> 80	Humid	1100 - 2700	1200 – 2000
II	65-80	Sub-humid	1000 - 1600	1300 – 2100
III	50-65	Semi-humid	800 - 1400	1450 – 2200
IV	40-50	S.humid - S.arid	600 - 1100	1500 – 2200
V	25-40	Semi-arid	450 - 900	1650 – 2300
VI	15-25	Arid	300 - 560	1900 – 2400
VII	< 15	Very arid	150 - 350	2100 – 2500

Notes: * R – Average rainfall; E_o- Average annual evaporation

The generalized AEZ categorization was found to be only suitable for decision making at higher ranks either international and policy levels. Thus a more differentiated system that incorporates crop yield probabilities was developed for Kenya (Jaetzold and Schmidt, 1982). The main factors considered in the new classification were temperature, water requirements for the main crop and the growing period. Temperature was based on the maximum temperature limits within which the main crop can successfully grow, which led to establishment of the temperature belts. These belts are lowlands, lower midlands, upper midlands, lower highlands and upper highlands all suited for different crops such as cashew and coconuts for lowlands. The temperature belts was followed by the main zones that considers the temperature and water requirements for the main crops for the area. A total of nine zones were created, namely, the maize, hybrid maize, wheat, unirrigated rice, irrigated rice, sorghum, finger millet, ground nuts and cotton zones. The main zones were further divided according to the annual and distribution of the growing period. The length of the growing

period is important in selecting crops for a given AEZ (Jaetzold and Schmidt, 1982). The start of the rainy season determines the beginning of the growing period and the period continues beyond the rainy season where the moisture reserves stored in the soil is considered (FAO, 1996).

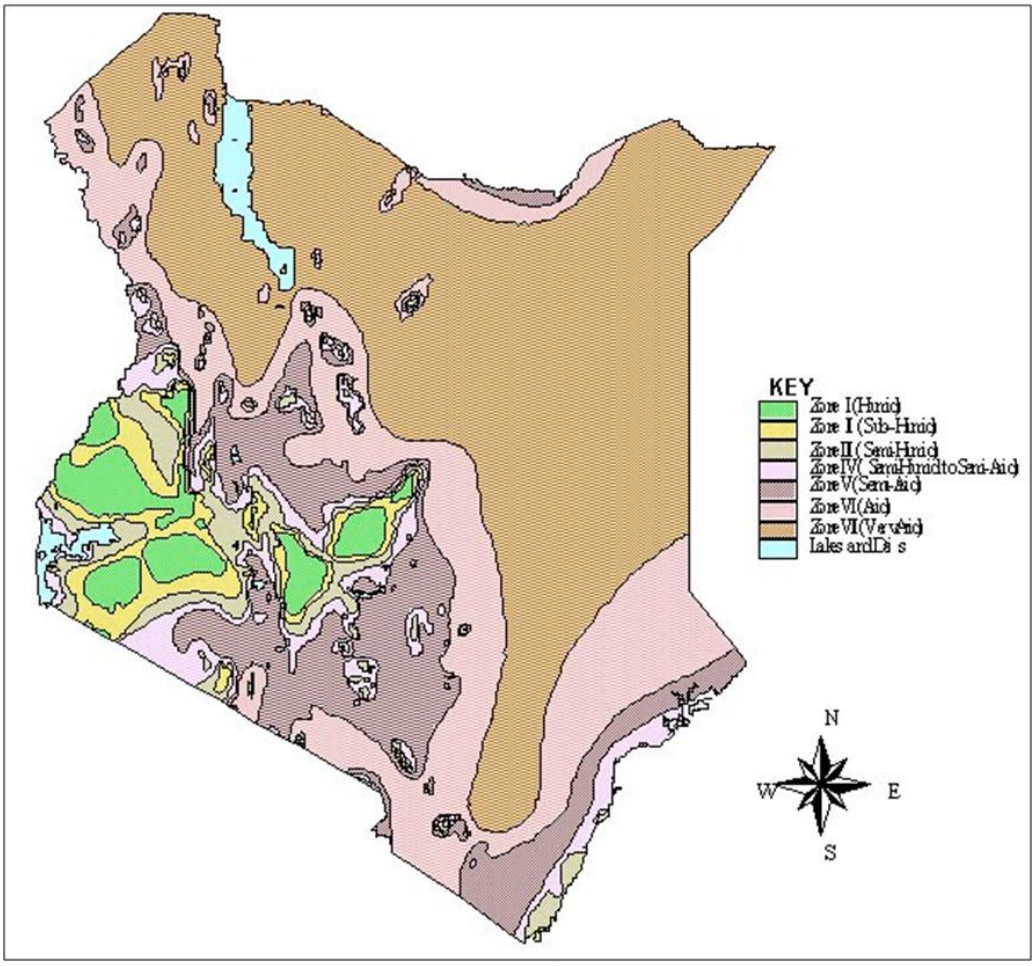

Figure 2.2: *Agro-ecological zones of Kenya*
Source: Kenya Soil Survey, 1980

CHAPTER THREE

CHARACTERIZATION OF ECOTYPES OF *CENCHRUS CILIARIS* L. AND *ERAGROSTIS SUPERBA* PEYR USING MORPHOLOGICAL AND NUTRITIVE VALUE TRAITS

3.1. Abstract

Characterization of forage germplasm is useful in providing information on variation among the ecotypes and in identification of traits of importance. To identify suitable germplasm for reseeding the rangelands, eleven ecotypes of *Cenchrus ciliaris* and nine for *Eragrostis superba* were characterized based on morphological traits and nutritive value components. The ecotypes were collected from three selected sites each in four Counties, namely Kilifi, Makueni, Taita Taveta and Kajiado. They were planted in the field at Kenya Agricultural and Livestock Research Organization (KALRO), Kiboko research station in five rows of four metres long per plot in randomized complete block design. Morphological data for stem, leaf and seed attributes and, crude protein (CP), crude fibre (CF), percent dry matter, ash and *in-vitro* digestibility of dry matter for nutritive values was recorded.

For *C. ciliaris*, significant variations in morphological traits were recorded. The eleven ecotypes clustered into two groups representing robust and small sized types using plant height, stem thickness, leaf length and leaf width. Two groups of late and early flowering ecotypes was formed using three flowering traits, namely, time to start flowering, time to 100 % plot flowering and flowering period. Ecotypes collected from Kilifi were earlier flowering while those from Kiboko were late flowering. Days to first flowering was positively correlated (r = 0.8) each with plant height, leaf length, leaf breadth and inflorescence length and negatively to percent fertile tillers (r

= -0.8). Positive and significant correlations were recorded between INVDMD with stem thickness (r = 0.6) and leaf breadth (r = 0.7) and, CP (r = 0.7) each for plant height, leaf length, and stem thickness and with first flowering time (r = 0.8). Crude fibre was negatively correlated (r = - 0.7) each with plant height and leaf length. For *E. superba*, clustering using 16 morphological traits, robustness related traits or seed yield traits resulted in similar groupings. KBK1 and KBK2 ecotypes collected from same geographical location of Makueni County with different management and use were clustered together as robust types while all other seven ecotypes were in one group. Nutritive value components resulted in two major groups where Kiboko (KBK2) and Kilifi (KLF1) ecotypes clustered together with significantly higher CP (11.0 %) than the cluster consisting of KLF2, KBK1, TVT1, TVT2, GBK and KLF3 ecotypes with 8.9 % CP.

There were early and late maturing ecotypes among the *C. ciliaris* collection and late flowering types were robust while early flowering types were small. The differences among the ecotypes in nutritive values could be exploited to meet different feed requirements. Late flowering and robust ecotypes of *C. ciliaris* were higher in CP. KBK2 and KLF1 ecotypes of *E. superba* should be selected for higher CP production. The correlated morphological traits can be used to select for high INVDMD or CP among and within *C. ciliaris* ecotypes. Selection for low CF should target shorter ecotypes of *C. ciliaris* with shorter leaves.

Key words: *Cenchrus ciliaris*, ecotype, *Eragrostis superba*, flowering, grass reseeding

3.2. Introduction

Grasses form the main source of nutrients to livestock production in the Arid and Semi-Arid Lands (ASALs) and majorly contributes to the daily dietary requirements. *Cenchrus ciliaris* and *E.*

superba are preferred by farmers in the ASALs with preference shifting from *E. superba* to *C. ciliaris* as aridity increases (Ndathi et al., 2012). The two grass species are commonly planted in mixtures in the Kenyan rangelands for improved natural pastures. The species are also commonly found among various vegetation types in a wide range of habitats in the ASALs in Kenya.

Cenchrus ciliaris is found in areas with 100 to 1000 mm rainfall and with an elevation of zero to 2500 metres above sea level. The species is well adapted to the ASALs, persistent, tolerant to grazing and drought and with good response to both small and large rainfall amounts (Cook et al., 2005). The species is very competitive due to its extensive rooting system and allelopathic effect that impedes successful establishment of other plants around it (Franklin et al., 2006).

Cenchrus ciliaris has been evaluated in various studies for reseeding potential (Bogdan and Pratt, 1967; Boonman, 1993; Opiyo, 1997), allelopathic effects (Kirwa et al., 2012), performance in mixtures (Mganga, 2010a) and seed and herbage yields (M'seddi et al., 2002; Visser et al., 2008). The species can produce two to nine tons/ha dry matter yield and seed yield of 150 – 500 kg/ha and is mostly preferred by livestock due to the high nutritive value and herbage yield (Cook et al., 2005). Differences in chemical composition among ecotypes of *C. ciliaris* were reported by Garcia-Dessommes et al., (2003), Morales et al., 2006; Saini et al., (2007) and Ashraf et al., (2013). Sufficiency to meeting daily dietary requirements for selected nutrients was found to differ between the *C. ciliaris* ecotypes (Garcia-Dessommes et al., 2003; Saini et al., 2007). Garcia-Dessommes et al., (2003) reported deficiency in molybdenum mineral in all six genotypes except hybrid Nueces.

Eragrostis superba is a poor competitor when compared to *Enteropogon macrostachyus* or to *C. ciliaris,* due to a high seedling mortality and associated low tiller development and low percent foliage (Opiyo et al., 2011). The species was least affected by frost burns and was recommended for use in pasture production in Australian subtropics due to significantly high yields during cool seasons (Strickland, 1973). Different studies from the Southern rangelands of Kenya reported varied dry matter yields for the species such as, 1899 to 2434 kg/ha by Opiyo et al., (2011), 896 kg/ha by Mganga (2010b) and 2750 kg/ha by Musimba et al., (2004).

Although the two species are widely cultivated in the agro-pastoral parts of the Southern rangelands of the Kenya for both seed and herbage production, their seeds are harvested from the wild establishments since there are no certified seeds. Due to high spatial variability of rangelands, there is a likelihood of existence of ecotypes among the indigenous grass species that could negatively affect seed quality.

Characterization of forage ecotypes could provide knowledge on the variations present among the collections which in turn may help in decision making on possible collections that could be made or the ecotypes to be recommended for evaluation and utilization. The clustering of ecotypes may also be used to identify possible parental types in breeding program. Phenotypic variation has been observed in agro-morphological traits and nutritive value components of *C. ciliaris* (Jorge et al., 2008; Ashraf et al., 2013). Studies done on morphological characterization of accessions of *C. ciliaris* resulted in various groupings particularly when variation in plant size and flowering time were measured. The species reproduces predominantly by aposporous apomixes. Due to the apomictic nature, there is little intra-variety variation and selection of true breeding genotypes from ecotypes that are highly variable in ecological and agronomic traits have been done in *C.*

ciliaris (Boonman, 1993; Arshad et al., 2007). Selection for *C. ciliaris* genotypes with improved spring yield in Australia was done by Hacker and Waite (2001) guided by previously characterized collections. Examples of commonly known cultivars from selections include the American and Gayndah for medium height and early flowering and Biloela and Molopo as tall and late flowering varieties.

There was no characterization results that had been reported on *Eragrostis superba* grass species by the time of carrying out this study. Eleven ecotypes of *Cenchrus ciliaris* and nine of *Eragrostis superba* were characterized using morphological and nutritive value components in order to identify unique important ecotypes that may be used in reseeding.

3.3. Materials and Methods

3.3.1. Collection sites

Collection of grass seeds from the wild was conducted in four agro-ecological zones (AEZ), represented by Kilifi, Taita Taveta, Makueni and Kajiado Counties for AEZ III, IV, V and VI, respectively (Orodho, 2006). One target site was purposively identified per county where collection was done. These were Kilifi Township, Taveta, Kiboko and Magadi site for Kilifi, Taita Taveta, Makueni and Kajiado Counties, respectively, and, recorded as sites of ecotype origin in this document. Three locations were sub-sampled in each of the target sites of ecotype origin where actual collection of germplasm was done.

Seeds were harvested from samples of 20 plants using randomly stratified technique (Guarino et al., 1995) in July – September 2012. Where seeds were not available or available in small quantities, tuft splits were uprooted as collections instead of seed. The germplasm was taken to

KALRO Kiboko where the seeds were tested for germination capacity (GC). Table 3.1 shows the list of ecotypes collected for *C. ciliaris* and *E. superba* grass species while Figure 3.1 shows the map of collection sites.

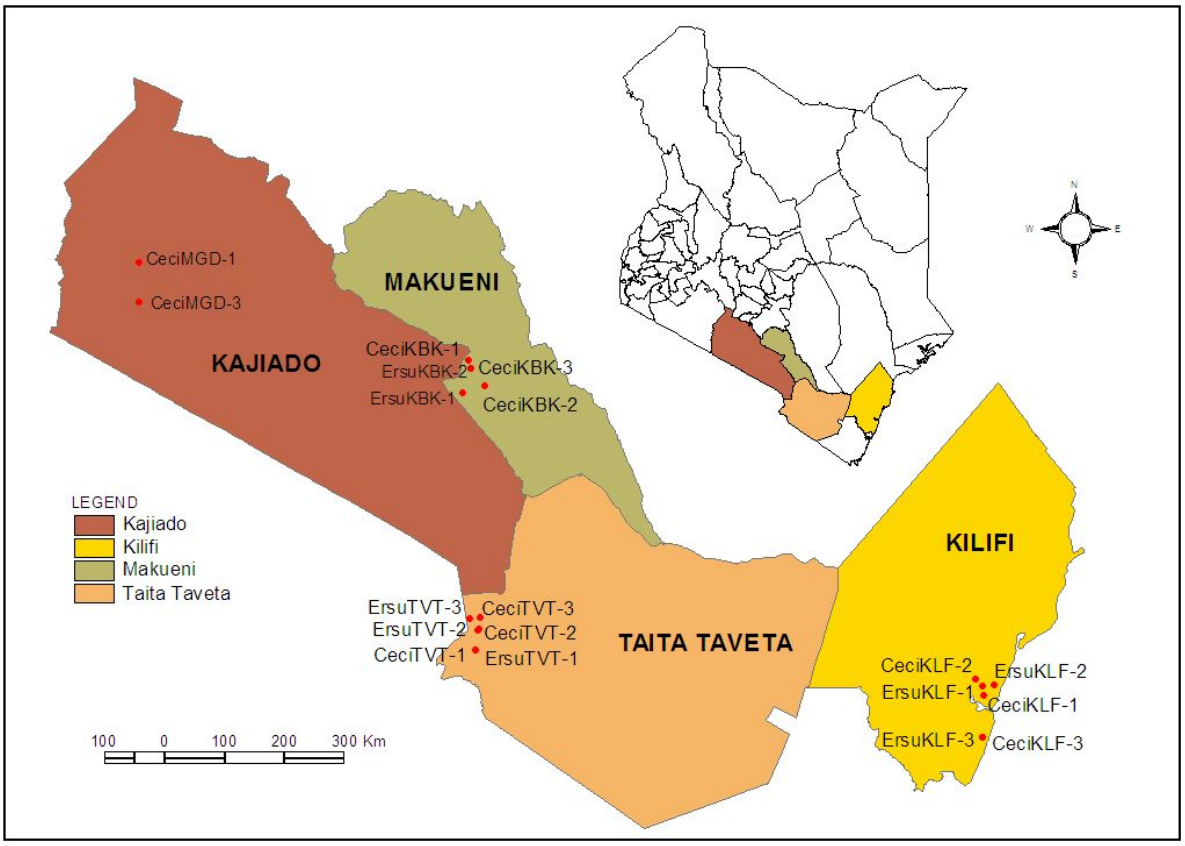

Figure 3.1*: Map of Kenya showing sampling Counties and collection sites*

Table 3.1: *Ecotypes of Cenchrus ciliaris and Eragrostis superba and description of site of origin*

Ecotype	GPS point	Altitude (m)	Management/use	Site of origin and environmental description
***Cenchrus ciliaris* ecotypes**				
Kiboko 1 (KBK1)	37M 0356997 UTM 9754961	950	Controlled grazing land	Kiboko (Rainfall 575 mm; temperature range 14 – 35 ^{o}C; $^{\#}$AEZ V)
KBK2	37M 0364664 UTM 9742932	1059	Riverine, grazing land	
KBK3	37M 0358340 UTM 9751011	900	Controlled grazing land	
Kilifi 1 (KLF1)	37M 0592230 UTM 9602470	49	Uncontrolled, overgrazed individual land	Kilifi (Rainfall 1200 mm; temperature range 20 -31 ^{o}C; AEZ III)
KLF2	37M 0588462 UTM 9609848	97	Edge of cultivated land	
KLF3	37M 0591436 UTM 9583080	32	Frequently mowed sisal farm	
Magadi 1 (MGD1)	37M 0206621 UTM 9799034	822	Along a dry riverbed, open communal grazing land	Magadi (Rainfall 600 mm; temperature range 28.6 -32.9 ^{o}C; AEZ VI)
MGD2*	37M 0209795 UTM 9805824	856	Open communal grazing land	
MGD3	37M 0206631 UTM 9781498	810	Controlled communal grazing land, flooded plain	
Taveta 1 (TVT1)	37M 0360211 UTM 9623156	770	Edge of cultivated land	Taveta (Rainfall 440 mm; temperature range 20 -30 ^{o}C; AEZ IV)
TVT2	37M 0361675 UTM 9632568	908	Open grazing land	
TVT3	37M 0362386 UTM 9637556	922	Edge of irrigation canal with minimal grazing	
***Eragrostis superba* ecotypes**				
E_KBK1	37M 0354247 UTM 9739790	1120	Chyulu hills national game reserve, controlled grazing	Kiboko temperature range 14 – 35 ^{o}C; $^{\#}$AEZ V)
E_KBK2	37M 0358340 UTM 9751011	900	Frequently mowed area	
E_KLF1	37M 0591450 UTM 9606252	80	Edge of cultivated land with minimal grazing	Kilifi (Rainfall 1200 mm; temperature range 20 -31 ^{o}C; AEZ III)
E_KLF2	37M 0597165 UTM 9607150	14	Edge of cultivated land	
E_KLF3	37M 0591436 UTM 9583054	32	Frequently mowed sisal farm	
E_TVT1	37M 0360260 UTM 9623140	761	Edge of cultivated land	Taveta (Rainfall 440 mm; temperature range 20 -30 ^{o}C; AEZ IV)
E_TVT2	37M 0361124 UTM 9632038	904	Open grazing land	
E_TVT3	37M 0357469 UTM 9637472	918	Open grazing land	
E_GBK		-		Magadi crossroads, Referenced as GBK^{+}

***Excluded in the analysis on account of lack of establishment;** $^{\#}$*AEZ = agro-ecological zone* $^{+}$Genebank of Kenya

3.3.2. Study sites

The experiment was done at KALRO's Arid and Rangelands Research Institute (ARLRI) - Kiboko Centre pasture plots located in Makindu Sub-County in the semi-arid county of Makueni, Kenya. Kiboko Research Centre is located 160 km South East of Nairobi in agro-ecological zone V. The site receives a bimodal rainfall pattern with the long rains occurring in March – May and short rains in October – December. The dry seasons come in the months of January -February (short dry season) and June to October (long dry season). The annual mean rainfall and temperature are 534 mm and 23.4ºC, respectively (Ndathi et al., 2011). The weather data for the Kiboko during the study period is shown in Table 3.2.

Table 3.2: *Weather data for KALRO - Kiboko for year 2013 and 2014*

	Year 2013				Year 2014			
	TEMPERATURE(°C)		R.H	RAINFALL	TEMPERATURE(°C)		R.H	RAINFALL
Month	Max	Min	%	mm	Max	Min	%	mm
Jan	31.00	18.06	89.45	29.5	30.73	17.05	90.39	0
Feb	33.13	17.27	81.43	0	32.54	18.43	89.18	55
Mar	33.02	20.15	82.35	58.3	31.94	19.29	85.61	186.5
Apr	31.67	19.53	91.77	228.2	31.40	19.47	89.23	42.5
May	29.97	16.50	90.65	36	30.18	16.56	86.61	12.4
June	27.75	14.80	90.90	0	28.88	15.53	85.43	4
July	27.53	14.18	87.52	3	27.90	14.02	85.94	0.4
Aug	28.55	14.94	84.77	0	29.55	14.19	80.71	0
Sep	30.67	15.58	86.10	0	30.27	16.13	79.93	1.5
Oct	32.90	17.00	83.58	2	32.92	18.15	75.13	2.5
Nov	30.47	18.70	87.90	102	31.03	18.73	84.43	186.5
Dec	29.07	18.00	89.06	84.5	29.60	18.35	88.39	199.5

R.H-relative humidity

3.3.3. Field conditions and layout

Seed bed preparation was done by ploughing and leveling the ground to a fine tilth using a rake. The plots consisted of five rows of four metres long with a distance of one metre between plots in a randomized complete block design with three replicates.

Seeds were planted in plastic germination trays and seedlings transplanted after one month to the plots as per above described design (Plate 3.1). Individual plants were spaced at one metre between rows and at 0.5 metre within rows. In cases where tuft splits had been collected, they were directly transplanted to the experimental plots. The tufts were split into several plantlets/splits and an estimated two splits were sown per hill. Planting was done during the short rains in October 2012.

Plate 3.1: *from Left: seedling establishment in germination trays of 280 holes; transplanting of seedlings to the plots*

Plots were kept free of weeds through hand weeding using a hoe. The plots were irrigated after every seven days during the dry seasons. The amount of water was measured by placing rain gauges at even intervals in the plots and the amount of water recorded after every irrigation period. The number of hours of irrigation was also recorded. Fertilizer was applied according to recommendations by Boonman (1993) as single superphosphate at a rate of 50 kg/ha/yr P at the

time of planting and once a year top dressing, and 100 kg N per ha at seven to ten days after every cutting of all herbage to five centimeters above the ground. Weeding was done using a hand hoe after every cutting before application of fertilizer.

The first cutting, which was a standardization cut was done in May 14th 2013 and repeated in September 2013 to even out the possible differences in stand establishment arising from use of seeds and splits (Jorge et al., 2008). The standardization cut involved cutting of all above ground plant material to a height of five centimeters in all the plots. A third cutting was done in November 2013 at the start of the short rains to allow for characterization data collection during the wet season.

3.3.4. Data collection

Germination data was recorded for the seeds harvested at maturity stage from the different agro-ecological zones during exploration expeditions. Four replicates of 25 caryopsis each were placed on moistened filter papers in plastic petri dishes and germinated at room temperature at KALRO Kiboko laboratory. Germination, defined as the appearance of a root, was counted and recorded daily from first day to fourteen days after germination.

Flowering data were collected on the regrowth following successive cutting of all above ground herbage material in all the plots. The first, second and third cut (C1, C2 and C3) were done in September 2013, November 2013 and February 2014, respectively (Table 3.3). The first data (C1) was collected during the dry season while the second (C2) and third (C3) were done during rainy seasons. The third cut received high intensity rainfall, although it was expected to be a short dry season. The time to cut the above ground material was dependent on the conditions of the

subsequent season, as described by Visser et al., (2008). For instance, the second cut was done at the beginning of the short rains, so as to prepare the plot for data collection during the rainy period.

Table 3.3: *Cutting dates, rainfall amounts and length of preceding growing periods at Kiboko*

	First Cut (C1)	**Second Cut (C2)**	**Third cut (C3)**
Cutting date	11-09- 2013	6-11-2013	14-02- 2014
Number of days between cuts	55	99	67
Total rainfall amounts (mm)	Irrigated	172.9	203.6

Data for flowering phenology were collected across the three cuts. Flowering, defined as emergence of an inflorescence per plant, was recorded daily from the date of each cut shown in Table 3.3. The total number of plants that had flowered per plot was recorded daily until 100 % plot flowering. The days from cutting date recorded as day 0, to when the first plant flowers in a plot was recorded as the days to first flowering (DSF). The days from day 0 to when all the plants in the plot have flowered was recorded as days to 100 % plot flowering or days to full plot flowering (DFF). Flowering period (FP) was recorded as the period between the days to the first flowering (DSF) to the days of 100 % plot flowering (DFF).

For other morphological traits, data was recorded according to descriptors used by Jorge et al., (2008) and M'seddi et al., (2002) (Table 3.4). The morphological data were collected during the second cut following procedures by IBPGR (1985), Van de Wouw et al., (1999) and Jorge et al., (2008). Measurements were done from ten randomly selected plants per ecotype as also recommended by Van de Wouw et al., (1999). Where measurements targeted parts of a tiller such as leaf or stem thickness then ten observations were done on ten randomly selected plants. Leaf attributes were recorded on the second leaf below the flag leaf.

Table 3. 4: *List of morphological characteristics used in data collection and their descriptions*

Morphological characteristic	Description	Units	No. of observations
Growth stage: Full bloom			
1. Flag leaf length	From the ground to the tip of the flag leaf	Cm	10 Plants
2. Plant height	From the ground to the tip of inflorescence	Cm	10 plants
3. Stem thickness	Average culm diameter above the lowest node	Mm	10 observations
4. Number of nodes	Count of all nodes in 1 randomly selected tiller per plant	No.	10 observations
5. Leaf length	Ligule to the tip of the leaf	Cm	10 observations
6. Leaf breadth	Width of leaf at widest point	Mm	10 observations
7. Leaf ratio	leaf length divided by width	Ratio	
8. Days to start & at 100% flowering	Daily record of no. of flowering plants per plot from the time of cutting	No.	whole plot
9. Flowering period (FP)	Days between flower initiation and 100% flowering	No	
10.Awn density	Awn presence on panicles (score of 1- sparse -3-dense)	Scale	whole plot
11. Total tiller number	Count of all tillers on a plant	No.	10 plants
Growth stage: Seed maturity			
12. % Fertile tillers	Tillers with Inflorescence as a percentage of all tillers on the particular plant	%	% of 10 plants
13. Plant height	From the ground to the tip of inflorescence	Cm	10 plants
14. Inflorescence length	From the lowest cluster to the top of bristle	Cm	10 observations
15. Inflorescence width	Width at the widest point	Cm	10 observations
16. Inflorescence ratio	Inflorescence length divided by inflorescence width		
17. Inflorescence shape	Scale 3 (tapering), 5 (parallel), 7 (fusiform)	Score	whole plot
18. Inflorescence density	Abundance of spikelets (score of 1- sparse -3-dense)	Score	whole plot
10. Inflorescence count	Count of all inflorescence on a plant	No.	10 plants
20. Aerial tillers	Count of branching tillers on main tillers	No	10 plants
21. Basal tillers	Count of all tillers only at the base	No	10 plants
22. Spikelet number	Count of all spikelets on an inflorescence	No.	10 observations
23 Ease to drop seed	Ease to dislodge seed, Scale of 1-5	Score	whole plot
24. Caryopsis Number	Count of all caryopsis in 100 spikelets	No	5 samples
25. 1000 Caryopses weight	calculated from mean weight of 100 caryopses	g	3 samples
26. Caryopsis per spikelet	Count of caryopses in individual spikelets from 25 spikelets	No.	4 samples
27. Spikelet weight	Mean weight of 100 spikelets	g	5 samples

Some of the traits with visually observable difference were recorded for *C. ciliaris* ecotypes which included different colors of inflorescence, stems and leaves.

Data collection for nutritive values was done on 18/12/2013 during the short rains season of October – December 2013 (Cut 2) at Kiboko. At six weeks post cutting of the plots, twelve plants were randomly sampled from three replicates of each ecotypes and clipped to five centimeter level. The samples were bulked into one sample per ecotype and dried in the oven for 24 hours at 60 ^{o}C to obtain the air dry matter (ADM). The ADM samples were ground through a 1.0 mm sieve hammer mill and analyzed in duplicates for ash content, crude protein (CP), crude fibre (CF), percent dry matter yield (%DM) and in-vitro digestibility of dry matter at the Animal Production Laboratory, University of Nairobi. The procedure of bulking of harvested plant samples and analyzing in duplicates has been done by Mtui et al., (2009) and Strickland (1973).

The ash, crude protein, crude fibre, and dry matter yield of individual grass ecotypes were analyzed using the standard procedures of AOAC (2005). Ash content was determined by burning two grams of air dried samples in a high temperature muffle furnace at 600^{o}C for two hours, removed, cooled and weighed immediately to give percent ash. Crude protein content (CP) was determined using Kjeldahl method where the sample was digested in H_2SO_4 with $CuSO_4$ as a catalyst, distilled, titrated and percent nitrogen was calculated. One gram of oven dried sample was put in a digestion flask then added 16.7 g K_2SO_4, 0.01 g anhydrous $CuSO_4$, 0.6 g TiO_2, 0.3 g pumice, 0.5 g alundum granules and 20mL H_2SO_4. Heat was adjusted to bring 250mL water at 25^{o} to rolling boil in five minutes then the sample was heated at the five minutes boil rate until dense fumes clear bulb of the flask. The flask was swirled gently and heated for additional 40 minutes. 250 mL water was added and cooled to room temperature. The product was distilled and excess acid titrated with NaOH standard solution, corrected for blank and percent nitrogen was calculated. The nitrogen levels obtained was multiplied with a factor of 6.25.

To determine percent dry matter (DM), the air dried samples were oven dried at 105^{o}C overnight, weighed in grams and the new weight used to calculate percent DM. The in-vitro digestibility of dry matter (INVDMD) was determined using the two stage technique by Tilley and Terry (1963). In the first stage, five grams of the ground oven dried samples were incubated anaerobically with rumen fluid in a buffered solution at 38^{o}C for 48 hours in the dark. For the second stage, the sample was digested in pepsin and hydrochloric acid at 38^{o}C for 48 hours.

3.3.4. Data analysis

Data analysis was performed using Genstat 15th edition analysis tools (Payne et al., 2012). A univariate analysis of variance for the replicated morphological traits was done to assess variation among ecotypes. Analysis of variance was carried out on flowering data for *Cenchrus ciliaris* and nutritive value data for *E. superba* and *C. ciliaris*. The flowering data included days to first flowering (DSF), days to full plot flowering (DFF) and flowering period (FP) for the three cuts while the nutritive value data was for CP, CF, % DM, ash and INVDMD. The means were separated using least significant difference (LSD) at $p \leq 0.05$ in Genstat 15th edition. The ANOVA model used was $Y_{ij}=\mu+\tau_i+\epsilon_{ij}$ where μ was the grand mean, τ_i are the deviations from the grand mean due to the treatment levels, that is the ecotypes, site of ecotype origin, or the cuts and ϵ_{ij} the error terms.

Means of all recorded morphological data sets were analysed for phenotypic correlation using Spearman's ranks correlation coefficient that was then used in principal components analysis and to develop a similarity matrix whose output was used in hierarchical cluster analysis (Jorge et al., 2008). For correlation, means for each trait listed in Table 3.4 per ecotype was first generated. The means were then subjected to correlation analysis in Genstat 15th edition where correlation

coefficients for each trait against the other traits was generated. The correlation output also gave the significant levels for each correlation coefficient which indicated whether the relationship was statistically significant at $p<0.05$ or at $p<0.001$ level. Where the correlation coefficient was more than $r=0.7$ the correlation was rechecked and if the relationship was due to overlap in the kind of data recorded, one of the attributes was omitted in Principal component analysis. This was to avoid indirect weighting because inclusion of more correlated attributes would over emphasize a particular direction in principal component analysis. An example of when an attribute could be omitted is a high correlation in plant heights measured in different forms such as height to inflorescence tip and height to the flag leaf.

Cluster analysis was done on morphological and nutritive value attributes. For cluster analysis to be done, first a similarity matrix was formed using the data set of means per ecotype. The matrix was generated using all the given data of means or selected few depending on identified criteria, such as nutritive value, flowering, seed yield or robustness related traits. The similarity matrix gives the percent relationship between one ecotype to another for all the ecotypes for the given traits. The matrix was then used to generate the dendrograms using the hierarchical cluster analysis. The dendrograms displayed the different groupings or clusters of ecotypes as a result of the selected traits.

3.4. Results

3.4.1. Germination capacity of *Cenchrus ciliaris*

Figure 3.2 and 3.3 shows germination results for seeds of *C. ciliaris* and *E. superba* ecotypes collected from different agro-ecological sites. For *C. ciliaris,* KLF1 had the highest germination

capacity (50%) and MGD3 ecotype had the lowest (20%). *Eragrostis superba* KLF3 ecotype had the highest germination capacity (60%) and TVT3 had the lowest at 31%.

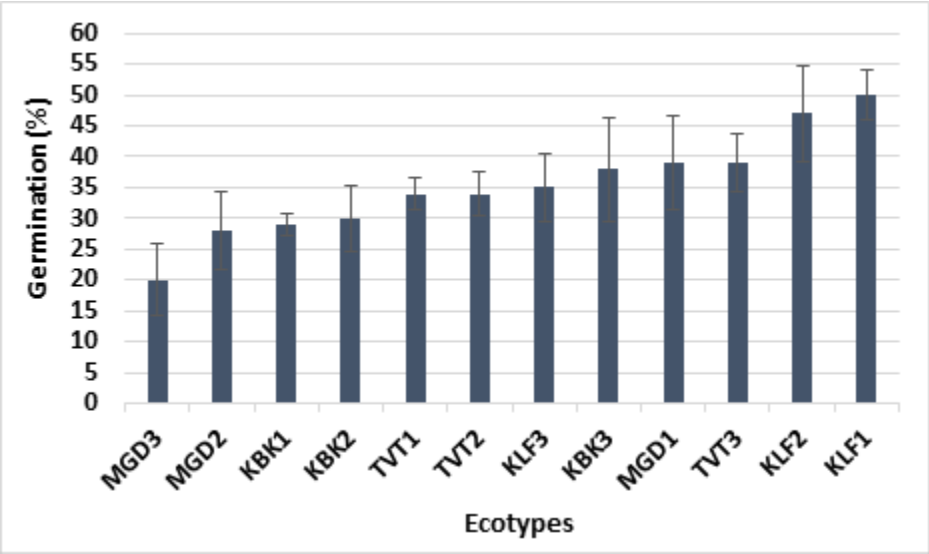

Figure 3.2: *Germination capacity Cenchrus ciliaris ecotypes seeds harvested during germplasm collection. KBK=Kiboko; KLF=Kilifi; MGD=Magadi and TVT=Taveta*

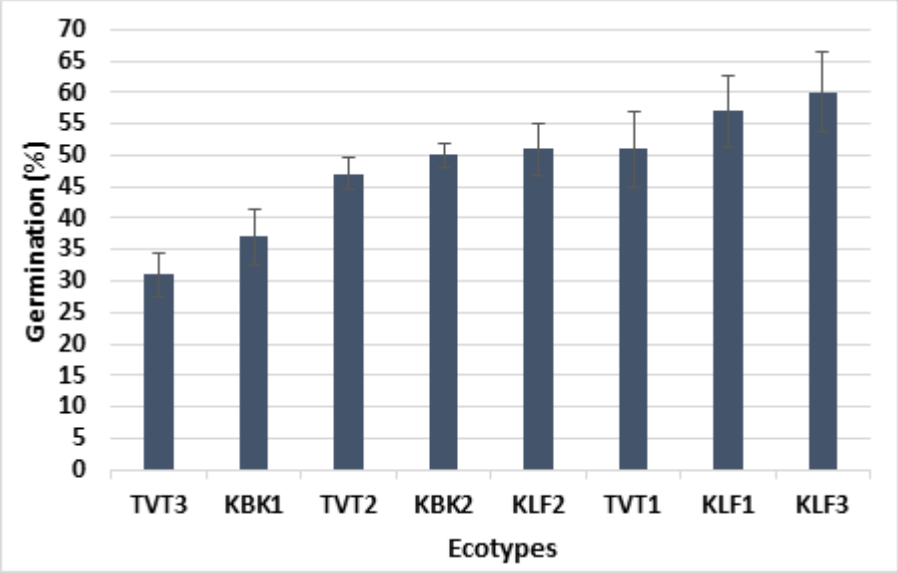

Figure 3.3: *Germination capacity Eragrostis superba ecotypes seeds harvested during germplasm collection. KBK=Kiboko; KLF=Kilifi and TVT=Taveta*

3.4.2. Phenotypic characterization of *Cenchrus ciliaris* ecotypes

Table 3.5 shows the mean range recorded among morphological traits for *C. ciliaris* ecotypes where wide ranges were recorded in all measured traits. Ecotypes KLF1 and TVT2 were among the shortest types while KBK1, KBK2 and KBK3 were among the tallest ecotypes. KBK2 ecotype recorded highest measurements in plant height to inflorescence tip, stem thickness, number of nodes and, leaf length and breadth. Ecotypes KLF1, TVT1 and TVT2 recorded the thinnest leaves while all Kiboko ecotypes and TVT3 had the widest leaves. KLF1 and KBK2 ecotypes had the highest spikelet weight with the lowest weight being recorded by KBK3 ecotype.

Table 3. 5: *Mean range, P_value and least significant difference at 0.05 ($LSD^{0.05}$) and % coefficient of variation (CV) of morphological attributes among Cenchrus ciliaris ecotypes*

No.	Attribute	Ranges	P_value	$LSD^{0.05}$	%CV
1.	Flag leaf height (cm)	69.8 – 122.4	<.001	9.6	11.8
2.	Inflorescence height at peak flowering (cm)	77.0 – 120.4	<.001	7.58	9.4
3.	Number of nodes	8.4 – 11.0	<.001	1.08	16.7
4.	Stem thickness (mm)	1.5 – 3.0	<.001	0.44	18.9
5.	Leaf length (cm)	13.5 – 39.6	<.001	5.22	19.2
6.	Leaf breadth (mm)	4.9 – 10.5	<.001	1.00	16.0
7.	Leaf ratio	2.4 – 5.5	0.032	0.64	19.8
8.	Inflorescence height at seed maturity (cm)	85.5 – 150.9	<.001	14.3	13.2
9.	Inflorescence number per plant	105.1 – 336.7	<.001	71.43	19.5
10.	Fertile tillers per plant (%)	22.5 – 73.2	<.001	0.64	18.5
11.	Inflorescence length (cm)	7.5 – 13.8	<.001	0.8741	9.1
12.	Inflorescence width (cm)	1.3 – 2.5	<.001	0.196	14.8
13.	Inflorescence ratio	4.1 – 11.3	<.001	0.96	13.8
14.	Spikelets per Inflorescence (Number)	60.3 – 302.4	<.001	25.35	18.2
15.	Days to start flowering	17.3 – 25.7	<.001	3.37	9.3
16.	Days to full flowering	24.0 –34.7	<.001	4.77	9.9
17.	Flowering period	6.3 – 11.3	ns	2.70	19.6
18.	Caryopses weight (1000 seed weight)	0.4 – 0.9	<.001	0.15	17.4
19.	Spikelet weight (1000 weight)	1.4 – 3.7	<.001	0.28	8.5
20.	Caryopses per spikelet (Number)	0.31 – 1.05	<.001	0.190	18.9
21.	Empty spikelets (%)	23 – 72	<.001	7.4	14.8

Figure 3.4 and 3.5 shows results for principal component and cluster analysis for ecotypes of *C. ciliaris* when analyzed using 27 morphological traits. The traits are listed in Table 3.4. Two main clusters were observed as shown in Figure 3.5. KLF3, KLF1, MGD1 and TVT2 formed one cluster and the rest of the ecotypes formed another cluster.

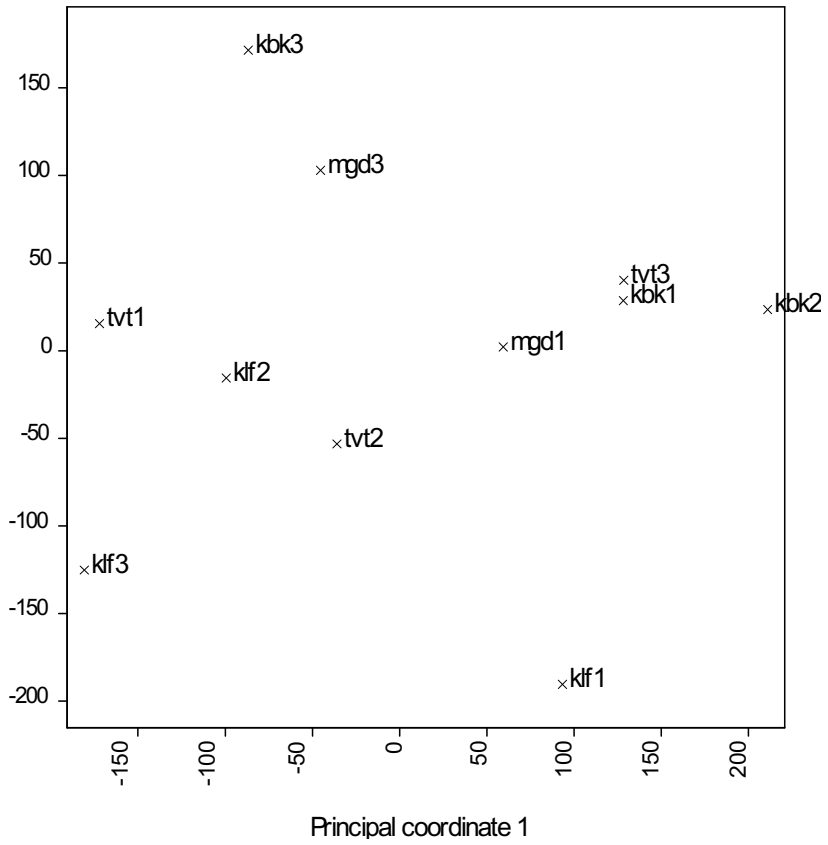

Figure 3.4*: PCA scatter plot of eleven ecotypes of Cenchrus ciliaris developed using with 27 morphological traits*

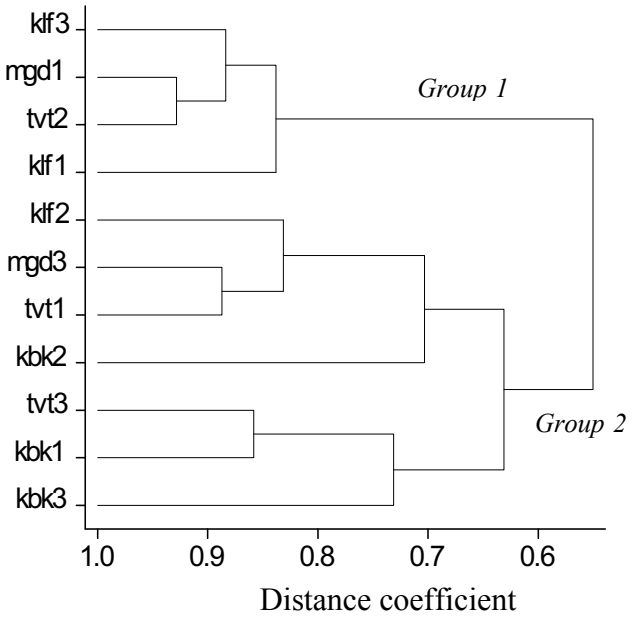

Figure 3.5: *Dendrogram of eleven ecotypes of Cenchrus ciliaris developed using 27 morphological traits*

The observed differences in colors of inflorescence, stems and leaves among *C. ciliaris* ecotypes are shown in Plates 3.2 to 3.4. Two of the ecotypes, KBK1 and TVT3, had the entire plants parts bluish in color while TVT1 had purple colored stems. The different forms of inflorescence colors observed included purple color at peak flowering for KBK3, KLF1 and KLF2 and KLF1 retained the purple color even at seed maturity.

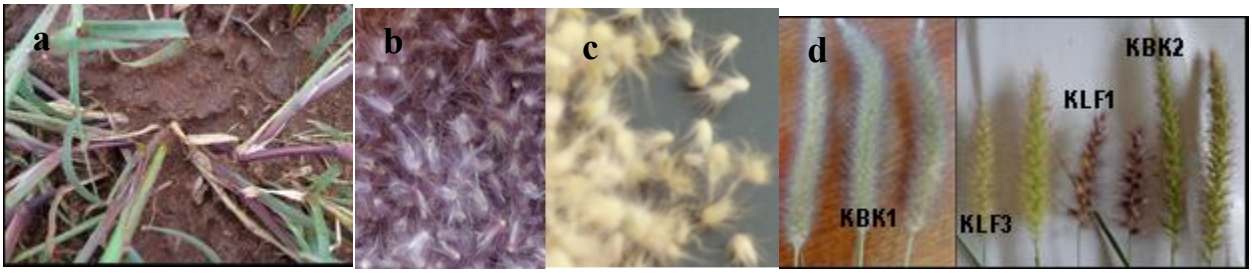

Plate 3.2*: Different morphologies for Cenchrus ciliaris ecotypes. From left: (a)Purple stems of TVT1; (b)purple dried KLF1 seeds, (c)brown dried KBK3 seeds and (d)different colours and sizes of inflorescences.*

Plate 3.3: *Cenchrus ciliaris characterization plots at KALRO Kiboko showing height variation among the ecotypes, December 2013*

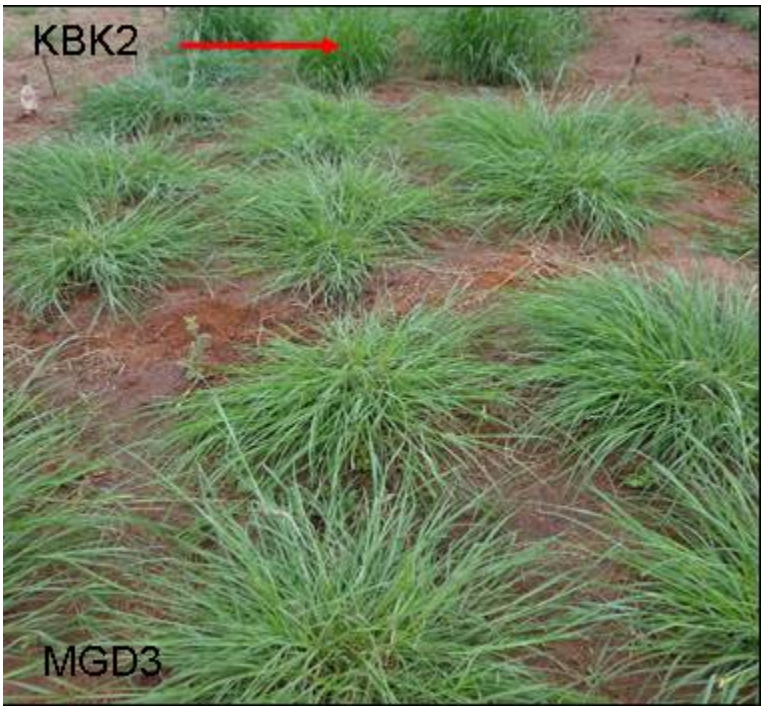

Plate 3.4: *Different growth forms of MGD3 (prostrate) and KBK2 (upright) at two weeks post clipping at KALRO*

Figure 3.6 shows a clustering of *C. ciliaris* ecotypes based on robustness related traits, namely, plant height, leaf length, leaf width and stem thickness. Two main clusters were formed similar to Figure 3.5. In Figure 3.6, KLF2 shifted to join the rest of Kilifi collections in cluster one and MGD1 shifted to cluster two.

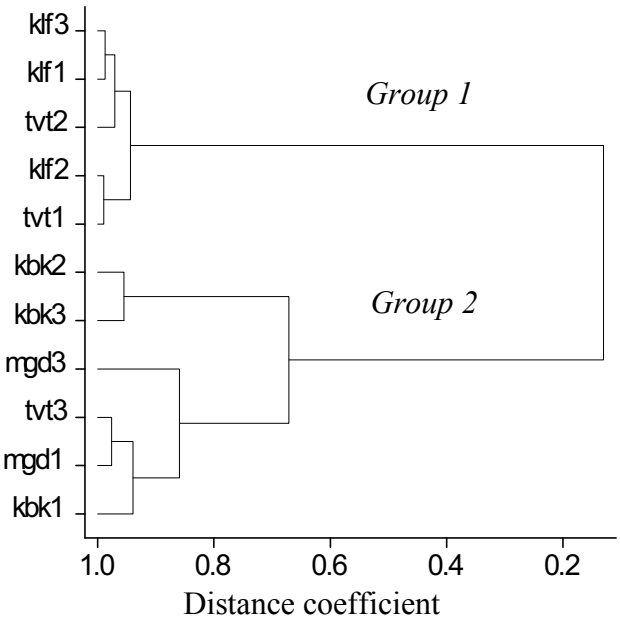

***Figure 3.6**: Dendrogram of eleven ecotypes of Cenchrus ciliaris based on robustness related characteristics*

Figure 3.7 shows a dendrogram of *C. ciliaris* ecotypes generated using three flowering related traits, namely, days to start flowering, days to 100 % plot flowering and flowering period. All KLF collections, TVT1, TVT2 and MGD1 were clustered together in group one. All KBK collections, TVT3 and MGD3 were clustered together in the second group. The difference between Figure 3.7 and 3.6 was the shifting of MGD1 from group two to one.

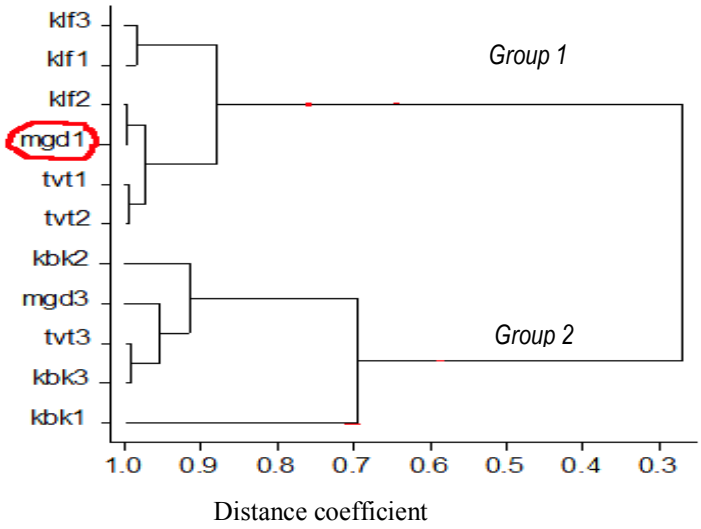

Figure 3.7: *Dendrogram of eleven ecotypes of Cenchrus ciliaris based on flowering traits*

Table 3.6 shows analysis of variance among three flowering traits, namely, days to first flowering, days to 100 % plot flowering and flowering period, between the two groups formed in Figure 3.7. There were significant differences at $p \leq 0.05$ in DSF and DFF between group one and two. There was no significant difference between group one and two in FP.

Table 3.6: *Mean and ranges of DSF, DFF and FP for two major clusters of Cenchrus ciliaris ecotypes developed using hierarchical cluster analysis*

Group	Ecotypes	DSF	DFF	FP
Group 1	KLF1, KLF2, KLF3, TVT1, TVT2 and MGD1	17.2 (17 - 20.7)*	25.6 (24 - 26.7)	7.4 (6.3 - 8.7)
Group 2	KBK1, KBK2, KBK3, MGD3, TVT3	24 (22. - 25.7)	31.6 (29.7 - 34.7)	8.7 (7 - 11.3)
	Grand mean	**21.4**	**28.3**	**8.0**
	P_value	<.001	<.001	ns
	$LSD^{0.05}$	1.98	2.28	1.69
	CV (%)	6.8	5.9	15.4

**Range of the mean Key: DSF - Days to start flowering; DFF - Days to 100% plot flowering; FP - Flowering period; ns – not significant*

3.4.3. Variation in flowering phenology of *Cenchrus ciliaris* between cuts

There were significant differences in days to first flowering (DSF) among the three cuts with Cut 1 at 26.1, Cut 2 at 21.7 and Cut 3 at 16.4 ($P \leq 0.001$; coefficient of variation (CV) =11.2%). For days to 100 % flowering (DFF), Cut 3 with 24.4 days reached full plot flowering earlier than Cut 1 and Cut 2 with 30.9 and 29.7 days, respectively ($P \leq 0.001$; CV=9.9%).

Figure 3.8 shows means of DSF for the different sites of collection. Cut 3 was shorter than Cut 1 for all the sites. There was a trend of reduction in DSF from Cut 1 to Cut 2 and from Cut 2 to Cut 3. Figure 3.9 shows means of DFF for the different sites of collection. Differences were observed in Cut 2. Kiboko collections had significantly higher DFF (35.7) than KLF (25) in Cut 2. Similar results were recorded with DSF in Cut 2 and Cut 3. Kilifi collections started to flower earlier by 13 days ($p \leq 0.05$) than Kiboko with 20.7 days. The Taveta collections also flowered earlier by 14.7 days than Kiboko in Cut 2.

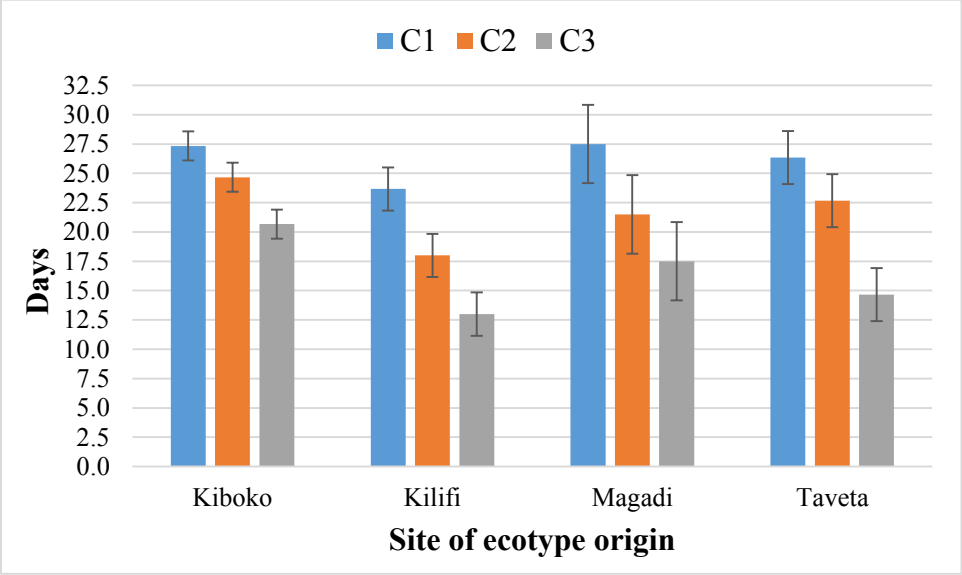

Figure 3.8: *Mean of DSF and $LSD^{0.05}$ for sites of ecotype origin at different, C1=cut 1, C2=cut 2 and C3 = cut 3; LSD – least significant difference, DSF - days to the start of flowering,*

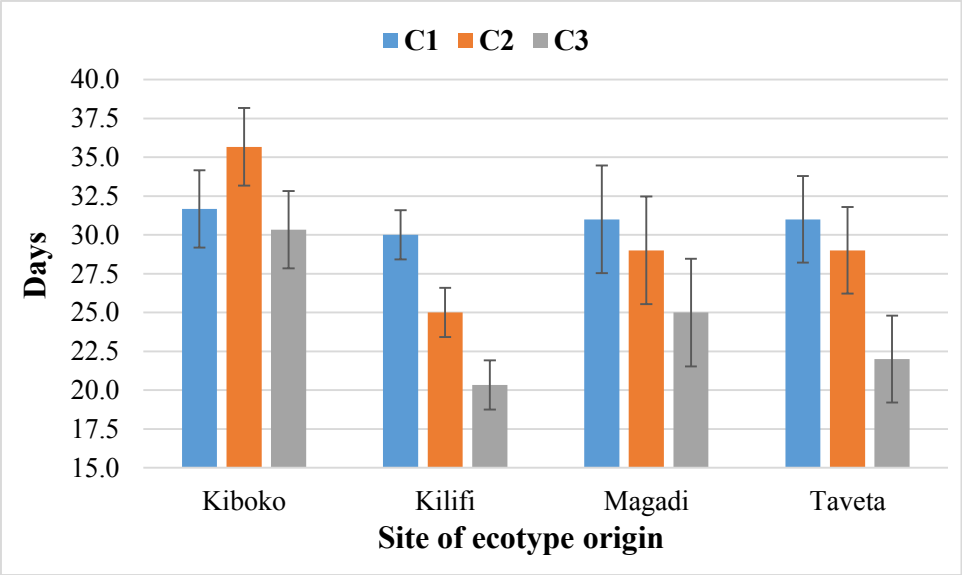

Figure 3.9: *Mean of DFF and $LSD^{0.05}$ for sites of ecotype origin at different cuts. C1=cut 1, C2=cut 2 and C3 = cut 3; LSD – least significant difference, DFF- days to full plot flowering*

3.4.4. Variation in flowering phenology between sites of collections of *Cenchrus ciliaris*

Table 3.7 shows comparison between sites of ecotype origin in relation to DSF, DFF and FP. Kilifi (KLF) collections with 18.2 days started to flower earlier (P≤0.001) than Kiboko (KBK) collections with 24.2 days. Magadi (MGD) and Taveta (TVT) collections were not different in DSF to either KLF or KBK collections. For DFF, Kiboko collections had the highest (32.6 days) and Kilifi collections the least with 25.1 days. There was no difference (P>0.05) between the sites in FP.

Table 3.7: *Mean of DSF, DFF and FP in relation to the site of origin of Cenchrus ciliaris ecotypes*

SITE	DSF	DFF	FP
Kiboko	24.2	32.6	9.5
Magadi	22.2	28.3	7.8
Taveta	21.2	27.3	7.3
Kilifi	18.2	25.1	7.3
P_value	<.001	<.001	ns
$LSD^{0.05}$	2.46	3.27	1.64
CV (%)	11.2	11.3	19.9

Key: CV – coefficient of variation, LSD – least significant difference, DSF - days to the start of flowering, DFF- days to full plot flowering and FP - flowering period; ns - not significant

3.4.5. Variation in flowering phenology between ecotypes of *Cenchrus ciliaris*

Table 3.8 shows result of days to the start of flowering between ecotypes. The DSF ranged from 17 to 25.7 days for KLF3 and KBK2 respectively. The variation among ecotypes was a significant at $p \leq 0.001$. KLF1 and KLF3 flowered earlier ($p \leq 0.001$) than MGD3, KBK1 and KBK2. Ecotypes from the same site of origin did not differ in DSF.

Table 3.8: *Mean of days to start flowering for Cenchrus ciliaris ecotypes in different cuts*

ECOTYPE / CUT	KLF 1	KLF 2	KLF 3	TVT 1	TVT 2	TVT 3	MGD 1	MGD 3	KBK 1	KBK 2	KBK 3
Sep-Oct	22	27	22	29	24	26	26	29	27	29	26
Nov-Dec	18	18	18	19	23	26	18	25	25	26	23
Feb-Mar	12	16	11	14	13	17	16	19	21	22	19
Mean*	**17.3**	**20.3**	**17**	**20.7**	**20**	**23**	**20**	**24.3**	**24.3**	**25.7**	**22.7**

CV = 9.3%; $LSD^{0.05}$=3.37 days; Key: CV – coefficient of variation, LSD – least significant difference

Table 3.9 shows the days to full plot flowering among ecotypes of *C. ciliaris*. Significant difference ($p \leq 0.001$) between the ecotypes in DFF was observed. KLF1 (24 days) and KLF3 (24.7 days) had full plot flowering significantly earlier than KBK1 (34.7 days) and KBK2 (33.3 days). There was no difference between ecotypes from the same site of origin.

Table 3.9: *Mean of days to full flowering for Cenchrus ciliaris ecotypes in different cuts*

ECOTYPE/ CUT	KLF1	KLF2	KLF3	TVT1	TVT2	TVT3	MGD1	MGD3	KBK1	KBK2	KBK3
Sep-Oct	29	31	30	32	29	32	30	32	33	32	30
Nov-Dec	25	25	25	25	29	33	25	33	38	35	34
Feb-Mar	18	24	19	22	18	26	24	26	33	33	25
Mean	**24**	**26.7**	**24.7**	**26.3**	**25.3**	**30.3**	**26.3**	**30.3**	**34.7**	**33.3**	**29.7**

CV = 9.9%; $LSD^{0.05}$= 4.771; Key: CV – coefficient of variation, LSD – least significant difference

Figure 3.10 shows the flowering patterns of the ecotypes in cut 3 where KBK1 and KBK2 ecotypes were isolated as late flowering types.

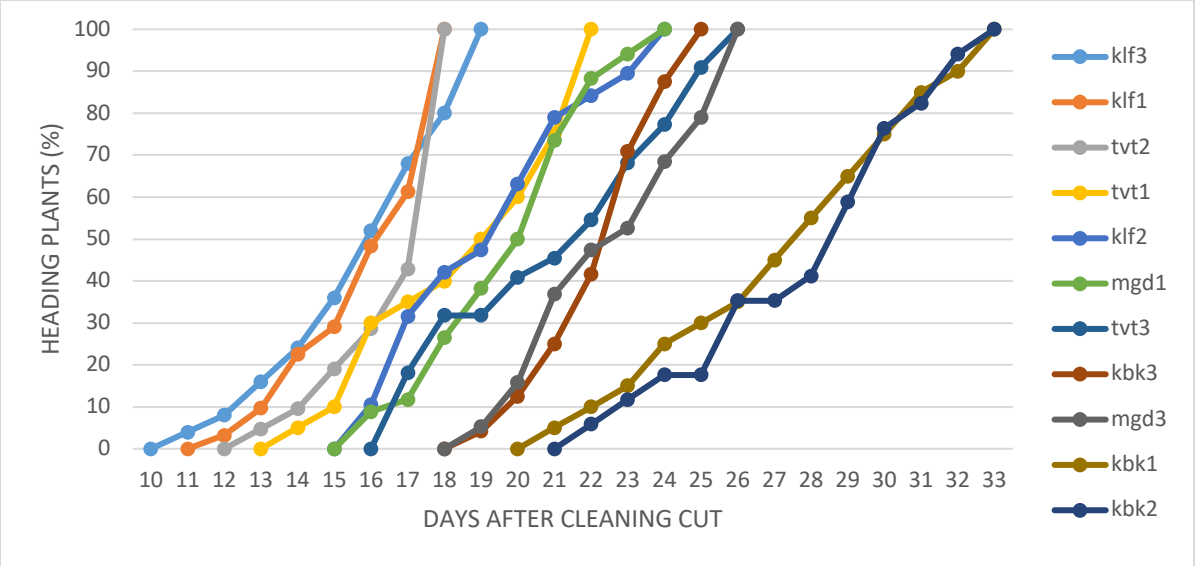

***Figure 3.10**: Heading patterns in spaced plants' plots of Cenchrus ciliaris ecotypes from the time of cleaning cut in mid Feb. 2014 to days to full flowering*

Table 3.10 shows flowering period (FP) results for ecotypes of *C. ciliaris*. There was no difference between the ecotypes in FP at $p \leq 0.05$. The mean flowering period ranged from 6.3 days for TVT2 to 11.3 days for KBK1.

***Table 3.10:** Mean of flowering period for Cenchrus ciliaris ecotypes in different cuts*

Ecotype / Cut	KLF 1	KLF 3	TVT 2	KLF 2	TVT 1	TVT 3	MGD 1	MGD 3	KBK 3	KBK 1	KBK 2
Sep-Oct	8	9	6	5	4	7	6	4	5	7	4
Nov-Dec	8	8	7	8	7	9	8	9	12	14	10
Feb-Mar	7	9	6	9	9	10	9	8	7	13	12
Mean	**7.7**	**8.7**	**6.3**	**7.3**	**6.7**	**8.7**	**7.7**	**7**	**8.6**	**10.7**	**9.3**

P>0.05; CV = 19.6%; $LSD^{0.05}$ = 2.70. Key: CV – coefficient of variation, LSD – least significant difference,

3.4.6. Correlation analysis between traits for *Cenchrus ciliaris*

Table 3.11 shows the correlation matrix for different traits among ecotypes of *C. ciliaris*. Days to the start of flowering was strongly and positively correlated with days to full plot flowering (r=0.9; $p \leq 0.001$). Days to full plot flowering was positively correlated with flowering period (r=0.7; $p \leq 0.05$). Flowering period was positively correlated to days to full plot flowering (0.7) and leaf breadth (0.6). Days to the start of flowering was positively correlated to plant height, leaf length and breadth, stem thickness, number of nodes, inflorescence length, the number of spikelets per inflorescence (r = 0.8, 0.8, 0.8, 0.9, 0.6, 0.8, 0.7, respectively). Days to the start of flowering was negatively correlated to percent fertile tillers (r = -0.8) and number of inflorescence per plant (r = -0.8).

Plant height to the flag leaf was significantly and positively correlated with stem thickness, spikelet number, leaf length, leaf breadth, leaf ratio, inflorescence length and plant height to the inflorescence tip (r=0.9, 0.9, 1, 0.8, 0.7, 0.8 and 0.9, respectively). Inflorescence length was negatively correlated with spikelet weight and awn density with r=-0.7 for both traits and was positive to ease to drop, stem thickness and leaf attributes.

Table 3.11: *Phenotypic correlations (correlation matrix for morphological traits for Cenchrus ciliaris)*

	%FT	SWT	AD	BT	CWT	DFF	DSF	EDS	FP	FLH	ITH	ID	IL	LR	LB	LL	NOD	SNO	ST
%FT	-																		
SWT	0.3	-																	
AD	0.3	-0.4	-																
BT	-0.1	-0.2	0.0	-															
CWT	0.1	0.6	-0.5	0.0	-														
DFF	-0.6*	-0.4	-0.3	-0.1	-0.1	-													
DSF	-0.8***	-0.3	-0.5	-0.1	0.0	0.9***	-												
EDS	-0.1	-0.1	-0.3	0.4	0.3	0.2	0.2	-											
FP	0.0	-0.4	0.2	-0.1	-0.3	0.7*	0.4	0.1	-										
FLH	-0.6	-0.4	-0.3	0.0	0.1	0.7**	0.8**	0.6	0.4	-									
ITH	-0.5	-0.2	-0.3	-0.1	0.2	0.8**	0.7**	0.5	0.5	0.9***	-								
ID	-0.4	-0.9***	0.3	0.5	-0.6*	0.2	0.1	0.2	0.2	0.2	-0.1	-							
IL	-0.7*	-0.2	-0.7*	0.1	0.2	0.7**	0.8**	0.6*	0.2	0.8**	0.8**	0.1	-						
LR	-0.6*	-0.2	-0.5	0.4	0.2	0.4	0.6	0.5	-0.2	0.7*	0.5	0.3	0.7*	-					
LB	-0.5	-0.3	-0.3	-0.4	0.1	0.8**	0.8**	0.3	0.6*	0.8**	0.8**	0.0	0.7*	0.1	-				
LL	-0.7*	-0.3	-0.5	0.0	0.2	0.8**	0.8**	0.5	0.2	1.0***	0.9***	0.1	0.9***	0.8**	0.7**	-			
NOD	-0.3	0.4	-0.5	-0.3	0.4	0.6*	0.6*	0.2	0.4	0.3	0.5	-0.4	0.5	0.1	0.6*	0.5	-		
SNO	-0.6*	-0.6*	-0.1	0.0	-0.2	0.7*	0.7*	0.3	0.4	0.9***	0.7*	0.4	0.7*	0.6	0.7*	0.8**	0.1	-	
ST	-0.7*	-0.4	-0.3	-0.2	0.0	0.9***	0.9***	0.3	0.4	0.9***	0.9***	0.1	0.8**	0.5	0.9***	0.9***	0.5	0.8***	-

****= p≤0.001; **=p≤0.01 and *= p≤0.05*

Table legend:

No.	Abbrev.	Meaning	No.	Abbrev.	Meaning	No.	Abbrev.	Meaning	No.	Abbrev.	Meaning
1	%FT	Percent fertile tillers	6	DFF	Days to full flowering	11	ITH	Inflorescence tip height	16	LL	Leaf length
2	SWT	Spikelet weight	7	DSF	Days to start flowering	12	ID	Inflorescence density	17	NOD	Number of nodes
3	AD	Awn density	8	EDS	Ease to drop seed	13	IL	Inflorescence length	18	SNO	Spikelet number
4	BT	Basal tillers	9	FP	Flowering period	14	LR	Leaf ratio	19	ST	Stem thickness
5	CWT	Caryopsis weight	10	FLH	Flag leaf height	15	LB	Leaf breadth			

3.4.7. Analysis of nutritive value components among sites of origin for *Cenchrus ciliaris* ecotypes

Table 3.12 shows the mean nutritive value contents among sites of ecotype collection for *C. ciliaris*. Kiboko site with 10.5 % CP was higher ($p \leq 0.05$) than Kilifi with 8.0 %. There were no differences ($p > 0.05$) between sites in the other components, that is, crude fibre (CF), percent dry matter (% DM), in-vitro digestibility of dry matter (INVDMD) and ash content.

Table 3.12: *Mean nutritive value contents of Cenchrus ciliaris ecotypes from Kiboko, Kilifi, Magadi and Taveta collection sites*

Site	N	CF	CP	INVDMD	Ash	%DM
Kiboko	6	35.2	10.5	51.6	14.3	91.3
Kilifi	6	36.7	8.0	49.5	13.0	91.9
Magadi	4	34.7	9.1	50.9	13.9	91.4
Taveta	6	36.7	9.1	48.6	14.4	91.5
P_value		ns	0.03	ns	ns	ns
$LSD^{0.05}$		2.68	1.72	3.25	1.64	1.28
CV (%)		5.82	14.6	5.05	9.37	1.32

KEY; CF - crude fibre; CP – crude protein; % DM - percent dry matter; INVDMD – in-vitro digestibility of dry matter; N – sample size; ns – not significant

3.4.8. Comparison of nutritive value components among ecotypes of *Cenchrus ciliaris*

Table 3.13 shows the mean nutritive value contents among twelve ecotypes of *C. ciliaris* species. For % DM, KBK1 (93.1 %) ecotype was higher than KBK2 (90.5 %) and KBK3 (90.3 %). Various levels of statistical differences were observed with ash content. KBK2 and KLF2 ecotypes with 15.2 and 15.3 % ash content, respectively, were the highest while KLF3 with 11.2 % was the least. For INVDMD, KBK2 (55 %) was significantly higher ($p \leq 0.05$) than TVT1 (45.6 %). KBK1 (38.4 %), KLF1 (37.5 %), KLF3 (38 %) and TVT2 (37.8 %) had significantly the highest CF while KBK3 and MGD3 had the least each with 32.4 % CF. There was no differences between the ecotypes in CP levels at $p \leq 0.05$.

Table 3. 13: *Mean values for nutritive contents of Cenchrus ciliaris ecotypes*

Ecotype	%DM	Ash	INVDMD	CP	CF
KBK1	93.1	14.0	48.2	10.5	38.4
KBK2	90.5	15.2	55.0	10.2	35.0
KBK3	90.4	13.9	51.7	10.9	32.4
KLF1	92.4	12.5	48.7	8.9	37.5
KLF2	91.7	15.3	50.0	8.3	34.9
KLF3	91.5	11.2	49.9	6.6	38.0
MGD1	91.7	13.2	50.8	8.4	37.1
MGD3	91.2	14.6	51.1	9.8	32.4
TVT1	90.6	14.2	45.6	9.6	37.3
TVT2	92.1	11.9	49.5	8.2	37.8
TVT3	91.8	14.2	50.9	9.5	35.3
Total mean	**91.5**	**13.7**	**50.1**	**9.17**	**36.0**
P_value	0.032	<.001	0.03	ns	<.001
SEM	0.464	0.212	1.28	0.996	0.364
CV (%)	0.7	2.2	3.6	15.4	1.4

KEY; CF - crude fibre; CP – crude protein; % DM - percent dry matter; INVDMD – in-vitro digestibility of dry matter; ns - not significant

Table 3.14 shows correlation matrix between the nutritive value components and selected herbage related traits for *C. ciliaris* ecotypes. There was a positive correlation ($p \leq 0.05$) between CP and first flowering time (DSF) (r = 0.6), plant height (r = 0.7), stem thickness (r = 0.7), leaf length (r = 0.7) and ash content (r = 0.7). In-vitro digestibility of dry matter was significantly and positively correlated with stem thickness (r=0.6) and leaf breadth (r=0.7). Crude fibre was significantly and positively correlated with %Dm (r=0.7) and negatively with plant height (r = -0.7) and leaf length (r = -0.7).

Table 3. 14: *Pearson correlation results between nutritive content and selected herbage related traits in Cenchrus ciliaris ecotypes*

Attribute	%DM	Ash	INVDMD	CP	CF
%DM	1	-.343	-.375	-.232	**.654***
Ash	-.343	1	.279	**.661***	-.156
INVDMD	-.375	.279	1	.162	-.526
CP	-.232	**.661***	.162	1	-.291
CF	**.654***	-.156	-.526	-.291	1
Robust group[1]	-.131	.473	.550	**.652***	-.476
Flowering group[2]	-.167	.538	.492	**.770****	-.575
Days to first flowering	-.268	.397	.446	**.634***	-.521
Plant height	-.480	.509	.551	**.719***	**-.650***
No of nodes	-.083	.242	.340	.226	-.028
Stem thickness	-.371	.438	**.608***	**.712***	-.505
Leaf length	-.578	.482	.560	**.675***	**-.720***
Leaf breadth	-.247	.487	**.684***	.571	-.357

***. *Correlation is significant at the 0.01 level and * at 0.05 level.* [1] *1 small and 2 robust;* [2] *1 early and 2 late flowering. KEY; CF - crude fibre; CP – crude protein; % DM - percent dry matter; INVDMD – in-vitro digestibility of dry matter*

3.4.9. Phenotypic characterization of *Eragrostis superba* ecotypes

Table 3.15 shows the varied ranges recorded among morphological attributes in *E. superba* ecotypes. Wide ranges were recorded in all measured traits. The Taveta ecotypes, TVT2 and TVT3, were the only ones with unique panicles. Plate 3.5 shows the differences in panicles. Differences at $p \leq 0.05$ were detected in all traits except number of nodes, stem thickness, number of tillers at peak flowering and percent fertile tillers.

Table 3.15: *Mean range, % coefficient of variation (CV), P_value and least significant difference at 0.05 ($LSD^{0.05}$) of morphological attributes among Eragrostis superba ecotypes*

No.	Attribute	Ranges	P_value	$LSD^{0.05}$	%CV
1.	Flag leaf height (cm)	66.6 – 96.1	<.001	12.45	17.3
2.	Inflorescence height at peak flowering (cm)	86.0 – 108.5	<.001	9.66	11.2
3.	Inflorescence height at seed maturity (cm)	115.5 – 138.8	<.001	10.5	11.43
4.	Number of nodes	3.0 – 3.5	ns	0.388	15.3
5.	Stem thickness (mm)	2.8 – 3.4	ns	0.495	18.2
6.	Leaf length (cm)	21.3 – 33.4	0.004	1.137	12.4
7.	Leaf breadth (mm)	8.9 – 11.3	<.001	4.886	19.7
8.	Leaf ratio	2.1 – 3.0	<.001	0.431	17.8
9.	Tiller number at peak flowering	206 -266	ns	56.08	27.3
10.	Tillers number at seed maturity	131.6 – 224.8	0.028	55.15	34.4
11.	Inflorescence number per plant	105.3 – 176.7	0.030	44.27	34.6
12.	Fertile tillers per plant (%)	77.4 – 83.7	ns	10.78	15
13.	Inflorescence length (cm)	29.2 – 36.8	0.009	4.966	17.1
14.	Panicle branches (Number)	11.7 – 13.9	0.011	1.443	12.5
15.	Inflorescence shape*	1 – 3	-	-	-
16.	Spikelets per Inflorescence (Number)	115.5 – 191.9	<.001	15.59	24
17.	Caryopses weight (1000 seed weight)	0.34 – 0.51	<.001	0.072	13.5
18.	Spikelet weight (1000 weight)	7.4 – 12.3	<.001	0.743	5.6
19.	Caryopses per spikelet (Number)	2.0 – 4.3	<.001	0.596	13.8

**(1) normal loose branches or (2) unique compact branching along branches/ rachilla or (3) closed and all grouped along main branch/rachis; ns= not significant*

Plate 3.5*: Images showing two different types of inflorescence for Eragrostis superba ecotypes, from left: loose branching and compact branching of inflorescence*

Figure 3.11, 3.12 and 3.13 shows two major groups of *E. superba* ecotypes formed using a combination of 16 morphological traits, seed yield and robustness related traits, respectively. The seed yield traits used were spikelets per inflorescence, % fertile tillers, inflorescence number per plant, inflorescence length and panicle branching. The robustness traits were plant height, stem thickness, leaf length and leaf width. In Figures 3.11, 3.12 and 3.13, Kiboko collections (KBK 1 and KBK 2) were closely related and distant to the other ecotypes.

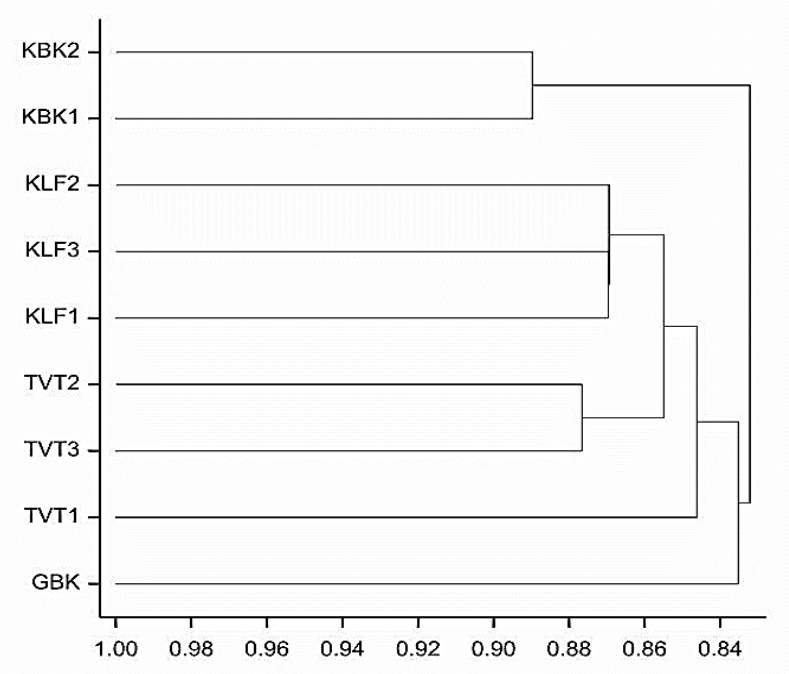

Figure 3.11*: Dendrogram of nine Eragrostis superba ecotypes developed using 16 morphological traits*

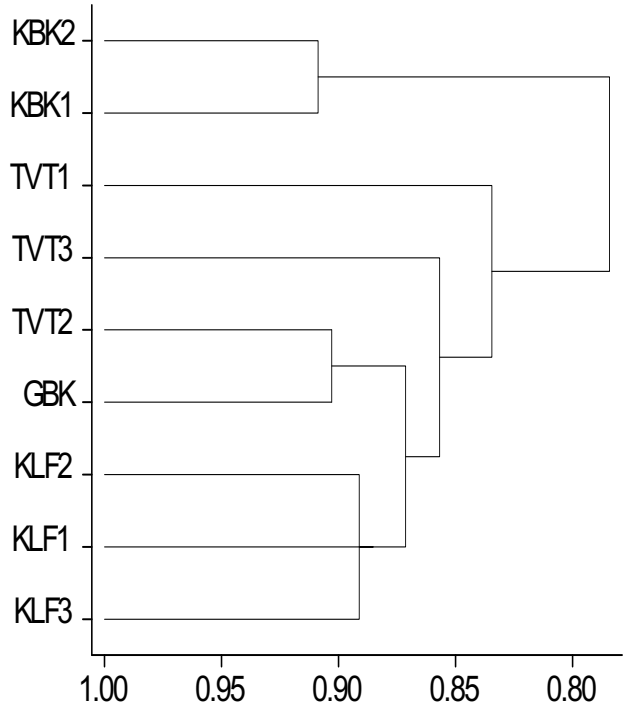

Figure 3.12: *Dendrogram of nine Eragrostis superba ecotypes developed using five seed yield traits*

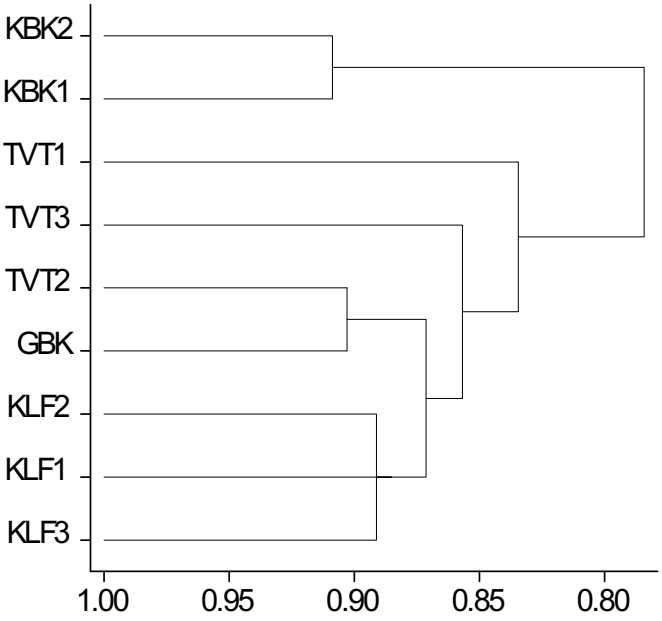

Figure 3.13: *Dendrogram of nine Eragrostis superba ecotypes developed using robustness related traits*

Table 3.16 shows variation in trait means between the two groups formed in Figures 3.11, 3.12 and 3.13. These were group one for KBK1 and KBK2 and group two consisting of the rest of the

ecotypes, that is TVT1, TVT2, TVT3, KLF1, KLF2, KLF3 and GBK. There were significant differences ($p<0.05$) between the two groups in inflorescence length, stem thickness, height to flag leaf at full bloom stage and height to inflorescence tip at seed maturity. Group one recorded higher scores in all traits.

Table 3.16: *Mean of each trait between clusters formed using traits for robustness among Eragrostis superba ecotypes*

Group	Inflorescence Length (cm)	Stem thickness (mm)	Flag leaf height (cm)	Inflorescence tip height (cm)
Group 1	35.45	3.34	91.9	132.4
Group 2	31.03	2.90	75.2	116.6
Mean	32.50	3.05	80.8	121.9
P_value	0.02	<.001	0.003	0.003
$LSD^{0.05}$	3.476	0.1373	8.86	8.385
CV (%)	6.4	2.7	6.6	4.1

3.4.10. Correlation analysis between traits for *Eragrostis superba*

Table 3.17 shows the different correlations observed between morphological traits among *E. superba* ecotypes. Plant height measured at different stages (the first three attributes in Table 3.14) were positively and significantly correlated with each other. Leaf length was positively and significantly correlated with leaf ratio (r=0.82). Inflorescence shape was positively and significantly correlated with caryopsis number per spikelet (r=0.73) and with 1000 spikelet weight (r=0.68). Primary panicle branching, which is the count of the number of main branches on an inflorescence was not correlated with other traits though had a strong positive correlation with rainfall at site of collection (r=0.68).

Table 3.17: Phenotypic correlations (*correlation matrix for morphological traits for Eragrostis superba ecotypes)*

	ITH 2+	ITH 1+	FLH	LB	LL	NOD	ST	TN 1+	TN 2+	CWT	CPS	%FT	IS	IN	IL	LR	PB	SNO	SWT	RCS
ITH 2+	-																			
ITH 1+	0.81 **	-																		
FLH	0.79 **	0.98 ***	-																	
LB	0.21	0.07	0.17	-																
LL	0.39	0.71*	0.76*	0.48	-															
NOD	-0.28	-0.49	-0.52	0.48	-0.23	-														
ST	0.81 **	0.76*	0.84 **	0.15	0.44	-0.55	-													
TN 1+	-0.42	-0.09	-0.18	-0.49	0.06	-0.19	-0.53	-												
TN 2+	-0.30	0.02	0.09	0.30	0.49	-0.15	-0.17	0.41	-											
CWT	0.16	0.41	0.25	-0.61	0.07	-0.14	-0.08	0.56	-0.17	-										
CPS	0.11	0.19	0.27	-0.46	-0.08	-0.79 **	0.56	-0.13	0.00	-0.07	-									
%FT	-0.06	-0.01	-0.12	-0.47	-0.05	-0.21	-0.35	0.79 **	-0.04	0.50	-0.22	-								
IS	-0.10	0.08	0.12	-0.57	0.08	-0.73*	0.18	0.43	0.15	0.20	0.73*	0.35	-							
IN	-0.30	0.04	0.09	0.23	0.49	-0.15	-0.24	0.52	0.99 ***	-0.05	-0.08	0.10	0.15	-						
IL	0.66*	0.76*	0.74*	-0.32	0.32	-0.85 **	0.69*	0.06	0.09	0.30	0.57	0.17	0.42	0.11	-					
LR	0.30	0.77*	0.76*	-0.10	0.82 **	-0.56	0.40	0.39	0.38	0.49	0.21	0.23	0.46	0.42	0.56	-				
PB	0.27	0.23	0.19	0.44	0.30	0.19	0.02	-0.04	-0.11	-0.14	-0.63	0.27	-0.55	-0.05	-0.05	0.03	-			
SNO	0.81 **	0.67*	0.66*	0.47	0.45	0.15	0.61	-0.42	-0.31	0.12	-0.28	-0.15	-0.46	-0.31	0.22	0.21	0.54	-		
SWT	-0.28	-0.21	-0.25	-0.50	-0.09	-0.14	-0.28	0.62	-0.15	0.38	0.10	0.68*	0.68*	-0.09	-0.12	0.21	-0.24	-0.33	-	
RCS	-0.37	-0.44	-0.44	0.52	-0.00	0.37	-0.52	-0.03	-0.04	-0.40	-0.77*	0.08	-0.57	-0.01	-0.74*	-0.36	0.68*	0.06	-0.04	-

*+1 done at peak of flowering and 2 is at seed maturity; ***= p≤0.001; **=p≤0.01 and *= p≤0.0*

Abbrev.	Meaning	Abbrev.	Meaning	Abbrev.	Meaning	Abbrev.	Meaning
ITH	Inflorescence tip height	ST	Stem thickness	IS	Inflorescence shape	SNO	Spikelet number
FLH	Flag leaf height	TN	Tiller number	IN	Inflorescence number	SWT	Spikelet weight
LB	Leaf breadth	CWT	Caryopsis weight	IL	Inflorescence length	RCS	Rainfall at collection site
LL	Leaf length	CPS	Caryopsis per spikelet	LR	Leaf ratio		
NOD	Number of nodes	%FT	Percent fertile tillers	PB	Panicle branching		

3.4.11. Analysis of nutritive value components between sites of origin for *Eragrostis superba* ecotypes

Table 3.18 shows the mean nutritive value contents among sites of ecotype collection for *E. superba*. There were no differences between the sites in CF, CP, INVDMD, ash and % DM at $p \leq 0.05$.

Table 3.18: Mean n*utritive value contents of Eragrostis superba ecotypes from Kiboko, Kilifi, Magadi and Taveta*

Site	N	CF	CP	INVDMD	Ash	%DM
Kiboko	4	40.1	10.1	54.1	8.89	92.3
Kilifi	6	40.0	9.94	50.1	8.53	91.5
Magadi	2	38.8	9.39	49.2	8.07	91.4
Taveta	6	38.2	9.09	48.7	8.83	91.6
P_value		ns	ns	ns	ns	ns
$LSD^{0.05}$		2.03	2.04	4.74	0.873	2.02
CV (%)		3.31	13.5	6.02	6.46	1.41

KEY: CF - crude fibre; CP – crude protein; % DM - percent dry matter; INVDMD – in-vitro digestibility of dry matter; ns – not significant; N- number of samples

3.4.12. Comparison of nutritive value components among *Eragrostis superba* ecotypes

Table 3.19 shows the mean nutritive value contents among nine ecotypes of *E. superba* grass species. There were differences ($p \leq 0.05$) in all the measured attributes resulting in several groups within a component. The Kilifi ecotype, KLF1 with 41.6 %, had the highest ($p \leq 0.05$) crude fiber than all other ecotypes except KBK2 (40.4 %) and KLF2 (40.7 %). The lowest CF was recorded by TVT3 ecotype at 37 %, which was lower than all except KLF3 (37.7 %) and TVT2 (38.5 %) at $p \leq 0.05$. For CP, KBK2 and TVT3 both with 11.2 % were the highest while TVT2 with 7.7 % was the lowest. KBK1 had higher INVDMD (56.1 %) than all ecotypes except KBK2 (52.1 %), KLF1 (54.7 %) and KLF2 (50.4 %) at $p \leq 0.05$. KLF3 had the least INVDMD at 45.4 %. TVT3 ecotype was higher in ash content (9.5 %) than all ecotypes except KBK1 (9.3 %) and KLF2 (9.3 %). KLF3 ecotype and GBK had the lowest ash content where each had 8.1 %. KBK1 (92.7 %) and KLF1 (93.1 %) were higher in % DM %) ($p \leq 0.05$) than all ecotypes except KLF2 that had 92.5 %. KLF3 recorded the least % DM at 88.9 %.

Table 3.19: *Mean nutritive contents of Eragrostis superba ecotypes*

Ecotype	CF	CP	INVDMD	Ash	%DM
KBK1	39.8	9.04	56.1	9.25	92.7
KBK2	40.4	11.2	52.1	8.53	91.9
TVT1	39.2	8.36	48.9	8.92	90.8
TVT2	38.5	7.68	47.5	8.13	91.9
TVT3	37.0	11.2	49.7	9.45	91.9
KLF1	41.6	10.8	54.7	8.18	93.1
KLF2	40.7	9.68	50.4	9.32	92.5
KLF3	37.7	9.27	45.4	8.08	88.9
GBK	38.8	9.39	49.2	8.07	91.4
Total mean	**39.3**	**9.64**	**50.4**	**8.66**	**91.9**
P_value	<.001	<.001	0.001	<.001	<.001
SEM	1.286	0338	1.09	0.103	0.138
CV (%)	1.0	5.0	3.0	1.7	0.2

KEY: CF - crude fibre; CP – crude protein; % DM - percent dry matter; INVDMD – in-vitro digestibility of dry matter

Figure 3.14 shows the clustering of *E. superba* ecotypes based on nutritive value contents. Two clusters (A and B) were observed while TVT3 was not clustered. Cluster A consisted of KLF2, KBK1, TVT1, TVT2, GBK and KLF3 while Cluster B consisted of KBK2 and KLF1.

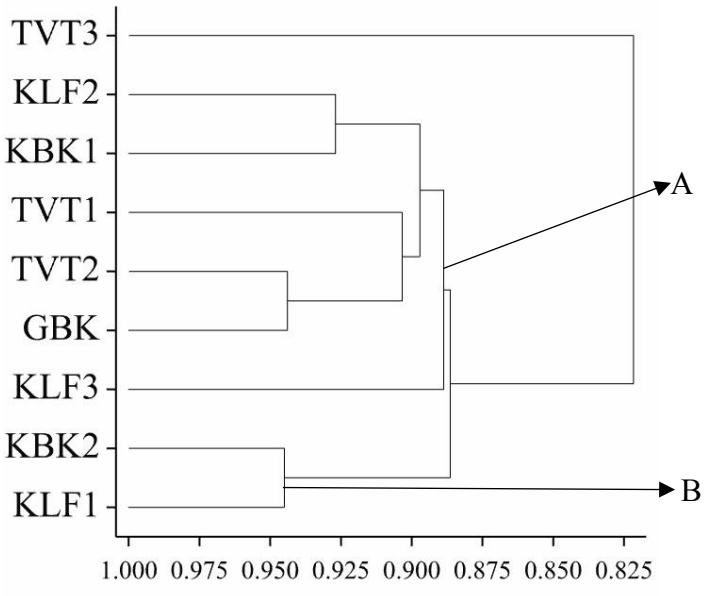

Figure 3.14: *Dendrogram of 9 ecotypes of Eragrostis superba based on five nutritive value components*

Table 3.20 shows the mean values for the nutritive components for the two clusters formed in Figure 3.14. Significant differences were observed in CP at $p \leq 0.05$ and not in CF, ash content, percent dry matter and INVDMD. Cluster B had higher CP (11.0 %) than cluster A which had 8.9 % CP.

Table 3.20: *Mean nutritive content for three clusters of Eragrostis superba ecotypes*

Cluster	Ecotypes	Crude Protein	Crude Fibre	Ash content	% Dry matter	In-vitro digestibility
A	KLF2, KBK1, TVT2, TVT1, GBK, KLF3	8.9	39.1	8.6	91.4	49.6
B	KBK2, KLF1	11.0	41.0	8.4	92.5	53.4
Mean		**9.5**	**39.9**	**8.63**	**921**	**51.3**
P_value		0.009	ns	ns	ns	ns
SED		0.56	0.83	0.46	1.08	2.77
CV (%)		7.3	2.6	0.7	1.4	6.7

KEY; ns – not significant

3.5. Discussion

Cenchrus ciliaris KLF1 ecotype had the highest mean germination capacity as shown in Figure 3.2, which could be a survival mechanism. The ecotype was the shortest in plant height during collection and grouped as small sized and early flowering. The low germination capacity for MGD3 ecotype could be attributed to adaptation to environmental conditions at the site of collection. Ecotype MGD3 was collected from a dry season preserved grazing area in Magadi within agro-ecological zone six where the ecotype dominated an expansive area of over 10 ha. The presence and dominance of MGD3 in the area could be attributed to the existence of traditional structures for controlling access and use of the natural resources among the pastoral Maasai community. *Eragrostis superba* KLF3 had the highest mean germination capacity, which could be attributed to the type of management at the site of collection. Mode of pasture utilization and management affects the behavioural pattern of plants (N'Guessan and Hartnett (2011). The ecotype was collected from sisal plantation where regular mowing is done.

Different types of *C. ciliaris* were observed in the field. Phenotypic characterization of *C. ciliaris* using 27 traits did not give distinct groupings as shown in scatter diagram in Figure 3.4. There were clearer groupings based on robustness and flowering related traits, respectively, as shown in Figures 3.6 and 3.7. The small sized ecotypes were grouped as early flowering types and the robust types as late flowering except for MGD1 that was robust and early flowering. Table 3.6 shows that the group consisting of all Kilifi collections, MGD1, TVT1 and TVT2 ecotypes, had significantly fewer days to 100% full plot flowering than group two made of robust ecotypes. The prevailing environmental conditions and management at the site of ecotype collection may have contributed to the different groupings. Although the site of origin for KLF1, KLF2 and KLF3 received the highest rainfall as compared to all the other sites and had potentially longer growing periods (Table 3.1), grazing for KLF1 and KLF2 and frequent mowing for KLF3 could have led to early flowering. Table 3.1 shows that KLF1 and KLF2 were collected from unprotected grazing areas adjacent to cultivated areas and KLF3 from a sisal plantation that is periodically mowed. The robust ecotypes were commonly collected in areas with controlled grazing or a long wetter areas such as flood plain (MGD3), irrigation canal (TVT3) or riverine (KBK1 and MGD1). This is because plants in long growing seasons devoid of disturbance like grazing or mowing would spend more time in exponential growth phase, hence accumulate disproportionately more vegetative resources (Franks et al., 2006). Long term defoliation by either grazing or mowing was observed to reduce leaf length, leaf width, stem width, stem diameter and the overall plant size of *Leymus chinensis* grass species by Li et al., (2015) which supports the observation on the small sized ecotypes in this study. Development of short stature was also observed by McKinney and Fowler (1991) with *Cenchrus incertus* due to grazing.

Results in Figure 3.8 and 3.9 and, Table 3.7 further supports the hypothesis that site of ecotype collection does influence flowering time. Figure 3.8 and Table 3.7 shows that all Kiboko collections started to

flower (DSF) later and had longer periods to reach full plot flowering (DFF) than Kilifi. Days to first flowering (DSF) is related to the prevailing environmental conditions of the site of ecotype origin (Boonman, 1993) and has a strong relationship with the length of growing season. Early flowering is adaptive in environments with highly variable resource availability and allows successful allocation of resources to reproduction before the onset of the harsh environmental conditions (Latta and McCain, 2009; Levin, 2009). Early flowering is an escape mechanism to a predictable disturbance, such as drought (Franks et al., 2006), grazing (Wissman, 2006) or light in case of other plant's canopy cover that is described as a conservative strategy in "bet-hedging evolutionary theory" (Childs et al., 2010) and plant species optimize fitness with regard to prevailing environmental conditions.

Early flowering has been observed to occur in certain ecotypes as an adjustment to terminal drought occurrences. For instance, Franks et al., (2006) reported that in an extreme drought event in 2000 to 2004 that resulted in a shortened growing season, descendant ecotypes of *Brassica rapa* significantly shifted to early flowering when compared to their ancestors. Craufurd and Wheeler (2009) reported late flowering genotypes of sorghum that reduced their optimum flowering time by about 20 days due to reduced rainfall amounts and consequently the growing seasons.

The early flowering of Kilifi collections could also be attributed to the latitude of the site where the ecotypes were collected. Kilifi collections were from the more southern latitudes (3.6^{o} S) and, therefore, more distant from the equator compared to Kiboko (2.2^{o} S) collections. The latitude observation is similar to several other previous findings (Rathcke and Lacey, 1985; Stinchcombe et al., 2004). Rathcke and Lacey, (1985) and Stinchcombe et al., (2004) reported close relationship between the sites of ecotype origin, especially latitude, with flowering attributes. The results of the current study agree with those of

Stinchcombe et al., (2004) who found ecotypes of *Arabidopsis thaliana* from the more Northern latitudes flowering later than those from less Northern latitudes.

Tables 3.8, 3.9 and 3.10 shows results of variation in DSF, DFF and FP among the ecotypes. The delayed DSF for Kiboko collections, (KBK1 and KBK2) as compared to Kilifi collections (KLF1 and KLF3) could be due to differences in plant sizes as shown in the inverse relationship between plant size and flowering time. The small plant size and early flowering are described as features for dehydration-avoidant phenotype by Blum (2005) resulting from a trade-off between allocation of resources to vegetative production and reproduction (Gardner and Latta, 2008). Plants growing under drought conditions have their leaves mature at smaller size than well-watered plants (Chaves et al., 2003). Late flowering grasses are associated with superior herbage yields (Boonman, 1993) and also with higher leaf tissue density Craine et al., (2011).

The expected relationship between plant size and days to first flowering (Zopfi, 1995; Colautti and Barrett, 2010) did not occur in MGD1. The ecotype which was collected from the edges of a dry sandy stream in association with short *Acacia* and *Aristida* species about 10 kilometres from Lake Magadi exhibited early flowering and robust related traits. Although MGD1 was collected from an arid agro-ecological zone VI and seemed to exhibit a moisture stress avoidance trait represented by early flowering, the ecotype should not be assumed to be drought tolerant especially because of its special habitat. The early flowering nature could be due to the arid conditions of the site of ecotype origin. High evaporation rates probably results in soil moisture being available only during the short periods of rains leading to an escape through early flowering. On the contrary, MGD3 ecotype collected at the end of a flooding valley in Magadi, was late flowering probably because it was grazed late since the area was strictly used as dry season grazing land for the pastoral Maasai community (Personal communication, exploration guide).

While selecting ecotypes for *Chloris gayana* at Kitale Research Station in Kenya, Boonman (1993) found a strong relationship between flowering time and rainfall patterns in sites of population origin and not to drought tolerance. Ecotypes from semi-arid zones (*Kapedo*, *Mpwapwa* and *Rongai)* flowered earlier than humid zone collections (*Pokot* and *Masaba)* due to the short rainy seasons in their sites of origin. The former were from Zone III/IV while the latter were from Zone II/III corresponding to semi-arid and humid conditions, respectively. *Pokot Rhodes*, a robust and late heading variety, was initially thought to be drought tolerant due to arid conditions of collection region, though the variety was collected from the moist cool parts of the region. The MGD1 results indicated that the ecotype is possible to select for early and robust ecotypes of *C. ciliaris*. Further studies on extent of tolerance to drought for MGD1 needs to be done.

Table 3.11 showed that DSF and DFF were positively correlated (r=0.9) among the *C. ciliaris* ecotypes. This implies that ecotypes with delayed DSF, took longer to reach DFF and the FP was also longer. This finding was supported by the curves in Figure 3.10 and the positive correlation between DFF and FP. It can be concluded that there is a trend of increasing FP with increase in DFF.

DSF was positively correlated to plant height, leaf length and breadth, stem thickness and number of nodes (r=0.8, 0.8, 0.8, 0.9, 0.6, respectively), which implies strong relationship between flowering time and plant size. The positive correlation between DSF and inflorescence length ($r= 0.8$; $p\leq0.01$) and the number of spikelets per inflorescence ($r= 0.7$; $p\leq0.05$) implies that the late flowering ecotypes produce more spikelets per inflorescence than early flowering ecotypes. This is further an indication of a mechanism by the early flowering to compromise in resource allocation to growth. Faba beans are known to escape droughts through early flowering and short grain filling periods to optimize production under unfavourable conditions (Kuol, 2004). In other studies on flowering time genes in rice, *Heading-date 1*

(*Hd1*) and *Early heading date 1* (*Ehd1*) were found to reduce the number of primary branches in a panicle, resulting in reduced spikelet numbers per panicle (Naokuni and Izawa, 2011).

The positive correlation between DSF with the number of nodes (r=0.6) is similar to results by Zopfi (1995) and Pleines et al., (2013) who worked with *Rhinanthus* spp. Zopfi (1998) noted that the number of internodes was a stable trait that is not affected by management regimes.

Days to first flowering (DSF) was strongly negatively correlated at $p \leq .0.001$ with percent fertile tillers (r = -0.8) and the number of inflorescence per plant (r = -0.8). This means that ecotypes that flowered early had higher percent fertile tillers and that early flowering plants allocate more resources to reproduction related traits than production. The high number of fertile tillers in early flowering ecotypes is not in agreement with findings by Zopfi (1995) where there was a trade off on the number of flowers with early flowering in *Rhinanthus glacialis* herb.

Table 3.12 indicated that Kiboko ecotypes of *C. ciliaris* species had higher CP than Kilifi collections at $p \leq 0.05$. This could be due to the very low levels of 6.6 % CP recorded by KLF3 which is one of Kilifi collections. The highest CP content among the ecotypes was for KBK3 at 10.9% and lowest was KLF3 with 6.6% (Table 3.13). The difference between Kiboko and Kilifi in crude protein may be attributed to differences in flowering time between Kiboko and Kilifi. Table 3.6 showed that Kilifi collections flowered significantly earlier than Kiboko ecotypes. Nutritive value of grasses reduces as the plant matures due to reduced leaf stem ration (Hintz et al., 1985). The CP levels for the Kilifi and other small sized ecotypes were already on the downfall given that full plot heading had been achieved by end of the fourth week. Full plot heading among the robust group was achieved by end of fifth week. Heading or flowering time can be used to estimate the period for obtaining optimum CP for different ecotypes of this species. Further studies are necessary to ascertain the optimum levels for individual ecotypes.

The range of CP levels for *C. ciliaris* ecotypes was higher than 9 % that was recorded by Morales et al., (2006) and lower than the minimum recorded by Ashraf et al., (2013) at 13.1 %. Although Morales et al., (2006) and Ashraf et al., (2013) were working with *C. ciliaris* species, variation in the time of sampling, ecotypic variation or environmental factors could have led to differences between the studies. Nutritive quality of forages varies depending on species type, variety, plant maturity stage and management practices such as fertilizer use and frequency of cutting. Significant differences in CP content among *C. ciliaris* cultivars was recorded by Morales et al., (2006), Saini et al., (2007) and Ashraf et al., (2013). The CP levels in all eleven *C. ciliaris* ecotypes were above the minimum 7 % required to sustain rumen functions except KLF3 that had 6.6 % (Garcia-Dessommes et al., 2003).

The positive correlation between robustness related traits and CP in Table 3.14 could imply that tall, long leaved and thick stemmed ecotypes have higher CP than small and early flowering ecotypes at six weeks post harvesting. These traits may be targeted in selection for higher CP among and within the ecotypes. Crude protein was significantly correlated (r=0.7) with ash content. This could be due to the effect of some of the minerals contained in ash. Ash is mainly made up minerals such as calcium, magnesium, potassium and phosphorus (Undersander, 2009). Calcium levels, like CP, decreases as the plant matures and fiber content increase (Hintz et al., 1985). The stage of harvest for the late flowering ecotypes could have also influenced the results implying that their CP levels may not have reached the optimal levels where the nutrient starts declining with maturity and ash content increased due to lignification.

Despite lack of difference between sites in ash content for *C. ciliaris* (Table 3.12), differences were recorded between ecotypes (Table 3.13). KBK2 and KLF2 had higher ash content ($p \leq 0.05$) than all ecotypes except MGD3. The differences in ash content between the ecotypes may have been due to differences in mineral contents among them. Possibly differences in rooting system among the ecotypes

may have influenced their efficiency in absorbing soil nutrients leading to variation among them in mineral contents. Flores et al., (2012) recorded significant differences in stem ash content in two genotypes of elephant grass, Roxo with 9.4 % and Paraiso with 4.5 % which was attributed to higher potassium content in Roxo (39.7 g kg^{-1}) than Paraiso with 17.9 g kg^{-1}. The different ash content results recorded in this study were higher than those reported by Onyeonagu and Eze (2013) who recorded 6.21 and 5.77 g kg^{-1} for rainy and dry seasons with selected grass species. Onyeonagu and Eze's grass species were robust grasses, mainly fodder types, with higher stem content which could explain the difference. Higher stem content has been noted to result in low ash content in plants (Roger and Bano 1998). The ash content level for *C. ciliaris* in this study was higher than the six percent recommended by Undersander, (2009) for grasses. Ash represents the total mineral content in the sample, which includes both endogenous and exogenous mineral content. The endogenous content includes calcium, magnesium, potassium, phosphorus, salt and trace elements, which is valuable to livestock and is about six percent. The exogenous ash represents contamination with soil contents among others. Probably the recorded levels above the 6% was due to contamination.

The overall mean of 91.5 % DM shown in Table 3.12 for all the *C. ciliaris* ecotypes was lower than 92.1 % recorded by Ndathi et al., (2012) with *C. ciliaris* grass samples from farmers' fields. The difference in the two studies could be as a result of differences in stage of plant growth during data collection or management of the crop.

Table 3.13 shows that crude fiber levels ranged from 38.4 to 32.4% for KBK1 and KBK3 or MGD3, respectively. The highest CF of 38.4 % compared well with that of Ashraf et al., (2013) which was 39.5 %. KBK1 had the highest level of CF probably because the ecotype, which is blue in color, has tough stems and rough leaves. Crude fibre was negatively correlated with all the traits and to significant levels

($p \leq 0.05$) with plant height ($r = -0.65$) and leaf length ($r = -0.72$). This indicated that tall and long leaved ecotypes, namely KBK2, KBK3 and MGD3 that essentially had longer leaves than the rest of the ecotypes ($p \leq 0.05$), had lower crude fibre at six weeks post clipping. Selection for lower CF should target the taller ecotypes with longer leaves.

INVDMD for the *C. ciliaris* ecotypes had positive and significant correlation with stem thickness ($r = 0.61$) and leaf breadth ($r = 0.68$). The correlation results indicated that ecotypes with thicker stems and wider leaves had higher INVDMD which probably gave them a higher intake. Selection within an ecotype for improved INVDMD is possible.

Some of the *C. ciliaris* ecotypes in the collection that have promising results include KBK2, KBK3 and MGD3 which recorded high CP and lower CF. KBK2 also had higher INVDMD and was lower in dry matter yield probably due to its bulkiness with high moisture content. The ecotype was clustered as a robust type as shown in Figure 3.6. The ecotype is significantly tall with long and wide leaves. Bulkiness is misinterpreted to mean high yield in forage production (Boonman 1993). Despite being bulky, Elephant grasses have the lowest % DM yield of all cultivated East African grasses such as Guineagrass and Rhodesgrass.

Differences were observed for the different morphological attributes measured among *E. superba* ecotypes as shown in Table 3.15. The different inflorescence shape recorded was due to two Taveta ecotypes, TVT1 and TVT2. The two ecotypes had the sub-branches on the inflorescence aligned or closed up along the main branch resulting in a compact inflorescence. Figures 3.11, 3.12 and 3.13 shows Kiboko collections (KBK1 and KBK2) of *E. superba* being grouped separately from the other ecotypes irrespective of the types of traits used. These indicate the Kiboko ecotypes as a stand-alone group. The two Kiboko ecotypes were collected from distinct environments, especially in terms of utilization as

shown in Table 3.1. Table 3.16 shows that group one that consisted of only Kiboko collections had significantly higher mean stem thickness, inflorescence length and plant height. This indicates that Kiboko collections are bigger in size than the rest of the ecotypes.

The clustering by plant size in both *C. ciliaris* and *E. superba* may help in selection among the ecotypes for either hay production or grazed pastures. For instance, the robustness in Kiboko ecotypes of *E. superba* may be used to select them for hay production and their thicker stems would aid against lodging. The second group of smaller sized ecotypes of *E. superba* may be selected for grazing. The thinner stems of small sized ecotypes allows for more animal intake.

Phenotypic correlation analysis for *E. superba* shown in Table 3.17 implies that taller ecotypes had thicker stems (r=0.84), longer leaves (r=0.76), longer inflorescence (r=0.0.74) and more spikelets per inflorescence (r=0.66) than shorter plants. The correlation results reflects the overall plant robustness and supports the results in Table 3.16 showing analysed difference between the robust and small sized plants of *E. superba*. Positive correlation coefficients between plant height with stem thickness, leaf length, inflorescence length, spikelet number per inflorescence were observed by Yamada et al., (2004) in Ryegrass. Plant height and stem thickness have been found to have a positive relationship with grain yield in maize (Sabiel et al., 2014). Spikelet number per inflorescence which is one of the components that accounts for seed yield in grasses is highly heritable and selection for the trait may result in better yielding genotypes (Boelt and studer, 2010). Selection for the traits correlated to spikelet number per inflorescence such as plant height and stem diameter may increase the potential seed yield among *E. superba* ecotypes. Indirect effect of plant height on seed yield was observed by Fang et al., (2004) while evaluating a full-sib family of Meadow fescue.

The significant correlation between inflorescence shape with caryopsis per spikelet (r=0.73) and with 1000 spikelet weight (r=0.68) in *E. superba* may imply that the uniquely compact shaped inflorescence have more pure seeds that are heavier than the common shaped type with loose branches. Ecotypes with compact heads may be better in seed production, although studies to evaluate performance in seed yield would be necessary to confirm this observation. The significantly negative correlation between the number of nodes with caryopsis per spikelet (r = -0.79) and inflorescence length (r = -0.85) could be used to select ecotypes that are high in seed yield especially because the internodes is a trait that is not influenced by management (Zopfi, 1998). In this case, selection for higher number of caryopsis per spikelet may be done indirectly using the number of nodes.

Percent fertile tillers was significantly positively correlated (r=0.79) with the number of tillers at peak flowering. Given that percent fertile tillers data was collected at seed maturity stage, the relationship implies that the percent heading tillers is linked to the total number of tillers at peak flowering stage and not the tillers present at seed maturity. Early formed tillers are mainly responsible for producing inflorescence, particularly larger ones (Boelt and studer, 2010).

Table 3.18 showed that there was no difference between the sites of *E. superba* collection in CF, CP, INVDMD, ash and % DM at $p \leq 0.05$. This could be attributed to the significant differences among ecotypes within a site and close ranges between ecotypes from different sites. Singular outlying ecotypes have been found to significantly affect potential utilization of group means (M'seddi et al., 2002). This was explained by results in Table 3.19 that shows that the two Kiboko ecotypes significantly differed in % DM, although the two did not differ to ecotypes collected from other sites such as Taveta or Kilifi. KBK1 ecotype with 92.7 % DM was significantly higher than KBK2 at 91.9 %. The KBK1 ecotype was

not significantly different to KLF1 (93.1%) and KLF2 (92.5) while KBK2 was not different to TVT2 (90.8 %), TVT2 (91.9 %), KLF2 and GBK (91.4 %).

Table 3.19 shows *E. superba* KLF1, KBK2 and KLF2 had the highest levels of CP and were among the highest in CF. Positive correlation between CF and CP have previously been recorded by Ashraf et al., (2013) while working on *C. ciliaris* collections from Pakistan.

The recorded INVDMD for *E. superba* ecotypes ranged from 45.4 to 56.1%. This range was higher than that recorded by Ndathi et al., (2012) on *E. superba* grass growing in farmers' fields. The variation could be attributed to the difference in the time of harvesting in the two studies. Sampling of plant materials was done at seed maturity by Ndathi et al., (2012) while in this study it was done at peak flowering time. Plant leaf-stem ratio reduces as the plant matures leading to reduced digestibility due to being stemmy. There is increased lignification as the forage plant advances in maturity which results in increased fibre content. De Santis et al., (2004) recorded a decline of leaf INVDMD from 658 to 515 g kg^{-1} during the growing season of Berseem clover.

Except in ash content, KLF1 and KBK2 ecotypes of *E. superba* featured highly in all the measured attributes and were clustered in group B using all the nutritive value components as shown in Figure 3.14. The clustering allowed easy comparison of the ecotypes across all the nutritive components recorded as shown in Table 3.20. Table 3.20 shows that cluster B, consisting of KLF1 and KBK1, had higher CP than clusters A. The KBK2 ecotype was taller (96.1 cm) with longer leaves ($p \leq 0.05$) than KLF1 (66.6 cm) and against an overall mean height of 80.8cm. TVT3 ecotype was distant from the rest of the ecotypes probably due to its significantly high CP (11.2 %) and lowest CF (37 %). The significant variation among the *E superba* ecotypes provides an opportunity for selection targeting specific nutritive value components.

3.6. Conclusion

Cenchrus ciliaris ecotypes were classified into small-early maturing types and robust-late maturing types except a unique type from Magadi, MGD1 that was early flowering and robust. Selection for maturity time and plant size is possible with the collections of *C. ciliaris* grass species. Selection for improved CP in *C. ciliaris* may be done indirectly using the correlated traits such as plant height, leaf length, stem thickness and time to start flowering (DSF). The robust types recorded higher CP than the small types. The positive significant correlation between INVDMD with leaf width and stem thickness may allow selection for improved INVDMD within *C. ciliaris*.

Kiboko collections of *E. superba* were closely related and distant to the rest of the ecotypes in terms of agro-morphological characterization. KBK1 and KBK2 ecotypes were grouped together as robust types while the rest (KLF1, KLF2, KLF3, TVT1, TVT2, TVT3 and GBK) were grouped as a smaller sized group. Selection for higher crude protein at peak flowering stage may target KBK2 and KLF1 ecotypes of *E. superba* that clustered together with higher mean CP than the rest of the ecotypes except TVT3 ecotype. KBK2 ecotype was found to be robust and with higher CP.

CHAPTER FOUR

CHARACTERIZATION OF ECOTYPES OF *CENCHRUS CILIARIS L.* AND *ERAGROSTIS SUPERBA* PEYR. USING SEQUENCE RELATED AMPLIFIED POLYMORPHISM (SRAP)

4.1 Abstract

Cenchrus ciliaris and *E. superba* are drought tolerant grass species widely introduced in rangelands for rehabilitation of degraded lands and to improve forage production. Knowledge on genetic diversity among grass populations being introduced for rehabilitation is important for successful establishment. Genetic diversity among 36 and 15 ecotypes of *C. ciliaris* and *E. superba*, respectively, was evaluated using Sequence Related Amplified Polymorphism (SRAP) markers. Total DNA was extracted from bulked leaf material of 10 plants per ecotype and used in SRAP marker analysis. In *C. ciliaris*, the percentage polymorphic loci ranged from 16 to 55% and Nei's gene diversity index from 0.089 to 0.217 with a mean of 30.6 and 0.142, respectively. Kajiado population of *C. ciliaris* recorded the highest diversity indices. Analysis of molecular variance (AMOVA) indicated significant genetic differences (<0.01) with proportion of variation among and within populations at five and 95%, respectively. The genetic differentiation value (F_{st}) was 0.053 indicating low genetic differentiation among the *C. ciliaris* populations. Principal coordinate analysis and Nei's unbiased genetic distance analysis indicated Kilifi and Narok populations as the most distant. For *E. superba*, there was significant ($p<0.07$) genetic variation among and within populations at 24 and 76 %, respectively. Genetic differentiation value (F_{st}) was 0.237 while the observed mean Shannon diversity index and Nei's genetic diversity index were I=0.357 and h=0.223, respectively. Geographical distances between sites of ecotypes collection may have contributed to the significant genetic differences among the populations in the two grass species. Compositing of ecotypes collected from varied ecological environments within a county may have

resulted in higher within population genetic diversity like in Kajiado population of *C. ciliaris*. Management and conservation of the various populations, particularly for *E. superba* is necessary for maintenance of genetic diversity. There is need for more collection and characterization of *C. ciliaris* and *E. superba* ecotypes from different agro-ecological areas to expand the genetic bases of available collections. Sequence related amplified polymorphism markers successfully revealed genetic diversity analysis of *C. ciliaris* and *E. superba* grass species.

Keywords: *Cenchrus ciliaris*, *Eragrostis superba*, genetic diversity, grasses, SRAP

4.2 Introduction

The Arid and Semi-Arid Lands (ASALs) are very varied environments in time and space possibly resulting in development of ecotypes with beneficial genetic variance that allows for adaptation to certain localities. Ecological factors such as micro and macro climatic factors have been found to influence selection of genotypes in wild barley for specific niche environments (Owuor et al., 1997). Marshall et al., (2012) observed large intra-species variation in *C. ciliaris* that was thought to be associated with differences in environmental tolerance.

Genetic diversity studies on different populations of *C. ciliaris* have been done (Gutierrez-Ozuna et al., 2009; Al-soqeer 2011; Kharrat-Souissi et al., 2011; Burson et al., 2015). *Cenchrus ciliaris* is highly polymorphic in various morphological traits which has been attributed to existence of different ploidy levels (Burson et al., 2012; Kharrat-Souissi et al., 2013) and the mode of reproduction of cloning by seed resulting in progenies that are uniform and identical to the parents (Hignight et al., 1991). Burson et al., (2012) observed tetraploids (2n=4x=36), pentaploids (2n=5x=45), hexaploids (2n=6x=54), septaploids

(2n=7x=63) and various aneuploids in 568 accessions of *C. ciliaris* where 308 of the accessions were tetraploids. The most dominant ploidy level for the species is the tetraploid.

Phenotypic variation among *C. ciliaris* ecotypes was also observed in this study which heightened the need to understand the genetic diversity among collections of *C. ciliaris* from Kenyan ASALs as well as for *E. superba*. No molecular studies have been undertaken on *E. superba* collections from despite the grass species being widely used in Kenyan rangeland restoration programmes. Genetic diversity analysis would help in management of germplasm collection for the target species for conservation and utilization in rangeland rehabilitation. Low genetic diversity in a population may affect successful establishment due to genetic bottlenecks.

Molecular markers are useful tools in genetic diversity analysis because they are not influenced by variable environmental conditions or plant phenology, and are a basis for discriminating among cultivars with similar morphological characteristics (Beebe et al., 2000). Many techniques have been developed and are able to assess genetic variation more accurately, quickly and cheaply (Spooner et al., 2005). Sequence Related Amplified Polymorphism (SRAP) is useful in genetic studies, such as biodiversity evaluation. The marker has been used in genetic studies of various plant species including fungi (Li and Quiros, 2001; Ferriol et al., 2003; Ma et al., 2010). The marker has been used successfully in characterization of genetic diversity of different grass species that include Bufallograss (Budak, et al., 2004a), turfgrass species (Budak et al., 2004b) and *Hemarthria compressa* grass (Huang et al., 2012).

A total of 36 samples of *C. ciliaris* and 15 of *E. superba* grass species collected from different counties in Kenyan ASALs were evaluated for genetic diversity using SRAP markers.

4.3 Material and methods

4.3.1. Germplasm acquisition

Acquisition of nine *E. superba* and eleven *C. ciliaris* ecotypes was done in 2012 as shown in Table 4.1. Additional twenty ecotypes of *C. ciliaris* were collected in 2013 from Narok, Kajiado and Taita Taveta Counties by scientists from KALRO Kiboko, KALRO-Genetic Resources Research Institute and National Museums of Kenya. Six *E. superba* and four *C. ciliaris* ecotypes were acquired from ILRI gene bank, Ethiopia. Therefore a total of 36 ecotypes of *C. ciliaris* and 15 for *E. superba* were used in this study.

The procedure of acquisition of germplasm in terms of seeds harvesting or plantlets during germplasm collection is as described in Section 3.3.1 of this study. All collections for 2012 and 2013 were established as ecotypes at the Kenya Agricultural and Livestock Research Institute (KALRO) Kiboko research station. The seeds from ILRI Ethiopia genebank were planted in petri dishes in a greenhouse at KALRO Biotechnology (Plate 4.1).

Plate 4.1: *ILRI accessions in pots at KALRO Biotech, Kabete*

Table 4.1: *Collection details of 36 ecotypes of Cenchrus ciliaris and 15 of Eragrostis superba used in the study*

No.	Code	Place of collection	Latitude	Longitude	Region/County	Collection date
Cenchrus ciliaris ecotypes						
1	MGD3	Magadi	37M 0206631 UTM 9781498		Kajiado	2012
2	TVT3	Ziwani ranch	37M 0362386 UTM 9637556		Taita Taveta	2012
3	KBK2b	Makindu River	37M 0364664 UTM 9742932		Makueni	2012
4	KLF1	Kilifi	37M 0592230 UTM 9602470		Kilifi	2012
5	KBK1	Kiboko River	37M 0356997 UTM 9754961		Makueni	2012
6	KBK2	Makindu River	37M 0364664 UTM 9742932		Makueni	2012
7	KLF2	Kilifi	37M 0588462 UTM 9609848		Kilifi	2012
8	MGD1	Oldonyonyokie	37M 0206621 UTM 9799034		Kajiado	2012
9	TVT1	Kimala	37M 0360211 UTM 9623156		Taita Taveta	2012
10	TVT2	Ziwani ranch	37M 0361675 UTM 9632568		Taita Taveta	2012
11	KBK3	KALRO Kiboko	37M 0358340 UTM 9751011		Makueni	2012
12	KLF3	Rea Vipingo farm	37M 0591436 UTM 9583080		Kilifi	2012
13	6645	Ethiopia	ILRI genebank		Ethiopia	1982
14	6646	Ethiopia	ILRI genebank		Ethiopia	1983
15	6652	Ethiopia	ILRI genebank		Ethiopia	1983
16	7143	Ethiopia	ILRI genebank		Ethiopia	1982
17	TVT4	Taveta	-	-	Taita Taveta	2013
18	MBRKN1	Mbirikani Ranch	02º30.962	037º31.706	Kajiado	2013
19	ILBSL1	Il Bisil	02º07.262	036º47.966	Kajiado	2013
20	MGD5	Olkramatian	01º55.687	036º04.553	Kajiado	2013
21	MBRKN2	Mbirikani Ranch	02º31.092	037º38.318	Kajiado	2013
22	TVT6	Taveta	-	-	Taita Taveta	2013
23	MOS2	Mosiro	01º20.363	036º06.520	Narok	2013
24	MBRKN3	Mbirikani Ranch	02º30.787	037º42.592	Kajiado	2013
25	KBK4	Kiboko	-	-	Makueni	2013
26	MKS2	Machakos	-	-	Machakos	2013
27	ILBSL2	Il Bisil	02º07.497	036º48.037	Kajiado	2013
28	SUS	Suswa	01º04.157	036º18.270	Narok	2013
29	MOS3	Mosiro	01º20.363	036º06.520	Narok	2013
30	MGD4	Oldonyonyokie	01º45.167	036º23.466	Kajiado	2013
31	ISYA	Isinya	01º31.796	036º40.827	Kajiado	2013
32	MKS1	Machakos	01º29.715	037º04.038	Machakos	2013
33	MTTE	Mwatate	-	-	Taita Taveta	2013
34	MGD2	Magadi	01º47.308	036º03.402	Kajiado	2013
35	TVT5	Taveta	-	-	Taita Taveta	2013
36	MOS1	Mosiro	01º17.876	036º07.575	Narok	2013
Eragrostis superba ecotypes						
1	KBK1	Chyulu hills	37M 0354247 UTM 9739790		Kiboko	2012
2	KBK2	KALRO Kiboko	37M 0358340 UTM 9751011		Kiboko	2012
3	KLF1	Kilifi	37M 0591450 UTM 9606252		Kilifi	2012
4	KLF2	Kilifi	37M 0597165 UTM 9607150		Kilifi	2012
5	KLF3	Rea Vipingo	37M 0591436 UTM 9583054		Kilifi	2012
6	TVT1	Kimala	37M 0360260 UTM 9623140		Taita Taveta	2012
7	TVT2	Ziwani ranch	37M 0361124 UTM 9632038		Taita Taveta	2012
8	TVT3	Ziwani ranch	37M 0357469 UTM 9637472		Taita Taveta	2012
9	GBK	Magadi	Muguga genebank		Kajiado	1989
10	12755	Wajir	ILRI genebank		North Eastern	1984
11	12777	Takaungu	ILRI genebank		Kilifi	1984
12	13122	Uasingishu	ILRI genebank		Rift Valley	1984
13	13289	Marsabit	ILRI genebank		North Eastern	1984
14	16595	Outjo	ILRI genebank		Namibia	1991
15	16619	Kaokoland	ILRI genebank		Namibia	1991

4.3.2. Plant leaf material collection

Plant leaf material was collected and bulked from 10 plants per ecotype at KALRO Kiboko experimental plots and immediately preserved in silica gel in zip-locked polythene bags and taken to KALRO Biotechnology laboratory for storage at -80^{o}C (Plate 4.2). Protective gloves were worn and disinfected with 70 % ethanol after harvesting from every ecotype to avoid contamination.

Plate 4.2: *Collection of plant leaves at KALRO-Kiboko pasture plots*

4.3.3. DNA extraction

Total DNA was extracted from approximately 0.5 g of leaf tissues using a modified CTAB method (Doyle and Doyle, 1987). The tissues were ground to a fine powder using a genogrinder and incubated at 65°C for 60 min in 2 ml tubes with 3% CTAB isolation buffer [100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 3% hexadecyltrimethylammonium bromide (CTAB), 2% β-mercaptoethanol and 1% polyvinlypyloridone (PVP)]. Next, an equal volume of chloroform-isoamyl alcohol (24:1) was added, and tube was mixed by inversion for 10 min and centrifuged twice. The supernatant was mixed with about 1000µL ice-cold isopropanol to pellet the DNA. The DNA was washed twice with 70% ethanol, air-dried at room temperature and resuspended in about 0.2 mL 0.1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The quality and concentration of the DNA were confirmed by electophoresis on 0.8% agarose gel.

4.3.4. Primer selection and SRAP-PCR amplification

A total of 48 primer pairs generated from the six forward and eight reverse SRAP primers shown in Table 4.2 were screened for polymorphism with the *C. ciliaris* and *E. superba* ecotypes. A sub-sample of three ecotypes per grass species was used in the screening. Primers pairs were ranked based on the number and clarity of bands produced and distinction in pattern. Six primer pairs were selected for use in analysis of genetic diversity in *C. ciliaris* and seven for *E. superba* (Table 4.3).

Table 4.2: *List of forward and reverse Sequence –related amplified polymorphism (SRAP) primer information for this study*

	Reverse primers		**Forward primers**	
Item No		**Sequence**		**Sequence**
1	EM_5	GACTGCGTACGAATTAAC	ME_1	TGAGTCCAAACCGGATA
2	EM_7	GACTGCGTACGAATTCAA	ME_2	TCCTCCGCTTATTGATATGC
3	EM_9	GACTGCGTACGAATTCGA	ME_5	CTTGGTCATTTAGAGGAAGTAA
4	EM_10	GACTGCGTACGAATTCAG	ME_8	GCTGCGTTCATCGATGC
5	EM_11	GACTGCGTACGAATTCCA	ME_11	GCATCGATGAACAACGCAGC
6	EM_12	GACTGCGTACGAATTATG	ME_12	GAGAGTTTGATCCTGGCTCAG
7	EM_15	GACTGCGTACGAATTTAG		
8	EM_17	GACTGCGTACGAATTGTC		

Table 4.3: *List of forward and reverse Sequence –related amplified polymorphism (SRAP) primer pairs used in genetic diversity analysis of ecotypes of Eragrostis superba and Cenchrus ciliaris*

Item No.	***Eragrostis superba***	**Item No.**	***Cenchrus ciliaris***
SRAP 24	EM_17 and ME_5	SRAP 4	EM 10 and ME_1
SRAP 44	EM_10 and ME_12	SRAP 5	EM_5 and ME_1
SRAP 30	EM_12 and ME_8	SRAP 6	EM_12 and ME_1
SRAP 46	EM_12 and ME_12	SRAP 7	EM_15 and ME_1
SRAP 42	EM_7 and ME_12	SRAP 20	EM_10 and ME_5
SRAP 20	EM_10 and ME_5	SRAP 48	EM_17 and ME_12
SRAP 48	EM_17 and ME_12		

The protocol for SRAP analysis was based on Li and Quiros (2001). Each 20-µL PCR mixture consisted of 40 ng genomic DNA, 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.5 µM primer, 1X PCR buffer, and 1 U Taq polymerase. Samples were subjected to the following thermal profile: the first five cycles were run at

94°C for 1 min, 35°C for 1 min, and 72°C for 1 min, for denaturing, annealing and extension, respectively. The annealing temperature was then raised to 50°C for another 35 cycles, followed by another extension step of 10 min at 72°C. The holding temperature was 4°C. PCR products were mixed with 2 µl loading buffer and separated on 1.5 % agarose gel pre-stained with ethidium bromide. One (1) kb gene ruler ladder was included as a marker of band sizes. The gel was run on 0.5X TBE buffer at 100 V constant voltage for 1 hour and 30 minutes. The gel was then visualized under UV light in a UV trans-illuminator.

4.3.5. Data analysis

Presence or absence of SRAP-amplified fragments was scored as one or zero, respectively. The resulting presence/absence data matrix was analyzed using GenAlex 6.5 statistical package (Peakall and Smouse, 2009) to compute a genetic distance matrix. The genetic distance matrix was used in analysis of molecular variance (AMOVA) which partitioned the total SRAP variation into within-population and between-population (Excoffier et al., 1992). Variance components, the sum of all squared differences, and analogues of F-statistics between populations were calculated to estimate the population differentiation, which was the equivalent of the Wright F_{ST} index (Wright, 1965). Principal coordinate analysis (PCoA) was also computed using GenAlex statistical package based on genetic distances to visualize the genetic relatedness between individuals in each population in a two dimensional figure. Assuming Hardy-Weinberg equilibrium, Tools for Population Genetics Analysis (TFPGA) (Miller, 1997) was used to estimate genetic diversity parameters which included the percentage of polymorphic loci and Nei's gene diversity (H) and generate the genetic distances in *C. ciliaris*. A dendrogram was constructed with the unbiased Nei's genetic distance matrix to display population relationships using the unweighted pair-group mean algorithm (UPGMA) of TFPGA. For *E. superba*, PopGen32 (Yeh et al., 1999) was used to analyse for genetic diversity indices such as Nei's genetic diversity index, polymorphic

loci, percent polymorphic loci and Shannon diversity index between the populations. DAWin5 software version 5.0.158 was used in cluster analysis of plant samples and to generate the dendrogram.

4.4 Results

Table 4.4 shows a summary of genetic diversity estimates of seven *C. ciliaris* populations. The percentage polymorphic loci ranged from 16.1 for Machakos to 51.5 % for Kajiado population. For heterozygosity (Nei's gene diversity) values, Kajiado population also recorded the highest value of h= 0.217 while Ethiopia had the lowest heterozygosity h=0.086 among the *C. ciliaris* samples.

Table 4.4: *Genetic diversity estimates for Cenchrus ciliaris populations generated from six SRAP markers*

Populations	Sample size (N)	Polymorphic loci (%)	Heterozygosity (h)
Makueni	5	35	0.165
Kilifi	3	21.9	0.108
Taita Taveta	7	39.4	0.180
Ethiopia	4	19.7	0.086
Kajiado	11	51.1	0.217
Narok	4	30.7	0.146
Machakos	2	16.1	0.089

Plate 4.3 shows an example of gel electrophoresis pattern resulting from SRAP EM5 - ME1 primer combination.

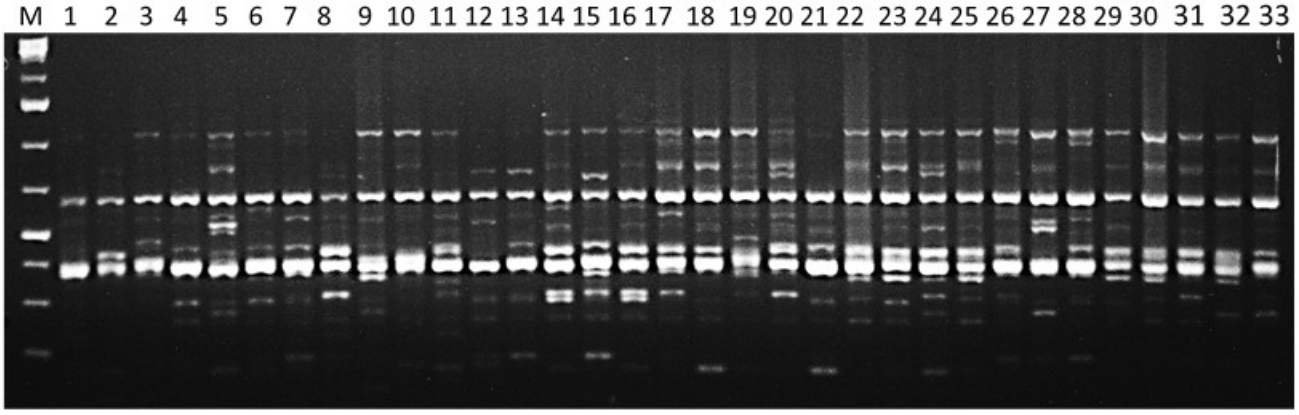

Plate 4.3: *SRAP amplification image of EM5 - ME1 primer combination in the first 33 Cenchrus ciliaris ecotypes as recorded in Table 4.1.*

Table 4.5 shows AMOVA results for seven populations of *C. ciliaris* grass species. Significant differentiation was observed among the seven populations using SRAP markers at $p<0.01$. The genetic

differentiation F_{st} value was 0.053 among the populations. AMOVA results generated from genetic distance matrix for *C. ciliaris* samples partitioned the overall variation into two levels. The variation within populations was 95% while among populations was only 5%.

Table 4.5: *Summary of analysis of molecular variance (AMOVA) results for Cenchrus ciliaris populations collected from Kajiado, Narok, Makueni, Taita Taveta, Kilifi, Machakos and Ethiopia*

Source of variation	df	SS	MSD	% variation	F_{ST}	P_value
Among populations	6	86.8	14.5	5%	0.053	<0.01
Within populations	29	328.9	11.3	95%		
Total	35	415.7		100%		

Degrees of freedom (df), sum of squares (SS), mean of square deviation (MSD), % variation, Genetic differentiation among populations (F_{ST}) and P_value

Figure 4.1 shows the Principal coordinate analysis for seven *C. ciliaris* populations.

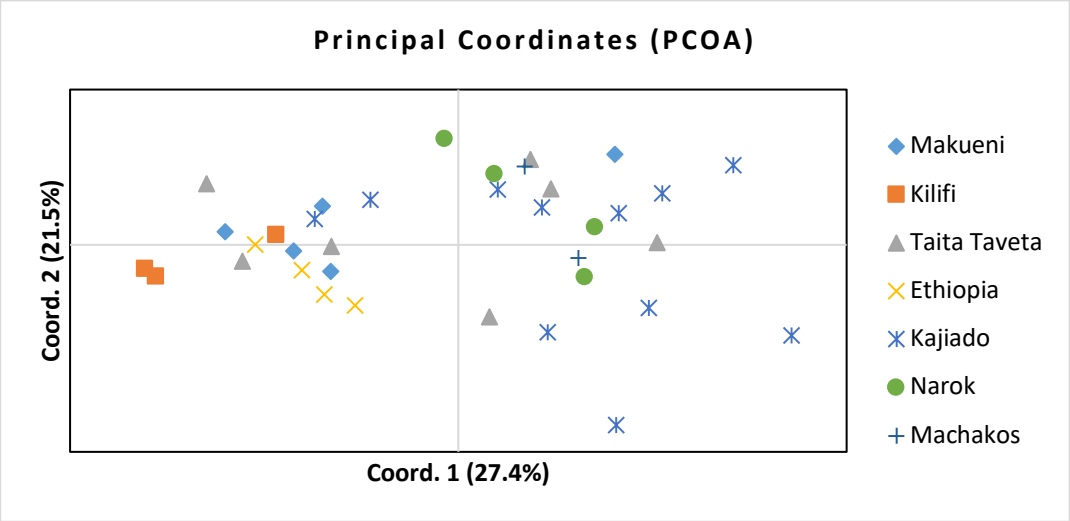

Figure 4.1: *Principal coordinates analysis using seven SRAP markers on seven Cenchrus ciliaris populations collected from Makueni, Kilifi, Taita Taveta, Ethiopia, Kajiado, Narok and Machakos Counties*

The dimensions of the first two PCoA axes accounted for 48.9 % of the total variation with first and second axis accounting for 27.4 % and 21.5 %, respectively. Kilifi and Ethiopian populations of *C. ciliaris* were aggregated on the opposite sides of the Machakos populations on the axis explaining 27.4%

variation. Narok and Kajiado populations aggregated on the opposite side of the Kilifi and Ethiopian populations, except for two ecotypes for Kajiado and one for Narok population. Individuals clustering in one side were closely related as opposed to those on different sides of the PCoA.

Table 4.6 shows Nei's unbiased measures of genetic distance between seven populations of *C. ciliaris*. The smallest genetic distance among the *C. ciliaris* populations was between Kajiado and Taita Taveta populations at 0.036 while the largest distance was between Narok and Kilifi populations at 0.128.

Table 4 6: *Nei's (1972) unbiased genetic distance between seven populations of Cenchrus ciliaris generated by SRAP*

Population	Makueni	Kilifi	Taita Taveta	Ethiopia	Kajiado	Narok	Machakos
Makueni	*****						
Kilifi	0.0675	*****					
Taita Taveta	0.0364	0.0684	*****				
Ethiopia	0.0590	0.0804	0.0698	*****			
Kajiado	0.0644	0.1268	**0.0361**	0.1067	*****		
Narok	0.0883	**0.1278**	0.0557	0.1021	0.0554	*****	
Machakos	0.1030	0.1192	0.0700	0.1146	0.0760	0.0478	*****

Figure 4.2 shows cluster analysis results of seven populations of *C. ciliaris* grass species.

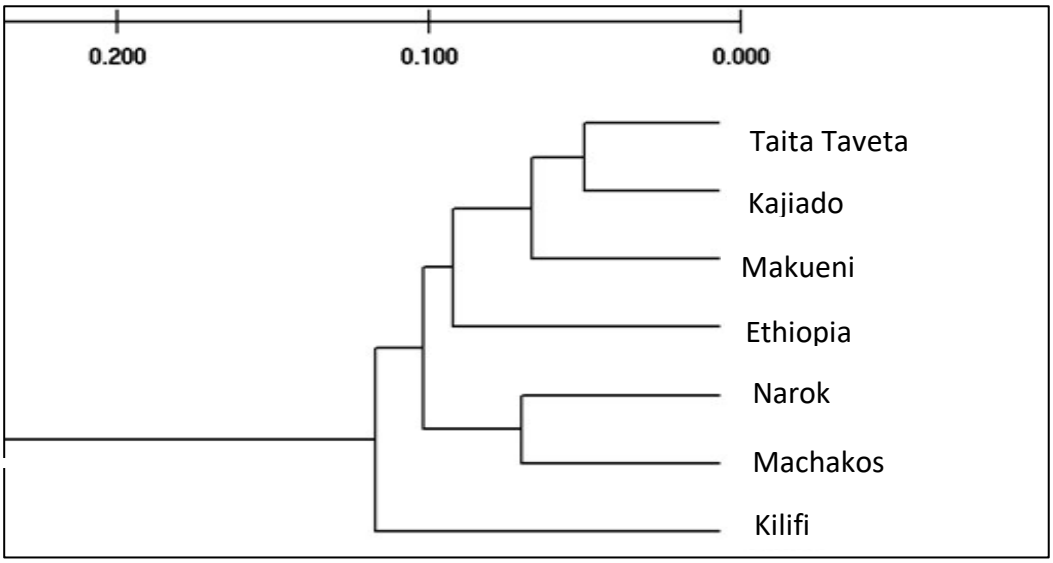

Figure 4.2: *Clustering of seven populations of Cenchrus ciliaris obtained using unweighted pair group method with arithmetic average (UPGMA) using SRAP data*

Taita Taveta, Kajiado and Makueni populations appeared to be the most closely related among the *C. ciliaris* populations with Kilifi population being most distant from them.

Table 4.7 shows different genetic diversity indices for five populations of *E. superba*. Nei's gene diversity index ranged from 0.053 (North Eastern) to 0.194 Kiboko) with a mean of 0.111 while Shannon information index ranged from 0.077 for North Eastern to 0.283 for Kiboko with a mean of 0.163. The percent polymorphic loci for *E. superba* populations ranged from 12.8 for North Eastern to 46.8 % for Kiboko with a mean of 27.7%. Kiboko population recorded the highest levels of the different indices while North Eastern had the lowest among all the populations.

Table 4.7: *Genetic diversity estimates for Eragrostis superba populations generated from seven SRAP markers*

Population	Sample Size	h	I	p	% P
Kiboko	2	0.194	0.283	22	46.8
Kilifi	4	0.123	0.184	16	34.0
Namibia	2	0.106	0.154	12	25.5
North Eastern Kenya	2	0.053	0.077	6	12.8
Taita Taveta	3	0.081	0.117	9	19.2

h = Nei's gene diversity index; I = Shannon's Information index; p=polymorphic loci and %p=percent polymorphic loci

Table 4.8 shows the results of analysis of molecular variance (AMOVA) for *E. superba* populations. Significant level of differentiation ($p<0.007$) was observed among populations. The genetic differentiation Fst value was 0.237 among the populations. The overall variation was partitioned into two levels with 76 % variation being between individuals within populations and 24 % between the populations.

Table 4.8: *Summary of analysis of molecular variance (AMOVA) results for Eragrostis superba populations collected from Kiboko, Kilifi, Taita Taveta, North Western Kenya and Namibia*

Source of variation	df	SS	MSD	% variation	F_{ST}	P_value
Among populations	4	34.8	8.7	24%	0.237	<0.007
Within populations	8	39.0	4.9	76%		
Total	12	73.8		100%		

Gene flow (Nm) = 0.805

Degrees of freedom (df), sum of squares (SS), mean of square deviation (MSD), % variation, genetic differentiation among populations (F_{ST}) and P_value

Table 4.9 shows Nei's unbiased measures of genetic distance between five populations of *E. superba*. The smallest genetic distance among the *E. superba* populations was between Kiboko and Taita Taveta at 0.057 while the largest distance was between Kiboko and North Eastern at 0.338.

Table 4.9: *Nei's (1972) unbiased genetic distance between five populations of Eragrostis superba*

	Kiboko	Kilifi	Namibia	North Eastern	Taita Taveta
Kiboko	****				
Kilifi	0.148	****			
Namibia	0.159	0.121	****		
North Eastern	**0.338**	0.115	0.249	****	
Taita Taveta	**0.057**	0.074	0.181	0.205	****

Figure 4.3 shows the principal coordinate analysis for five *E. superba* populations. The dimensions of the first three PCoA axes accounted for 62.3% of the variation where first, second and third axis represented 25.0, 23.8 and 13.5% variation, respectively. Taita Taveta and Kilifi populations were closely related except for one Kilifi ecotype that clustered with North Eastern population. The two ecotypes of Kiboko population, KBK1 and KBK2, were distantly related as shown by the second axis accounting for 23.8 % variation.

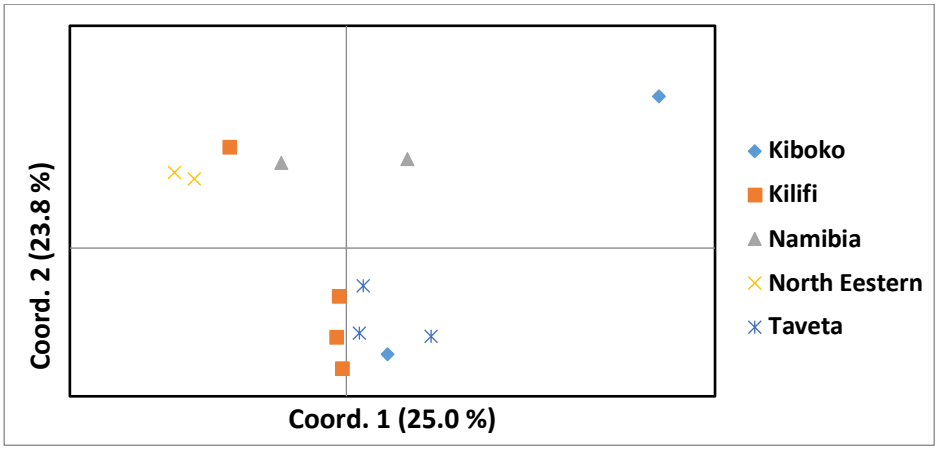

Figure 4.3: *Principal coordinates analysis of five Eragrostis superba populations using SRAP data.*

Figure 4.4 shows a dendrogram of *E. superba* ecotypes, which includes two ecotypes, Magadi (GBK) and 13122 collected from Uasin-gishu that were not included in population analysis since there was not enough samples to represent a population.

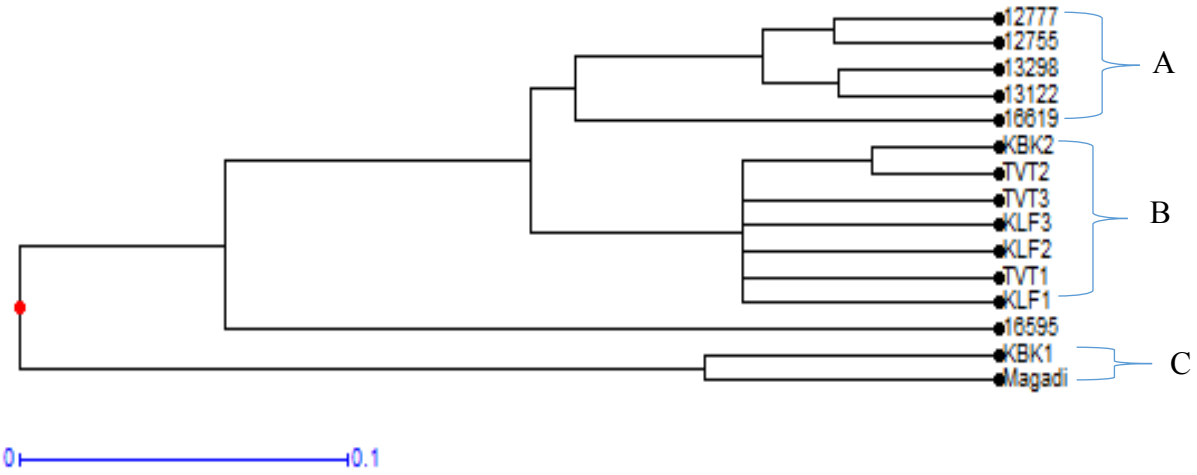

Figure 4.4: *UPGMA dendrogram showing relationship among Eragrostis superba populations using SRAP markers; KBK – Kiboko, KLF – Kilifi and TVT – Taveta ecotypes*

Three main clusters A, B and C were observed while Namibian ecotype 16595 was not clustered and distant to 16619, the other Namibian ecotype. KBK1 and Magadi were clustered in C and distantly related to the rest of the ecotypes. Five of the ILRI Ethiopia genebank ecotypes were grouped in cluster A. The

Kilifi ecotype 12777 received from ILRI was isolated from Kilifi ecotypes that were all clustered in B. The ecotype was the isolated Kilifi ecotype in principal coordinate analysis shown in Figure 5.3.

4.5 Discussion

As shown in Table 4.4, the percentage polymorphic loci values for the *C. ciliaris* populations ranged from 16% to 51%. Kajiado population of *C. ciliaris* was the most diverse with the highest values of both heterozygosity (0.217) and percentage of polymorphic loci (51.1%). These results could be associated with the large number of sample size from Kajiado. Probably the high diversity indices for Kajiado population of *C. ciliaris* could be due to the existence of diverse agro-ecological zones in the county that range from zones IV to VI from which collections were done. These values were similar to those obtained by Gutierrez-Ozuna et al., (2009) working with *Pennisetum ciliare* in Mexico who obtained a range of 22.2 to 51.9 % percent polymorphic loci with Inter Simple Sequence Repeat (ISSR) markers. *Pennisetum ciliare* is the synonym of *Cenchrus ciliaris* (FAO, 2012). Al-soqeer (2011) observed 14.6 and 17.6 % polymorphic bands with Random Amplified Polymorphic DNA (RAPD) and ISSR, respectively, while working with *C. ciliaris* genotypes.

Significant genetic variation ($P<0.01$) among populations of *C. ciliaris* observed with the SRAP markers using AMOVA as shown in Table 4.5. The observed variation between and within populations was five and 95 %, respectively. The five % variation implies low genetic variability between the populations that was confirmed by the moderate genetic differentiation value of $F_{st} = 0.053$ indicating low variance in allele frequency among the populations. The genetic variation between the populations could be attributed to the differences in geographical locations. The observed genetic variability between populations was explained by the PCoA and Nei's unbiased genetic distance analysis in Figure 4.1 and Table 4.6, respectively. The PCoA showed that Kilifi and Ethiopia populations were aggregated

separately from Machakos and majority of Narok and Kajiado populations along the first principal coordinate that explained 27.4% variability. This implies that Narok and Machakos populations are closely related and distant to Kilifi and Ethiopia populations. Nei's unbiased genetic distance between Kilifi and Narok populations was the largest at 0.128. The difference was also observed with the cluster analysis shown in Figure 4.2 where Kilifi population was clustered distant from the other populations.

Adaptation in the *C. ciliaris* populations could be possibly the cause of the significant differentiation among them. Kilifi population was distant from the rest of the population probably because of climatic conditions in its collection sites which are in AEZ III with higher rainfall amounts of up to 1200 mm per annum and low altitude of below 100 m above sea level (Table 3.1). The smallest genetic distance was between Kajiado and Taita Taveta (0.036) probably because most of the samples collected from Taita Taveta and Kajiado counties were from Taveta and Loitoktok sub-counties that are bordering each other. The Ethiopian *C. ciliaris* population clustered together with the Kenyan populations regardless of the large geographical distance between the two countries. This could be an indication of sharing common ancestry. Al-soqeer (2011) observed low levels of genetic diversity among population of *C. ciliaris* in Central Arabia with genetic similarity ranging from 0.89 to 0.97. Gutierrez-Ozuna et al., (2009) found significant levels of genetic differentiation (22.2 %) between pasture and roadside populations of *Pennisetum ciliare*.

AMOVA results shown in Table 4.5 indicate a high variation within *C. ciliaris* populations of 95%. The results in this study may have been influenced by compositing of county-wide collections from different environmental conditions into one population. Although the partitioning within population was high, such partitioning has been observed in *C. ciliaris* and other grass species. Gutierrez-Ozuna et al., (2009) observed 77.4% with *C. ciliaris* while Wanjala, et al., (2013) found 91 % partitioning while working on

Pennisetum purpureum populations in East Africa. The high level of within population variability for *C. ciliaris* which is an apomictic grass species indicates a possibility of sexual reproduction. Outcrossing has higher levels of variation within populations which is contrary to selfing (Rossetto et al., 1995). Occasionally, sexual reproduction has been identified in *C. ciliaris* species (Fisher et al., 1954; Bray 1978; Sherwood et al., 1980). Studies made by Hignight et al., (1991) found that *C. ciliaris* can reproduce through facultative apomixes.

This was the first time genetic diversity analysis on ecotypes of *E. superba* was being done and comparisons of findings was be based on other grass species. Table 4.7 shows that the number of polymorphic bands ranged from six to 22 and percent polymorphic loci ranged from 12.8 to 46.8 %, which was low probably because of the low number of markers used as well as small sample sizes per population. Nei's genetic diversity index ranged from 0.053 to 0.194 with Kiboko population recording the highest and North Eastern the lowest. Kiboko population recorded the highest Shannon diversity index of 0.283 against a mean of 0.163. The high diversity indices in Kiboko population was probably because the two Kiboko ecotypes were collected from distinct environments. Ecotype KBK1 was collected from Chyulu hills with controlled grazing while KBK2 was collected from a homestead at Kiboko research station with frequent mowing. Morphologically the Kiboko ecotypes looked distinct in terms of spikelet size with KBK1 having uniquely small sized spikelets compared to the rest of the ecotypes. The recorded mean Shannon diversity index of 0.357 is similar to 0.352 recorded by Huang et al., (2012) while working with 12 populations of *Hemarthria compressa* grass species with twenty pairs of SRAP markers. The range observed in percent polymorphic loci is similar to that of *C. ciliaris* in this study as well as that of Gutierrez-Ozuna et al., (2009).

AMOVA analysis results shown in Table 4.8 shows significant variation ($p<0.007$) between populations of *E. superba*. The variation within populations was 76 % while among population was 24 % with a genetic differentiation of 0.237. The significant and relatively high genetic variation between populations could be due to the geographical distance between them such as the Southern Kenya populations (Kiboko and Taita Taveta) against North Eastern Kenya or the Kenyan against the Namibian populations. This observation was supported by Nei's unbiased measures of genetic distance shown in Table 4.9 where the largest distance was between Kiboko and North Eastern population at 0.338. Principal coordinate analysis shown in Figure 4.3 places the NE population as isolated from the other Kenya populations, that is, Kiboko, Taita Taveta and Kilifi. The relation between genetic distance and geographical location was observed by Ondabu et al., (2017) and Wanjala et al., (2013). Ondabu et al., (2017) attributed the high genetic distance between Alupe and Kitui populations of *Brachiaria* species to geographical distance while Wanjala et al., (2013) associated the low genetic distance of 0.0001 to 0.0897 to close proximity of collection sites of their 21 Napier grass populations. The variation between *E. superba* populations (24 %) was higher than recorded on *C. ciliaris* (5 %) in this study with SRAP markers and similar to Gutierrez-Ozuna et al., (2009) who had 24 % with *P. cilliarie* with ISSR markers. Contrary to this study, Huang et al., (2012) observed a higher genetic variation between populations (53.4 %) than between individuals (46.7 %) in a population of *Hemarthria compressa.*

Figure 4.4 shows that clustering of the *E. superba* ecotypes was not mainly based on their geographical origin except for Kilifi and Taita Taveta ecotypes that clustered in one group, cluster B. Ecotype 12777 that had been combined with Kilifi collections as one population due to its proximity in geographical origin, was distant to other Kilifi ecotypes in cluster analysis and PCoA. The 12777 ecotype had been collected from the same locality with KLF3 in Kilifi. This results indicates that despite being of close proximity in geographical location of collection, the ecotypes can be genetically distantly related. The

distant relatedness could probably be due to the long period of establishment of 12777 in an ex situ genebank at ILRI Ethiopia. Wanjala et al., (2013) observed clustering not based on geographical origins with nappier cultivars. The Namibian ecotypes, particularly 16595, were not clustered during cluster analysis. The close relatedness of the ILRI genebank ecotypes collected from different regions in Kenya, as shown by clustering in A, could indicate exchange of genetic material at ILRI field genebank probably due to the cross pollinated nature of *Eragrostis superba* species (Busey, 1976).

4.6 Conclusion

Significant genetic differences were recorded among the *C. ciliaris* ecotypes. The SRAP markers revealed that the total variation was mainly influenced by the within population variance at 95 %. Kajiado population was the most diverse as revealed by high values of heterozygosity and percent polymorphic loci. Relatively low genetic differentiation (5.3 %) was observed among the *C. ciliaris* populations with the SRAP markers. Kilifi population of *C. ciliaris* was distantly related to the rest of the populations.

Preliminary results indicate that SRAP markers can be used to analyse genetic diversity among *E. superba* ecotypes. Significant genetic variation was recorded among and between the *E. superba* populations. The Kiboko collection was the most diverse among the populations with the highest genetic diversity indices such as 46 % polymorphic loci. Ecotypes KBK1 and KBK2 were distantly related though they were from the same locality. The high genetic differentiation and a low exchange of genetic materials between the *E. superba* populations could have been as a result of the geographical distance between sites of ecotype collection. Effective maintenance of genetic diversity in *E. superba* requires conservation of the different populations. Long term establishment of *E. superba* ecotypes in common gardens may result in exchange of genetic materials due the out-crossing nature of the species.

CHAPTER FIVE

EVALUATION FOR STABILITY IN DRY MATTER AND SEED YIELD AND RELATED TRAITS OF *CENCHRUS CILIARIS* ECOTYPES

5.1 Abstract

Cenchrus ciliaris is widely promoted as a choice grass species for reseeding Arid and Semi-arid Lands (ASALs). Due to the high variability in ASAL environments, there is need to evaluate ecotypes of *C. ciliaris* with potentially higher yields in dry matter (DM) or seed that may possibly be selected and promoted for rangeland reseeding initiatives. Nine ecotypes of *C. ciliaris* collected from selected sites in Kenyan ASALs were evaluated for seed and DM yield and related traits in three KALRO centres (Kiboko, Buchuma and Mtwapa). Stability analysis of DM yield was done using AMMI stability value (ASV) and yield stability index (YSI) that combines ASV and mean DM yield across sites. Using two seasons' data per site, ASV ranked KBK3 and MGD3 as first and second most stable ecotypes respectively and KLF1 the least stable while YSI ranked KBK3 as the highest DM yielder followed by TVT3. Ecotype KBK1, the highest mean DM yielder across sites with 10, 864.3 $kgha^{-1}$ was ranked among the lowest with ASV and third with YSI implying that highest yielders are not necessarily the most stable ecotypes. Buchuma site (208.4 kg/ha) had higher ($p \leq 0.05$) mean seed yield than Kiboko and Mtwapa with 87.3 and 104.2 kg/ha, respectively. Seed yield varied among ecotypes at Mtwapa and not at Kiboko and Buchuma. Seed yield was significantly and positively correlated with caryopsis per spikelet (r = 0.78) and, significantly and negatively correlated with percent empty spikelets (r = -0.75). Dry matter yield was significantly positively correlated with plant height at seed maturity (r = 0.84) and empty spikelets (r = 0.8) and, negatively correlated to caryopsis per spikelet (r = -0.74). KLF1 ecotype, a high seeder with lowest DM yield, had the highest mean germination capacity at 71 % and MGD3 had the least with 22.5%. Ecotype MGD3 collected from a seasonally flooded preserved grazing area in agro-

ecological zone six recorded the poorest germination characteristics across all sites. Adaptation to the prevailing environmental conditions and management and utilization of grazing lands at sites of ecotype collection may have influenced the observed DM yield and seed characteristics and shorter ecotypes may be lower DM yielders. Ecotype KBK1 was the best suited ecotype for Kiboko and Mtwapa and MGD1 for Buchuma. Ecotype KBK3 was the most stable in all the environments.

Key words: *Cenchrus ciliaris,* dry matter yield, grass ecotype, reseeding, seed yield

5.2 Introduction

Cenchrus ciliaris, is a perennial grass among the species preferred by farmers for grass reseeding in the Southern rangelands of Kenya and beyond. Due to the challenges of seed availability, approaches such as the community based forage seed system (CBFSS) were established to aid in seed bulking and increase access of quality seeds through farmer trainings (Kimitei et al., 2010). Seed bulking of *C. ciliaris* in the rangelands is opportunistic mainly targeting wild establishments. Due to high spatial variability of rangelands particularly in moisture availability, there is a likelihood of existence of ecotypes among the indigenous grass species due to adaptations to local environmental conditions (Jorge et al., 2008). This could result in variation in seed yield and quality among the ecotypes that could compromise the success of programmes such as the CBFSS.

Seed production in terms of quantity and quality is of major interest in successful establishment of *C. ciliaris* grass species in reseeding programmes. Seed yield is affected by both genetics, environment and their interactions. Soil moisture, management aspects such as row spacing and fertilizer levels are among the factors that can influence seed yield in grasses. Genotype x environment interactions were recorded by Waldron et al., (2006) in Western Wheatgrass. Kumar et al., (2008) found that seed yield of Marvel

grass was depressed by heavy rains while supplementation with irrigation enhanced seed yield among range grass species (Koech et al., 2014). Narrow spacing depressed seed yield in *C. ciliaris* with 40 cm (75.9 kg/ha) and 60 cm (83.7 kg/ha) row spacing yielding less than 75 cm that yielded 97 kg/ha (Kumar et al., 2005).

It is necessary to establish the potential relationship between seed yield and related traits such as seed number per spikelet, seed weight and seed germination that may influence seedling establishment and hence rangeland rehabilitation. The traits could be used in indirect selection for seed yield. Seed weight have been found to affect seed yield and seed germination in Buffelgrass (Rajora et al., 2011). Grass seed germination is also affected by prevailing environmental conditions such as rainfall and temperature that may lead to development of adaptive traits such as seed dormancy. High seed dormancy has been blamed for poor stand establishment in *C. ciliaris* during the first year in CBFSS initiative although with more seedling recruitment during the subsequent year. Seed dormancy due to seed coverings allows for accumulation of a persistent soil seed bank. Variation in germination capacity and seed dormancy among ecotypes of *C. ciliaris* have been recorded by Venter and Rethman, (1992) who observed enhanced germination in shelled seeds of *C. ciliaris* ecotypes.

Dry matter yield is an essential trait in grass forage performance evaluation. Dry matter yield is a complex trait that is highly variable between seasons and varieties or species. Dry matter yield changes with plant growth where the trait increases with delayed harvesting time (Boonman, 1993). *Cenchrus ciliaris* has been found to yield higher dry matter than *Eragrostis superba* in previous studies by Mganga et al., (2010b) and Ndathi et al., (2012). Evaluation for dry matter yield on ecotypes and cultivars of *C. ciliaris* have previously been done and dry matter yield varied between accessions and environments (Hacker et al., 1995; Hacker and Waite, 2001; Al-Dakheel and Hussain, 2016). The objective of the study was to

evaluate the performance of *C. ciliaris* ecotypes for dry matter and seed yield and their related traits in different environments.

5.3 Materials and methods

5.3.1 Study Area

The study involved nine of the twelve ecotypes of *Cenchrus ciliaris* indicated in Table 3.1 in chapter three of this study. Ecotypes KBK2, MGD2 and TVT2 were omitted in the study for not being able to germinate in at least two of the study sites.

The study was conducted in three sites, Kiboko, Buchuma and Mtwapa research stations of the Kenya Agricultural and Livestock Research Organization (KALRO). Table 5.1 gives some of the descriptions for the three study sites while Figure 5.1 shows the monthly and total annual rainfall for 2013 and 2014. Mtwapa and Buchuma stations are in the coastal Kenya and located in agro-ecological zones III and V, respectively. Rainfall is bimodal in distribution at Mtwapa and Buchuma with the short rains occurring in October-December. The long rains occur in March-August in Mtwapa and in March-June in Buchuma.

***Table 5.1**: Description of the study sites, Kiboko, Mtwapa and Buchuma*

Site	Longitude	Latitude	Elevation (m a.s.l)	Temperature (°C)		Long term Annual rainfall (mm)	Soil type
				Min.	Max.		
Kiboko	37º 83'E	02º 28'S	975	14	35	575	Rhodic Ferralsols
Mtwapa	39° 44'E	03° 50'S	15	20	31	1200	Acrisol/Luvisol
Buchuma	38° 51'E	03° 42'S	400	18	37	560	Cambisols/Luvisol

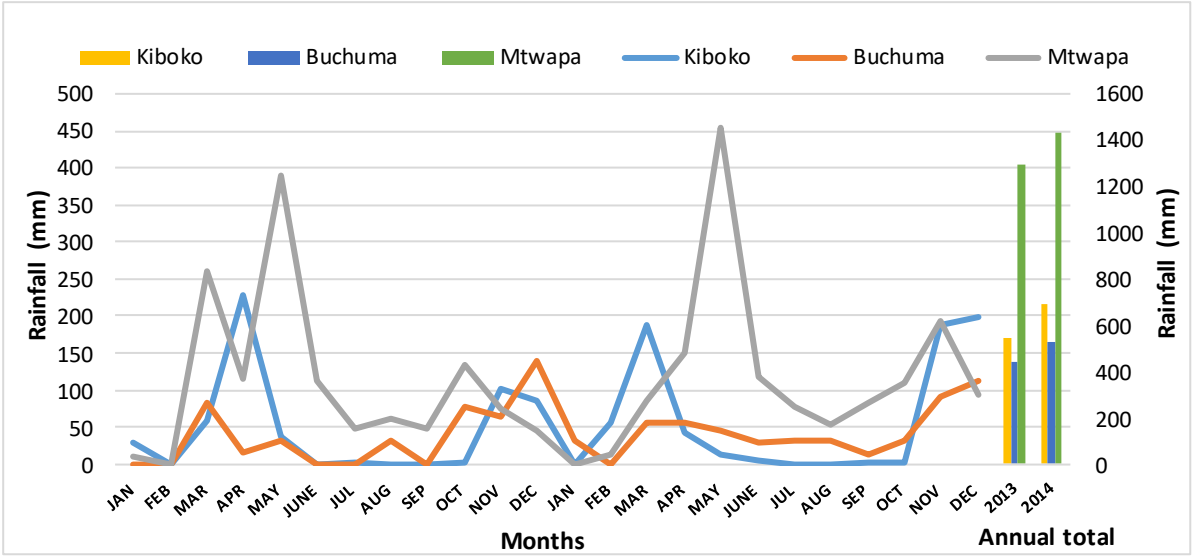

Figure 5.1: *Monthly and total annual rainfall (mm) for Kiboko Buchuma and Mtwapa study sites for 2013 and 2014*

5.3.2 Experimental Layout and Design

The experimental design was randomized complete block design with three replicates. Seed bed preparation was done by ploughing and levelling the ground to a fine tilth using a rake. The plots consisted of five rows of four metres long with a distance of one metre between plots. The planting of Kiboko plots is as described in Section 3.3.3. Planting for Buchuma and Mtwapa sites was done during the long rains in May 2013. Seedlings established in plastic trays at Kiboko were transplanted to the plots. Tuft splits were used in cases where there was shortage of seeds. Fertilizer and weed management was done as is described in Section 3.3.3

Standardization by cutting of herbage to 5 cm stubble in each plot per site was done at the beginning of short rains in early November 2013.

5.3.3 Data collection

Data on herbage yield was collected for two seasons per site on ratoon crop of the ecotypes as previously used in sugarcane genotypes by Sengwayo et al., (2017). These were, the short rains of 2013 as season one and the long rains for 2014 as season two for all sites. The rest of the data set was collected in one

season only during the short rains due to lack of funds to visit the sites. These were plant height, seed yield, germination capacity, germination rate, length of germination time and mean germination time.

For plant height data, four randomly selected plants per replicate were used. Plant height was measured in centimeters from the ground to the tip of the tallest inflorescence at peak of flowering and seed maturity. Herbage yield data was recorded after all seeds were harvested per plot per site where all above ground herbage was cut to 5 cm stubble and weighed at the field as wet herbage yield in kilograms. A sub sample of about 200 g was picked, weighed and oven dried at 65^{o}C to a constant weight. The oven dried weights were used to calculate the dry matter yield from the recorded total wet herbage yield per plot. The plot dry matter yield was extrapolated to per hectare yield as shown below in equation 4.1:

$$1 \text{ ha DM yield (kg)} = \frac{\text{plot DM yield (kg)}}{16 \text{ m}^2} \times 10{,}000 \text{ m}^2$$

Seed harvesting was done by stripping all the seeds on a seed head. All seed heads were harvested per plot at the end of the short rains season in January 2014 from three study sites and taken to KALRO Kiboko station for processing. Due to the variation and subsequent spread in seed maturity between tillers, seed harvesting was done thrice for three consecutive weeks. After cleaning by removing all plant material and remaining with only spikelets, each seed sample was weighed using an electric balance (Scout Pro SPU601, Ohaus Corporation, USA). The initial seeds harvested from the three replicates of each ecotype were mixed and sub-sampled for germination analysis. Four replicates of 25 caryopsis each were placed on moistened filter papers in plastic petri dishes and germinated at room temperature. Germination, defined as the appearance of a root, was counted daily from day 1 to 14. Counting of caryopsis number per spikelet was done by picking four samples of 25 spikelets each and scarifying each spikelet then counting the number of caryopsis contained.

5.3.4 Data analysis

Data for plant height, seed and dry matter yield, germination capacity, germination time, coefficient of velocity and length of germination were analysed for variance as unbalanced data in Genstat 15th edition. The (analysis of variance) ANOVA model was as shown in equation 4.2:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta_{ij}) + \varepsilon_{ijk}$$

Where Y_{ijk} is mean for the variable of i^{th} ecotype in j^{th} environment with k^{th} interaction effect, μ is the overall mean, α_i is i_{th} ecotype effect, β_j is j_{th} environmental effect, $\alpha\beta_{ij}$ is the site and genotype interaction effect and ε_{ijk} is the error.

The DM yield per ha was analyzed for genotype by environment interaction using the AMMI model in Genstat 15th edition. The AMMI model was as in equation 4.3 below:

$$Y_{ij} = \mu + g_i + e_j + \sum_{n=1}^{N} \lambda_k \gamma_{ik} \delta_{jk} + \rho_{ij} + \varepsilon_{ij}$$

Y_{ij} is DM yield of i^{th} ecotype in j^{th} environment, μ is the overall mean, g_i is i_{th} ecotype effect, e_j is j_{th} environmental effect, Λ_k is the eigen value of the principal component analysis (PCA) axis k, γ_{jk} and δ_{ik} are j_{th} environment and i_{th} ecotype PCA scores for PCA axis k, ρ_{ij} is the residual G X E interaction, ε_{ij} is the error and n the number of Principal components retained in the model.

AMMI Stability Value (ASV) was used to quantify and rank the ecotypes. The ASV is the distance from the origin in a two dimensional scatter diagram of interaction principal component analysis axis 1 scores (IPCA1 scores) against IPCA2 scores (Purchase et al., 2000). IPCA1 is weighted against the relative

contribution of PCA1 to PCA2 to the interaction sum of squares due to its major contribution to G x E sum of squares.

The following formula number 4.4 was used to calculate AMMI stability value (ASV):

$$\sqrt{\left[\frac{IPCA1_{ss}}{IPCA2_{ss}}(IPCA1_{score})\right]^2 + IPCA2_{score}}$$

Where $IPCA1_{ss}$, $IPCA2_{ss}$, $IPCA1_{score}$ and $IPCA2_{score}$ are the interaction principal component analysis sum of squares (ss) and scores for a specific ecotype at principal component analysis axis one and two.

Yield stability index (YSI) was calculated as the sum of rank in mean yield across environments and the ASV rank as in equation 4.5 below:

$YSI_i = RASV_i + RDMY_i$, where $RASV_i$ is the ranking of the AMMI stability value and $RDMY_i$ the ranking of mean dry matter yield in all environments. The YSI gives the ecotype with higher ranking in dry matter yield as well as in ASV.

Germination data was analysed for germination capacity (GC), length of germination (LG), germination time (MGT) and the germination rate. Germination capacity was expressed as the percentage of the total number of germinated seeds relative to the total number per replicate. Length of germination (LG) was the days from the start of germination to the end per replicate. Mean germination time (MGT), which is the average length of time required for maximum germination of the seeds was calculated as indicated by Ranal and Santana (2006) (equation 4.6):

$$\acute{t} = \sum_{i=1}^{k} ni\ ti\ / \sum_{i=1}^{k} ni$$

Where t_i is the number of days from the start of the experiment to the ith observation and n_i number of seeds germinated on day i.

Germination rate was analysed using Kotowski's coefficient of velocity (CoV) which is an estimate of the germination rate and measures the distribution of germination regarding the number of seeds germinated in time (Cervantes et al., 1996). The germination rate was derived as $CoV = 100(\sum n_i/\sum n_i t_i)$ where n_i was the number of seeds germinated on the i^{th} day and t_i the number of days by the i^{th} day when counted from day zero.

The analysis of variance (ANOVA) for the different seed traits was performed and means separated using Least Significant Difference (LSD) at $p \leq 0.05$ in Genstat 15th edition. Genstat 15th edition was used in correlation analysis on the recorded data set.

5.4 Results

Table 5.2 shows the mean height measurements for *C. ciliaris* ecotypes at Kiboko, Mtwapa and Buchuma study sites at two growth stages. At full bloom stage, the mean height at Buchuma (103 cm) and Mtwapa (104.1 cm) was higher than that at Kiboko of 88.8 cm. At seed maturity stage, the mean height at Kiboko and Mtwapa was higher than that at Buchuma.

Table 5.2: *Mean plant heights for Kiboko, Buchuma and Mtwapa at two growth stages for ecotypes of Cenchrus ciliaris ecotypes*

	Growth stage	
Site/ Season	**Full bloom (cm)**	**Seed maturity (cm)**
Kiboko	88.8	126.2
Buchuma	103.0	128.4
Mtwapa	104.1	110.0
P_value	0.025	0.034
$LSD^{0.05}$	5.19	6.28
CV (%)	17.70	17.16

Key: CV – coefficient of variation, LSD – least significant difference

Table 5.3 gives the mean height measurements between *C. ciliaris* ecotypes within three sites and at two growth stages. KBK1 with 110.7 cm and KBK3 with 116.5 cm had the highest mean height at full bloom stage while at seed maturity KBK1 with 140.2 cm and TVT3 141.1 cm were the tallest ecotypes. KLF1 and TVT1 ecotypes were the shortest in the two growth stages.

Table 5.3: *Mean heights for within sites and two growth stages for ecotypes of Cenchrus ciliaris ecotypes*

Growth stage	Full bloom stage (cm)				Seed maturity stage (cm)				
Ecotype	Kiboko	Buchuma	Mtwapa	Stage mean	Kiboko	Buchuma	Mtwapa	Stage mean	Overall mean
KBK1	106.2	-*	115.1	110.7	150.2	-	130.2	140.2	125.4
KBK3	110.6	122.9	116.1	116.5	141.3	130.8	120.2	130.8	123.6
KLF1	76.6	-	83.5	80.1	99.9	-	86.5	93.2	86.6
KLF2	81.1	93.6	102	92.2	107.6	116.4	104.6	109.5	100.9
KLF3	78.7	-	104	91.3	111.8		103.4	107.6	99.5
MGD1	92.9	109.2	-	101.1	140.3	135.7	-	138	119.5
MGD3	92.9	88.2	-	90.6	130.0	134.1	-	132.1	111.3
TVT1	80.8	80.3	86.9	82.7	113.5	103.9	92.4	103.3	93
TVT3	79.0	123.7	121.1	107.9	140.8	149.7	132.9	141.1	124.5
Site Mean	**88.8**	**103.0**	**104.1**		**126.2**	**128.4**	**110**		
P_value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
$LSD^{0.05}$	5.85	10.63	8.96	7.47	7.28	16.3	8.15	7.66	8.8
CV %	8.0	12.7	10.6	14.6	7.3	15.7	9.1	12.0	17.7

**missing values for unestablished plot; Key: CV – coefficient of variation, LSD – least significant difference*

Table 5.4 shows the mean dry matter yield per ecotype per site. Ecotype, site and site x ecotype effects were significant. Mtwapa site with 7379 kg/ha DM was higher than Kiboko and Buchuma with 5356 and 4207.3 kg/ha DM, respectively (p=0.006; CV=8.8 %). Ecotype KBK1 was the highest DM yielder at Kiboko (8222 kg/ha) and Mtwapa (14, 996.9 kg/ha) while MGD1 was the highest at Buchuma with 8649.7 kg/ha. Ecotype TVT1 was the lowest DM yielder at Kiboko (2704 kg/ha) and Buchuma (2322 kg/ha) while KLF1 was the lowest at Mtwapa with 2494.6 kg/ha.

Table 5.4: *Mean dry matter yield (kg/ha) for Cenchrus ciliaris ecotypes at Kiboko, Mtwapa and Buchuma sites*

	Dry matter yield (kg/ha) per site			
Ecotype	**Kiboko**	**Mtwapa**	**Buchuma**	**Overall mean**
KBK1	8222.0	14996.9	-*	10864.3
TVT3	7888.6	9908.3	6137.6	7943.3
KBK3	6792.0	7481.7	5420.0	6441.7
MGD1	6576.6	-*	8649.7	7656.0
KLF3	6067.4	11668.1	-	8165.8
KLF1	5714.8	2494.6	-	3890.5
KLF2	4841.7	6011.7	2722.7	4295.4
MGD3	2792.5	-	3040.9	2910.7
TVT1	2704.0	6081.4	2322.7	3365.1
Grand mean	**5358.0**	**7379.0**	**4207.3**	**5553.9**
P_value	0.013	<.001	0.047	<.001
SED	1857.3	1836.0	1890.4	2136
CV (%)	7.0	5.7	9.5	7.9

**missing values for unestablished plots; Key: CV – coefficient of variation, LSD – least significant difference*

Figure 5.2 shows GGE biplot for mega environments and the best genotype for each environment. There were two mega environments where site one (Kiboko) and three (Mtwapa) were grouped as environment one and site two (Buchuma) as second environment. Ecotype KBK1 was at the apex of mega environment one while MGD1 was for environment two. The ecotype at the apex of an environment is the most suitable for the particular area.

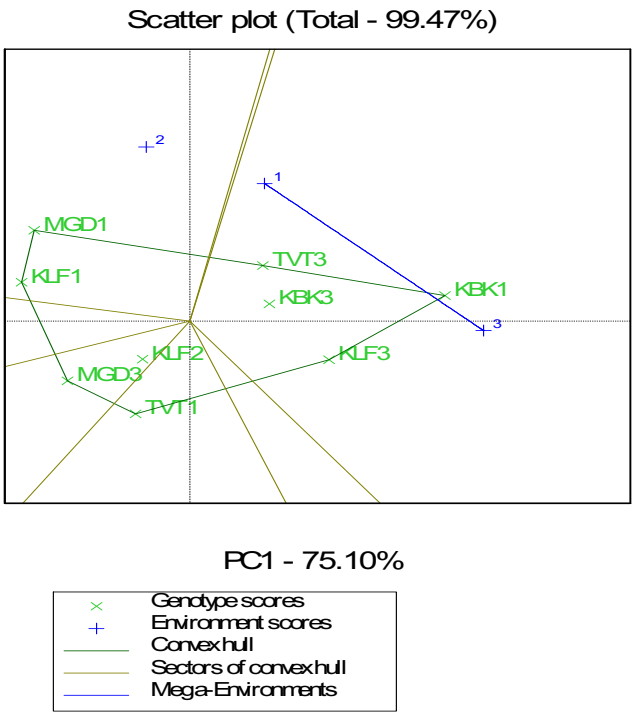

Figure 5.2: *GGE bi-plot showing two mega environments for Cenchrus ciliaris ecotypes established at Kiboko, Buchuma and Mtwapa. Site 1=Kiboko, 2=Buchuma and 3=Mtwapa*

Table 5.5 shows the results for AMMI analysis of variance for dry matter yield among ecotypes of *C. ciliaris*. Genotype, environment and genotype by environment effects were significant.

Table 5.5: *AMMI analysis of variance for dry matter yield (Kg/ha) of Cenchrus ciliaris ecotypes*

Source	DF	SS	MS	F	P_value
Treatments	26	1526364639	58706332	5.66	**
Genotypes	8	1048510995	131063874	12.63	**
Environments	2	251893307	125946654	6.12	**
Block	6	123478776	20579796	1.98	ns
Interactions	11	225960337	20541849	1.98	*
IPCA1	9	204036959	22670773	2.19	*
IPCA2	7	21923377	3131911	0.3	ns
Residuals	-5	0	0	0	
Error	104	1078896252	10374002		
Total	161	2728739667	16948694		

**, ** and ns represents significance at 1%, 5% and not significant, respectively*

Table 5.6 shows mean DM yield across sites, AMMI stability value (ASV) and yield stability index (YSI) and rankings for nine *C. ciliaris* ecotypes. Ecotype KBK1 had the highest mean DM yield of 10864.3

kg/ha across the sites. KBK3 was ranked the first using ASV and KLF1 the last. As for YSI, KBK3 was ranked first and KLF1 was the last.

Table 5.6: *Dry matter yield (Kg/ha), IPCA scores and stability parameters generated using AMMI model for ecotypes of Cenchrus ciliaris*

Genotype	Ecotype code	Mean DM yield (kg/ha)	Rank	IPCAg[1]	IPCAg[2]	ASV	ASV Rank	YSI	YSI Rank
KBK1	1	10864.3	1	34.96	2.87	325.33	8	9	3
KBK3	2	6441.7	5	-1.87	29.06	18.13	1	6	1
KLF1	3	3890.5	7	-56.56	-5.62	526.33	9	16	9
KLF2	4	4295.4	6	-18.89	4.34	175.80	6	12	8
KLF3	5	8165.8	2	28.50	2.36	265.28	7	9	3
MGD1	6	7656.0	4	11.05	-29.94	102.68	5	9	3
MGD3	7	2910.7	9	3.21	-9.26	29.69	2	11	6
TVT1	8	3365.1	8	7.07	3.98	65.86	3	11	6
TVT3	9	7943.3	3	-7.47	2.21	69.69	4	7	2

Key: DM yield – dry matter yield; IPCAg[1] and IPCAg[2] – interaction principal components axes 1 and 2 scores, respectively; ASV – Ammi stability value; YSI – yield stability value

Table 5.7 shows the mean seed yield (kg/ha) for ecotypes of *C. ciliaris* established at Kiboko, Mtwapa and Buchuma. Site effect was significant for seed yield while ecotype effect and ecotype x site interaction were not significant. Buchuma (208.4 kg/ha) had higher mean seed yield than Kiboko and Mtwapa with 87.3 and 104.2 kg/ha, respectively ($p<0.001$, CV=11.34%). Significant difference in seed yield was recorded at Mtwapa site only where ecotype KLF2 with 160.3 kg/ had significantly higher yield than the rest of the ecotypes except KLF1 with 144.2 kg/ha. There were no statistical differences between ecotypes at Kiboko and Buchuma sites ($p\geq0.05$).

Table 5.7: *Mean seed yield (kg/ha) for Cenchrus ciliaris ecotypes at Kiboko, Buchuma and Mtwapa*

Ecotype	Kiboko	Buchuma	Mtwapa (kg	All sites Mean
KLF 1	199.1	-*	144.2	169.4
KLF2	142.6	220	160.3	171.4
KBK3	113.2	139.9	79.4	107.9
MGD3	109.1	227.0	-	157.4
KBK1	88.1	-	88.5	88.3
KLF3	60.0	-	104.0	79.1
TVT3	65.9	381.1	85.9	129.6
TVT1	54.8	207.0	92.7	101.6
MGD1	44.4	149.3	-	81.7
Mean	**87.3**	**208.4**	**104.2**	**120.6**
P_value	Ns	ns	0.010	Ns
CV (%)	13.6	8.8	4.8	11.9

**missing values for unestablished plots; Key: ns – not significant at p≥0.05; CV – coefficient of variation, LSD – least significant difference*

Figure 5.3 and Table 5.8 shows the mean caryopsis number per spikelet per ecotype for Kiboko, Buchuma and Mtwapa. All ecotypes had overall mean of less than one caryopsis per spikelet except MGD3 (1.39), KLF1 (1.14) and KLF2 (1.08). Ecotype KBK1 (0.33) had the lowest mean number of caryopsis per spikelet and the highest percent empty spikelets (70 %). There were differences in caryopsis number within and between sites. Mtwapa site was the highest in mean caryopsis number with 0.99 while Kiboko was the least with 0.66 ($LSD^{0.05}$ = 0.0869).

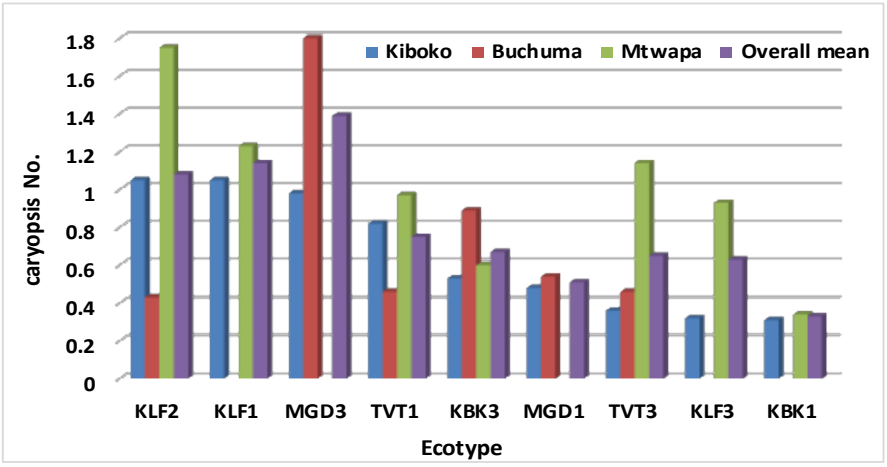

Figure 5.3: *Mean caryopsis number per spikelet for ecotypes of Cenchrus ciliaris at Kiboko, Buchuma and Mtwapa*

Table 5.8: *Mean caryopsis number per spikelet per site and percent empty spikelets among Cenchrus ciliaris ecotypes*

	Mean caryopsis number per spikelet				
Ecotype	**Kiboko**	**Buchuma**	**Mtwapa**	**Total Mean**	**Empty spikelets (%)**
KLF2	1.05	0.43	1.75	1.08	33.7
KLF1	1.05	-*	1.23	1.14	20.0
MGD3	0.98	1.80	-	1.39	24.5
TVT1	0.82	0.46	0.97	0.75	40.7
KBK3	0.53	0.89	0.6	0.67	43.0
MGD1	0.48	0.54	-	0.51	59.5
TVT3	0.36	0.46	1.14	0.65	49.7
KLF3	0.32	-	0.93	0.63	51.0
KBK1	0.31	-	0.34	0.33	69.5
Site Means	**0.66**	**0.76**	**0.99**	**0.79**	**43.5**
P_value	<.001	<.001	<.001	<.001	<.001
$LSD^{0.05}$	0.190	0.277	0.178	0.181	8.79
CV (%)	18.9	24.1	12	18.9	13.9

**missing values for unestablished plots; Key: CV – coefficient of variation, LSD – least significant difference*

Figure 5.4 shows the mean percent occurrence of caryopsis number per spikelet. The number of caryopsis in a spikelet ranged from 1-2 (KBK1, KBK3 and MGD1), 1-3 (KLF2, KLF3, TVT1 and TVT3) and 1-4 KLF1 and 1-7 for MGD3. Over 50 % of sampled spikelets were empty for four ecotypes, namely, KBK1 (70 %), MGD1 (60 %) and KLF3 (51%) and TVT3 (50 %).

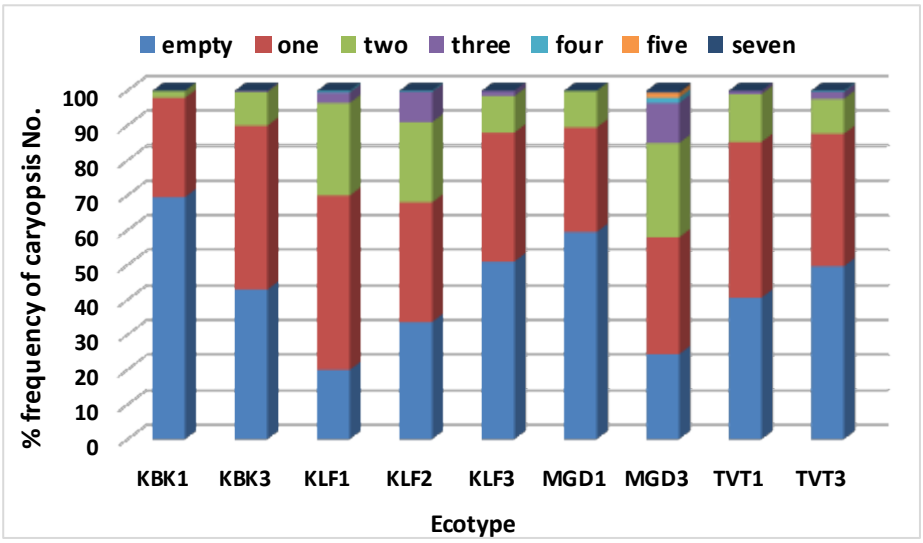

Figure 5.4: *Mean percent frequency of number of caryopsis occurring per spikelet in Cenchrus ciliaris ecotypes*

Table 5.9 shows ANOVA table for germination capacity. Ecotype effect was significant (p<.001) for percent germination capacity while site effect was not significant at p≤0.05. The interaction of ecotype x environment was significant at p≤0.0.5.

Table 5.9*: Anova table for ecotype germination capacity for seeds of Cenchrus ciliaris ecotypes harvested from Kiboko, Buchuma and Mtwapa*

Change	d.f.	s.s.	m.s.	v.r.	P_value
+ Ecotype	10	10881.3	1088.1	8.66	<.001
+ Site	2	198.0	99.0	0.79	ns
+ Ecotype. Site	13	3874.7	298.1	2.37	0.010
Residual	78	9804.0	125.7		
Total	103	24758.0	240.4		

Key: ns – Not significant at p≤0.05

Figure 5.5 shows the mean percent germination capacity for *C. ciliaris* ecotypes from Kiboko, Buchuma and Mtwapa study sites. KLF1 was the highest in germination capacity at Kiboko (67 %) and Mtwapa (75 %), the only sites that the ecotype successfully established. MGD1 recorded the highest germination capacity at Buchuma at 69 %. The overall mean germination capacity ranged from 22.5 % for MGD3 to 71.0 % for KLF1 ((p <0.001; 21.8% CV and average $LSD^{0.05}$ of 10.2 %).

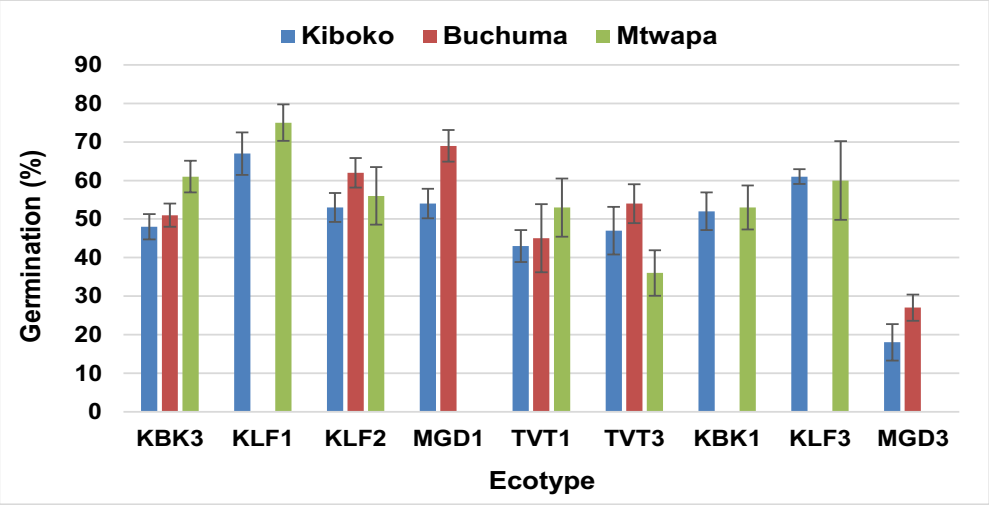

Figure 5.5*: Mean percent germination capacity ± SE (P<0.001) of seeds of Cenchrus ciliaris ecotypes harvested from Kiboko, Buchuma and Mtwapa study sites*

Figure 5.6 shows the mean daily germination for seeds of *C. ciliaris* ecotypes harvested from Kiboko, Buchuma and Mtwapa in January-February, 2014. Ecotype MGD3 was the only ecotype with no germination occurring within the first 24 hours of the trial. The peak mean daily germination of the ecotype occurred in day three and was the lowest peak at 6 %.

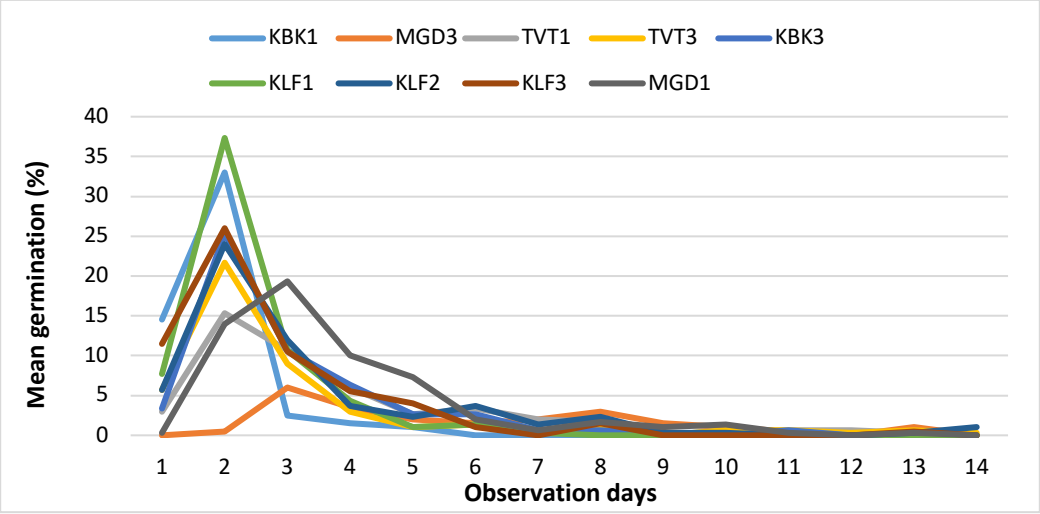

Figure 5.6: *Mean daily germination (%) trend for seeds of Cenchrus ciliaris ecotypes harvested from Kiboko, Buchuma and Mtwapa in Jan-Feb 2014*

Table 5.10 shows the mean germination rate, germination time and length of germination for *C. ciliaris* ecotypes at Kiboko, Buchuma and Mtwapa. The columns shows the mean germination rate, germination time and length of germination per site. For site x ecotype interaction, the data was compared along rows showing performance of each ecotype across the three sites.

Table 5.10: *Mean Coefficient of velocity (CoV), mean germination time (MGT) and length of germination (LG) of seeds of Cenchrus ciliaris ecotypes harvested from Kiboko, Buchuma and Mtwapa*

Site/	KIBOKO			BUCHUMA			MTWAPA		
Ecotype	**COV**	**MGT**	**LG**	**COV**	**MGT**	**LG**	**COV**	**MGT**	**LG**
KBK 1	68.4	1.5	2.5	-*	-	-	44.7	2.3	3.3
KBK 2	43.4	2.3	5.5	-	-	-	-	-	-
KBK 3	40.7	2.5	5.6	27.5	3.7	9.0	35.9	2.9	5.3
KLF 1	56.3	1.8	3.8	-	-	-	40.1	2.5	4.5
KLF 2	27.6	3.7	10.8	37.1	2.7	6.0	26.8	3.9	11.5
KLF 3	52.7	2.0	5.3	-	-	-	29.3	3.5	8.7
MGD 1	30.1	3.5	8.0	24.7	4.1	8.5	-	-	-
MGD 3	21.5	5.0	7.3	15.1	6.8	11.3	-	-	-
TVT 1	27.7	3.7	11.3	26.3	4.0	7.5	25.6	4.0	8.5
TVT 2	27.2	4.5	10.0	-	-	-	-	-	-
TVT 3	39.0	2.6	4.8	43.7	2.4	6.0	20.4	4.9	11.3
Mean	**39.5**	**3.0**	**6.8**	**30.1**	**3.8**	**7.6**	**31.2**	**3.5**	**7.7**
P_value	<.001	<.001	<.001	<.001	<.001	0.016	<.001	<.001	<.001
$LSD^{0.05}$	10.3	1.47	3.37	8.2	1.09	3.43	7.2	0.78	3.44
CV%	18.1	34	34.5	18.5	19.6	30.7	15.8	15.5	30.6

**missing values for unestablished plots at the site*

KBK1 recorded the highest germination rate (CoV) at Kiboko and Mtwapa with 68.4 and 44.7 %, respectively. In both sites, the ecotype had the least mean germination time of 1.5 and 2.3 days for Kiboko and Mtwapa, respectively.

Ecotype x environment interaction was significant for germination rate ($p < .001$; 17.8% CV and average $LSD^{0.05}$ of 8.9). KBK3 recorded higher germination rates at Kiboko (40.7 %) than Buchuma (27.5 %). KLF2 had higher germination rates at Buchuma (37.1 %) than Kiboko (27.6 %) and Mtwapa (26.8 %). Mean germination rate for TVT1 was not statistically different in all the sites.

Ecotype x environment interaction was significant for mean germination time ($p = 0.004$; 24.5% CV and average $LSD^{0.05}$ of 1.2). Mean germination time for TVT3 was longer at Mtwapa (4.9 days) than Kiboko

(2.6 days) and Buchuma (2.4 days). Mean germination time for TVT1 was not different across the three sites, that is, 3.7, 4.0 and 4.0 days for Kiboko, Buchuma and Mtwapa, respectively.

Table 5.11 shows the correlation coefficients and the significance levels for the mean seed yield (Kg/ha) and means of other seven traits recorded from *C. ciliaris* ecotypes established at Kiboko, Buchuma and Mtwapa. Seed yield per hectare was positively and significantly correlated with number of caryopsis per spikelet (r = 0.8) and negatively with percent empty spikelets (r = -0.8). Mean number of caryopsis per spikelet negatively and significantly correlated with dry matter yield (kg/ha; r = -0.7 and with number of empty spikelets (r = -0.9). Mean germination capacity of the seed lot under the study (GC2014) positively correlated (r = 0.8) with germination capacity of the seeds collected from the wild at the time of germplasm collection.

Table 5.11: *Correlation matrix between seed yield and related traits of Cenchrus ciliaris grass ecotypes*

	DMY	GC2012	GC2014	HGT2	SY	C-SPK	E-SPK
DMY	-						
GC2012	-0.18	-					
GC2014	0.05	0.82**	-				
HGT2	0.84**	-0.50	-0.44	-			
SY	-0.40	0.25	-0.30	-0.13	-		
C-SPK	-0.74*	0.02	-0.35	-0.43	0.78**	-	
E-SPK	0.84**	-0.22	0.15	0.60	-0.75*	-0.94***	-

***= $p \leq 0.001$; **= $p \leq 0.01$ and *= $p \leq 0.05$; DMY=Dry matter yield; GC2012= germination capacity for 2012 seeds; GC2014= germination capacity for 2014 seeds; HGT= Plant height at seed maturity; SY = seed yield; C_SPK = caryopsis number per spikelet E_SPK = % empty spikelets;

5.5 Discussion

Plant height data shows that KBK1, KBK3 and TVT3 were among the tallest at peak flowering and seed maturity growth stages while TVT1 and KLF1 were the shortest. This results were in line with characterization data in this study where KBK1, KBK3 and TVT3 were grouped as robust types and

KLF1 and TVT1 were grouped as small sized types of *C. ciliaris*. Existence of small and robust types of *C. ciliaris* have been reported in other studies (Jorge et al., 2008). Released cultivars such as *Biloela* are known to be robust and American believed to have been collected from Turkana desert in Kenya is a small type. The Mtwapa and Buchuma sites had taller plants with a mean of 104.1 and 103 cm, respectively, compared to Kiboko with 88.8 cm. The trend changed by the time of seed maturity where Kiboko had taller plants with 126.2 cm mean height than Mtwapa plants with 110 cm. The change in trend was associated with delay in taking measurements at both Buchuma and Mtwapa during the full bloom stage due to financial constraints and as a result the full bloom data was not included during correlation analysis and only height at seed maturity was used. The shorter height at Mtwapa by seed yield stage could be associated with the lower rainfall amounts received during the study. Buchuma received 140.1 mm compared to the Kiboko (84.5 mm) and Mtwapa (45.5 mm).

AMMI stability value (ASV) and Yield stability index (YSI) ranking in Table 5.6 shows that ecotypes with lower ASV were the most stable across environments. Ecotype KBK3, MGD3, and TVT1, with ASV of 18.3, 29.7 and 65.9, respectively, were ranked as the first, second and third most stable ecotypes in dry matter yield across the study sites. Ecotype KLF1 with 526.3 ASV was the most sensitive to environmental change. Using the YSI, KBK3 and TVT3 were ranked as the first and second best performers in dry matter yield and KLF1 was the last. These results indicates that the highest yielder in mean dry matter yield, such as KBK1, may not necessary be the most stable across environments. Ecotype KBK1 was ranked third when stability and DM yield were combined in YSI. Although KBK3 was ranked the highest in both ASV and YSI, the ecotype was affected by a disease of brown spotting of the leaves followed by yellowing of entire foliage in all the three sites, particularly during the rainy seasons (Plate 5.1). Similar observation was made with KLF3 though at a lower incidence than KBK3 ecotype where all the plants were affected in all three replicates.

Plate 5.1: *Diseased leaves of KBK3 ecotype of C. ciliaris at KALRO Kiboko, Jan, 2014*

Ecotype KLF1 was among lowest yielders in mean dry matter yield as well as the most unstable ecotype based on ASV. The low dry matter yield of KLF1 could be because of its prostrate growth form and small stature where the ecotype is the shortest among all the ecotypes. Jorge et al., (2008) recommended small sized ecotypes of *C. ciliaris* for habitat rehabilitations and robust tall types for hay production.

Figure 5.2 shows GGE biplot for mega environments where Kiboko and Mtwapa were grouped as one mega environment. These results imply that dry matter yield for Kiboko and Mtwapa were similar or correlated. Ecotype KBK1 was the best yielder in Kiboko-Mtwapa environment while MGD1 was the best for Buchuma, which was an isolated environment. Table 5.4 shows that KBK1 ecotype was the highest dry matter yielder at Kiboko (8,222 kg/ha) and Mtwapa (14,996.8 kg/ha) while MGD1 was the highest at Buchuma (8,649.7 kg/ha). The high dry matter yield for KBK1 could be associated with the ecotype's rhizomatous growth form that results in large tufts per plant. Ecotype KBK1 was also probably able to take advantage of the high rainfall amounts in Mtwapa especially the long rains in season two. The ecotype was collected along a riverine vegetation and two farmer groups out of three noted the

ecotype's preference to wetter environments during focused group discussions to assess farmer knowledge on *C. ciliaris* ecotypes (Chapter six). The two ecotypes, KBK1 and MGD1, were clustered as robust and late flowering types during morphological characterization. Late flowering grasses are associated with superior herbage yields (Boonman, 1993).

Previous studies on *C. ciliaris* found variation in dry matter yield among accessions, which is similar to this study (Hacker et al., 1995; Hacker and Waite, 2001; Al-Dakheel and Hussain, 2016). Al-Dakheel and Hussain, (2016) found significant variation in herbage yield for salt tolerance in *C. ciliaris*. Hacker et al., (1995) study was done under irrigation while Hacker and Waite (2001) was rainfed in multi-environments. Inconsistencies were observed between the two studies where high performers under the first study did not maintain high yield during rainfed evaluation. Molopo ranked highly under Hacker et al., (1995) and not in Hacker and Waite (2001) while the opposite occurred with Bella type. Hacker and Waite (2001) recommend the use of larger data in environments with highly variable rainfall between seasons and across years. Given the varied performance of the ecotypes in this study, long term evaluation may be necessary particularly for selection among the robust types.

This study was done using spaced plants which has been noted not to directly translate to performance in a sward for complex traits such as dry matter yield (Casler and Santen, 2010). Forage production in a sward is influenced by several factors including plant competition, mortalities and seedling recruitment, which are controlled in spaced plants. The recorded dry matter yield per ecotype in this study may vary when evaluated under sward conditions.

The seed yield results shown in Table 5.7 indicate that Buchuma (208.4 kg/ha) had higher yields than Kiboko with 87.3 and Mtwapa with 104.2 kg/ha. The high seed yield at Buchuma could be associated to variation in rainfall amounts between sites during the study period. Higher total rainfall amounts were

received in the month of December (140.1 mm) at Buchuma compared to the Kiboko (84.5 mm) and Mtwapa (45.5 mm). Flowering of the plots occurred in December when there was adequate amounts of rainfall that could have supported better seed setting at Buchuma than the other sites. Although heavier rains have been found to depress seed yield in Marvel grass (Kumar et al., 2008), the amounts at Buchuma may have just been adequate. Studies by Koech et al., (2014) indicated that addition of soil moisture increases seed yield in grasses. Koech et al., (2014) recorded depressed seed yield in *C. ciliaris* under rainfed conditions (21.6 kg/ha) compared to irrigation to 80, 50 and 30 % field capacity soil moisture content that yielded 150.5, 136.6 and 156.6 kg/ha, respectively. The lack of differences in seed yield across sites for KLF2 and KBK3 could imply stability to environmental effect. The two ecotypes successfully established in the three study sites.

Mean caryopsis number per spikelet is shown in Table 5.8. Caryopsis number per spikelet is an important seed trait. The trait determines how much pure seed is available per seed lot. The results indicated that KBK1 had the highest percent empty spikelets (70 %) and lowest number of caryopsis per spikelet (0.33) against total mean of 0.79. The ecotype ranked among the lowest in seed yield. These results could be because the ecotype is a high yielder in dry matter. The ecotype was found to be late flowering with the lowest percent fertile tillers (22 %) compared to others like KLF1 with 78 % during characterization. Delayed head emergence and lower density of flowering tillers was found to contribute to low seed yield in grasses (Boonman, 1993). Awad et al., (2013) observed a significant positive correlation (r=0.55) between grain yield and number of panicles per plant with Sudangrass (*Sorghum sudanense*). Similarly, spike density per unit area had positive and significant influence on seed yield in Buffelgrass for three different seasons (Rajora et al., 2011). Table 5.11 shows that seed yield was positively correlated with caryopsis number (r =0.8) and negatively to percent empty spikelets (r = -0.8). High percentage of empty spikelets for KBK1 may have translated to lower seed yield.

Seeds of MGD3 had the lowest germination capacity at Kiboko (18 %) and Buchuma (26 %) (Figure 5.5). Table 5.10 shows MGD3 ecotype with the longest mean germination time at Kiboko (5.0 days) and Buchuma (6.8 days). The ecotype also recorded the lowest germination (22.5 %) among the seeds harvested from the wild during germplasm collection (Figure 3.2; Chapter three). The low germination attributes of MGD3 could be due to the significantly low caryopsis weights of the ecotype. The ecotype recorded the lowest seed weight (0.4 g) against a total mean of 0.7 g when seeds harvested from Kiboko were analysed. Casler and Santen, (2010) observed that bigger or heavier seeds are known to have more rapid germination.

The low germination capacity in some of the ecotypes could also be attributed to dormancy characteristics that adapted them to the climatic conditions of their origin (O'Connor and Everson, 1998). The low germination capacity and other germination characteristics of MGD3 may be attributed to adaptation to the flooding conditions and controlled grazing at the site of collection, near Lake Magadi. Species found in frequently flooded areas require alternating temperatures to germinate which is associated with detection of end of floods (Cornaglia et al., 2009). Cornaglia et al., (2009) found that successful establishment of seedlings is achieved when flooding and grazing conditions are followed by high moisture seasons. The high dormancy in MGD3 seeds could have been selected for by the normally succeeding long dry seasons. Use of alternating temperatures should be tested in breaking dormancy in MGD3 seeds. Long term germination studies are necessary to identify potential peak in germination of MGD3 seed lots. The controlled grazing also allowed time for the plants to develop sufficient foliage during wet-flooded seasons resulting in lower mortalities of mature plants unlike the continuous grazing of KLF1 where recruitment was necessary. Sala (1988) and Wissman (2006) observed higher number of smaller plants in grazed lands compared to few large ones in ungrazed areas. This was observed during

collections where KLF1 was characterized by very many small sized plants, less than 30 cm tall while MGD3 had tall robust sparsely spaced plants (Kirwa, Personal observation).

Seeds of KLF2 ecotype had higher germination rates at Buchuma (37.1 %) than Kiboko (27.6 %) and Mtwapa (26.8 %) (Table 5.10). The ecotype had no statistical differences in seed yield across the three sites. This could infer efficient seed setting at Buchuma due to adequate soil moisture as was observed with seed yield. Ecotype TVT1 did not exhibit ecotype x environment effect in germination capacity, mean germination time and germination rate. The ecotype successfully established in all the three sites and ranked third in ASV ranking. Production of TVT1 seeds from varied environments with similar conditions as the study sites may not affect the quality of the seeds.

Correlation analysis results for seed yield and related traits as shown in Table 5.11 indicates that germination capacity (GC2014) was positively correlated (r=0.8) with germination for the original seeds harvested from the site of ecotype origin (GC2012) at the time of germplasm collection. This indicates that the observed germination capacity was not influenced by the new establishment sites. In both trials (2012 and 2014), KLF1 was the highest in germination capacity and MGD3 lowest.

5.6 Conclusion

Ecotype KBK1 was the highest yielder in mean dry matter across the sites. KBK3 was ranked the most stable and highest yielding ecotype across the three environments, Kiboko, Buchuma and Mtwapa, using AMMI stability value (ASV) and the yield stability index (YSI). Ecotype TVT3 ranked second best across sites with YSI. KLF1 was ranked the most unstable and the lowest yielding ecotype. Ecotype KBK1 was the best suited for Mtwapa and Kiboko in dry matter yield and MGD1 for Buchuma.

Ecotype KLF1 presented better seed quality based on the measured germination attributes and the higher seed yield. Seeds of MGD3 had very poor germination characteristics across all sites. The seasonal flooding and controlled grazing at the site of collection may have induced the observed characteristics. Seed germination attributes, namely, mean germination capacity, germination time and germination rate for TVT1 did not vary across the three sites

CHAPTER SIX

PARTICIPATORY IDENTIFICATION AND SELECTION OF ECOTYPES OF *CENCHRUS CILIARIS* IN THE SOUTHERN RANGELANDS OF KENYA

6.1. Abstract

A study was carried out through Focused group Discussions (FGDs) to evaluate the farmer knowledge and perceptions regarding ecotypes of *C. ciliaris* grass species. Selection of farmer preferred ecotypes was done at KALRO Kiboko research Station using the ribbon technique of participatory variety selection. Farmers selected among twelve established ecotypes following their own developed farmer criteria of a good and bad grass. Through FGDs, it was observed that farmers were knowledgeable on the existence of the ecotypes of *Cenchrus ciliaris* whose occurrence were similar in the three different farmers' groups interviewed. Three main ecotypes were identified by all groups; the small type with purple colored flowers, the robust bluish type and robust green type. The small type with purple flowers was noted as the most preferred by all groups during the FGDs. The ecotype was said to be a heavy seeder dropping a lot of purple colored seeds on the ground which enhanced its spread and establishment in different habitats. Also, the ecotype is perceived to be tolerant to droughts and heavy grazing. The criteria for selection of ecotypes varied depending on the type of utilization of the pasture. KLF1 and TVT1 ecotypes received the highest hits from the farmers due to their small stature and hence perceived to be good as grazing types and drought tolerant.

Key words: *Cenchrus ciliaris,* forage production, participatory variety selection, reseeding

6.2. Introduction

Cenchrus ciliaris is a tufted perennial grass species that is widely cultivated for forage production in the tropics. The grass species is adapted to a wide variety of soils, is drought tolerant with some ecotypes having the potential to withstand temperatures as low as -10 ^{o}C and as high as 46.5 ^{o}C (Griffa et al., 2006; Arshad et al., 2007). The species is able to utilize both low and high rainfall amounts by branching of the existing tillers or development of new tillers, respectively (Visser et al., 2008). The grass is highly tolerant to grazing pressure (FAO, 2012), which could be attributed to its varied forms of tiller developments (Visser et al., 2008), or the extensive deep rooted system that may exceed two metres. Wide adaptation has resulted in many ecotype variants. The species has been promoted widely for natural pasture improvement and rehabilitation programs in the arid and semi-arid lands (ASALs) of Kenya (Manyeki et al., 2015). This study describes a participatory selection experiment that was conducted on *C. ciliaris* ecotypes that had been established at KARO-Kiboko to evaluate farmer preference.

Participatory variety selection (PVS) is the involvement of farmers in the selection of non-segregating, described products of plant breeding, for example in form of lines, hybrids or clones. Involvement of farmers in selection of varieties builds in a sense of ownership (Ceccarelli, 2009) as it includes testing and selecting of the varieties either in farmer's fields or on-station (Almekinders et al., 2006). Participatory variety selection is commonly initiated after genetic variability has been reduced in the selection materials in the first steps of the plant breeding cycle, although, evaluation and selection for varieties from natural populations or ecotypes in grass species has also been successfully conducted (Savidan et al., 2001). *Cenchrus ciliaris*, the grass species in the study, is a non-segregating obligate apomict (Griffa et al., 2006). Apomixis is common in perennial forage grasses and grass cultivars derived

directly from natural populations are widely sown (Savidan et al., 2001). For instance, the Kentuckey bluegrass has highly variable cultivars due to its apomictic mode of reproduction (Huff, 2010).

The aims of the present study were to establish the knowledge and perceptions of farmers on ecotypes of *C. ciliaris* and test the use of participatory techniques in selection of the grass ecotypes.

6.3. Methodology

The study was in two parts. First, the use of Focused Group Discussions as the tool to collect information from the farmers on their knowledge and perception on the ecotypes of *Cenchrus ciliaris* and develop the criteria for selecting grasses was conducted. Secondly, participatory selection of established ecotypes in the field using the ribbon technique was carried out.

6.3.1. Focused group discussions (FGDs)

Three FGDs with each targeting eight farmers of mixed gender were held in late October 2014. Two of the groups (GP2 and GP3) were made up of the agro-pastoral Kamba farmers from Kibwezi and Makindu Sub-counties while one had pastoral Maasai community from Mashuru Sub-county. The GP3, involved farmers that were in leadership who actively participated in pasture improvement activities. Previously identified questions were used to guide the discussion (Table 6.1). Samples of some of the ecotypes were also availed to ensure the farmers were talking about the species of interest. Pairwise ranking was used during the discussions to rank the different criteria as well as the ecotypes of choice. At the end of the sessions, each group was also asked to identify the criteria for a good and bad grass. These were to be used in the selection of ecotypes in the field. A good grass was defined as the one that the farmer would want to take home to improve their pasture production while a bad grass was the one not to be promoted in their region.

Table 6.1: *List of questions used during focused group discussions*

No.	Question
1	Which ecotypes of *C. ciliaris* are you aware of?
2	Which ones are preferred by animals?
3	Which parts are preferred by animals
4	Does the preference change with change in season or growth stage? (If so why?)
5	How does the ecotype affect animal performance?
6	Any other benefits of this ecotypes?
7	Have you noticed these ecotypes disappearing with time? (If so why?)
8	Which grass would you prefer for promotion in pasture improvement?
9	What would you say about ease of establishment of each ecotype

6.3.2. Participatory selection of ecotypes of *C. ciliaris* using the ribbon technique

Eleven ecotypes of *C. ciliaris* were previously established at KALRO-Kiboko research station pasture plots between October 2012 and April 2014 for morphological characterization. The selected field site was similar in environmental conditions to the targeted farmer region because farmer selection is environment dependent (Ceccarelli, 2009). The experiment was arranged in a randomized complete block design replicated three times. Subsequently, replication was ignored and only one block was used because use of replications was found to have no significant difference and farmers complained of replication being too tedious.

This activity targeted the same farmer groups that attended the FGDs. Twenty four farmers (eleven for GP1, eight for GP2 and five for GP3) participated in the activity. Each of the three groups selected two different colored ribbons representing a good and bad ecotype (Table 6.2).

Table 6.2: *Number of participating farmers and ribbons colors selected*

Farmer group	Farmers (No.)	Good grass		Bad grass	
Pastoral Maasai (GP1)	11	Orange		Maroon	
General Kamba (GP2)	8	Blue		Purple	
Key informant Kamba (GP3)	5	Green		Red	
TOTAL	**24**				

The women in the groups had their ribbons tied to form knots to distinguish theirs from the men during the counting. Each farmer was given five ribbons for a good ecotype and three for a bad one. Each group went round the plots to first select the good ecotype by tying each of the five ribbons to ecotypes of choice. Poles were erected at the edge of each plot to facilitate the process. The same process was repeated for selection of the bad ecotype with each group going in at different times. Counting of the total number of ribbons per ecotype based on color and gender was done at the end of the exercise.

6.4. Results

6.4.1. Identification of ecotypes of *C. ciliaris*

The identified ecotypes during the FGDs are listed in Tables 6.3, 6.4 and 6.5 below. All the identified ecotypes were among the collections established at KALRO-Kiboko research station. The pastoral group (GP1) indicated that they knew 3 ecotypes, namely, robust green (*Entomonyua*), small with purple flowers (*Enkamba*) and the bluish type. Similar ecotypes were recorded by GP2 except with an additional black headed ecotype. The small purple flowered and bluish types were also identified by GP3. The GP3 members indicated that they knew of two types of the robust green based on flower color, that is, the purple flowered and the green flowered ecotypes. All the groups indicated that the small purple flowered is the most preferred by animals and all its parts were palatable. The ecotype is also reducing overtime in composition and size due to overutilization, frequent droughts and land fragmentation. Based on farmer description of the ecotype, the ecotypes was found to be similar to Kilifi 1 (KLF1) collection from Kilifi County, a sea-coast ecology.

Table 6.3: *List of Cenchrus ciliaris ecotypes identified by group one consisting of pastoral farmers from Mashuru sub-county*

1. *Entomonyua* (robust green)	2. *Enkamba (Small purple flowered)*	3. *Bluish type (blue)*
• Does not dry easily (especially stem). Still wet even at the peak of the dry season • Persistent • Found in valleys, near water collections, along rivers (specific areas- KARI, Chyulu, Ngulia) • Provides good habitat for tse-tse fly • Can grow up to 2 m with good rains or along riverine areas	• Very small, prostrate, roots not deep • Inflorescence purplish and length about 1/3 of the E*ntomonyua* • Tastes salty • Liked by animals • Found in black cotton soils (*Engusero*) • Regrowth with start of rains is very fast (faster than the robust green) • Not found in dry seasons due to overutilization	• Only found during rainy season especially on riverbanks • Rare • Easily disappears especially in reduced rains
Preference by animal:		
• Utilized though not preferred • Has a bitter taste to the animal and smells bad	• Most preferred because of salty taste even when dry (more preferred than *Digitaria macroblephara** (*rikaru)* during wet season)	• Utilized, although rarely accessed and measure of preference was difficult
Parts preference		
• Flowers and leaves selected and the stem is never utilized to below 20 cm unless during extreme drought.	• All parts utilized small and easily uprooted because roots not strongly held to the ground, thus whole plant utilized	• Since rare and not easily found, easily depleted
Preference changes with change in season or growth stage		
• Not liked during the wet season though selected as moisture content reduces (through yellowing of leaves).	• Animals like it all the time (wet or dry). They like it even at advanced growth stage	
Effect on animal performance		
• Utilized for a short period, thus difficult to gauge its effect on animal performance • Causes East Coast Fever disease[#]	• Increase milk production, • Fattens the animals	
Other benefits		
• Good for erosion control. Persistent and roots strongly held in the soi Difficult to eradicate if it spreads into cropland	• Other benefits were not so clear because the ecotype is not present during dry season and roots not deep	
Disappearance with time		
• Does not change and still found in the same habitats	• Composition has reduced a lot in the area. Currently found only during wet seasons. The species used to dominate the area in the past	
Which grass was preferred for promotion		
• Not selected	• Selected by all participants because of being "small with all parts liked by animals irrespective of the season"	• Not selected

*the *Digitaria macroblephara* is the most referred grass among the pastoral community from the area. Women indicated that they specifically targeted it for feeding calves because of being "sugary, soft (not hard stem), fattens the calves and intake is higher" compared to *C. ciliaris*.

The farmers indicated that during wet seasons there is a watery foam-like collection on the leaf node which acts as breeding grounds for tse tse fly. Consequently, the animals get sick when they graze on the grass. The symptoms of the sickness include dots appearing on the skin with fur flattened, a wet look from afar, and sometimes the animals lose hair. Later, swollen lymph nodes, teary eyes, drying around the nose and a cough develop. This problem is particularly common during heavy rains.

Table 6.4: *List of Cenchrus ciliaris ecotypes identified by group 2 comprising of agro-pastoral farmers from Makindu and Kibwezi sub-counties*

1. Bluish type	**2. Green, robust**	3. ***Small, purple flowers***	4. ***Black head type***
• Milky colored leaves • Very tall, many tillers and wide tussock (robust) and upright • Grows well in wetter areas and riverbanks, does well in croplands along the terraces though not common in the grazing areas. **Remarks:** Said to have been introduced within *C. ciliaris* seeds given by KALRO.	• Tall (about 130 cm) • Normally looks healthy (big green leaves). • Can only grow in places with water e.g. riverine areas **Remarks**: Identified by all	• Very small, about 60 cm tall, prostrate, • Inflorescence purplish • Liked by animals including goats, everything eaten though not uprooted, roots not deep • Forms a good ground cover (bushy), grazed to the ground and although may seem finished, resprouts immediately after rains • Very good in spreading due to high seeding • Common everywhere, adapted to low moisture conditions **Remarks**: Identified by all	• Very leafy with deep green leaves • Leaf shape like majority of big green • Tufted/upright • Found along Makindu river • Thought to have been introduced (by an NGO) **Remarks:** It was identified by only one farmer; no discussions on this ecotype
Parts preference and changes with change in season or growth stage			
• Leaves and flowers selected; one farmer insisted that the flowers are not utilized because they prick the animals. During wet seasons, it produces high herbage and animals get stomach full faster; low intake at maturity.	• Flowers and leaves selected and not stems	• All parts utilized because stems not tough and preference does not change with growth stage or season	
Effect on animal performance: Not fed them separately therefore may not be able to tell benefits of either			
Other benefits			
• Good in erosion control • Makes good brooms	• Good in erosion control • Good for thatching	• Good in erosion control better than the rest because the ecotype is prostrate giving full ground cover • Roots are pound and boiled or directly chewed for cough in children • Liked by bees • A problem in cropland- spreads easily and difficult to eradicate – may have to relocate the crop	
Disappearance with time			
• Not reducing	• Not reducing	• Reducing because of not being obviously seen as before • Also becoming smaller in size	
• **Ease of establishment:** Not able answer because they have not participated in grass reseeding activities.			

Table 6.5: *List of Cenchrus ciliaris ecotypes identified by group 3 (GP3) comprising of agro-pastoral farmers from Makindu and Kibwezi sub-counties*

1. ***Green, purple awns***	***2.*** **Green, green awns**	**3.** ***Bluish type***	4. ***Small, purple flowers***
• Tall (150cm), less leaves and thinner than No. 2 • Soft leaves, liked by animals • Does not easily disappear • Lower herbage than No.2	• Tall about 150cm, wide leaves, • Stems tough when mature • Does not easily disappear • High herbage yield -liked by animals • Remarks: Both No. 1 and 2 did not differ; therefore recorded as **robust green**	• Very tall – about 200 cm depending on soil & moisture • Stem tough when mature • Quite persistent • Rough leaves • Best in black cotton soils • Head pure white when dry • Easy to strip whole head as a bunch (all spikelets attached)	• Small, about 100 cm, prostrate, shallow roots • Short, soft liked by animals • Disappears with extreme drought or if overutilized due to shallow roots • High disease incidence • High and early seeder and drops seeds as the dry season approach • Seeds spread far and germinate easily thus higher species composition in pasturelands
Preference			
• Preferred only before seed maturity. • Stems are left or utilized at the top only		• Not preferred although intake increases when chopped and mixed with molasses • Not selected when other grasses are available including annuals.	• Most preferred • All parts grazed • The ecotype is utilized even when mature because of soft leaves and stems. • Height makes it preferred since animals do not like tall forage.
Other uses			
• Reduce soil erosion		• Reduce soil erosion	• Reduce soil erosion
• Has strong rooting system (thus nicknamed "Kiemobunie" since the ecotype is stronger than someone well fed). Meaning that the ecotype not easily uprooted			• Has high ground cover and easy to establish
• Good for hay baling; used in tying other animal feed during harvesting or when preparing for preservation		• Good for hay baling	
• Used in thatching houses and making nest for hens to lay eggs		• Used in thatching houses and making nest for hens to lay eggs	
• Liked by bees; produces gum like substance that attracts bees			
Ease of establishment			
• Does not spread easily from the place of establishment.			• Easy to spread and establish even with little rainfall.

1. *Green, purple awns*	*2.* **Green, green awns**	**3.** *Bluish type*	4. *Small, purple flowers*
			• Also re-sprouts faster with little rains
Disadvantages			
• Liked by termites; probably the roots have high moisture content. • Liked by snakes			• A weed in croplands – Though not a significant problem if ploughing or weeding is not done during rains
Disappearance of ecotype			
• Reducing due to overgrazing, land fragmentation and frequent droughts			• Reducing due to overgrazing, land fragmentation and frequent droughts

Robust green: Its stem does not loose water quickly and re-sprout when grazed or clipped even during the dry season. This was observed in some environments, especially in black cotton soils; not the case in red soils.

6.4.2. Selection of identified ecotypes for promotion in grass reseeding

All the participants in the pastoral Maasai group (GP1) selected only the small purple flowered because the ecotype is small with all parts liked by animals irrespective of the season. Tables 6.6 and 6.7 shows the ecotypes selected by GP2 and GP3 for promotion. The small purple flowered ecotype was selected for promotion by the majority of participants in the other two groups. The green robust and the bluish type was not selected by GP2 and GP3, respectively.

Table 6.6: *Ecotype selected for promotion and reasons for the nomination by GP2*

Small, Purple flowered (selected by 6 out of 8 participants)	**Bluish type** (selected by 2 out of 8 participants)
- Liked by animals	-High herbage yield and animal gets enough
-Adapted to low soil moisture thus good for our area	easily, more milk realized,
-Spreads easily through both seed and rhizomes	-Spreads easily through rhizomes
-Resprouts very fast with onset rains- including very low amounts	-Easy to harvest seeds
-Forms a good ground cover	

Table 6.7: *Ecotype selected for promotion and reasons for the nomination by GP3*

Small, Purple flowered (4 participants)	Robust green (3 participants)
- spreads and re-sprouts easily with little rains	-High herbage yield
- drops seeds easily	- Re-sprouts after use even during dry season
- liked by animals	- Easy to make hay
- reduce soil erosion better by forming a good ground cover	- High in other benefits listed above e.g. thatching
	- persistent with grazing or droughts

6.4.3. Development of criteria for selection of grasses

It was difficult to agree on the criteria with GP1 because the group based their decisions mainly on the animal preference or performance such as increased milk yield or fattening of the animals. For instance, *D. macroblephara* was said to increase milk yield and to fatten across seasons. In addition, they seemed to base their selection on seasons. For instance, during the dry season, a

good grass does not fill up the animal stomach allowing the animal to remain light (fit) and not tire easily. Grasses that fill up the animal stomach quickly were preferred during the wet seasons since they quickly fatten the animal for the market. Wet season grasses were believed to be high herbage yielding species. Three criteria were finally agreed upon for commonly preferred good grasses. These were thin stems, short and prostrate grass for grazing. Ranking was not done since the group maintained only the three criteria and indicated that the opposite applied for a good grass under wet conditions. Consideration of the semi-nomadic lifestyle seemed to have influenced their seasonally based choices.

Table 6.8 shows the criteria identified for a good grass by GP2. Through pairwise ranking they identified the three most important criteria as high seed yield, many leaves and small plant size, particularly leaves.

Table 6.8: *Pairwise ranking results for selection criteria as identified by group 2*

Criteria	**1**	**2**	**3**	**4**	**5**	**Rank**
1. Not stemmy with big sized leaves	-	3	4	5	6	**5**
2. Resistant to drought (small leaves and size)		-	3	5	6	**3**
3. Big stems (robust)			-	5	6	**4**
4. Leafy (Many leaves)				-	6	**2**
5. High seed yield (a lot of seed on inflorescence)					-	**1**
Count	**0**	**2**	**1**	**3**	**4**	

High yield of seed was ranked highly because the farmers insisted that their main target was the business value in dealing with grass seeds. Therefore, germination capacity that had been listed in the criteria was removed after agreeing that a measure of the trait could not be applied in the field except if laboratory results were included in the target selection. The high ranking of small sized and leafy type was in line with their selection of the small purple flowered type among their

ecotypes. They indicated that a tall, bushy grass is not well grazed and animals would keep moving in the field selecting leaves instead of keen grazing. Farmers that targeted cut and carry system preferred a robust type grass since they believed chopping would increase intake. Group two (GP2) also identified criteria for a "bad" (meaning un-preferred) grass as hairy, thick stems, spaced or few leaves (less leafy) and low amount of seed yield. This was to be done through evaluation of seed amounts on the flower heads and observed number of seeding heads.

Table 6.9 shows five factors identified GP3 for a good grass. Through pairwise ranking they ranked the three most important criteria for a good grass as many leaves, soft stems and many tillers. The group also listed the criteria for a "bad" grass as rough leaves (hairy or rough to touch), hard or tough stems and low biomass through observed herbage yield in the field.

Table 6.9: *Pairwise ranking results for selection criteria as identified by group 3*

Criteria	1	2	3	4	5	RANK	
1. Many leaves		-	1	1	1	**1**	**1**
2. Soft stems			**-**	**2**	**2**	**2**	**2**
3. High seed yield				**-**	**4**	**5**	**5**
4. Many tillers					**-**	**4**	**3**
5. Taller						**-**	**4**
COUNT		**4**	**3**	**0**	**2**	**1**	

6.4.4. Selection of good ecotypes using the ribbon technique

Table 6.10 shows farmer preference and selection of *C. ciliaris* ecotypes at KALRO Kiboko Research Station. The smaller ecotypes received significantly higher number of ribbons for a good grass. TVT1 (91.7 %) and KLF1 (83.3 %) ecotypes had the highest percent ribbons while TVT3 and KBK3 each with 8.3 % had the least each with 8.3 %. All men present selected TVT1 as their

preferred grass ecotype. For female, KLF1 and KLF3 ranked highly each with nine ribbons out of ten although KLF2 and TVT1 each received eight ribbons

According to field plenary discussions, TVT3 which was a bluish type, received the least ribbons because of its big and hard stems, low leaf to stem ratio (less leafy) and course leaves when touched. KBK1 that is bluish in colour received 25 % because the ecotype is leafier than the other robust ecotypes.

Table 6. 10: *Percent number of ribbons per group, gender and of total participants indicating preference to Cenchrus ciliaris ecotypes by farmers*

Ecotype	GP1	GP2	GP3	Female	Male	% of total participants
MGD1	54.5	0.0	80.0	2	8	**41.7**
TVT3	18.2	0.0	0.0	1	1	**8.3**
MGD3	18.2	100.0	100.0	6	9	**62.5**
KBK2	18.2	0.0	80.0	1	5	**25.0**
KLF2	45.5	100.0	0.0	8	5	**54.2**
KLF3	63.6	100.0	20.0	9	7	**66.7**
KLF1	81.8	100.0	60.0	9	11	**83.3**
KBK3	0.0	0.0	40.0	1	1	**8.3**
TVT2	63.6	0.0	20.0	3	5	**33.3**
KBK1	45.5	0.0	20.0	2	4	**25.0**
TVT1	90.9	100.0	80.0	8	14	**91.7**

6.5. Discussion

The farmers were aware of existence of ecotypes of *C. ciliaris*. There were similarities in three of the ecotypes identified. These were the small-purple flowered, bluish type and robust green. Group three were able to identify two types from robust green, i.e. the purple head (due to purple awns) and pure green head with no tint of purple colour. All the mentioned ecotypes have been observed among the collections at KALRO- Kiboko Centre. The small purple flowered type is actually similar to KLF1 ecotype. In a previous study, Millar and Curtis (1997) found that farmers in

Australia were knowledgeable in perennial grasses in terms of animal performance, drought and persistence.

The majority of the farmers in each FGD group nominated the smaller ecotype due to its soft stems and leaves as well as being short, prostrate and perceived preference by the animals. Shortness and prostrate growth form confers the plants tolerance to grazing (Milchunas et al., (1988). Li et al., (2015) observed reduced plant size with long term defoliation in *Leymus chinensis* grass species. Farmers that targeted harvesting for baling preferred the robust ones implying that the mode of utilization determines the ecotypes to select. The appropriateness of the intended use is an important consideration while planning pasture production (Barnhart, 2011).

The criteria for a good grass were relatively similar among the groups. Robust related traits were not preferred especially height due to poor grazing habits by animals and thick stems due to associated toughness with maturity. The pastoral group (GP1) based their criteria development on seasons. They had special reasons for dry season preferred grass. This was to allow for fitness in the animals for ease of movement. This implies that promotion of grass species for reseeding pastoral Maasai areas should consider grazing tolerant species of both small and big types

As was shown in Table 6.10, KLF1 and TVT1 were the most preferred because of being small, leafy and with thin stems as indicated in selection criteria. TVT1 is medium in height and semi prostrate while KLF1 is short and prostrate. The KLF1 ecotype was the small purple flowered ecotype identified by farmers during the FGDs as shown in Tables 6.3, 6.4 and 6.5 while TVT1 was identified by only one farmer in GP2 as the black headed ecotype. The two ecotypes had no significant difference in regards to crude protein, crude fiber, in-vitro dry matter digestibility

(INVDMD) and percent DM (Kirwa et al., 2015). Ecotype TVT1 had the least INVDMD of 45.6 % among twelve evaluated ecotypes.

Among the robust ecotypes, the Magadi collections, MGD1 and MGD3 with 41.7 and 62.5 %, respectively, received higher number of ribbons compared to the Kiboko collections, that is, KBK1 and KBK2 with 25 % and KBK3 with 8.3 %. The preference was mainly from GP2 and GP3 farmers targeting hay production in their selection. The recorded good ribbons for MDG1 from GP1, the Maasai community, may be as a result of its stems and height that did not seem to differ with the small types. The ecotype height and stem thickness did not differ significantly to the small types during characterization. The ecotype is also leafy compared to the other robust types.

Some of the characteristics depicted by the highly selected ecotypes such as high tiller numbers, small size and prostrate growth form are associated with grazing effects. The grass plant developed some of these traits due to the long term interaction with the grazer as a way of adapting to grazing (Sala 1988). N'Guessan and Hartnett (2011) observed increased tiller recruitment and development of meristems in different positions of the little bluestem grass leading to a more prostrate position as a response to frequent grazing. High seed production is also a survival trait in the grass family allowing a species to maintain persistence in competitive environments, including croplands (Simpson, 1990). Table 6.4 indicated that the small purple flowered ecotype was common in agro-pastoral areas since the ecotype produces a lot of seeds and was a problem in croplands. The high seed yield trait was expressed by KLF1 ecotypes during evaluation for seed yield.

6.6. Conclusions

The farmers were knowledgeable on the existence of varied ecotypes of *C. ciliaris* in their environs. They were able to give details on their preferences as well as their reasons for the same. The criteria for selection of ecotypes varied depending on the type of utilization of the pasture. The small ecotypes received the highest percent ribbons as good grass for grazing. This means cut and carry or baling of hay as techniques for utilization of pastures were yet to be fully embraced. Therefore, successful development and promotion of grass varieties needs to consider the mode of utilization by the target group. TVT1 and KLF1 were highly preferred by the farmers.

CHAPTER SEVEN:

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

It is concluded that there were phenotypic differences among ecotypes of *C. ciliaris* and *E. superba* ecotypes from morphological characterization. For *C. ciliaris*, there were two major groups of small sized and robust types of ecotypes The small sized ecotypes of *C. ciliaris* were early flowering while the robust types were late flowering and a robust type, MGD1, was found to be early flowering. All Kiboko collections were robust and late flowering while all ecotypes from Kilifi were small and early flowering. For *E. superba*, Kiboko collections, KBK1 and KBK2, were robust and clustered separately from the rest of the nine ecotypes. Selection for maturity time and plant size is possible with the collections of *C. ciliaris* grass species. Kilifi collections may be selected for the early flowering, Kiboko ones for late flowering traits and MGD1 as an early maturing and robust type. As observed with Magadi ecotypes of *C. ciliaris* that did not depict characteristics of a dry environment, consideration and proper description of specific site of collection during germplasm explorations particularly special niches is important to avoid generalization of plant attributes such as drought tolerance.

The nutritive value differences among the ecotypes of *C. ciliaris* and *E. superba* provides opportunities for selection targeting specific components within and between ecotypes. Selection for higher crude protein at peak flowering stage may target KBK2 and KLF1 ecotypes of *E. superba*. There was positive correlation between CP and INVDMD to other plant traits such as stem thickness and plant among *C. ciliaris* ecotypes, which provides opportunities for selection of

high yielding lines among the ecotypes. For *E. superba*, distinct clusters were formed representing different levels of nutritive value components. KBK2 and KLF1 were grouped together with high CP levels.

Genetic variation among the *C. ciliaris* and *E. superba* ecotypes was recorded. Kajiado population of *C. ciliaris* was the most diverse group while Kilifi and Narok populations were the most distantly related. The genetic variation between *E. superba* populations could be attributed to geographical distance between sites of collections, adaptation of ecotypes in new environments such as the Malindi ecotype established at ILRI Ethiopia versus the Kilifi collections and variation in niche habitats such as for the Kiboko collections.

The multi-environment evaluation of *C. ciliaris* ecotypes on biomass yield indicated that KBK1 was the most suitable for Kiboko and Mtwapa environments while MGD1 was the most stable for Buchuma. Ecotype KBK3 was the most stable across all sites according to ASV as well as ranked first by yield stability index.

The farmers were well-informed on the presence of diverse ecotypes of *C. ciliaris* in their grazinglands. They also had their own preference among the ecotypes based on their perceived benefits. The information on farmer preference would be useful in identifying and collecting particular ecotypes of importance to farmers that may not have been captured in previous collection expeditions. The farmer criteria for selection of ecotypes varied depending on the type of utilization of the pasture where the grazing-type ecotypes were most preferred. TVT1 and KLF1 were highly preferred by the farmers due to their perceived preference by the grazing animals and tolerance to drought.

7.2 Recommendations

- Selection for late maturing types in C. *ciliaris* should be done for all the Kiboko collections, MGD1, MGD3 and TVT3 ecotypes while for early maturity should be among all Kilifi collections, TVT1 and TVT2.
- KBK1 ecotype of *C. ciliaris* should be promoted for biomass production at Kiboko, Mtwapa and similar environments and MGD1 for Buchuma environments. KBK1 at Kiboko and similar environments should be supplemented with irrigation during dry seasons
- KBK3 ecotype of *C. ciliaris* that was ranked highly in biomass production across the three sites should be evaluated for disease management before promotion.
- KBK2 ecotype of *E. superba* should be selected expected higher biomass yield and forage quality due to bulkiness and higher CP.
- TVT1 and KLF1 ecotypes of *C. ciliaris* that were highly preferred by the farmers should be selected for evaluation on performance and adaptability in the farmer target areas before their promotion.
- Further germplasm collection expeditions for *C. ciliaris* and *E. superba* covering more parts of Kenyan ASALs should be carried out.

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