PHENOTYPIC ASSESSMENT AND MOLECULAR DIVERSITY EVALUATION USING SIMPLE SEQUENCE REPEATS OF KENYAN SORGHUM GERMPLASM UNDER DROUGHT STRESS CONDITIONS

BY

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DECLARATION

I declare that this is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

This thesis is dedicated to all those who believe in the richness of learning.

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ABSTRACT

In terms of tonnage, sorghum is Africa's second most important cereal. The continent produces about 20 million tonnes of sorghum per annum, about one-third of the world crop. The crop is widely grown in the semi arid tropics where local farmers depend on the natural rainfall and the crop is subject to un-predictable drought stress factors.

The objective of this study was to assess the genetic diversity and the wide range of sorghum accessions grown in Kenya under drought stress conditions. A total of 139 accessions were characterized and evaluated for drought tolerance and stay green during the dry seasons of 2007 and 2008 at the University of Nairobi, Kibwezi farm. All accessions were grown in 2 m entry rows using an alpha lattice design. Both qualitative and quantitative traits were used to assess the phenotypic variability. There was significant phenotypic variability in the diverse traits of sorghum measured. There was a clear indication of the suitability of the genotypes to specific environments. The early maturing accessions exhibiting an adaptation to drought escape, originated mainly from dry and marginal North-eastern and eastern provinces of Kenya. In both seasons, water stress caused decrease in plant height. Under drought stressed conditions, grain yield was significantly correlated to days to 50% flowering, and also to the number of productive tillers. Grain yield, Days to flowering, rates of senescence (15, 30 and 45 DAF) showed high and significant broad-senescence heritabilities under drought conditions indicating that these traits are highly heritable and can be selected for under drought stress conditions. Green leaf area or 'stay-green' levels was higher in the season with less drought stress than in the more stressed one. Correlations for stay-green measured either at 15, 30 or 45 days after flowering were highly significant. Accessions with high green leaf area yielded higher than those with lower green leaf areas in both seasons. Accessions clustered in groups C and A gave useful higher grain yields, seed mass and also stay green than those clustered in groups B and D. Principal Component Analysis bi-plots identified a total of three similar groups. One group was mainly influenced by days to 50% flowering, the other one was mainly attributed to the green leaf area (stay

green) and the last group was mainly influenced by yield, 100 seed mass, plant height and lodging. Kenyan sorghum germ-plasm can therefore be categorized as having been selected for early maturing drought escaping types, drought tolerant stay-green types and high yielding drought susceptible genetic materials.

Molecular data on diversity demonstrates that the Kenyan sorghum accessions contain a great deal of genetic diversity as indicated by the the Nei's genetic diversity. The high genetic diversity value among the sorghum accessions indicates that the level of genetic diversity was not influenced by selection.

The F_{ST} values observed in this study are relatively low, indicating a reduced degree in allelic fixation. The cluster analysis based on genetic similarity among the bulked sorghum accessions examined showed a clear demarcation of the germplasm mainly according to their breeding origin.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Sorghum (*sorghum bicolar* (L.) Moench) is the fifth most important cereal crop world wide (http://apps.fao.org/default.jsp) as well as an important source of feed, fibre, and biofuel (Dogget, 1988). Sorghum is in the panicoid grass subfamily of East African origin and is closely related to maize, millet and especially sugarcane, and is more distantly related to wheat and rice. It is a crop of worldwide importance and much of the developing world because of it's superior tolerance to arid and water logged areas. Sorghum, like maize and sugarcane, carries out C_4 photosynthesis, a specialization that make these grasses well adapted to environments subject to high temperature and water limitation (Edwards *et al.*, 2004).

Because of it's relatively small genome (approximately 818 *Map*) (Price et al., 2005) among the C_4 grasses, sorghum has therefore become an important target for genome analysis. The cultivated species is diploid (2n =20) and the sorghum germplasm is diverse (Dje *et al.*, 2000; Menz et al., 2004).

It is a dietary staple food for more than 500 million people in more than 30 countries with only rice, wheat, maize and potatoes feeding more people than sorghum. In Africa, it is processed into a wide variety of nutritious attractive, traditional foods such as semi-leavened bread, cous, dumplings and fermented and non-fermented porridges. It has also been used as a key ingredient for brewing African beers. Currently, technology has enabled it to be processed into a myriad of products such as instant soft porridge and malt extracts. In essence the sorghum economy is predominantly found among subsistence, smallholder-farming communities where most production is consumed directly as food (porridge, ugali) and the stover is fed to livestock (Devries and Toenniessen.,2001). On a world basis, sorghum represents 3.5% of the total cereal production and while this figure is small, it is of great importance to most

African countries; Botswana (84.4%) Sudan (71.6%) Burkina Faso (52.8%), Rwanda (51.5%), Chad (41%), Cameroon (39.9%).In Africa as a whole, the proportion is 17.9% (Dendy, 1995). In Eastern Africa region an average of approximately 7 million ha yr^{-1} has been harvested, and 5.4 million tons of sorghum grain produced over the past three years (FAO, 2001).

Sorghum grain yields in Kenya are however low and highly variable from year to year. The crop is predominantly traditionally grown in Eastern, Western, and Nyanza provinces. Farm production went up from 118,227 tonnes in 2002 to 126,433 tonnes in 2003.Generally sorghum productivity is influenced by rainfall. As an indigenous Kenyan crop, sorghum provides food security and is becoming a suitable alternative in many places where maize crop fails (MOA, 2003). After soil nutrient deficiencies, drought stress is the most important abiotic constraint to sorghum for the farmer preferred sorghum variety in Kenya (Ochuti). Ochuti variety of sorghum is a variety that originated from Nyanza in Kenya. It is late maturing but is high yielding and least attacked by birds and therefore preferred by most farmers in Kenya over the others such as Seredo, Mureta and Andiwo. Due to unfavorable weather characterized by short and erratic rains Ochuti has largely disappeared in areas where it's largely grown. (Eyzaguirre, and Iwanaga.1991).These conditions have been found to reduce grain yield of this variety especially at the grain filling stage.

1.2 Problem Statement

Drought-stress is a major constraint to sorghum productivity world wide. However, sorghum is one of the most drought tolerant grain crops and is an excellent model for evaluating mechanisms of drought tolerance. Screening for drought tolerance and resistance with conventional methods has been slow because the physiological and genetic mechanisms that mediate drought tolerance are poorly understood (Blum, 2004). For instance, although ability of leaves to delay senescence has a genetic basis in sorghum (Van oosterom et al., 1996) expression of this characteristic is strongly influenced by environmental

factors. The trait expresses best in environmental factors in which crop is dependent upon stored soil moisture but where there is sufficient moisture to meet only a part of transpiration demand. Sufficient expression of this trait for selection is dependent upon occurrence of prolonged period of drought stress during grain filling period of sufficient severity to accelerated normal leaf senescence but not of sufficient magnitude to cause premature death to plants. Due to this precise requirement for this trait expression, field environment do not offer ideal conditions for selections and molecular markers associated with this trait may offer the better alternative (Crasta et al., Xu *et al.,* 1999).

Molecular markers have been used to characterize several different traits in sorghum including plant height and maturity (Pereira and lee, 1995), characters concerned with domestication (Patterson *et al.*, 1995) and drought tolerance (Tuinstra *et al.,* 1996, 1997, 1998).

In addition the DNA marker systems based on polymerase chain reaction (PCR) are particularly suited to select specific genomic regions without excessively reducing genetic variability in the rest of the genome.

Hence, sorghum breeders have recently been concentrating their efforts on selection of secondary traits that confer a yield advantage under drought and have developed a criteria for selection of these traits (Rosenow *et al.*, 1983; Henzel *et al.*, 1992). Stay green or delayed foliar senescence is one such trait that is being exploited to breed for drought tolerance in sorghum.

1.3 Justification of the study

Sorghum is the main staple crop to over 100 million people in the semi-arid tropical regions of sub-Saharan Africa. In Eastern Africa per capita being the second most important cereal crop after maize (*Zea mays L.*) that contributes immensely to domestic food supply and rural household incomes, with a total acreage of 8,199,741 ha (FAO, 2004).

Sorghum is a suitable alternative, owing to superior drought tolerance and adaptability to poor soils. Sorghum is better adapted to water limiting environments compared with most other crops (Doggett, 1988; Ludlow and Muchow, 1990; Mullet *et al.*, 2001; Sanchez *et al.*, 2002). This attribute is of great importance as the demand for food and water supplies increases due to world population growth (Khush, 1999; Gleick, 2003). In Kenya for example there has been an accelerated migration of farmers to the drylands due to lack of agricultural land in high potential areas (M'ragwa and Kanyenji, 1987). This has created the need for the development of suitable crops for the drylands. In such situations the most practical method for a farmer is to adopt drought tolerant cultivars that are adapted to these arid environments. Such improved sorghum varieties ensure substantial and a fairly sustained yields for the farmers.

1.4 Objectives of the study

1.4.1 Overall objective

To develop methods that speed up breeding for resistance / tolerance to drought through phenotypic screening and genotyping.

1.4.2 Specific objectives

1. To characterize sorghum accessions in Kenya for drought tolerant traits and genetic diversity using phenotypic variation.
2. To characterize and assess genetic diversity of Kenyan accessions for drought related traits using microsattelite variation.

1.5 Hypothesis

1. Will there be differences in yield between accessions?
2. Are differences going to be noted in yield from season to season?
3. Are there differences between Kenyan sorghum based on their geographic origins and genotype?

CHAPTER TWO: LITERATURE REVIEW

2.0 Breeding strategies for drought

Sorghum is probably indigenous to Africa, it is one of the longest cultivated plants of warm regions there and also in Asia, especially India and China. The crop is grown on 44 million ha in 99 countries in Africa, Asia, Oceania, and the Americas. Ethiopia is considered a probable centre of origin (Dogget and Prasada Rao, 1995). Grain sorghum is well known for its capacity to tolerate conditions of limited moisture and to produce during periods of extended drought, in circumstances that would impede production in most other grains. Sorghum leaves roll along the midrib when moisture stressed, making the plant more drought resistant than other grains plants. Like corn, sorghum can be grown under a wide range of soil and climatic conditions. Unlike corn however, sorghum yields under different conditions are not so varied (darwin.nmsu.edu/~molbio/plant/ sorghum.html).

Developing crops that use water more efficiently is one of the greatest challenges facing crop scientists today. In the face of diminishing water resources, the world is expected to consume twice as much food in the next 50 years as it has in the past 10,000 years. To meet this demand, world grain production will have to increase by 40% by 2020 (Dupont, 2000). Crops such as sorghum are specially adapted to semi-arid regions, and contain various mechanisms enabling these crops to escape and resist drought.

Understanding the genetic, physiological, molecular and biochemical basis of such drought-resistance mechanisms is fundamental to development of new strains that are better adapted to dry conditions. Keeping leaves alive longer is a fundamental strategy for increasing crop production, particularly under water limited conditions. Recent studies in Australia have examined two sources of stay-green. B35 and Ks19, derived from sorghum lines native to Ethiopia and Nigeria, respectively. Early in crop growth, stay green hybrids partition more carbon and Nitrogen to leaves compared with their senescent counterparts, resulting in higher Specific Leaf Nitrogen (SLN). It is hypothesized that the higher SLN initiates a chain of responses, including enhanced radiation use efficiency (RUE) and

transpiration efficiency (TE), which enable the plant to set a higher yield potential by anthesis. After anthesis, higher SLN delays the onset and reduces the rate of leaf senescence and this is associated with stay green crops taking up more nitrogen from the soil compared with senescent crops. These processes lead to increased grain yield and lodging resistance in stay green lines under post-anthesis drought.

2.1 Selection of drought tolerant sorghum

Drought is a major constraint in sorghum production worldwide and drought tolerance improvement has become an important objective in many breeding programs. However, selection for drought tolerance is difficult because of inconsistency in testing improvements and interaction between stages of plant growth and environment. The genetic mechanisms that condition the expression of drought tolerance in crop plants are poorly understood. Since drought tolerance is a complex trait controlled by many genes, and is dependent on the timing and severity of moisture stress, it is one of the most-difficult trait to study and characterize.

To deal with this complexity, the breeder has to make the appropriate decision to choose the right breeding strategy. Two main strategies have been employed by breeders in attempting to improve drought resistance in sorghum; direct selection for yield in drought environments and indirect selection for traits known to be associated with yield under drought.

Sorghum is generally grown in regions that commonly experience water deficit. As a result, most breeding programs practice direct selection for yield under drought to greater or lesser extent. However, there is little published work to indicate that selection for drought resistance genes has contributed to any genetic gain in yield when direct selection for yield has been practiced. Problems of low heritability and G x E interaction suggest that most progress can be attributed to improvement in general adaptation of drought environments. New tools have emerged with the potential to enhance the capacity of sorghum breeders to directly select for yield under drought. One such tool that addresses the G x E is the combining of

simulation modeling (Hammer and Muchow,1994) and a long-term weather data to calculate a water stress index for specific environments, grouped by pattern analysis(Chapmanet al.,2000) This approach not only identifies particular environment types, but also indicates how frequently these environments occur in target population of environments (TPE). Podlich and Cooper (1998) suggested that genetic gain could be enhanced by using this information to weight the data from particular tests, depending on how frequently the environment of the particular test occurred in the TPE. Such an approach could be used to more successfully select for specific adaptation to a particular pattern of water stress. Although this relatively new tool has significant potential, there is no indication at this time of its effect on the genetic gain in sorghum drought resistance.

The selection of the candidate traits can be assisted by considering the environment types and their frequency of occurrence in the TPE, as suggested by (Chapmanet al.,2000).While genetic variation exists for many candidate drought resistance traits that have been proposed, such as root architecture (Jordan and Miller,1980) ,osmotic adjustment (Shackel *et al.*,1982),stay green (Rosenow, 1977), pre-anthesis traits (Rosenow and Clark,1981) and transpiration efficiency (Hammer *et al.*,1997) few are used as a selection criteria in applied breeding programs ,either because of a lack of large-scale selection methodologies or because the association between the trait and yield under drought has not been well established.

2.2 Drought adaptation in sorghum

2.2.1 Components of drought adaptation

Sorghum is adapted to tolerate water-limited conditions and this ability can be assessed in terms of drought escape and drought resistance. The latter can be further partitioned into dehydration avoidance and dehydration tolerance (Levitt,1980;Ludlow and Muchow,1990). Sorghum will only contribute to economic yield if plants can survive intermittent short-term water deficits. In the case of terminal drought, the longer the plant survives during grain filling, the more likely the plant will contribute to yield, either directly by supplying carbon to the

developing grains, or indirectly by reduced lodging (Ludlow and Muchow,1990).The fundamental issue is how traits associated with drought resistance influence yield by their effects on growth and survival.

Drought escape is the evasion of periods of water deficit by limiting either their life cycle duration or rate of water use (Begg and Turner,1976). For instance, in arid environments, desert ephemerals and short season annuals germinate after rain, ,grow rapidly, flower, then set seed before exhausting the available soil water and activate tissue dehydration. The cost of these strategies is low yield in better than average years.

Some longer season annual and perennial plants survive water deficit conditions through one of the two drought resistance strategies: avoiding or tolerating dehydration. Under hot and dry conditions, some plants can maintain cell turgor and cell volume, thereby avoiding water deficits in tissues. Dehydration avoidance can be achieved by maintaining water uptake, reducing water loss, or changing tissue characteristics such as osmotic adjustment or increased tissue elasticity (Ludlow and Muchow,1990).Dehydration avoidance differs from drought escape; but not in the latter. Alternatively, superior proplasmic tolerance of desiccation enables some plants to tolerate dehydration.

2.3 Traits to combat drought

2.3.1 Drought escape

Matching duration of the crop cycle to water supply via genotypic variation in phenology is one of the most obvious means of drought escape. When rainfall is limited during grain filling, early flowering generally results in higher yield and greater stability compared with later flowering. The harvest index will also be improved if early flowering enables a genotype to escape drought during the critical reproductive stages. There may be a cost associated with short season genotypes in unpredictable environments because potentially transpirable water may be left in the soil at maturity in wetter years (Ludlow and Muchow,1990), as demonstrated in sorghum by Jordan et al. (1983).

Genetic variation for phenology in sorghum is associated with variation in responses to both temperature and photoperiod (Hammer *et al.*,1989). Sorghum is a quantitative short day plant (rate of progress towards flowering is faster under shorter days.) and therefore it flowers earlier as daylight periods become shorter, depending on the maturity of the genotype (Blum, 2004)

2.3.2 Shoot attributes

2.3.2.1 Leaf movements

Leaves move to help shed incident radiation, thereby reducing leaf temperatures and water loss (O'toole *et al.*,1979). Leaf movements have evolved in the Graminae as a means of protecting the leaf from excessive radiation load when this radiation cannot be dissipated by transpiration, i.e., when turgor is lost (Blum, 2004). If leaves remain unrolled, yet do not transpire, their temperature will rise to a point that will kill them. Blum and Arkin (1984) found that older sorghum leaves lose their capacity to roll, and they suggest that this could be one reason why older leaves desiccate first when water deficit develops.

Leaf movements include rolling,folding and wilting (Rawson,1979; Begg,1980, Wilson *et al.*,1980; Ludlow and Bjorkman,1984) and they increase avoidance of dehydration (Begg,1980; Ehleringer and Forseth,1980).Leaf movements should therefore, contribute to stability of yield in conditions of intermittent water stress by increasing the survival of plants until the next rainfall (Ludlow and Muchow,1990).Genetic variation in leaf rolling has been observed in sorghum (Begg,1980;Santamaria et al.,1986 and rice (Chang *et al.*,1974; Turner *et al.*,1986).

Leaf rolling in sorghum is a simple indicator of turgor loss, since rolling consistently occurs at a relative water content (RWC) very close to the stomatal closure (Blum, 2004). Differences in leaf rolling between various sorghum genotypes under the same soil moisture stress is often observed in the field. Such differences may be due to differences in either leaf water potential (LWP) or osmotic adjustment (OA),since both determine the leaf turgor potential at any given time (Blum, 2004). This means that delayed leaf rolling in the field indicates

a greater capacity to maintain higher LWP or better OA. It does not mean that leaf rolling is a negative trait *per se* (Blum, 2004).

2.3.2.2 Leaf surface characteristics

Leaves vary considerably in the extent to which they reflect visible light from both ad- and abaxial surfaces. Increasing the leaf reflectance lowers the leaf temperature, the leaf-air vapor pressure difference, and water loss (Johnson *et al.*,1983). Reflectance can be caused by the presence of epicuticular wax (Ludlow and Muchow,1990); such wax on sorghum leaves is visually recognized as the waxy bloom on the abaxila surfaces on the leaf lamina and leaf sheath (Blum, 2004). At a microscopic level, these depositions appear amorphic, flacky, or star-shaped on the leaf lamina and leaf sheath (Blum,1975; Tarumoto *et al.*,1981;Maiti *et al.*,1984; Traore *et al.*,1989). The trait termed *bloomed* in sorghum, compared with non bloomed or bloomless (Ludlow and Muchow,1990), and the depositions are genetically controlled by the B*m* gene locus. The recessive allele(s) (*bm*) reduce the amount of leaf lamina epicuticular wax to about one-quarter of the wild type (Blum,1975; Ebercon *et al.*,1977) and sometimes reduces cuticle thickness and weight (Jenks et al.,1994). Water deficit reduces epicuticular wax load (Jordan *et al.*,1983; Premachandra *et al.*,1992), and the deposition is promoted by high irradiance, high temperature, high vapor pressure deficit and soil moisture deficit.

2.3.2.3 Stomatal control and Carbon Assimilation

Reductions in stomatal conductance to conserve water inevitably lower photosynthesis because stomata regulate the influx of CO_2 in the leaves as well as the loss of water vapor (Ludlow and Muchow,1990). This trade off between loss of production and the need to prevent dehydration determines the usefulness of reduced stomatal conductance as a drought resistance mechanism. The primary role of reduced stomatal conductance is to avoid dehydration (Blum *et al.*,1981; Ludlow *et al.*,1983)

2.4.0 Integrated traits

2.4.1 Leaf Area Maintenance (Stay green)

The genetic mechanisms that condition the expression of drought tolerance in crop plants are poorly understood. Since drought tolerance is a complex trait controlled by many genes, and is dependent on the timing and severity of moisture stress, it is one of the most-difficult traits to study and characterize.
In spite of this, two distinct drought-stress responses have been identified in sorghum (Rosenow and Clark, 1981, 1995; Rosenow 1983); a pre-flowering drought response that occurs prior to anthesis and a post-flowering water limitation that occurs during the grain-filling stage.

Symptoms of post-flowering drought-stress susceptibility include pre-mature leaf and plant senescence, stalk lodging and charcoal rot, and a reduction in seed size (Rosenow and Clark, 1995). Sorghum genotypes that exhibit resistance to pre-flowering and / or post-flowering drought have been identified (Rosenow and Clark, 1995). Genotypes resistant to post flowering drought stress are called 'stay green' types because these plants retain chlorophyll in their leaves and maintain the ability to carry out photosynthesis longer than 'senescent' genotypes under terminal drought conditions. This phenotype is distinct from 'cosmetic' stay green, which is characterized by senescing leaves that retain chlorophyll but lose the capacity to carry out photosynthesis (Thomas and Smart, 1993; Thomas and Howarth, 2000; Cha *et al.*, 2002). The stay green genotypes also exhibit reduced stalk lodging (Woodfin *et al.,* 1998) and resistance to Charcoal rot (Rosenow, 1983).

The physiological basis of the sorghum stay green trait remains to be clarified. Stay green genotypes have been found to contain higher cytokinin levels (McBee, 1984; Ambler *et al.*, 1987) and more stem sugars (Duncan *et al.*, 1981; McBee and Miller, 1982; Dahlberg, 1992) than senescent genotypes under certain conditions. In addition staygreen hybrids assimilate more Nitrogen and base higher specific leaf Nitrogen than senescent hybrids, suggesting a link between

nitrogen status and stay green trait (Borell and Hammer, 2000; Borell *et al.*, 2001). However, it is unclear if these traits are a cause or a consequence of the stay green trait, or are secondary traits that are associated with the general adaptation of stay green genotypes to their agro-ecological zones. While the precise physiological basis of stay green remains unclear, the positive impact of this trait on yield under terminal drought has been confirmed in several studies (Borell et al., 2000b; Jordan et al., 2003). Moreover, this trait has little, if any, yield penalty when plants are grown under conditions where water is not limiting (Borrell *et al.*, 2000b).

Several sorghum genotypes have been identified that exhibit the stay green trait (BT x 642, SC56, E36-1) (Rosenow, 1983; Kebede *et al.*, 2001; Haussmann *et al.*, 2002b). The genotype BT x 642 (formerly B35) has been an especially useful source of stay green for research (Tuinstra *et al.*, 1997, 1998; Crasta *et al.*, 2000; Xu *et al.*, 2000) and the development of commercial hybrids (Henzell *et al.*, 2001). BT x 642 is derived from is 12555, a durra sorghum from Ethiopia. Genetic studies showed that the BT x 642 genes conferring the stay green trait act with varied levels of dominance (Walulu *et al.*, 1994) or additive fashion if the onset of senescence was analyzed (Van Oosterom et al., 1996). Several stay green QTL mapping studies have been conducted using BT x 642 as one of the parents (Tuinstra *et al.*, 2000; Tao *et al.*, 2000; Xu *et al.*, 2000).
These studies identified four major QTL designated Stage 1, stage 2, stage 3 and stage 4 and additional minor QTL that can modulate expression of the stay green trait.

2.4.2 Physiology of the stay green trait

Stay green can be defined as extended foliar greenness during grain-filling under post-anthesis drought, and can be viewed as a consequence of the balance between N demand by the grain and N supply during grain filling (Borrell *et al.*, 2001).

Three mechanisms of functional expression of stay-green have been identified (Borell *et al.*, 2000a; Thomas and Howarth, 2000) delayed onset of senescence, reduced rate of senescence, and increased LAI at anthesis. In sorghum, the expression of stay-green during grain filling can be viewed as a consequence of the balance between demand for Nitrogen (N) by the grains and supply of N through soil N-uptake and translocation from vegetative plant parts, including stems and leaves. Leaf –N translocation occurs if grain-N demand cannot be met through soil-N uptake and stem-N translocation (Van Oosterom *et al.*, 2005b). The onset of leaf senescence can be delayed by increased soil-N uptake during grain filling, as observed under terminal drought stress by Borrell and Hammer (2000). Such increased N-uptake could be associated with either increased water uptake (i.e., transpiration, T) or increased transpiration efficiency (TE) (A.K Borrell, pers. Comm.) as at least one of these is required to explain enhanced biomass accumulation and yield of stay green types. Another mechanism that delays onset of leaf senescence is through increased availability of stem-N- for translocation, although this mechanism might compromise the leaf-N status if increased stem-N is not matched by increased total N-uptake.

Increased specific leaf Nitrogen (SLN) or leaf area at anthesis can also reduce the rate of leaf senescence. Once leaf –N translocation starts, the amount of leaf area that needs to senescence in order to meet the N demand declines with improving leaf-N status (Van Oosterom *et al.*, 2005b).

Genotypic variation in maximum SLN under optimum conditions has been observed for sorghum, and was associated with differences in leaf size (Van Oosterom *et al.*, 2000a). Increased leaf area at anthesis can be achieved through increased partitioning of dry matter and N to the leaves (Borrell and Hammer, 2000), although there are also indications that increased LAI of stay green hybrids is due to inability of other hybrids to compensate for their smaller area on the main shoot through tillering, particularly if resource availability is limited (Van Oosterom *et al.*, 2005a). This frame work can explain genotypic differences and effects of

genotype-environment interaction on the expression of stay green. Phenotypic expression becomes an emergent consequence of the interplay of differences in underlying traits like leaf size, leaf SLN, dry matter partitioning, N uptake and possibly transpiration or transpiration efficiency.

Stay-green and yield were positively associated in a range of studies conducted in both Australia (Borrell *et al.*, 2000) and India (Borrell *et al.*, 1999), highlighting the value of retaining green leaf area under post-anthesis drought. Grain yield is the product of grain number and grain size. Grain number is generally the main determinant of differences in grain yield, and this has also been observed for sorghum, grown under post-anthesis drought stress in southern India (Borrell *et al.*, 1999). In wheat, grain number is a function of the spike weight around anthesis (Fischer 1993, Miralles *et al*, 1998), and similar results have been obtained for sorghum (Van Oosterom and Hammer, unpublished data). Factors, related to the stay-green mechanisms of B35 and KS19, that can potentially increase the panicle growth rate around anthesis, and hence can have a positive effect on grain number, include increased LAI 'Leaf Area Index' (Borrell *et al*, 1999), increased SLN, as that increases RUE (Muchow and Sinclair, 1994) and competition for assimilate from the stem (Borrell et al, 1991, Fischer and Stockman, 1986).

Grain size is a secondary yield determinant and is often negatively associated with grain number, e.g. Bidinger et al., (2001) for a set of 93 pearl millet hybrids. Hence, grain size is independent of green leaf area of anthesis (Borrell *et al*, 1999). However, the retention of photosynthetic capacity under water-limited conditions of stay-green hybrids ensures continued availability of new assimilates and is associated with increased N-uptake during grain filling (Borrell and Hammer, 2000), potentially improving grain size. This was illustrated in the recombinant inbred line study, where Borrell *et al,* (1999) found that grain size was correlated with relative rate of leaf senescence during grain filling such that reducing rate of leaf senescence from 3 to 1% loss of leaf area per day resulted in doubling grain size from about 15 to 30 mg. Thus stay-green can potentially increase grain yield by improving grain number and grain filing ability. Recent

genetic improvements in post-flowering drought tolerance of sorghum have been achieved through the stay-green trait (Squire, 1993).

Stay-green is the best-characterized component of terminal drought tolerance available in sorghum, (Borrell and Hammer, 2000; Borrell *et al.*, 1999, 2000a,b, Mahalakshmi and Bidinger, in press; Thomas and Howarth, 2000; Van Oosterom *et al.,* 1996; Walulu *et al* 1994; and Xu *et al.*, 2000a; 2000b). Drought resistance sources have been identified and exploited. Resistance has been shown to be highly heritable (Rosenow *et al.,* 1996). Broad-sense and narrow-sense heritabilities of 0.8 and 0.6 respectively have been reported (Walulu *et al.,* 1994). Resistance in some stay-green sources (SC35 and SC56) has been shown to be dominant while in others (BT x 625 x B35 derivative) it has been reported to be recessive (Rosenow *et al.,* 1996). Some 220 polymorphic sequence tagged microsatellite (STMS) marker loci (Tamarino et al., 1997; Kong *et al*, 2000; Tao *et al*, 2000; Bhattramakki *et al.*, 2000) have recently been added to the restriction fragment length polymorphism (RFLP) based genetic linkage map of sorghum (Peng *et al.*, 1999). These STMS marker loci are highly polymorphic and suitable for use in a backcrossing programme in which selection is based on high-throughput DNA marker genotyping.

2.5 Genetic diversity in sorghum using phenotypic traits

Analysis of genetic relationships in crop species is an important component of crop improvement programs, as it serves to provide information about genetic diversity, and is a platform for stratified sampling of breeding populations. Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including; analysis of genetic variability in cultivars (Smith, 1984; Cox *et al.*, 1986), identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kid-well, 1998), and introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al.*, 1998).

Morphological characterization is the first step in the classification and description of any crop germplasm. Quantification and classification of diversity in germplasm collections is important for plant breeders. Based on extensive collection and exploration, Vavilov (1951) observed that some areas of the world contained much greater phenotypic variability than others. These areas of greater variability were termed as centers of origin and diversity. Understanding the diversity of germplasm collections in major repositories is crucial to effective exploitation of crop genetic resources.

The genetic diversity present within a species is one of the components of biological systems allowing for further development and adaptation to changing environmental conditions. It is also the raw material available to plant breeders.

Assessment of the genetic variability within cultivated crops has a strong impact on plant breeding strategies and conservation of genetic resources (Dean *et al.*, 1999; Simioniuc *et al.*, 2002). It is particularly useful in the characterization of individuals, accessions, and cultivars in determining duplications in germplasm collections and for the choice of parental genotypes in breeding programs (Davila *et al.*, 1998; Ribaut and Hoisington, 1998). In the past, indirect estimates of similarity based on morphological information have been widely used in many species including sorghum (Ayana and Bekele, 1999). However, morphological variation does not reliably reflect the real genetic variation because of genotype-environment interactions and the largely unknown genetic control of polygenically inherited morphological and agronomic traits (Smith and Smith, 1992).

A further analysis on molecular diversity (see chapter 4) was therefore necesary so as to be able to reliably reflect the real genetic variation of the sorghum accessions collected. Further more, DNA based technologies are the most reliable and allow not only the assessment of genetic variability but also for individual DNA typing (Bling, 2000). Molecular markers based on DNA variations are also abundant in number, independent of environmental effects, can be assayed easily at any stage of the plant's development, and are free from pleiotropic or epistatic effects.

CHAPTER THREE: PHENOTYPIC SCREENING OF LOCAL GERMPLASM FOR PREFLOWERING AND POST FLOWERING DROUGHT TOLERANCE

ABSTRACT

A total of thirty six accessions were characterized and evaluated for drought tolerance and stay green during the dry seasons growing periods of 2007 and 2008. All accessions were grown in 2 m rows using an alpha lattice design. Both qualitative and quantitative traits were used to access the genetic variability. There was significant phenological variation ($P< 0.05$) in the sorghum accessions and also between the two seasons. This was an indication of the suitability of the genotypes to specific environments. Generally, accessions in season one flowered much earlier than season two. For both seasons there was a significant correlation between yield and days to 50 % flowering. Yield and the green leaf area levels were also higher in 2007 than 2008. Cluster C and A for season one and two respectively formed a group of accessions that could be useful for stable yields, a high seed mass and also a high level of stay green. From the PCA biplots, a total of three similar groups were identified in both 2007 and 2008. One group was mainly influenced by days to 50% flowering, the other one was mainly attributed to the green leaf area (stay green) and the last group was mainly influenced by yield, 100 seed mass, plant height and lodging.

3.1 Introduction

Sorghum is an important crop in some parts of East Africa. In Kenya, trials during the short rainy season showed that an improved sorghum variety KAT 369 yielded 4.1 tonnes/ha in comparison to the 3.2 tonnes/ha for maize. (ICRISAT, 1994) The precise reasons for sorghum's environmental tolerance are not fully understood, and are undoubtedly multi-factorial. Certain sorghum varieties posses "stay green" genes that enable them to continue to photosynthesize, post-flowering during drought. Non-senescence or "stay-green" is the delayed or reduced rate of normal plant senescence as it approaches maturity. Senescence is mainly expressed in the breakdown of leaf chlorophyll, reduced photosynthesis, and the general reduction in cellular capacity for various life functions. The normal progress of senescence is accelerated when drought occurs during the late developmental stage. There are several known major genes and quantitative trait loci (QTL) that delay the onset of senescence or reduce its rate. These generally do not require drought for their expression. However, when normal senescence is amplified by drought, the state of these genes is more pronounced phenotypically. Thus, constitutive non-senescence becomes more effective towards plant production when stress occurs as compared with non-stress conditions, but these genes are expressed irrespective of stress. Further research into the mechanisms of sorghum's environmental tolerance will clearly be highly beneficial.

3.2 MATERIALS AND METHODS

3.2.1 Plant Materials and Experimental site

This study was initiated to characterize the accessions, screen for drought tolerance and determine the extent of genetic diversity among sorghum accessions for various quantitative traits and qualitative traits.

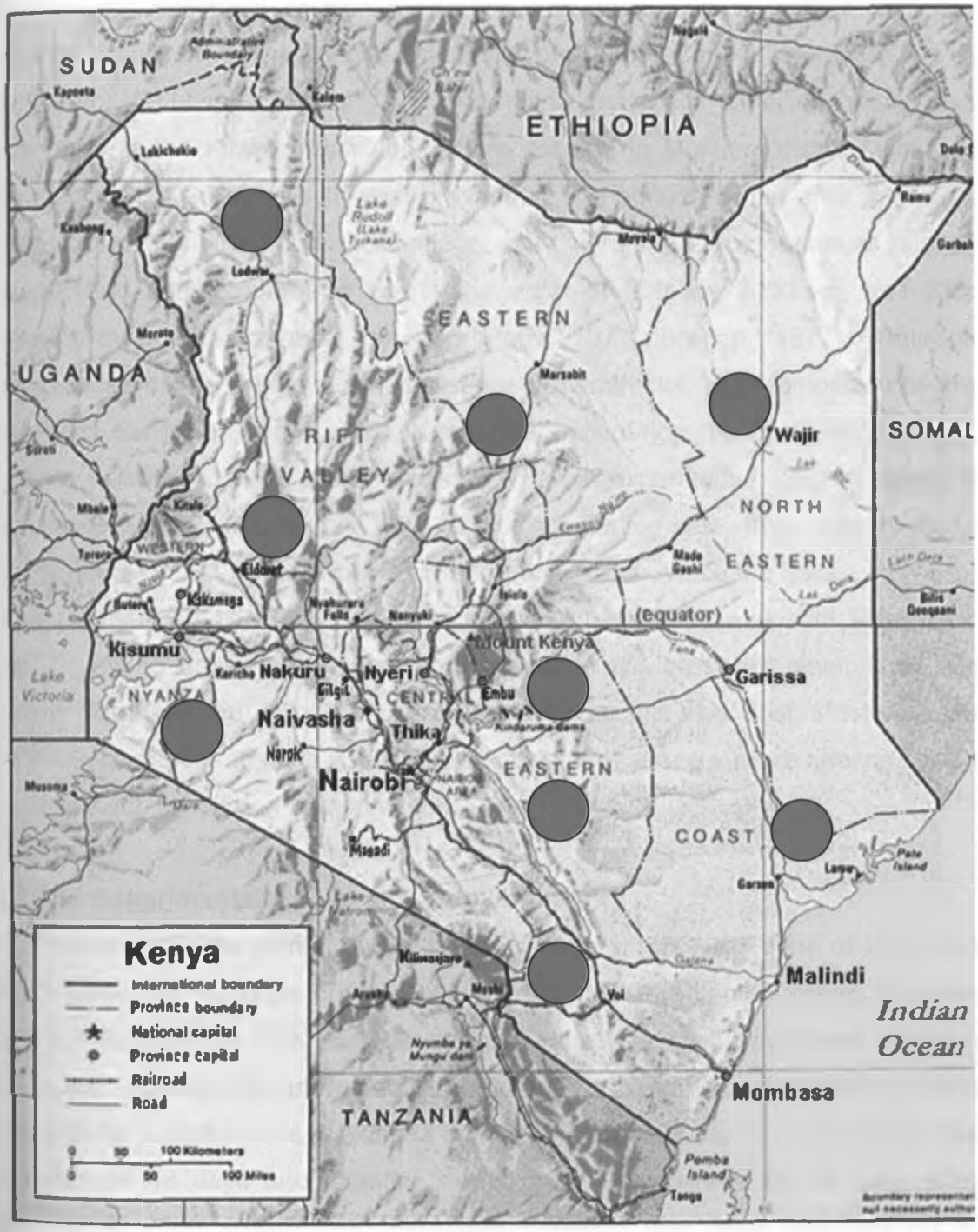


Figure 1: Map of Kenya showing the collection sites for the Landraces evaluated in this study.

- Regions where collections were done.

Germ-plasm collection of local landraces was conducted in various sorghum growing areas of Kenya by the University of Nairobi College of Agriculture & Veterinary Sciences (Figure 1).All the study materials collected were then evaluated at the Kibwezi University farm, located in Makueni district, which is situated about 200Km south east of Nairobi. The altitude of the area is around 600m a.s.l. The site is agro-climatic zone V with an average annual rainfall, evaporation and temperature are in the order of 600mm, 2000mm, and 23^0c respectively (Michieka and Van Der Pouw 1977; Braunn, 1977). Although temperature varies with altitude, the area is generally hot. High temperatures are expected during day time and low temperatures during nights. During the dry periods, between May and October the area experiences intense heat. It experiences less wind and a high evapotranspiration rate. The highest mean temperatures (32-33^0c) prevail during February- March, while the lowest (15-16^0c) during July-August (MDDP 1993). According to Michieka and Van Der Pouw (1977), red to brown sandy clay soils prevail in the erosional plain. They are mainly Ferralsols, but Nitosols, Luvisols, and Cambisols also exist. Most of these soils are compact and have a massive structure with strong surface sealing, which causes much runoff during heavy rains.

3.3 The Experimental design and crop husbandry

The experiment was carried out in the dry months of May and June of 2007 and 2008 respectively and the crop mainly relied on the stored soil moisture.This was a rain free period in both seasons and therefore furrow and overhead irrigation was used to germinate and establish the crop. The field was irrigated to full field capacity for 3 days before planting in both 2007 and 2008 seasons. The crop was irrigated for 18 days after planting during 2007 season and for 25 days after planting in 2008 season and thereafter irrigation was then with-held up to harvesting. Terminal drought stress was induced by allowing the accessions to depend on the residual soil moisture. The accessions were planted in three replicates using an alpha lattice design. The row plots were 2m in length, with inter and intra row spacing of 1m and 0.75m respectively with four seeds/hill.

Thinning was done to two plants per hill after two weeks. Weeding was done three times across plots. The plants were sprayed using the recommended insecticides *bull-dock* and *duduthrin (Karate)* to prevent insect pest damage after emergence and also before flowering.

3.4 Agro-morphological traits, data Collection and analysis

Characterization of the accessions and the screening for drought was done using 9 qualitative and quantitative traits based on IBPGR/ICRISAT Descriptors (1993) during the crop growth and development. Rainfall and temperature data for the experimental site during the cropping season were also recorded. Three uniform plants were selected and tagged in every plot for data collection. Measurement of the traits was done as follows;

Visual and quantitative scoring of stay green was done on a scale of 1 – 9 on an individual plant basis using the lead and plant death (LPD) scores at physiological maturity, on a plot basis. Score 1 indicates very slightly senescent whereas 9 indicates completely senescent (leaves and stalk dead).

Quantitative measurement of stay green was also recorded. At the time of emergence of the flag leaf, three uniform plants in each plot were tagged, the length and breadth of the upper six leaves measured, and the area of each estimated as: (Leaf length x leaf width x 0.70). All these measurements were done at 15, 30 and 45 days after flowering. The rate of senescence was calculated by plotting values of stay green from 15 days to 45 days after flowering and the slope was determined.The formular used to calculate the rate of senescence in this study is as shown below;

$$\text{Rate of senescence } (cm^2\ plant^{-1} day^{-1}) = \frac{(GLA15 - GLA45)}{(45\text{days} - 15\text{ days})}$$

Where, GLA15; Green leaf area/ stay green at 15 days after flowering

GLA45; Green leaf area/ stay green at 45 days after flowering

Lodging susceptibility was recorded whereby a scale of 3 indicated low lodging susceptibility and a scale of 1 indicated high lodging susceptibility.

Data on flowering was recorded as the number of days from planting to when 50% of the plants in each plot flowered. Plant height was also measured in centimeters from the base of the plant to the tip of the parnicle. Ten representative plants were sampled in each plot for this trait.

Analysis of variance (ANOVA) was done using Genstat Release 9.1 at 5 % level of significance and for the treatment effects that were significant, mean separation was done by Fisher's Least Significant Difference (L.S.D) method.

Broad-sence heritability was also calculated using both genotypic and phenotypic variances. The genetic (or genotypic) variance in a cultivar trial is the variance of the cultivar effects, is denoted by σ^2_G.The phenotypic variance in a cultivar trial is the variance of cultivar means across replications. It is denoted σ^2_P. because means are based on plot measurements, which contain both G_i's and e_j's, σ^2_P contains both the genetic variance and a portion of the residual variance:

$$\sigma^2_P = \sigma^2_G + \sigma^2_e/r$$

Where, σ^2_e is the plot residual or error variance from the ANOVA, and r is the number of reps. σ^2_G and σ^2_e are estimated from the ANOVA as follows:

Source of variation	**Df**	**Mean square**	**Expected mean square**
Replicates	r-1		
Genotypes	g-1	MS_G	$r\sigma^2_G + \sigma^2_e$
Residual	(r-1)(g-1)	MS_E	σ^2_e

Thus, to estimate σ^2_G:

$$\sigma^2_G = (MS_G - MS_E)/r$$

σ^2_e is estimated directly as the error variance of the experiment.

The repeatability of a variety trial is the proportion of the variation among line means that is due to the variation in genotype effects. This statistic, that also denoted broad-sense heritability (H), is calculated as:

$$H = \sigma^2_G / \sigma^2_p$$
$$= \sigma^2_G / [\sigma^2_G + \sigma^2_e / r]$$

H is an important measure of the precision (reliability) of an experiment.

A cluster analysis was carried out using coordinates derived from evaluation of each accession based on Euclidean distance matrix. The weighted distance aggregation criterion in an ascending hierachical way (Spark, 1973; Fundora Mayor, 2004) was calculated using average linkage analysis in Genstat 9.0. Quantitative traits from this study were subjected to Principal Component Analysis (PCA) using Genstat 9.0. Multivariate-principal component analysis was conducted between variance-covariance matrix to determine patterns of variation and major traits contributing to the delineation.

3.5 RESULTS

3.5.1 Drought related quantitative traits

3.5.1.1 Days to 50% flowering

This is a key trait in evaluating drought escape. Significant differences ($P < 0.05$) were detected among the genotypes and also across seasons with accessions in 2007 flowering much earlier than 2008. (Appendix 4), It was revealed from Table 1 that days to flowering in 2007 ranged from 47 to 107 days for accessions UON48381 and UON 43324 respectively. The mean value for days to flowering for 2007 was 73 days. Two sorghum accessions, UON 48381 and UON 32 flowered in less than 50 days (Early materials) that is, 47 and 49 days respectively. A total of 10 accessions flowered within a range of 50 to 69 days, 19 accessions flowered within 70 to 100 days. UON 43964, UON 43024 and UON 43324 flowered within 101,105 and 107 days respectively (late materials).

During the year 2008, the days to flowering varied from 63 days to 133 days in accessions UON16 and UON 43964 respectively (Table 2). The mean value for days to flowering was 89 days. Among some of the earliest materials were UON 16, UON 32, UON 959, UON 48381, UON 998 that flowered in 63,67,67,68 and 69 days respectively. 21 accessions flowered between 70 and 100 days thus forming the bulk of the materials. The rest, 8 accessions flowered late at 102 days to 133 days.

Early flowering enables a genotype to escape drought during the critical reproductive stages. The early materials identified in 2007 and 2008 can be used as good sources for selection and breeding for earliness.

Table 1:Table of means of drought related traits for the accessions evaluated at the Kibwezi field station during the dry season of 2007

Entry (Uon)	Days to flowering	Lodging	Plant Height (cm)	100 seed mass(g)	Tillers	Yield (Kg/ha)	GLA 15daf(Cm^2 Plant^{-1})	GLA 30daf(Cm^2 Plant^{-1})	GLA 45daf(Cm^2 Plant^{-1})
15	54	8	108	2	5	599	0	0	0
16	57	5	102	5	6	65	1817	1329	907
28	77	8	99	3	10	279	0	0	0
31	72	5	155	2	8	2162	952	668	329
32	49	6	121	2	4	505	966	678	341
47	71	5	141	6	12	2686	1101	776	528
50	71	4	146	4	14	2606	1083	765	651
59	89	5	132	4	21	153	1185	882	496
62	70	5	126	2	15	2449	1403	990	658
66	70	6	103	6	8	1727	1080	830	333
98	71	6	148	1	10	794	1093	771	455
412	87	6	173	5	15	1905	1141	806	481
445	72	3	121	6	8	3062	1156	835	432
959	51	5	161	6	18	1325	1525	1040	509
998	53	5	145	5	28	1523	1119	791	341
999	90	6	203	3	9	801	1149	810	383
32248	71	5	138	3	9	2518	1518	1048	464
32358	100	3	85	1	6	163	969	685	269
43024	105	6	167	1	7	565	1104	851	472
43063	90	5	141	5	14	1943	1023	1199	599
43323	81	4	139	2	8	574	1536	1089	595
43324	107	4	113	1	10	56	1105	910	450
43723	88	4	65	2	16	217	1527	1079	531
43738	69	2	83	6	5	848	1640	1246	713
43964	101	5	138	1	13	706	1082	811	425
43971	81	11	208	2	20	1518	1101	777	466
44043	54	11	132	2	9	1877	694	676	328
44044	57	18	168	1	13	1516	0	0	0
48367	92	5	153	6	5	328	1206	870	402
48378	53	6	119	4	12	2439	1676	1160	629
48381	47	6	135	2	14	993	1294	784	352
48383	50	6	91	2	9	2656	851	608	348
48387	52	5	131	3	8	1913	0	0	0
48392	74	8	117	5	10	1478	0	0	0
Mean	72.78	5.89	132.6	3.34	11.11	1322	1032	752	413
$LSD_{0.05}$ Variety (V)	4.95	3.74	37.67	0.23	3.3	367.5	223.6	184	123.62
$LSD_{0.05}$ Season (S)	1.2	0.91	9.14	0.06	0.8	89.1	54.2	44.6	29.98
$LSD_{0.05}$ (VxS)	7	5.29	53.27	0.33	4.6	519.7	316.3	260.2	174.83
CV (%)	5.4	73.8	21.8	7.6	39.9	22.5	17.3	19	20.9

Table 2: Table of means for drought related traits for the accessions evaluated at the Kibwezi field station during the dry season of 2008

Entry (Uon)	Days to flowering	Lodging	Plant Height (cm)	100 seed mass(g)	Tillers	Yield (Kg/ha)	GLA 15daf (Cm^2 $Plant^{-1}$)	GLA 30daf (Cm^2 $Plant^{-1}$)	GLA 45daf (Cm^2 $Plant^{-1}$)
15	70	4	157	2	4	2086	1006	882	531
16	63	3	136	3	3	1091	574	473	264
28	85	3	162	2	3	1625	1613	1250	921
31	94	3	174	2	4	1015	2270	1828	1338
32	67	3	159	2	3	1061	566	430	216
47	88	3	164	3	3	1928	1809	1414	1021
50	92	3	159	2	3	2313	1201	920	636
59	83	3	174	1	4	972	1513	1094	743
62	82	3	160	2	4	1900	1284	857	493
66	92	3	98	3	3	1559	2056	1480	942
98	84	3	175	3	3	1798	1125	796	522
412	100	3	166	2	3	1643	0	0	0
445	80	3	177	2	3	3400	1384	983	706
959	67	3	187	2	3	1366	1139	824	537
998	69	3	166	3	3	1300	0	0	0
999	102	3	367	2	3	349	0	0	0
32248	90	3	156	3	4	2016	811	641	362
32358	114	3	153	2	3	403	1022	810	474
43024	107	3	160	2	4	1199	1449	1134	852
43063	102	3	162	1	4	1836	2154	1553	829
43323	98	3	174	1	4	2038	2238	1722	1262
43324	123	3	168	2	3	1586	551	417	273
43723	103	3	169	2	3	728	1100	937	574
43738	92	3	174	2	3	947	1124	988	577
43964	133	3	168	2	3	1586	1029	835	489
43971	122	3	168	2	4	1586	1831	1369	1000
44043	70	3	161	2	4	1813	1830	1397	855
44044	70	3	173	2	4	2717	1132	799	546
48367	99	3	137	2	3	1165	1446	1103	738
48378	77	3	160	3	4	419	1760	1331	1032
48381	68	3	149	2	3	2748	920	721	437
48383	70	3	172	3	3	1884	1548	1226	856
48387	72	3	157	3	3	1587	945	745	455
48392	83	3	155	2	4	2254	1336	944	675
Mean	88 61	3	167.6	2.17	3.37	1586	1228	938	622
$LSD_{0\,05}$ Variety (V)	4 95	3.74	37.67	0.23	3.3	367.5	223.6	184	123 62
$LSD_{0\,05}$ Season (S)	1.2	0.91	9.14	0.06	0.8	89.1	54.2	44.6	29 98
$LSD_{0\,05}$ (VxS)	7	5.29	53.27	0.33	4.6	519.7	316.3	260.2	174.83
CV (%)	5.4	73.8	21.8	7.6	39.9	22.5	17.3	19	20.9

Table 3: Table of combined means for the drought related traits measured in the 2007 and 2008 season.

Entry (Uon)	Days to flowering	Lodging	Plant Height (cm)	100 seed mass (g)	Tillers	Yield (Kg/ha)	GLA 15daf (Cm^2 $Plant^{-1}$)	GLA 30daf (Cm^2 $Plant^{-1}$)	GLA 45daf(Cm^2 $Plant^{-1}$)
15	62	6	132	2	4	1342	503	441	265
16	60	4	119	4	5	578	1195	901	586
28	81	6	131	2	7	952	806	625	530
31	83	4	165	2	6	1589	1611	1248	833
32	58	5	140	2	4	783	766	554	279
47	79	4	153	4	7	2307	1455	1095	775
50	82	4	153	3	9	2460	1142	843	644
59	86	4	153	3	13	563	1349	988	619
62	76	4	143	2	10	2174	1343	924	575
66	81	4	101	5	6	1643	1568	1155	638
98	78	4	162	2	6	1296	1109	783	488
412	94	4	170	3	9	1774	571	403	241
445	76	3	149	4	5	3231	1270	909	569
959	59	4	174	4	11	1346	1332	932	523
998	61	4	156	4	16	1412	559	396	171
999	96	5	285	3	6	575	575	405	192
32248	81	4	147	3	6	2267	1165	844	413
32358	107	3	119	1	4	283	996	748	371
43024	106	5	164	2	6	882	1277	993	662
43063	96	4	152	3	9	1890	1589	1376	714
43323	90	3	157	2	6	1306	1887	1405	928
43324	115	3	140	2	7	821	828	663	362
43723	96	4	117	2	9	472	1314	1008	553
43738	81	2	128	4	4	897	1382	1117	645
43964	117	4	153	2	8	1146	1055	823	457
43971	102	7	188	2	12	1552	1466	1073	733
44043	62	7	146	2	6	1845	1262	1036	592
44044	64	11	171	2	8	2117	566	400	273
48367	95	4	145	4	4	746	1326	987	570
48378	65	5	139	4	8	1429	1718	1245	831
48381	58	4	142	2	9	1871	1107	753	394
48383	60	5	131	3	6	2270	1199	917	602
48387	62	4	144	3	6	1750	473	373	227
48392	78	6	136	3	7	1866	668	472	338
Mean	80.2	4.44	151.1	2.66	7.27	1427	1130	848	517.4
$LSD_{0.05}$ Variety (V)	4.95	3.74	37.67	0.23	3.3	367.5	223.6	184	123.62
$LSD_{0.05}$ Season (S)	1.2	0.91	9.14	0.06	0.8	89.1	54.2	44.6	29.98
$LSD_{0.05}$ (VxS)	7	5.29	53.27	0.33	4.6	519.7	316.3	260.2	174.83
CV (%)	5.4	73.8	21.8	7.6	39.9	22.5	17.3	19	20.9

3.5.1.2 Lodging

This trait is associated with stay green and drought tolerance. Significant variation was detected in lodging among the genotypes ($P<0.05$) as well as across seasons.(Appendix 5) During the dry season of 2007, the number of plants lodged ranged from as low as 2 per accession to as high as 18 in accessions UON 43738 and UON 44044 respectively (Table 1).The mean value of plants lodged per accession was 6 per plant. UON 43738,UON 445, UON 32358 had the least lodged plants that is, 2 plants,3 plants and 3 plants respectively, 74 % the accessions ranged between 4 to 6 lodged plants.

During the 2008 season, there was more uniformity with majority of the accessions, 97 % having a total of 3 lodged plants (Table 2). Only one accession UON 15 had 4 lodged plants.

3.5.1.3 Plant Height

Plant height is a key trait in reflecting drought escape, biomass apportioning and yield There was sufficient genetic variability observed ($P<0.05$) in case of plant height among genotypes and also from season to season (Appendix 6). Plant height during the year 2007 varied from 65 to 208 cm in accessions UON 43723 and UON 43971 respectively (Table 1). The mean height for the accessions was 132.6 cm with 4 accessions noted to be of short stature of less than 91cm, they included UON 48383, UON32358, UON 43738, UON 43723, with heights of 91cm, 85cm, 83cm and 65cm respectively. A fairly large number of accessions (24) ranged between 100cm to 155cm. Only two accessions; UON 999 and UON 43971 were over 200cm with a height of 203 and 208cm respectively.

The plant height ranged from 98cm to 367cm in season two for UON 66 and UON 999 respectively. The accessions were much taller in this season with a mean height of 167cm.The bulk of the accessions (31) fell between 136cm and 177cm. Only UON 999 recorded a height of 200cm with a plant height of 367cm.

3.5.1.4 100-seed weight

A 100 seed mass is important in evaluating desiccation tolerance of crops. According to the ANOVA table (Appendix 7), significant ($P<0.05$) differences among the accessions were detected. The statistics in Tables 1and 2 showed that 100 grain weight in season one ranged from 1 gram in accessions UON 43964, UON 43324 UON 43024, UON 32358, UON 44044 and UON98 to 6 grams in accessions UON 959 UON 48367, UON 66, UON 47, UON 43738 and UON 445. The mean for this season was 3.34 grams with almost a third (10) of the accessions attaining a weight of 2 grams. This was followed by two other groups of accessions (6 per group) with weights of 6 grams and 1 gram respectively. Accessions with 4 grams formed the smallest group with only 3 accessions.

During 2008 100 grain weight ranged from1 gram in accessions UON(43063,43323,59) to 3 grams in accessions UON (48387, 998, 32248, 48378, 66,47,48383,98,16)and a mean 100-seed weight of 2.17 grams was noted. The largest group of accessions (22) had a 100-seed weight of 2 grams. Only 3 accessions had a 100-seed weight of 1 gram, they include UON 43063, UON 43323 and UON 59. These accessions that were large seeded under moisture stress can be said to be tolerant accessions and can thus be used in the selection of drought.

Table 4: Summary of ANOVA for all the traits measured

Trait	m.s	v.r	F pr.
Days to flower	825754	21.53	<.001 **
Lodging	466091	17.19	<.001 **
Plant height	6194	5.7	<.001 **
100 seed mass	7.49	182.07	<.001 **
Number of tillers	40.39	4.79	<.001 **
Yield	3051793	29.49	<.001 **
15 DAF	825754	21.53	<.001 **
30 DAF	466091	17.19	<.001 **
45 DAF	203889	17.4	<.001 **

Photo 1: Phenotypic diversity of Sorghum accessions grown at the Kibwezi University Farm during the 2007 and 2008 seasons

Photo 2: Stay green accessions grown at Kibwezi University farm during the 2007 and 2008 seasons.

3.5.1.5 Number of tillers per plant

The number of productive tillers is a good measure of a crop's resilience under water limited conditions. Differences in the number of tillers between the two seasons and among genotypes were significant (P 0.05) (Appendix 8). During the season of 2007, the number productive tillers per plant ranged from 4 tillers for accession UON 32 to 28 in accession UON 998 with a mean value of 11 (Table 1) This was followed by accessions with only 5 productive tillers that is, UON 48367, UON 32358 and UON 15. Most of the other accessions had 8 to 15 tillers per plant. Only UON 998, UON 59 and UON 43971 had more than 20 tillers per plant with 20, 21 and 28 tillers respectively.

The second season of 2008 had a range of 3 tillers in accession UON 47 to 4 tillers in accession UON62 (Table 2). The mean number of productive tillers per plant was 3.37. In this season only, two distinct groups were clearly visible; one large group consisting of 21 accessions with 4 tillers per plant and the second group of 13 accessions that had 4 tillers per plant. Materials that had productive tillers can be termed to be responsive to drought and can be utilized in drought selection.

3.5.1.6 Yield

Yield is a key component in the measurement of drought. The variation in yield during the 2007 season was significant ($P<0.05$) among the genotypes and also from season to season (Appendix 9). The yield of the accessions ranged from 56 Kg/ha for accession 43324 to 3062 Kg/ha for accession 445 (Table 1). The mean yield per accession was 1322Kg/ha.The best performing accession was UON 445, followed by accessions UON47 and UON 48383 with yield of 3062 Kg/ha,2686 Kg/ha and 2656 Kg/ha respectively. Three groups were fairly distinguishable in terms of yield with the first one containing 16 accessions that had yields of less than a 1000Kg/ha, the second group of 10 accessions had yields ranging from 1325 to 1943 Kg/ha and lastly, the third and smallest group of 8 accessions had yields of more than 2000Kg/ha.

During the second season of 2008 the yield varied from as low as 349 Kg/ha in accession UON 999 to as high as 3400 Kg/ha in accession UON445.(Table 2). Some of the best performing accessions were UON 445, UON 48381, and UON 44044 with yields of 3400 Kg/ha,2748 Kg/ha and 2717 Kg/ha respectively. During this year, the bulk of the accessions (20) had yields ranging from 1000 to 2000 Kg/ha. Only UON 445 had yields of more than 3000Kg/ha. Accessions UON 999, UON 32358 and UON 48378 had the least yields of 367 Kg/ha,403 Kg/ha and 419 Kg/ha respectively. In general, average yields for year 2008 were higher than the 2007 season. This is because in season one the crops were more highly stressed than season two.

Accessions such as UON 445, UON 50) showed consistency in high yields in both seasons (Table 1, 2 and 3). Such materials can be quite useful in future drought screening programs.

3.5.1.7 Green Leaf Area (Stay green)

Retention of green leaf area in grain sorghum under post-anthesis drought also known as stay green, is associated with greater biomass production, lodging resistance and yield.

3.5.1.7.1 Stay green 15 days after flowering

At 15 days after flowering, the green leaf area of the accessions was significant ($P<0.05$) among the genotypes and also across the seasons for both seasons (appendix 10). The green leaf area for the accessions vary from as low as 0 cm^2 plant-1 to as high as 1817 cm^2 plant-1 in accessions UON 15 and UON 16 respectively. The mean for green leaf area at 15 days was 1032 cm^{-2} $plant^{-1}$. UON 16, UON 48378 and UON 43738 had the highest levels of green leaf area at fifteen days at 1817 cm^2 $plant^{-1}$,1676 cm^2 $plant^{-1}$ and 1640 cm^2 $plant^{-1}$ respectively. The bulk of the accessions (17) mainly clustered within the range of 1023 $cm^{-2}plant^{-1}$ to 1403 cm^2plant^{-1}. UON (1528, 44044, 48387 and 48392) had the lowest green leaf area at 15 days after flowering that is, 0 $cm^{-2}plant^{-1}$.

During the second dry season, the green leaf area of the accessions at 15 days after flowering ranged from as low 0 cm^2 $plant^{-1}$ to as high as 2270 cm^2 $plant^{-1}$ in accessions UON 412 and UON 31 respectively. The mean green leaf area at 15 days after flowering was 1228 cm^2 plant-1. Among the accessions with the highest green leaf area at 15 days after flowering were UON 31, UON 43323, UON 43063 and UON 66 with green leaf areas of 2270 cm^2 plant-1,2238 cm^2 $plant^{-1}$,2154 cm^2 $plant^{-1}$ and 2056 cm^2 $plant^{-1}$ respectively. A good number of accessions (21) fell between the range of 1006 cm^2 plant-1 and 1831 cm^2 $plant^{-1}$. Most of these materials were from North Eastern,Rift Valley and Eastern parts of Kenya. UON 412, UON 998 and UON 999 had the lowest green leaf area at 15

days that is, 0 cm^2 $plant^{-1}$. These materials were mostly from Rift Valley and Central parts of Kenya.

Materials that had a fairly high level of stay green at 15 days after flowering in both 2007 and 2008 could be attributed to a lower rate of senescence or delayed onset of senescence for the accessions under drought.

3.5.1.7.2 Stay green 30 days after flowering

From the ANOVA tables (Appendix 11), there were significant differences ($P<0.05$) among the genotypes and also from one season to another. The green leaf area at 30 days ranged from 0 cm^2 $plant^{-1}$ to 1329 cm^2 $plant^{-1}$in accessions UON 15 and UON 16 respectively (Table 1). The mean green leaf area at 30 days after flowering was 752 cm^2 $plant^{-1}$.Accessions with the highest green leaf area at 30 days after flowering were UON16, UON 43738, UON 43063 and UON 48378 with green leaf areas of 1329 cm^2 $plant^{-1}$, 1246 cm^2 $plant^{-1}$, 1199 cm^2 $plant^{-1}$ and 1160 cm^2 $plant^{-1}$ respectively. UON (15, 28, 48387, 48392 and 44044) had the lowest green leaf area of 0 cm^2 $plant^{-1}$.

The green leaf area during the second season ranged from as low as 0 cm^2 $plant^{-1}$ to as high as 1828 cm^2 $plant^{-1}$ in accessions UON 412 and UON 31 respectively (Table 2). The mean green leaf area at 30 days after flowering was 938 cm^2 $plant^{-1}$.UON 31, UON 43323 and UON 43063 had the highest levels of green leaf area at 30 days after flowering that is, 1828 cm^2 $plant^{-1}$,1722 cm^2 $plant^{-1}$ and 1553 cm^2 $plant^{-1}$ These materials were from Rift Valley-west pokot, Transzoia and Western-Kakamega respectively. UON 412, UON 998 and UON 999 had the lowest green leaf area at 15 days that is, 0 cm^2 $plant^{-1}$. These accessions were from south Nyanza, Eastern Kitui and Eastern Kitui respectively.

Materials that had a fairly high level of stay green at 30 days after flowering in both 2007 and 2008 could be attributed to a lower rate of senescence or delayed onset of senescence for the accessions under the drought conditions.

3.5.1.7.3 Stay green 45 Days after flowering

Significant differences ($P<0.05$) were detected among the genotypes and across the seasons (Appendix 12).In the year 2007,green leaf area at 45 days ranged from 0 cm^2 $plant^{-1}$ to 907cm^2 $plant^{-1}$in accessions UON (15, 44044,48387, 48392,28) and UON 16 respectively (Table 1). Accessions that had the highest levels of green leaf area at 45 days included UON 16,UON 43738,UON 62,UON 48378 with leaf areas of 907 cm^2 $plant^{-1}$,713 cm^2 $plant^{-1}$,658 cm^2 $plant^{-1}$,651 cm^2 $plant^{-1}$ and 629 cm^2 $plant^{-1}$ respectively.

In the 2008 season, the green leaf area at 45 days ranged from as low as 0 cm^2 $plant^{-1}$to 1338 cm^2 $plant^{-1}$in accessions UON 412 and UON 31 respectively (Table 2) Accessions with the highest levels of stay green included UON 31, UON 43323,UON 48378 and UON 47 with green leaf area at 45 days of 1338 cm^2 $plant^{-1}$,1262 cm^2 $plant^{-1}$,1032 cm^2 $plant^{-1}$,1021 cm^2 $plant^{-1}$ and 1000 cm^2 $plant^{-1}$respectively. These accessions were from West Pokot, Keiyo and North Eastern parts of Kenya. UON 412, UON 998 and UON 999 had the lowest green leaf area at 15 days that is, 0 cm^2 $plant^{-1}$. The accessions were from South Nyanza and Eastern itui and Eastern Kitui. Extended foliar greenness (stay green) in grain sorghum has been associated with higher yield levels under water limited conditions. This was observed to be true for accessions UON 48373, UON 43323 and UON 47 that were consistent in both seasons for both of these traits.

3.5.1.7.4 Rate of senescence

This trait is a factor of stay green measured as explained in the preceding sections. It is a measure of green leaf retention (stay green) over a 45 day period after flowering. The rate of senescence was calculated as follows;

$$\text{Rate of senescence } (cm^2\ plant^{-1} day^{-1}) = \frac{(GLA15 - GLA45)}{(45\text{days} - 15\text{ days})}$$

Where, GLA15; Green leaf area/ stay green at 15 days after flowering

GLA45; Green leaf area/ stay green at 45 days after flowering

Table 5.0: Rates of senescence for year 2007 and 2008

Accession	Season one rate of senescence ($cm^2\ plant^{-1}day^{-1}$)	Season two rate of senescence ($cm^2\ plant^{-1}day^{-1}$)	combined rate of senescence ($cm^2\ plant^{-1}day^{-1}$)
UON15	-	15.8	7.9
UON16	30.3	10.3	20.3
UON28	-	23.1	9.2
UON31	20.8	31.1	25.9
UON32	20.8	11.7	16.2
UON47	19.1	44.2	22.7
UON50	14.4	32.5	16.6
UON59	23	19.5	24.3
UON62	24.8	23.6	25.6
UON66	24.9	24.2	31
UON98	21.3	23	20.7
UON412	22	25.7	11
UON445	24 1	19.9	23.4
UON959	33.8	16.1	27
UON998	25.9	16.4	13
UON999	25.5	22	12.8
UON32248	35.1	26.3	25.1
UON32358	23.4	18.8	20.8
UON43024	21.1	26.4	20.5
UON43063	14.1	37.1	29.1
UON43323	31.4	20.1	32
UON43324	21.8	-	15.5
UON43723	33.2	22.6	25.4
UON43738	30.9	20.1	24.6
UON43964	21.9	-	19.9
UON43971	21.1	-	24.4
UON44043	12.2	15	22.4
UON44044	-	18.3	9.8
UON48367	26.8	32.5	25.2
UON48378	34.9	9.2	29.6
UON48381	31.4	17.5	23.8
UON48383	16.7	18.2	19.9
UON48387	-	18	8.2
UON48392	-	27.7	11
Means	20.8	20.2	20.4

From Table 5.0 above, accessions UON 28, UON 44043 and UON 43063 showed the least rate of green leaf area senescence for the 15 days after the onset of

flowering with rates of 12.2 cm^2 $plant^{-1}day^{-1}$, 14.1 cm^2 $plant^{-1}day^{-1}$ In the same season accessions UON 959, UON 48378 and UON 32248 were observed to have the highest rates of stay green senescence at 33.8 cm^2 $plant^{-1}day^{-1}$, 34.9 cm2 $plant^{-1}day^{-1}$ and 35.1 cm^2plant^{-1} day^{-1} respectively.

In the 2008 season, accessions UON 48378, UON 16 and UON 32 were observed to have the lowest rates of stay green senescence of 9.2 cm^2 $plant^{-1}$ day^{-1}, 10.3 cm^2 $plant^{-1}day^{-}$ and 11.7 cm^2 $plant^{-1}day^{-1}$ respectively. Accessions UON 50, UON 43063 and UON 47 were observed to have the highest rates of stay green senescence of 32.5 cm^2 $plant^{-1}day^{-}$, 37.1 cm^2 $plant^{-1}day^{-1}$ and 44.2 $cm^2plant^{-1}day^{-1}$ respectively.

3.6 Correlation among the traits

Table 6: Correlation table for the quantitative traits in 2007

	Dtf	Lodging	Plant height	100 seedmass	Tillers	Yield	15daf	30daf	45daf	Rate of senescence
Dtf	1									
Lodging	-0.27	1								
Plant height	0.13	0.37 (*)	1							
100 seedmass	-0.18	-0.26	0.01	1						
Tillers	-0.03	0.1	0.31	0.13	1					
Yield	-0.38 (*)	0.06	0.18	0.31	0.16	1				
SG15daf	0.15	-0.56	-0.01	0.25	0.14	-0.01	1			
SG30daf	0.22	-0.54	-0.02	0.27	0.13	-0.01	0.97	1		
SG45daf	0.17	-0.5	-0.02	0.27	0.12	0.03	0.9	0.92	1	
Rate of senescence	0.12	-0.55	-0.01	0.21	0.14	-0.06	0.96	0.89	0.74	1

* Correlation is significant at the 0.05 level (1-tailed)

Table 7: Correlation table for the quantitative traits in 2008.

	Dtf	Lodging	Plant height	100 seed mass	Tillers	Yield	15daf	30daf	45daf	Rate of senescence
Dtf	1									
Lodging	-0.27	1								
Plant height	0.13	0.37 (*)	1							
100 seed mass	-0.18	-0.26	0.01	1						
Tillers	-0.03	0.1	0.31	0.13	1					
Yield	-0.38 (*)	0.06	0.18	0.31	0.16	1				
SG15daf	0.15	-0.56	-0.01	0.25	0.14	-0.01	1			
SG30daf	0.22	-0.54	-0.02	0.27	0.13	-0.01	0.97	1		
SG45daf	0.17	-0.5	-0.02	0.27	0.12	0.03	0.9	0.92	1	
Rate of senescence	-0.08	-0.23	-0.07	-0.13	-0.15	0.14	0.97	0.98	0.99	1

* Correlation is significant at the 0.05 level (1-tailed)

Table 8: Correlation table for the quantitative traits in 2007 and 2008.

	Dtf	Lodging	Plant height	100 seed mass	Tillers	Yield	15daf	30daf	45daf	Rate of senescence
Dtf	1									
Lodging	-0.38 (*)	1								
Plant height	0.19	0.02	1							
100 seed mass	-0.40 (*)	-0.2	-0.09	1						
Tillers	-0.09	-0.08	0.22	0.23	1					
Yield	-0.34 (*)	0.07	0.02	0.26	0.15	1				
SG15daf	0.11	-0.65	0	0.29	-0.04	0.09	1			
SG30daf	0.11	-0.64	-0.04	0.28	-0.07	0.13	0.98	1		
SG45daf	0.12	-0.63	-0.06	0.25	-0.1	0.14	0.95	0.96	1	
Rate of senescence	0.18	-0.2	-0.05	-0.11	-0.2	0.24	0.39	0.42	0.34	1

* Correlation is significant at the 0.05 level (1-tailed)

Dtf-days to 50% flowering,SG15daf-stay green 15 days after 50% flowering, SG 30daf-Stay green 30 days after 50% flowering,SG45daf-Stay green 45 days after 50% flowering.

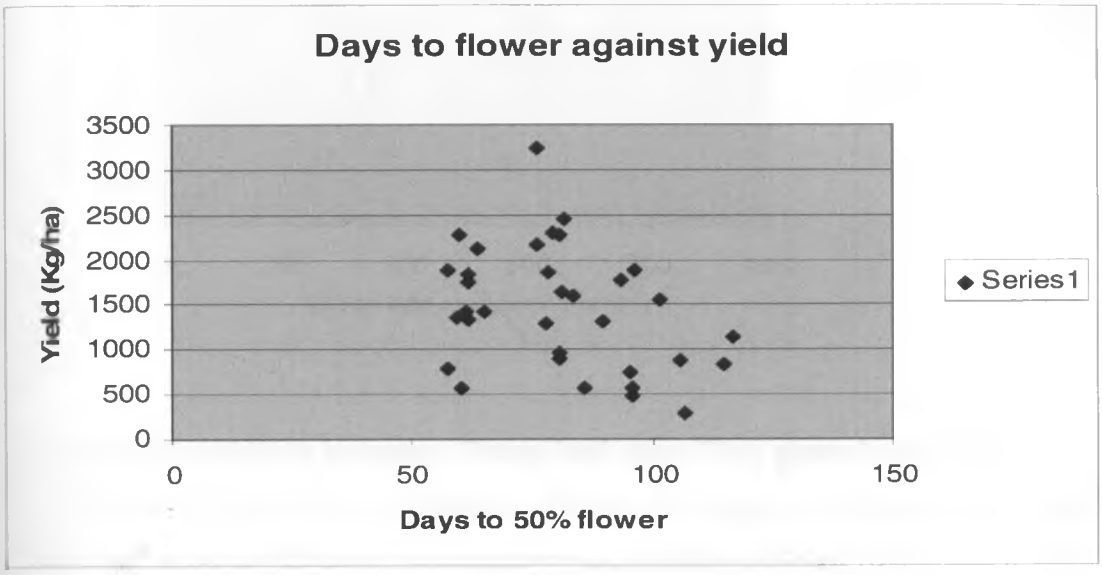


Figure 2: Correlation between days to 50% flowering and yield

Results from Table 8.0 and Figure above showed significant negative (-0.34) correlations between yield and the days to 50% flowering. Early flowering was noted to be a key factor in relation to high yields. Accessions flowering between 50 to 80 days were observed to have good yield ranging from around 1400 Kg/ha to as high as 2500 Kg/ha. Most of the accessions that flowered after a 100 days were observed to have reduced yields. This could partly be attributed to more photosynthates being apportioned to biomass production of the plants rather than seed production.

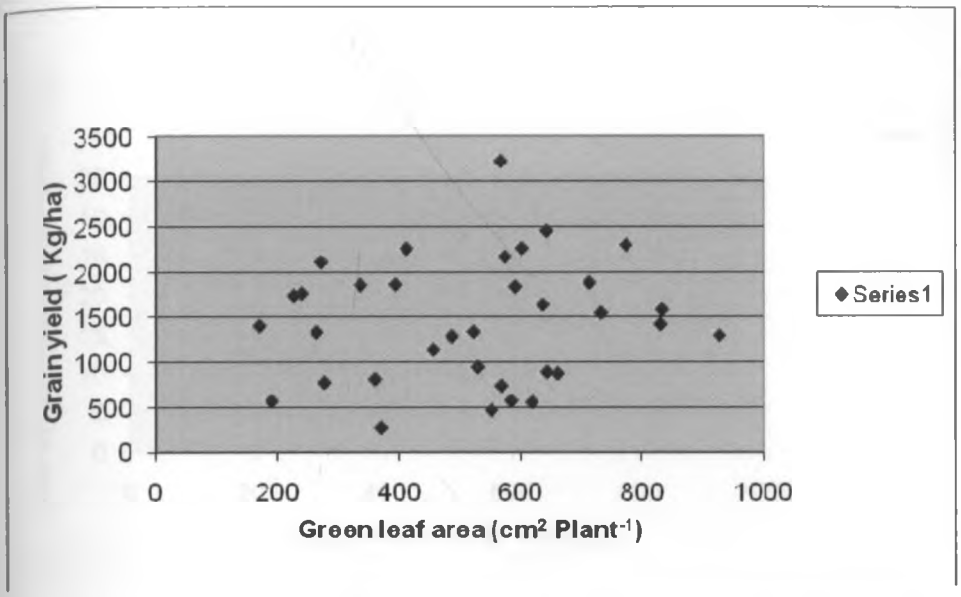


Figure 3: Correlation between Green leaf area (Stay green) and yield

As shown in Table 8.0 and Figure 3 above, correlations between yield and the green leaf area at 45 days (stay green) was slightly positive (0.14) but it was not significant. Highest yields were recorded between stay green levels of 200 to around 620 cm^2 $plant^{-1}$. Plants that were observed to have stay green levels of more than 620 cm^2 $plant^{-1}$ did not show increased levels of yield. These yield increases by stay green genotypes can be attributed directly to maintenance of photosynthetic capability during grain filling period.

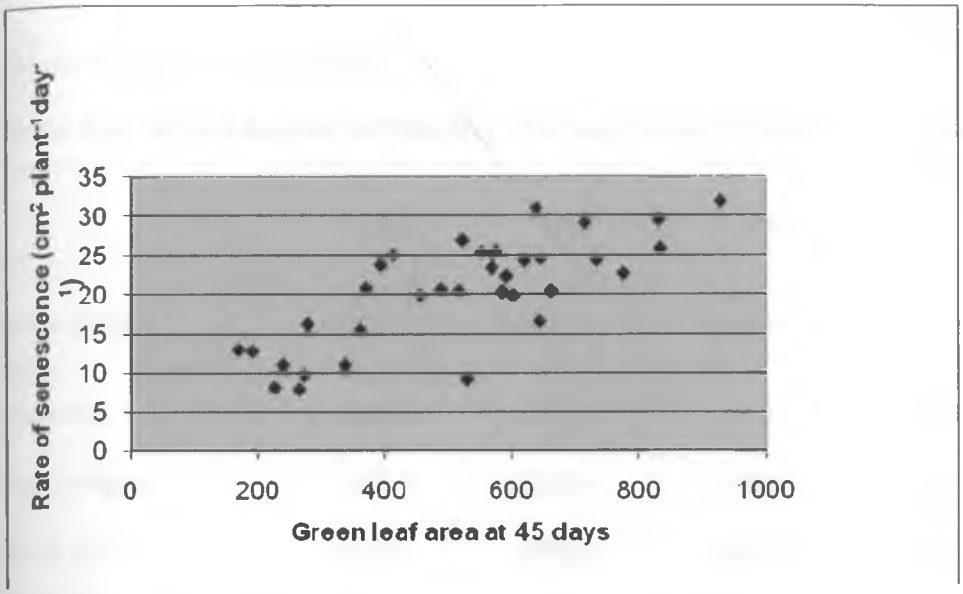


Figure 4: Correlation between Senescence and green leaf area at 45 days

As shown in Figure 4 above, a direct and positive correlation was observed between the rate of senescence and the green leaf area between the sorghum accessions. Positive correlations were observed between rate of senescence and green leaf area at 45 days. The average rate of senescence was observed to be at 20.4 cm^2 per $plant^{-1}$. At this rate, green leaf area senescence was highest for most of the accessions. It could be concluded that, the higher the stay green levels the higher the levels in the rates of senescence for the genotypes.

3.7: Broad-sence heritability

Table 9.0: Broad-sence heritability for key drought traits

	δ_g^2	δ_{ph}^2	δe^2	H
Grain yield	982771	11892731	103480	0.82
Days to flowering	582.98	601.79	18.81	0.97
Plant height	567.4	2269.4	1087	0.25
SG15 DAF	262465	300822	38357	0.87
SG30 DAF	154379	180331	295952	0.85
SG45 DAF	64056	75776	11720	0.85

SG15daf-Stay green/ Green leaf area15 days after 50% flowering, SG30daf-stay green/ Green leaf area 30 days after 50% flowering, SG 45daf- Stay green 45 days/ Green leaf area after 50% flowering

From Table 9.0 above, the six traits; grain yield, days to 50% flowering, plant height and the green leaf area (Stay green) at 15, 30 and 45 days showed very high broad-sense heritabilities of 0.87, 0.85 and 0.85 respectively. The respective estimates of broad-sense heritabilities ranged from as low as 0.25 to as high as 0.97. The highest heritability was observed in the days to 50% flowering and the lowest was plant height.

3.8 Qualitative traits

In this study the key traits that showed polymorphism were analyzed and are tabulated in table 10.

Table 10: Frequency distribution of qualitative traits in sorghum accessions during year 2007 and 2008.

	Trait /Scale	Category	Frequency (%)
Season one	**senescence**		
	1	Very slightly senescent	7
	3	Slightly senescent	88
	5	Intermediate	3
	6	mostly senescent	2
	Drought		
	1	Tolerant	44
	2	Fairly tolerant	15
	3	Moderately tolerant	35
	4	Susceptible	3
	5	Very susceptible	3
	Preflower stress		
	1	Tolerant	49
	2	Moderately tolerant	10
	3	Susceptible	39
	4	Very susceptible	2
Season two	**Senescence**		
	1	Very slightly senescent	47
	3	Slightly senescent	16
	5	Intermediate	26
	7	mostly senescent	13
	Drought		
	1	Tolerant	78
	2	Fairly tolerant	6
	3	Moderately tolerant	7
	4	Susceptible	11
	Preflower stress		
	1	Tolerant	80
	2	Moderately tolerant	11
	3	Susceptible	2
	4	Very susceptible	9

From Table 10 above, senescence, drought and pre flowering stress indices were used for both seasons in the analysis of qualitative traits for the accessions. For season 2007, 7% of the accessions were very slightly senescent, 88% were slightly senescent, 3% were intermediates and 2 % were categorized as mostly senescent. In the case of drought, 44% of the accessions were categorized as tolerant, 15% were fairly tolerant, 35% moderately tolerant, and 3% each for both susceptible and very susceptible accessions respectively. 49 % of the accessions that were categorized as tolerant to pre flowering stress, 10% were moderately tolerant, 39% susceptible and 2 % were categorized as very susceptible.

During the 2008 season, 46 % of the accessions were categorized as very slightly senescent, 16 % as slightly senescent, 25% as intermediate and 13 %as very susceptible. The drought score, categorized 77 % of the accessions as tolerant, 6% as fairly tolerant, 6 % as moderately tolerant and 11 % as susceptible. The pre flowering stress score categorized 79 % of the accessions as tolerant, 11 % as moderately tolerant, 2% susceptible and 8 % of the accessions were categorized as very susceptible.

3.8 Multivariate analysis

Multivariate analyses are helpful in describing phenotypic variation among the accessions. Two complimentary procedures namely cluster analysis (CA) and principal component analysis (PCA) were used in this study.

3.8.1 Cluster analysis

Euclidean dissimilarity coefficient matrix was constructed based on agro-morphological traits of the sorghum accessions. The phenogram regarding data observed for various traits during the year 2007 was constructed and is presented in Fig.5.0.The matrix was beyond the scope of its presentation in tabulated form; nevertheless it was observed that dissimilarity ranged from a minimum of 2 between accessions UON 50 and UON62 to a maximum of 24 between UON

43063 to UON 999.The cluster diagram proposed two major groups. The critical examination of the phenogram revealed 5 clusters (A,B,C,D and E). The number of accessions in each cluster ranged from 1 in cluster A to 16 in cluster C.

Cluster A only had one accession UON 998 from Eastern Kitui which represented 2.9 % of the whole population. This was one of the accessions that was classified as early maturing. Cluster B was made up of three accessions UON (959, 43971 and 59) that accounted for 8.8% of the whole population. The accessions in this cluster were early maturing , tall and had a high tillering capacity.

Cluster C contributed 47.05 % to the population and consisted of 16 accessions. The accessions for this cluster form source materials for stable yields, earliness and stay green. Cluster D represents 11.8 % of the population. The accessions of this cluster had the lowest number of lodged plants, they were of medium stature and were also early maturing to some extent. Cluster E made up 34 % of the entire population. Accessions in this cluster were mainly average performers in terms of yield, days to flowering and seed mass. However there were a number of accessions noted that could be good sources of stay green such as accession UON 48378 from North-Eastern Kenya. Another accession UON 43063 was also identified as having good yields.

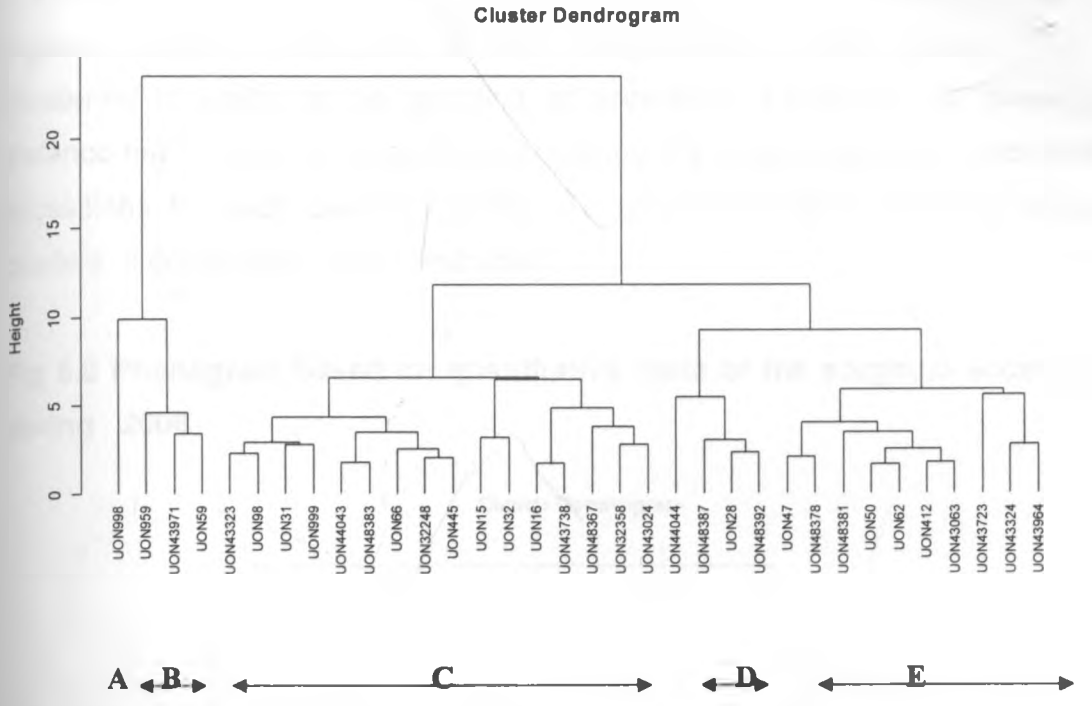


Fig 5.0 Phenogram based on quantitative traits of the sorghum accessions during 2007

The Euclidean dissimilarity matrix for the sorghum accessions during the year 2008 for the agro-morphological traits exhibited a dissimilarity and a phenogram was constructed which is presented in Fig.6.0.The cluster diagram revealed four main groups with each group having clusters ranging from one to three.

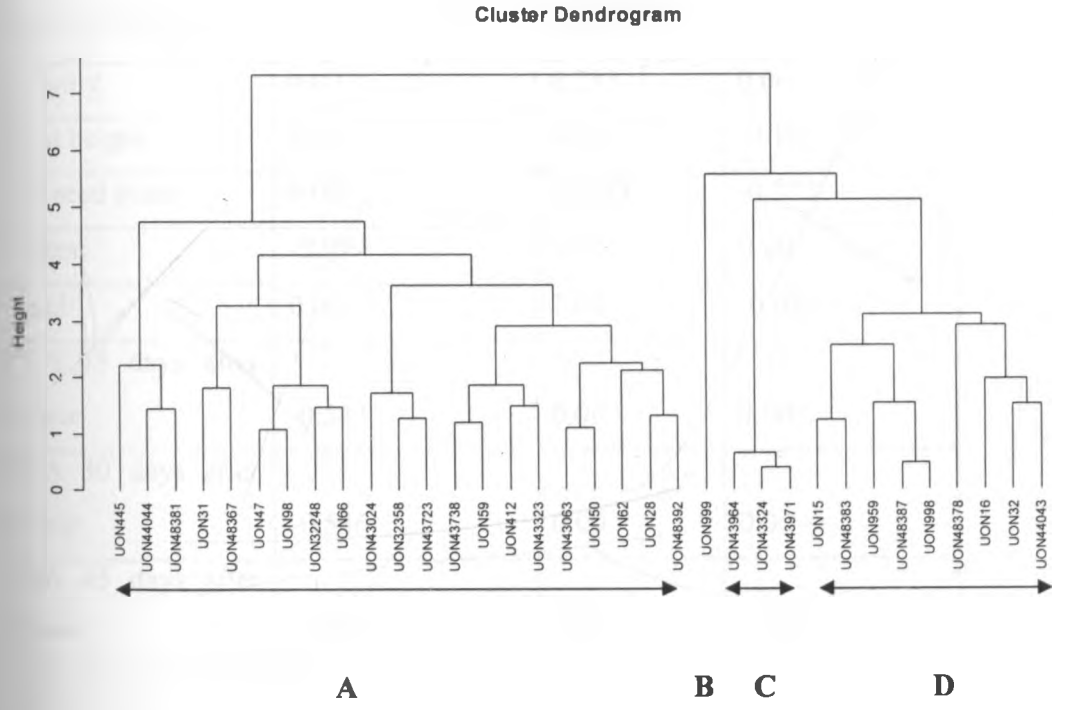
Cluster A contributed to 61.8 % of the whole population. It was observed that this cluster had accessions with the highest yield of up to 3400Kg/ha, a high number of tillers and also a 100 seed mass of between 2 to 3 grams. Some of the accessions in this cluster were also noted to be one of the tallest at a plant height of 177cm. Cluster B accounted for 2.9 % of the whole population. This cluster was characterized by very low yields (102 Kg/ha), late flowering and also lodging.

Cluster C contributed to 8.8% of the whole population. Materials in this cluster can mainly be classified as late maturing at 123 to 133 days, fairly tall(168cm) and fairly good yielding accessions at 1199 to 1586 Kg/ha. Cluster D made up 26.5 % of the entire population. Accessions in this cluster could be classified as early in

this season, but in comparison to season two they would be classified as medium. A good number of these accessions also had good yields (2086 Kg/ha).

Clustering is useful in the grouping of accessions based on the phenotypic distance matrix which is created by calculating the distance between each pair of accessions for each quantitative trait. To show the overall similarity between clusters, a dendrogram was constructed.

Fig 6.0 Phenogram based on quantitative traits of the sorghum accessions during 2008



3.8.2 Principal Component Analysis Based on Agro-Morphological Traits

The variation among accessions was studied through Principal Component Analysis and the specific patterns that defined the way the variables were associated were identified (Table 11). The data revealed that three principal components having greater than 1 eigen

Table 11.0: Principal components for the Kenyan sorghum accessions in 2007

	PC1	PC2	PC3
Eigen value	25.88	2.48	1.48
Proportion of variance	0.81	0.08	0.05
Cumulative variance	0.81	0.88	0.93
loadings			
Days to 50% Flower	0.00	-0.15	0.541
Lodging	0.00	0.253	0.00
Plant height	0.00	0.00	-0.102
100 seed mass	0.00	-0.239	-0.512
Tillers	-0.997	0.00	0.00
Yield	0.00	0.00	-0.657
GLA 15 days after flower	-0.543	0.00	0.00
GLA 30 days after flower	-0.546	0.00	0.00
GLA 45 days after flower	-0.508	0.00	0.00

values contributed 93% of the total variation among the accessions of sorghum. It was found that principal component 1 (PC1) contributed 81 %, whereas PC 2, PC 4 contributed 8%, 5 % of the total variation respectively.

There were no traits that contributed positively for PC 1, days to 50 % flowering, lodging, plant height and yield showed non significant genetic variance. It was also observed that number of tillers per plant and green leaf area at 15,30 and 45 days had a negative loadings to this component at -0.997,-0.543, -0.546 and

-0.508 respectively. Genetic variance for PC2 was contributed by loadings from 50 % days to flowering (-0.15), lodging (0.253) and 100 seed mass (-0.239).A high positive loading of 50% to flowering (0.541), Plant height (-0.102),100 seed mass (-0.512) and yield (-0.657) PC3.

From PCA grouping based on the quantitative variables, the bi-plot of the PC scores (PC1 and 2) distributed the accessions as scatter diagrams to observe the relationships between clusters as shown in Figure 7.0.

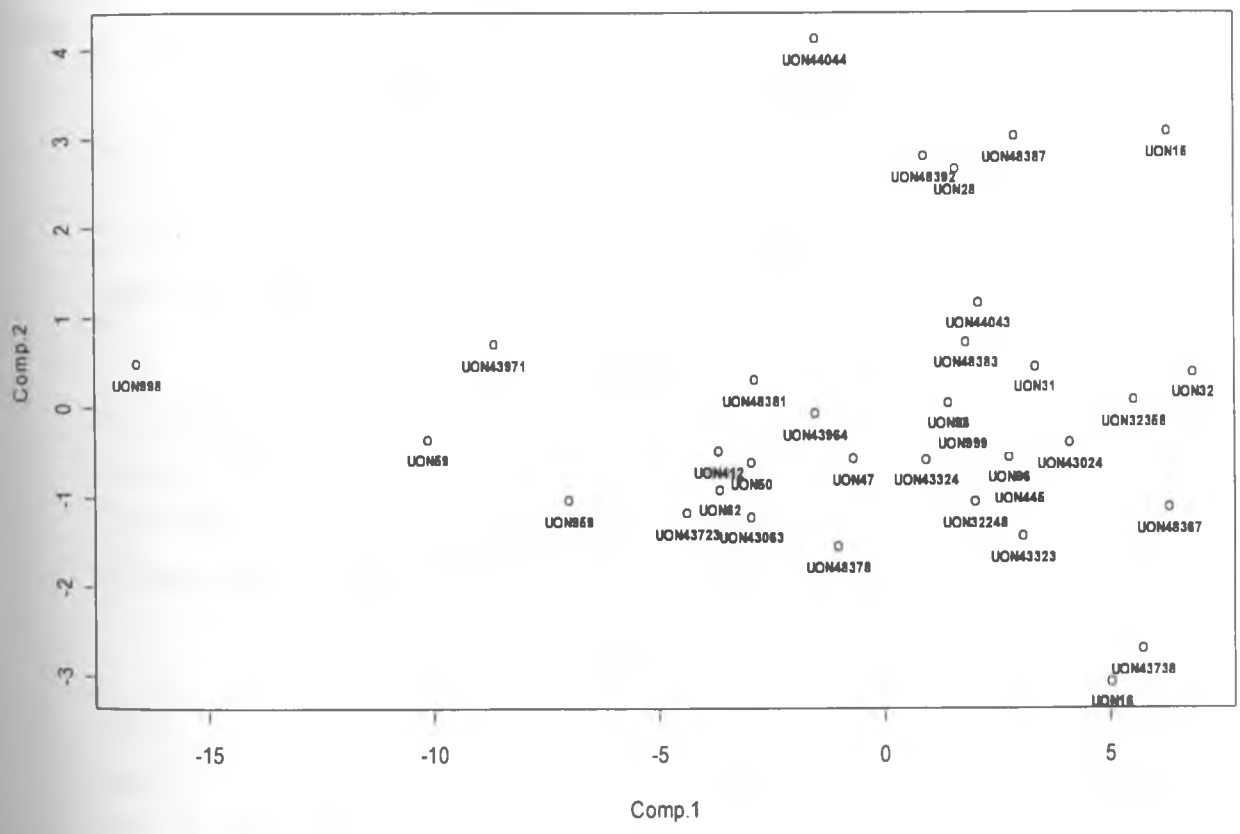


Figure 7.0.Scatter diagram for the first two principal components based on the quantitative traits for the Kenyan sorghum accessions in 2007.

During 2008, two components for which eigen values were greater than 1 (Table 12.0), contributed 66 % of the variation among the sorghum accessions

evaluated. It was found that principal component 1 contributed 46 % and principal component 2, 20% of the total genetic variation of this set of germplasm.

The characters that contributed to PC1 were days to 50 5flowering (-0.105), green leaf area at 15,30 and 45 days at 0.586,0.558 and 0.556 respectively. PC2 contribution towards total explained variation was as a result of the high positive loading of days to 50% flowering (0.718), lodging (-0.239), plant height (0.181), 100 seed mass (-0.572) and yield (-0.241)

Table 12.0: Principal components for the Kenyan sorghum accessions in 2008

	PC1	**PC2**
Eigen value	2.94	1.31
Proportion of variance	0.46	0.20
Cumulative variance	0.46	0.66
Loadings		
Days to 50% Flower	-0.105	0.718
Lodging	0.00	-0.239
Plant height	0.00	0.181
100 seed mass	0.00	-0.572
Loadings		
Tillers	0.00	0.00
Yield	0.00	-0.241
GLA 15 days after flower	0.586	0.00
GLA 30 days after flower	0.558	0.00
GLA 45 days after flower	0.556	0.00

The first two components that contributed to 66% of the total variance were plotted graphically as scatter diagrams to observe the relationships between the clusters as shown in figure 7.0.

PCA uses a covariance-covariance matrix to determine patterns of variation and major traits contributing to the delineation. PCA is thus a data reduction technique that reduces the number of variables in a data set while retaining the variability in the data. The proportions of these variances have been highlighted in tables 11 and 12.

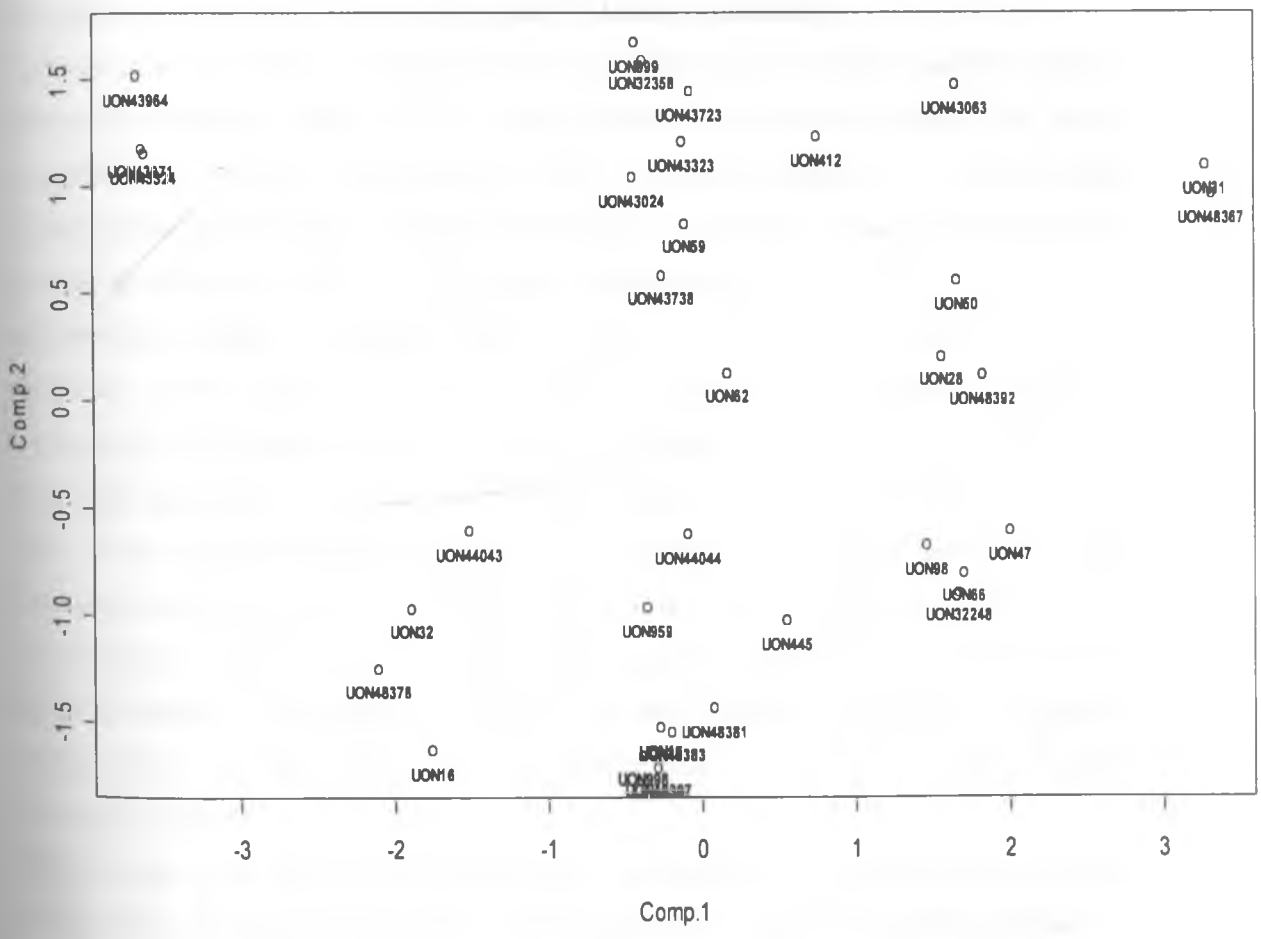


Figure 8.0: Scatter diagram for the first two principal components based on the quantitative traits for the Kenyan sorghum accessions in 2008.

3.9 Discussions

The sorghum accessions were characterized and evaluated for nine agro-morphological characters.A wide range of variability was recorded in both qualitative and quantitative characters. The use of both qualitative and quantitative traits for phenotypic analysis has been applied in crop germplasm. Bajracharya *et al.*, (2006) reported using forty-two qualitative and quantitative traits to assess rice landrace accessions with 10 different names. In a related study, genetic variation of 11,402 pigeonpea germplasm accessions collected from 54 countries were analysed using 14 qualitative and 12 quantitative traits (Upadhyaya *et al.*, 2007). The use of these morphological markers can only be reliable if they can give a higher variation between accessions.

Farmers use quantitative traits like earliness, plant height, seed mass and yield to determine desirable traits that can be retained for future cropping. The highly significant phenological variabilities in the sorghum accessions and between accessions and the seasons are an indication of suitability of certain genotypes to specific environments.The results agree with findings of Bramwel *et al.*, (2004) who evaluated 638 accessions from 4 districts in Andhra Pradesh and found significant differences in quantitative traits among the accessions, between accessions and between districts. Grain yield, days to 50% flowering, green leaf area (stay green) at 15, 30 and 45 days all showed high heritability values in this study. This is a good indication that the traits chosen for drought screening were good phenotypic markers. The relative importance of a genotype as a determinant of phenotypic value is given by the ratio of genotypic to phenotypic variance, Vg/Vp (Falconer, 1996). Most of the traits were mainly influenced by genetic factors. Plant height showed low heritability and, therefore, it is not a good phenotypic marker.

Water stress may reduce or extend days to flowering. Studies done in wheat (Hurd 1964) showed that water stress shortened days to heading. Cases of hastened maturity have been reported in sorghum.

Genotypic differences were observed in days to 50 % flowering in the two seasons. On average, accessions in the year 2008 took longer to flower with a

mean average of 89 days compared to 73 days in year 2007. This could be attributed to the fact that the year 2007 was more drought stressed than the year 2008 hence triggering the sorghum accessions to flower much earlier. Increased growing season allows the crop to accumulate higher thermal time (^{0}Cd) Thermal time affects the crop development; the high cumulative thermal time contributes to higher yields as observed in wheat Hongyong *et al.*, (2007) This could be a likely indication as to why accessions in the 2008 season performed much better in terms of yield compared to season 2007. On the other hand, accessions such as UON 48381 and UON 32 which flowered in 47 and 49 days respectively in season one (2007) were early maturing and possibly adapted to escaping drought. The two accessions were noted to be from North Eastern and West Pokot parts of Kenya. Shortening of life cyle allows the crop to escape late season drought (Turner, 1979). Earliness also assists in creating asynchrony between plant development and the occurrence stress. This phenomenon has also been reported by Rao *et al.*,(1979) in tropical sorghum hybrids and varieties. Earliness is therefore effective when stress becomes important during later stages of growth, as observed in the second season. The negative correlations between 50% days to flowering to lodging could partly be attributed to the fact that early maturing varieties apportion less of the carbohydrates to the grains than the stems compared to late maturing genotypes.

Delayed senescence (stay green) in sorghum is considered a valuable trait, as it improves genotype adaptation to post flowering drought stress, particularly in environments in which the crop depends largely on stored soil moisture to fill and mature grain (Rosenow *et al.*,1977). Specifically, stay green has been associated with reduced lodging (Mughogho and Pande,1984), higher levels of stem carbohydrates both during and after grain filling (McBee,1984). The correlations between stay green at 15 days with stay green at 30 days and 45 days were all positive and strongly related. This is a good indication that selection of stay green genotypes is independent to the time when the measurements were taken. Similar positive and strong correlations between the rate of senescence and stay green at 15, 30 and 45 days were also observed. This is an indication that the rate of

senescence was higher in the genotypes that had higher green leaf area (stay green) levels at the beginning of the season. These observations were noted for accession such as UON 16, UON 43738, and UON 48378 from Laikipia, Bungoma and North Eastern parts of Kenya respectively. The genotypes with lower green leaf area (stay green) levels were noted to also have lower rates of senescence. Such genotypes included UON 32358, UON 44043, UON 43964 from Kwale, Baringo and Siaya respectively. The correlations also indicated a higher rate of senescence at 15 days after flowering in comparison to measurement of the same rate at 45 days flowering. These higher rates of senescence may be explained by the early vulnerable stages of a crop under moisture stress before it adapts to drought tolerant or escape mechanisms.

The high levels of heritability indicated that the sampling done for stay green estimation was good and can be reliably used as phenotypic markers. The correlations for stay green at 15, 30 and 45 days after flowering and lodging were all negative and strong. This is in agreement with the observations made by Mughogho and Pande,(1984). This could be explained by the fact that materials that had lower levels of stay green received lesser portions of stem carbohydrates compared to the stay green genotypes. The correlations to grain yield and 50 % to flowering were however not significant. Similar observations were made by W.G Wenzel,(1999). Improved grain filling and grain yield under stress has been associated with stay green (Rosenow and Clark, 1981). Because of these benefits, selection for enhanced stay green has been an important component in breeding programs. This was evident in accessions such as UON 43738, UON 43323, from Bungoma and Keiyo valley all of which showed consistency in all traits, though tall, the accessions showed little lodging in both seasons, a fairly high level of stay green (green leaf area at 45 days after flowering), average good yields and also a high number of tillers, another important trait for drought tolerance. These results may indicate the occurrence of yield stability genes in these accessions. Such accessions might prove useful in introgressive hybridization programmes.

Water stress has been shown to cause decrease in plant height. During the 2008 season, the sorghum accessions attained more height than the previous year. This could be due to the early rains during the vegetative phase. The mean height for the accessions in the 2008 season was 167 cm compared to 132.6 in 2007. Plant height is considered to be an important character for adaptation in dry areas. One of the main effects of a dry spell during the growing season is a drastic reduction of stem elongation of straw yield. Total biomass increased with growth duration. Late genotypes such as UON 43964, UON 43324, and UON 43971 were much taller compared to early accessions and had high biomass yields especially in the second season (2008). The correlations between plant height and stay green at 15, 30 and 45 days were negative though not sufficiently strong. This could be an indication that stay green is indirectly associated with plant height.

During both seasons, high level of genetic variation was observed for 100 seed mass. The mean 100 seed weight in 2008 was however lower compared to the year 2007. This could be an indication that the moisture stress levels in 2008 during flowering were likely to be much higher compared to the previous season.This could also be supported by the fact that the 100 seedmass range in 2008 was between 1 to 3 grams yet in 2007 the range was between 1 gram to as high as 6 grams in certain accessions. This trait could be utilized efficiently for tailoring a new sorghum crop variety according to the need of different regions of a country.

Tillering as induced by moisture stress can not be viewed as a drought resistance mechanism. It is instead a response mechanism for evolutionary survival. Under severe moisture stress especially during the flowering period, excessive tillering may have a negative effect on grain yield. Non tillering cultivars are required for precision planting. Under near optimum condition of tillers to grain yield may be small (Gerik and Neely, 1987)

Genotypic effects were observed to be significant for yield in both seasons (Appendix 9). This meant that there was inherent differences among the genotypes. In general water stress caused a decline in yield especially in the 2007

season. Negative and significant correlations were observed between yield and days to flowering. Similar observations were made in a study of wheat by Derera *et al.*,(1969), where they noted negative correlations between grain yield and days to ear emergence. They concluded that 40 to 90 % of variation in yield in early flowering genotypes studied could be ascribed to earliness. Fischer and Maurer (1978) observed yield increase owing to earliness was greater when deficits were more severe. This was true for accessions such as UON 48383, UON 48378, and UON 48383 from North Eastern Kenya. However with adequate moisture, positive correlation has been observed between maturity and yield (Dalton 1967). Season 2007 was noted to be more stressed than 2008 and therefore the mean yields for 2008 was observed to be higher (Table 1 and 2). The correlations between yield and lodging were not sufficiently strong indicating that the two components are not strongly associated. However the correlations between 100 seed mass and yield were positive and fairly strong. This was an indication that a higher seed mass is likely to be associated with good yields in sorghum genotypes. The broad-sense heritability for yield, 15 GLA, 30 GLA45 GLA, 100 seed weight, and lodging was quite high thus indicating that these traits were less influenced by environmental factors, had higher genetic variance compared to phenotypic variance and could therefore be used as phenotypic markers of selection drought tolerance.

The poor performance of the late genotypes such as UON 43324, UON 32358 and UON 48367, may be due to their greater stover production and their large area and number, and water requirement. Low harvest index and reduced assimilate portioning to the parnicle also appear to characterize the tall and late endemic sorghums of Africa (Willey and Basiime, 1973)

One aspect that drought stress reduces is leaf area and hence the amounts of photosynthates produced (Wein, 1979). This is an observation that was made in all genotypes. The only difference was that, certain genotypes such as UON 15 and UON 44044 from Laikipia (Rift Valley) and Nyeri (central) were more susceptible compared to an accession such as UON 48378 from North Eastern Kenya. Similarly, Borrell et al. (2000a), in a study of nine sorghum hybrid based

on two different sources of stay green (B 35 and KS 19), reported that KS 19 hybrids had delayed onset and reduced rate of senescence. KS 19 hybrids had a delayed onset and reduced rate of senescence, whereas the B35 hybrids had only delayed onset of senescence. The KS 19 hybrids had a smaller leaf area at flowering than the B 35 hybrids, however; the green leaf areas at maturity for both were similar. The nitrogen concentration in the green leaves of both the B35 and KS 19 hybrids was also reported to be higher than the senescent hybrids at mid grain fill and maturity, yet this was associated with thicker leaves only in hybrids with the b 35 source (Borrell and Hammer, 2000).

From this study, a number of accessions showed a good level of stay green at 45 days after flowering however only a few showed consistency in both seasons and also ranked very well for other important traits such as yield, 100 seed mass and number of plants lodged. These accessions include UON 48373, UON 43323 and UON 47. Although the ability of the leaves to delay senescence has a genetic basis in sorghum (Van Oosterom *et al.*,1996), the expression of the character is strongly influenced by the environment factors. The trait expresses best in environments in which the crop is dependent upon stored soil moisture, but where there is sufficient moisture to meet only a part of the transpiration demand. Sufficient expression of the trait for selection is thus dependent upon the occurrence of prolonged period of drought stress during the grain filling period of sufficient severity to accelerate normal leaf senescence, but not sufficient magnitude to cause premature death of plants. The high broad-sence heritabilities for stay green at 15, 30 and 45 days after flowering shows the trait can be used as a phenotypic marker for drought.

Most of the traits that were scored for indirect selection to improve drought tolerance in the sorghum accessions were insufficiently correlated with grain yield, which is an important selection index as reported by (Richard & Thurling, 1979). Therefore these traits could together with stay-green be used as selection criteria for drought tolerance. The high heritbilities observed for grain yield is an indication that ,yield which is generally difficult to select for under drought stress conditions, can be a useful selection criteria under low moisture conditions.

Climatic and ecotypic differences at a site play an important role in developing and sustaining variability within a population (Bennet, 1999). This best explains the grouping for this data. Season 2007 separated into five clusters compared to four clusters in the 2008 season. Accessions of importance were those of cluster C in the 2007 season and cluster A in 2008 season. Though most were from the Rift valley and western regions, accessions from Eastern, Northeastern and Coastal region were also included. These accessions were particularly stable yielding, had a high seed mass and were also good sources of stay green.The cross region clustering could be as a result of seed exchanges between farmers either through relative, markets, relief food/seed as reported by Manyasa *et al.*, (2007). It therefore follows that, agro-ecological/topographic conditions in a given location usually determine farmers' selection strategies as indicated by the above results.

The PCA performed revealed similar important traits to explain variability both at individual level and across the accessions. The study showed that only three components contributed to variability in 2007 while in 2008 only two components formed the bulk of the variability. This is supported by Kaiser (1960) in Rojas *et al.*, (2000) who reported that values greater than 1 can be used for delineation of accessions. The first PC in season one, grouped together accessions that were high numbers of tillers and the green leaf area (GLA). The second PC differentiated the accessions based on 50 % flowering, lodging and 100 seed mass while PC 3 grouped together the accessions based on yield, plant height and 50 % flowering. In season two, the loadings in PC1 were mainly attributed to, 50% days to flowering and the remaining components were attributed to PC2. From the PCA grouping, the bi-plot of the PC scores (PC 1 and 2) for season one distributed the accessions into one large group with tillering being the main influence to the genetic variability. On further analysis without the inclusion of the number of tillers in 2008 an interesting observation was noted. The accessions in both seasons distributed themselves into three major groups. One group was mainly influenced by 50 % days to flowering, the other one was mainly attributed to the green leaf area and the largest groups in both seasons delineated itself

based on yield, 100 seed mass, plant height and lodging. This could be attributed to the high selection pressure by farmers based on some of these desirable traits, either for feed in the case of green leaf area, food and seed size in respect to yield and seed mass or drought escape in the case of days to flowering. Principal component analysis depicted phenotypic variation among accessions belonging to different regions of Kenya. Broschat (1979) also considered PCA as a powerful technique for data reduction which removes interrelationships among components. PCA and clustering are thus useful techniques in describing the phenotypic variation among genotypes.

In general, one of the main characteristics of semi-arid areas is the large variability of environmental conditions across seasons and across locations as was noted in this study between the two seasons. This was also reflected in the large range for grain yield obtained as well as other similar studies conducted in East African dryland areas (Jowett,1972).

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CHAPTER FOUR: MOLECULAR DIVERSITY OF LOCAL GERMPLASM USING SIMPLE SEQUENCE REPEATS (SSRs)

Abstract

Analysis of genetic relationships in crop species is an important component of crop improvement programs, as it serves to provide information about genetic diversity, and is a platform for stratified sampling of breeding populations. Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including, analysis of genetic variability in cultivars, identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and introgressing desirable genes from diverse germplasm into the available genetic base.

A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines, and populations. These methods have relied on pedigree data, morphological data, agronomic performance data, biochemical data, and more recently molecular (DNA-based) data. DNA based markers provide powerful and reliable tools for discerning variation within crop germ-plasm.

In this study, the major objective was to assess the genetic diversity of sorghum accessions collected from different parts of Kenya using SSRs .The specific objective was to determine the patterns of relationships among the study materials. One hundred and thirty nine accessions were genotyped as bulks and individuals using 11 microsatellite markers and genetic diversity estimated. The markers showed a wide range of differences in quality index from 0.005 to 0.39, Polymorphic information content ranged between 0.21-0.82 while, the number of alleles detected by the markers were between 2-16. Cluster and principal coordinate analysis revealed five distinct groups Further statistical analysis also revealed that the level of heterozygosity varied from as low as 0.000 to as high 0.89, the gene diversity ranged from 0.24 to 0.84 and the Nei genetic distance varied from 0.1 to 1.0. Analysis of molecular variance indicated that differences

among populations explained 6.48% of the variation. The Fst value 0.0648 indicated a moderate level of differentiation.

4.1 Introduction

Biological diversity embraces the variability within and between all the species of plant and animals and ecological systems, which they inhabit. The genetic diversity found in the plant species, which provide food, shelter and medicine for the worlds' population, is vital component of this biodiversity. The magnitude and distribution of genetic variation in wild plants are major factors for a continuous evolutionary process in nature. The access of variable genetic material is a prerequisite for early domestication, adaptation to new areas or habitats and further progress in modern plant breeding. The basic mechanisms of mutation, recombination and selection are operating under domestication and breeding whereas the speed of changes has increased considerably under cultivation in comparison to nature (Zaheer *et al.*, 2005).

Plant genetic resources therefore play an important role in generating new crop varieties with high yield potential and resistance to biotic and abiotic stresses. The germplasm of a particular crop collected from the local sources provides greater genetic variability and can provide useful traits to broaden the genetic base of the crop species. Morphological, biochemical and molecular procedures are currently being employed in evaluating plant genetic resources. Until recently, most of the characterization and evaluation has been based on the recording of either qualitative or quantitative morphological characters.

DNA based markers provide powerful and reliable tools for discerning variation within crop germplasm and to study evolutionary relationships (Gepts, 1993). PCR based techniques have been used successfully in DNA fingerprinting of plant genomes and in genetic diversity studies. These techniques include RAPD (Randomly Amplified Polymorphic DNA), RFLP (Restriction Fragment Length

Polymorphism), SSR (Simple Sequence Repeat) AFLP (Amplified fragment Length Polymorphism).

Existing genetic marker (genotyping) technologies mostly developed for applications in human health have also been applied successfully to agricultural species but their cost remains prohibitive for most agricultural applications. This is particularly true for species for which no molecular data and very limited resources are available. Because of the limitations of existing marker technologies, Diversity Array Technology (DArT) has recently been developed as a novel method to discover and score genetic polymorphic markers. DArT is a sequence-independent, high throughput method that is able to discover hundreds of markers in a single experiment. DArT markers are typed in parallel, using high-throughput platforms, with low cost per data point. With DArT, plant breeders, plant ecologists, as well as the managers of the germplasm collections, will be able to perform genetic analysis in a cost effective and high-throughput manner. DArT fingerprints will be useful for accelerating plant breeding, and for the characterization and management of genetic diversity in domesticated species as well as in their wild relatives. DArT has successfully been developed for rice, sorghum, barley, wheat, and cassava and work is in progress to establish DArT platforms for other species of importance to tropical agriculture such as pigeon pea and chicken pea (Wenzl *et al.*, 2004; Huttner *et al.*, 2005).

4.2 Morphological and phenological characteristics

Morphological and phenological methods discriminate between individuals based on physical characteristics, e.g. maturity cycle, growth habit, leaf shape, hairiness, nature of corolla and panicle/pod/fruit size (Van der Maesen, 1990). These methods have many shortcomings. For example, these characters may not be significantly distinct hence require that plants grow to full maturity prior to identification (Ratnaparklhe *et al.*, 1995). In addition, the characters are often influenced by environmental factors resulting in differences of expression that confound the interpretation of results. Because different genes are expressed at

different developmental stages or in different tissues, the same type of material must be used for all experiments. Furthermore, there may also be limited polymorphism in cultivated germplasm if these methods are used (Matus and Hayes, 2002). Nevertheless, morphological and phenological characteristics are still important measures of genetic variation..

4.3 DNA based Molecular markers

Molecular markers are recognized as significant tools to orient plant genetic resource conservation management, providing a means to accurately estimate the genetic diversity and genetic structure for species of interest (Hamrick and Godt 1997). They have been used, in the context of the *ex situ* conservation of domesticated species, to assess the pattern of genetic diversity in large germplasm collections (Lubbers *et al.*, 1991; Zhang *et al.*, 1992), to suggest priorities in future sampling missions, or to optimize the assembly of core collections (Schoen and Brown 1995). In the context of the *in situ* conservation of landraces, molecular markers could be useful to facilitate the selection of optimum sites, as well as to monitor ongoing changes in the pattern of diversity in the course of conservation practices (Newbury and Ford-Lloyd, 1997). Currently, the pattern of genetic diversity within large germplasm collections is well characterised for most crops (Morden *et al.*, 1989), but this does not necessarily reflect the extant genetic structure of landraces under cultivation conditions. Several factors could account for discrepancies between genetic-diversity estimates based on direct held samples and on accessions taken from germplasm collections: (1) most studies involve extremely low sample sizes at the accession level less than ten seeds per accession in most cases (Doebley et al. 1985; Morden et al. 1989); (2) the plant material in germplasm collections is likely to have passed through genetic bottlenecks because of sampling and regeneration procedures. For these reasons, investigations on the pattern of genetic diversity of landraces *in situ* are urgently needed in order to orient *in situ* conservation programmes.

Molecular markers are also rapidly being adopted by crop improvement researchers globally as an effective and appropriate tool for basic and applied

studies addressing biological components in agricultural production systems (Jones *et al.*, 1997; Mohan *et al.*, 1997; Prioul *et al.*, 1997). Molecular markers offer specific advantages in assessment of genetic diversity and in trait-specific crop improvement. Use of markers in applied breeding programs can range from facilitating appropriate choice of parents for crosses, to mapping/tagging of gene blocks associated with economically important traits (often termed "quantitative trait loci" (QTLs)). Gene tagging and QTL mapping in turn permit marker-assisted selection (MAS) in backcross, pedigree, and population improvement programs (Mohan *et al.*, 1997). This is especially useful for crop traits that are otherwise difficult or impossible to deal with by conventional means. The near-isogenic products of marker-assisted backcrossing programs provide genetic tools for crop physiologists and crop protection scientists to use in improving our understanding of the mechanisms of various abiotic stress tolerances (Jones *et al.*, 1997; Prioul *et al.*, 1997) and resistances to biotic production constraints such as diseases, insect pests, nematodes, and parasitic weeds such as *Striga*. QTL mapping of yield and quality components, as well as components of other physiologically or biochemically complex pathways, can provide crop breeders with a better understanding of the basis for genetic correlations between economically important traits (linkage and/or pleiotropic relationships between gene blocks controlling associated traits; e.g., flowering time and biomass; inflorescence size and inflorescence number). This can facilitate more efficient incremental improvement of specific individual target traits. Further, specific genomic regions associated with QTLs of large effect for one target trait can be identified having minimal effects on otherwise normally correlated traits, permitting an improvement in the first trait that need not be accompanied by counterbalancing reductions in others. Finally, these molecular marker tools can also be used in ways that allow us to more effectively discover and exploit the evolutionary relationships between organisms, through comparative genomics.

4.4 DNA markers

Most points on molecular marker-based genetic linkage maps are anonymous DNA polymorphisms (e.g., restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and micro satellite markers) and do not correspond to any gene of known function.

However, some molecular markers (including coding DNA (cDNA) and expressed sequence tag (EST) markers, as well as the protein markers described above) do pinpoint individual genes. Anonymous DNA markers are generated by a wide variety of techniques, differing greatly in their reliability (repeatability and robustness), difficulty, expense, and the nature of the polymorphism that they detect. Because of these differences, they also vary greatly in their suitability for various uses. They may be gel based (e.g., RFLP), or polymerase chain reaction (PCR) based (e.g., RAPD and AFLP); they may detect single locus, oligo-locus, or multiple locus differences; and the markers detected may be inherited in a presence/absence, dominant, or co-dominant manner. Brief descriptions of each of a number of the more widely used DNA marker groups are given further on this chapter based on information contained in the Plant Genome website (http://www.nal.usda.gov/pgdic/tutorial/lesson4.htm) and recent reviews of molecular markers useful in mapping plant genomes (Karp *et al.*, 1997; Malyshev and Kartel, 1997; and Mohan *et al.,* 1997).

4.5 Microsatellite (SSR) markers

Microsatellites are simple sequence tandem repeats (SSRs) of nucleotides. The repeat units are generally mono-, di-, tri-, tetra- or pentanucleotides repeat types e.g. AA--, or AG--, CGA short stretches of DNA that are "hypervariable". They are ubiquitously distributed throughout the genome of eukaryotes and abundant in genomes of plants where they are thought to be a source of genetic variation (Mahalakshmi *et al.*, 2002). They tend to occur in non-coding regions of the DNA, although a few human genetic disorders are caused by (trinucleotide) microsatellite regions in coding regions. On each side of the repeat unit are

flanking regions that consist of "unordered" DNA. The flanking regions are critical because they allow us to develop and find locus-specific microsatellites (Saghai *et al.*, 1994). They are also variable because their mutations occur in a fashion very different from that of "classical" point mutations. The mutation processes in microsatellites occur through what is known as slippage replication.

SSR markers have proved a valuable asset for breeding programs and have been used for a wide range of applications mostly in measuring genetic diversity (Xiao *et al.*, 1996), and assigning lines to heterotic groups (Senior *et al.*, 1998). They have been used in genetic analysis of breeding schemes (Kejun *et al.*, 2003), genetic distance analysis (Chen *et al.*, 1997), estimation of genome size (Smith *et al.*, 1997), population genetics, fingerprinting for legal protection of cultivars and parental lines and in establishing genome relationships in species with putative inter-specific parents. SSR markers have been developed in pigeon pea and have been used to assess the degree and distribution of genetic diversity in pigeon peas landraces from Andhra Pradesh (Newbury *et al.*, 2004; Buhariwalla and Crouch, 2004; Bramel *et al.*, 2004). Polymorphisms have been observed with this kind of marker in loquat (Sorriano *et al.*, 2005), peanut (Krishna *et al.*, 2004), perennial ryegrass (Kubik *et al.*, 2001), rice (Liu *et al.* 2000), maize (Senior *et al.*, 1998; Senior and Heun, 1993) and in barley (Shaghai *et al.*, 1994). SSRs have also been found to occur in other plant genomes including soyabean (*Glycine max* L.) (Akkaya *et al.*, 1992), rice (*Oryza sativa* L.) (Wu *et al.*,1993) and barley (*Hordeumn vulgare* L.) (Saghai *et al.*,1994). Sorghum and pearl millet (Taramino *et al.*, 1997). Variation in allele frequencies at many unlinked loci is the preferred method of assessing genetic diversity and differentiation and estimation of the strengths of the various forces shaping them (Fregene *et al.*, 2003). Simple sequence repeat (SSR) markers are particularly attractive for studying genetic differentiation because they are co-dominant and abundant in plant and animal genomes (Folkertsma *et al.*, 2005).

4.6 Advantages and limitations of microsatellites as genetic markers

The method is relatively simple and can be automated (Kresovich *et al.*, 1995; Mitchell *et al.*, 1997). Most of the markers are locus-specific (in contrast to multi-locus markers such as minisatellites or RAPDs) and show Mendelian inheritance (Saghai *et al.*, 1994; www.fao.org/BIOTECH/ docs/Korzun). They are highly informative and PCR-based, this means that only tiny amounts of tissue are needed and they work even on highly degraded or "ancient" DNA (Rafalski and Tingey, 1993). In sorghum, numerous SSR markers have been developed and mapped (Brown *et al.*, 1996; Taramino *et al.*, 1997; Kong *et al.*, 2000; Bhattramakki *et al.*, 2000; Schloss *et al.*, 2002). A high number of public SSR primer pairs are available and are cost-effective per genotype and primer (similar to that of RAPD) (Korzun, 2001). A few of these public domain markers have been employed to analyse the genetic diversity in subsets constituted from the ICRISAT sorghum collection (Grenier *et al.*, 2000), the USDA sorghum collection (Dean *et al.*, 1999) and from collections originating from single countries (Dje *et al.*, 1999; Ghebru *et al.*, 2002).

Since microsatellites are co-dominant markers, heterozygotes can be distinguished from homozygotes, in contrast to RAPDs and AFLPs which are dominant . In addition, SSRs are highly polymorphic (Weber, 1990; Doldi *et al.*, 1997; Schug *et al.*, 1998) and thus the level of polymorphisms in plant species studied has been greater than that found with other markers. The markers are useful at a range of scales from individual identification to fine-scale phylogenies. Despite their efficiency, SSRs have some limitations, thus they are probably rarely useful for higher-level systematics. That is because the mutation rate is too high. Across highly divergent taxa two problems arise. First, the microsatellite primer sites may not be conserved (that is the primers we use for species A may not even amplify in species B). Secondly, the high mutation rate means that homoplasy becomes much more likely; we can no longer safely assume that the two alleles identical in state are identical by origin (www.fao.org/ BIOTECH/ docs Korzun).

4.7 Objectives of the study

To assess the genetic diversity of 139 different *Sorghum bicolor* landraces and establish the phylogenetic relationships among them.

4.8 MATERIALS AND METHODS

4.8.1 Plant materials

This work was conducted at the Biosciences Eastern and Central Africa (BecA) laboratories located at the International Livestock Research Institute (ILRI), Kenya. A total of 139 sorghum accessions were selected for this study (Table 13.0). All the accessions were landraces collected from different parts and diverse geographic origins in Kenya (Figure 1)

Table 13.0: 139 sorghum accessions selected for molecular characterization

SERIAL NO.	VARIETY	LOCALITY
1	UON 043723	RIFT VALLEY-KEIYO
2	UON 000055	WESTERN-BUNGOMA
3	UON 000003	EASTERN-MERU
4	UON 043321	RIFT VALLEY-KEIYO
5	UON 000032	RIFT VALLEY-WEST POKOT
6	UON 000034	RIFT VALLEY-WEST POKOT
7	UON 000062	WESTERN-BUNGOMA
8	UON 000017	RIFT VALLEY-LAIKIPIA
9	UON 0000031	RIFT VALLEY-WEST POKOT
10	UON 000024	RIFT VALLEY-WEST POKOT
11	UON 048373	NORTH EASTERN-WAJIR
12	UON 000028	RIFT VALLEY-WEST POKOT
13	UON 000022	RIFT VALLEY-WEST POKOT
14	UON 000016	RIFTVALLEY-LAIKIPIA
15	UON 000050	RIFTVALLEY-TRANSZOIA
16	UON 000047	RIFTVALLEY-TRANSZOIA

17	UON 000051	RIFTVALLEY-TRANSZOIA
18	UON 000014	RIFTVALLEY-LAIKIPIA
19	UON 000057	WESTERN-BUNGOMA
20	UON 000027	RIFT VALLEY-WEST POKOT
21	UON 043378	RIFTVALLEY-KEIYO
22	UON 000060	WESTERN-BUNGOMA
23	UON 032248	RIFTVALLEY-WESTPOKOT
24	UON 043757	WESTERN-BUNGOMA
25	UON 032222	RIFTVALLEY-BARINGO
26	UON 034699	EASTERN-MAKUENI
27	UON 000440	RIFTVALLEY-KERICHO
28	UON 000432	NYANZA-SIAYA
29	UON 000434	NYANZA-SIAYA
30	UON 000929	EASTERN-KITUI
31	UON 000059	WESTERN-BUNGOMA
32	UON 034698	EASTERN-MAKUENI
33	UON 000080	WESTERN-BUSIA
34	UON 000376	NYAZA-KISII
35	UON 000099	WESTERN-SIAYA
36	UON 000097	WESTERN-SIAYA
37	UON 000066	WESTERN-BUNGOMA
38	UON 000096	WESTERN-SIAYA
39	UON 000376	NYANZA-KISII
40	UON 000067	WESTERN-BUNGOMA
41	UON 000073	WEATERN-SIAYA
42	UON 000077	WESTERN-BUSIA
43	UON 000079	WESTERN-BUSIA
44	UON 000075	WESTERN-SIAYA
45	UON 000064	WESTERN-BUNGOMA
46	UON 000070	WESTERN-BUSIA
47	UON 043967	NYANZA-SIAYA
48	UON 043978	WESTERN-BUSIA
49	UON 043097	NYANZA-SIAYA
50	UON 043977	WESTERN-BUSIA
51	UON 043975	WESTERN-BUSIA
52	UON 034691	EASTERN-MAKUENI

53	UON 000413	NYANZA-S.NYANZA
54	UON 000377	NYANZA-KISII
55	UON 048372	NORTH EASTERN-WAJIR
56	UON 000977	EASTERN-KITUI
57	UON 000412	NYANZA-S.NYANZA
58	UON 000069	WESTERN-BUSIA
59	UON 048367	EASTERN-MOYALE
60	UON 48383	NORTH EASTERN-WAJIR
61	UON 000447	NYANZA-S.NYANZA
62	UON 048382	NORTH EASTERN-WAJIR
63	UON 000959	EASTERN-KITUI
64	UON 000422	NYAZA-S.NYANZA
65	UON 048375	NORTH EASTERN-WAJIR
66	UON 000957	EASTERN-KITUI
67	UON 000366	NYANZA-KISII
68	UON 000078	WESTERN-BUSIA
69	UON 000107	NYANZA-KISUMU
70	UON 000425	NYANZA-SIAYA
71	UON 048388	NORTH EASTERN-WAJIR
72	UON 043971	NYANZA-SIAYA
73	UON 048390	NORTH EASTERN-WAJIR
74	UON 042992	WSTERN-KAKAMEGA
75	UON 043987	WESTERN-KAKAMEGA
76	UON 048389	NORTHEASTERN-WAJIR
77	UON 048385	NORTH EASTERN-WAJIR
78	UON 042990	WESTERN-KAKAMEGA
79	UON 000946	EASTERN-KITUI
80	UON 000437	NYANZA-SIAYA
81	UON 000955	EASTERN-KITUI
82	UON 044083	EASTERN-MERU
83	UON 048377	NORTH EASTERN-WAJIR
84	UON 000993	EASTERN-KITUI
85	UON 000020	RIFTVALLEY-BARINGO
86	UON 000998	EASTERN-KITUI
87	UON 000056	WESTERN-BUNGOMA
88	UON 000433	NYANZA-SIAYA

89	UON 048369	EASTERN-MOYALE
90	UON 043100	NYANZA-SIAYA
91	UON 000949	EASTERN-KITUI
92	UON 44112	COAST-TAITA TAVETA
93	UON 043323	RIFT VALLEY-KEIYO
94	UON 001001	EASTERN-KITUI
95	UON 000936	EASTERN-KITUI
96	UON 000951	EASTERN-KITUI
97	UON 000962	EASTERN-KITUI
98	UON 043063	WESTERN-KAKAMEGA
99	UON 043040	WESTERN-KAKAMEGA
100	UON 000058	WETERN-BUNGOMA
101	UON 043102	NYANZA-SIAYA
102	UON 048381	NORTH EASTERN-WAJIR
103	UON 044043	RIFTVALLEY-BARINGO
104	UON 048025	EASTERN-MOYALE
105	UON 048386	NORTHEASTERN-WAJIR
106	UON 048316	EASTERN-MOYALE
107	UON 000436	NYANZA-SIAYA
108	UON 000018	RIFTVALLEY-LAIKIPIA
109	UON 000947	EASTERN-KITUI
110	UON 034692	EASTERN-MAKUENI
111	UON 000015	RIFTVALLEY-LAIKIPIA
112	UON 043018	WESTREN-KAKAMEGA
113	UON 034596	COAST-TAITA TAVETA
114	UON 044044	RIFT VALLEY-BARINGO
115	UON 044051	RIFT VALLEY-TRANS NZOIA
116	UON 048379	NORTHEASTERN-WAJIR
117	UON 000418	NYANAZA-S.NYANZA
118	UON 044045	RIFTVALLEY-SAMBURU
119	UON 000426	NYANZA-SIAYA
120	UON 00953	EASTERN-KITUI
121	UON 044111	COAST-TAITA TAVETA
122	UON 044116	COAST-TAITA TAVETA
123	UON 000999	EASTERN-KITUI
124	UON048393	NORTH EASTERN-MARSABIT

125	UON 044042	RIFTVALLEY-BARINGO
126	UON 044117	COAST-TAITA TAVETA
127	UON 044072	CENTRAL-KIRINYAGA
128	UON 048368	EASTERN-MOYALE
129	UON 000435	NYANZA-SIAYA
130	UON 048387	NORTH EASTERN-WAJIR
131	UON 000445	NYANZA-S.NYANZA
132	UON 048374	NORTH EASTERN-WAJIR
133	UON 000962	EASTERN-KITUI
134	UON 044081	RIFTVALLEY-SAMBURU
135	UON 048370	NORTH EASTERN-WAJIR
136	UON 034598	COAST-TAVETA
137	UON 048378	NORTHEASTERN-WAJIR
138	UON 048392	NORTH EASTERN-MARSABIT
139	UON 048371	NORTH EASTERN-WAJIR

4.8.2 DNA extraction

After incubating the sorghum seeds for a few days to hasten the development of roots and shoots, the seeds were then surface sterilized then ten to fourteen plants per accession were harvested and pooled into eppendorf tubes.

DNA was extracted from shoot and root samples of 3-4 days old sorghum seedlings using a modified CTAB protocol (Mace *et al.*, 2004). Two beads were put in each of the wells of the geno-grinder plate then kept in an ice bucket with liquid nitrogen. Leaves were cut and put in the wells and hot CTAB buffer added then geno-ground for 10mins. The macerated substance was transferred to fresh microfuge tubes and incubated for 10 minutes at 65°C with occasional mixing. Solvent extraction was done by adding 450µl chloroform: isoamylalcohol (24:1) to each tube and inverted twice to mix. The tubes were centrifuged at 12,000rpm for 10 minutes and the upper layer transferred to fresh microfuge tubes. 0.7 volume of isopropanol was added (stored at -20°C) and inverted once to mix and the tubes centrifuged at 12000rpm for 15 minutes. This was necessary to precipitate the crude DNA pellet. The supernatant was decanted and the pellet air-dried for

30 minutes. Low salt TE buffer (200µl) with 3µl RNase A (10mg/ml) was added to each of the samples and incubated for 30 minutes or overnight at room temperature (in the dark). A second solvent extraction was done by adding 200µl phenol: chloroform: isoamylalcohol (25:24:1) to each sample and inverted twice to mix then centrifuged at 12,000 rpm for 10 minutes. The volume was transferred to fresh Eppendorf tubes and chloroform: isoamylalcohol (24:1) added to each tube then inverted twice to mix and centrifuged, after which the aqueous layer was transferred to fresh Eppendorf tubes. To purify the DNA, 31.5 ml ethanol: Sodium acetate solution was added to each sample and placed in -20°C for 5 minutes then centrifuged at 12,000 rpm for 5 minutes. The supernatant from each sample was decanted and the pellets washed with 200µl 70% ethanol. The tubes were centrifuged at 12,000 rpm for 5 minutes and the supernatant from each sample decanted and the pellet air-dried for approximately 1hr. The pellet was re-suspended in 100µl low salt TE and stored at 4°C.

4.8.3 DNA quality, quantity and purity checks

Once the DNA was isolated, its quality and quantity was determined since PCR reactions using SSR markers were optimized for a specific DNA concentration. Therefore knowing the concentration of the DNA samples was essential to enable dilution of the DNA to the required concentration. In addition, PCR reactions needed high quality DNA that had not been degraded during DNA extraction. Thus, checking the quality of DNA before embarking on a genetic diversity project was extremely important to ensure high quality results. DNA quality checks were done using agarose (0.7%) gel electrophoresis stained with ethidium bromide (10 mg/ml) at a voltage of 70V for 30 minutes. The quantity and purity of the DNA were checked using a nano-drop spectrophotometer.

4.8.4 DNA normalization

Using the nana-drop readings and the agarose 0.8% gel images, all DNA samples were diluted to the required concentration (5ng/µl). A 1:10 dilution was made for

all DNA samples by mixing it with 40 µl distilled water. This was necessary to ensure uniformity in results. DNA samples (5µl) were mixed with bromo-phenol blue dye and lambda DNA used as standards. Different concentrations of lambda DNA standards, that is, 2.5ng, 5ng, 10ng, 20ng, 50ng and 100ng were filled in the outer wells at the left edges of submerged gels in an electrophoresis unit, containing 1 x TBE buffer, for comparison. An electric current of 120 V was applied for 30 minutes. The gel was stained with ethidium bromide (1%), thus the DNA fragments emitted a luminous glow. The emittance was detected under UV light and photographed using a video capture system (Flowgen IS 1000). The concentration of the DNA fragments was estimated according to the thickness of the band in comparison with the Lambda DNA standards at the edge of the gel. After the gel was visualized and quantified, samples were diluted to different concentrations accordingly to obtain a final concentration of approximately 5ng/µl of DNA (Figure 9.0).

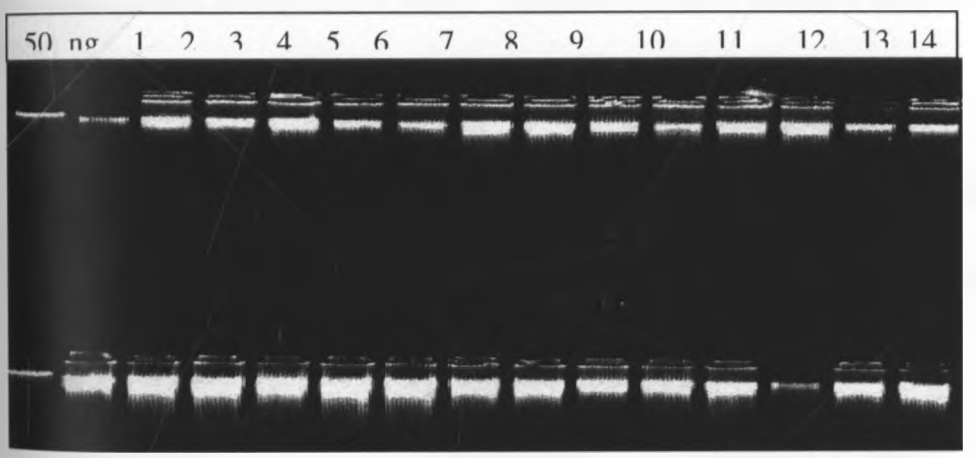


Figure 9.0: Normalized DNA samples.50ng DNA standard- lambda.

4.8.5 Optimization of PCR conditions

A set of 11 sorghum SSR primers was used for genotyping (Table 14.0). SSR markers were chosen based on three criteria: genome position, repeat size (ranging from di-nucleotide to hexa-nucleotide repeats) and the number of previously reported alleles (ranging from two to six). Optimization of the primers was necessary to avoid non amplifications and stuttering, a problem of un-

optimized PCR. All PCR reactions for the 11 primers were ran using the BTX623 standard DNA and the products were ran on 2.5% agarose gel to check for amplification. Optimization was done by varying the PCR components as in Table 15. The annealing temperatures of the 11 primers were optimized using the touchdown PCR amplification procedure.

4.8.6 PCR and capillary electrophoresis

Upon dilution of DNA samples to 5 ng/µl, a 5µl PCR mix consisting 5 ng of DNA, 10 X reaction buffer, 10 mM $MgCl_2$, 2 mM dNTPs, 2 pmols of forward and reverse primers, 0.5 U *Taq* polymerase and 2.23µl of deionised water was prepared for each genotype and amplified in GeneAmp PCR system 9700 (Applied Biosystems, Foster City, Ca, USA).

Table 14.0: Summary of the 11 SSR markers, primer multiplex and co-loading sets used in this study (to get primer sequences check from the references on the table).

Marker	Dye label	Multiple set	Repeat Motifs	References
mSbCIR300	PET	5	(GT)9	unpublished, Agropolis-Cirad-Genoplante
mSbCIR329	VIC	2	(AC)8.5	unpublished, Agropolis-Cirad-Genoplante
Xcup02	PET	1	(GCA)6	Schloss et al., 2002
Xcup14	FAM	10	(AG)10	Schloss et al., 2002
Xisep0310	PET	6	(CCAAT)4	unpublished, ICRISAT
mSbCIR223	NED	3	(AC)6	unpublished, Agropolis-Cirad-Genoplante
mSbCIR240	FAM	7	(TG)9	unpublished, Agropolis-Cirad-Genoplante
Xtxp012	VIC	3	(CT)22	Kong et al., 2000
Xtxp021	PET	4	(AG)18	Kong et al., 2000
Xtxp114	NED	2	(AGG)8	Bhattramakki et al., 2000
Xtxp141	FAM	3	(GA)23	Bhattramakki et al., 2000

Temperature cycling was carried out using the GeneAmp PCR systems 9600 (PE-Applied Biosystems) and touch-down PCR amplification: one 15min denaturation cycle, followed first by ten cycles of 94°C for 10s, 61°C for 20s (ramp of 1 per cycle) and 72°C for 30s, then by 31cycles of 94°C for 10s, 54°C for 20s and 72°C for 30s. After completion of the 31 cycles, a final extension of 20 min at 72°C was included to minimize the +A overhang (Smith *et al.*, 1995).

After the PCR, a few accessions in each primer were randomly selected and their PCR products run on a 2.5% agarose gel to check for amplification. Genotyping was carried out by capillary electrophoresis using the ABI PRISM 3730 (Applied Biosystems), a fluorescent based capillary detection system that uses polymer as the separation matrix.

Table 15.0: PCR optimization conditions for the primers used in the study.

Marker	Label	Primer 2p/ul	$Mgcl_2$ 10mM/ ul	dNTP S 2mM/ ul	DNA 5ng/ ul	Enzy me 5U/ul	Buffer 10X	Water
mSbCIR300	PET	0.5	0.75	0.25	0.75	0.02	0.5	2.23
mSbCIR329	VIC	0.5	0.75	0.25	0.75	0.02	0.5	2.23
Xcup02	PET	0.5	0.75	0.25	0.75	0.02	0.5	2.23
Xcup14	FAM	0.5	0.75	0.25	0.75	0.02	0.5	2.23
Xisep0310	PET	0.5	0.75	0.25	0.75	0.02	0.5	2.23
mSbCIR223	NED	0.5	0.75	0.25	0.75	0.02	0.5	2.23
mSbCIR240	FAM	0.5	0.75	0.25	0.75	0.02	0.5	2.23
Xtxp012	VIC	0.5	0.75	0.25	0.75	0.02	0.5	2.23
Xtxp021	PET	0.5	0.75	0.25	0.75	0.02	0.5	2.23
Xtxp114	NED	0.5	0.75	0.25	0.75	0.02	0.5	2.23
Xtxp141	FAM	0.5	0.75	0.25	0.75	0.02	0.5	2.23

This facilitated the accurate sizing of the microsatellite alleles to within ± 0.3 base pairs (Buhariwalla and Crouch, 2004).

PCR products were co-loaded post-PCR based on dye label, fragment size and fluorescence to reduce the unit cost of high throughput genotyping (Table 14.0). 0.5-1μl of labelled PCR products (depending on the intensity of the bands on agarose gel) were loaded mixed with formamide (PE-Applied Biosystems) and ROX-labelled GS500LIZ-3730 size standard (PE-Applied Biosystems). DNA fragments were denatured and size-fractioned using capillary electrophoresis on an ABI 3730 automatic DNA sequencer (PE-Applied Biosystems). The peaks were sized and the alleles called using GeneMapper software and the internal ROX GS500LIZ-3730 size standard. This system has the advantages of automated filling of capillaries, automated sample loading and rapid electrophoresis (Buhariwalla and Crouch, 2004). To verify the repeatability of each PCR and each capillary eletrophoresis run, a control sample (accession BTx623) was included during the PCR of each SSR marker and during each capillary electrophoresis run. Allelobin software was used for checking the quality of markers.

4.8.7 Data analysis

All SSR markers showed high reproducibility, with high consistency in the amplified product between the PCR and ABI runs of the control, BTx623. Therefore, all 11 markers were included in the analysis. Alleles were called and scored using the GeneMapper version 3.7 software, and then the data was subjected to Allelobin software to check the quality of the SSR markers that were used in the study. The data generated from Allelobin was analysed using PowerMarker version 3.25 to calculate the Polymorphic Information Content (PIC), heterozygosity and number of alleles for each marker, % of polymorphic loci estimates, genetic diversity among the accessions and their genetic distances . PIC values give the information that each marker impacts into the study, which is the measure of the usefulness of each marker in distinguishing one individual from another. Allele and genotype frequencies were calculated using haplotype diversity values calculated according to Nei (1978) (PowerMarker version 3.25).

Darwin Version 5.0 software was used to calculate the principle component analysis (PCA) and clustering among the accessions. To determine the genetic relationships and differentiation, the 139 sorghum accessions were clustered based on the matrix of genetic similarities using the Un-weighted Pair Group Method using Arithmetic Averages (UPGMA) clustering algorithm. Dissimilarity was calculated from allelic data, where a dissimilarity index was calculated by simple matching. The distances were computed for microsatellite data (11 loci) and trees constructed using the neighbour- joining method using Darwin Version 5.0 software. A second means of cluster analysis was performed by converting the presence or absence of alleles into binary data where a "1" indicated the presence of a specific allele and "0" indicated its absence, this was done using ALS – Binary software. The data was then transformed using BinGeno software then to Darwin version 5.0. PCA was then performed based on the variance-covariance matrix calculated from the transformed data.

The principle component scores were not standardized and thus had variance equal to the corresponding Eigen values. The UPGMA results were used to generate dendrograms. The robustness of the phylogenies was evaluated by bootstrapping (1000 permutations) over all loci. An exact test was used to determine possible deviations from Hardy-Weinberg Equilibrium and the existence of non-random associations of genotypes across polymorphic co-dominant loci (Weir, 1990). Analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) was used to partition SSR variation among groups. Significance levels for variance component estimates were computed by a non-parametric permutation procedure, using 100 permutations. AMOVA and F_{st} indices were calculated using the ARLEQUIN program, version 3.11 (http://cmpg.unibe.ch/software/arlequin3).

4.9 RESULTS

4.9.1 DNA quality, quantity and purity checks

The quality and quantity of the extracted root DNA showed a lot of variation. The concentrations ranged from as low as 3 ng/ul in sample ID numbers 30,32,39,47,62 and 79 to as high as 6944 ng/ul in sample ID number 80. The DNA purity was checked at light wavelengths of OD 260/280. For most of samples extracted the Dna quality was was high (for pure DNA OD 260/280 should be within a range of 1.8 - 2.0) except for samples ID numbers shown below that had OD260/280 below or above the required range.

Table 16.0: List of low quality samples according to the light wavelength of OD 260/280

Sample ID	Wavelength OD 260/280
74	1.21
86	1.25
68	1.29
75	1.31
51	1.33
140	1.35
85	1.39
98	1.39
89	1.44
80	1.51
39	1.59
91	1.6
5	1.61
135	1.64
64	1.66
99	1.71
132	1.71
58	1.72
84	1.74

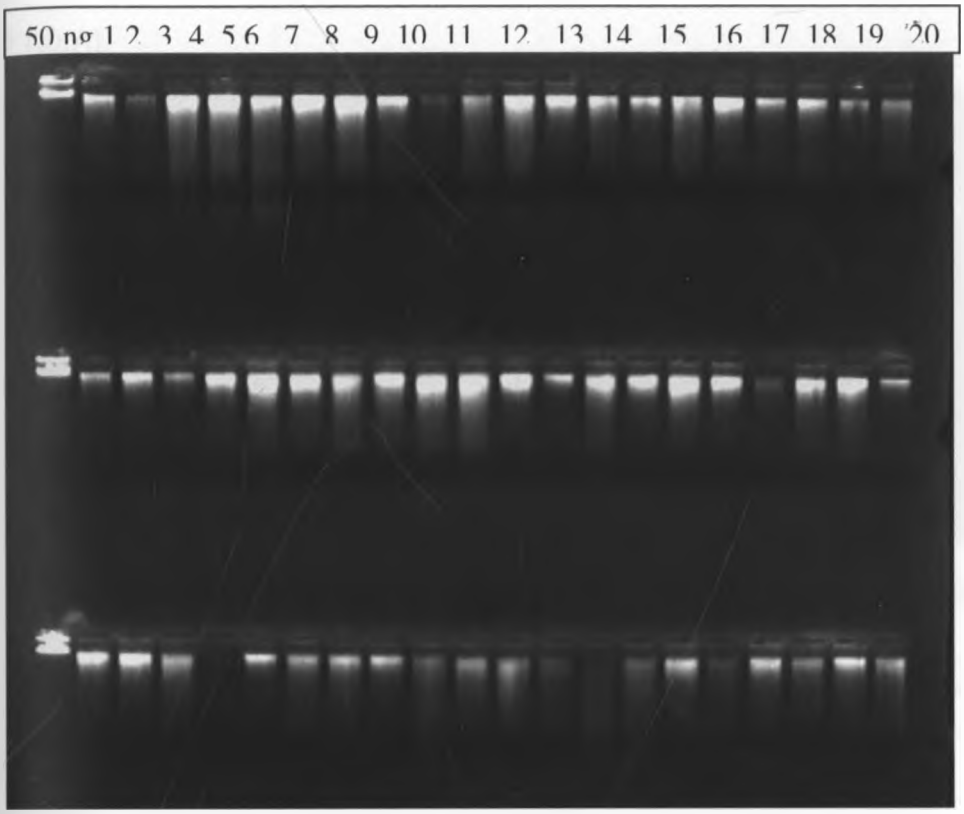


Figure 10.0: 0.8 % Agarose gel images for some of the DNA samples (2ul of DNA + 3ul 6x Loading Dye +1ul double distilled water) 50ng is a DNA standard of diluted Lambda DNA.
Voltage used; 100V for 30 minutes

4.9.2 Optimization of the primers

All the primers were optimized to specific conditions (Table 15.0). The PCR products were then ran on 2.5% agarose gel (Figure 11.0) and all the primers amplified well. The PCR products were ran on the sequencer to check the quality and height of the peaks as revealed by the electrophenographs (Figure 12.0). The peak heights in the electrophenograms were high (over 500 Relative Frequency Units). There was no stuttering or data loss observed in all the 11 markers. Some

of the alleles were homozygous while other alleles were heterozygous (Figure 11.0.A).

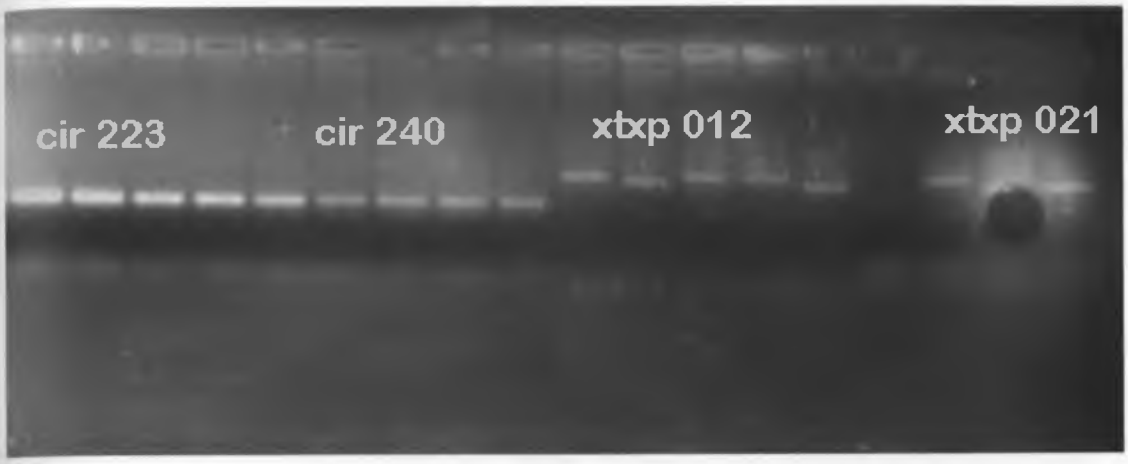
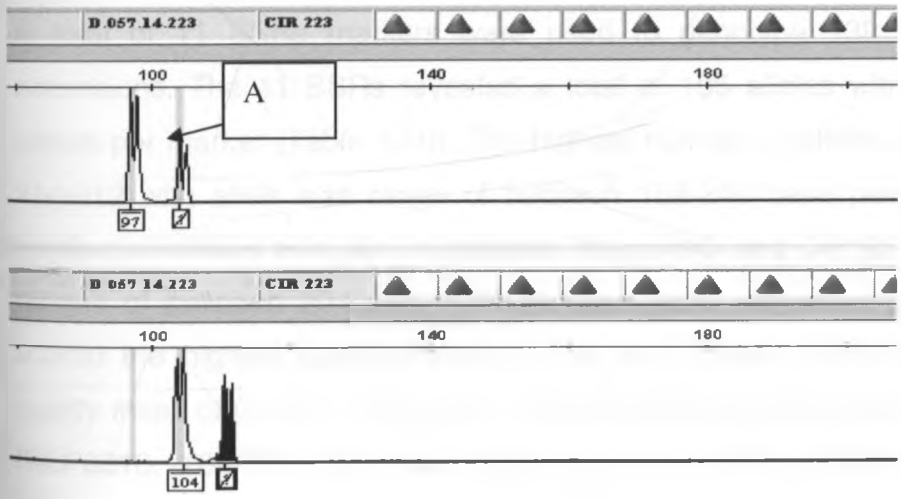


Figure 11.0: 2.5 % Agarose gel showing an optized PCR of the 11 SSR markers that were used for genotyping in this study.



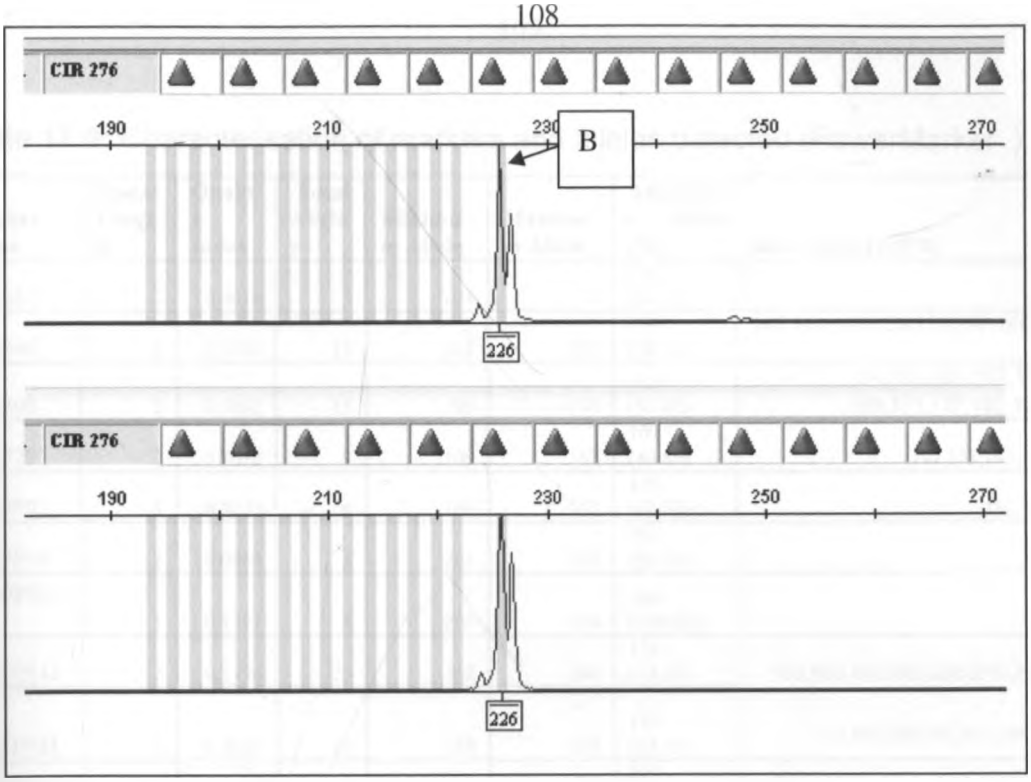


Figure 12.0: Genotype plot of a primer showing two allele states (A) heterozygote, (B) Homozygotes using Gene Mapper software

4.9.3 Marker characterization and allele frequencies

A total of 11 SSRs markers were used to genotype 139 Kenyan sorghum accessions. The 11 SSRs revealed a total of 105 alleles with a mean of 8.75 alleles per marker (Table 17.0). The highest number of alleles was 23 in marker Xtxp012 with allele size range of between 162-240 base pairs and the lowest number of alleles was two in markers Xisep0310 and CIR 223 with allele size ranges of between 204 and 104-114 base pairs respectively. Marker CIR300 scored the highest quality index of 0.39 and marker Xtxp114 had the lowest quality index of 0.0005. The highest percentage of abundant allele was in marker ISEP0310, 100.00%, and the lowest was for marker Xtxp012, 10.51%. The average number of rare alleles per SSR marker ranged from two alleles for markers CIR223, Xcup14 and Xtxp114 to 12 alleles for marker CIR300. Markers ISEP0310 did not have any rare alleles (Table 17.0). The PIC value over the 12 SSR markers averaged 0.62, ranging from 0.21 for marker ISEP0310 to 0.93 for marker Xtxp012. The mean level of heterozygosity per SSR marker was 0.17

Table 17.0: Characterisation of markers and alleles detected (PowerMarker)

Marker Name	repeat Length	Quality Index	Total Alleles	Minimum Allele	Maximum Allele	Abundant Allele (%)	Rare Alleles (<5%)
CIR223	2	0.0618	5	104	114	104 (44.27)	108,114
CIR240	2	0.2126	12	105	181	109 (58.03)	105,141,147,153,155,157,161,175,181
CIR300	2	0.3892	15	93	193	105 (51.82)	93,101,103,107,113,165,169,171,177,181,183,193
CIR329	2	0.1672	9	109	161	109 (40.65)	127,131,143,149,161
XCUP02	3	0.2177	6	160	202	193 (62.23)	160,190,196
XCUP14	2	0.0864	5	202	210	204 (60.53)	202,208
XISEP0310	5	0.1391	1	204	204	204 (100.00)	
XTXP012	2	0.1196	23	162	240	174 (10.51)	188,190,202,206,208,210,238,240
XTXP021	3	0.3647	10	168	228	171 (43.10)	174,180,186,192,201,228
XTXP114	3	0.0005	6	215	233	230 (32.20)	221,224
XTXP141	2	0.2655	13	135	165	149 (29.24)	135,143,153,155,159,000
Total			105				

Table18.0: Summary of markers and diversity indices (Power Marker ver 3.25).

Marker	Major allele Frequency	Genotype Number	Allele Number	Gene diversity	Heterozygosity	PIC
CIR 223	0.4427	7	5	0.6415	0.0992	0.5686
CIR 240	0.5657	20	14	0.6245	0.1971	0.588
CIR 300	0.5182	21	16	0.6903	0.2628	0.6671
CIR 329	0.4065	14	10	0.7255	0.0504	0.6845
XCUP 02	0.5863	10	9	0.5717	0.0647	0.513
XCUP14	0.6053	7	6	0.5523	0.0075	0.4911
XISEP 0310	0.8593	2	2	0.2419	0	0.2126
XTXP 012	0.1051	43	24	0.9352	0.1812	0.9313
XTXP 021	0.431	12	10	0.7289	0.0172	0.6938
XTXP 114	0.322	10	6	0.7373	0.8983	0.6906
XTXP 141	0.2924	24	13	0.8411	0.1102	0.8247
Mean	0.4668	15.4545	10.4545	0.6627	0.1717	0.6241

ranging from 0.00 for marker ISEP0310 to 0.89 for marker Xtxp114. Marker Xtxp012 had the highest gene diversity of 0.93 while marker ISEP0310 the lowest gene diversity of 0.24, the mean gene diversity per SSR marker was 0.66

4.9.4 Allele frequency

A total of 115 alleles were detected with an average of 10.45 allele per marker (Table 19). The number of alleles ranged from two Xisep 0310 to 24 in marker Xtxp 012. Of the total number of alleles detected, majority of them 49 (42.6%) were between the frequency range of 0.41-0.60. The lowest number of alleles were detected between the range of 0.81-1.00. (Figure 13 below)

In general, most of the alleles detected ranged between of 0.20-0.60.

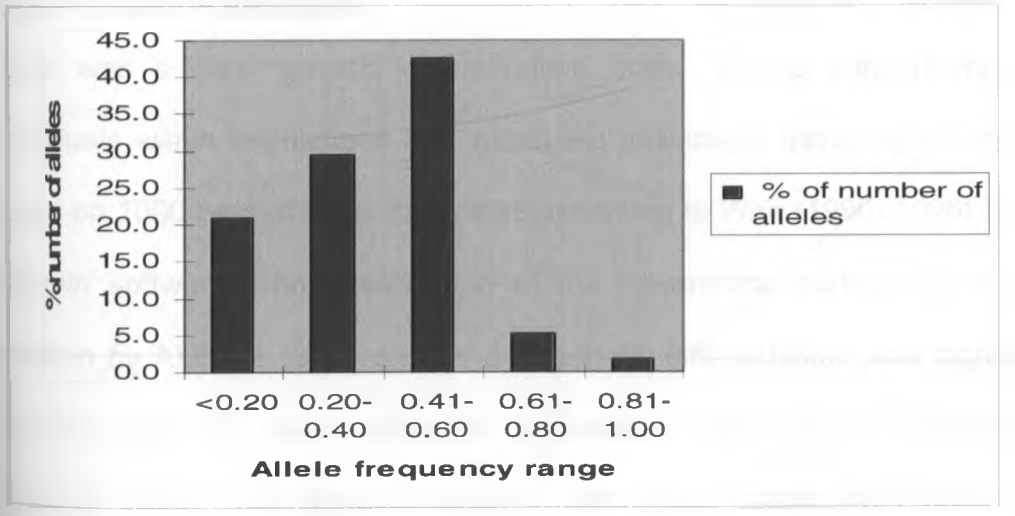


Figure 13.0: Allele frequency ranges and % of number of alleles

Table 19.0: Number of alleles per locus and their frequencies.

Marker	Number of Alleles	Allele Frequency
CIR 223	5.0000	0.4427
CIR 240	14.0000	0.5657
CIR 300	16.0000	0.5182
CIR 329	10.0000	0.4065
XCUP 02	9.0000	0.5863
XCUP14	6.0000	0.6053
XISEP 0310	2.0000	0.8593
XTXP 012	24.0000	0.1051
XTXP 021	10.0000	0.4310
XTXP 114	6.0000	0.3220
XTXP 141	13.0000	0.2924
Total	115	
Mean	10.4545	0.4668

4.9.5 Population structure

There was a clear genetic differentiation both among populations, among individuals within populations and within the individuals using significance tests based on 1000 permutations calculated according to Weir (1990; 1996) using the Arlequin software. The examination of the hierarchical partitioning of genetic variation by AMOVA demonstrated that genetic differentiation was significant at $P<0.00$ using the exact value for population differentiation (Raymond and Rousset, 1995). The genetic variation was higher within individuals (with a variance component of 0.37) than among populations with variance components of 0.13. The variation was highest among individuals within populations with a variance component of 1.5 of the total diversity, 6.48% was attributed to

population differences, 74.85% was attributed to differences among individuals within the population while 18.67% was attributed to differences within individuals. The Fst value was 0.06480, indicating a moderate level of genetic differentiation among the populations. The value of F, which is equivalent to F_{IT} and estimates the overall (total population) level of inbreeding, was 0.81 indicating a relatively high level of inbreeding while the value of theta (P) was 2.145 showing a high number of homozygotes as expected in sorghum (Table 20.0).

Table 20.0: AMOVA calculated according to weir, Weir, B.S. 1996

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	P-value	Fst
Among populations	6	49.62	0.13	6.48	0.020	0.0648
Among individuals within populations	132	445.39	1.50	74.85	< 0.001	
Within individuals	139	52.00	0.37	18.67	< 0.001	
Total	277	547.01	6.47	100.0		

4.9.5.0 Genetic relationships among the 139 accessions

4.9.5.1 Genetic distances

The Roger's modified genetic distance was calculated to determine the relationships among the 139 populations. Pair-wise genetic similarities between accessions were assessed based on Dice's genetic similarity co-efficients. The most distant accessions were UON000962, from Eastern Kitui, and UON048316 from eastern Moyale, UON048371 from North eastern Wajir, UON 048393 also

from North eastern Wajir, UON 000962 from eastern Kitui and UON 048316 from Eastern Moyale all of which had a genetic distance of 1. The closest accessions were UON044042 from Rift Valley Baringo and UON048025 from Eastern Moyale which had a genetic distance of 0 indicating they are one and the same. Other close related accessions included UON 000447 (South Nyanza), UON 000047 (Rift Valley-tranzoia), UON 000069 (Western Busia) and UON000014 (Rift Valley-Laikipia) which had a genetic distance of 0.15and 0.20 respectively.

The distance matrix of the pair-wise genetic distances between accessions was subjected to sequential agglomerative hierarchical nested (SAHN) with un-weighted pair-group analysis (UPGMA) using Dice's indices as provided in DARWin 4.0. The hierarchical cluster analysis (HCA) partitioned the accessions in accordance with their geographical regions. Major clusters were generated from Nei and Li (1979) genetic distance matrices. All accessions were distinctly placed in this dendrogram, and showed clustering into five groups (Figure14). As shown by the color codes it's clear that some accessions from same origins were scattered into different clusters while others from the same origin clustered together. The accessions are assumed to be grouped according to the geographical regions were they were collected.

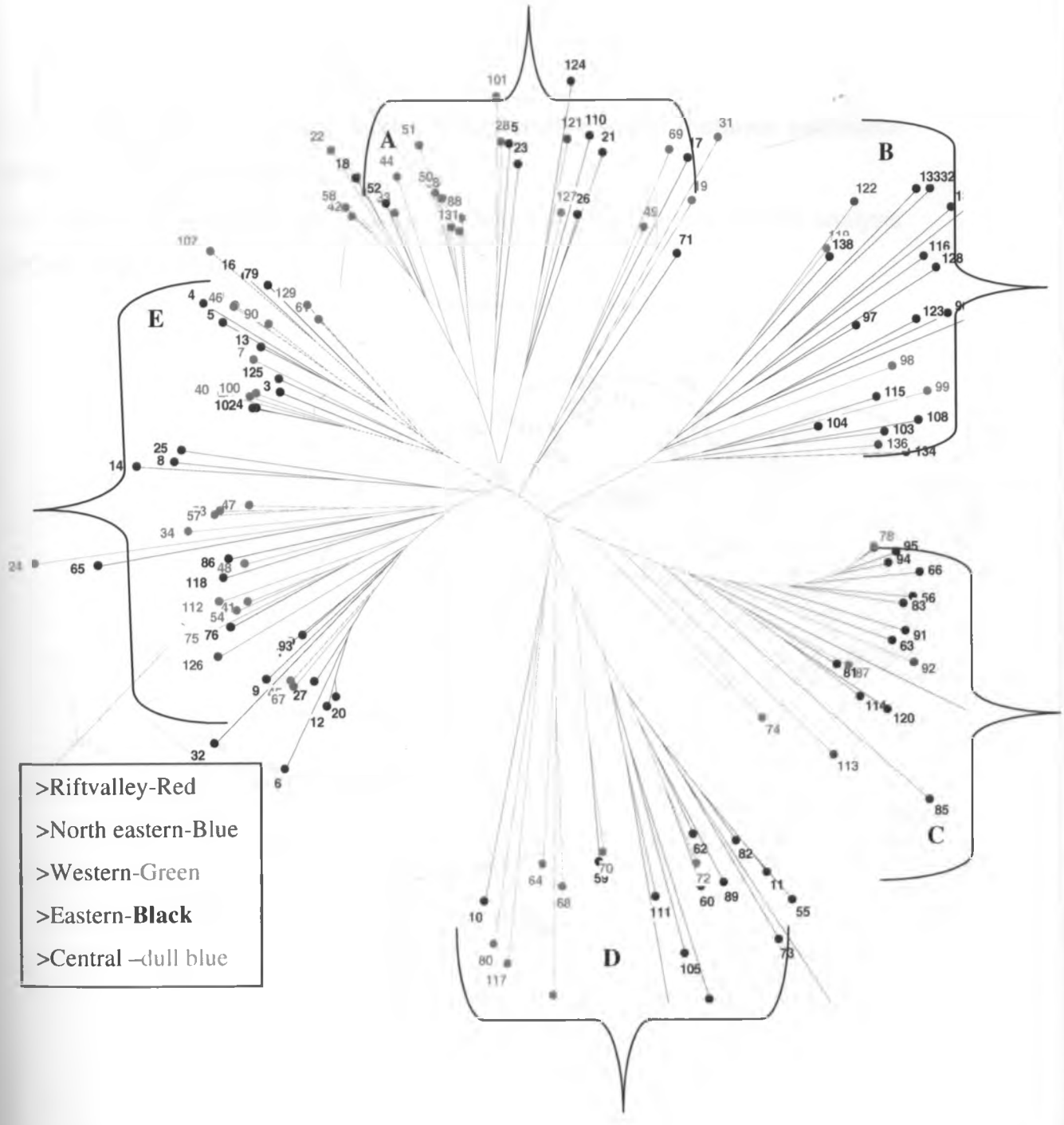


Figure 14.0: Neighbour joining tree showing relationships between 139 accessions from different parts of Kenya using the UPGMA method of Darwin Ver. 4.0.

4.9.5.2 Principal co-ordinate Analysis based on genetic distance estimates of the 139 Kenyan accessions

The patterns of cluster analysis were confirmed by principal co-ordinate analysis (PCoA) (Figure 15.0).

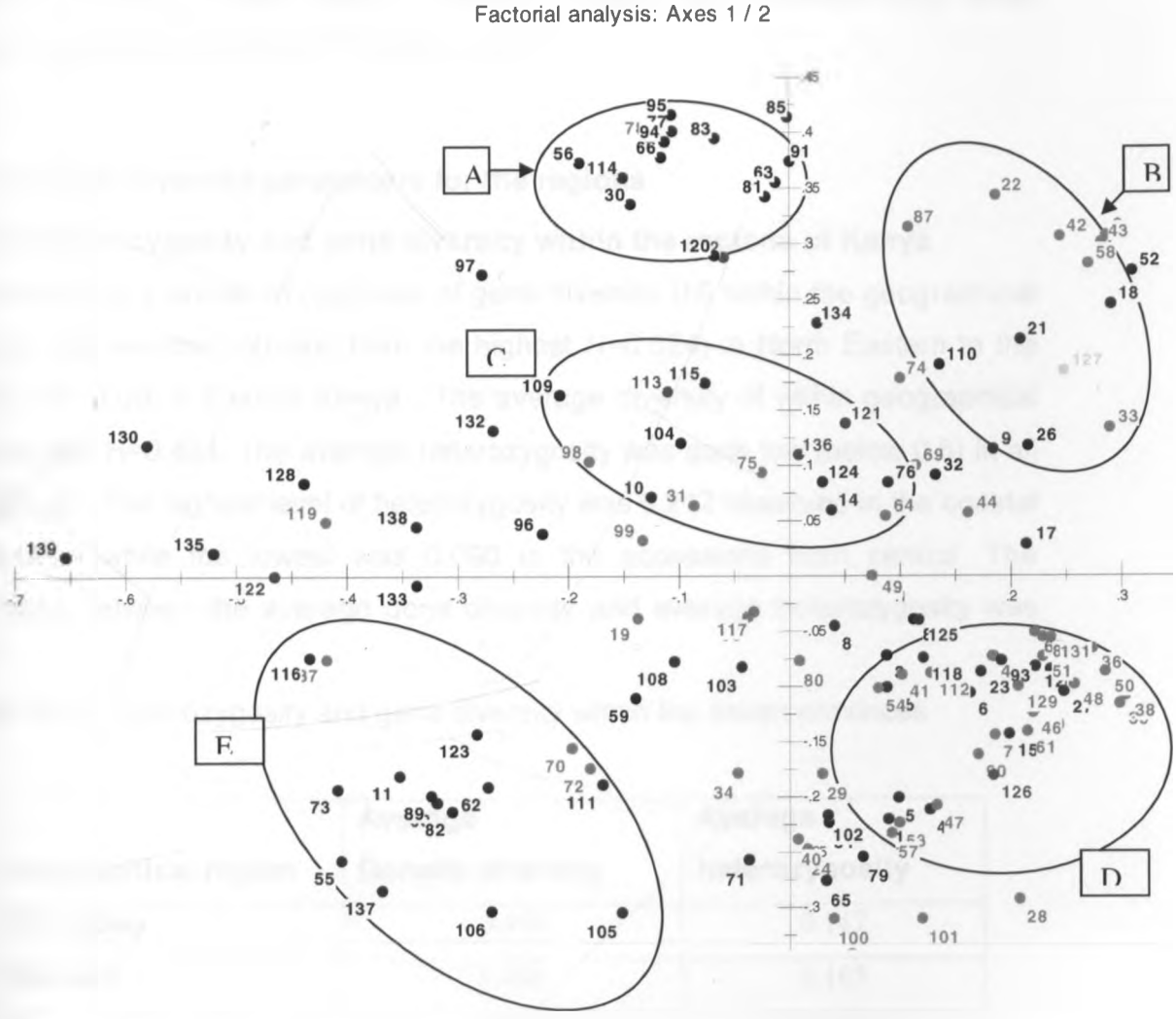


Figure 15.0: Principle co-ordinate scatter plot showing genetic distance estimates of 139 Kenyan accessions.

Western Kenya were distributed into three clusters B, C and D. Cluster D was the largest and was mainly comprised of materials from Rift valley, Nyanza and western Kenya.

A scatter plot of the first and second axes of non-metric multi-dimensional scaling (MDS) revealed five clusters of inter-relationships among accessions. The first Eigen vector explained 11.56% variation. The analysis showed that the accessions generally clustered on the basis of the geographical regions. Five clusters were clearly visible based on their geographical origins that were cluster A, B, C, D and E from Eastern, Western, Coastal, and Riftvalley and North Eastern regions respectively. Accessions from

4.9.6 Genetic diversity parameters for the regions

4.9.6.1 Heterozygosity and gene diversity within the regions of Kenya

The mean Nei's unbiased (estimate of gene diversity (*H*) within the geographical regions was variable, ranging from the highest *H*=0.624) in North Eastern to the lowest (*H*= 0.09) in Central Kenya. The average diversity of within geographical regions was H=0.484. The average heterozygosity was quite low (below 0.5) in all the regions. The highest level of heterozygosity was 0.212 observed in the coastal accessions while the lowest was 0.090 in the accessions from central. The difference between the average gene diversity and average heterozygosity was 0.32

Table 21.0: Heterozygosity and gene diversity within the seven provinces

Geographical region	Average Genetic diversity	Average heterozygosity
Rift Valley	0.494	0.147
Western	0.496	0.165
Nyanza	0.562	0.153
Eastern	0.602	0.210
Coastal region	0.523	0.212
Central	0.090	0.090
North eastern	0.624	0.172
Mean	0.484	0.164

The allele frequency based pair-wise genetic distances between the geographical regions calculated using PowerMarker version 3.25 (Liu and Muse, 2004) revealed the relatedness of accessions on a region by region basis (Table 21). The between regions pair-wise comparisons revealed that accessions from North Eastern and Central Kenya were the most distant whereas accessions from Western and Nyanza were the closest genetically. The accessions from Eastern and Coastal regions are also quite close.

Table 22.0: Genetic distance matrices between countries calculated according to Nei and Li (1979) using PowerMarker Ver. 3.25.

Geographical region	Rift Valley	Western	Nyanza	Eastern	Coast	Central	North eastern
Rift Valley	0.00	****	****	****	****	****	****
Western	0.03	0.00	****	****	****	****	****
Nyanza	0.02	0.01	0.00	****	****	****	****
Eastern	0.08	0.09	0.07	0.00	****	****	****
Coast	0.05	0.05	0.04	0.01	0.00	****	****
Central	0.12	0.15	0.10	0.18	0.09	0.00	****
North eastern	0.18	0.20	0.15	0.06	0.10	0.31	0.00

4.9.7 Discussions

The sorghum SSR markers that were used in this study revealed genetic polymorphism with a relatively high index (Table 17). Brown *et al.* (1996) as well as Anas and Yoshinda (2004), also working on sorghum, observed similar high levels of polymorphism. The mean diversity index per each SSR locus was 0.66 which allowed us to discriminate each of the 139 sorghum accessions. This suggests that the SSRs marker were useful as a tool in categorizing sorghum germplasm. The SSRs used in this study covered the sorghum genome (Dean *et al.*, 1999; Dje'*et al.*2000), fairlly well considering they were few in number. Thus, these markers should represent the genetic diversity among these sorghum lines (Anas and Yoshinda, (2004). All the 11 SSR loci were polymorphic as seen in previous studies of sorghum germplasm with diverse geographic origins (Grenier *et al.*, 2000b; Casa *et al.*, 2005).

The average number of alleles per locus identified in this study (10.45) was higher than the average of 5.9 previously reported in elite sorghum lines (Smith *et al.*, 2000). It was also higher than the average (7.8a/l) reported in the inbreds of sorghum (Menz *et al.*, 2004), (8.7a/l) reported in landraces from Southern Africa (Uptmoor *et al.*, 2003) and accessions from world germplasm collection (Dje *et al.*, 2000; Grenier *et al.*, 2000). As reported in a number of genetic diversity studies on other species and populations, SSR loci were able to uniquely identify each of our accessions. Thus confirming that the materials were quite diverse and also from diverse geographical regions.The number of observed alleles for most of the loci was higher than those observed by Schloterer (1998). This suggests that the sorghum accessions studied may be exceptionally polymorphic, providing more size variation within 30 accessions in the world collection (Brown *et al.*, 1996; Dean *et al.*, 1999; Dje *et al.*, 2000; Grenier *et al.*, 2000; Ghebru *et al.*, 2002). This could probably also be due to the inclusion of interracial varieties in the collections made.

Most of the 115 alleles detected in the 139 Kenyan sorghum accessions occurred between at a frequency of 0.60 or less translating to three out of five

chromosomes that carry an allele thus indicating a high allelic diversity. This allelic diversity reflects the high levels of polymorphism of the markers. The presence of many unique (rare) alleles may be an indication of the relatively high rate of mutation in the SSR loci analyzed. Mutations in SSR markers resulting in allele size differences are often caused by deletions or insertions of single or multiple repeat units due to unequal crossing-over followed by concerted evolution. The step wise mutation model (SMM) of Kimura and Crow (1964), assumes that alleles mutate back and forth by a small number of repeats and that the same allelic states are created repeatedly over time. An alternative model is the infinite alleles model (IAM) of Ohta and Kimura (1973), which assumes that each mutation creates a new allele in the population (Folkertsman *et al.*, 2005).

The observed inbreeding coefficient (F_{IS} = 0.81) was high, which would be expected under the mixed mating model as a consequence of self-fertilization at a rate of $S = 2 F_{IS}/ (1+F_{IS}) = 0.71$. A similar level of selfing has been reported for sorghum by Ellstrand and Foster (1983). Similar values of inbreeding coefficient (F_{IS} = 0.70) were obtained using both alloenzyme and microsatellite markers in cultivated sorghum sampled *in situ* in North-Western Morocco (Dje *et al.*, 1999). The coefficients obtained were higher than those of Dje *et al.*,(2000). The large F_{IS} values show the large degree of relatedness among the individuals within the sorghum accessions studied. This may be explained by the fact that there is a lot of selection for uniformity exercised by farmers in the collection areas (Ghebru *et al.*, 2002; Ghebru, personal interview of local farmers; Araya *et al.* 1997; Moa, 1999; Dje *et al.*, 2000).

Wright (1978) cited by Kiambi *et al.* (2005); Semagn *et al.* (2001) and Hartl (1987), suggested that an Fst range of 0-0.05 indicates little differentiation, 0.05-0.15 moderate and 0.15-0.25 large differentiation and above 0.25 indicates very large differentiation. In this study, the level of population differentiation was Fst =0.0648, which is moderate using the suggested parameters. This F_{ST} value was slightly lower than in other sorghum population genetics studies. Dje *et al.* (2000) reported Fst = 0.68 for landraces on the basis of only three different SSR loci. The Kenyan sorghum accessions show a lower level of allelic fixation than in

previously reported landrace populations. Apparently, this could be due to a reduced level of inbreeding and hence high levels of heterozygosity as reported by Ghebru *et al.* (2002).The F_{ST} values observed in this study are relatively low, indicating a reduced degree in allelic fixation. New alleles may be generated because of outcrossing and subsequent intralocus recombination, including gene conversion. Because local farmers practice little selection, effective populations stay large, thereby decreasing the opportunity for fixation of any alleles.

In this study, a high inter-population differentiation was found (74.85 %). Isoenzyme studies have shown that selfing species have nearly 60% of their genetic diversity distributed among populations while less than 25% of the genetic diversity of mixed mating and outcrossing species is found among populations (Semagn *et al.* 2001; Hamrick and Godt, 1997).

The Kenyan sorghum accessions showed a wide genetic background; the 139 accessions divided into five groups A –E. In this study, a clear tendency of clustering was observed based on the accessions breeding origin, similar clustering was also observed by Geleta *et al.* (2006). Heterozygosity cluster analysis dendrogram (Figure 12.0) clustered the sorghum accessions into five main groups. Most of the accessions given the same name, or similar identification characters by farmers were grouped together, similar results were also obtained by Ghebru *et al.*, (2002) based on the accession names (given by farmers) and again on the basis of their country of origin. Similar results were also obtained by Anas and Yoshinda (2004). In many instances, species exhibit a spatial structure of genetic variation across their ecogeographic range. Different levels of genetic diversity among regions may be due to several factors including mating systems, rate of mutation, migration and dispersal mechanisms, biotic and abiotic selection intensities which are determined by geographic location, climate and soil (Kiambi *et al.*, 2005).

Information about the relationship among breeding materials and the genetic diversity in the available germplasm is important for the choice of parents in breeding programs. This applies particularly to hybrid breeding where recognition and exploitation of heterotic patterns between different sources of germplasm are

important for success. The principal coordinate analysis PCoA based on genetic distance (GD) estimates determined by SSR data for the 139 individuals provided a distinct separation of lines from different germplasm groups in Kenyan sorghum. The cluster analysis based on genetic similarity among the 139 bulked sorghum accessions examined showed a clear demarcation of the germplasm mainly according to their breeding origin.

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CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study revealed that the characterization and evaluation of germplasm is a pre-requisite for the utilization of available diversity in the crop improvement programme. Hence, the accessions were characterized to assess the variability and identify the promising accessions for different traits. A wide range of variability was recorded for both quantitative and qualitative characters. A similar observation was reported by Bajracharya *et al.*, (2006) who used forty-two qualitative and quantitative traits to assess rice landrace accessions with 10 different names. Farmers use quantitative traits like earliness, plant height, seed mass and yield to determine desirable traits that can be retained for future cropping. The highly significant phenological variabilities in the sorghum accessions and between accessions and the seasons are an indication of suitability of certain genotypes to specific environments. The results agreed with findings of Bramwel *et al.*, (2004) who evaluated 638 accessions from 4 districts in Andhra Pradesh and found significant differences in quantitative traits among the accessions and between accessions and between districts.

The early maturing accessions in season one and two are possibly adapted for drought escape. Shortening of life cycle allows the crop to escape late season drought (Turner, 1979). Earliness also assists in creating asynchrony between plant development and the occurrence stress. This phenomenon has also been reported by Rao *et al.,1979* in tropical sorghum hybrids and varities.

Stay green has been associated with reduced lodging (Mughogho and Pande, 1984), higher levels of stem carbohydrates both during and after grain filling (McBee, 1984). This could explain the reason behind some of non senescent tall accessions that reported a few number of lodged plants. Such accessions include UON 48373, UON 43323, UON 47 and UON 43738.

In both seasons, plant height was observed to be highly influenced by moisture availability. This was quite evident in the year 2007 which was more stressed than 2008. The accessions in 2007 were observed to be of shorter statue than season two with a mean plant height of 132cm compared to 167cm in the subsequent season. A similar explanation could also be given to the observations made for the lower mean grain yields in 2007 than 2008. The mean for 100 seed mass in season two was low despite fairly high moisture levels at the beginning of the season. This could be attributed to the onset of moisture stress during grain filling. Similar observations were noted by Sionit and Kramer (1977) in soy bean. It was noted that tillering as induced by moisture stress can not be viewed as a drought resistance mechanism. It is instead a response mechanism for evolutionary survival. Under severe moisture stress especially during the flowering period, excessive tillering may have a negative effect on grain yield.

In the case yield, the poor performers may have been due to their greater stover production and their large area and number, and water requirement. Low harvest index and reduced assimilate portioning to the parnicle also appear to characterize the tall and late endemic sorghums of Africa (Willey and Basiime, 1973)

A number of genotypes were noted to possess the stay green trait, however it would be worth while noting that, sufficient expression of the trait for selection is dependent upon the occurrence of prolonged period of drought stress during the grain filling period of sufficient severity to accelerate normal leaf senescence, but not sufficient magnitude to cause premature death of plants. Because this precise requirement for the trait expression, field environments do not offer ideal conditions for selection and molecular markers associated with this trait may offer better alternative (Crasta et al., 1999; Xu et al., 2000)

Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including; analysis of genetic variability in cultivars, identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and

introgressing desirable genes from diverse germplasm into the available genetic base. As reported in this study, SSR loci were able to uniquely identify each of our accessions. The number of observed alleles for most of the loci was higher than those observed by Schloterer (1998). This suggests that the sorghum accessions studied may be exceptionally polymorphic, providing more size variation within 30 accessions in the world collection (Brown *et al.*, 1996; Dean *et al.*, 1999; Dje *et al.*, 2000; Grenier *et al.*, 2000; Ghebru *et al.*, 2002). This could probably also be due to the inclusion of interracial varieties in the collections made. Most of the 115 alleles detected in the Kenyan sorghum accessions occurred at a frequency of 0.60 or less translating to three out of five chromosomes will carry that allele thus indicating a high allelic diversity. This allelic diversity reflects the high levels of polymorphism of the markers. The presence of many unique (rare) alleles may be an indication of the relatively high rate of mutation in the SSR loci analyzed.

The Kenyan sorghum accessions showed a wide genetic background; the 139 accessions divided into five groups A –E. In this study, a clear tendency of clustering was observed based on the accessions race and breeding origin, similar clustering was also observed by Geleta *et al.* (2006). The between accession dendrogram (Figure 15.0) clustered the sorghum accessions into five main groups. Most of the accessions given the same name, or similar identification characters by farmers were grouped together, similar results were also obtained by Ghebru *et al.*, (2002) Anas and Yoshinda (2004) based on the accession names (given by farmers) and again on the basis of their country of origin. Different levels of genetic diversity among countries may be due to several factors including mating systems, rate of mutation, migration and dispersal mechanisms, biotic and abiotic selection intensities which are determined by geographic location, climate and soil

5.2 Conclusions

The characterization of genotypes for drought and the evaluation of their genetic inter-relationships require a multidisciplinary approach of both conventional and molecular breeding. To achieve an efficient breeding programme for drought resistance, a combination of several selection indices appears essential. In this study the accessions characterized showed a lot phenotypic and genotypic variability. This variability was quite useful in identifying promising accessions for different traits. Generally drought stress shortened the days to 50 % flowering in 2007 compared to 2008. Similar observations were also noted for yield, seed mass and the stay green levels at 15 days, 30 days and 45 days. This could be mainly attributed to the fairly higher moisture levels during the vegetative growth of the sorghum accessions in 2008 compared to 2007.Important correlations between grain yield and days to 50% flowering, and also between tillers and days to 50 % flowering were noted. High grain yield under water stress was mainly attributed to earliness. Among the accessions studied, cultivars UON 48723, UON 43323, UON 48378, UON 47, UON 445, UON 48381, UON 32 seem to be most adapted and stable accessions under drought conditions in the two seasons. These accessions scored well for most of the traits such as yield, lodging, earliness, seed mass and stay green. Such accessions could serve as a good source of germplasm for a drought improvement programme. Apparently a good number of these accessions were from North Eastern Kenya.

In a nutshell, accessions that were noted to be early maturing in both seasons were UON 48383, UON 48381, UON 959, UON 32, UON 48381 from North Eastern Kenya, North Eastern Kenya, Eastern Kitui, Rift Valley and Rift Valley respectively. The highest yielding accession in both seasons was UON 445 from Nyanza south. Most of the good yielding accessions that were observed to be mainly from RiftValley Pronvice mainly from Transzoia, Laikipia and Bungoma. Accession UON 48381 from North Eastern Kenya was also noted to be good yielding in both seasons. Accessions that were noted to be among the tallest in both seasons were mainly from Nyanza siaya, Eastern Kitui and also from Nyanza

south. Stay green in both seasons was noted to highest mainly from the accessions from Rift Valley, North Eastern Kenya and also from Western Kenya. Not all accessions observed to have high stay green were also high yielding or early flowering but a good number of accessions that were noted to have a high level of green leaf area (stay green) were also observed to be good yielders. The high heritability values for days to 50 % flowering, yield and the stay green at 15, 30 days and 45 days confirmed that the traits were stable for drought evaluation and affirmed the repeatability of the study.

Data on molecular diversity demonstrates that the Kenyan sorghum accessions contain a great deal of genetic diversity as indicated by the observed number of alleles. The high genetic diversity value among the sorghum accessions indicates that the level of genetic diversity was not influenced by breeding activities. The consistent clustering of most breeding entries close to each other in the present study apparently substantiates that the marker system used has a high potential in quantifying the level of similarity and relationships among sorghum germplasm.

The molecular data reaffirm the power of SSR markers to distinctly group closely related accessions. Hence, Kenyan sorghum deserve broader characterization at both molecular and Agricultural levels, Thus, the use of SSRs potentially could remove most, if not all, of the limitations in revealing polymorphism and in obtaining more complete genomic coverage for plants, as has been achieved already for the human genome. The utility of PCR-based markers such as SSRs for measuring diversity, for assigning genotypes to heterotic groups, and for genetic fingerprinting should prove valuable for sorghum breeding programs. Materials from North Eastern and Central Kenya showed the highest diversity and could thus good materials for polymorphism. Analysis of phenotypic performance in the field in combination with molecular analysis provides useful information to increase the efficiency plant breeding programs.

This study thus provides a detailed analysis and quantification of genetic diversity in sorghum accessions of Kenyan sorghum using both phenotypic and molecular markers.

5.3 Recommendations

There is need to include new tools that should enable breeders to better select for drought adaptation, both directly and indirectly. One such tool is simulation modeling which would involve using models with long-term weather data to classify the nature of water stress throughout the growing season in the target set of production environments. Genetic gain could be enhanced by using this information to weight the relative importance of data conducted in specific environment types. Other useful tools that would enhance the efficiency of drought breeding programs include bioinformatics and the field of transcriptomics.

More elaborate studies should be conducted to obtain preliminary information on the mode of gene action for the stay green trait in sorghum. The physiological mechanism of this trait is also not well understood stood in relation to moisture stress. This is also another avenue for more research.

Identifying and understanding the function of genes and gene networks that contribute to improved plant drought adaptation is a fundamental component that can improve sorghum breeding programs. New molecular technologies will enable discoveries to go beyond understanding of physiological function of genes, but ultimately discover the genes and the gene networks, responsible for the respective functions.

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APPENDICES

Appendix 1: Accessions and where they were collected.

ENTRY	SOURCE
UON15	Rift valley-laikipia
UON16	Rift valley-laikipia
UON28	Rift valley-Westpokot
UON31	Rift valley-Westpokot
UON32	Rift valley-Westpokot
UON32248	Riftvalley-west pokot
UON32358	Coast-kwale
UON412	Nyanza--south nyanza
UON43024	Western-kakamega
UON43063	Western-kakamega
UON43323	Riftvalley-keiyo
UON43324	Riftvalley-keiyo
UON43723	Riftvalley-keiyo
UON43738	western-Bungoma
UON43964	Nyanza-siaya
UON43971	Nyanza-siaya
UON44043	Riftvalley-baringo
UON44044	Riftvalley-baringo
UON44074	Central-nyeri
UON445	Nyanza-south nyanza
UON47	Rift valley-Transzoia
UON48367	Eastern-moyale
UON48378	Northeastern-wajir
UON48381	Northeastern-wajir
UON48383	Northeastern-wajir
UON48387	Northeastern-wajir
UON48392	Northeastern-Marsabit
UON50	Rift valley-Transzoia
UON59	Bungoma
UON62	Bungoma
UON66	Bungoma

UON959	Eastern-kitui
UON98	Western-siaya
UON998	Eastern-kitui
UON999	Eastern-kitui
UON15	Rift valley-laikipia
UON16	Rift valley-laikipia
UON28	Rift valley-Westpokot
UON31	Rift valley-Westpokot
UON32	Rift valley-Westpokot
UON32248	Riftvalley-west pokot
UON32358	Coast-kwale
UON412	Nyanza--south nyanza
UON43024	Western-kakamega
UON43063	Western-kakamega
UON43323	Riftvalley-keiyo
UON43324	Riftvalley-keiyo
UON43723	Riftvalley-keiyo
UON43738	western-Bungoma
UON43964	Nyanza-siaya
UON43971	Nyanza-siaya
UON44043	Riftvalley-baringo
UON44044	Riftvalley-baringo
UON44074	Central-nyeri
UON445	Nyanza-south nyanza
UON47	Rift valley-Transzoia
UON48367	Eastern-moyale
UON48378	Northeastern-wajir
UON48381	Northeastern-wajir
UON48383	Northeastern-wajir
UON48387	Northeastern-wajir
UON48392	Northeastern-Marsabit
UON50	Rift valley-Transzoia
UON59	Bungoma
UON62	Bungoma
UON66	Bungoma

UON959	Eastern-kitui
UON98	Western-siaya
UON998	Eastern-kitui
UON999	Eastern-kitui
UON15	Rift valley-laikipia
UON16	Rift valley-laikipia
UON28	Rift valley-Westpokot
UON31	Rift valley-Westpokot
UON32	Rift valley-Westpokot
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UON48383	Northeastern-wajir
UON48387	Northeastern-wajir
UON48392	Northeastern-Marsabit
UON50	Rift valley-Transzoia
UON59	Bungoma
UON62	Bungoma
UON66	Bungoma

UON959	Eastern-kitui
UON98	Western-siaya
UON998	Eastern-kitui
UON999	Eastern-kitui

Appendix 2: Kibwezi field station Weather data for the short season of 2008.

Month	Mean Max	Mean Min	Extreme Max	Extreme min	Rainfall(mm)
May	30.2	16.2	32.7	14.8	1.5
June	28.2	18.1	31.5	12.3	4.5
July	27.6	16.8	30.0	12.0	1.0
Aug	27.8	15.5	30. 0	9.5	0.0
September	30.9	16.6	32.8	13.6	0.0
October	31.9	17.8	35.2	14.5	0.0
November	30.6	19.4	34.8	15.8	202.3

Appendix 3: Kibwezi field station Weather data for the short season of 2007.

Month	Mean Max	Mean Min	Extreme Max	Extreme min	Rainfall(mm)
May	31.0	18.8	33.0	15.5	0.0
June	29.9	16.5	31.7	12.9	0.0
July	28.9	15.3	31.6	12.9	0.0
Aug	28.2	5.1	32.0	13.8	1.5
September	30.7	16.3	33.3	15.2	0.0
October	30.4	20.7	32.8	15.3	3.8
November	31.2	20.9	33.6	19.1	177.7

Appendix 4 : Analysis of variance (ANOVA) table for the effects of the season on the days to flowering during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	17.72	8.86	0.47	
Variety	33	58956.82	1786.57	94.99	<.001* *
Season	1	12769.59	12769.59	678.94	<.001* *
Variety*season	33	3406.75	103.23	5.49	<.001* *
Residual	134	2520.28	18.81		
Total	203	77671.16			

* = significant,* * = highly significant, ns = not significant

Appendix 5: Analysis of variance (ANOVA) table for the effects of the season on lodging during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	2.44	1.22	0.11	
Variety	33	417.29	12.66	1.18	0.255 ns
Season	1	424.29	424.29	39.49	<.001* *
Variety*season	33	392.66	11.90	1.11	0.334 ns
Residual	133	1428.92	1 0.74		
Total	202	2666.10			

* = significant,* * = highly significant, ns = not significant

Appendix 6 : Analysis of variance (ANOVA) table for the effects of the season on the plant height during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	1042	521	0.48	
Variety	33	204415	6194	5.70	<.001* *
Season	1	69421	69421	63.89	<.001* *
Variety*season	30	60852	2028	1.87	<.009* *
Residual	125	135821	1087		
Total	191	442026			

* = significant,* * = highly significant, ns = not significant

Appendix 7: Analysis of variance (ANOVA) table for the effects of the season 100 seedmass during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	0.85	0.43	10.36	
Variety	33	247.23	7.49	182.07	<.001* *
Season	1	93.15	93.15	2263.66	<.001* *
Variety*season	30	118.11	3.94	95.67	<.001* *
Residual	128	5.20	0.04		
Total	194	399.67			

* = significant,* * = highly significant, ns = not significant

Appendix 8: Analysis of variance (ANOVA) table for the effects of the season on the tilers during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	72.90	36.45	4.33	
Variety	33	1332.88	40.39	4.79	<.001* *
Season	1	3099.10	3099.097	367.89	<.001* *
Variety*season	33	1301.75	39.45	4.68	<.001* *
Residual	127	1069.85	8.42		
Total	196	6525.43			

* = significant,* * = highly significant, ns = not significant

Appendix 9: Analysis of variance (ANOVA) table for the effects of the season on the yield during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	871503	435752	4.21	
Variety	33	100709166	3051793	29.49	<.001* *
Season	1	2236642	2236642	21.61	<.001* *
Variety*season	30	33292097	1109737	10.72	<.001* *
Residual	128	13245450	103480		
Total	194	145124242			

* = significant,* * = highly significant, ns = not significant

Appendix 10: Analysis of variance (ANOVA) table for the effects of the season on stay green 15 days after flowering during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	57168	28584	0.75	
Variety	33	27249874	825754	21.53	<.001* *
Season	1	1963708	1963708	51.20	<.001* *
Variety*season	33	32907877	997208	26.00	<.001* *
Residual	134	5139864	38357		
Total	203	67318490			

* = significant,* * = highly significant, ns = not significant

Appendix 11: Analysis of variance (ANOVA) table for the effects of the season on stay green 30 days after flowering during the dry seasons of 2007 and 2008 at the Kibwezi field station.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	240207	120103	4.63	
Variety	33	14721013	466091	17.19	<.001* *
Season	1	1665848	1665848	64.19	<.001* *
Variety*season	33	18680287	566069	21.81	<.001* *
Residual	134	3477568	25952		
Total	203	38784924			

* = significant,* * = highly significant, ns = not significant

Appendix 12: Analysis of variance (ANOVA) table for the effects of the season on stay green 45 days after flowering during the dry seasons of 2007 and 2008 at the Kibwezi field station

Source of variation	d.f	s.s	m.s	v.r	F pr.
Block	2	78243	39121	3.34	
Variety	33	6728335	203889	17.40	<.001* *
Season	1	2241991	2241991	191.29	<.001* *
Variety*season	33	8476764	256872	21.92	<.001* *
Residual	134	1570506	11720		
Total	203	19095838			

* = significant,* * = highly significant, ns = not significant