Genetic diversity and post flowering drought tolerance analysis of Eritrean sorghum [*sorghum bicolour* (L) Moench] landraces using morpho-physiological and molecular markers

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DECLARATION

This thesis is my original work and has not been presented for a degree in any university.

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This work is dedicated to my late father Abraha Negash, who passed away while I was on study leave and who continuously supported and encouraged me but didn’t get the chance to be alive to see this outcome.

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ABBREVIATIONS AND SYMBOLS

µg	Micro gram
µl	Micro liter
Qt	Quintal = 100 kg
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BecA	Biosciences in eastern and central Africa
bp	Base pair
CTAB	Cetyl-trimethyl ammonium bromide
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
EG	Eritrean germplasm
FAO	Food and agriculture organization
FAOSTAT	Food and agriculture organization statistics
ICRISAT	International Crop Research Institute in Sem-Arid Tropics
IPGRI	Internation plant genetic resource institute
HAC	Hamelmalo Agricultural College
LG	Linkage group
LSD	Least significant diffence
MoA	Ministry of Agriculture
NARI	National Agricultural Research Institute
PC	Principal component
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PIC	Polymorphic information content
SSR	Simple sequence repeats

ABSTRACT

Sorghum (*Sorghum bicolour* (L.) Moench) is the most important staple food crop in Eritrea, mainly used for home consumption in the form of injera, bread, porridge and local alcoholic beverages. Sorghum grown under rain-fed conditions is usually affected by drought stress at different stages resulting in negative effect on yield. The assessment and quantification of morpho-physiological diversity for the traits contributing towards drought tolerance at these stages is of critical importance. The aim of this current study was to evaluate and identify sorghum landraces for post flowering drought stress tolerance and assess Eritrean sorghum landraces for diversity.The study was done in two parts: A two year field experiment on evaluation of sorghum landraces for post flowering drought tolerance in Eritrea, and a laboratory study on sorghum genetic diversity using SSR markers in Kenya. In a randomized field design experiment a total of 100 sorghum genotypes (96 landraces and 4 checks) were evaluated off-season (February – June, 2013) for drought tolerance and variation in morpho-physiological traits. Twenty genotypes selected from this rapid screening were proceeded to a replicated field evaluation trial at the Hamelmalo College field station, Eritrea in the off-season of March – June, 2014 using a split plot design and irrigation levels as the main plots and genotypes as the sub plots. Data collected on 16 different morpho-physiological traits were analysed using the analysis of variance, drought tolerance indices, estimation of genetic variability and heritability and principal component analysis. In the laboratory experiments the genetic diversity analysis of 98 Eritrean and 42 regional sorghum landraces was conducted using SSR markers.

Observation showed that the treatments under stress and irrigated conditions had significant genotypic differences at $P<0.05$ - <0.001. Based on grain yield, positive and significant correlations were recorded between yield under irrigated (Yi) and each of the parameters yield under drought stressed (Yr) conditions, mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI). The biplot and cluster analysis also grouped clearly the tolerant and susceptible landraces based on drought tolerance indices. High magnitude of phenotypic and genotypic coefficient of

variations for plant height, harvest index and biomass as well as high heritability for days to flowering, panicle length, days to maturity and over all agronomic score were recorded.. Principal component (PC) analysis showed that the first 4 PCs having eigen values >1 explained 74.6% of the total variation. Based on these analyses and drought indices, seven (7) accessions namely, EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711 were identified as promising genotypes for post-flowering drought tolerance that could be used by breeders in sorghum improvement programmes and small scale farmers. The SSR genotyping analysis also revealed high genetic variation among the landraces, especially among individual populations. Besides this, the results indicated that the landraces had unique alleles and higher levels of allelic richness with close genetic distance and isolated clustering that could indicate the Eritrean germplasm have not been introgressed with foreign genes and are a valuable resource for future breeding programmes.

Key words: Biplot analysis, Drought stress, Genetic diversity, Principal Component Analysis, Sorghum, SSR markers

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Sorghum [*Sorghum bicolour* (L.) Moench 2n = 20] is a food staple for more than 500 million people in the semi-arid tropics of Africa and Asia and more than 80% of the world area of production is confined to these two continents (Serna-Saldivar & Rooney, 1995). In sub-Saharan Africa, over 100 million people depend on sorghum as a staple (Smith & Frederiksen, 2000). Nutritionally, the grains are equal to or superior to other staple cereals and it is a good source of quality protein and various minerals. In marginal and medium agricultural zones, sorghum is a high priority staple in eastern African countries (Ketema, 2008). It is primarily a crop of resource-poor small-scale farmers and is grown predominantly in marginal lands located in arid to semi-arid environments. The crop is typically produced under adverse conditions such as low input and on marginal lands. It is well adapted to a wide range of precipitation and temperature levels and is produced from sea level to above 2000 meter above sea level. Due to its drought tolerance and adaptation attributes, this crop is grown in eastern Africa where agricultural and environmental conditions are unfavourable for the production of other cereal crops. All facts of evidence point to the north-east quadrant of Africa, mainly Ethiopia, Eritrea and Sudan, as the centre of domestication of sorghum. Therefore, the greatest genetic diversity for both cultivated and wild forms of sorghum is found in these north eastern African countries.

The average yield of sorghum in eastern Africa has been limited to only 0.6-1.5 t ha^{-1} compared to the worldwide average yield of more than 4.3 t ha^{-1} (Rohrbach, 2004). The low yields have been attributed to various biotic and abiotic constraints. The combined biotic stresses reduce sorghum grain yields by at least 60% while drought stress alone, under severe conditions, may cause total crop failure. Current and predicted climate change will likely result in increased temperatures and unreliable rainfall, and may lead to a larger diversity of pests and diseases attacking these crops. Sorghum production in

eastern Africa is expected to be greatly affected by the effects of climate change and the livelihoods of millions of people depending on this crop will be at high risk. Therefore, producing more resilient and drought tolerant varieties that are adapted to the changing climate as well as controlling diseases and pests through innovative biological systems is central to sustain the lives of millions of people in the region.

Crop improvement through conventional breeding is slow, especially for traits controlled by quantitative gene action like drought tolerance. Hence, the need to use modern crop improvement tools such as genomics to transfer genes from model species to the species of interest and genetic mapping in order to identify genes controlling traits of interest that can provide a more timely and robust response to crop production threats. It also provides added opportunities to develop crop varieties with multiple stress tolerance. Therefore, a crop's response to drought and/or pest attacks can be studied by the evaluation of traits that are related to these abiotic and biotic tolerances at the physiological, cellular, biochemical and molecular level (Praba et al., 2009).

Crop species of the Poaceae family, such as rice, sorghum, finger millet and pearl millet, display a remarkable level of genetic similarity despite their evolutionary divergence 65 million years ago (Gale & Devos, 1998; Devos, 2010). The high levels of conserved colinearity between different grass genomes can facilitate the exploitation of the information and resources available from sequenced genomes in cereal species to develop superior lines or genotypes that can perform well in drought prone drylands (Srinivasachary, Gale, & Devos, 2007).

Sorghum requires less moisture than other cereal crops and is more tolerant to drought prone and poorly drained soils, making production easier in most agro-ecological zones with limited rainfall areas which are unfavourable for most cereals (Maunder, 2002). Sorghum is an important food crop in Eritrea where it is widely grown in the mid lands, low lands and semi-arid regions of the country (Tesfamichael, 1999). Being an indigenous crop, a large amount of variability exists in the country, as a result, a large number of sorghum germplasm have been collected by the Eritrean Genetic Resource

since 1993. Many of these accessions have not been evaluated in the country using morphological, biochemical and DNA molecular markers (Tesfamichael et al., 2013).

Limited diversity studies on sorghum have been carried out in Eritrea, which, like in most countries, is threatened by loss of landraces due to introduction and development of improved varieties. Evaluating genetic diversity of germplasm can assist to identify accessions with novel traits which can be incorporated into crop improvement programmes. Besides, genetic distance estimates determined by phenotypic and molecular markers help identify suitable germplasm for incorporation into future plant breeding programmes.

1.2 Statement of the problem

Drought is one of the major constraints to crop production in Eritrea. Drought occurs as a result of inadequate, poor distribution and erratic rainfall and a short rain season which is associated with high temperature and high solar radiation. Drought is also unpredictable in its timing of occurrence, duration and intensity. Drought stress in Eritrea causes a severe yield reduction. In some years its effect can cause complete crop failure especially if it occurs at post flowering growth stage.

Sorghum is a major crop in Eritrea. The existence of the different sorghum landrace accessions, which can respond to the recurrent moisture stress, is expected to provide an opportunity in screening and identifying best drought tolerance accessions with relatively stable yield. Even though Eritrea has rich sorghum diversity, there has been no systematic evaluation that has looked into superior trait for drought resistance potentials of this crop. In addition, there is limited information on sorghum productivity and constraints to its production in the country. Even those little documented information available in the country were found scattered in different reports. The available information thus needs to be compiled for use by relevant stakeholders.

Evaluating sorghum germplasm genotypes and accessions based solely on a few discrete morphological characters for drought resistance may not provide an accurate indication of the genetic divergence among the cultivated genotypes/ landraces of

sorghum. The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. DNA markers have the potential to enhance the operation of a plant breeding programme through a number of ways, ranging from finger printing of elite genetic stocks, assessment of genetic diversity, increasing the efficiency of selection for difficult traits.

The Eritrean sorghum landraces have common names depending on the location. However, this doesn't mean that the landraces are genetically the same because of the common name in different regions. On the other hand, the fact that they have different names does not mean that they are genetically different. Besides, there has been no study to determine drought tolerance on the Eritrean sorghum landraces. Therefore to asses the variability of the landraces genetic and morphological evaluation is necessary using SSR markers for different traits.

Field evaluation of the selected Eritrean accessions assisted by SSR markers will lead into identifying superior genotypes that can be used for direct improvement or as parent material for further diversification in developing drought tolerant genotypes.

1.3 Justification

Sorghum is a major staple food and a leading cereal crop in Eritrea; more than 50% of the area under cereals and 45% of total yield comes from this crop. In extreme drought period, yield losses in sorghum in Eritrea are estimated to be between 70 - 100% (Tesfamichael et al., 2013). The general complexity of drought problem in Eritrea is often aggravated by low and erratic rainfall and short rainy season which is associated with high temperature, high level of solar radiation and poor soil characteristic. Previous research work indicates that genetic diversity plays a vital role in the success of any breeding programme (Ali et al., 2007). The local germplasm, which are the spine of agricultural production in Eritrea, are well adapted to stressful environments and farmers prefer these landraces due to their ability to produce some yield even in difficult conditions where modern cultivars are failed.

Identification of promising landraces through molecular technique and field morphological evaluation is a key feature for earmarking best ones. Different studies have indicated that simple sequence repeat markers give a successful tool in genotyping and assessing the genetic diversity of many plant species (Kong, Dong, & Wet, 2000). Using SSR markers promising landraces will be identified as possible source of sorghum enhancement that can bring change in the improvement of sorghum production and hence improve the farmers' livelihood in particular and food security of Eritrea in general.

1.4 General and specific objectives

General Objective

Identify superior drought tolerant sorghum landraces through morphological and molecular techniques to contribute and enhance food security in Eritrea.

Specific Objectives

1. Assess the current level of productivity, constraints to production and utilization of sorghum in Eritrea.
2. Evaluate the extent of diversity in sorghum landraces/ traits associated with drought tolerance.
3. Analyse of selected sorghum landraces for their performance under drought stress conditions of Eritrea.
4. Investigate sorghum drought tolerance, phenotypic and genetic variances as well as heritability and drought stress indices of various characters.
5. Evaluate Eritrean sorghum landraces for diversity using SSR markers.

1.5 Hypothesis

1. No difference exist among the sorghum landraces for drought tolerance
2. No correlation between the marker data generated and morphological field tests

CHAPTER TWO
LITERATURE REVIEW

2.1 Sorghum origin and botanical description

Sorghum (*Sorghum bicolour* (L.) Moench) (2n =20) belongs to the family Poaceae, genus *Sorghum* Moench, species *bicolour* (L.) Moench and tribe Andropogoneae. Sorghum is a C4 grass and a close relative of maize. It is the fifth most economically important cereal crop grown worldwide (Doggett, 1988). It is a staple food used in porridges and breads in several parts of Africa and Asia (Mann et al., 1983).

Linnaeus described three species of cultivated sorghum: Holcus sorghum, Holcus saccaratus and Holcus tricolour. In 1794, Moench distinguished the genus Sorghum from the genus Holcus, and in 1805 Person suggested the name Sorghum vulgare for Holcus sorghum (L.). In 1961, Clayton proposed the name *Sorghum bicolour* (L.) Moench as the correct name for cultivated sorghum and this is currently the accepted one (Doggett, 1988).

Sorghum includes three species; the rhizomatous taxa *S. halepense* and *S. propinquum* and all annual wild, weedy, and cultivated taxa belong to *S. bicolour*. Sorghum taxonomy based on Harlan and deWet, classifies Sorghum bicolour into five races based on spiklelet morphology. These races are Bicolour, Guinea, Caudatum, Kafir, and Durra (Figure 2.1) Because of the variability that is found in each race, and the existence of race intermediates, a classification scheme integrating Harlan and deWet's classification with working groups (sub-races) was established (Dahlberg, Burkeand Rosenow, 2004). Common names of sorghum vary from continent to country levels. The most encountered names are: 'kafferkoren', 'soedangras', 'suikergierst', or 'suiker-sorghum' (the Netherlands), 'kaoliang' (China), 'Mtama', 'shallu' or 'feterita' (East Africa), 'durra' (Egypt), 'chicken corn', sorghum or guinea corn (United Kingdom), 'jola', 'jowar', 'jawa', 'cholam', 'bisinga', 'durra' or 'shallu' (India), 'kaffir corn' (South Africa), 'milo', 'sorgo', 'sudangrass' or sorghum (USA), 'milo' (Middle East Africa)

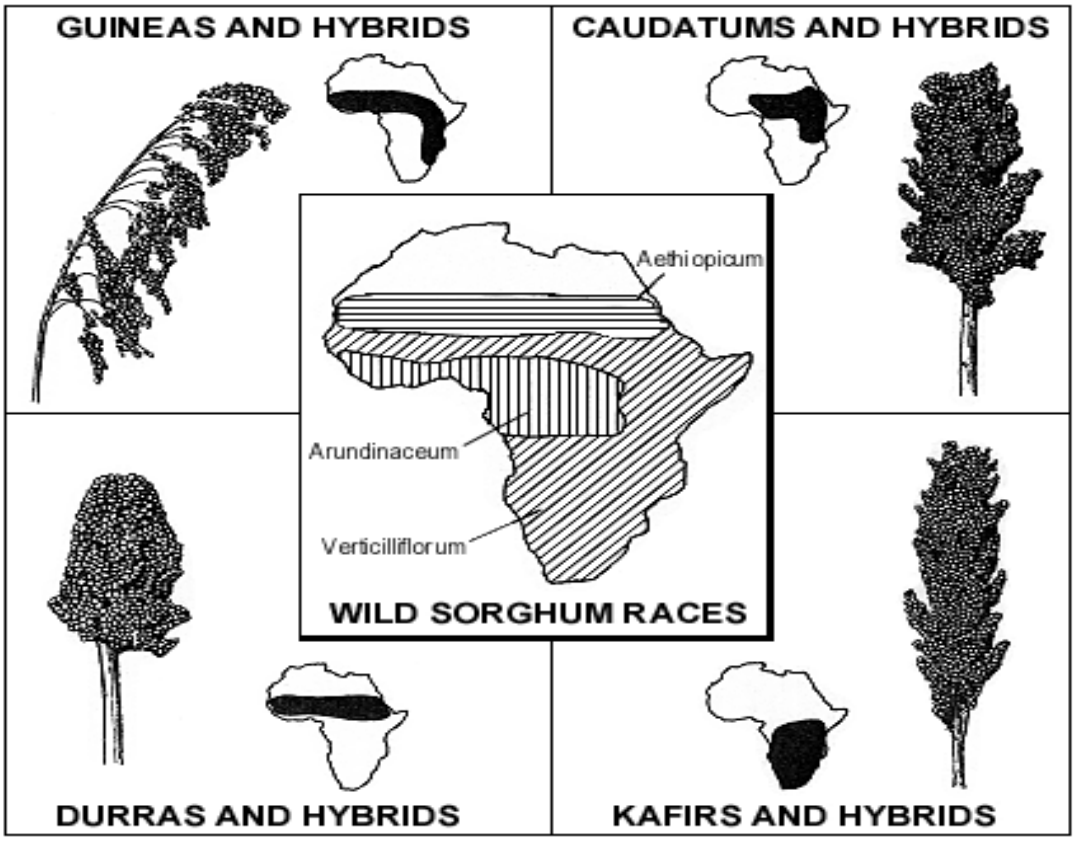


Figure 2.1. Races of sorghum based on spikelet morphology (Hancock, 2005).

and great millet, guinea corn, 'feterita', sorghum or 'sorgho' (West Africa) (Mamoudou et al., 2006). Doggett (1988) and (Zidenga, 2004) suggested that sorghum was domesticated and originated in the northeast quadrant of Africa, most likely in the Ethiopian-Sudanese border regions with domestication having taken place there around 5,000–8,000 years ago. The largest diversity of cultivated and wild sorghum is also found in this part of Africa. The presence of wild and weedy species as well as primitive races of bicolour in the south western of Eritrea especially the Goluj plains on the border with Ethiopia and Sudan could indicate that Eritrea is part of the primary centre of origin and diversity (ICRISAT, 2002). Diversity in Eritrean sorghum is based on maturity dates, adaptation to different soils and fertility levels, moisture regimes, panicle orientation, seed colour, seed size, disease and insect resistance and grain

quality (Tesfamichael et al., 2013). The presence of such a highly variable genetic pool with diverse agro-ecological adaptations poses an enormous challenge as well as opportunity for improvement of the crop (Doggett, 1988).

Certain varieties of sorghum posses "stay-green" genes that enable them to perform photosynthesis permanently. Sorghum is particularly adapted to drought prone areas: hot, semi-arid tropical environments with 400-600 mm of rainfall that are too dry for other cereals. Sorghum is also found in temperate tropical regions and at altitudes of up to 2300 meters above sea level. It is well suited to heavy soils commonly found in the tropics, where tolerance to water logging is often required. Sorghum is a vigourous grass that varies between 0.5 - 6 m in height. It has deep and spread roots with a solid stem. Leaves are long (0.3-1.4 m) and wide (1-13 cm) with flat or wavy margins. The flower is a panicle, usually erect, but sometimes re-curved to form a goose neck (Maiti, 1993). Grain or caryopse is usually covered by glumes. Glumes are the maternal plant tissues in the panicle that holds the developing caryopses after pollination.

2.2 Sorghum production and utilization

2.2.1 World sorghum production

Sorghum is one of the most important tropical cereal crops in the world (Anglani, 1998). In terms of cereal grains production, sorghum ranks fifth after wheat, rice, maize and barley (Smith & Frederiksen, 2000). In the year 2013 sorghum was cultivated in about 42 million hectares that produced approximately 61 million tons with an average productivity 1.5 tha^{-1} (FAOSTAT, 2014). More than 35% of sorghum produced is utilized as food and the balance is used primarily for animal feed, alcohol production and industrial products (Awika & Rooney, 2004; Dicko et al., 2006 b). In sub Saharan Africa sorghum is the second most important cereal crop after maize (*Zea mays* L) (Zidenga, 2004). It is the second most preferred cereal after tef (*Eragrostis tef* (Zucc.) Trotter) for preparing 'injera', which is the staple food in Eritrea and Ethiopia (Ayana, 2001).

2.2.2 Sorghum production in Eritrea and Kenya

Sorghum is the main food crop in Eritrea among cereals. The mean area of sorghum cultivation in the years 2000 to 2011 is about 224,000 hectares of land (Table 2.1). Nationally, sorghum accounts for more than 50% of total food crop production. Sorghum is particularly important in the lowlands of Eritrea where rainfall is erratic and crop failures are frequent, but it is also grown in nearly all regions of the country. Over 90% of the sorghum produced comes from subsistence farmers, who have small holdings and have not adopted improved production technologies.

Table 2.1. Area covered and production of cereals in the period 2000-2011, in Eritrea

Year	Area (ha)	Percentage of area cover	Average Production	Crop specific % of yield contribution
Sorghum	224089	53.8	114,703	**51.5**
P.millet	50714	12.2	15,901	**7.1**
Maize	17785	4.3	10,699	**4.8**
F.millet	23568	5.7	11,630	**5.2**
Barley	45217	10.8	33,650	**15.1**
Wheat	19631	4.7	14,379	**6.5**
Taff	29836	7.2	16,632	**7.5**
Hanfez	6059	1.5	5,077	**2.3**
Total	**416899**	**100**	**222671**	**100**

Source: Ministry of Agriculture, Eritrea (2012)

Average sorghum yield levels in Eritrea is below 1 t ha^{-1} (Table 2.2). The most common reasons for this low yield in sorghum is drought, pests, diseases, weeds (Striga), and lack of improved technology such as fertilizers and manure. (Tesfamichael et al., 2013).

Table 2.2. Area covered and production of Sorghum in the period 2000- 2010, in Eritrea

Year	Area (ha)	Production (Tons)	t ha^{-1}
2000	150,558	62,004	0.41
2001	165,821	78,758	0.47
2002	182,051	28,433	0.16
2003	200,933	64,061	0.32
2004	211,756	56,745	0.27
2005	233,134	184,271	0.79
2006	282,203	222,685	0.79
2007	282,909	302,515	1.07
2008	249,286	67,981	0.27
2009	250,971	59,188	0.24
2010	255,354	135,090	0.53
Average	**224,088.7**	**114,702.8**	**0.5**

Source: Ministry of Agriculture, Eritrea (2010)

Sorghum is a staple food crop for many low-income households in Kenya. It is typically grown by small-scale, resource-poor farmers and is mainly used for home consumption. As the only cereal species indigenous to Kenya, sorghum is produced throughout much of the country, even in areas with low agricultural potential. Sorghum can grow anywhere from sea level to 2,500 meters above sea level and requires a minimum rainfall of 250 mm per year and a minimum temperature of 10°C (Chemonics, 2010).

Most sorghum production is concentrated in Kenya's southwestern and south-central districts namely within the Eastern, Nyanza, Western and Rift Valley provinces, which accounted for about 43, 41, 9 and 7 percent respectively of Kenya's total sorghum production in 2011. Collectively, these provinces produce 99 percent of the country's sorghum (KMoA-ERA, 2012).

2.2.3 Utilization of sorghum in the world

Sorghum is grown in the United States, Australia, and other developed nations essentially for animal feed. However, in Africa and Asia the grain is used both for human food and animal feed. It is estimated that more than 300 million people from developing countries essentially rely on sorghum as a source of energy (Godwin & Gray, 2000). The main foods prepared from sorghum are: tortillas (Latin America), thin porridge, e.g. “bouillie” (Africa and Asia), stiff porridge, e.g. (West Africa), couscous (Africa), injera (Eritrea and Ethiopia), nasha and kisra (Sudan).

Traditional foods made from sorghum include unfermented and fermented breads, porridges, couscous and snacks, as well as alcoholic beverages. Sorghum blended with wheat flour has been used over the last two decades to produce baked products, including yeast-leavened pan, hearth and flatbreads, cakes, cookies, and flour tortillas. Sorghum flour alone is not considered as a bread making cereal because it lacks gluten, but addition of 20-50% sorghum flour to wheat flour produces excellent bread (Anglani, 1998; Carson, Setser, & Sun, 2000; Hugo, Rooney, & Taylor, 2000, 2003). Among interesting features of sorghum utilization is biscuits and other cooked products (Olatunji et al., 1989). In the USA and Japan, sorghum was considered as animal feed, however, utilization as human food is increasing because of its use in snacks and cookies (Rooney & Waniska, 2004). Sorghum has been intentionally introduced in China for food needs and it is becoming one of the most important crops in this country (Kangama & Rumei, 2005). The future promise of sorghum in the developed world is for wheat substitution for people allergic to gluten (Fenster, 2003). In addition, pasta products, such as spaghetti and macaroni made from semolina or wheat could be made with mixtures of composite flour consisting of 30-50% sorghum in wheat (Hugo et al., 2000, 2003). Pre-cooked sorghum flours mixed with vitamins and exogenous sources of proteins (peanuts or soybeans) are commercially available in many African countries for the preparation of instant soft porridge for infants. Sorghum can be puffed, popped, shredded and flaked to produce ready-to-eat breakfast cereals.

The grain sorghum plays a dominant role in the traditional beer brewing, at household and industrial levels (House et al., 2000). Grain sorghum is used to make products such as potable alcohol, malt, beer, liquids, gruels, starch, adhesives, core binders for metal casting, ore refining, and grits as packaging materials. Grains are a rich and cheap source of starch and have applications in the food, pharmaceutical, textile, and paper industries. Malt drinks and malt cocoa-based weaning food and baby food industries are popular in Nigeria (Chandel & Paroda, 2000). In Africa, sorghum is fermented to make beer, porridge, injera (fermented bread) and other products, to utilize the proteins it contains. Hard endosperm sorghum is used extensively in south-east Asia for noodles. Sorghum grain is one of the major ingredients in swine, poultry and cattle feed in the western hemisphere, China and Australia. The vegetative portions of plant are important sources of fuel for cooking and the stems of the wild varieties are used to make baskets or fish traps (Singh & Lohithaswa, 2006). The plant stem and foliage are used for green chop, hay, silage, and pasture. In some areas, the stem is used for hut making. Sweet sorghum is used to a limited extent in producing sorghum syrup and 'jaggery' (raw sugar) in India and has recently gained importance in ethanol production (Mamoudou et al., 2006). In South Africa, Nigeria and other African countries sorghum is industrially used for the production of lager beer (Taylor & Dewar, 2001).

2.2.4 Utilization of sorghum in Eritrea and Kenya

All sorghum grain produced annually in Eritrea is used for human consumption. The major part is used for injera. Injera, a leavened, round flat pancake, is the national dish of Eritrea. Second to Eritrean taff, sorghum is the cereal with the best quality for injera. But sorghum is also used for making bread. Especially the white grained and red grained are preferred for local bread, locally called kicha in Tigrigna language. The home-made beverage siwa, a nationally widespread local beer, can also be produced with sorghum. Sorghum is also used for porridge (geat), whole boiled grains (titko) and roasted grain (kolo) (Tesfamichael, 1999). The straw of sorghum is used for animal feed, fire wood, and construction.

Most sorghum grain in Kenya is consumed by rural households, who typically grind it into flour to make porridge, known as 'ugali'. Some sorghum grain is also processed into flour by commercial mills and sold in urban markets. In many cases, sorghum flour is used to enrich cassava flour before it is packaged and sold to consumers (Chemonics, 2010). The by-products from sorghum processing are typically used for animal feed production. In recent years, there has been growing demand for sweet sorghum within the brewing industry for use in beer production. On average 53 percent of the total sorghum supply in Kenya each year is consumed as food in the form of grain or flour, while 24 percent is processed to make other commodities (e.g. beer), 10 percent goes to the animal feed industry and 2 percent is used as seed for planting (FAOSTAT, 2012).

2.3 Quality and chemical composition of grain sorghum

2.3.1 Quality of grain sorghum for food and feeding

The quality of grain sorghum is determined by the status of visual quality, nutritional quality (including whole grain, protein and starch digestibility; nutrient bio-availability), and anti-nutritional factors such as tannins, processing characteristics, cooking quality and consumer acceptability (Hulse, Laing, & Pearson, 1980). Grains of most cereal species, such as wheat, maize and sorghum, provide food and are important economical commodities but contain inadequate amount of some essential amino acids, particularly lysine, threonine, tryptophan and methionine. A wide range of variability has been observed in the essential amino acid composition of sorghum protein, because the crop is grown under diverse agro-climatic conditions which affect the nutritional composition of the grain (FAO, 1995).

Seed colour is an important trait that affects grain quality in sorghum. The Sorghum caryopse is composed of three distinct anatomical components: seed coat (testa or pericarp), germ (embryo) and endosperm (storage tissue) (Figure 2.2). In some sorghum genotypes the testa is highly pigmented. The presence of a pigment and the colour is a character controlled by the R and Y genes (Waniska, 2000). The thickness of the testa

layer is not uniform and is governed by the Z gene. In some genotypes there is a partial testa, while in others it is not apparent or is absent.

The colour of sorghum grain varies greatly due to pericarp colour and thickness, presence of testa, and endosperm texture and colour. The relationship between sorghum colour and tannin content was previously reported (Hahn & Rooney, 1985). Phenolic compounds, particularly tannin, may change the pigmentation of the pericarp and testa in sorghum grain (Rooney & Miller, 1982).

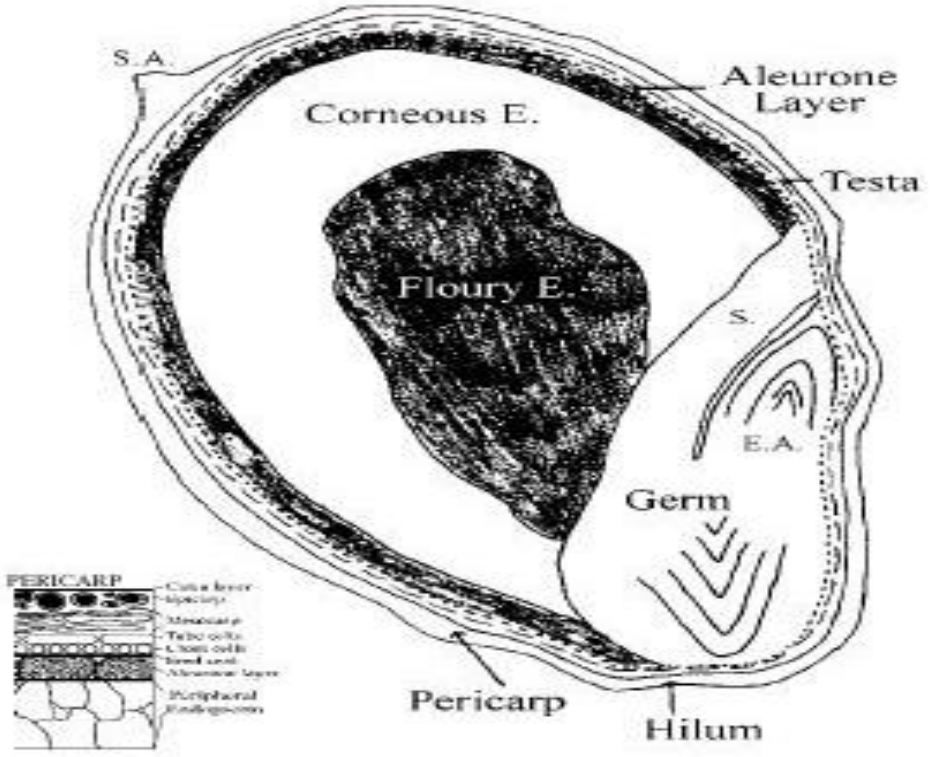


Figure 2.2. Diagram of sorghum caryopsis showing the pericarp [cutin, epicarp, mesocarp, tube cells, cross cells, testa, pedicel, and stylar area (SA)], endosperm (E) (aleurone layer, corneous, and floury), and germ [scutellum (S) and embryonic axis (EA)] (Source: Rooney and Miller 1982)

In sorghum, pericarp colour, secondary plant colour, endosperm colour, and the presence of a pigmented testa are factors affecting the colour and acceptability of food products (Waniska & Rooney, 2000). A large number of traditional food products (i.e., porridges, alcoholic and non-alcoholic beverages) are prepared using tannin sorghums.

Sorghums with a pigmented pericarp provide a unique opportunity to produce special food products with a natural, attractive dark colour, high levels of dietary fiber and antioxidants with a variety of phenols. Black and tannin sorghum brans have been added in to yeast-leavened bread formulas for production of food products with potential health benefits. For example, good-quality breads containing tannin sorghum bran have high phenols, antioxidant activity, and dietary fiber levels with a natural dark-brown colour and excellent flavor (Gordon, 2001; Rooney & Waniska, 2000). Healthy bread mixes containing tannin sorghum bran, barley flour, and flax seed hulls have also been developed (Rudiger, 2003).

The tannins in tannin pigmented sorghums provide a degree of resistance to bird predation in the field. Hence, the terms sometimes used for the tannin sorghums are ‘‘bird proof’’ or ‘‘bird resistant. ’’Birds can and do consume all sorghums and are not adversely affected by condensed tannins. In sorghum nurseries with white, red, and tannin sorghums, birds eat white sorghum first and then red sorghums before eating the Type II tannin sorghums and finally the Type III tannin sorghums (Rooney, 2005). Birds consume tannin sorghums when no other food is available, but definitely prefer other sorghums when given a choice (Bullard & Gebrekidan, 1989).

The grain sorghum is rich in protein, lipids, minerals and vitamin B; thus removal of the outer pericarp increases the protein and reduces the cellulose, lipid and mineral content of the grain. Sorghum is an important source of minerals that are located in the pericarp, aleurone layer and germ. Sorghum is a good source of potassium and an adequate source of magnesium, iron, zinc, and copper (Smith & Frederiksen, 2000). Grain sorghum is also rich in calcium and phosphorous (Hulse, Laing, & Pearson, 1980). The mineral composition of sorghum grain is highly variable. Other than genetic factors, environmental conditions prevailing in the growing region affect the mineral content of this food grain (FAO, 1995). Sorghum is a good source of vitamins, especially the B complexes (thiamin, riboflavin, pyridoxine), and the liposoluble vitamins A, D, E and K (Dicko et al., 2006a). Among the B group vitamins, concentrations of thiamin, riboflavin and niacin in sorghum were comparable to those in maize. Wide variations

have been observed in the values reported, particularly for niacin (Hulse, Laing, & Pearson, 1980).

Sorghum product quality is determined by endosperm texture and endosperm type which are important characteristics of the grain (Pushpamma and Vogel, 1982). Endosperm type refers to either a horny or floury endosperm (Dewar, Von Ascheraden, & Taylor, 1993), while endosperm texture is the proportion of horny to floury (soft) endosperm (Cagampang & Kirleis, 1984). Seed hardness, weight, and size are important parameters for assessment of sorghum grain quality (House, 1985). In addition to the average values for hardness, weight and size, uniformity of these characters is important in sorghum grain quality assessment because of its impact on processing. Grain size, shape, lustre and colour are the important grain quality traits that contribute to consumer preferences and acceptability. Grain size is more importantly influences both market and yield. Grain size has a positive correlation with grain yield (Potdukhe et al., 1994; Senthil & Palanisamy, 1995; Sankarapandian, Krishnadoss, & Devarathinam, 1996; Asthana et al., 1997; Muppidathi et al., 1999; Audilakshmi & Aruna, 2005), crude protein content (Hicks et al., 2002) and the stay-green trait in sorghum (Borrell et al., 2003). Furthermore, grain size, grain shape and lustre are also important quality characters that determine the market price of sorghum grain. Sorghum varieties with round and/or large grain have higher hulling yield (Audilakshmi & Aruna, 2005). Understanding the significant importance of gene effects is beneficial in choosing the breeding programmes and selection procedures to develop new sorghum cultivars with enhanced grain size, grain colour, round grain shape and a high degree of grain luster (Audilakshmi & Aruna, 2005).

Sorghum grain is an important ingredient in poultry diets as it has approximately 95% of the nutritional value of corn (Dowling, Arndt, & Hamaker, 2002). Tannin content in the pericarp is one of the most important factors affecting the feeding value of sorghum grain and adversely affects its metabolizable energy and protein utilization in poultry (Selle et al., 2010). Sorghum grain research indicated that its true metabolizable energy corrected for nitrogen content ranged between 3003 and 3899 kcal/kg (Sedghi et al.,

2011), and protein content ranges from 10.9 and 13.8% (Ebadi et al., 2011) in high- and low-tannin sorghum, respectively. Tannin can reduce feed intake (Oduhu & Baker, 2005), metabolizable energy (Perez-Maldonado & Rodrigues, 2009; Sannamani et al., 2010; Sedghi et al., 2011) and amino acid digestibility (Selle et al., 2010; Ebadi et al., 2011) in broilers when present in sorghum. Therefore, performance can be reduced when broiler are fed diets containing sorghum (Oduhu & Baker, 2005).

2.3.2 Chemical composition of grain sorghum

Carbohydrates

Carbohydrates are sugars and starches, which provide energy for humans and animals, and cellulose which make up many plant structures. The quality and quantity of carbohydrates present in sorghum are significantly important quality parameters that can influence consumer acceptance of the end product (Pushpamma & Vogel, 1982).

Starch is the primary carbohydrate and most abundant chemical component, while soluble sugars and crude fiber are low (Waniska & Rooney, 2002). Starch makes up about 60 to 80% of the normal, non-waxy, kernels of sorghum. Starch is structurally composed of two high molecular weight homo polysaccharides known as amylose, a straight chain and amylopectin, a branched chain polymer of glucose which are held together by hydrogen bonds and are arranged radically in spherical granules (Rooney & Pflugfelder 1986). Sorghum with low amylose content could be targeted for industrial brewing and infant porridge preparation (Dicko et al., 2006a).

Proteins

Sorghum protein is significantly important for the human diet in many countries in the world (Cecil, 1992; Gomez, 1993). The protein quality of sorghum is associated with the distribution of protein fractions in the grains which affects consumer acceptability (Pushpamma and Vogel, 1982), and nutritional composition (Serna-Saldivar & Rooney, 1995).

The average protein content of sorghum ranges from 11 to 12 % (Dendy, 1995). Lasztity, (1996) reported that the protein content varies from 6 to 25%. The protein content and amino acid composition in sorghum varies due to genotype and environmental conditions at which the crop is grown (water availability, soil fertility, temperatures and environmental conditions during grain development) (Taylor & Schussler, 1986; Frey, 1997) that affect the grain composition. Sorghum proteins are located in the endosperm (80%), germ (16%), and pericarp (3%) (Taylor & Schussler, 1986). Kafirins, or prolamins, and glutelins comprise the major protein fractions in sorghum. These fractions are located primarily within the protein bodies and protein matrix of the endosperm. Nitrogen fertilization significantly increases grain yield, kafirin accumulation and protein content (Warsi & Wright, 1973). Protein quality is critically important in developing countries where the human diet consists mainly of cereals grains.

In several cereal grains, including sorghum, an inverse correlation has been reported between grain yield and protein content (Frey, 1997). The protein content of the grain is significantly and inversely correlated with its weight and starch content (FAO, 1995). Likewise, the ash content and protein content of the sorghum grain are positively correlated with each other (Subramanian & Jambunathan, 1982). Grain protein and its amino acid composition in sorghum differ with the environmental conditions (Deosthale, Ngarajan, & Visweswar, 1972). Wide variability has been observed in the essential amino acid composition of sorghum protein (Hulse, Laing, & Pearson, 1980; Jambunathan, Singh, & Subramanian, 1984).

2.4 Sorghum phytochemicals and human health

Sorghum contains various phytochemicals (including phenolic compounds, plant sterols and policosanols) that are secondary plant metabolites or integral cellular components. Phenols help in the natural defense of plants against pests and diseases, while the plant sterols and policosanols are mostly components of wax and plant oils. The phytochemicals have gained increased interest due to their antioxidant activity,

cholesterol lowering properties and other potential health benefits in humans. The phenols in sorghums fall under two major categories; phenolic acids and flavonoids. The phenolic acids are benzoic or cinnamic acid derivatives (Waniska, Poe, & Bandyopadhyay, 1989), whereas the flavonoids include tannins and anthocyanins as the most important constituents isolated from sorghum (Krueger, Vestling, & Reed, 2003). Sorghum phytosterols are similar in composition to those from corn and contain mostly free sterols or stanols and their fatty acid/ ferulate esters (Singh, Moreau, & Hicks, 2003). The sterols and stanols are structurally similar, except for the presence of a double bond at position 5 in sterols, which is lacking in stanols.

These phytochemicals have potential to significantly impact human health. Sorghum fractions possess high antioxidant activity in vitro relative to other cereals or fruits (Prior et al., 1998). These fractions may offer similar health benefits commonly associated with fruits. Available epidemiological evidence suggests that sorghum consumption reduces the risk of certain types of cancer in humans compared to other cereals (Higdon & Frei, 2003). The high concentration of phytochemicals in sorghum may be partly responsible. Sorghums containing tannins are widely reported to reduce caloric availability and hence weight gain in animals. This property is potentially useful in helping reduce obesity in humans (Wyatt, 2003). Sorghum phytochemicals also promote cardiovascular health in animals and has potential in humans (Higdon & Frei, 2003; Anderson, 2003), since cardiovascular disease is currently the leading killer in the developed world.

2.5 Sorghum genetic diversity

Genetic diversity refers to the variation of heritable characteristics present among alleles of genes in different individuals of populations of species that serve an important role in evolution by allowing a species to adapt to a new environment (Weir, 1996; Kremer, Petit, & Pons, 1998). The ultimate source of genetic diversity is gene mutation, it is a permanent change in the DNA sequence, molded and shaped by selection, recombination, gene flow, genetic drift, and migration in heterogeneous environments

in space and time (Hartl & Clark, 1997). Natural selection chooses the best fit among and within a population; there can be no adaptive evolution without genetic variation (Ayana, 2001). Genetic diversity is an essential raw material for evolution, which enables populations of the crop species to survive, adapt to new circumstances, and evolve to produce new genetic variants, where some of them may become the most fit variants that meet long-term changes in the environment (Hedrick, 2000; Ayana, 2001).

Likewise, genetic diversity is vital in plant breeding for developing new and high yielding varieties and protecting the productivity of such varieties by integrating genes / traits for disease and insect pest resistance as well as tolerance to abiotic stresses (Allard, 1988) to address ever-increasing food requirement. So, the level of genetic diversity determines the evolutionary potential of a species and the rate of gain from human selection in breeder's materials. Therefore, a major focus of research in genetics has been to determine the amount of genetic variation in both natural and domestic populations and describing the possible mechanisms of maintaining such variability in meeting new climate change (Weir, 1996; Ayana, 2001).

Genetic resources have evolved as a product of domestication, intensification, diversification, and improvement through selection by farmers for different purposes. The local landraces and newly developed improved cultivars provide raw materials for crop improvement worldwide, for present and future generations (Rai, 2002). Therefore, it is important to conserve the diversity of crop species.

Genetic diversity can be expressed, through a large number of associations of genes which exist in individuals of a single species and are shown as characters that differ among cultivated varieties of the same plant species in growth pattern, resistance to disease and pests, tolerance to environmental conditions and productivity (Frankel & Brown, 1984). Genetic diversity is an important factor in breeding procedures that is aimed at improving crop varieties for desirable traits. It is crucial factor against climatic stress and pests.

Genetic diversity can be measured using different approaches within and between populations as the number of organisms differing from others and the relationships

among individuals of their relative frequency at genus, species, population, individual, genome locus and DNA base sequence levels (Kresovich & McFreson, 1992; Gaston, 1998). Although, the process of assessment needs to be interactive and dynamic, due to evolutionary changes (Gaston, 1998), genetic divergence acts as a vital role in the successful breeding programmes. Genetically diverse parents produce high heterozygotic effects and yield desirable segregates. Thus, quantitative assessment of genetic diversity is significantly important to determine the extent of genetic variabilities between and within crop species (Adugna, 2002).

Genetic variability within a taxon is of great importance for plant geneticists, breeders, physiologists, taxonomists and biosystematists (Prince et al., 1992). Diversity within a given plant population is a product of biotic factors, physical environment, artificial selection and plant characters such as size, mating system, mutation, migration and dispersal and the influence of man through domestication and selection (Allard, 1988).

The genetic diversity in the germplasm of a breeding programme affects the potential genetic gain through selection. Estimates of genetic diversity using new molecular tools, especially molecular markers have proven to be a useful way to delineate existing heterotic groups, identify new heterotic groups and assign inbreds of unknown genetic origin to established heterotic groups (Dubreuil et al., 1996; Pejic et al., 1998; Casa et al., 2002).

Eritrea is considered a centre for genetic diversity for many domesticated crop plant species such as sorghum, barley, sesame, okra and pearl millet, largely represented in the country by local landraces and wild types that are exceptionally adapted to adverse environmental conditions. Much of this crop diversity is found in the fields of small scale farmers, who have played a great role in the creation, maintenance and efficient utilization of these resources (Worede, Tesemma, & Feyissa, 2000).

In a country like Eritrea, which is characterized by highly varied agro-ecological and diverse growing conditions, the existence of genetic diversity is significantly important for the maintenance, conservation and enhancement of production and productivity in agricultural crops. Such diversity provides security for the farmer against biotic and

abiotic stresses. Genetic diversity grants farmers to exploit highly varied microenvironments differing in characteristics such as soil, water, temperature, altitude, slope, and fertility. Genetic diversity between and within species is especially significant as it represents an important genetic resource to the subsistence farming communities at country and regional level (Worede, Tesemma, & Feyissa, R. 2000). An intensive study of genetic diversity in sorghum local landraces based on race, latitude of origin, photoperiod-sensitivity, grain and nutritional quality, agro-morphological traits and DNA markers, has provided evidence that sorghum has appreciable genetic variation that has been poorly used in terms of crop improvement (Abu Assar et al., 2005; Deu, Rattunde, & Chantereau, 2006; Dillon et al., 2007).

Previously, genetic diversity of sorghum was studied using morphological traits. However, the advent of molecular marker technologies offer great potential to add to the genetic diversity studies in sorghum. In recent years, simple sequence repeats (SSRs) and amplified fragment length polymorphism (AFLPs) have been used effectively in marker assisted breeding of different crops and are often considered the molecular markers of choice. With respect to efficient breeding, the conservation and effective use of genetic resources is important, since different farmers' varieties provides greater genetic variability and furnish useful genes that are especially useful in resistance breeding and quality traits (Tanksley & McCouch, 1997). However, the success of genetic conservation and breeding programmes depend on understanding the distribution of genetic diversity and evolutionary relationships present in the gene pool (Zhang et al., 2000). Hence, the assessment of the genetic diversity and evolutionary relationships between and within local crop species could provide high potential use and ensure rapid adoption of the improved germplasm by growers (Van Leur & Gebre, 2003).

In general, knowledge of genetic diversity and evolutionary relationships among individual germplasm within a species or among different species and its potential merit would be beneficial to crop improvement programmes (Lee, 1996). Evaluation and characterisation of genetic diversity levels among germplasm provides the estimates of

genetic variation among segregating progeny for pure line development and the degree of heterosis in the progeny of certain parental combinations (Cox & Murphy, 1990; Barbosa-Neto et al., 1996).

Diverse taxonomic characteristics have been used to separate and assess patterns of phenotypic diversity in the relationships of species and germplasm collections of crops (Perry & MacIntosh, 1991; Rabbani et al., 1998). A great extent of variability exists in quantitative and qualitative traits among sorghum local landraces, such as maturity, yield, plant height, plant pigmentation, midrib colour, panicle length and width, panicle compactness and shape, glume colour, grain colour, size and weight and disease reaction (House, 1985; Mukuru, 1993).

2.6 Morphological traits

Traditionally, characterisation and evaluation of genetic diversity in crop species is based on variation in quantitative and qualitative characters (Vega, 1993; Schut, Qi, & Stam, 1997). Phenotypic estimates are used to present the degree of genetic relationship and difference between lines; it is presumed that similarity in phenotype characteristics reflects genetic similarity of genotypes (Cox et al., 1985). The application of agro-morphological traits has been used as a powerful tool in the classification and grouping of lines, to study taxonomic status, identification, determination of genetic variation and correlation of characters with agronomic potential (Millan & Cubero, 1995; Van Beuningen & Busch, 1997). Before the advent of DNA-technology, genetic diversity analysis was only studied using morphological and physiological descriptors (Liu & Furnier, 1993; Neinhuis, Trivang, & Skrotch, 1995). Characterization and studying evolutionary relationships of crop species involves the cultivation of sub-samples and their subsequent morphological and agronomic description (Vega, 1993). Therefore, it is of paramount importance to comprehend the nature of the interaction and relationships between genetic, physiological, morphological and physico-chemical characters, in order to employ intensive selection criteria effectively.

Morphological markers are also important in the study of genetic diversity and relationships in plant breeding programmes (Cox & Murphy, 1990; Van Beuningen and Busch, 1997) because (1) the existing data based on the germplasm collection or breeding stock can often be used for genetic analysis; (2) statistical procedures for morphological trait analysis are readily available; (3) morphological information is essential in understanding the ideotype performance relationships; (4) explanations of heterosis may be enhanced if morphological measures of distances are included as an independent variable. However, use of morphological traits for the study of genetic diversity and relationship has been criticized since the study of genetic relationship among germplasm using morphological characteristics is time consuming and costly process. Furthermore, the genetic control of morphological characters is complex, involving epistatic interactions (Smith & Smith, 1989). Thus, morphological appearance cannot adequately describe genotypes without extensive trials (Lin & Binns, 1994) and, therefore, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith & Smith, 1989). On the other hand, discrete morphological traits are the basis for description of identity, distinctness and uniformity of cultivars in plant variety protection and registration under the guidelines of the International Union for the protection of new varieties of plants (UPOV, 1980). Geleta et al. (2006) also indicated that although morpho-agronomical characterisation is influenced by the environment and is time consuming, in general among other disadvantages in relation to AFLPs and SSRs, it can still be an important and practical means of making progress in germplasm evaluation by conservationists and breeders. Furthermore, morphological traits are almost entirely used for crop diversity analysis in countries like Ethiopia where economy and trained manpower are the limiting factors to establish modern technologies for crop diversity analysis.

In sorghum, studying genetic diversity include concepts of Mendelian hereditary analysis of discrete morphological traits (Doggett, 1988) and statistical analysis of quantitative agro-morphological traits together with eco-geographic information (de Wet, Harlan, & Prince, 1976.; Murty, Arundachlam, & Saxena, 1976, Ayana, 2001).

Using ex situ and conserved sorghum germplasm accessions from Ethiopia and Eritrea, Ayana and Bekele (1998) reported that high and comparable levels of phenotypic variation exist between the regions of origin.

2.7 Molecular markers in sorghum diversity studies

Molecular genetic markers are defined as differences at the genotype level that can be used to answer and explain questions of genetics (Lokko et al., 2005). To be useful as a genetic marker, the marker locus has to show experimentally detectable variation among individuals (Sørensen et al., 2008).

Variation in nucleotide sequence is exploited to assess the genetic diversity and relationships in sorghum germplasm and other cereals. Molecular marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are associated with favourable trait of interest. With the development and availability of an array of molecular markers and dense molecular genetic maps in crop plants, MAS has become possible for traits governed by both major genes and by quantitative trait loci (QTL) (Singh & Lohithaswa, 2006).

Molecular markers have provided a powerful approach to analyse genetic diversity and evolutionary relationships among and within germplasm accessions in many crop species. Molecular markers are useful DNA techniques that complement morphological and physiological characterisation of cultivars since they are found in the whole genome, independent of plant tissue, influence of environmental and management practices and allow cultivar identification (Manifesto et al., 2001; Altintas et al., 2008). Molecular characterization of cultivars is also useful to evaluate potential genetic erosion due to the extensive selection, biotic and abiotic factors resulting in a reduction of genetic diversity.

The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. DNA markers have the potential to enhance the operation of a plant breeding programme in a number of ways, ranging from fingerprinting of elite genetic stocks, assessment of

genetic diversity, increasing the efficiency of selection for difficult traits, to making environment neutral selection possible. However, their greatest potential appears to be in accelerating the rate of gain from selection for desirable genotypes and in the manipulation of QTL that condition complex economic traits. DNA markers also permit plant breeders to correctly map or place the various interacting genes that condition complex agronomic traits (Ejeta et al., 1999). DNA markers are used to evaluate the genetic variation in genebanks as well as to identify phylogenetic and molecular structure of crops and their associated wild species. Molecular markers assisted genetic analysis provides a means to locate and select genes controlling important agronomic traits like pest resistance, stress tolerance, and food quality (Singh and Lohithaswa, 2006). Markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. In contrast to morphological markers, which are based on visible traits, and biochemical markers, which are based on proteins produced by genes, molecular markers rely on a DNA assay. Molecular markers have been used to identify and characterize QTL associated with several different traits in sorghum including plant height and maturity (Pereira & Lee, 1995), characters related to plant domestication (Patterson et al., 1995), diseases resistance (Gowda et al., 1995), and drought tolerance (Tuinstra et al., 1996, 1997a, 1998).

Compared to morphological and biochemical characteristics, the DNA markers provides a significantly more powerful source of genetic polymorphism. They allow direct comparison of genetic diversity to be made at the DNA level, have the potential to identify a large number of polymorphic loci with whole coverage of an entire genome, are phenotypically neutral, allow scoring of plants at any developmental stage and are not modified by environment and management practices (Messmer et al., 1993, Prabhu et al., 1997). They also render to detect the exact genetic constitution of an individual plant in a segregating population (Phillip, Wehling, & Wricke, 1994). DNA markers are now widely used in constructing genetic maps, QTL mapping, and diversity analysis and as tool for marker assisted selection in breeding programmes.

Molecular markers have the advantage of improving the effectiveness of conventional breeding through the selection of desirable characteristics based on the presence of molecular markers, which are linked to the particular trait in question (Lee, 1996). Molecular markers are discrete and non-deleterious and are unaffected by environmental conditions and free of epistatic interaction (Mclntyre et al., 2001). Molecular marker technology can greatly improve the efficiency and effectiveness of sorghum breeding programmes by helping to select genes for traits of interest that are otherwise difficult to measure or that require particular conditions for their expression. Molecular markers are laboratory based tests in which the presence or absence of bands on a gel is used to indicate the presence or absence of a favourable version of a gene for a particular trait (Jordan, 2006). DNA markers provide a possibility due to a favourable combination of circumstances to detect, monitor and manipulate genetic variation more precisely compared to morphological and biochemical markers (Yamamoto, Nishikawa, & Oeda, 1994).

2.8 Drought stress in plants

Water serves many vital roles in plants, including acting as a solvent, a transport medium, and an evaporative coolant (Boyer, 1982). Consequently, water limitation causes a decrease in whole plant growth and photosynthesis, wilting, stomatal closure, and is associated with changes in carbon and nitrogen metabolism (Sanchez et al., 2002).

Drought stress is a serious agronomic problem contributing to severe yield losses worldwide (Boyer et al., 2004). This agricultural constraint may nevertheless be addressed by developing crops that can tolerate drought prone environments. Physiologists have identified three general mechanisms of drought resistance involving avoidance, tolerance, and escape (Levitt, 1980). Drought avoidance mechanisms allow plants to maintain cell turgor and cell water content under water-limiting conditions. This is accomplished by maintaining water uptake by the roots and/or reduction of water loss from transpiration and other non-stomatal pathways such as the cuticle. Most

sorghum genotypes have a thick waxy cuticle that limits water loss during periods of water deficit. The possession of a deep, large root system, which has the ability to penetrate hard soil layers, is also often associated with plants that are able to maintain water supply during periods of low rainfall. C4 vs. C3 photosynthesis also improves water use efficiency especially at high temperatures where the oxygenase activity of rubisco is favoured over the carboxylation activity (Condon et al., 2006). C4 plants concentrate CO_2 in bundle sheath cells thus reducing photorespiration allowing these plants to decrease stomatal conductance and to conserve water without decreasing carbon fixation rates. Other types of avoidance mechanisms are based on leaf abscission, dormancy, and leaf angle/rolling that reduce water loss through transpiration. Reducing the evaporative surface area of the leaf is an effective means of decreasing transpiration.

Drought tolerance is a mechanism by which plants maintain metabolism even at low water potential. Two traits known to influence drought tolerance are osmotic adjustment and antioxidant capacity. Drought tolerance mechanisms allow plants to maintain metabolic activity during drought and under conditions of reduced plant water potential by osmotic adjustment and antioxidant capacity. Many plants can accumulate compatible solutes including sugars, organic acids, amino acids, sugar alcohols, or ions which accumulate in the cytosol, lowering the osmotic potential and maintaining turgor of both shoots and roots. Sorghum, for example, is known to accumulate glycine betaine and proline in response to water deficit (Buchanan et al., 2005). Antioxidant capacity is the ability of plants to detoxify reactive oxygen species (Scandalios, 2005). Drought escape refers to early completion of the plant's life cycle, essentially flowering prior to the onset of drought. Early maturing varieties of sorghum avoid water deficit that in some regions often occurs later in the growing season.

According to Pinto et al., (2010), breeding for drought adaptation has been strongly affected by drought escape based on development, whereby sensitive development stages do not coincide with the stress peak. For instance, flowering time tends to be associated with yield (Ludlow & Muchow, 1990) but in a rather unpredictable manner.

Accordingly, early flowering may be advantageous if it enables a cultivar to escape drought during the reproductive stages where as late flowering may be beneficial in the cases where drought stress occurs early in the season. In quantitative trait loci mapping for drought tolerance, unsynchronized phenology may result in the detection of escape-related quantitative trait loci, which arise mostly from variations in phenology (Pinto et al., 2010), translating into co-localization between phenology quantitative trait loci and those for yield and stay-green. In addition, because other relevant quantitative trait loci may be missed, quantitative trait loci with limited practical relevance for drought tolerance breeding may be detected if the confounding effect of phenology is disregarded.

Genes induced by water-stress encode proteins involved in protection and signal transduction (Mundree et al., 2002). A hormone that acts as a major signal of water deficit is abscisic acid (ABA). Most drought-responsive genes are induced by exogenous ABA treatment, and are included in the ABA-dependent signal transduction. An additional gene set is induced by drought, but not by ABA, providing evidence for a second, ABA-independent signal transduction pathway (Mundree et al., 2002). Promoters of ABA inducible genes contain sequence-specific ABA-responsive cis elements (ABRE's) with the sequence ACGTGGC (Mundree et al., 2002). These same cis-elements are found in sorghum genes that respond to ABA (Buchanan et al., 2005). Dehydrins, hydrophilic proteins thought to stabilize cell structures against dehydration, accumulate upon drought onset or ABA treatment and many studies have shown a positive correlation between the accumulation of dehydrins and drought tolerance (Cellier et al., 1998).

2.9 Drought tolerance in sorghum

Drought tolerance in sorghum is a complex trait influenced by many genes coding for various traits contributing towards drought tolerance (Blum, 1979). Over the past decades plant breeders have focused on some traits that were incorporated to plant survival under drought for instance lower leaf canopy and reduced transpiration

(Karamanos & Papatheohari, 1999), which are not essentially correlated with high yield and led the breeders to evolve cultivars with poor yield under stress condition.

Drought tolerance in sorghum depends on the plant developmental stage at the onset of the stress condition, which in sorghum may happen during the early vegetative seedling stage, during panicle development and in post-flowering, in the period between grain filling and physiological maturity (Rosenow & Clark, 1995; Rosenow et al., 1996). In particular, post-flowering drought stress can result in significant reductions in crop yield (Rosenow & Clark, 1995; Rosenow et al., 1996). Sorghum is a drought tolerant crop species and is an important model system for studying physiological and molecular mechanisms underlying drought tolerance (Doggett, 1988; Ludlow & Muchow, 1990; Mullet, Klein, & Klein, 2001; Sanchez et al., 2002). Incidence of drought stress at seedling stage may lead to higher dry root weights, longer roots, coleptiles and higher root: shoot ratios (Zekri, 1991). Post-flowering drought adaptation in sorghum is associated with the stay-green phenotype, which is characterized by the maintenance of green stems and upper leaves under water limitation after flowering (Subudhi et al., 2000).

2.10 Sorghum stay-green

The term ‘stay-green’ has been used to describe an important component of post-flowering drought response in sorghum (Rosenow & Clark, 1995). Stay-green refers to a drought tolerance mechanism that enables the sorghum plants to tolerate premature senescence under drought stress that occurs during grain filling. The stay-green trait results in greater functional photosynthetic leaf area during grain filling and even after physiological maturity. Recent physiological studies have highlighted the contribution of both onset and rate of leaf senescence to the stay-green phenotype. These differences in onset and rate of senescence can also be explained by differences in the nitrogen dynamics of the plant at leaf and whole-plant levels (Borrell & Hammer, 2000).

Thomas and Smart, (1993) have identified four classes of stay-green. The first two classes are functionally stay-green and may be a result of alteration of genes involved in

the onset of senescence and the regulation of its rate of progress. Stay-green in the other two classes is cosmetic, where in the plants are green but lack photosynthetic activity. This may be due to loss of photosynthetic capability that normally accompanies senescence combined with maintenance of leaf chlorophyll. In these stay-greens, the greater leaf greenness is related simply to higher initial chlorophyll content and thus, the decrease in chlorophyll content during senescence results in a slower reduction in leaf greenness.

Sorghum genotypes with the stay-green trait continue to fill their grain normally under drought stress (Rosenow & Clark, 1995) and exhibit resistance to charcoal rot and lodging (Rosenow, 1984). Stay-green genotypes also contain more basal stem sugars and cytokinins than senescent genotypes, which may reduce the rate of drought-induced senescence. Increased accumulation of soluble sugars found in stay-green genotypes may reduce the dependence on stored assimilates from the stem to fill the grains (Thomas & Smart, 1993).

Stay-green lines in sorghum produce two to three more basal tillers per plant at black layer, have a greater stem diameter, have higher sugar concentrations at the base of the stem, maintain greater green leaf area longer, have a greater leaf area index than senescent lines, have a higher leaf relative water content, have higher specific leaf nitrogen, contain a higher level of cytokinins, and have enhanced transpiration efficiency (Borrell & Hammer, 2000). Furthermore, stay-green genotypes do not show reduced yield under fully irrigated conditions, thus stay-green genotypes can be grown on both irrigated and non-irrigated land (Borrell et al. 2000).

2.11 Selection criteria for identifying drought tolerance in sorghum under stress condition

Tolerance to drought is a quantitative trait, with a complex phenotype, often confounded by plant phenology. Breeding for drought tolerance is further complicated since several types of abiotic stress, such as high temperatures, high irradiance, and nutrient toxicities or deficiencies can challenge crop plants simultaneously.

Sorghum genotypes with good tolerance during one of the developmental stages are typically found to be susceptible to drought during the other growth stages. This developmental interaction further complicates the phenomenon of drought tolerance and each of these has a different effect on the crop. In this study, the approach has been to break down the complex trait of drought tolerance into simpler components by studying drought-stress expressions at specific stages of plant development. Selection for drought tolerance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable drought stress conditions when a large number of genotypes can be evaluated efficiently (Ramirez & Kelly, 1998). Achieving a genetic increase in yield under these environments has been recognized to be a difficult challenge for plant breeders while progress in yield grain has been much higher in favourable environments (Richards, 2004).

Many studies have been carried out to set selection criteria for drought tolerance. Khan et al., (2004) reported that drought adapted plants are often characterized by deep and vigourous root systems. Some other scientists focused on morpho-physiological flag leaf related characters especially leaf water relations and their considerable interaction with drought tolerance. Selection based on plant developmental traits such as plant phenology (days to flowering and maturity), stay-green, leaf area, tillering, panicle size and peduncle exsertions are conducive for drought tolerance in sorghum genotypes (Ali et al., 2011).

Screening drought-tolerant genotypes based on drought indices is another selection criteria which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions (Mitra, 2001). These indices are based on either drought resistance or susceptibility of genotypes (Fernandez, 1992). Drought resistance is defined by Hall, (1993) as the relative yield of a genotype compared to other genotypes subjected to the same drought stress. Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988) whilst the values are confounded with differential yield potential of genotypes (Ramirez & Kelly, 1998). Rosielle and Hamblin (1981) defined stress

tolerance (TOL) as the differences in yield between the stress (Ys) and non-stress (Yp) environments and mean productivity (MP) as the average yield of Ys and Yp. Fischer and Maurer, (1978) proposed a stress susceptibility index (SSI) of the cultivar. Fernandez, (1992) defined a new advanced index, stress tolerance index (STI), which can be used to identify genotypes that produce high yield under both stress and non-stress conditions. Other yield based estimates of drought resistance are geometric mean (GM), mean productivity (MP) and TOL. The geometric mean is often used by breeders interested in relative performance since drought stress can vary in severity in the fields environment over years (Ramirez & Kelly, 1998). Clarke et al., (1992) used SSI for evaluation of drought tolerance in wheat genotypes and found year-to-year variation in SSI for genotypes and their ranking pattern. In spring wheat cultivars, Guttieri et al., (2001) using SSI criterion suggested that SSI more than 1 indicated above-average susceptibility to drought stress. Golabadi et al., (2006) and Sio-Se Mardeh et al., (2006) suggested that selection for drought tolerance in wheat could be conducted for high MP, GMP and STI under stressed and non-stressed environments. Fernandez, (1992) had divided genotypes reaction on the basis of their yields into 4 categories under stressed and non-stressed conditions: group A are genotypes which have high yield in both conditions; group B are genotypes which have a high yield under non-stressed conditions; group C genotypes which have a good yield under stressed conditions and finally group D are genotypes which have a low yield in both conditions. Selection of different genotypes under environmental stress conditions is one of the main tasks of plant breeders for exploiting the genetic variations to improve the stress-tolerant cultivars (Clarke et al., 1984).

The use of multivariate techniques was also another selection criterion that was employed to select several characters simultaneously which make it feasible to approximate the genetic divergence. These multivariate techniques include principal component and cluster analysis which have analogous efficacy to establish the most suitable selection combinations (Machado et al., 2000). In past, multivariate analysis had mostly been exploited to assess and differentiate the genotypes for various

morphological traits in sorghum (Teshome et al., 1997; Ayana & Bekele, 1999; Tesso, Claflin, & Tuinstra, 2005 and Chozin, 2007; Aruna & Audilakshm, 2008). However, Ahlawat et al., (2002) utilized multivariate analysis to ascertain diversity for stay-green character in 36 wheat genotypes. Similarly, Tesso et al., (2005) worked out multivariate analysis for drought tolerance in sorghum. Recently, Bibi et al., (2010), working on 80 sorghum genotypes, found osmotic potential as the most important physiological marker for drought tolerance in addition to root length.

CHAPTER 3

DOCUMENTATION OF SORGHUM (*Sorghum bicolour* L MOENCH) LANDRACES: PRODUCTION, UTILIZATION AND CHALLENGES IN ERITREA

Abstract

Grain Sorghum (*Sorghum bicolour* (L.) Moench) is the most important staple food crop in Eritrea. A study was conducted in four sub regions (Hamelmalo, Segeneyti, Tesseney and Goluj) of Eritrea determined farmers' perceptions on sorghum diversity, utilization, post harvest and production problems and their management practices using a semi-structured questionnaire and focused group discussions. A total of 190 sorghum growing farmers were randomly selected for this study. Results from the study showed that about 22 sorghum landraces were in active cultivation in the four sub regions, though there was a possible duplication in the naming of landraces. The naming of landraces was based on maturity dates, grain colour, plant height and utility. Grain sorghum was used for home consumption in the form of injera (90%), bread (5%) porridge (5%) and local alcoholic beverages (13%). Varieties with white and red grains were used mainly for injera and porridge while those with brown grains were used for local alcoholic beverages. Storage pests were the leading post harvest constraint in all the sub regions. Farmers reported various traditional pest management options which included treatment with ash and herbs; washing with water, sun drying and winnowing methods. Low yields of less than 1.0 t ha^{-1} were reported by farmers in all the sub regions. Drought was reported to be the leading production constraint (71%) followed by striga and diseases (17.9 %), and access to labour (3.2 %). Post flowering drought was the key yield reducing factor on farmers' fields. The use of early maturing landraces and good adaptation to marginal areas coupled with some agronomic practices are the main options used by the farmers to mitigate the effect of drought. The results also indicated that 85.8 % of the farmers used their own "saved" sorghum seed for planting. The main criteria for seed selection were panicle and seed size, grain

colour and maturity dates. The panicles to be used as seed were selected when the sorghum plants had reached physiological maturity.

***Key words*:** Diversity, Household survey, Landraces, Participatory Rural Appraisal, *Sorghum bicolour*,

3.1 Introduction

In Eritrea, sorghum (*Sorghum bicolour* (L.) Moench) is commonly grown under rain fed conditions by resource-poor subsistence farmers with very little or no capital inputs, such as fertilizers, pesticides, or irrigation. It is widely grown in the lowland and mid highland regions of the country where rainfall is low for the cultivation of other cereals (Tesfamichael, 1999). The most commonly grown sorghums are the local landraces that have diverse plant structure, panicle orientation, seed colour and maturity ranges. Local landraces have been chosen by farmers on the basis of their grain and stalk qualities and adaptation to specific ecologies (Mann et al., 1983). However, some sorghum landraces have disappeared from the farmers fields due to climatic variabilities. Though sorghum is well adapted to drought prone environments some late maturing varieties have been neglected due to their inability to cope with erratic rainfall and short growing season of the country.

The basis why farmers prefer growing sorghum landraces over improved varieties is their ability to adapt to various temperatures, rainfall, soil type, and ecological settings (Mekbib, 2006). In general, research efforts to breed improved varieties have primarily concentrated on more favoured and high-potential environments in which the increase in productivity and yield response to harmonizing inputs is high (Bellon, 2006). In contrast to improved varieties landraces are generally the product of farmer selection for adaptation to specific environments (FAO, 1998; Mekbib, 2006). High genotype-environment interactions can result in higher performance from landraces compared with improved varieties (Ceccarelli et al., 2001).

Improved sorghum varieties respond well to the input supply and other improved managements. However, they are generally susceptible to both biotic and abiotic stresses and have poor storage, processing and nutrition qualities as compared to landraces (Beta & Corke, 2001; Kenga, Alabi, & Gupta, 2004; Mgonja et al., 2005; Medraoui et al., 2007). Consequently; the rate of adoption of new varieties is low (Wubeneh & Sanders, 2006; McGuire, 2008). On the other hand, landraces perform well under sub-optimal conditions as they are well adapted to local stresses and

possessed farmers' preferable traits (Bantilan et al., 2004; Setimela, Monyo, & Bänziger 2004). It is, therefore, necessary to study the genetic relationships and document of these landraces and identify traits to be incorporated in the released varieties. Genetic characterization of genotypes gives descriptive information of the traits and helps in understanding the similarities and differences among genotypes (IBPGR & ICRISAT, 1993). Both morphological and molecular markers are used for this purpose.

The north eastern African region, to which Eritrea belongs, has been described as one of the centers of diversity and a possible area of domestication for sorghum (Vavilov, 1992). Although, some landraces have been collected from the country, very little information on these landraces is available. Some of the local landraces which were once widely cultivated in Eritrea are now grown only in some restricted areas or are potentially extincted. The farmers' indigenous knowledge on sorghum landraces has not been well documented. Moreover, a previous similar study conducted in Eritrea concentrated on general cereal production of the country and gave little attention to the sorghum sub-sector. Information specific to sorghum production systems, utilization and challenges was not documented and hence, this study was conducted. The objectives of the study were to document sorghum production systems, utilization and challenges as well as farmers' preferences and criteria for selection of the landraces to meet different needs.

3.2 Methodology

3.2.1 Study locations

The study was conducted in four sub regions of the country namely Goluj, Tesseney, Hamelmalo, and Segeneyti, where sorghum is a major crop (Figure 3.1). The cultivated areas in Goluj and Tesseney are flat with altitudes ranging from 500 to 700 m above sea level while those in Hamelmalo and Segeneyti are undulated with altitudes of 1280 and 2171 m above sea level, respectively. The soils in Goluj and Tesseney plains were dark clay loamy vertisols. Soils of Hamelmalo areas ranged from sandy to sandy loamy lithosol while those in Segeneyti were sandy and loamy leptosols with dark brown soils

(Table 3.1). All the sub regions have a short, single rainy season from June to September, followed by a long dry season. The total amount and distribution of the annual rainfall is highly variable from one year to another (Figure 3.2).

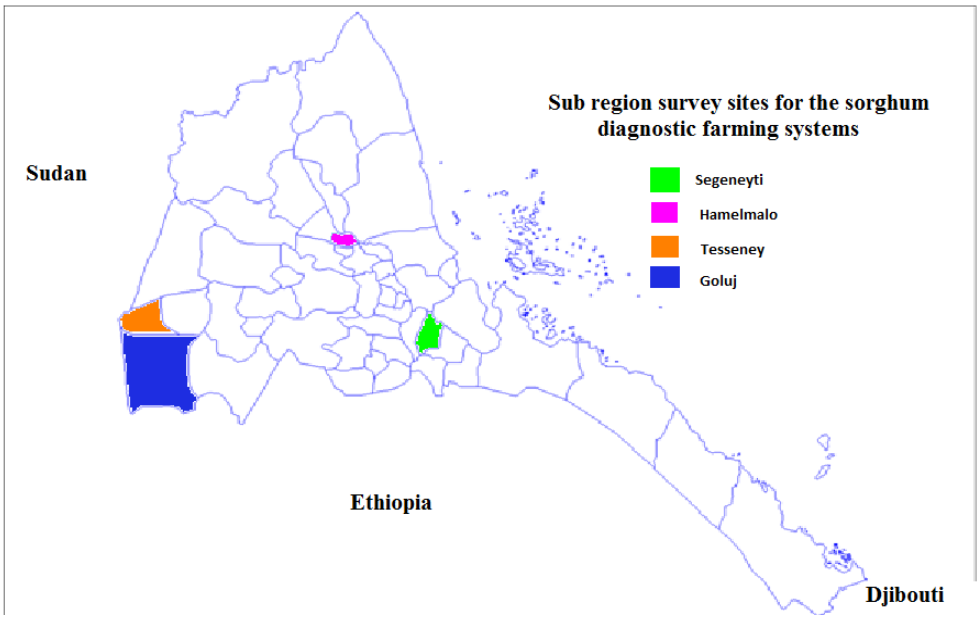


Figure 3.1 Map of Eritrea sub-regions and the locations of the surveyed sub regions

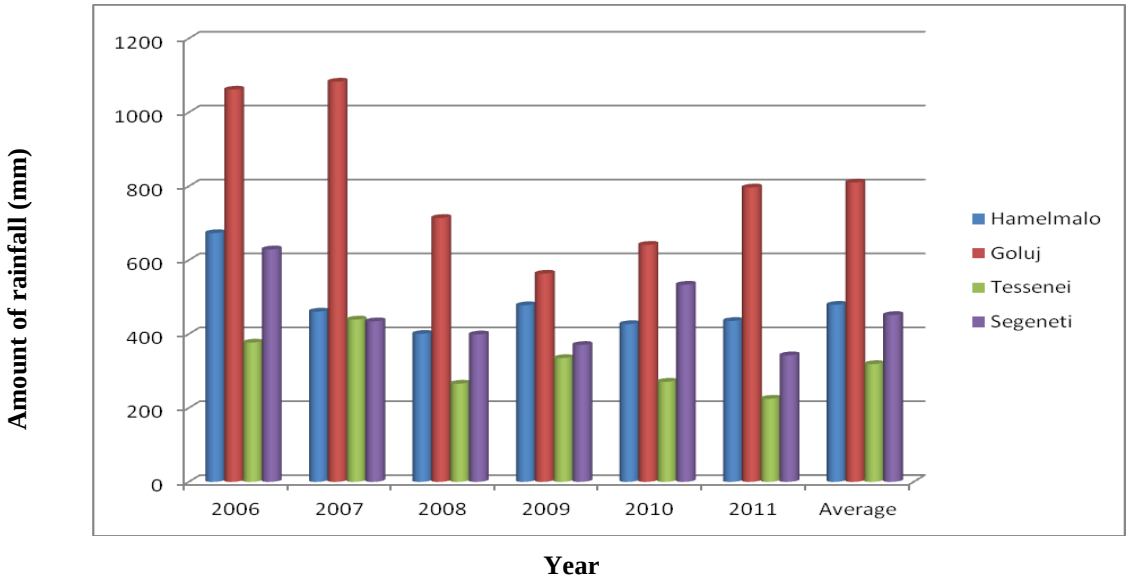


Figure. 3.2 Amount of rainfall and distribution in the four sub regions studied (Source: MoA, 2006-2011)

Table 3.1. Agro ecology, location, mean annual rainfall and soil types of the surveyed sub regions

Sub region	Agro ecology zones	Location			Mean annual Rainfall (mm)	Soil type
		Altitude (m)	Latitude	Longitude		
Hamelmalo	North Western Lowland	1280	16^0 01' N	38^0 20' E	479.2	Sandy and sandy loam with low water retention capacity
Segeneyti	Central highland	2171	15^0 05' N	39^0 19' E	451.5	Sandy and loamy, leptosols
Tesseney	South Western lowland	600	15^0 11' N	36^0 66' E	318.7	Dark clay loam, Vertisols
Goluj	South Western lowland	678	14^0 74' N	36^0 72' E	759.3	Dark clay loam, Vertisols

3.2.2 Research approach and sampling size

Formal Survey

Three villages from each of the four sub regions, giving a total of twelve villages, were selected for the study. The villages were selected with the assistance of agricultural extension staff on the basis of growing area sorghum as a major crop. A total of 190 sorghum growing households were randomly selected from the four sub regions. The number of households that were sampled from each village is given in table 3.2. A semi-structured questionnaire was used to collect information from these households on sorghum production, utilization and major constraints affecting production (appendix 1).

Focused group discussion

A focused group discussion with stakeholders was organized in each of the sub regions. A total of 25 participants that comprised of farmers, extension experts, researchers and

local administrators participated in the group discussions. In addition, field observations and secondary data reviews were used to enrich the study.

Table 3.2. Number of households sampled in the study sites

Region	Sub region	Sites/ villages	Number of Households	Sub region total
Anseba	Hamelmalo	Gizgiza	19	47
		Fledareb	16	
		Hamelmalo	12	
South	Segeneyti	Hadida	12	40
		Adi Hadid	13	
		Akrur	15	
Gash Barka	Tesseney	Tesseney	30	51
		Aligidir	11	
		Thalata	10	
Gash Barka	Goluj	Goluj	18	52
		Gergef	17	
		Omhager	17	

3.2.3 Data Analysis

The primary data collected in this study was entered into an Excel spread sheet and analysed using XLSTAT 2012. The data were summarized into averages, percentages and frequencies. The data analysed was presented in the form of tables, pie charts and graphs.

3.3 Result and Discussion

3.3.1 Rainfall distribution, major crops grown and land suitability in the sub regions

The rainfall records in Eritrea at village level were not available, however, at sub region levels rainfall records were collected by the Ministry of Agriculture (Table 3.3). The rainy season in Eritrea is influenced by the northward and southward movement of the Inter-tropical Convergence Zone (ITCZ). The wettest months in the four surveyed sub regions are those from June to September, with maximum rainfall usually occurring either in July or August. These two months contribute about 58 to 64 percent of the total average rainfall of Hamelmalo, Segeneyti, Goluj and Tesseney as it was calculated using the historic rainfall record. In sub region Segeneyti some early rains may start in the months of April and May. Rainfall occurs mainly as thunder showers of one or two hour's duration. Rainfall in Goluj (Table 3.3) and areas south of Goluj such as Ghergef and Omhajer is relatively high and has permitted the expansion of dry farming concession, where land is plain and level (Plate 3.1); however, such farming is more uncertain in Tesseney areas where less rainfall causes lower yields though the land is level (Plate 3.2). The rainfall situation in sub regions Hamelmalo and Segeneyti is enough for the growth of the rainfed crops grown in the area.

Table 3.3. Mean annual rainfall of Hamelmalo, Segeneyti, Goluj and Tesseney in the period 2006-2011

Sub region	2006	2007	2008	2009	2010	2011	Average	Major rainfed crops
Hamelmalo	673.1	460.8	400.6	477.9	427	435.5	479.2	sorghum, pearl millet, ground nut
Goluj	1061	1083	714.2	563.4	641.5	796.8	810.1	Sorghum, sesame, pearl millet, pea nut
Tesseney	376.9	439.2	265.7	335	270.9	224.9	318.8	Sorghum, sesame pearl millet, peanut
Segeneyti	629	434.5	399	370.5	533.5	342.5	451.5	Barley, wheat, sorghum, maize, taf and finger millet

Source : Ministry of Agriculture (MoA), Eritrea (2012)

The major problem of these two sub regions were that the land is ragged and shallow soils which are less suitable for cultivation due hilly and undulated nature (Plates 3.3 and 3.4) and soils have very low water retention capacity thus there is high runoff (MoA, 2012).

Plate 3.1 Goluj sub region land suitability and high rainfall patterns that allow successful rainfed crop production

Plate 3.2 Sub region Tesseney, land is suitable for crop growth but frequent crop failures due to rainfall scarcity and distribution

Plate 3.3 Sub region Segeneyti land is hilly and undulated not suitable for farm mechanization

Plate 3.4 Sub region Hamelmalo where land is fragmented, small holding, undulated and hilly which is exposed to water erosion

3.3.2 Landholding and production status of sorghum

Results from both the household survey and the group discussion indicated that about 95% of the farmers in sub regions Segeneyti and Hamelmalo had land holdings of 0.5 to 2.5 hectares while in Tesseney and Goluj land size ranged from 1.3 to 8 hectares. Five percent of the farmers in the study were commercial farmers who owned land between 40 to 100 hectares in sub region Goluj. The average land holding of all farmers in this study was 2.5 hectares (Table 3.4). These results were in agreement with those of Bekuretsion (2005) who reported that the average land holding size in the South and Anseba regions was 0.25 to 4 hectares while in Gash Barka it ranged from 1.3 to 40

hectares. The average proportion of farm land allocated to sorghum cultivation in the study area was 96%.

Results from this study showed that the size of land holding was not proportional to family size and therefore, household food security was not guaranteed for the majority of the families. Consequently, most of the households were dependent on allied agricultural activities, such as animal rearing, wage earning and trading.

Table 3.4. Landholding and land ratio allocation for sorghum in the study sites

Sub region	Village Administration	No. of Household	Total HH landholding size	Average landholding	Land allocated for sorghum	Total sorghum ratio to landholding
		(No.)	(ha)	(ha)	(ha)	(%)
Hamelmalo	Gizgiza	19	19.25	1.0	19.2	100.0
	Fledareb	16	32.00	2.0	29.7	93.0
	Hamelmalo	12	33.75	2.8	27.3	80.0
Segeneyti	Hadida	12	12.75	1.1	10.3	80.4
	Adi Hadid	13	18.00	1.4	15.2	84.7
	Akrur	15	16.00	1.1	9.8	60.9
Tesseney	Tesseney	30	139.00	4.6	137.0	98.6
	Aligidir	11	27.00	2.5	24.0	88.9
	Thalat Ashir	10	12.50	1.3	12.0	96.0
Goluj	Goluj	18	84.00	4.7	82.0	97.6
	Gergef	17	547.00	32.2	547.0	100.0
	Omhager	17	142.00	8.4	131.0	92.3

Where: HH = Household

The area under sorghum cultivation in Eritrea is increasing but the yield levels are stagnant (MoA, 2012.). Based on a review of secondary data, 33% of the total area under sorghum cultivation and 26 % of total national production comes from the four surveyed sub regions (Table 3.5). Sub region Goluj is a leading sorghum producer and

covers the highest area under sorghum cultivation in the country. Sorghum productivity in this region was 0.6 t ha^{-1}, a figure that is similar to the national average. Sub region Hamelmalo had the lowest sorghum productivity (0.35 t ha^{-1}) and sub region Segeneyti recorded the highest value for sorghum productivity (1 t ha^{-1}).

Table 3.5. Average area of sorghum cultivation and production in the study sites for the period 2006-2011

Sub region	Area and production of sorghum		
	Area under sorghum (ha)	Production (tons)	Productivity (t ha^{-1})
Hamelmalo	2,197.7	790.1	0.4
Segeneyti	1,257.6	1,369.2	1.0
Tesseney	23,398.0	9,487.5	0.4
Goluj	60,120.8	30,516.0	0.6
Sub regions total	86,974.1	42,162.8	0.5
National Total	264,144.0	157,492.0	0.6
Sub regions contribution (%)	33.0	26.7	

Source: Ministry of Agriculture (2012)

3.3.3 Sorghum utilization and preferences

The majority of the farmers in the surveyed area produced sorghum both for home consumption (98.9%) and seed for the next growing season (85.8%). About 41% of the farmers produced sorghum for sale in smaller quantities when they have excess as grain and 10.5% as seed (Table 3.6). Some farmers (16.8%) use sorghum for exchange with their neighboring farmers or relatives. The farmers often do such seed exchange when there is a variety they want to grow but they don't have when they need for modern improved varieties.

Sorghum grain in Eritrea is used primarily in the home to prepare local foods such as 'injera', thick porridge (*Geat*), bread (*kicha*) and other dietary functions. *Injera* is a leavened, round and flat pancake which is national dish of Eritrea. The type of grain sorghum used for injera, bread and porridge significantly differed from region to region and sub regions as described by the household farmers. White-grained sorghum generally is preferred for food in the highlands such as in sub region Segeneyti because it gives the desired colour, while Red and Brown grains are preferred for brewing a local alcoholic beer called '*siwa'*. In the lowlands areas such as sub regions Hamelmalo, Tesseney and Goluj the White and Red grain sorghum are equally important for injera, bread and porridge making while Brown grain is for the preparation of homemade drinks.

Table 3.6. Frequencies of household responding on the main use of sorghum

	Utilization					
Sub region	**Grain sell**	**Seed sell**	**Seed exchange**	**Own seed**	**Home consumption**	**Animal feed**
Goluj	30	6	17	46	52	0
Hamelmalo	9	13	5	32	46	0
Segeneyti	3	1	8	38	39	0
Tesseney	37	0	2	47	51	1
Total number	**79**	**20**	**32**	**163**	**188**	**1**
Total (%)	**41.6**	**10.5**	**16.8**	**85.8**	**98.9**	**0.5**

NB: The sum total number of household respondants for all sub sub regions = 190

The red grain sorghum was preferred for injera (45.8%), local alcoholic beverage (13.2%) while brown sorghum was preferred for local alcoholic beverage preparations and has longer storage life after harvest. The home made drink 'siwa' is only prepared by Christian household (Table 3.7). In general, households are slightly inclined to use red sorghum for injera and bread in the lowlands. More importantly farmers both in the

household interviewed and group discussion confirmed that they used sorghum for more than one specific form of food preparations.

Table 3.7. Preference of grain colour of sorghum for injera, local drinks and seed storage life

	Sorghum Seed Colour					
Preference and storage length (%)	Red	White	Brown	Chalky White	Red, White, Brown and Chalky White	**Total**
Injera making	45.8	27.4	9.5	8.9	8.4	**100.0**
Siwa making †	13.2	15.8	14.2	7.9	6.8	**57.9**
Better storage life	45.3	15.3	36.3	3.1	0.0	**100.0**

† 42.1% of the household do not prepare local alcoholic beverages

About 93 % of the households use sorghum in multiple ways (Figure 3.3). It is only 2.6% and 4.7% of the of the household farmers that use sorghum for injera and porridge respectively.

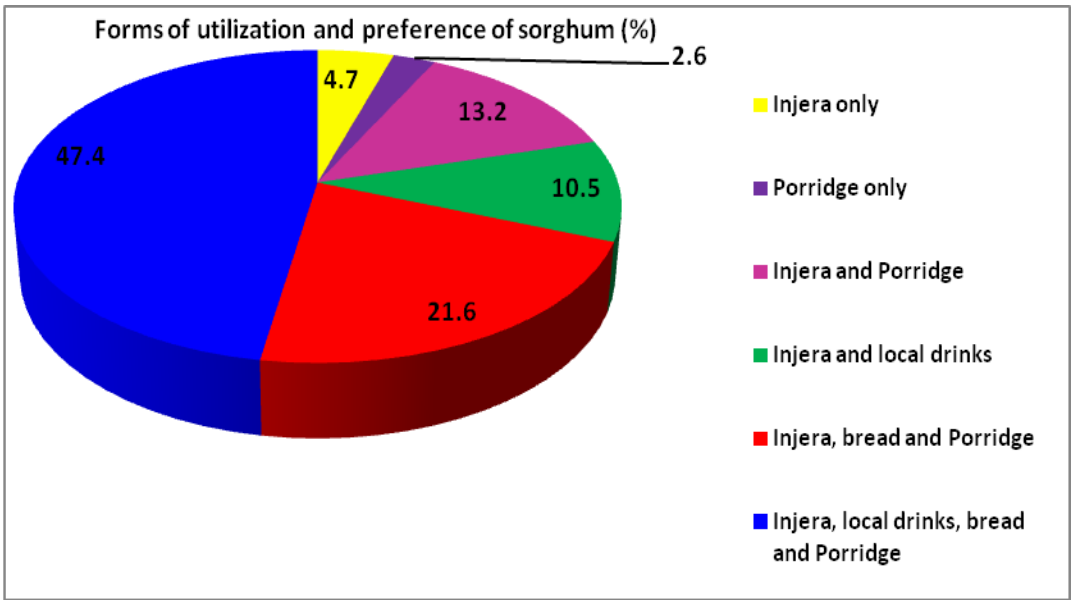


Figure 3.3 forms of sorghum utilization by households in percentage

3.3.4 Agronomic practices and cropping calendar for sorghum

The cropping patterns practiced in the surveyed sub regions included sole cropping, intercropping, and crop rotation. 'Fallowing' was not practiced in the surveyed sub regions due to shortage of farmland. The results indicated that intercropping was not common among the farmers except in sub region Hamelmalo (Table 3.8). However, inter-cultivation (*Gusia*) was generally practiced in all sub regions.

Majority of the households reported that they practiced crop rotation (Table 3.8). The pattern of crop rotation followed by farmers in all sub regions was cereals after pulses and oil crops or vice versa. For instance a field planted with one of the pulse crops (Chick pea, Faba bean or Grass pea) was allotted for growing sorghum or finger millet in the next season. The rotation cycle basically differs from one sub region to another based on the crop type and agro-ecology. The commonly used rotation cycles are: *Sorghum - Ground nut - Pear millet* (Hamelmalo), *Finger millet – Sorghum – Taff – Fallow* or *Sorghum – Barley –Taff - Chick pea - Finger millet* (Segeneyti) and *Sorghum - Sesame - Pearl millet* (Tesseney and Goluj).

Generally, the soils in Eritrea are poor in fertility and eroded by rain and wind. This is especially true in the highlands (Segeneyti) and mid lowlands (Hamelmalo) areas where the land holdings of the households are generally small and undulated (Negassi et al., 2002). In these two sub regions the land is redistributed among the families of the village every 6-7 years, a land tenure system called 'diesa'. In this system farmers often do very little soil maintenance and cultivation is very intensive because of high population pressure leading to the soils becoming poorer and poorer. In the sub regions Tesseney and Goluj soil fertility is generally better.

In all sub regions, commercial fertilizer for sorghum cultivation is very rarely used by the farmers (Table 7). However, some farmers in Hamelmalo and Segeneyti sub regions, applied farm yard manure (FYM) in their fields located near the homestead.

Table 3.8. Number of household respondents on application to different sorghum agronomic practices

Sorghum agronomic practices	Responses for the practices (Yes/No)	Sub Region				Total respond (No.)	HH responses (%)
		Hamelmalo	Segeneyti	Teseney	Goluj		
Weeding	**Y**	47.0	40.0	51.0	52.0	**190**	**100**
	N	0.0	0.0	0.0	0.0	**0**	**0.0**
Inter Cultivation	**Y**	47.0	40.0	19.0	42.0	**148**	**77.9**
	N	0.0	0.0	32.0	10.0	**42**	**22.1**
Fertilizer	**Y**	37.0	35.0	3.0	4.0	**79**	**41.6**
	N	10.0	5.0	48.0	48.0	**111**	**58.4**
Intercropping	**Y**	31.0	9.0	2.0	6.0	**48**	**25.3**
	N	16.0	31.0	49.0	46.0	**142**	**74.7**
Crop rotation	**Y**	45.0	40.0	29.0	44.0.	**158**	**83.2**
	N	2.0	0.0	22.0	8.0	**32**	**16.8**

The operations and cropping calendar in the surveyed area is categorized into two forms, highland and lowland cropping calendars. Sub region Segeneyti represents the highland and Hamelmalo, Goluj and Tesseney for the lowlands. In the lowlands, land preparation and planting start in the middle of June, normally just after the first rains while in the highlands land preparation and planting for sorghum depends on the onset of rain normally between March to April (Table 3.9). The other farm activities normally coincide with each other and are the same in all sub regions.

Table 3.9. Cropping calendar of farm activities for sorghum in the surveyed area

Sub region	Altitude	Cropping activities						
		Land pre-paration	Sowing	Weeding	Cultivation	Bird scaring	Harvesting	Threshing
Hamelmalo	**Lowland**	May –Jun.	Jun.-Jul.	Jun-Aug.	Jun-Aug.	Sept.	Oct.-Nov.	Nov.-Dec
Tesseney	**Lowland**	May –Jun.	Jun.-Jul.	Jun-Aug.	Jun-Aug.	Sept.	Oct.-Nov.	Nov.-Dec
Goluj	**Lowland**	May –Jun.	Jun.-Jul.	Jun-Aug.	Jun-Aug.	Sept.	Oct.-Nov.	Nov.-Dec
Segeneyti	**Highland**	Mar.-Apr.	May	Jun-Aug.	Jun-Aug.	Aug.	Oct.-Nov.	Nov.-Dec

3.3.5 Seed sources, selection practices and criteria

In each cropping season, the household head decides which variety and how much seeds of a given variety were to be planted. In most of the cases, farmers in the study area used their own saved seeds (unless unpredicted factors such as drought acts otherwise) although they may obtain seeds through exchange, gift or purchase. In the present study, 47.4% of the respondent farmers retained seed from their produce, while 9.5 % obtained from the market, 7.9% from neighbors, and 1.6% from Ministry of Agriculture and the rest from a combination of the four sources (Figure 3.4).

Very few respondent farmers used improved sorghum varieties provided by the Ministry of Agriculture. This was probably due to the absence or poor distribution of modern varieties or farmers were reluctant to use them and preferred their own landraces. Majority of respondent farmers do seed selection before harvesting based on a number of traits such as big and long panicles, seed size and colour, early maturing types and disease free plants.

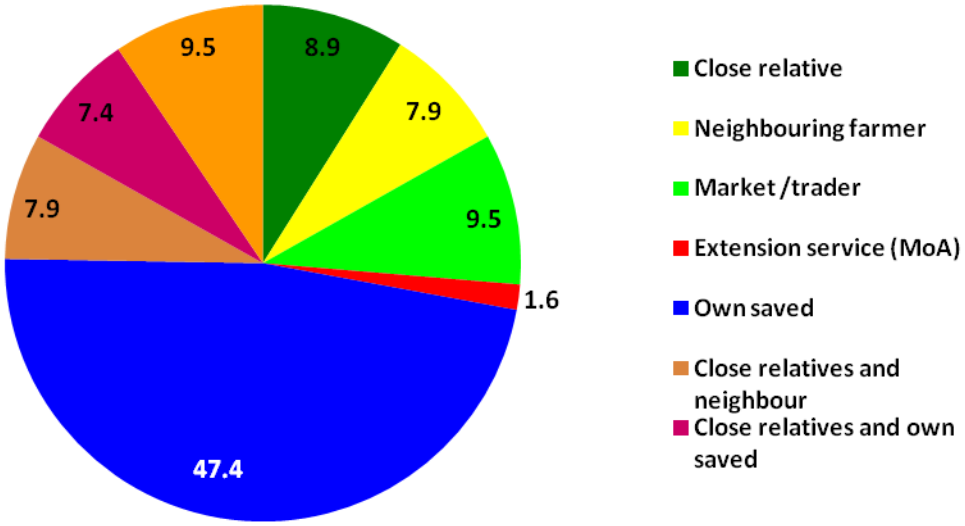


Figure 3.4 Sources of sorghum seed for the households in percentage

3.3.6 Post harvest constraints and management

Majority of the farmers stored sorghum grain in containers such as sisal and polyethylene sacks. In Segeneyti areas few farmers used the traditional store called 'Kofo'. Kofo is an above ground raised structure made of clay and cow dung mixtures. About 63% of the farmers indicated that storage pests such as weevils (*Sitophilus oryzae*) were major constraints (Table 3.10). The combination of storage pests, poor threshing facilities and high moisture content (MC) was ranked second in importance. A few farmers (4.7%) responded that they did not face post harvest problems. The main argument given by these few farmers were that '*post harvest problems were considered as an indicator of carelessness of the farmer themselves'*. If one could have avoided the main factors that lead to post harvest loss of sorghum then the loss wouldn't have occurred. According to the elderly farmers, the causes of post harvest deterioration could be prevented in advance by keeping optimum moisture content at maturity, care at the time of harvesting not to include immature plants, leaving the panicles at threshing floor for some days to dry well before threshing, ensuring the storage environment is clean and if a problem of post harvest is encountered then timely care should be taken such as drying and winnowing.

Table 3.10 Number of household respondents for major post harvest factors affecting sorghum

	Factors of post harvest problem					
Sub region	**Storage pests**	**Poor threshing**	**High MC**	**Grain mold**	**Storage pests, poor threshing and high MC**	**No post harvest problem faced**
Goluj	25	0	0	0	24	3
Hamelmalo	26	0	1	0	16	4
Segeneyti	35	0	0	0	3	2
Tesseney	35	2	4	0	10	0
Post harvest Problem %	**63.7**	**1.1**	**2.6**	**0.0**	**27.9**	**4.7**
Rank	**1**	**5**	**4**	**6**	**2**	**3**

The post harvest management practices used by farmers could be categorized into traditional, mechanical and physical controls. Farmers have traditionally used plants and

tree leaves inside and around their store as sources of insecticides. However, there is very little direct evidence which demonstrates these botanical plants used by the farmers are effective grain protectants. Majority of the farmers do mechanical techniques such sieving, winnowing combined with sun drying, water traps and baits that are considered as an insect control in storage. Addition of dusts such as ash is practiced by few farmers that provide a barrier to insect movement and damage the insect cuticle causing death by dehydration.

3.3.7 Variation of sorghum landraces and seed systems in the surveyed sub regions

Subsistence farmers in the survey area generally gave high value on their landraces because of the specific and distinct roles these landraces played in relation to adaptation and yield insurances. The choice of landrace to be grown depends on several factors. In field conditions some of the factors that were observed to affect the diversity of landraces include precipitation, temperature, growing season, crop types, farmers' selection criteria and intensity of cropping activities. These factors are directly or indirectly associated with altitude. Sorghum grows at slower rate in the high altitudes. Much of the Eritrean sorghum diversity therefore occurs in the mid and lower altitude areas such as in Gash Barka and Anseba regions (personal experience). During the survey, it was noted that there were landraces that grew both in the mid and lower altitudes but not in the cool highlands above 1700 m. Sorghum landraces such as Zengeda and Amal are examples of those that are adapted to cooler environments. Many of the recorded lowland landraces did not grow in the highlands because they were adapted to the lowland environmental conditions (Table 3.11).

During the group discussion farmers indicated that they did not rely on only one landrace for production and consumption preferences, rather they have more than one cultivar of choice. This is in agreement with Bellon, (1996) and Smale et al., (2001), who pointed out that there is no one single variety that is able to satisfy farmers both in production and consumption needs. Hence, farmers demand multiple varieties to meet a

range of objectives. Though farmers have multiple varieties of sorghum in their hand, some of the landraces however are steadily being neglected due to climatic variability such as drought stress and growing season variabilities. The late maturing varieties are the ones that are most vulnerable to extinction. Erratic rainfall conditions made the cultivation of long duration varieties very risky. Under such circumstances, only early maturing crop varieties can be grown. Such a situation could gradually lead to a loss of biodiversity.

The naming of local landraces was mainly based on their maturity period, grain colour or their use. It became evident that similar cultivars had different names due to differences in ethnicity and locality, thus, there were duplications within the landraces listed by the farmers during group discussion. For example, Hariray grown in Gash Barka looks to be similar to Red Hillo and Embulbul in South and Anseba regions respectively (Table 3.11).

The study revealed that 63% of the interviewed farmers were aware of the existence of improved varieties. However, their adoption rates were very low. Nearly 90% of the households (170 households) cultivated mainly landraces, and only 10% of the households adopted improved varieties. The main reasons for not adopting the improved varieties were: risks associated with late maturity, poor tolerance to adverse climatic conditions and seed availability. Few farmers around Hamelmalo area indicated that they used an improved variety called *Hamelmalo* that was released by Hamelmalo Agricultural College in 2010. Improved varieties S*hambuko, Bushka* and *ICSV 111 IN* are also used by some farmers in sub regions Goluj and Tesseney. However, the general tendency of the farmers who participated in the group discussion in these sub regions indicated that the improved varieties are late in maturing and susceptible to striga and birds. The source of seed of improved varieties was the national research systems in the country.

Table 3.11 Sorghum landraces recorded during the survey with their localities and status of cultivation

S.No	Landraces	Region	Place of cultivation		Status	Remark
			Sub region	Village/ town		
1	Baryay Red	Anseba	Hamelmalo	Gizgiza	Cultivated in specific area	
2	Baryay white	Anseba	Hamelmalo	Gizgiza	Cultivated in specific area	
3	Embulbul	Anseba	Hamelmalo	Gizgiza	Cultivated in specific area	
4	Kibra	Anseba	Hamelmalo	Fledareb	Limited cultivation	
5	Hariray	Anseba/ Gash Barka	Hamelmalo, Goluj and Tesseney	Hamelmalo Fledareb, Tesseney, Goluj and Gergef	Cultivated in specific area	
6	Red Hillo	Anseba and South	Hamelmalo and Segeneyti	Fledareb, Hadida and Adi Hadid	Cultivated in specific area	
7	White Hillo	Anseba and South	Hamelmalo and Segeneyti	Fledareb, Hadida and Adi Hadid	Cultivated in specific area	
8	Gimbilu	South	Segeneyti	Hadida, Adi Hadid, Engela and Akrur	Cultivated in specific area	
9	Zengeda	South	Segeneyti	Hadida, Adi Hadid, Engela and Akrur	Widely cultivated	
10	Kinibiba	Gash Barka	Tesseney	Thalata Ashir and Aligidir	Limited cultivation	
11	Amal	South	Segeneyti	Hadida and Engela	Cultivated in specific area	

Table 3.11 cont.,

12	Anseba	South	Segeneyti	Akrur and Hadida	Limited cultivation	
13	Wediaker short	Gash Barka	Tesseney	Thalata ashir and Omhajer	Cultivated in specific area	
14	Wediaker tall	Gash Barka	Tesseney	Thalata ashir and Omhajer	Cultivated in specific area	
15	Feterit	Gash Barka	Tesseney	Tesseney and Goluj	Limited cultivation	
16	Arfaegedam	Gash Barka	Tesseney	Tesseney Gergef and Omhager	Limited cultivation	
17	Wedifereg	Gash Barka	Tesseney/ Goluj	Tesseney, Gergef, Goluj and Omhager	Cultivated in specific area	
18	Ugana/ Bazenay	Gash Barka	Tesseney	Aligidir	Cultivated in specific area	
19	Koden short	South /Gash Barka	South/Tesseney	Hadida and Tesseney	Cultivated in specific area	
20	Koden Tall	South /Gash Barka	South/Tesseney	Hadida and Tesseney	Cultivated in specific area	
21	WediArbaa	Gash Barka	Tesseney	Aligidir	Cultivated in specific area	
22	Aklamoya	Gash Barka	Tesseney	Aligidir	Extinct	drought
23	Brown Chimro	Gash Barka	Tesseney	Aligidir	Limited cultivation	
24	White Chimro/ (Habarat)	Gash Barka	Tesseney	Thalata ashir	Limited cultivation	
25	Ajebsidu	Gash Barka	Goluj	Omhajer	Extinct	drought
26	Hugurtay	Gash Barka	Goluj	Goluj, Gergef, Omhajer and Tesseney	Widely cultivated	
27	Korokora	Gash Barka	Goluj	Goluj and Gergef	Limited cultivation	
28	Gunseber	Gash Barka	Tesseney	Aligidir	Extinct	drought

A key issue affecting the demand for improved and traditional landrace varieties is their ability to grow and give yield in marginal conditions. The risk management characteristics, such as good adaptability, early maturity, and drought resistance were considered as the most desirable attributes for farmers to use as selection criteria for good varieties. 47% of the household consider as good variety if it is early maturing, 32% if it gives reasonable yield during unfavourable condition and 21% good adaptability (Table 3.12).

Table 3.12 Farmers' consideration of most desirable attributes in local sorghum varieties

Sub region	Desirable sorghum characteristics			Total
	Reasonable yield in bad years	Good adaptability	Early maturing	
Hamelmalo	9	16	22	47
Segeneyti	26	10	4	40
Tesseney	18	10	23	51
Goluj	8	4	40	52
Good attribute	**32**	**21**	**47**	**100**

3.3.8 Sorghum production constraints

Sorghum production in Eritrea is affected by many factors. This study collected extensive information on this issue to identify the farmer's main sorghum production limiting factors and their prioritization. The current study indicated that the two major sorghum production constraints in the surveyed sites were drought and the parasitic weed, striga. Across all the four sub regions drought stress, (71%) ranked first followed by striga and diseases (17%) (Table 3.13). Drought stress occurs when rainfall is generally low and its distribution is erratic and sometimes leads to complete yield lose. Drought stress affects sorghum at different developmental stages such as seedling, vegetative, flowering and post-flowering. The results indicated drought stress that occurring at post flowering stage of growth was the most important in influencing sorghum production.

Table 3.13 Sorghum production constraints across the four surveyed sub regions

Production constraints	Sub region				Total	Constraints (%)	Rank
	Hamelmalo	Segeneyt	Tesseney	Goluj			
Drought stress	29.0	27.0	40.0	39.0	**135.0**	**71.1**	**1**
Striga and diseases	8.0	11.0	7.0	8.0	**34.0**	**17.9**	**2**
Access to Seed	0.0	2.0	0.0	1.0	**3.0**	**1.6**	**5**
Access to Labour	3.0	0.0	0.0	3.0	**6.0**	**3.2**	**3**
Access to Credit	2.0	0.0	2.0	1.0	**5.0**	**2.6**	**4**
Access to Land	1.0	0.0	2.0	0.0	**3.0**	**1.6**	**5**
Access to fertilizer	3.0	0.0	0.0	0.0	**3.0**	**1.6**	**5**
Access to market	1.0	0.0	0.0	0.0	**1.0**	**0.5**	**6**

Farmers in sub regions Tesseney and Goluj, however, expressed that the occurrence of drought stress at post flowering stage was the most common phenomenon. Seed loss due to total sorghum crop failures by drought has been observed once in every three years. This problem was more serious in sub region Tesseney where the amount of rainfall is much lower than the other sub regions.

The management methods practiced by the farmers to alleviate drought stress differ from one sub region to another. In areas like sub region Hamelmalo and Segeneyti where the landscape is hilly and undulated, farmers have established terraces and bundings to harvest the available rainfall inside their fields. Basically, to establish such structures is very expensive for resource poor farmers and they are assisted by the Ministry of Agriculture and the local administration. The land and crop fields of Goluj and Tesseney sub regions are flat and farmer's drought control practices were mainly focused on establishing soil bunds and flood water diversions. Few farmers who have the capacity to make such activities in their fields shared experiences and the yield advantage they got during the group discussion.

The other most commonly used approach to overcome droughtstress was selection of crop variety that fits into the short growing period. Majority of the interviewed household farmers know using drought escaper and early maturing sorghum is at the expense of yield. However, early maturing sorghum landraces assured them of some yields during bad years.

Conclusion

Sorghum is the most important staple crop in Eritrea and a crucial crop to achieve food security under the area's difficult weather conditions. The study brought important information interms of production, utilization and constraints for this crop.

The main conclusion from the survey study includes:

- Household farmers use sorghum landraces for food in different forms of utilization. Based on the result, sorghum grain is used primarily in the home to prepare local foods such as 'injera', thick porridge (*Geat*) and bread (*kicha*).
- White and red grain sorghums are generally preferred for injera making while brown and red grains for for local brewing.
- Most of the grain sorghum landraces are selected by farmers on the basis of good food quality, taste, storage life and brewing quality.
- Drought stress that occurs during post flowering stage of the crop is a major challenge of sorghum production.
- Farmers mitigate drought stress through agronomic practices such as terracing and bunding; terracing, bunding and water harvesting
- Framers recognized that drought escaping and early maturing varieties were the most common desirable traits to overcome drought stress.
- Farmers' selections for desirable agronomic traits are major forces in shaping of the sorghum cultivars to be used on a farmland.
- Majority of the farmers who participated in this study saved their own seed for next season planting. Seed selection while the crop was in the field was practiced by most farmers who deliberately selected them on the basis of panicle and seed size, seed colour and well matured plants.

Recommendation

- The existence of diverse sorghum landraces in the surveyed regions could be manipulated for value addition and diversified utilization. The preparation of traditional home made drinks from red and brown grain sorghum for instance can

be changed into industry based brewing factory. Sorghum can be thought also as a new potential substitute for barley, which can not only resolve the ingredient problem, but also raise economic status of the small scale farmers in particular and the country at large.

- The sorghum improvement programme of the country needs to push towards developing promising sorghum varieties with good yield and resistant to drought**.**
- To protect from in danger of extinction the Eritrean sorghum landraces, recollection exercises are necessary where genetic erosion is common, primarily due to natural disasters.
- Farmers selected landraces on the basis of phenotypic appearances. However, due to the existence of wild and semi cultivated sorghum progenitors, high rate of out crossing and gene flow from wild to cultivated thus contamination is expected in the surveyed regions. This is especially true in sub regions Goluj and Teseney where there is high existence of wild sorghum and shatter canes. In this regard the national breeding programme have to intervene in disseminating improved sorghum varieties with enough seed supply and create awareness on their advantage without endangering the local landraces.
- This study brings a future research outlook and opportunity to explore in depth and document the entire diversity of sorghum that is available in Eritrea.

CHAPTER FOUR

MORPHOLOGICAL EVALUATION OF ERITREAN SORGHUM LANDRACES FOR DROUGHT TOLERANCE

Abstract

Grain sorghum is an important food crop of Eritrea. The crop incurs heavy yield loses resulted from abiotic stresses such as flowering and post-flowering drought stress. The present study was conducted to evaluate and identify sorghum landraces for post flowering drought stress tolerance. Two years field experiment was conducted during the off-season of 2013 and 2014 at Hamelmalo Agricultural College. During the 1st year rapid screening experiment, 100 genotypes were evaluated under stress managed and fully irrigated control while in the 2nd year, 25 selected accessions were evaluated in split plot design with three replication. Fully irrigated and drought stress treatments assigned in main plot and the landraces in sub plot. Data on 16 different morpho-physiological traits were recorded and analysed using the analysis of variance, drought tolerance indices, and estimation of genetic variability, heritability and principal component analysis. Based on the statistical analysis the rapid screening experiment selected 20 superior genotypes for post flowering drought tolerance. The analysis of variance in the second year experiment showed significant genotypic variation in both stress and irrigated treatments at $P<0.05$ - <0.001. Based on grain yield under stress and irrigated conditions positive and significant correlation were recorded between yield under irrigated (Yi) and moisture stressed (Yr) conditions and mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI). The biplot and cluster analysis also grouped tolerant and susceptible landraces based on the selection indices. High magnitude of phenotypic and genotypic coefficient of variations for plant height, harvest index and biomass as well as high heritability for days to flowering, panicle length, days to maturity and over all agronomic score were recorded. Principal

component (PC) analysis showed first 4 PCs having Eigen value >1 explaining 74.6% of the total variation with grain yield, biomass, stay-green, leaf area, peduncle exsertion and days to flowering and maturity being the most important characters in PC1 and PC2. Overall, this study demonstrated that the amount of diversity present for the characters among the landraces and could be exploited to execute a breeding programme aimed at improving drought stress tolerance. Moreover, this research showed drought stress reduced the yield of some genotypes while that of others was not affected suggesting genetic variability of drought tolerance in this material. Accessions EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711 were identified as promising genotypes for post-flowering drought tolerance that could be used by breeders in sorghum improvement programmes.

Key words: Biplot analysis, Drought Tolerance, Principal Component Analysis, Sorghum

Introduction

Drought tolerance was defined by Hall, (1993) as the major relative yield of a genotype compared with other genotypes subjected to the same drought stress. Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988), while the values are confounded with the differential yield potentials of genotypes (Ramirez & Kelly, 1998). Enhancing drought tolerance is an important objective in many crop improvement breeding programmes. However, selection for drought tolerance is difficult because of inconsistency in testing environments and interaction between stages of plant growth and environment. The genetic mechanisms that condition the expression of drought tolerance in crop plants are also 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 (Kebede et al., 2001).

Understanding plant responses to drought is of great importance and also a fundamental part of making crops stress tolerant (Reddy, Chaitanya, & Vivekananda, 2004). The relative yield performance of genotypes in drought-stressed and favourable environments seems to be a common starting point in the identification of desirable genotypes for unpredictable rainfed conditions (Mohammadi et al., 2010). However, some researchers believe in selection under favourable conditions (Betran et al., 2003), others in a target stress condition (Rathjen, 1994) while others yet have chosen a mid-point and believe in selection under both favourable and stress conditions (Byrne et al., 1995).

Sorghum (*Sorghum bicolour*) is one of the major cereal crops grown in the semi-arid tropics where prolonged droughts are frequent. Although sorghum has an ability to cope with many types of stresses, including heat, drought, salinity and flooding (Ejeta & Knoll, 2007) but in arid and semi-arid regions, this crop is usually affected by drought stress at the reproductive stage particularly post flowering stage (Tuinstra et al., 1997a; Kebede et al., 2001).

Stay-green or non-senescence is an important trait associated with drought tolerance (Rosenow, 1977). Stay-green trait is the ability of the plant to retain greenness during grain ripening under water limited conditions (Borrell et al., 2000; Xu et al., 2000). Sorghum genotypes with the stay-green trait continue to fill their grains normally even under limited water or drought stress conditions (Borrell et al., 2000). Delaying the onset of leaf senescence and reducing its rate offer an effective strategy for increasing grain production, and grain crop residues particularly under water limited conditions. This trait is also reported to be associated with increased cytokinin concentration (McBee, 1984). This phenomenon enables the plant to exhibit drought tolerance and resistance to stalk lodging and charcoal rot (Woodfin et al., 1998). Other traits related to drought tolerance in sorghum include early maturity and increased root density. Attempts to exploit these genetic variations for drought tolerance in sorghum through conventional plant breeding methods have been slow and arduous.

Drought tolerance depends on the plant developmental stage at the onset of the stress condition, which in sorghum may happen during the early vegetative seedling stage, during panicle development and in post-flowering, in the period between grain filling and physiological maturity. The post-flowering drought stress in particular can result in significant reductions in crop yield (Rosenow & Clark, 1995; Rosenow et al., 1996).

Food security in the 21st century will rely increasingly on the release of cultivars with improved resistance to drought conditions and with high yield stability (Borlaug, 2007; Pennisi, 2008). The presence of significant genetic variability for these traits among the sorghum germplasm genotypes suggests an opportunity for improvement of grain yield and drought tolerance through hybridization of genotypes related to divergent groups and subsequent selection from the segregating generations.

Genetic variability for agronomic characters is a key component of breeding programmes for broadening the gene pool of crops (Ahmad et al., 2011). Success for breeding under drought stress depends on understanding of genetic basis of drought tolerance in crop plants based on various morpho-physiological to evolve superior genotype (Mitra, 2001). Knowledge of heritability influences the choice of selection

procedures used by the plant breeder to decide which selection methods would be most useful to improve the character, to predict gain from selection and to determine the relative importance of genetic effects (Waqar et al., 2008; Laghari et al., 2010). Evaluation of the components of variation and heritability are therefore among characters that will facilitate improvement of crops such as sorghum.

In the Eastern Africa, sorghum is the second most important cereal crop after maize. In this region, sorghum is grown on approximately 7 million hectares per year (FAO, 2010). It is mostly cultivated in the semi-arid and arid areas that extend from Southern and western lowlands of Eritrea, Northern Ethiopia, through North-eastern Kenya, Northern Uganda, and Central and Southern Tanzania. As these countries are located in the arid and semi arid tropics, drought contributes heavily to the constant food insecurity and rampant poverty characteristic of these zones. Water stressed plants produce inferior grain, low yields or no grain yield at all. Evolution of sorghum under pressures of drought has resulted in favourable physiological properties of the crop such as metabolic suppression and structural adjustment. The eastern Africa region is the origin of sorghum where the crop exhibits high genetic variability. The utilization of such genetic variability could contribute to the improvment of yield limiting factors such as drought stress.

Sorghum productivity in Eritrea is of less than 1 t ha^{-1} which is below the average global production (1.5 t ha^{-1}) (MoA, 2010). This low productivity of sorghum is due to drought, striga and poor understanding on the potentials of the genetic diversity in the country (Tesfamichael et al., 2013). Post-flowering drought stress is the most important factor that severely reduces the yield. Even though Eritrea has rich sorghum genetic diversity, no assessment has been done to take the advantage of these landrace diversities to develop drought tolerant sorghum varieties and for other yield limiting factors.

Objectives

The specific objectives of the present study were to:

1. Screen and select sorghum landraces for drought tolerance under managed drought stress conditions in Eritrea
2. Evaluate selected sorghum landraces for post flowering drought stress and correlate the tolerant with phenotypic and genetic variances as well as heritability and drought stress indices of various characters.

4.1 Rapid screening of sorghum landraces for post-flowering drought tolerance in Eritrea

4.1.1 Materials and methods

4.1.1.1 Location of the experiment

The site for the experiment was Hamelmalo Agricultural College, 12 km to the north of Keren city on the Keren-Nakfa road along Anseba River in Anseba region. Hamelmalo Agricultural College farm is located at 15^0 52'15"N latitude and $38^0$27' 55" E longitudes with an altitude of 1,274 meters above sea level in semi-arid agro-ecological zone of Eritrea. The experiment was conducted in a sandy and clay loam soil, during the dry off season from March to June in 2013. The average maximum and minimum air temperatures during the experimental period were 37.5^0C and 19 ^{0}C respectively. Soil moisture content before sowing and after stress imposition was taken.

4.1.1.2 Experimental material

Ninety six sorghum accessions (Table 4.1) along with two controls (E 36 and B 35 from ICRISAT) and two improved varieties (ICSV 111IN and Hamelmalo) from the national breeding programme were used in this study. The two ICRISAT varieties (B-35 and E-36) are known for their drought tolerance and stay-green traits.

4.1.1.3 Treatments and experimental lay out

The sorghum accessions were grown in randomized block design under two treatments: post-flowering drought stressed and full irrigation control. The stressed and irrigated blocks were divided each into ten sub blocks in which each sub block was divided into ten plots. Each plot had an accession. The plot size used was 3m x 2m with 4 m rows length. A spacing of 75 cm and 20 cm were used for the between and within rows respectively, giving 60 plants in each plot. Drought stress was imposed in the stress treatment blocks for two weeks by appropriately withholding water at flowering and

post-flowering stage of growth while the control block continued to receive irrigation at 3-5 days intervals. Soil moisture analysis was conducted by the department of land resources of Hamelmalo Agricultural College at different stages of crop growth before and after drought stress imposed. Besides plant and leaf symptoms were observed to differiantate the landraces for their post flowering drought tolerance.

Table 4.1. Sources of 96 sorghum landraces used in this study by region and sub region

Region	Sub region	Number of accessions	Region	Sub region	Number of accessions
Gash	Laelay Gash	17	**South**	Segeneyti	5
	Goluj	17		Dubaruba	6
	Logo anseba	3		Areza	3
	Shambuko	3		Tserona	4
	Barentu	2		Mendefera	1
	Tesenai	4		Adi Kehi	3
	Molqi	2		Senafe	1
	Gash sub total	**48**		Adi Quala	1
Anseba	Hagaz	7		**South sub total**	**24**
	Halhal	6	**NRS**	Shieb	6
	Elabered	1		Afabet	4
	Anseba Sub	**14**		**NRS sub total**	**10**

Where, NRS= Northern Red Sea Region

4.1.1.4 Data recorded

Agronomic traits that contribute to drought tolerance were assessed. The agronomic data recorded included seedling vigour which was recorded at three weeks after germination (visual observation of the seedling in 1-5 scale where 1 poor and 5 highly vigour), panicle orientation (visual description of the panicles), time to 50% flowering (the date from planting to the date when 50 percent of the plants produced flowers), days to maturity (the date from planting to the date when 90 percent of the plants are physiologically matured), number of leaves (counting number of leaves on the main plant stem), plant height (height of the plant from the base of the plant to the tip of the panicle in centimeter at maturity), leaf area (measurement of leaves (cm^2) using LI 3000 C Portable area meter from 5 randomly selected plants), peduncle exertion (the average length of the node between the flag leaf and the base of the panicle measured in cm

from 5 randomly selected plants at maturity), panicle length (length measurement (cm) from the base of the panicle to the tip from five randomly selected plants per plot at maturity), panicle width (panicle width measurement in the widest diameter of the panicle on five randomly selected plants per plot at maturity), over all plant agronomic scores (5 = most desirable and 1= least desirable), grain yield (total grain weight per plot in kilogram after threshing then converted into tons per hectare), 100-grain weight, harvest Index (the ratio of grain weight to the total biomass in percentage computed from the two middle rows) and stay-green scores at maturity based on visual ratings (Wanous, Miller, & Rosenow, 1991) using 1 to 5 scale (1 = < 10% leaves stay-green and 5 = >75% leaves stay-green and most desirable) based on the proportion of leaf area of normal sized leaves that had greenness and dried. The stay-green trait was recorded two times; first two weeks after flowering and second at the time of physiological maturity of the grain.

4.1.1.5 Data Analysis

The primary data collected in this study was entered into an excel spread sheet and analysed using Genstat® 14th Edition. The data were summarized into means and percentages. Phenotypic correlation coefficients were computed to examine the degree of association among the morphological traits. The analysed data was presented in the form of tables, charts and graphs.

4.1.1.6 Selection criteria of drought tolerance accessions

Selection for drought tolerance was based on morphological traits that focused on the post flowering drought stressed treatment accessions. The morphological traits were weighed for each accession on the basis of 1-5 scale where 1 is less desirable and poorly performanced and 5 is desirable and best performed genotype for that specific character. The maior morphological traits used in assessing the genotypes for drought stress tolerance were: their rate of stay-greenness and senescence under drought stress condition. Days to flowering, plant height, leaf area, number of leaves, over all plant vigour and yield parameters were also used for selection and ranking criteria of the accessions.

4.1.2 Results and Discussions

Out of the 96 sorghum accessions used in this study, only 42 accessions (42%) reached grain filling and maturity stage after being exposed to post flowering drought stress (Table 4.2). These accessions were mainly the early and medium flowering types. All the data presented and analyzed here are therefore based on those accessions that were able to reach physiological maturity.

4.1.2.3 Plant Height

The mean analysis for plant height indicated that the accessions differed significantly under drought stress conditions. Accession EG 537 recorded the highest plant height (220 cm) followed by EG 883 (210 cm). The shortest height was recorded by accessions EG 797 and EG 1257 with 105 and 110 cm respectively (Table 4.2). In general, there was a decrease in plant height from 0 - 30% for all accessions under post flowering drought stress compared to the fully irrigated controls.

4.1.2.4 Stay-green scores and leaf area measurements

In the current experiment, phenotypic variation was observed for stay-green trait. The mean stay-green rating for the accessions was 3.8 with a range of 1.0 -5.0. The two average observation on stay-green scores indicated that 10 accessions had good stay-green attributes ($\geq$ 4.5) (Table 4.2) and the majority were categorized under medium stay-green and few under senescence. It was also noted that those accessions with good stay-green attributes also recorded high scores for leaf area. The experimental data in Table 4.2 also indicated that the mean number of leaves, which ranged from 7-12 in moisture stress significantly differed each other.

Highest number of leaves was recorded by accession EG 584 having 12 leaves which was followed by accessions EG 481, EG 537, EG 794, EG 836, EG 881, EG 1157 and EG 885 with 11 numbers of leaves. The data in table 4.2 revealed that accession with higher number of leaves and high leaf area recorded high score of stay-green. However, high stay-green, leaf area and number of leaves not associated with plant height.

Table 4.2 Mean values for plant height and leaf and panicle characteristics recorded on sorghum accessions grown under post flowering drought stress at HAC, 2013 offseason

		Leaf characterstics			Panicle characterstics				
Cultivar name	**Plant Height (cm)**	**number of leaves**	**Leaf area (cm^2)**	**Stay-green score (1-5)**	**Panicle orientation**	**Peduncle exertion (cm)**	**Panicle length (cm)**	**Panicle width (cm)**	**Productive tillers**
EG 469	175.1	11.0	389.0	5.0	SLE	9.4	20.1	10.0	2.4
EG 473	130.3	8.0	98.2	2.2	SCE	15.1	8.4	6.2	3.2
EG 481	150.0	8.1	171.0	4.2	CB	11.1	9.2	7.1	2.1
EG 497	140.7	10.3	237.8	2.1	CE	10.0	9.5	6.4	1.3
EG 526	170.3	9.2	193.0	4.2	SCE	7.4	20.1	7.2	2.0
EG 537	220.2	11.4	327.2	5.0	SCE	10.5	20.3	13.2	0.0
EG 546	155.2	8.2	163.8	4.3	CE	12.2	10.4	7.3	2.0
EG 557	130.0	8.1	189.4	4.2	CE	10.4	9.3	7.3	1.1
EG 584	200.4	12.3	261.0	4.3	SCE	11.0	20.2	12.1	3.3
EG 711	140.6	8.1	183.8	4.1	CE	15.2	6.1	4.5	1.0
EG 756	135.3	8.0	180.2	4.3	CE	10.3	9.1	6.2	2.0
EG 782	140.5	10.1	255.0	3.4	SCE	5.1	10.3	7.1	1.1
EG 783	170.6	8.2	278.2	4.2	CB	14.4	8.3	6.4	2.0
EG 786	140.3	7.4	110.8	4.4	CE	13.2	9.3	5.5	3.2
EG 787	160.1	9.1	204.6	4.2	CB	10.2	10.1	9.1	2.2
EG 789	130.7	8.4	239.2	3.4	CE	15.0	13.3	5.4	0.0
EG 791	150.4	9.3	294.4	4.1	CE	8.4	18.4	6.1	2.1
EG 794	170.0	11.2	247.4	4.2	SCE	10.3	15.2	6.3	0.0
EG 797	105.1	9.5	282.6	5.0	CE	8.3	13.2	5.4	2.2
EG 806	165.8	8.0	174.8	5.0	CB/CE	15.1	15.3	7.5	1.2
EG 813	150.2	8.5	196.8	5.0	CE	14.4	10.4	5.2	1.1
EG 815	155.1	9.2	272.8	4.1	CE	15.2	18.1	8.5	1.4
EG 830	135.5	8.3	181.2	4.2	CE	7.5	10.3	5.4	1.1
EG 836	150.2	11.3	226.0	5.0	CE	8.0	8.3	5.3	0.0
EG 845	125.3	6.1	193.0	4.2	CE	11.2	10.2	5.3	1.0
EG 849	120.3	11.2	248.0	5.0	CE	8.4	12.2	9.3	1.0
EG 870	140.5	9.3	263.0	1.2	CE	3.5	16.1	5.1	0.0
EG 875	120.1	10.3	305.2	3.2	LD	5.4	15.1	5.6	0.0
EG 881	150.4	11.4	254.8	4.4	LD	3.7	26.3	4.4	0.0
EG 883	210.6	10.0	309.0	5.0	CE	11.1	20.2	10.1	0.0
EG 885	155.0	11.3	238.5	4.5	CB	9.0	10.4	6.2	2.4
EG 889	195.1	10.0	300.5	4.3	VL	6.1	20.1	3.2	0.0
EG 890	170.3	8.2	291.5	4.1	SLE	7.5	18.1	4.5	0.0
EG 893	170.5	10.1	283.5	4.2	CE	5.2	10.1	3.6	0.0
EG 896	160.0	8.3	189.5	5.0	LE	12.4	20.1	5.2	2.2
H/malo	110.6	10.4	445.0	4.5	CE	1.6	15.3	6.3	0.0
EG 1157	130.2	11.0	276.5	4.1	CE	1.0	19.3	6.5	0.0
EG 1224	125.1	10.2	320.0	4.3	CE	10.3	15.4	7.2	0.0
EG 1256	160.7	9.3	176.0	1.1	SCE	6.9	12.1	6.1	0.0
EG 1257	110.0	10.3	271.5	3.1	CE	8.3	13.2	6.5	0.0
EG 1261	130.2	7.2	114.2	2.0	CE	7.1	8.2	5.2	3.4
EG 2457	155.1	8.4	170.5	2.3	CE	13.5	10.2	5.2	0.0
Mean	**159.3**	**9.4**	**243.3**	**4.0**		**9.5**	**13.7**	**6.6**	**1.1**

Where, CE -Compact Erect, CB -Compact Bent, SLE -Semi Loose Erect, LE -Loose Erect, SCE -Semi-compact Erect

4.1.2.5 Panicle characterstics

The panicle length and width differed significantly both in irrigated and post flowering stressed accessions. There was also significant difference within the stressed accession on panicle length and width where the highest panicle width (12 cm) and length (20 cm) was recorded by EG 584 and lowest width (4 cm) and length (6 cm) by EG 711 (Table 4.2 and Plate 4.1). Accessions with higher panicle width and length were observed to score better grain yield as compared to those with lowest panicle width and length.

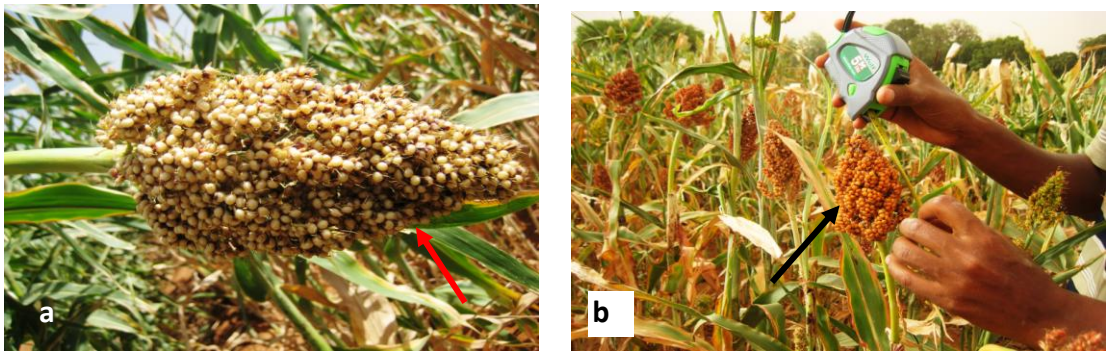


Plate 4.1 Drought stress has significantly reduced the length and width of panicles of the different accessions. This picture indicates some landraces were less affected as compared to susceptible genotypes with the same magnitude of drought treatment [highest panicle length and width EG 584 (**a**) and lowest size by EG 711 (**b**)

4.1.2.6 Days to 50% Flowering and physiological maturity

The accessions differed significantly for days to 50% flowering and maturity. Time to 50% flowering of the accessions ranged from 45.3 to 72 days and for physiological maturity it ranged from 72.2 to 103.4 days (Table 4.3). The data indicated that accessions could be categorized into four maturity groups; extra early (65-75 days), early (76-80 days), medium (81-95 days) and late (> 95 days) maturing. The extra early and early accessions have short life cycle in which they escape the drought, however they recorded low grain yields.

Table 4.3 Mean values for days to flowering, maturity and yield component traits recorded on sorghum accessions grown at HAC under post flowering drought stress

	Plant aspects			Yield components			
Cultivar	**Days to 50% flowering**	**Plant Vigour score (1-5)**	**Days to Maturity**	**Dry Panicle weight (kg)**	**Grain Weight (t ha^{-1})**	**100 seed wt. (gm)**	**Harvest Index (%)**
EG 469	60.0	4.0	81.4	1.2	2.7	2.9	19.3
EG 473	45.3	3.0	72.2	0.6	1.1	4.1	20.2
EG 481	51.5	3.0	77.3	1.0	2.1	3.5	27.8
EG 497	57.6	3.0	80.2	0.8	1.7	3.1	23.0
EG 526	60.2	4.0	86.3	0.9	1.7	3.0	29.5
EG 537	70.0	3.5	93.3	1.2	2.6	3.2	14.0
EG 546	56.8	3.5	77.0	0.6	1.2	3.0	24.7
EG 557	53.4	3.0	78.3	0.7	1.9	3.5	33.2
EG 584	77.5	3.0	89.3	1.4	2.6	2.8	15.9
EG 711	52.4	3.0	80.2	0.8	1.5	3.4	20.7
EG 756	58.3	3.0	81.5	0.8	1.5	3.5	16.8
EG 782	68.5	3.5	95.7	1.0	1.7	2.7	15.4
EG 783	66.7	3.0	82.2	0.9	2.5	3.3	25.9
EG 786	45.7	3.0	72.2	0.7	1.3	3.4	25.1
EG 787	54.7	2.5	78.1	0.9	1.8	3.2	20.4
EG 789	67.2	2.0	85.5	0.5	0.7	3.6	16.6
EG 791	62.6	3.5	92.4	8.0	1.4	4.2	12.3
EG 794	62.1	3.5	85.3	0.7	1.5	2.5	25.4
EG 797	78.5	3.5	89.2	0.9	1.4	2.5	17.4
EG 806	51.2	4.0	87.3	0.9	2.2	3.3	37.3
EG 813	56.3	4.0	85.1	0.9	1.6	2.9	26.2
EG 815	57.3	3.5	90.4	0.9	1.7	3.4	17.6
EG 830	58.4	3.5	84.6	0.8	1.6	3.6	30.1
EG 836	64.2	3.5	90.0	1.0	1.9	3.1	17.4
EG 845	56.5	3.5	83.2	0.6	1.5	3.8	40.5
EG 849	58.7	4.0	87.3	1.4	3.1	3.2	23.2
EG 870	74.7	1.5	97.1	1.0	1.1	4.1	11.3
EG 875	70.1	2.0	100.5	1.0	1.1	3.0	10.7
EG 881	68.2	2.5	95.0	1.1	2.2	2.3	16.0
EG 883	70.6	3.5	93.2	1.5	2.9	3.7	19.3
EG 885	64.4	3.5	85.3	1.0	2.0	3.4	22.7
EG 889	59.0	3.5	87.5	1.0	1.8	3.1	15.9
EG 890	66.3	2.5	86.2	0.9	1.3	2.8	16.3
EG 893	70.3	2.5	100.6	1.0	1.5	2.9	12.3
EG 896	54.8	3.5	78.2	0.7	1.5	3.0	22.5
H/malo	68.2	3.5	80.3	1.0	2.0	2.7	16.7
EG 1157	69.3	2.0	103.4	1.1	2.2	2.4	17.9
EG 1224	63.5	3.5	99.0	1.1	2.3	4.3	33.2
EG 1256	72.0	1.0	101.4	1.1	1.7	2.5	14.3
EG 1257	67.1	3.0	96.5	0.9	1.2	3.3	17.3
EG 1261	49.3	3.5	67.3	0.7	1.2	3.5	22.3
EG 2457	60.6	3.5	86.7	0.8	1.8	3.6	17.3
Mean	**57.7**	**3.1**	**86.8**	**1.1**	**1.7**	**3.2**	**21.0**

4.1.2.7 Yield Parameters

There were significant differences among the accessions for grain yield. Grain yield of the accessions under the post flowering drought stress ranged from 0.7 tha^{-1} (EG 789) to 3.1 tha^{-1} (EG 849) (Table 4.3). Overall mean analysis for grain yield indicated that post flowering drought stress greatly affected productivity of the accessions when compared with the fully irrigation treatments (Plate 4.2).

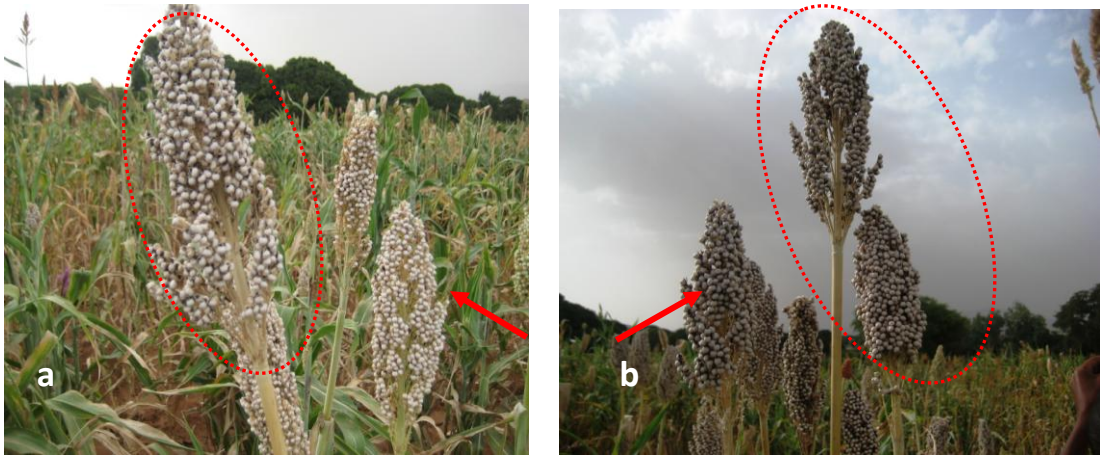


Plate 4.2 The effect of post flowering droguht stress on sorghum was highly pronounced on seed setting, peduncle exsertion and yield that showed reduced under stress demonstrated by EG 789 under post flowering stress (**a**) as compared to fully irrigated (**b**).

However, few accessions performed well and recorded superior grain yield in both post flowering drought stress and full irrigation. Accessions EG 537, EG 584, EG 849 and EG 1224 were among the high yielding genotypes and had good agronomic performance under both post flowering drought stress and irrigated conditions (Table 4.4 and Plate 4.3).

Table 4.4 Mean values for plant height, days to lowering and maturity, grain yield and harvest index, recorded on sorghum accessions grown under full irrigation and post flowering drought stress conditions at HAC in 2013 offseason

Accession	Plant height (cm)		Days to 50% Flowering (Days)		Days to maturity (Days)		Grain Yield (t ha^{-1})		Harvest Index (%)	
	Irr.	Str.	Irr.	Str.	Irr.	Str.	Irr.	Str.	Irr.	Str.
EG 469	210.3	175.1	58.1	60.0	81.4	87.4	36.7	2.8	15.3	19.3
EG 473	140.4	130.3	41.5	45.3	72.2	82.6	19.5	1.1	26.6	20.2
EG 481	165.3	150.0	48.2	51.5	77.3	78.5	27.4	2.1	30.5	27.8
EG 497	170.3	140.7	54.3	57.6	80.2	98.2	25.5	1.7	22.5	23.0
EG 526	180.4	170.3	58.4	60.2	86.3	89.3	17.6	1.7	16.0	29.5
EG 537	230.0	220.2	66.3	70.0	93.3	97.0	27.1	2.6	15.9	14.0
EG 546	170.2	155.2	51.0	56.8	77.0	80.1	12.9	1.2	18.4	24.7
EG 557	140.4	130.0	53.3	53.4	78.3	81.3	19.4	1.9	24.3	33.2
EG 584	200.1	200.4	65.0	77.5	89.3	97.4	28.2	2.6	17.2	15.9
EG 711	150.2	140.6	52.4	52.4	80.2	84.4	15.6	1.5	16.1	20.7
EG 756	160.5	135.3	53.4	58.3	81.5	84.2	19.8	1.5	19.8	16.8
EG 782	170.0	140.5	58.3	68.5	95.7	96.3	17.2	1.7	17.2	15.4
EG 783	170.4	170.6	58.3	66.7	82.2	95.5	27.1	2.5	21.4	25.9
EG 786	150.2	140.3	41.0	45.7	72.2	68.1	12.8	1.3	24.0	25.1
EG 787	180.3	160.1	54.3	54.7	78.1	83.0	29.0	1.8	20.7	20.4
EG 789	185.4	130.7	61.4	67.2	85.5	90.1	21.9	0.7	20.6	16.6
EG 791	165.2	150.4	59.1	62.6	92.4	95.2	22.0	1.4	20.0	12.3
EG 794	205.1	170.0	60.1	62.1	85.3	87.4	21.3	1.5	18.3	25.4
EG 797	106.2	105.1	59.2	78.5	89.2	90.3	17.9	1.4	21.4	17.4
EG 806	170.4	165.8	51.6	51.2	87.3	89.1	23.8	2.2	22.3	37.3
EG 813	160.2	150.2	50.8	56.3	85.1	87.2	16.5	1.6	24.8	26.2
EG 815	160.1	155.1	54.3	57.3	90.4	93.0	20.1	1.7	25.1	17.6
EG 830	140.5	135.5	52.2	58.4	84.6	88.4	18.8	1.6	22.6	30.1
EG 836	170.3	150.2	60.5	64.2	90.0	95.2	19.1	1.9	23.9	17.4
EG 845	140.7	125.3	53.1	56.5	83.2	88.1	17.3	1.5	21.6	40.5
EG 849	170.8	120.3	58.1	58.7	87.3	89.3	31.9	3.1	23.3	23.2
EG 870	155.3	140.5	57.3	74.7	97.1	97.4	13.7	1.1	18.7	11.3
EG 875	145.1	120.1	69.2	70.1	100.5	102.0	10.8	1.1	13.5	10.7
EG 881	200.3	150.4	68.5	68.2	95.0	100.5	22.1	2.2	15.8	16.0
EG 883	210.5	210.6	65.7	70.6	93.2	93.2	23.6	2.9	15.7	19.3
EG 885	175.3	155.0	58.8	64.4	85.3	82.4	16.0	2.0	19.2	22.7
EG 889	200.4	195.1	59.0	59.0	87.5	88.1	15.6	1.8	11.4	15.9
EG 890	170.6	170.3	60.0	66.3	86.2	89.0	13.0	1.3	13.9	16.3
EG 893	180.9	170.5	67.3	70.3	100.6	104.3	15.1	1.5	14.2	12.3
EG 896	195.2	160.0	51.5	54.8	78.2	78.2	16.0	1.5	32.0	22.5
H/malo	115.1	110.6	65.2	68.2	80.3	82.4	18.6	2.0	12.4	16.7
EG 1157	150.5	130.2	68.4	69.3	103.4	106.3	21.0	2.2	22.5	17.9
EG 1224	160.2	125.1	60.4	63.5	99.0	102.2	24.0	2.3	36.0	33.2
EG 1256	195.4	160.7	67.1	72.0	101.4	104.6	18.9	1.7	16.6	14.3
EG 1257	140.0	110.0	66.6	67.1	96.5	98.7	16.6	1.2	21.6	17.3
EG 1261	140.	130.2	45.4	49.3	67.3	72.1	12.0	1.2	20.0	22.3
EG 2457	188.5	155.1	57.2	60.6	86.7	89.2	19.0	1.8	21.9	17.3
Mean	**168.7**	**150.3**	**57.7**	**61.9**	**86.8**	**90.2**	**20.1**	**1.7**	**20.4**	**21.0**

Where, Irr = fully irrigation and Str = stress treatment

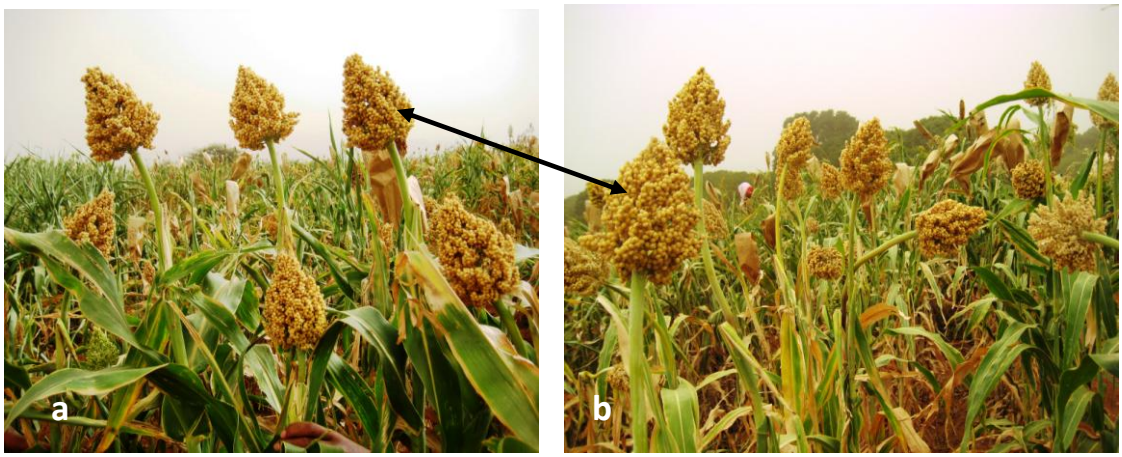


Plate 4.3 Some accessions of sorghum performes well both in drought stressed and irrigated control. Accessions **EG 849** was among the consistent and best performing cultivar under both fully irrigated (**a**) and post flowering drought stress (**b**)

4.1.2.8 Drought tolerant varieties based on selections criterion

Although most of the accessions performed better than the check varieties, only 20 accessions were earmarked as promising to proceed into second year experiment. The accessions were selected based on their response to drought parameters such as stay-green, flowering and maturity dates, leaf area, panicle sizes, grain yields and their overall agronomic performances (Table 4.5). Based on the selection criteria the accessions that showed superior performance under post flowering drought stress were: EG 469, EG 849, EG 537, Hamelmalo, EG 806, EG 782, EG 797, EG 791, EG 815, EG 836, EG 883, EG 885, EG 889, EG 1224, EG 526, EG 584, EG 783, EG 813, EG 830 and EG 481.

Table 4.5 Ranking of sorghum accessions for drought tolerance based on agronomic traits

S.No	Accessions	SG (1-5)	DFL (1-5)	PLHT (1-5)	NL (1-5)	LA (1-5)	OAS (1-5)	DM (1-5)	GW (1-5)	Sum Total	Ranking
1	**EG 469**	5	5	3	4	5	4.0	4	5	35.0	1
2	**EG 849**	5	5	3	4	4	4.0	5	5	35.0	1
3	**EG 537**	5	4	2	5	4	3.5	5	4	32.5	3
4	**Hamelmalo**	4	4	4	4	5	3.5	4	4	32.5	3
5	**EG 806**	5	5	3	3	3	4.0	5	4	32.0	5
6	**EG 782**	4	5	3	4	4	3.5	5	3	31.5	6
7	**EG 797**	5	5	4	3	4	3.5	5	2	31.5	6
8	**EG 791**	4	5	4	3	4	3.5	5	2	30.5	8
9	**EG 815**	4	5	3	3	4	3.5	5	3	30.5	8
10	**EG 836**	5	4	3	4	3	3.5	5	3	30.5	8
11	**EG 883**	5	2	2	4	4	3.5	5	5	30.5	8
12	**EG 885**	4	5	3	4	3	3.5	4	4	30.5	8
13	**EG 889**	4	5	2	4	4	3.5	5	3	30.5	8
14	**EG 1224**	4	5	3	4	4	3.5	3	4	30.5	8
15	**EG 526**	4	5	3	3	3	4.0	5	3	30.0	15
16	**EG 584**	4	4	2	4	4	3.0	5	4	30.0	15
17	**EG 783**	4	5	3	3	4	3.0	4	4	30.0	15
18	**EG 813**	5	5	3	3	3	4.0	4	3	30.0	15
19	**EG 830**	4	5	4	3	3	3.5	4	3	29.5	19
20	**EG 481**	5	3	4	3	3	3.0	4	4	29.0	20
21	**EG 497**	3	5	4	4	3	3.0	4	3	29.0	20
22	**EG 557**	4	5	4	3	3	3.0	4	3	29.0	20
23	**EG 711**	4	5	4	3	3	3.0	4	2	28.0	23
24	**EG 756**	4	5	4	3	3	3.0	4	2	28.0	23
25	**EG 546**	4	5	3	3	3	3.5	4	2	27.5	25
26	**EG 787**	4	5	3	3	3	2.5	4	3	27.5	25
27	**EG 794**	4	4	2	4	4	3.5	4	2	27.5	25
28	**EG 845**	4	5	4	2	3	3.5	4	2	27.5	25
29	**EG 881**	4	2	2	4	4	2.5	5	4	27.5	25
30	**EG 890**	4	4	3	3	4	2.5	5	2	27.5	25
31	**EG 896**	5	5	2	3	3	3.5	4	2	27.5	25
32	**EG 2457**	2	5	3	3	3	3.5	5	3	27.5	25
33	**EG 789**	3	5	4	3	3	2.0	4	1	25.0	33
34	**EG 1157**	5	2	4	4	3	2.0	1	4	25.0	33
35	**EG 1257**	3	2	4	4	4	3.0	3	2	25.0	33
36	**EG 473**	5	3	4	3	1	3.0	3	2	24.0	36
37	**EG 870**	2	5	4	3	4	1.0	3	2	24.0	36
38	**EG 786**	4	3	4	2	2	3.0	3	2	23.0	38
39	**EG 893**	4	2	3	4	4	2.5	1	2	22.5	39
40	**EG 875**	3	2	4	4	4	2.0	1	1	21.0	40
41	**EG 1261**	2	3	4	2	2	3.5	2	2	20.5	41
42	**EG 1256**	1	2	3	3	4	1.0	1	3	18.0	42

Where, selection weight is based on scale 1-5, where 5 = very good and 1= poor performance for the trait indicated; SG = stay-green, DFL = days to 5-% flowering, PLHT = plant height, NL = number of leaves, LA = leaf area, OAS = overall agronomic score, MD = Days to maturity and GW = grain weight

4.1.2.9 Simple correlation analysis among morphophysiological parameters

Correlations analysis revealed that the accessions with medium flowering produced more grain yield, higher number of leaves and higher leaf area (positive correlation) as compared to extra early and early accessions. However, early flowered accessions scored higher harvest index (negative correlations) than medium maturing. In addition the results showed that higher number of leaves and greater leaf area (positive association) produced higher grain yield. Accessions with better agronomic and stay-green (positive correlation) traits produced significant more yield as compared with those poor agronomic performance and senescence ones. Except for accession EG 849 plant height has also shown positive association with grain yield (Table 4.6).

Table 4.6. Correlation among agronomic traits recorded on sorghum accessions grown at HAC, 2013, HAC

Trait	DF	GY	HI	LA	NL	OAS	SG	PH
DF	1.00							
GY	0.390*	1.00						
HI	-0.506**	0.156	1.00					
LA	0.714**	0.389*	-0.389*	1.00				
NL	0.695**	0.524**	-0.442**	0.622**	1.00			
OAS	-0.338*	0.342*	0.459**	0.036	0.002	1.00		
SG	0.047	0.480**	0.278*	0.281*	0.192	0.604**	1.00	
PH	0.192	0.438**	-0.136	0.147	0.284	0.128	0.265	1.00

Where, DF – Days to 50% Flowering, GY - Grain Yield, HI – Harvest Index (%), LA – Leaf Area, NL - number of Leaves, AS – Overall Agronomic Score, SG – Stay-green, PH- plant height,

4.1.2.10 Panicle and seed color varaition among accessions

The accessions showed great panicle variations between and within accessions with regards to panicle orientations, seed colours and sizes (Plate 4.4). The common panicle orientations recorded on the experimental accessions were, compact erect and bent; semi loose erect and compact erects and some were droopy and very loose panicles. The existence of such panicle variability gives good opportunity for selection and improvement of this crop.

Plates 4.4 Plates (**a** to **h**) showing the variability of panicle size and orientation, seed colour and peduncle exsertions that could give good selection opportunity for sorghum breeding programmes

Conclusion

Grain sorghum is an important food crop of Eritrea especially in the regions of Gash Barka, South, Anseba and Northern Red Sea. Some abiotic stresses such as terminal drought stress reduce the yield levels achieved by the farmers. Information on the genotypic variation of traits related to drought resistance is required.

Phenotypic and physiological factors in sorghum were used to determine which cultivars are more tolerant to drought stress than others. The major criteria used for selecting the accessions that responded well to drought stress condition were based on phenotypic data such as stay-green, maturity dates, and leaf area and yield parameters. Taking into consideration of these criteria:

- Ten sorghum accessions namely EG 469, EG 849, EG 537, Hamelmalo, EG 806, EG 782, EG 797, EG 791, EG 815 and 836 were categorized as the most drought tolerant
- Ten accessions namely EG 883, EG 885, EG 889, EG 1224, EG 526, EG 584, EG 783, EG 813, EG 830 and EG 481 classified as medium drought tolerant.

- Results on fully irrigated treatment indicated that 5 accessions (EG 537, EG 584, EG 849, EG 469 and EG 1224) had superior grain yield and stay-green characters.
- The current study resulted in the selection of 20 superior sorghum accessions which were advanced into the second year experiment to be evaluated under managed drought stress condition.

4.2 Evaluation of selected sorghum landraces for tolerance to post-flowering drought stress

4.2.1 Materials and methods

4.2.1.1 Plant materials and trial site

The germplasm used in this study comprised 25 sorghum genotypes that include 21 accessions selected from 2013 rapid screening experiment, Two improved varieties (B-35 and Hamelmalo) from ICRISAT and National programme respectively, and 2 susceptible sorghum germplasm accessions from the Eritrean sorghum improvement programme (Table 4.7).

Table 4.7 Sorghum accessions used in the study with their sources and local names

S.No.	Germplasm identifier	Area of collection (administration region)	Local Name	Status
1	EG 469	Gash Barka	Tseda Bazenay	Landrace
2	EG 849	Gash Barka	Hugurtay	Landrace
3	EG 537	South	Anseba	Landrace
4	Hamelmalo	Anseba/Gash Barka	Hamelmalo	Released cultivar
5	EG 806	Gash Barka	Hiriray	Landrace
6	EG 782	South	Tseda Hele	Landrace
7	EG 797	Gash Barka	Wedi-Aker	Landrace
8	EG 791	Gash Barka	Korekora	Landrace
9	EG 815	Gash Barka	Estif	Landrace
10	EG 836	Anseba	Hugurtay	Landrace
11	EG 883	Gash Barka	Kinabiba	Landrace
12	EG 885	Gash Barka	Duruta	Landrace
13	EG 889	Gash Barka	Kileaentu	Landrace
14	EG 1224	Gash Barka	Mahagen	Landrace
15	EG 526	Anseba	Wedi-Aker	Landrace
16	EG 711	Anseba	Embulbul	Landrace
17	EG 783	Gash Barka	Aklamoy	Landrace
18	EG 813	Anseba	Wedi-Ferej	Landrace
19	EG 830	Gash Barka	Wedi-Arba	Landrace
20	EG 481	Anseba	Wedi-Susa	Landrace
21	H-35-1	South	Tseda mashela	Landrace
22	B-35 (DT)	ICRISAT	B-35	Released cultivar
23	EG 870	Gash Barka	Ajebsidu	Landrace
24	EG 473 (S)	South	Keih Hele	Landrace
25	EG 843 (S)	South	Koden	Landrace

The experiment was conducted under managed drought stress condition at Hamelmalo Agricultural College (HAC) farm from March-June, 2014 dry season period. Geographically the trial site is located at 15^0 52'15"N latitude and $38^0$27' 55" E longitudes with an altitude of 1,274 meters above sea level in a semi-arid agro-ecological zone of Eritrea. The research site is located 12 km the north of Keren city on the way Keren-Nakfa road along Anseba River in Anseba region. The soil type of the experimental site was sandy clay loam with an average maximum and minimum air temperatures during the experimental period reached 38 ^{0}C and 20 ^{0}C respectively. Soil moisture content before sowing and after imposing drought stress were taken.

4.2.1.2 Experimental design and treatments

Split plot design was used by setting two main plots, fully irrigated and stress plots with three replications. The spacing between the irrigated and stressed replications was three meters. The sub plots were the 25 genotypes that were planted in plots of four rows with a spacing of 75 cm x 20 cm between and within rows respectively and three meter row length.

In order to impose drought stress, the accessions were subjected to two conditions: non-stressed (with normal irrigation) and drought stressed (irrigation withheld) at reproductive phase. All the accessions in both irrigated and drought stress treatments were fully irrigated until booting to early flowering stage. At flowering stage water was withheld for 14 days for the drought stress treatment, while the control treatment received regular irrigation throughout the experiment. Normal watering was resumed when the flowered plants showed visual signs of wilting. Soil moisture was measured twice in both the stress and control treatments; at the time water was withheld and before the water stress was relieved.

4.2.1.3 Phenotypic data records

Phenotypic data (Table 4.8) were recorded for the assessment of the sorghum drought tolerance at post flowering.

Table 4.8 Full names, Abbreviations and descriptions of the traits investigated in the study:

S. No	Traits name	Abbreviations	Description
1	Seedling Vigour	SV	Visual observation of the seedling in 1-5 scale where 1 poor and 5 highly vigour
2	Days to 50% flowering	DFL	The date when 50 percent of the plants produced flowers was recorded and converted in number of days from date of planting up to date of heading
3	Plant height	PLHT	Height of the plant from the base of the plant to the tip of the panicle in cm at maturity
4	Total number of leaves	TNOL	Number of leaves on the main plant stem
5	Leaf Area	LA	Measurement of leaves (cm^2) using LI 3000 C Portable area meter from 5 randomly selected plants in each replication during early morning hours when leaves were fully turgid.
6	Stay-green	StG	Stay-green scores at maturity based on visual ratings (Wanous, Miller, & Rosenow, 1991) using 1 to 5 scale (1 = < 10% leaves stay-green and 5 = >75% leaves stay-green and most desirable)
7	Total number of tillers	TNOT	Counts of the total number of productive tillers
8	Peduncle exsertion	PEX	The average length of the nod between the flag leaf and the base of the panicle measured in cm from 5 randomly selected plants at maturity
9	Panicle length	PL	Panicle length measurement (cm) from the base of the panicle to the tip from five randomly selected plants per plot at maturity

Table 4.8 Continued

10	Panicle width	PW	Panicle width measurement in the widest diameter of the panicle on five randomly selected plants per plot at maturity
11	Panicle orientation	PO	Visual observation of the inflorescence compactness and shape at maturity
12	Days to maturity	DM	The date when 90 percent of the plants are physiologically mature counting in days taken from planting up to physiological maturity
13	Plant agronomic score	OAS	Over all plant agronomic scores (5 = most desirable and 1= least desirable)
14	Grain weight	GW	Total grain weight per plot (kg) after threshing then converted into tons per hectare
15	Biomass	BM	The total weight of the plants in the two middle rows (Kg), 15 plants/ row
16	Harvest index	HI	The ratio of grain weight to the total biomass (%) computed from the two middle rows
17	Seed colour	SC	Description of the seed colour after threshing

4.2.1.4 Data Analysis

4.2.1.4.1 Analysis of Variance (ANOVA)

The data on yield and yield components and morphological characteristics related to drought and phenotypic correlation between drought incidences were calculated by the analysis of variance and least significant difference of the mean using the Genstat 14 Statistical software (Payne *et al.,* 2011). For the analysis of variance the following statistical model was fitted:

$$\mathbf{Y_{ijkl} = \mu + E_i + Y_j + EY_{ij} + R_{k(ij)} + G_l + GE_{il} + GY_{jl} + GEY_{ijl} + \epsilon_{ijkl}}$$

Where:

Y_{ijkl} = observed landrace response; μ = overall population mean; E_i = Effect of the i^{th} environment; G_l = Effect of the l^{th} genotype, $Y_{j=}$ Effect of the j^{th} year, EY_{ij} = Interaction effect of i^{th} environment j^{th} year, $R_{k(ij)}$ = Effect of the k^{th} replication in the i^{th} environment; GE_{il} = interaction effect of l^{th} genotype and i^{th} environment; GY_{jl} = interaction effect of the l^{th} genotype and j^{th} year; GEY_{ijl} = interaction effect of the l^{th} genotype, i^{th} environment and j^{th} year and ϵ_{ijkl} = Experimental error.

G was considered as fixed and E, Y, GE and GEY were considered as random effect.

In addition principal component analyses (PCA) were calculated after standardization to mean of zero and variance of one using the Genstat statistical software. Cluster analysis was also done using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis (Sokal and Michener, 1958) and dendrograms were constructed using the SAHN programme.

4.2.1.4.2 Analysis of genetic variability and estimation of coefficients of variations

Genetic parameters were estimated to identify genetic variability among the lines and determine genetic and environmental effects on various characters. These genetic parameters were estimated with the methods illustrated by (Assefa et al., 1999). Accordingly, to identify the major traits contributing to the overall phenotypic variation among the germplasm accessions and to estimate the broad sense heritability, cluster and principal component analysis for the various morpho-physiological traits in sorghum under drought stress and control condition were analysed using the following formulae:

i. Genotypic variance, GV= (MSg –MSe)/r, where MSg = mean square of genotypes, MSe = mean square of error, and r = number of replications
ii. Phenotypic variance, PV= GV + MSe, where GV = genotypic variance and MSe = mean square of error

iii. Phenotypic coefficient of variation, PCV = $\sqrt{(PV)/\bar{x}}$ x 100, where PV = phenotypic variance and $\bar{x}$ = mean of the character

iv. Genotypic coefficient of variation, GCV = $\sqrt{(GV)/\bar{x}}$ x 100, where GV = genotypic variance and $\bar{x}$ = mean of the character

v. Heritability (Broad sense heritability), H = GV/PV, where GV and PV are genotypic phenotypic variances respectively

4.2.1.4.3 Susceptibility and tolerance indices for the sorghum accessions

Stress tolerance index was calculated to identify germplasm accessions with high stress tolerance and overall good agronomic performances. The drought stress indices were calculated with method as described by Agili et al 2012.

i. Stress Susceptible Index (SSI) = [1-(Ys/Yi)]/SI, Where SI = 1- ($\overline{Y}$s/$\overline{Y}$i)

ii. Mean Productivity (MP) = (Yi+Ys)/2

iii. Tolerance (TOL) = Yi-Ys

iv. Stress tolerance index (STI) = Yi x Ys/$\overline{Y}$i ^{2}

v. Geometric Mean Productivity (GMP) = $\sqrt{Yi \times Ys}$

vi. Yield Index (YI) = Ys/$\overline{Y}$s

vii. Yield Stability Index (YSI) = Ys / Yi

Where:

- Yi = Yield of accessions in normal irrigation condition
- Ys = Yield of accessions in water stress condition
- $\overline{Y}$i = Mean yield in normal irrigation condition
- $\overline{Y}$s = Mean yield in water stressed condition

4.2.2 Results

4.2.2.1 Assessment of seedling vigour and leaf related traits

Analysis of variance showed statistical differences among the local landraces studied for various seedling and leaf related traits. High genotypic variations recorded for seedling vigour, number of leaves, leaf area and stay-green traits. The genotypes also exhibited difference under irrigation and drought stressed conditions. Mean scores for seedling vigour under drought stress condition were 1.2 – 4.0. EG 883, EG 836, E G711, EG 783 and EG 791 were among vigous genotypes at seedling in stress treatment. The genotypes also differed in relation to the leaf area. The accessions under stress treatment showed significant reduction in their leaf area compared to the fully irrigated ones (Table 4.9). The range of leaf area measurement under stress conditions was 114.7 – 323.7 cm^2 while in the fully irrigated leaf area was 127.7 to 433.3 cm^2. Under stress treatment, EG 473 scored lowest while EG 883 scored highest in terms of leaf areas. Hamelmalo cultivar scored 433.3 cm^2, highest in the irrigated treatment. The genotypes tested also showed significant difference among each other. Overall performance of the sorghum genotypes indicated that stay-green score ranged from 2.3 to 4.7 (mean 3.3; SE+0.50). Among the controls, B-35 showed an average score of 3.3 whereas EG 836, EG 883 and EG 885 scored the highest stay-green value with 4.7, 4.3 and 4.3 respectively. Among the genotypes evaluated, 16 recorded stay-green scores more than or equal to the mean and 9 of them had score less than the mean. Five promising genotypes EG 469, EG 489, Hamelmalo, EG 836 and EG 711 were selected on the basis of their stay-green score that are associated with higher yield attribute medium flowering dates and overall agronomic desirability in this trial.

Table 4.9 Mean genotype values for seedling vigour, and leaf related traits under drought stress and control conditions, Hamelmalo Agricultural College 2014

Genotypes	SV		NoL		LA		StG
	Stress	Control	Stress	Control	Stress	Control	Stress
EG 469	1.7	1.2	10.3	11.3	285.0	361.7	4.0
EG 849	2.0	1.3	11.0	10.3	269.3	289.0	4.3
EG 537	2.0	1.7	12.3	12.3	253.0	301.7	3.0
Hamelmalo	1.7	1.3	12.0	12.0	285.7	433.3	4.0
EG 806	2.3	1.3	9.7	10.0	191.0	205.0	3.0
EG 782	2.2	1.5	10.3	11.3	191.3	206.0	3.3
EG 797	2.8	1.7	10.0	10.0	267.7	295.7	2.7
EG 791	1.5	1.5	10.3	10.3	266.7	293.3	3.7
EG 815	4.0	3.3	9.7	10.0	262.3	279.7	2.0
EG 836	1.3	1.2	10.3	11.3	243.3	270.0	4.7
EG 883	1.2	1.3	11.7	11.3	323.7	358.0	4.3
EG 885	2.0	1.5	10.7	11.0	216.0	233.7	4.3
EG 889	1.8	1.2	10.0	11.3	214.7	224.7	3.0
EG 1224	2.3	2.3	10.0	9.3	205.3	218.7	2.7
EG 526	2.2	2.0	9.0	10.0	196.0	199.7	3.7
EG 711	1.5	1.3	10.0	9.7	176.7	197.0	4.0
EG 783	1.5	1.3	11.0	12.3	236.3	303.7	3.3
EG 813	2.7	1.3	10.0	10.0	180.0	198.3	2.7
EG 830	1.7	1.5	9.7	9.3	194.3	206.3	4.0
EG 481	2.3	1.7	9.7	9.3	195.3	205.0	4.0
B35-1	1.8	1.0	11.3	11.0	174.0	196.3	3.3
B-35	1.7	1.5	13.0	13.0	174.3	182.3	4.0
EG 870	2.5	1.8	11.3	10.7	237.0	244.7	2.7
EG 473	2.0	1.5	8.3	8.3	114.7	127.7	3.3
EG 843	1.7	1.2	11.0	12.7	199.3	206.0	2.3
Mean	**2.0**	**1.5**	**10.5**	**10.7**	**222.1**	**249.5**	**3.3**
LSD $_{0.05}$	**1.2**	**0.7**	**1.2**	**1.0**	**57.2**	**41.6**	**1.4**
CV%	**19**	**17**	**6.2**	**4.5**	**13.4**	**8.8**	**17.5**
Fprob	******	*******	*******	*******	*******	*******	*****

Where, SV = Seedling vigour, NoL = Number of leaves, LA = Leaf area (cm^2) and StG = Stay-green score, LSD = Least significant differences, CV (%) = Coefficient of variance and Fprob = F probability differences at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

4.2.2.2 Assessment of plant height and panicle related traits

A wide range of variation was recorded in plant height, which ranged from 92 to 227.7 cm under drought stress, while under the control conditions it ranged from 97.3 to 266.7 cm. The overall mean plant height of the accessions was lower under drought stress (175 cm) than under the control treatment (191.8 cm).

The mean values for the panicle related traits such as peduncle exsertion, (PEX), panicle length (PL) and panicle width (PW) showed significant reduction under the drought stress conditions. With regard to PEX, the mean values ranged from 3.3 to 12.3 cm scored by Hamelmalo and EG 711 accessions under drought stress conditions respectively (Table 4.10).

In the fully irrigated condition, the range was 4.3 to 18.3 which recorded by Hamelmalo and EG 526 respectively. There were no significant differences recorded among the accessions for PW under the fully irrigated conditions, while there were highly significant differences under the drought stress conditions. Under drought stress conditions the values for PW ranged from 5 to 9.3 cm in EG 889 and EG 469 respectively.

Panicle length and productive tillers slightly fluctuated between the stressed and irrigated accessions because some accessions perform better under drought stress condition than under fully irrigated condition. Genotype EG 469 scored the highest PL (28 cm) and lowest by EG 836 (9 cm) under drought stress condition (Table 4.10). With regard to productive tillers EG 526, EG783 and EG 870 accessions scored highest tillering capacity under stress condition and lowest by EG 469, EG 849 and EG 481.

4.2.2.3 Assessments of yield related characters, days to flowering and maturity

Significant effects of genotypes were recorded for days to 50% flowering (DFL), days to maturity (DM), overall agronomic score (OAS), grain yield (GY), total biomass (BM) and harvest index (HI) under both treatments.

Table 4.10 Mean genotype values for Panicle related traits and plant height under drought stress and control conditions, Hamelmalo Agricultural College 2014

Genotype	PEX		PaW		PaL		PTil		PLHT	
	Str	Con	Str	Con	Str	Con	Str	Con	Str	Con
EG 469	6.3	9.7	9.3	13.3	28.0	29.3	1.0	1.0	202.3	223.7
EG 849	7.3	9.7	8.7	11.3	12.7	14.0	1.0	1.0	169.7	184.7
EG 537	7.7	14.7	8.3	9.3	24.7	28.3	1.3	2.0	227.7	266.7
Hamelmalo	3.3	4.3	6.0	7.7	19.7	20.7	1.3	1.3	129.7	133.7
EG 806	9.7	12.0	7.3	11.0	11.7	14.0	2.0	2.0	176.7	198.3
EG 782	9.3	12.3	8.3	14.0	14.0	15.3	2.0	1.7	175.0	189.7
EG 797	5.7	13.3	5.0	8.3	17.3	19.3	1.3	2.0	124.7	135.7
EG 791	9.0	13.7	6.7	9.0	21.7	21.3	1.7	2.0	177.7	192.7
EG 815	4.7	10.7	6.0	10.0	22.3	22.3	2.0	3.0	161.3	187.7
EG 836	7.0	12.0	6.3	9.7	9.0	10.7	2.0	1.0	202.7	210.0
EG 883	9.7	13.0	8.0	9.3	19.3	17.0	1.3	2.0	218.3	236.7
EG 885	7.3	11.0	6.3	9.3	10.7	11.0	2.0	2.0	181.7	202.3
EG 889	8.3	15.7	5.0	6.7	29.7	24.0	1.0	1.0	220.3	244.0
EG 1224	6.7	11.3	5.7	10.0	17.3	24.7	2.0	1.7	126.0	171.0
EG 526	10.7	18.3	5.7	10.7	20.0	23.0	2.3	2.3	170.3	193.3
EG 711	12.3	15.7	7.0	8.0	13.3	9.7	2.0	1.7	188.3	189.3
EG 783	6.3	10.0	6.0	11.0	10.0	9.7	2.3	2.0	187.0	197.3
EG 813	9.3	12.7	7.7	10.3	13.7	14.3	1.7	2.3	178.0	197.7
EG 830	10.0	14.7	8.0	12.7	12.0	13.7	2.0	2.0	177.3	189.3
EG 481	11.0	14.0	6.3	8.7	9.7	9.7	1.0	1.0	156.3	159.3
B35-1	11.7	15.0	7.3	14.0	25.0	30.3	1.3	1.7	188.7	203.3
B-35	5.7	11.3	6.3	13.3	22.7	25.7	1.0	1.0	92.0	97.3
EG 870	5.3	6.3	7.0	10.0	22.3	22.7	2.3	2.0	183.3	198.0
EG 473	12.0	15.7	6.7	9.3	11.0	10.3	1.3	2.0	153.7	159.7
EG 843	5.7	11.3	6.7	10.7	30.0	27.0	1.3	2.0	212.7	234.3
Mean	**8.1**	**12.3**	**6.8**	**10.3**	**17.9**	**18.7**	**1.6**	**1.7**	**175.3**	**191.8**
LSD $_{0.05}$	**4.9**	**5.1**	**2.5**	**4.7**	**3.5**	**3.8**	**0.6**	**0.5**	**20.9**	**19.3**
CV%	**15.8**	**15.0**	**19.1**	**24.8**	**9.1**	**8.8**	**11.3**	**11.1**	**2.6**	**4.2**
Fprob	*	***	*	NS	***	***	***	***	***	***

Where, PEX = Peduncle exsertion (cm), PaW = Panicle width (cm), PaL, Panicle length (cm), PTil = Number of Productive tillers, PLHT = Plant height (cm), LSD = Least significant diffences, CV (%) = Coefficient of variance and Fprob = F probability differences at * $P \leq 0.05$, *** $P \leq 0.001$, NS = not significant

Days to 50% flowering under drought stress ranged from 54 to 80 days after sowing, whereas the range under control conditions was 50 to 74 days after sowing. Under stress condition, EG 473, EG 711, EG 481, EG 830 and EG 813 were among the early flowering genotypes that took 54, 57, 58 and 59 days to flower respectively while EG 843 (80) was the latest genotype to flower. Days to maturity under stress ranged from 95 to 115 days after sowing, where as the range under the control conditions was 93 to 107 (Table 4.11). Under the drought stress conditions, delays in flowering and maturity were also observed in most of the accessions when compared with the fully irrigated; values ranged from 3 to 9 days (DFL) and 1 to 12 days (DM). The grain yield of accessions varied significantly under drought stress, ranging from 0.8 to 2.9 t ha^{-1} with an average of 2.1, similarly under the control condition the yield level of the genotypes varied from 1.4 to 3.3 t ha^{-1} (Table 4.11). Among the highest yielding genotypes under drought stress and control condition includes EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711.

Table 4.11 Mean genotype values for plant phenology and yield related component traits under drought stress (Str) and control (Con) conditions, Hamelmalo Agricultural College

Genot.	DFL		DM		OAS		GY		BM		HI	
	Con	Str	Con	Str	Con	Str	Con	Str	Con	Str	Con	Str
EG 469	62.7	70.7	94.7	100.3	3.5	3.5	2.9	2.7	19.9	16.7	15.2	7.5
EG 849	59.3	62.3	94.7	97.0	3.3	3.5	3.3	2.4	14.8	11.0	25.2	5.0
EG 537	66.0	71.0	102.3	106.7	1.7	1.0	2.2	2.2	25.4	17.8	8.6	8.0
H/malo	63.3	68.3	97.0	100.7	4.0	3.5	2.5	2.6	22.4	18.1	11.2	8.2
EG 806	54.7	61.3	94.7	99.7	3.8	3.5	2.4	2.3	14.2	7.9	19.0	3.5
EG 782	60.3	68.7	95.7	104.0	3.8	3.5	2.1	1.8	16.4	15.6	12.4	7.0
EG 797	61.3	67.0	96.0	104.7	3.5	3.5	2.2	1.6	11.1	10.4	19.2	4.7
EG 791	62.3	71.3	96.3	104.7	3.7	4.0	2.1	2.3	13.5	12.5	16.7	19.3
EG 815	62.0	70.3	103.0	110.7	1.2	1.5	1.9	0.8	18.1	8.9	13.1	9.2
EG 836	61.3	67.0	95.3	99.0	3.2	3.7	3.2	2.7	21.1	17.3	15.9	16.2
EG 883	64.7	68.3	96.3	99.7	3.3	3.0	3.1	2.6	20.6	17.0	15.5	15.5
EG 885	63.0	66.7	95.3	107.7	3.3	3.0	3.0	2.9	17.0	14.4	18.1	23.0
EG 889	62.0	68.0	103.0	111.3	2.8	1.8	1.9	1.5	20.4	12.6	9.7	12.3
EG 1224	63.3	68.0	102.3	110.3	2.3	1.3	1.5	1.0	14.8	9.6	9.5	11.2
EG 526	61.0	67.7	101.0	108.0	2.3	2.5	2.3	2.2	19.4	13.7	11.7	16.3
EG 711	52.0	57.0	93.3	96.3	3.5	3.7	2.9	2.6	11.6	8.0	26.6	37.3
EG 783	60.7	65.3	95.7	100.0	2.8	3.7	3.0	2.6	19.9	14.7	15.5	17.9

Table 4.11 Cont.,

EG 813	53.7	58.7	93.3	94.7	4.0	3.3	2.6	2.0	10.4	6.4	27.7	31.4
EG 830	52.0	59.3	94.7	99.7	3.0	3.5	2.4	2.2	9.9	7.0	26.8	32.6
EG 481	50.3	58.0	94.7	97.3	3.8	3.0	3.1	2.7	12.2	8.7	29.3	33.6
B35-1	69.0	71.0	104.0	113.7	4.0	3.0	2.5	2.1	24.6	20.0	10.1	10.3
B-35	69.7	75.3	106.7	114.3	3.7	3.2	1.4	1.6	17.8	14.1	8.3	10.9
EG 870	61.7	69.7	101.0	107.3	2.0	2.3	1.9	1.6	17.9	10.4	11.2	15.7
EG 473	50.0	53.7	94.7	96.0	3.8	3.0	2.3	2.0	7.1	5.3	35.5	38.8
EG 843	74.0	79.7	103.0	114.7	1.3	1.5	1.4	1.8	15.0	13.3	10.3	13.4
Mean	**60.8**	**67.0**	**97.9**	**104**	**3.1**	**2.9**	**2.4**	**2.1**	**16.6**	**12.5**	**16.8**	**19.5**
LSD $_{0.05}$	**4.4**	**4.3**	**3.1**	**6.9**	**0.6**	**0.8**	**0.8**	**0.9**	**5.1**	**5.7**	**10.1**	**10.3**
CV (%)	**3.1**	**3.6**	**1.7**	**3.4**	**8.1**	**15.8**	**18.0**	**21.4**	**2.0**	**24.7**	**12.4**	**25.2**
Fprob	***	***	***	***	***	***	**	***	***	***	***	***

Where, DFL = Days to 50% flowering, DM = Days to physiological maturity, OAS, Over all agronomic score, GY = Grain yield (t ha^{-1}) BM = Total biomass (tha^{-1}), HI (%) = Harvest index, LSD = Least significant differences, CV (%) = Coefficient of variance and Fprob. = F probability differences at ** P $\leq$ 0.01, *** P $\leq$ 0.001

The genotypes varied on the overall agronomic scores in both stress and control conditions. The value under drought stress ranged from 1.3 to 4.0, whereas under control conditions the value varied from 1.2 to 4.0. No significant difference were recorded on combined analysis among the drought stress and control conditions with respect to the fresh total biomass (data not shown) while individually the stress and control the genotypes varied greatly. The values for the fresh biomass ranged from 5.3 to 20 t ha^{-1} under drought stress and 7.1 to 25.4 t ha^{-1} in control conditions.

Table 4.12 Mean genotype values for selected traits under stress (Str) and control (Con) conditions, Hamelmalo Agricultural College, 2014

Genotype	StG (1-5)		LA (cm^2)		GY (t ha^{-1})		OAS (1-5)	
	Con	Str	Con	Str	Con	Str	Con	Str
EG 469	-	4.0	285.0	361.7	2.9	2.7	3.5	3.5
EG 849	-	4.3	269.3	289.0	3.3	2.4	3.3	3.5
EG 537	-	3.0	253.0	301.7	2.2	2.2	1.7	1.0
Hamelmalo	-	4.0	285.7	433.3	2.5	2.6	4.0	3.5
EG 806	-	3.0	191.0	205.0	2.4	2.3	3.8	3.5
EG 782	-	3.3	191.3	206.0	2.1	1.8	3.8	3.5
EG 797	-	2.7	267.7	295.7	2.2	1.6	3.5	3.5
EG 791	-	3.7	266.7	293.3	2.1	2.3	3.7	4.0
EG 815	-	2.0	262.3	279.7	1.9	0.8	1.2	1.5
EG 836	-	4.7	243.3	270.0	3.2	2.7	3.2	3.7
EG 883	-	4.3	323.7	358.0	3.1	2.6	3.3	3.0
EG 885	-	4.3	216.0	233.7	3.0	2.9	3.3	3.0
EG 889	-	3.0	214.7	224.7	1.9	1.5	2.8	1.8
EG 1224	-	2.7	205.3	218.7	1.5	1.0	2.3	1.3
EG 526	-	3.7	196.0	199.7	2.3	2.2	2.3	2.5
EG 711	-	4.0	176.7	197.0	2.9	2.6	3.5	3.7
EG 783	-	3.3	236.3	303.7	3.0	2.6	2.8	3.7
EG 813	-	2.7	180.0	198.3	2.6	2.0	4.0	3.3
EG 830	-	4.0	194.3	206.3	2.4	2.2	3.0	3.5
EG 481	-	4.0	195.3	205.0	3.1	2.7	3.8	3.0
B35-1	-	3.3	174.0	196.3	2.5	2.1	4.0	3.0
B-35	-	2.3	174.3	182.3	1.4	1.6	2.7	2.2
EG 870	-	4.0	237.0	244.7	1.9	1.6	2.0	2.3
EG 473	-	3.3	114.7	127.7	2.3	2.0	3.8	3.0
EG 843	-	2.7	199.3	206.0	1.4	1.8	1.3	1.5
Mean	-	**3.5**	**222.1**	**249.5**	**2.4**	**2.1**	**3.1**	**2.9**
LSD $_{0.05}$	-	**1.4**	**57.2**	**41.6**	**0.8**	**0.9**	**0.6**	**0.8**
CV (%)	-	**17.5**	**13.4**	**8.8**	**18.0**	**21.4**	**8.1**	**15.8**
Fprob		*	***	***	**	***	***	***

Where, StG = Stay-green score, LA = Leaf area (cm^2), GY = Grain yield (t ha^{-1}), OAS = Over all agronomic, LSD = Least significant differences, CV (%) = Coefficient of variance and Fprob. = F probability differences at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Four selected traits were taken to categorized and identify genotypes with best performance. Table 4.12 showed the genotypes performance under stress and control condition with stay-green, leaf area, grain yield and overall agronomic score. In the stress treatments among the top six accessions EG 885, EG 469, EG 836 and EG 481 were superior in yield which showed 51.7-72.3 % increase over the susceptible accession (Table 4.13). Similarly stay-green in the top six ranged from 4.0 to 4.7 while the six bottom susceptible ranged between 1.2 to 2.7 of stay-green scores.

Table 4.13 Mean values for top six and bottom six genotypes based on stay-green, grain yield, leaf area and overall agronomic score under stress and control condition

	Stressed condition				Non stressed (control) condition			
Genotype	**StG**	**GY**	**LA**	**OAS**	**StG**	**GY**	**LA**	**OAS**
Top six genotypes					**Top six genotypes**			
EG 885	4.3	2.9	216.0	3.4	-	3.0	233.7	3.5
EG 469	4.0	2.7	285.0	3.5	-	2.9	361.7	3.5
EG 849	4.3	2.4	269.3	3.5	-	3.3	289.0	3.3
Hamelmalo	4.0	2.6	285.7	3.5	-	2.5	433.3	4.0
EG 836	4.7	2.7	243.3	3.7	-	3.2	358.0	3.2
EG 481	4.0	2.7	195.3	3.2	-	3.1	205.0	3.8
Bottom six genotypes					**Bottom six genotypes**			
EG 815	2.0	0.8	262.3	1.5	-	1.9	279.7	1.2
EG 889	3.0	1.5	214.7	1.8	-	1.9	224.7	2.8
EG 1224	2.7	1.0	205.3	1.3	-	1.5	218.7	2.3
EG 870	2.7	1.6	237.0	2.3	-	1.9	244.7	2.0
EG 843	2.3	1.4	199.3	1.5	-	1.8	206.7	1.3
B-35	2.3	1.6	174.3	2.2	-	1.4	182.3	2.7

Where, StG = Stay-green score in 1-5, GY = Grain yield (t ha^{-1}), LA = leaf area (cm^{2}) and OAS, Over all agronomic in 1-5 scores

4.2.2.4 Correlation analysis among morphophysiological parameters under drought stress

Overall agronomic score was positively associated with stay-green, grain yield and harvest index and negatively associated with days to 50% flowering and physiological maturity and panicle length. Grain yield was strongly correlated with stay-green and overall agronomic scores but negatively associated with days to maturity and panicle length. Correlations analysis revealed that the genotypes with early flowering produced more grain yield and score higher harvest index (significantly negative correlation). The total biomass showed positive association with days to flowering, number of leaves and leaf area but negatively associated with harvest index (Table 4.14).

Table 4.14 Correlation analysis among morphophysiological parameters under post flowering drought stress

	BM	DFL	DM	GY	HI	LA	NoL	OAS	PEX	PLH	PaL	PaW	StG
BM	1.00												
DFL	0.65***	1.00											
DM	0.36	0.81***	1.00										
GY	0.31	-0.30*	-0.58***	1.00									
HI	-0.54**	-0.66***	-0.47*	0.28	1.00								
LA	0.46*	0.37	-0.04	0.17	-0.52***	1.00							
NoL	0.56**	0.53**	0.33	0.11	-0.41*	0.41*	1.00						
OAS	-0.01	-0.40**	-0.61**	0.60**	0.21	0.03	-0.09	1.00					
PEX	-0.28	-0.58**	-0.34	0.28	0.58**	-0.55**	-0.48*	0.23	1.00				
PLH	0.29	0.40*	-0.07	0.33	0.04	0.21	-0.04	-0.19	0.24	1.00			
PaL	0.40*	0.75***	0.69**	-0.40*	-0.46*	0.25	0.30	-0.53*	-0.32	0.23	1.00		
PaW	0.07	-0.09	-0.33	0.30	-0.01	0.08	0.09	0.17	0.28	0.43*	-0.01	1.00	
StG	0.24	-0.28	-0.51**	0.78***	0.26	0.29	-0.02	0.48*	0.22	0.32	-0.37	0.27	1.00

Where, *, ** and *** significant at the 5%, 1% and 0.1% level of probability respectively

4.2.2.5 Assessment of heritability, phenotypic and genotypic coefficients of variations

The data were subjected to the analysis of variance components to estimates the extent or magnitude of genetic variations among the traits. The results revealed that all the traits had considerable values of phenotypic and genotypic coefficients of variation (PCV and GCV respectively) among the accessions, but in general the PCV was slightly higher than the GCV. High PCV and GCV values were obtained for majority of the characters studied except for days to flowering, days to maturity and number of leaves. However, PLHT gave rise to the highest coefficients of variation (both PCV and GCV) followed by HI, PaL and SV. On the other hand parameters such as GY, BM, PaW, PEXS, OAS and StG showed moderate phenotypic and genotypic coefficient of variation (Table 4.15).

Table 4.15 Estimates of means, % of reduction, genotypic and phenotypic variation, genotypic and phenotypic coefficients of variation and heritability for yield and yield components under stress and control conditions

Traits	Mean		% of reduction	δ^2p	δ^2g	PCV (%)	GCV (%)	h^2_{BS} (%)
	Control	Stress						
Seedling vigour	1.5	2.0	-33.3	0.7	0.4	46.5	35.1	57.1
Days to flowering	60.8	67.0	-10.2	74.8	67.7	13.5	12.9	90.6
Days to naturity	97.9	104.0	-6.2	57.2	46.5	7.5	6.8	81.3
Grain yield	2.4	2.1	12.5	77.7	46.2	39.0	30.1	59.5
Biomass	16.6	12.5	24.7	8.9	6.7	45.8	39.7	75.2
Harvest index	16.8	19.5	-16.1	157.8	119.0	66.8	58.0	75.4
Plant height	191.8	175.3	8.6	65409.0	51061.6	139.3	123.1	78.1
No. of leaves	10.7	10.5	1.9	2.6	2.1	15.2	13.6	80.7
Panicle length	18.7	17.9	4.3	89.5	84.4	50.6	49.1	94.3
Panicle width	10.3	6.8	34.0	7.5	2.2	31.8	17.1	29.0
Peduncle exsertion	12.0	8.0	33.3	19.6	10.3	43.4	31.5	52.6
Agronomic score	3.1	2.9	6.5	1.4	1.2	39.9	37.0	86.0
Productive tiller	1.7	1.6	5.9	0.4	0.3	39.3	34.6	77.6
Stay-green	-	3.5	-	1.1	0.3	30.4	15.2	25.0

Where, δ^2p = phenotypic variation, δ^2g = genotypic variation, GCV (%) = Genotypic coefficient variance, PCV (%) = Phenotypic coefficient variance and h^2_{BS} (%) = Heritability in broad-sense

Broad sense heritability estimates were medium to high for most of the morp-physiological traits among the local landrace accessions. The highest heritability was recorded for days to flowering, days to maturity, number of leaves, panicle length and overall agronomic score traits while it was moderate for seedling vigour, grain yield, biomass, harvest index, plant height, peduncle exsertions and productive tillers. The lowest heritability was scored for panicle width and stay-green score (Table 4.16).

Table 4.16: Category and estimates of broad sense heritability of the sorghum Accessions

Characters	Broad sense heritability (h_{BS}%)	Class interval	Category
Panicle length	94.3	>85	Very high
Days to 50% flowering	90.6		
Overall agronomic score	86.0		
Days to maturity	81.3	80-85	High
Number of leaves	80.7		
Plant height	78.1		
Productive tillers	77.6		
Harvest index	75.4	50-79	Medium
Biomass t	75.2		
Grain yield	59.5		
Seedling vigour	57.1		
Productive tillers	52.6		
Panicle width	29.0	<50	Low
Stay-green	25.0		

4.2.2.6 Principal component analysis of various morpho-physiological traits in sorghum

Principal component (PC) analysis showed that the first 4, out of the 7 PCs explained majority of the total variation. These four PCs with Eigen value >1 contributed 74.6% of the total variability amongst the sorghum genotypes assessed for various morpho-physiological traits (Table 4.17). The remaining 3 components contributed only 15.4% towards the total morphphysiological diversity for this set of sorghum genotypes. The PC I contributed maximum towards the variability (32.8%) followed by PC II (22.8%), PC III (10.98%) and PC IV (8.0%). The most important characters in PC I was due to variations among the accessions mainly for days to 50% flowering, days to maturity, harvest index, peduncle exsertion and panicle length. Besides, days to flowering, days to maturity and panicle length had considerable positive factor loadings on PC I. Similarily the PC II was related to diversity among sorghum genotypes due to specific biomass, grain yield, seedling vigour, stay-green and Leaf area. The PC III was

explained mainly by variation among genotypes resulting from plant height, peduncle exsertion, overall agronomic score and panicle length. In this principal component plant height, peduncle exsertion and panicle length have positive factor while overall agronomic scores contributed negatively. The fourth (PC IV) was explained negatively by the variations resulting from leaf area, plant height, number of productive tillers, panicle width and seedling vigour (Table 4.17).

Table 4.17 Principle component analysis of various morpho-physiological traits in sorghum under water stress at post-flowering stage

	PC I	PC II	PC III	PC IV	PC V	PC VI	PCVII
Eigen value	4.9	3.2	1.6	1.2	0.9	0.8	0.6
% total variance	32.8	22.9	10.9	8.0	6.5	5.0	3.9
Cumulative variance	32.8	55.7	66.6	74.6	81.1	86.1	90
Factor loading by various traits							
Biomass	0.22	**0.39**	0.02	0.13	0.19	0.09	0.21
Days to 50%	**0.40**	0.13	0.06	0.03	0.22	0.17	0.06
Days to maturity	**0.38**	-0.10	0.17	0.21	0.29	0.14	0.02
Grain yield	-0.25	**0.40**	-0.04	0.09	0.06	-0.07	-0.03
Harvest index	**-0.34**	-0.12	0.19	0.24	0.09	-0.22	-0.45
Leaf area	0.16	**0.31**	-0.29	**-0.43**	-0.09	-0.33	0.03
Number of leaves	0.24	0.27	-0.17	0.19	-0.14	0.26	-0.67
Overall agronomic	-0.27	0.19	**-0.35**	0.14	-0.02	0.33	0.41
Peduncle exsertion	**-0.30**	-0.039	**0.45**	0.17	-0.02	0.15	0.17
Plant height	-0.02	0.26	**0.52**	**-0.39**	0.18	-0.21	-0.07
Number of	-0.14	-0.16	-0.12	**-0.39**	0.70	0.36	-0.14
Panicle length	**0.36**	0.05	**0.32**	0.03	-0.10	-0.13	0.24
Panicle width	-0.10	0.22	0.29	**-0.39**	-0.42	0.58	-0.10
Seedling vigour	0.09	**-0.39**	-0.14	**-0.40**	-0.18	-0.04	0.04
Stay-green	-0.24	**0.37**	-0.06	-0.07	0.19	-0.24	0.01

4.2.2.7 Morphological cluster analysis

The percentage similarity between accessions ranged from 90 to 99% (Figure 4.6). The resulting phenetic dendrogram revealed three main clusters (I, II and III) at a genetic distance of 0.9. Cluster I contained seven accessions and further classified into two sub clusters, EG 469, EG 883 and EG 849 in one subgroup and EG 537, EG 843, EG 889 and B-35-1 in the second at a genetic similarity of 0.92. All accessions in cluster I were from Gash Barka and South region and were characterised by flat seed, small to medium grain size with red and brown grain colour and tall in their height. Furthermore, accessions in this cluster known to have semi-compact elliptic panicle and non-lustrous as well as elliptical grain shape. Accessions EG 469, EG 883 and EG 889 were known as Bazenay family in Gash Barka region.

Cluster II contained the majority of accessions (Figure 4.1) and of those 15 accessions 8 were from Gash Barka, 5 from Anseba and 2 from South regions with varied morphological characters. All accessions in this cluster were characterised by early flowering, medium plant height, round grain shape, red grain colour, and compact to semi-compact bent type of panicle. Two accessions, EG 830 and EG 711 clustered very closely from the remaining accessions in cluster II and were the most similar accessions at a genetic similarity coefficient of 0.99, indicating a higher morphological similarity.

Cluster III contained three genotypes B-35, Hamelmalo and EG 797. This genotypes were B-35 from ICRISAT, Hamelmalo recently released variety in Anseba region and EG 797 accession from Gash Barka. These genotypes were characterised by short plant height, red glume colour, round grain shape, chalky white and brown grain colour. The ICRISAT Variety B-35 was separated within the sub cluster III indicating that it had some differences in the traits.

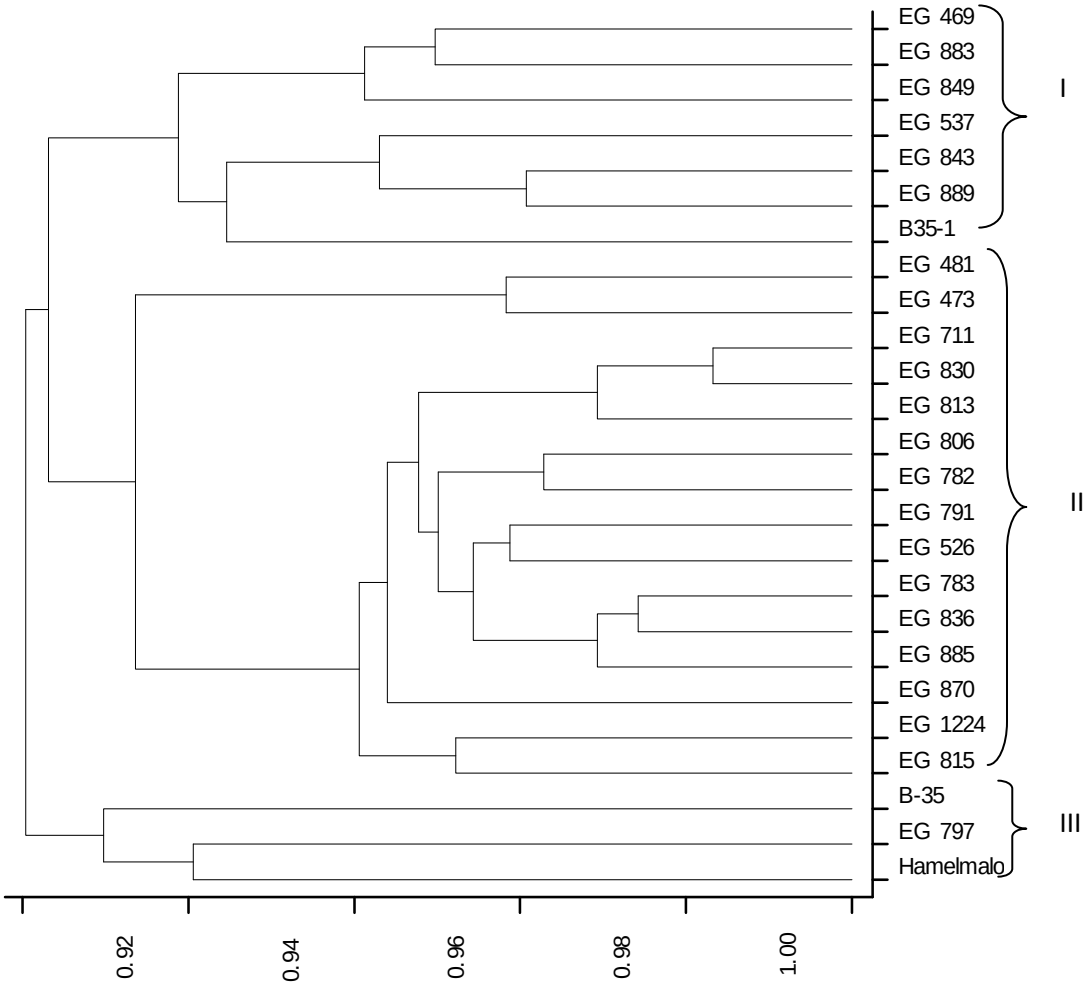


Figure 4.1 Phenetic dendrogram generated using morphological data of 25 sorghum accessions depicting their relationships based on UPGMA clustering comparisons

4.2.2.8 Drought tolerance indices and their correlation with yield in sorghum genotypes

The data on frought tolerance indices showed that drought stress in sorghum can significantly reduce grain yield. The accessions EG 849, EG 836, EG 481, EG 883, EG 885, EG 783 and EG 469 showed higher grain yield under irrigated conditions, with yield averages higher than 290 g/m^2. Accessions EG 885, EG 481, EG 836, EG 469, EG 883, EG 783 and Hamelmalo recorded higher grain yield in stress condition, with values as high as 260 g/m^2. The genotypes EG 481, EG 836, EG 885, EG 883 and EG

469 showed good performance under both irrigated and drought stressed conditions (Table 4.18).

The result of drought tolerance indices showed great variation among accessions with respect to yield reduction and the value of stress tolerance index (STI) ranged from 0.25 to 1.54 (Table 4.18). Genotypes EG 836 (1.54) scored the highest value of STI followed by EG 885, EG 481 and EG 849 with STI values 1.51, 1.47 and 1. 37 respectively which were considered to be tolerant genotypes. EG 1224, EG 815 and EG 843 scored the lowest STI value 0.25, 0.26 and 0.44 and were considered as drought susceptible. The values of geometric mean productivity (GMP) ranged from 121.6 to 298.9 g/m^2 and the genotypes EG 836 and EG 885 were the most productive (>296 g/m^2). Stability tolerance index (STI) ranged from 0.26-1.54; (values $\geq$ 1 indicate high stress tolerance). Genotypes EG 836, EG 885, EG 481, EG 883, EG 783 and EG 469 had higher values of > 1.35, suggesting that these genotypes were the most tolerant. YI ranged from 0.36 to 1.35, with genotypes EG 885, EG 836, EG 481, EG 883 and EG 783 with the higher index (>1.23). The YI selected the same genotypes as in Y_s (r = 1.00) and showed a moderate correlation with Y_{ir} (r = 0.79). SSI values varied from -2.49 -5.05, which were negatively correlated with yield under drought stress (Y_s) and positively associated with the TOL index. YSI ranged from 0.39-1.30; (a higher rate indicated greater stability). Genotypes that showed higher stability indices include EG 843, B-35 and EG 791 whose values were greater than 1.13 (Table 4.18). Besides the mean productivity (MP) and geometric mean productivity (GMP) showed similar ranking pattern as in STI. In both indices, the top five genotypes with highest value of MP and GMP were EG 836, EG 885, EG 481, EG 883 and EG 849. Similarly, those genotypes that showed lower SSI values also scored higher yield stability index (YSI) whereas yield index (YI) have similar ranking with STI values.

To determine the most desirable drought tolerance measures, the correlation coefficient between Y_{ir}, Y_s, and other quantitative indices of drought tolerance were estimated (Table 4.19).

Table 4.18 Mean values of yield in stressed (Y_s), yield in irrigated (Y_{ir}), tolerance index (TOL), mean productivity (MP), stress susceptibility index (SSI), geometric mean productivity (GMP), stress tolerance index (STI), yield index (YI) and yield stability index (YSI) in sorghum

Accessions	Y_{ir} (g/m^2)	Y_s (g/m^2)	TOL	MP	SSI	GMP	STI	YI	YSI	Ranking
EG 849	334.00 (**1**)	238.60 (**9**)	95.40 (**2**)	286.30 (**5**)	2.35 (**3**)	282.30 (**5**)	1.47 (**3**)	1.13 (**9**)	0.71 (**23**)	5
EG 836	329.30 (**2**)	271.30 (**3**)	58.00 (**4**)	300.30 (**1**)	1.45 (**7**)	298.90 (**1**)	1.54 (**1**)	1.28 (**2**)	0.82 (**19**)	1
EG 481	313.90 (**3**)	271.60 (**2**)	42.30 (**10**)	292.80 (**3**)	1.11 (**11**)	292.00 (**3**)	1.47 (**4**)	1.28 (**3**)	0.87 (**15**)	2
EG 883	309.10 (**4**)	263.60 (**5**)	45.50 (**6**)	286.40 (**4**)	1.21 (**9**)	285.40 (**4**)	1.40 (**5**)	1.25 (**5**)	0.85 (**17**)	4
EG 885	307.00 (**5**)	285.80 (**1**)	21.20 (**16**)	296.40 (**2**)	0.57 (**18**)	296.20 (**2**)	1.51 (**2**)	1.35 (**1**)	0.93 (**8**)	3
EG 783	303.80 (**6**)	260.50 (**6**)	43.30 (**8**)	282.20 (**6**)	1.17 (**10**)	281.30 (**6**)	1.36 (**6**)	1.23 (**6**)	0.86 (**16**)	6
EG 469	292.70 (**7**)	267.10 (**4**)	25.60 (**13**)	279.90 (**7**)	0.72 (**16**)	279.60 (**7**)	1.35 (**7**)	1.26 (**4**)	0.91 (**9**)	7
EG 711	289.90 (**8**)	259.30 (**8**)	30.60 (**12**)	274.60 (**8**)	0.87 (**15**)	274.20 (**8**)	1.29 (**8**)	1.22 (**8**)	0.89 (**11**)	8
EG 813	259.00 (**9**)	199.60 (**17**)	59.40 (**3**)	229.30 (**13**)	1.89 (**5**)	227.40 (**13**)	0.89 (**13**)	0.94 (**17**)	0.77 (**21**)	11
Hamelmalo	250.20 (**10**)	260.10 (**7**)	-9.90 (**22**)	255.20 (**9**)	-0.33 (**22**)	255.10 (**9**)	1.12 (**9**)	1.23 (**7**)	1.04 (**4**)	9
B35-1	247.90 (**11**)	211.00 (**15**)	36.90 (**11**)	229.50 (**12**)	1.22 (**8**)	228.70 (**12**)	0.90 (**12**)	1.00 (**15**)	0.85 (**18**)	13
EG 830	243.00 (**12**)	222.10 (**12**)	20.90 (**18**)	232.60 (**11**)	0.71 (**17**)	232.30 (**11**)	0.93 (**11**)	1.05 (**12**)	0.91 (**10**)	12
EG 806	242.80 (**13**)	233.30 (**10**)	9.50 (**19**)	238.10 (**10**)	0.32 (**19**)	238.00 (**10**)	0.98 (**10**)	1.10 (**10**)	0.96 (**7**)	10
EG 473	227.40 (**14**)	201.90 (**16**)	25.50 (**14**)	214.70 (**17**)	0.92 (**13**)	214.30 (**17**)	0.79 (**17**)	0.95 (**16**)	0.89 (**12**)	15
EG 526	226.90 (**15**)	220.70 (**13**)	6.20 (**20**)	223.80 (**14**)	0.22 (**20**)	223.80 (**14**)	0.86 (**14**)	1.04 (**13**)	0.97 (**6**)	14
EG 537	215.60 (**16**)	215.70 (**14**)	-0.10 (**21**)	215.70 (**16**)	0.00 (**21**)	215.60 (**16**)	0.80 (**16**)	1.02 (**14**)	1.00 (**5**)	17
EG 797	215.00 (**17**)	158.30 (**21**)	56.70 (**5**)	186.70 (**19**)	2.17 (**4**)	184.50 (**19**)	0.59 (**19**)	0.75 (**21**)	0.74 (**22**)	18
EG 782	205.50 (**18**)	182.60 (**19**)	22.90 (**15**)	194.10 (**18**)	0.92 (**14**)	193.70 (**18**)	0.65 (**18**)	0.86 (**19**)	0.89 (**13**)	19
EG 791	204.50 (**19**)	230.90 (**11**)	-26.40 (**24**)	217.70 (**15**)	-1.06 (**23**)	217.30 (**15**)	0.81 (**15**)	1.09 (**11**)	1.13 (**3**)	16
EG 815	199.20 (**20**)	77.00 (**25**)	122.20 (**1**)	138.10 (**24**)	5.05 (**1**)	123.80 (**24**)	0.26 (**24**)	0.36 (**25**)	0.39 (**25**)	21
EG 889	197.00 (**21**)	154.00 (**23**)	43.00 (**9**)	175.50 (**20**)	1.80 (**6**)	174.20 (**20**)	0.52 (**20**)	0.73 (**23**)	0.78 (**20**)	20
EG 870	185.10 (**22**)	164.00 (**20**)	21.10 (**17**)	174.60 (**21**)	0.94 (**12**)	174.20 (**21**)	0.52 (**21**)	0.77 (**20**)	0.89 (**14**)	22
EG 1224	146.10 (**23**)	101.20 (**24**)	44.90 (**7**)	123.70 (**25**)	2.53 (**2**)	121.60 (**25**)	0.25 (**25**)	0.48 (**24**)	0.69 (**24**)	24
EG 843	140.80 (**24**)	183.40 (**18**)	-42.60 (**25**)	162.10 (**22**)	-2.49 (**25**)	160.70 (**22**)	0.44 (**22**)	0.87 (**18**)	1.30 (**1**)	23
B-35	138.60 (**25**)	158.20 (**22**)	-19.60 (**23**)	148.40 (**23**)	-1.16 (**24**)	148.10 (**23**)	0.38 (**23**)	0.75 (**22**)	1.14 (**2**)	25
Mean	**240.97**	**211.67**	**29.30**	**226.35**	**0.92**	**224.93**	**0.92**	**1.00**	**0.89**	

High significant correlations were found between grain yield under stress condition and the drought indices MP, GMP, STI and YSI. The result also indicated that genotypes with high STI have high difference in yield.

The indices GMP, MP and STI were very similar to the selection based on Y_{ir} and Y_s. This was confirmed by the high correlations between Yir and GMP (r = 0.94), MP (r = 0.95), and STI (r = 0.95) and the correlation between Ys and GMP (r = 0.96), MP (r = 0.94) and STI (r = 0.93) (Table 4.19). MP is the mean production under both stress and non-stress conditions, and was highly correlated with yield under both conditions. Thus, MP can be used to identify cultivars in the tolerant group. Similar to the SSI and TOL, correlations between YSI and GMP, STI and MP were low (r = 0.10, r = 0.05 and r = 0.06 respectively), indicating that similar genotypes were not selected. The correlation between STI and GMP was nearly one and these two were positively correlated with MP but not with SSI. SSI was found to be highly negatively correlated with YSI and positively correlation with TOL (Table 4.19).

Table 4.19 Genotypic correlation of yield in non-stressed (Yi), yield in stressed (Ys), tolerance index (TOL), mean productivity (MP), stress susceptibility index (SSI), geometric mean productivity (GMP), stress tolerance index (STI), yield stability index (YSI) and yield index (YI) in sorghum

	Yi	Ys	YSI	MP	GMP	TOL	SSI	STI	YI
Yi	1.00								
Ys	0.798 ***	1.00							
YSI	-0.233	0.382*	1.00						
MP	0.952***	0.945***	0.068	1.00					
GMP	0.939***	0.956***	0.103	0.999***	1.00				
TOL	0.410	-0.222	-0.956***	0.111	0.073	1.00			
SSI	0.233	-0.382	-1.000***	-0.068	-0.103	0.956***	1.00		
STI	0.951***	0.935***	0.053	0.995***	0.993***	0.125	-0.053	1.00	
YI	0.798***	1.000***	0.382*	0.945***	0.956***	-0.222	-0.382*	0.935***	1.00

Where, *, and *** significant at the 5% and 0.1% level of probability respectively

Biplot analysis was also conducted and identified superior genotypes for both stress and non-stress conditions. In this biplot a strong negative association was recorded between SSI and TOL with YSI, as indicated by the large angles between their vectors (Figure 4.2).

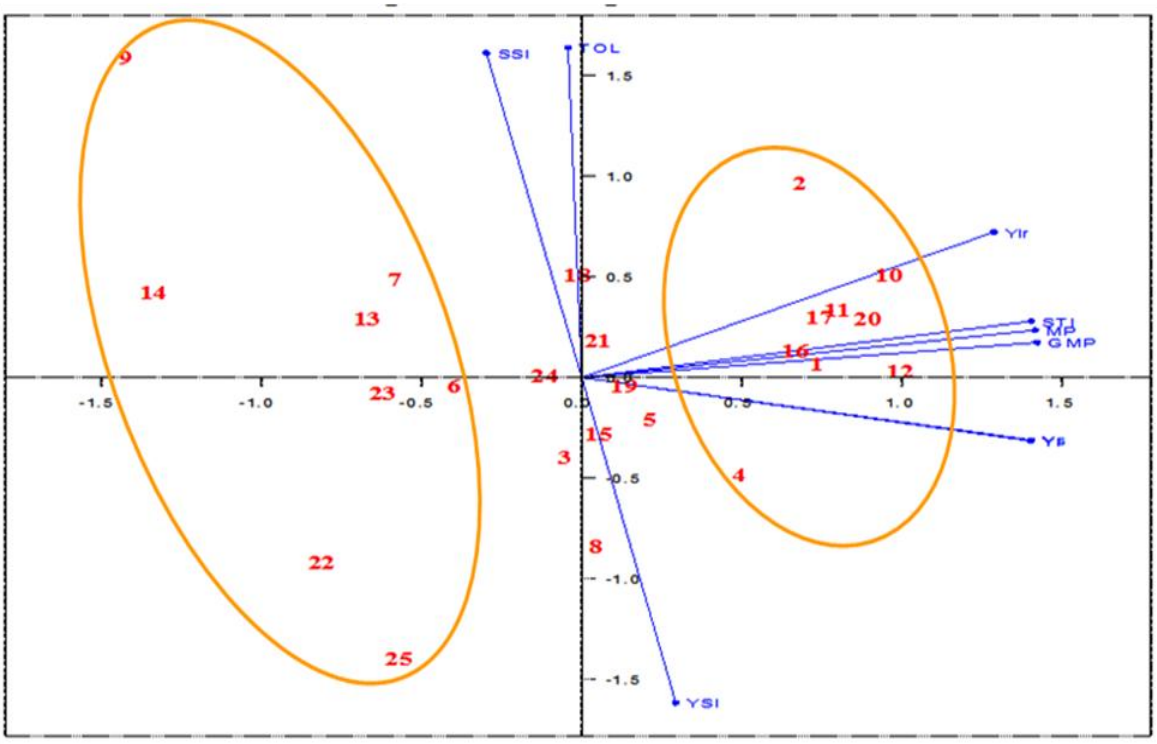


Figure 4.2 Biplot diagram of 25 sorghum genotypes and 8 drought indices. The indices are indicated using uppercase letters (see Table 2, for abbreviations), and each accession is represented with numbers (Table 4.7).

Nearly zero correlation was also recorded between SSI with GPM, MP, HM, and STI, as well as SSI and TOL with Y_s and YI, as indicated by the nearly perpendicular vectors. Besides, positive association between Y_{ir} and Y_s with MP, GMP, HM, and STI was observed as indicated by the acute angles. The results obtained from the biplot graph confirmed the correlation analysis results in Table 4.19.

Using the drought indices unweighted pair group with arithmetic mean cluster analyses were conducted to categorize the genotypes (Figure 4.3). The results were consistent with those of biplot analysis (Figure 4.2). The advantage of this approach is that it can be used to calculate distances between genotypes. The genotypes were clustered into 5 main groups with different size of genotype grouping. The top cluster grouped were genotypes with higher yield, while the lower clusters included genotypes with lower yield.

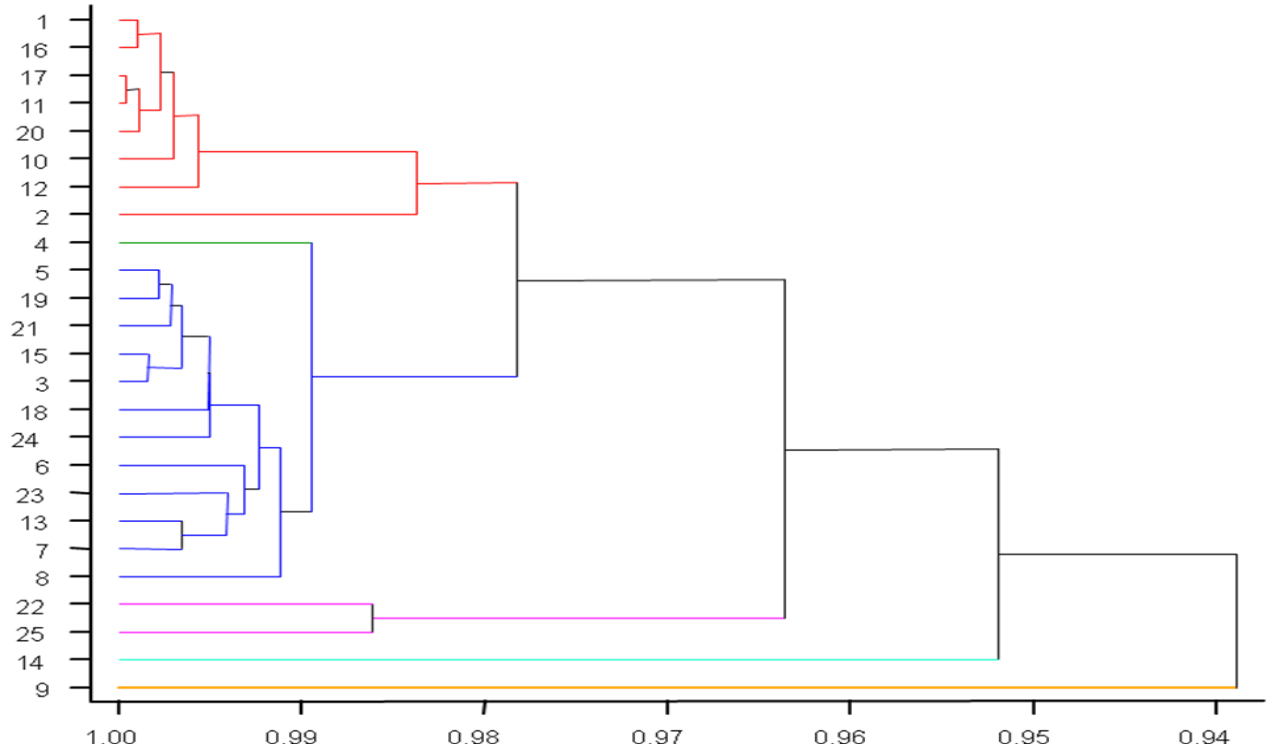


Figure 4.3. Dendrogram from UPGMA cluster analysis of genotypes based on drought tolerance indices (Y_s, Y_{ir}, GMP, MP, STI, YI, TOL, YSI and SSI) and grain yield of sorghum accessions, in both irrigated and drought stress condition (for genotype codes: see Table 4.7).

4.2.3 Discussion

Drought is one of the most damaging abiotic stresses affecting crop yield especially when it occurs during the reproductive stage. The water requirement increases from the boot stage after anthesis. The impact of drought stress on crop plants can be partly mitigated through genetic improvement. Genetic improvement for drought tolerance in sorghum will require selection for tolerant germplasm and deeper understanding of the physiological and genetic responses to stress.

The results of the present study showed significant variation for most of the traits studied under non-stressed and drought stressed conditions. Performance of the accessions under drought stress for traits like seedling vigour and number of leaves had low variability when compared with the non stressed control. However, between the genotypes there appeared greater differences indicating that there was high variability among these accessions for these traits. Stay-green had very strong association with grain yield and with overall agronomic score implying that genotypes with high stay-green and good agronomic performance gave high grain yield. However, stay-green was negatively correlated with days to maturity. Early maturing genotypes had better stay-green value and gave good yield.

Leaf area had a significant role in drought tolerance evaluations in sorghum. In the current study accessions with medium value of leaf area exhibited higher yield. Accessions EG 849 (269.3 cm^2), Hamelmalo (285.7 cm^2), EG 836(262 cm^2), EG 885 (216 cm^2) and EG 469 (285 cm^2) recorded medium value of leaf area but with good yield attributes. However, few accessions such as EG 481 (195.3 cm^2), EG 711 (176.7 cm^2) and B-35-1 (174.3 cm^2) were observed to have lower leaf area but scored high yields. This study is in agreement with the study by Tsuji et al. (2003) who reported drought tolerance in sorghum is associated with smaller leaf area. However, under drought conditions optimum leaf area (LA) is also important for optimum photosynthetic activity. The genotype Hamelmalo with 433.3 cm^2 gave the highest leaf area under irrigation while it reduced its leaf area to 285.7 cm^2 under drought stress conditions. This indicated that drought considerably reduced leaf area in this genotype

to save loss of water through evapo-transpiration. However, such reduced leaf area may cause lower photosynthetic activity (Khaliq, Irshad, & Ahsan, 2008) which is also unwanted. Moreover, traits like reduced leaf area and prolonged stomata closure, decrease water loss, but result in reduced dry matter production and, therefore, reduced final yield (Karamanos & Papatheohari, 1999). Hence, optimum leaf area is important for producing high dry matter as well as grain yield under water stressed situations.

In this study, plant height exhibited significant positive correlation with days to flowering and negative correlation with harvest index. A similar relationship was reported in earlier studies by Murray et al. (2008), Ritter et al., (2008) and Zhao et al., (2009) in sorghum. These authors concluded that taller sorghums have the advantage of accumulating more biomass due to greater translocation of photosynthates from the vegetative tissues resulting in late maturity and low grain yield. Genotypes of this group may utilize the available soil water for vegetative development, leaving no moisture for the grain filling stage concomitant with lower current photosynthesis during post-flowering stages and decreased grain yield.

This study also showed that drought tolerant accessions produced higher grain yield and better agronomic performance which is in agreement with Cooper et al., (2006), who reported that a drought-tolerant genotype produces higher yields than a drought-susceptible genotype under water-stressed environments. The results here suggest that tolerant accessions may be utilized to develop best breeding lines in sorghum improvement programmes. Delay to maturity is a strong indication of sensitivity and is caused by growth retardation during soil drying in response to stress as reported by Blum et al., (1999). Results in the current study for delayed to flowering supported those of Sellamuthu et al., (2011), who reported that delay in flowering during the reproductive stage in rice could affect starch accumulation in grains by reducing photosynthesis and altering sink structure; these changes would reduce grain weight, and in turn, grain yield. This study therefore confirmed that early and medium maturing genotypes have better grain yield. In general grain was positively associated with

overall agronomic score, stay-green and panicle width under post flowering stress condition.

The total biomass of the accessions failed to show significant differences under drought stress and non stress treatments. This could be due to the fact that water was withheld in the stressed treatment at the time of early flowering where by that time the plants were fully established that contribute equal biomass as in the control condition.

Most of the economic characters (grain yield) are complex in inheritance and are greatly influenced by several genes interacting with various environmental conditions, the study of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) is not only useful for comparing the relative amount of phenotypic and genotypic variations among different traits but also very useful to estimate the scope for improvement by selection. The reliability of a parameter to be selected for breeding programme among other factors is dependent on the magnitude of its coefficient of variations (CV) especially the GCV. However, the differences between genotypic and phenotypic coefficient of variability indicate the environmental influence. The current result on variance component showed that the phenotypic variances (σ^2p) and PCVs were slightly higher than the genotypic variance (σ^2g) and GCV for all the characters, suggesting the least influence of environment in the expression of these characters. Generally the results depicted that high to moderate values of PCV and GCV for all the traits except for days to flowering (DFL), days to maturity (DM) and number of leaves (NoL). These results proved that selection can be effective for these traits but also indicated that the genotypes have a broad base genetic background and existence of substantial variability among the accessions, ensuring ample scope for their improvement through selection. These observations are in agreement with the findings by Rafique et al., (2004) and Rafiq et al., (2010) in maize. In addition, the characters showed higher phenotypic and genotypic variance estimates than the error variance estimates indicating that expressions for most of the characters were genetic, which can be exploited in breeding programmes. This finding is in agreement with the findings of Basu, (1981) and Abu-Gasim and Kambal, (1985) for several quantitative characters in

sorghum genotypes. High heritability estimates recorded in some characters such as panicle length, number of leaves per plant, days to 50% flowering and days to physiological maturity indicated that these characters could respond to selection pressure. This result is in agreement with finding of Bello et al., (2010) in sorghum studies of genetic variability. Stay-green and grain yield showed low heritability and implied selection for such trait reduce the effectiveness of phenotypic selection. Selection for such low heritability quantitative traits such as yield and stay-green is a common problem encountered in conventional breeding programmes. This low heritability can be partially overcome through the use of markers linked to QTLs for the target traits that enables individuals to be scored based on their genetic makeup rather than their phenotypic features. Genetic studies of stay-green have generally indicated a complex pattern of inheritance, both dominant and recessive expression have been reported by Tuinstra et al. (1997b).

In this experiment, the PC analysis divided the total variance into 7 PCs out of which first 4 PCs contributed main attribute of diversity among the genotypes due to different characters studied. Considering a minimum threshold Eigen value of one, the four principal components (PCs) accounted for a cumulative of about 74% of the whole phenotypic diversity observed among the germplasm lines. This result was similar to the report by Mujaju and Chakuya, (2008) and Ali et al., (2011) who worked on different agro-morphological traits in sorghum. Moreover, the principal components analysis also showed that the variation in the germplasm lines can not be explained on basis of few characters. This, in turn, implies that a number of traits were involved in explaining the gross variance among the accessions. In order of diminishing importance, the explanation of greater proportion of the entire phenotypic diversity involved main ones's were panicle traits (i.e. its panicle width and peduncle exsertion), leaf traits (it's stay-green and leaf area), yield related traits (grain weight and biomass) and plant phenology (plant height, days to flowering and maturity). This further confirmed the previous results that have also described the importance of these traits in

contributing towards the overall diversity of the sorghum germplasm landraces (Ayana & Bekele, 1999).

Morphological cluster analysis confirmed the presence of variation among genotypes. Besides, the accessions in cluster I was also known for their drought tolerance, and high yielding which is also confirmed in selections from the two years field phenotypic evaluation for drought tolerance. The accessions were clustered together based mainly on geographical sites and pedigree relationship. Likewise, Bucheyekei et al., (2009), Dean et al., (1999) and Ghebru et al., (2002) detected clustering of sorghum accessions based on their collection site and pedigree relationship. Similarly, Geleta and Labuschagne, (2005) found the existence of morphological variation among sorghum accessions collected from eastern parts of Ethiopia using 10 morphological traits and concluded that the variation among the sorghum germplasm implies the need for the genetic resource collection and maintenance. Teshome et al., (1997) evaluated 117 sorghum accessions from North Shewa and South Welo regions of Ethiopia based on 14 morphological traits and reported extensive variation of the accessions. Grenier et al., (2004) reported morphological diversity among sorghum accessions as well as a high level of diversity within region and was distributed with geographical origin using 2017 Sudanese sorghum landraces.

Genotypic correlation coefficient between Y_{ir}, Y_s and other quantitative indices were the most desirable drought tolerance criteria to determine the performance of sorghum landraces. The strong positive association of the yield under irrigation (Y_{ir}) and yield under stress (Y_s) conditions depicted that genotypes giving high yield under the best possible conditions could also do so under stress conditions. This means that genotypes under drought stressed condition have a good response under irrigated conditions. The accessions that give superior yield in both irrigated and drought stressed treatment conditions include EG 885, EG 469, EG 836, EG 481, and EG 883 as examples of high yielding genotypes. However, there were few accessions EG 537, Hamelmalo, EG 797 and B-35 that gave better yield under stress condition only and accessions EG 836, EG

481, EG 849 and EG 813 gave superior yield under irrigation indicated that they were the better predictors of potential yield under stress and irrigation respectively.

Y_{ir}, Y_s, STI, GMP and MP were strongly correlated with yield under both conditions, suggesting that these parameters are suitable for screening drought tolerant and high yielding genotypes in both drought stressed and irrigated conditions. Similar results were reported by Agili et al. (2012) in sweet potato, Fernández (1992) in mungbean, Farshadfar and Sutka (2002) in wheat and maize, Golabadi et al. (2006) in durum wheat, Sio Se-Mardeh et al. (2006) and Mohammadi et al. (2010) in wheat, all of whom found these parameters to be suitable for discriminating the best genotypes under drought stress and irrigated conditions. STI was significantly correlated with Y_{ir} and Y_s and calculated based on the GMP index. High positive correlation was observed between these indices (0.963), which is in agreement with Fernández (1992) and Mozaffari et al., (1996). TOL appears to be useful for selecting genotypes with high yield under drought stress, but failed to select genotypes with good yield in both conditions. Similar results were reported by different authors in several crops such as barley Rizza et al. (2004), wheat Sio-Se Marde et al. (2006), durum wheat Talebi, Fayaz, and Naji, (2009); Shiri, Choukan, and Aliyev (2010), and chickpea Talebi et al. (2011). The significant positive correlation found between SSI and TOL, indicated that these indices are able to select susceptible genotypes.

The biplot vectors for the indices MP, STI, and GMP remained between the Y_{ir} and Y_s vectors, indicating that these indices are very similar for drought selection. In the current research, MP, STI and GMP appeared to be the best indices for dividing the angle symmetrically between Y_{ir} and Y_s. Therefore, these factors can be used to select for genotypes that are better adapted to both conditions. Similar results were reported by Yarnia et al. (2011) in rapeseed. Darvishzadeh et al., (2010) examined sunflower in one location, and found that tolerant indices including MP, STI and GMP were suitable for drought-tolerant genotype selection. However, based on the biplot presented by these authors, GMP is the most appropriate index for selection under stressed and non-stressed conditions. Kharrazi and Rad (2011) suggested that MP and STI are useful

indicators for selecting tolerant genotypes. In the cluster analysis, the high yielding and drought tolerant genotypes (1 = EG 469; 2 = EG 849; 10 = EG 836; 11= EG 883; 12 = EG 885; 16 = EG 711; 17 = EG 783 and 20 = EG 481) were grouped in one cluster while the susceptible and low yielding genotypes (9 = EG 815; 14 = EG 1224; 22 = B-35 and 25 = EG 843) grouped in the bottom cluster indicating the efficiency of the drought indices for classifying genotypes under both stress and non-stress conditions.

Conclusion

In this study, a set of sorghum accessions were screened under drought stress conditions at post-flowering stage and superior cultivars were identified based on their yield and yield components, stay-green and overall agronomic performances.

- The results of the analysis of variance for the different morpho-physiological traits indicated that genotypic differences were significant and the analysis was able to differentiate the accessions with respect to drought tolerance.
- Grain yield under drought stress was influenced by many reproductive growth processes such as leaf area, panicle exsertion, panicle length and width as well days to flowering and maturity.
- Yield and yield-related traits under drought stress conditions were positively correlated of yield and yield-related traits under irrigated conditions.
- STI, GMP and MP were used to identify tolerant genotypes that produced high yield under both conditions.
- YSI and YI were useful indices that could discriminate resistant genotypes that are stable in different conditions and produce high grain yield under stressed conditions.
- The genotypes with high TOL and SSI had high yield only under irrigated conditions.
- The dendrogram demonstrated variation of accessions based on morphological traits, collection region and pedigree could be a valuable source for the sorghum improvement programmes in the three geographical regions, Gash Barka, Anseba and South in particular and Eritrea in general.

Recommendation

This study showed that drought stress significantly reduced the yield of some sorghum genotypes while others were tolerant to drought, which indicated genetic variability for drought tolerance in these accessions. Therefore, breeders can choose better genotypes, under drought stress, and compare their performance under normal condition based on some indices (e.g. MP, GMP and STI) and a combination of different methods of selection such as genetic and phenotypic coefficient of variability and heritability. Based on these different methods of selection the current study identified seven outstanding genotypes (EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711) for post-flowering drought tolerance that could be used by breeders in sorghum improvement programmes. Besides small scale farmers could be benefitted in adopting one or two sorghum landraces depending on the sorghum growing region.

CHAPTER FIVE
GENETIC DIVERSITY ANALYSIS OF ERITREAN SORGHUM GERMPLASM USING SSR MARKERS

Abstract

Eritrea is considered a center of origin for sorghum, the main cereal crop in terms of area under cultivation and production in the country. There have been very little genetic diversity studies done on the Eritrean sorghum to date. To improve this crop, genetic diversity estimation is needed on the available germplasm. The aim of this study was therefore to asses the extent of genetic diversity within and among 98 sorghum genotypes collected from Eritrea alongside 42 regional reference accessions from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) using a set of 29 Simple Sequence Repeat (SSR) markers. The data generated from the gene Mapper were analyzed for polymorphic information content (PIC), allele number and frquencey using Power marker; produce principal coordinate analysis (PCoA), and analysis of molecular variance (AMOVA) by Genealex and allele matching and cluster analysis using DARwin software. An average of 4.8 alleles per marker was recorded. The mean PIC value for the SSR loci was 0.52. The Analysis of Molecular Variation revealed that 12% of the variation resulted from the difference among populations, 31% within individual populations and 57% among individual accessions of the sub populations. Neighbor joining phylogeny tree based on genetic similarity coefficient revealed three distinct groups of clustering with the Eritrean populations further sub clustered into three groups. The Eritrean sorghum accessions from Gash Barka and South regions and South Sudan accessions recorded the highest private alleles. The results of PCoA also classified the sorghum accessions into three major groups. Genetic distance matrix revealed that the Eritrean accessions are more related to each other compared to the regional accessions. The existence of higher level of allelic richness, close genetic distance and an isolated clustering of the Eritrean population indicates that

the accessions have not been introgressed with foreign genes and are valuable resource for future breeding programmes of this crop.

Key words: Sorghum, SSR markers, Germplasm, Analysis of molecular variance, Principal coordinate analysis

5.1 Introduction

Sorghum (2n=20) belongs to the family Poaceae, genus Sorghum Moench, species *Sorghum bicolour* (L.) Moench and tribe Andropogoneae. This species includes the annual sorghums, namely grain sorghum, sorgos, broomcorn and Sudan grass (Prasad & Scott, 2008). Sorghum is the fifth most important cereal crop worldwide after wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*) and barley (*Hordeum vulgare)* (FAOSTAT, 2012). It forms the most important dryland cereal crop for the semi-arid tropics together with maize and pearl millet (*Pennisetum glaucum* (L.). It is grown in at least 86 countries, in an area of 38 million hectares and with annual grain production of about 58 million tonnes. The average productivity reaches 1.5 tha^{-1} (FAOSTAT, 2012).

Grain sorghum is the most important staple food crop in Eritrea where the grain is used for human consumption in different forms. Grains are ground into flour and used to make '*Injera*', bread and local drinks, while the leaves and stalks are commonly fed to animals. Sorghum is mainly grown under rain fed conditions by resource-poor subsistence farmers with very little or no capital inputs, such as fertilizers, pesticides, or irrigation (Tesfamichael et al., 2013). It is widely grown in the lowland and mid-highland regions of the country where rainfall is low for the cultivation of other cereals. This crop is cultivated annually in Eritrea on an average area of 230,000 hectares producing approximately 135,000 tons of grain and with productivity of less than 1 $t\ ha^{-1}$ which is below the average global productivity (MoA, 2010). This low productivity is due to drought, striga and lack of knowledge on the benefits of genetic diversity in the country.

The eastern African region, to which Eritrea belongs, has been described as one of the centers of diversity and possible area of domestication for sorghum (Ghebru, Schmidt, & Bennetzen, 2002). Although Eritrea is a home to a large number of sorghum landraces, very little information on the genetic diversity of these landraces is available. Previous studies at the National Agricultural Research Institute of Eritrea indicated that in the last 15 years, the countrys' sorghum improvement programme has relied on adopting exotic

improved cultivars. Relying on improved exotic cultivars has brought the risk of eroding the genetic diversity of the local landraces of sorghum (Engels & Hawke, 1991). However, small-scale farmers in Eritrea commonly grow sorghum landraces that have wide variation in plant structure, panicle orientation, seed colour and maturity periods (Tesfamichael et al., 2013). Landraces have been selected and continued to be grown by the farmers for several years on the basis of their grain and stalk qualities and adaptation to specific ecologies (Mann *et al*., 1983). Successful plant-breeding programmes depend on the availability of a wide crop genetic diversity. In the search for diverse breeding material, farmer cultivar or landraces (locally adapted populations bred through traditional methods of direct selection) are usually the major sources of genetic variation for solving different production constraints (Ghebru, Schmidt, & Bennetzen, 2002).

There are different DNA markers that have been used for diversity assessment in sorghum and other crops. Among the different DNA markers, simple sequence repeats or SSRs are the most commonly used because they are hyper-variable, co-dominant, robust, and multi-allelic in nature (Rakshit et al., 2012). SSR markers are widely used for diversity assessment in several cultivated crop species including sorghum (Dje et al., 2000; Ghebru, Schmidt, & Bennetzen, 2002.; Agrama & Tuinstra, 2003). The main aim of this current research was to assess the genetic diversity and genetic relationships within and among the Eritrean accessions with reference set of germplasm from eastern and central Africa. This study has a paramount importance to address the knowledge gap and facilitate the utilization and documentation of the extent of landrace diversity for sorghum breeding programmes in Eritrea for the benefit of small scale farmers in solving the low productivity of this crop.

5.2 Materials and Methods

5.2.1 Plant germplasm

A total of 96 Eritrean landraces (Annex 1) along with two released cultivars were selected based on the Eritrean gene bank characterization information and agro-

ecological representation. The seeds of these accessions were obtained from the Plant Genetic Resource unit of Eritrea. In addition, 42 sorghum germplasm (Annex 2) from the eastern and central African (ECA) countries were obtained from the International Crop Research Institute for Semi Arid tropics (ICRISAT), Kenya regional collection and included as a reference set. All the 140 accessions were planted in the greenhouse at the Biosciences eastern and central Africa (BecA) –ILRI hub, Nairobi, Kenya.

5.2.2 Genomic DNA extraction

The seeds of each selected germplasm were planted in a plastic tray with 2.8 cm base and 4 cm of top diameter with a height of 4 cm per single hole. Each hole was then filled with sterile soil, irrigated as required and seeds planted at Biosciences for eastern and central Africa (BecA) greenhouse. The seedlings were irrigated as required and maintained at temperatures between 21^0C to 25^0C. Tender leaf tissues from three plants per accession were harvested from 14 day-old seedlings and bulked for genomic DNA extraction. DNA was extracted using Cetyl-trimethyl Ammonium Bromide (CTAB) method according to Mace et al., (2004). Determination of the quality and concentration of the isolated DNA was done using agarose (2%) gel electrophoresis stained with GelRed^{TM} (Biotium, USA) (2.4µl/100ml) and a Nanodrop® 2000_C spectrophotometer respectively. All the DNA samples were diluted to a final concentration of 20ng/µl.

5.2.3 PCR amplification

A total of 29 labeled SSR markers previously described by Menz et al., (2002) were used for this study (Table 5.1). The preparation of PCR was done in 10 µl reaction volume consisting of 2 mM $MgCl_2$, 1x PCR buffer, 0.20 µM reverse primer, 0.20 µM forward primer labeled with either 6 FAM, VIC, PET or NED, 0.04 mM of each of the four dNTPs and 0.2 U Taq DNA polymerase (Sibenzyme®), 30 ng template DNA and topped up with sterile distilled water. GeneAmp® PCR system 9700 (PE-Applied Biosystems) was used for temperature cycling as follows: 5 min at 94°C followed by 35 cycles of 30 seconds at 94°C, 1 min at 55°C and 2 min at 72°C with a final extension of

15 minutes at 72 ºC. Following PCR, two reaction products from each SSR marker were randomly selected to confirm proper amplification and PCR product concentration on a 2% (w/v) agarose gel. Samples that amplified well were subjected to capillary electrophoresis to determine their sizes.

PCR amplified products of 3-4 individual primer pairs were co-loaded based on the florescent dye, fragment size and dye florescence strength, to reduce the unit cost of high throughput genotyping. 2.0 µl labeled PCR products were mixed with 7.85µl Hi-Di formamide (Applied Biosystems), 0.15µl GeneScan Liz 500 size standard (Applied Biosystems) and denatured at 94^0C for 5 min before analysis by capillary electrophoresis using the ABI PRISM 3730 (Applied Biosciences).

Table 5.1. List and characteristics of the SSR markers used for the sorghum diversity analysis in this study

No	Marker	LG	Forward_Primer	Reverse_Primer	Annealing_Tm	Size (bp)
1	gpsb067	8	TAGTCCATACACCTTTCA	TCTCTCACACACATTCTTC	49	160-190
2	gpsb123	8	ATAGATGTTGACGAAGCA	GTGGTATGGGACTGGA	50	284-304
3	mSbCIR223	2	CGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT	55	104-124
4	mSbCIR238	2	AGAAGAAAAGGGGTAAGAGC	CGAGAAACAATTACATGAACC	55	69-129
5	mSbCIR240	8	GTTCTTGGCCCTACTGAAT	TCACCTGTAACCCTGTCTTC	55	104-180
6	mSbCIR246	5	TTTTGTTGCACTTTTGAGC	GATGATAGCGACCACAAATC	55	86-114
7	mSbCIR248	10	GTTGGTCAGTGGTGGATAAA	ACTCCCATGTGCTGAATCT	56	79-111
8	mSbCIR262	7	GCACCAAAATCAGCGTCT	CCATTTACCCGTGGATTAGT	57	208-446
9	mSbCIR276	3	CCCCAATCTAACTATTTGGT	GAGGCTGAGATGCTCTGT	53	222-252
10	mSbCIR283	7	TCCCTTCTGAGCTTGTAAAT	CAAGTCACTACCAAATGCAC	54	111-157
11	mSbCIR300	5	TTGAGAGCGGCGAGGTAA	AAAAGCCCAAGTCTCAGTGCTA	61	74-118
12	mSbCIR306	1	ATACTCTCGTACTCGGCTCA	GCCACTCTTTACTTTTCTTCTG	55	118-126
13	mSbCIR329	10	GCAGAACATCACTCAAAGAA	TACCTAAGGCAGGGATTG	54	73-121
14	Xcup14	3	TACATCACAGCAGGGACAGG	CTGGAAAGCCGAGCAGTATG	54	209-251
15	Xcup53	1	GCAGGAGTATAGGCAGAGGC	CGACATGACAAGCTCAAACG	54	182-202
16	Xcup61	3	TTAGCATGTCCACCACAACC	AAAGCAACTCGTCTGATCCC	54	189-204

Table 5.1 continued

17	Xcup63	2	GTAAAGGGCAAGGCAACAAG	GCCCTACAAAATCTGCAAGC	54	127-163
18	Xtxp010	6	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC	50	119-155
19	Xtxp012	4	AGATCTGGCGGCAACG	AGTCACCCATCGATCATC	55	143-215
20	Xtxp015	10	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	55	197-273
21	Xtxp021	4	GAGCTGCCATAGATTTGGTCG	ACCTCGTCCCACCTTTGTTG	60	151-227
22	xtxp040	5	CAGCAACTTGCACTTGTC	GGGAGCAATTTGGCACTAG	55	108-144
23	Xtxp057	9	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	55	213-285
24	Xtxp136	10	GCGAATAGCATCTTACAACA	ACTGATCATTGGCAGGAC	55	240-246
25	Xtxp141	7	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	55	133-175
26	Xtxp145	9	GTTCCTCCTGCCATTACT	CTTCCGCACATCCAC	55	204-278
27	Xtxp265	9	GTCTACAGGCGTGCAAATAAAA	TTACCATGCTACCCCTAAAAGTGG	55	168-246
28	Xtxp278	5	GGGTTTCAACTCTAGCCTACCGAACTTCCT	ATGCCTCATCATGGTTCGTTTTGCTT	50	225-318
29	Xtxp321	8	TAACCCAAGCCTGAGCATAAGA	CCCATTCACACATGAGACGAG	55	180-252

Note: The SSR primers were from Generation Challenge Programme (GCP) global marker elaborated and described by Menz et al., 2002 and Kim et al., 2005, LG = linkage groups

5.2.4 Data Analysis

Twenty nine SSR markers were used for this study. These SSR markers are selected from GCP markers that are the world bench mark known for sorghum diversity genotyping. The peaks were sized and the alleles were scored using GeneMapper version 4.1 software (Applied Biosystems). The data was analysed using Power-Marker version 3.25 (Liu & Musa, 2005) to calculate PIC for an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. The power marker analysis also includes heterozygosity, number of alleles identified for each marker, the extent of genetic diversity among the accessions and their genetic distances.

The data generated from the gene Mapper were analyzed using Genealex version 6.4 (Peakall & Smouse, 2012) to produce Principal Coordinate Analysis that helped to establish the relationship among individuals of the sorghum populations, Analysis of Molecular Variance (AMOVA) was used to compute the differences of variance among the genotypes and for calculating percentage of polymorphism, number of private alleles and genetic distances. Dissimilarity indices were estimated using allelic data by simple allele matching and cluster analysis based on unweighted neighbor-joining (Gascuel, 1997) were carried using DARwin 5.0 dissimilarity analysis software (Perrier & Jacquemoud, 2006).

5.3 Results

5.3.1 SSR marker categorization and extent of genetic diversity

The twenty nine SSR markers generated a total of 140 alleles which were used to estimate the genetic diversity among the 140 sorghum genotypes. The number of alleles revealed by each marker ranged from two (gbsb123, Xcup61 and mSbCIR262) to eight (mSbCIR283 and Xtxp141) with an average of 4.8 per marker (Table 5.2). The PIC value for the SSR loci ranged from 0.06 (mSbCIR262) to 0.74 (Xtxp265) with a mean of 0.52. In the current study nineteen SSR markers revealed PIC values of more than 0.50. The mean level of heterozygosity per SSR marker was 0.22 ranging from 0.02 for marker mSbCIR262 to 0.74 for Xtxp136. Marker Xtxp265 had the highest gene diversity (0.77) and while mSbCIR262, with a value of 0.07, had the lowest.

Table 5.2 Summary evaluation of 29 SSR markers on major allele frequency, number of alleles identified, gene diversity, heterozygosity and polymorphism information

Marker	Major Allele	Number of Allele	Gene Diversity	Heterozygosity	PIC†
gbsb67	0.58	5	0.56	0.16	0.48
gbsb123	0.72	2	0.40	0.12	0.32
mSbCIR223	0.69	4	0.44	0.24	0.36
mSbCIR238	0.36	6	0.70	0.21	0.64
mSbCIR240	0.47	6	0.61	0.26	0.53
mSbCIR246	0.82	3	0.29	0.07	0.26
mSbCIR248	0.78	6	0.36	0.31	0.33
mSbCIR262	0.97	2	0.07	0.02	0.06
mSbCIR276	0.48	3	0.63	0.30	0.56
mSbCIR283	0.30	8	0.76	0.15	0.72
mSbCIR300	0.51	6	0.69	0.09	0.66
mSbCIR306	0.57	5	0.61	0.08	0.57
mSbCIR329	0.42	5	0.71	0.12	0.67
Xcup14	0.56	4	0.59	0.13	0.52
Xcup53	0.45	6	0.66	0.23	0.60
Xcup61	0.75	2	0.38	0.09	0.31
Xcup63	0.72	4	0.43	0.40	0.38
Xtxp010	0.38	4	0.69	0.35	0.63
Xtxp12	0.44	7	0.74	0.28	0.71
Xtxp15	0.46	4	0.67	0.09	0.61
Xtxp21	0.59	4	0.58	0.40	0.53

Table 5.2 continued

Xtxp40	0.56	4	0.52	0.08	0.41
Xtxp57	0.51	5	0.65	0.46	0.60
Xtxp136	0.49	3	0.62	0.74	0.54
Xtxp141	0.40	8	0.71	0.24	0.66
Xtxp145	0.51	7	0.67	0.11	0.64
Xtxp265	0.36	7	0.77	0.21	0.74
Xtxp278	0.61	4	0.54	0.23	0.48
Xtxp321	0.38	6	0.70	0.27	0.64
Mean	**0.55**	**4.8**	**0.57**	**0.22**	**0.52**

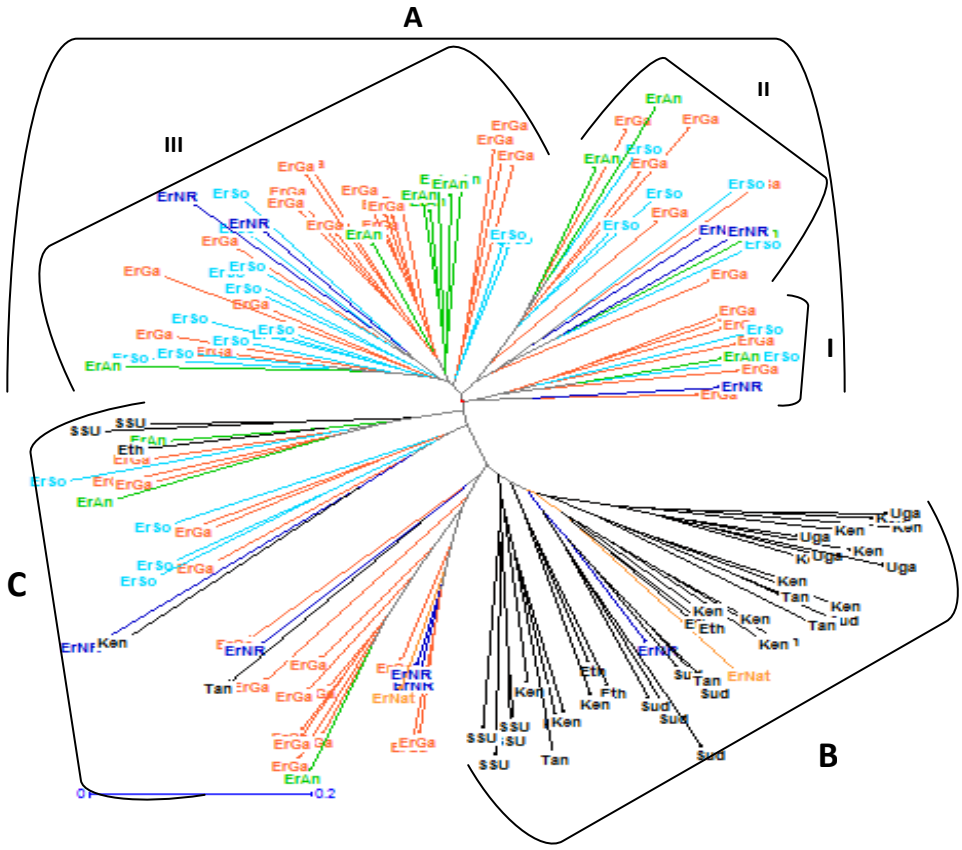
Where, †PIC, Polymorphic Information Content

5.3.2 Patterns of genetic differentiation

Cluster analysis was carried out independently for the 11 populations of Eritrean and regional reference accessions. Based on unweighted neighbor-joining cluster analysis put the 140 sorghum accessions into three major clusters, 'A', 'B' and 'C' (Figure 5.1). Cluster 'A', consisted of 66 Eritrean accessions, cluster 'B' consisted of 39 accessions from Tanzania, Uganda, Kenya, Ethiopia, Sudan and South Sudan. Cluster 'C' consisted of 35 accessions from regional and Eritrean populations. Cluster 'A' was further subdivided into three sub clusters I, II and III. Ten accessions were grouped in sub cluster I that comprised genotypes from Gash Barka, South, Anseba and Northern Red Sea. Sub cluster II comprised 16 accessions from Gash Barka, Anseba, South and Northern Red Sea while sub cluster III was the largest cluster with 40 accessions that composed of accessions from the 4 regions of Eritrea (Figure 1). Cluster 'B' is mainly from the regional accessions of Uganda (4), Kenya (15), Tanzania (4), and Sudan (6), Ethiopia (4), South Sudan (4), Northern Red Sea (1) and national released cultivars of Eritrea (1). Cluster 'C' consists of mixed populations from South Sudan (2), Ethiopia (1), Kenya (1), and Tanzania (1), Anseba (3), Gash Barka (18), South (4) and Northern Red Sea (5) regions.

The genetic relationships among the Eritrean and regional accessions were further investigated using principal co-ordinate (PCoA) analysis (Figure 5.2). The PCoA

classified the 140 accessions into three major groups based on the Eritrean origin accessions and regional reference



The different cluster populations are high-lighted with different colours.
ErGa = red,
ErAn = green,
ErSo = light blue,
ErNR = deep blue and
ErNat = Orange
All regional accessions from ECA are in black

Figure 5.1 Unrooted neighbor joining tree showing genetic relationship among the populations. The different populations are highlighted with different colours. Majority of the Eritrean accessions are grouped in A, most regional accessions grouped in B and cluster C comprises of intermediates from both Eritrean and regional accessions.

accessions from Eastern Africa countries where the Eritrean populations and regional groups indicated by I and II, respectively. The pattern of clustering was also similar to those detected by cluster analysis except some nine germplasm accessions from Uganda and Kenya that remained distinctly outlier and forming a solitary group in the PCoA that categorized as group III. These accessions clustered far apart from all other germplasms indicating their dissimilarity with other groups.

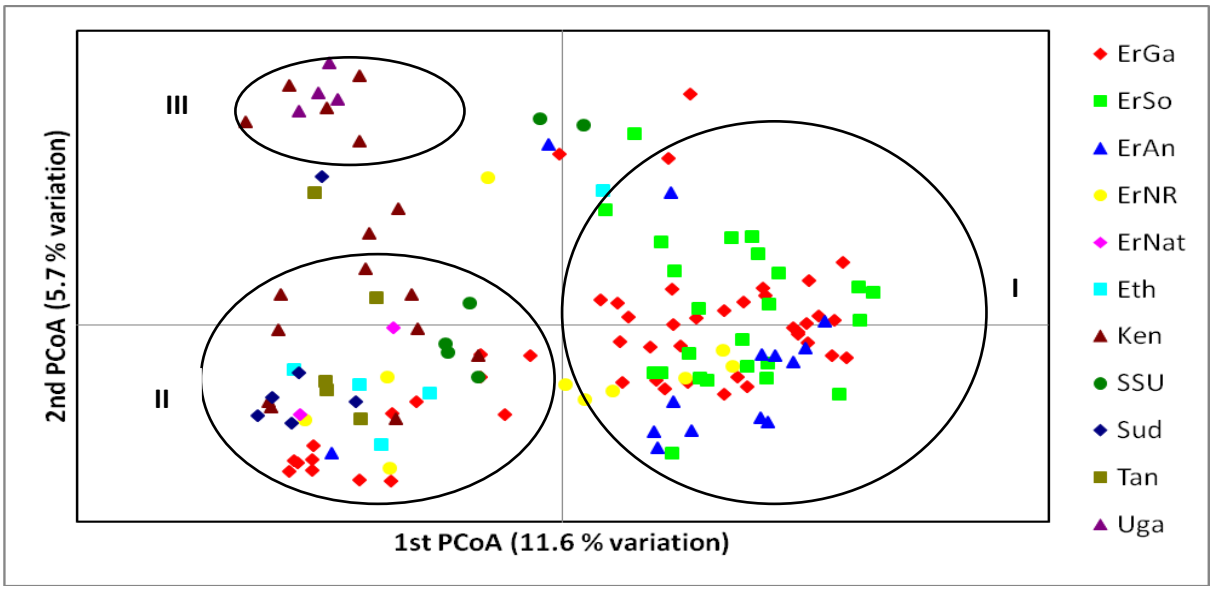


Figure 5.2 Principal Coordinate plot describing genetic relationship among the Eritrean accessions and regional germplasm which are highlighted by different colours. Majority of the Eritrean populations of Gash Barka (ErGa), South (ErSo), Anseba (ErAn), and Northern Red Sea (ErNR) clustered in I; regional accessions from Ethiopia (Eth), Kenya (Ken), Sudan (Sud), South Sudan (SSU), Tanzania (Tan) and few from Eritrean populations of Gash Barka and Anseba clustered in II and few Kenya and Uganda accessions grouped in III.

5.3.3 Population structure analysis

All the variance components of the sorghum accessions under study had highly significant differences ($P < 0.001$) among populations, among individuals and within individuals. The Analysis of Molecular Variation (AMOVA) revealed that 12% of the variation resulted from the difference among populations, 31% within-individuals population and 57% among individual accessions of the sub populations (Table 5.3). The variations for the within-populations mainly contributed from the Eritrean populations of Gash Barka, South, Anseba and Northern Red Sea regions germplasm.

Table 5.3 AMOVA partitioning SSR variation, among populations, among individuals within populations, and within individuals in 140 sorghum accessions

Source	Degree of freedom	Sum of squares	Mean of squares	Variance components	Percentage of variation	P-value
Among Populations	10	405.3	40.53	1.16	12	***
Among individual sub population	129	1810.7	14.04	5.53	57	***
Within individual sub population.	140	415.5	2.97	2.97	31	***
Total	279	2631.5	57.54	9.66		

5.3.4 Allelic richness and pattern of genetic diversity

The SSR markers used in this study were able to structure both the Eritrean and regional accessions. Comparing the allelic richness of the different populations the data provides significant variation among the populations. Private or rare allele per population ranged from 0 to 13. The Eritrean Gash Barka accessions were observed to have highest private allele with 13 that came from 9 accessions followed by the South Sudan with 8 and South region of Eritrea and Kenya each having 5 private alleles. The percentage of polymorphic loci indicated that the populations of Gash Barka, Anseba and Northern Red Sea regions had the highest percentage of polymorphic loci with 100% (allelic frequency >5%). The lowest was observed for the Ugandan and National programme populations. The mean observed gene diversity with the geographical populations was variable, ranging from 0.093 for Tanzanian to 0.253 for Eritrean South populations (Table 5.4).

Table 5.4 Population estimates on percentage polymorphism, number of private alleles, gene diversity on expected and observed heterozygosity and inbreeding index

Population Name	N	%PL	N^P	N^S	Na (SE)	H_o (SE)	uH_e (SE)	F_{is} (SE)
Gash Barka (ErGa)	48	100.0	13	9	4.8 (0.3)	0.21 (0.03)	0.61 (0.03)	0.6 (0.05)
South (ErSo)	24	96.6	5	4	4.4 (0.2)	0.25 (0.03)	0.55 (0.03)	0.5 (0.06)
Anseba (ErAn)	14	100.0	2	1	3.8 (0.2)	0.21(0.03)	0.54 (0.03)	0.6 (0.06)
Northern Red Sea	10	100.0	2	1	3.6 (0.2)	0.21(0.04)	0.56 (0.03)	0.6 (0.08)
National (ErNat)	2	56.6	0	0	1.6 (0.1)	0.12 (0.04)	0.38 (0.06)	0.7 (0.11)
Ethiopia (Eth)	5	83.3	1	1	2.5 (0.1)	0.20 (0.04)	0.48 (0.04)	0.6 (0.08)
Kenya (Ken)	16	96.6	5	4	3.6 (0.2)	0.17 (0.02)	0.53 (0.03)	0.7 (0.05)
South Sudan (SSU)	6	86.6	8	6	2.6 (0.2)	0.13 (0.03)	0.49 (0.04)	0.7 (0.07)
Sudan (Sud)	6	83.3	0	0	2.5 (0.1)	0.13 (0.04)	0.45 (0.04)	0.7 (0.09)
Tanzania (Tan)	5	83.3	0	0	2.5 (0.1)	0.09 (0.03)	0.54 (0.05)	0.8 (0.07)
Uganda (Uga)	4	63.3	1	1	1.9 (0.1)	0.11 (0.03)	0.33 (0.05)	0.7 (0.08)

Where, N = Population size; N^P = No. of private alleles; N^S = No. samples contributed to private alleles; % PL = percentage of Polymorphic loci; Na = Number of different alleles; uH_e = Unbiased expected heterozygosity, H_o = Observed heterozygosity and F_{is} = inbreeding index, SE = standard error in parenthesis

The data in general revealed that the Eritrean populations showed higher observed heterozygosity compared to the regional populations. Population specific F-statistic indices showed higher values of inbreeding index (Fis) for all populations which ranged between 0.5 and 0.8 with a mean of 0.65 (Table 5.4).

The Nei unbiased genetic distance matrix was calculated to estimate the relationship among the 11 populations that comprises 140 accessions. The furthest distance was between accessions from Uganda and those from South Eritrea and Anseba regions with genetic distance of 0.674 and 0.668 respectively. The closest populations were for accessions from Gash Barka, Anseba, South and Northern Red Sea with genetic distance ranging from 0.042 to 0.085. The Ethiopian, South Sudanese, Kenyan and Tanzanian also showed close genetic distance among each other ranging from 0.068 to 0.171. In general all the Eritrean germplasm accessions were far-off in their genetic distances from the regional accessions of Ethiopia, Kenya, Sudan Tanzania, South Sudan and Uganda. However, the regional accessions had close genetic distance and clustered to each other with the exception of the Ugandan and some Kenyan accessions (Table 5.5).

Table 5.5 Pairwise Population Matrix of Nei Unbiased Genetic Distance on 11 sorghum populations

	ErGa	ErSo	ErAn	ErNR	ErNat	Eth	Ken	SSU	Sud	Tan	Uga
ErGa	0.000										
ErSo	0.042	0.000									
ErAn	0.085	0.052	0.000								
ErNR	0.043	0.078	0.131	0.000							
ErNat	0.336	0.485	0.544	0.247	0.000						
Eth	0.148	0.324	0.283	0.180	0.152	0.000					
Ken	0.295	0.415	0.444	0.250	0.283	0.139	0.000				
SSU	0.314	0.335	0.476	0.335	0.484	0.357	0.268	0.000			
Sud	0.174	0.489	0.427	0.332	0.371	0.171	0.168	0.355	0.000		
Tan	0.273	0.374	0.318	0.213	0.296	0.093	0.091	0.276	0.068	0.000	
Uga	0.541	0.674	0.668	0.527	0.592	0.355	0.182	0.545	0.392	0.319	0.000

Where, ErGa = Eritrea Gash Barka; ErSo = Eritrea South; ErAn = Eritrea Anseba; ErNR = Eritrea Northern Red Sea; ErNat = Eritrea National; Eth = Ethiopia; Ken = Kenya; SSU = South Sudan; Sud = Sudan; Tan = Tanzania; Uga = Uganda

5.4 Discussion

The mean number of alleles per SSR locus (4.8) detected on the 140 sorghum accessions in the current study was similar to that detected in sorghum that employed 28 SSR primers by Agrama and Tuinstra, (2003) with mean allele per locus of 4.3 but lower than those reported on sorghum by Smith et al., (2000), with mean allele per locus (5.9). The gene diversity observed in current studied populations (0.57) is also very similar to the diversity value (0.58) reported by Smith et al., (2000) in sorghum, but lower than the diversity value (0.62) reported by Agrama and Tuinstra, (2003). The high levels of gene diversity of SSR markers observed in this study was probably due to the presence of an extensive genetic diversity in these sorghum accessions that represented different races and geographic regions. Nineteen SSR markers recorded PIC values more than 0.50 indicating their usefulness in discriminating the genotypes. Similar PIC value results with more than 0.50 were also reported by Smith et al., (2000) and Rakshit et al., (2012) in *sorghum bicolour*.

The accessions that originated or that were collected from close geographic regions were generally clustered together by the unrooted neighbor joining population structure. The fact that 63% of the Gash Barka, 79% of the Anseba, 83% South and 50% of the Northern Red Sea accessions clustered together in 'A' and further sub clustered into 3 groups indicates that the Eritrean populations though similar but have some degree of variability among each other. When the Eritrean accessions were closely examined, smaller clusters were observed that represented from all the four administration regions of Eritrea within the clusters. This could be due to different naming of the accessions in the different regions for the same accession or sharing common gene pool in their ancestry. Two years field experiment records on phenotypic and morphological evaluation in the Eritrean sorghum accessions also elucidate similar charateristics and this speculation is reflected in the clusters. However, compared to the East African reference sets the Eritrean populations have high degree of dissimilarity and are closely related to each other indicating the uniqueness of the Eritrean accessions. On the other hand, it can't be ignored that few accessions from the Eritrean populations are still

closely clustered with the regional references. For instance 18 accessions of the population from Gash Barka region which has a border with Sudan and Ethiopia grouped in one major cluster 'C'. This indicates some degree of germplasm exchange and common gene pool sharing between these regions and neighboring countries i.e. Sudan and Ethiopia. The sorghum accessions from Gash Barka grow in an area bordering the Sudan and Ethiopia and often near to wild sorghums, providing an additional opportunity for the introgression of foreign genetic material. Similar studies by Epperson, (2004) and Ghebru et al., (2002) indicated that seed exchange and pollen dispersal causes similarity between neighboring populations, whereas distant populations differ for the studied autocorrelation. Interestingly, the two cultivars released by the National programme in Eritrea indicated here as National programme population, were clustered within the regional set of references 'B'. The main reason for these released cultivars to be clustered within the regional populations is that the pedigrees for the crosses of these varieties were originated from Sudan, Ethiopia and Kenya. The results of PCoA were also similar to those of the neighbor joining method. However, four accessions from Uganda namely IS 8193, Serena, Seredo and 5DX 160 formed a solitary group with five accessions from Kenya: Teso #1, Asinge local, Siaya #42 Siaya #82 and Makueni. These accessions, though clustered on the basis of their geographical regions, they showed a higher degree of relatedness.

In this study all the variation components confirmed that there is reasonable genetic diversity among individual accessions within the population (57%) than among populations (12%) and within individual accessions (31%) of the given populations. In agreement with the current results, Ghebru et al., (2002) reported the existence of high genetic diversity in a separate study of 28 Eritrean sorghum landraces. The presence of relatively higher percent of variation among individuals accessions within a population could be due to the selection practice of local farmers, where each farmer keeps and maintains more than one landrace for various uses as reported by Tesfamichael et al., (2013) and Tiny et al., (2014) in sorghum. This practice of separately maintaining several landraces increases the total sorghum genetic diversity within a given

geographic area but does not increase the within population genetic diversity, due to the self pollination nature of sorghum. The occurrence of fair inbreeding index values may come as a result of shared common alleles and genetic drift. This is especially true with the Eritrean populations where farmers have the tradition of selecting panicles while the crop is in the field and retaining their own seeds. Another reason for high genetic variation among accessions of population could also be due to high informal seed exchange and open sorghum marketing across localities within the administrative regions (Tesfamichael et al., 2013).The inbreeding index obtained in the current study were slightly lower than those of Dje et al., (2000) with Fis = 0.68 but higher than obtained by Ghebru et al., (2002) with Fis = 0.45 in *sorghum bicolour*.

The most noticeable result in the current study was the occurrence of high level of private allelic richness actually observed in the sorghum populations of Gash Barka and South regions of Eritrea which could be beneficial to sorghum breeding programme and further diverse types of population-genetic studies as it may be linked to unique traits. The presence of high allelic richness in the accessions of Gash Barka and South regions of Eritrea could be due to the fact that most of the collection of sorghum germplasm in the country comes from these two geographical areas. Besides, further and specific conservation effort may be necessary to maintain this unique diversity in their area of cultivation. The list of Eritrean accessions with higher private and unique alleles include 'white Bazenay', 'Hugurtay', 'Koden short' and 'Koden tall', 'Embulbul', 'Ajeb Sidu', 'Kine Dirga' and 'Kine Biba' from Gash Barka population. Accessions 'Kehi Mashela', 'Anseba', 'Koden loose' and 'Koden compact' are from Eritrea South population. 'Hijeri' and 'Wedi-Susa' are accessions with rich private alleles from Northern Red Sea and Anseba populations respectively. Accessions from South Sudan with rich private alleles include 'Jeri', 'Medenga', 'Okabir', 'Deri', 'Kodu Kine' and 'Oderi'.

The mean gene diversity (H_e =0.50) for the current sorghum populations is slightly lower than the value estimated for cultivated sorghum of Kenya (0.59) by Mutegi et al., (2011). The H_o values were generally lower than the H_e values, indicating deviations from the random mating and low cross pollination rate due to the isolation among the

different accessions of each population or among the diverse geographical sampling. However, the observed heterozygosity (H_o) on the Eritrean sorghum populations of Gash Barka (0.25) and South (0.21) is relatively higher than the other populations. This higher observed heterozygosity in the two Eritrean population's landraces may be because they are in a continuous segregation that could be due the free gene flow and cross administrative regional seed genetic exchange. Based on the current results, it could be noted that the most relevant population for further improvement and selection of this crop is the sorghum populations from Gash Barka and South regions.

The Nei's unbiased pairwise genetic distance between population showed variable genetic distances. The Eritrean populations have shown lower genetic distance to each other indicating that they are geographically nearest to each other and genetically similar. In the same way, the regional reference sets showed lower genetic distance to each other except those from Uganda that showed higher genetic distance to the Eritrean and other regional accessions. This could be due to geographical isolation with the Eritrean accessions and could have unique characters in which the Eritrean accessions might not have. Thus introducing some selected Ugandan accessions could be beneficial to the Eritrean sorghum breeding programme.

Conclusion

- Eritrean accessions showed good genetic diversity which is contributed from among individual population (51%).
- The existence of unique alleles and higher levels of allelic richness, close genetic distance and an isolated clustering of the Eritrean population indicates that the Eritrean germplasm have not been introgressed with foreign genes and are a valuable resource for future breeding programs.

Recommendation

- The Eritrean germplasm accessions therefore can be exploited in breeding programmes for improvement of the sorghum crop in Eritrea.
- To retain this rich and unique genetic diversity special conservation effort for all the Eritrean sorghum population in general, and the Gash Barka and South region populations in particular, may be necessary to safeguard and reap maximum benefits associated with the rich genetic diversity.

CHAPTER SIX
GENERAL DISCUSSION AND CONCLUSION

Information on the levels and patterns of genetic diversity is valuable for efficient management of germplasm and for effective documentation, utilization and identification of materials in breeding programmes to meet the ever-changing needs of growers and consumers in the face of changing and unpredictable environmental challenges. A few sorghum landraces have been documented in the course of this study in relation to utilization, challenges and constraints of sorghum production in Eritrea. Based on the group discussion with the farmers in the different sub regions farmers indigenous knowledge is vital to discover the existing sorghum diversity and cultivation in the country. In addition, it became clear that farmers have played an important role in the dynamics of creation, perpetuation and extinction of this crop to fit with the ever varying climatic changes. Besides, the group discussion with the farmers elucidated that farmers provided opportunities for selection and hybridization by bringing together geographically and ecologically isolated landraces with desirable agronomic traits. Such practices help the farmers in shaping of this crop plant population on the farmland.

The existences of diverse and important morphological traits associated to sorghum were the result of farmer's indigenous knowledge of selection and adaption to their areas in the surveyed four sub regions of Eritrea. Those landraces we see them today may not exist after some years as happened to the neglected cultivars. The study concluded that the necessity of recollection for sorghum landraces in the major sorghum growing area of Eritrea where genetic erosion is common, primarily due to natural disasters.

The primary resource of plant breeding programmes are the genetic variability available within germplasm closely related to the crop of interest. However, the success of crop improvement programmes is highly reliant on the power and efficiency with which this genetic variability can be manipulated. Besides being the major important economic crop, a large amount of genetic variation is present in cultivated and wild sorghum

germplasm of Eritrea. However, the genetic diversity available in sorghum germplasm of Eritrea has not been fully investigated. So far, sorghum germplasm improvement strategies in Eritrea have been based only on traditional breeding. Under the present situation, superior genetic resources and technology are required to improve yield and reduce the risk of loss from biotic and abiotic stresses in sorghum. A prerequisite for the genetic improvement programme of sorghum is knowledge of the extent of genetic variation present among accessions and their genetic distances between them. This could be achieved through characterisation and assessment of germplasm using field morphological evaluation and DNA markers.

Sorghum accessions were obtained from Eritrean national gene bank of the National Agricultural Research Institute (NARI) and International Crop Research Institute in Semi-Arid Tropics (ICRISAT) for evaluation based on field phenotypic assessment for post flowering drought tolerance and SSR markers for diversity analysis. The combination of both field and marker evaluation has proven that the Eritrean germplasm accessions have high genetic variability that can exploited for breeding programmes. Estimates of molecular variation, genetic distances matrix and allelic richness on 140 sorghum accessions were examined. As a result this molecular analysis, it has proved that Eritrean sorghum germplasm accessions are still isolated and contain a great deal of genetic diversity with higher level of allelic richness. The genetic analysis study has identified some Eritrean accessions with rich private and unique alleles that include '**white Bazenay**', '**Hugurtay'**, 'Koden short' and 'Koden tall', 'Embulbul', 'Ajeb Sidu', '**Kine Dirga'** and 'Kine Biba' from Gash Barka region and accessions 'Kehi Mashela', '**Anseba**', 'Koden loose' and 'Koden compact' are from Eritrea South region as well as 'Hijeri' and 'Wedi-Susa' accessions from Northern Red Sea and Anseba regions respectively. These unique and distinct sorghum accessions could be used in breeding programmes and for direct use by farmers.

Climate change has increased the occurrence and severity of drought incidence due to the higher evapo-transpiration and rising temperatures. Drought stress has emerged as one of the most severe constraint faced by the sustainable crop productivity all over the

world. Drought stress diversely affects various developmental stages and ultimately affects the yield. Drought stress at different growth stages causes various physiological changes in the plants. For example, drought conditions both pre-flowering and post-flowering stages have the most adverse effect on yield during and after anthesis. Drought stress at post-flowering has been identified in Eritrea as one of the yield reducing factor. Sorghum phenotypic field evaluation for post flowering was conducted for two years in Eritrea using 96 sorghum accessions from the national gene bank with 4 checks from ICRISAT and national programme. The first year study was rapid screening that categorizes the 100 genotypes into three maturity dates (early, medium and late) and drought responses (tolerant, moderate and susceptible) based on stay-green. Using selection index 20 genotypes has been selected and promoted into advance yield testing for the following year under stress managed condition. Ten sorghum accessions namely EG 469, EG 849, EG 537, Hamelmalo, EG 806, EG 782, EG 797, EG 791, EG 815 and EG 836 has been identified as the most drought tolerant.

During the second year field evaluation of 20 selected genotypes with five drought susceptible and tolerant checks were evaluated. The different analysis of variance, genotypic and phenotypic variations, heritability, drought selection indices and principal component analysis has confirmed that the Eritrean accessions possessed a great deal of variability for post-flowering drought tolerance. In this study, grain yield has revealed significat and positive correlation with stay-green and overall agronomic performance of the genotypes. This confirmed that stay-green has positive impact on yield under terminal drought stress. The analysis also proved that yield and yield-related traits under drought stress conditions are independent of yield and yield-related traits under irrigated conditions. Besides, the study was also confirmed the mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI) are good yield selection indices under moisture stress and compare this with performance under normal condition in combination with other different methods of selection such as genetic and phenotypic coefficient of variability and heritability. Based on these different methods of selection, the current study has idientified seven outstanding

genotypes (EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711) for post-flowering drought tolerance that can be used by breeders in sorghum improvement programme.

In conclusion, the overall combined genetic diversity analysis and phenotypic evaluation has identified five common accessions consistently superior in all experiments as having unique alleles and drought tolerance traits. The genotypes that show promising results for allelic richness and drought tolerance traits include: EG 469 (White Bazenay/ Kinedirga), EG 849 (Hugurtay from Gash Barka), EG 836 (Hugurtay from Anseba), EG 537 (called Anseba in south region) and Hamelmalo. The first three are very popular landraces in Gash Barka region for their yield and quality preferences. EG 537 (Anseba) is popular landrace with tall plant height and high yielding in the South region of Eritrea. Hamelmalo has recently released cultivar known for its striga and drought tolerance from the national programme. It is therefore recommended that these five genotypes can be used as breeding material for further improvement of the crop in Eritrea. Some of the local landraces which were once widely cultivated in Eritrea are now grown only in some restricted areas or extincted. This study, thus recommended retaining this rich and unique genetic diversity by having special conservation effort for all the Eritrean sorghum population in general, and the Gash Barka and South region populations in particular to safeguard and obtain maximum benefits associated with the rich genetic diversity.

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APPENDICES

Appendix 1 Questionaire on diagnostic farming system survey of sorghum production, utilization and constraints of production

HOUSEHOLD SURVEY QUESTIONNAIRE

Date of Interview		Questionnaire ID	
Name of the Interviewer			

LOCATION OF HOUSEHOLD

01	Region		02	Kebabi	
03	Sub-Region		04	Village	

HOUSEHOLD STRUCTURE

05	Name of the respondent			
06	Age (Approx)		07	Male ☐ or Female ☐

TYPE OF CROPS AND LAND AREA CULTIVATED BY HOUSEHOLD

08. Type of farming: Rain-fed ☐ Irrigated ☐ Mixed ☐

09 Which of these crops does the household grow? (Rank from 1 = most important to 6 = least important)	Crop	Ranking	1	2	3	4	5	6
	Sorghum	Ranking (1 to 6)	☐	☐	☐	☐	☐	☐
	Ground Nuts	Ranking (1 to 6)	☐	☐	☐	☐	☐	☐
	Pearl Millet	Ranking (1 to 6)	☐	☐	☐	☐	☐	☐
	Maize	Ranking (1 to 6)	☐	☐	☐	☐	☐	☐
	Taf	Ranking (1 to 6)	☐	☐	☐	☐	☐	☐
	Sesame	Ranking (1 to 6)	☐	☐	☐	☐	☐	☐
	Finger millet	Ranking (1 to 6)	☐	☐	☐	☐	☐	☐

10 Total land area	Hectare		11 Own area cultivated	Hectare	
12 Land rented	Hectare		13 Rent paid to owner	Nakfa	
14 Area allocated for sorghum	Hectare		15 Is the area under sorghum is increasing	Y/N	Y ☐ N ☐

SORGHUM UTILIZATION AND POST HARVEST HANDLING

16. What percentage of the crop you produce:

Crop	Sell as a grain	Sell as a seed	Exchange as a seed	Keep as own seed	Give as free seed	Use for home consumption
Sorghum						
Pearl Millet						
Maize						
Barley						
Wheat						
Taff						
Finger						
Sesame						
Ground nut						

17. If sorghum is used for home consumption in what form do you use it?

Injera ☐ Kicha ☐ Siwa ☐ mixed with other crop for Injera ☐

18	If you use sorghum for Injera and Kicha making only, which type of sorghum do you preferred for such use?	Red grain sorghum	☐
		White grain sorghum	☐
		Mixed Grains	☐
		Chalky white grain	☐
		Yellow grain	☐

19	What type of sorghum do you use for the preparation of local drinks (Siwa/ Daga)?	Red grain sorghum	☐
		White grain sorghum	☐
		Mixed Grains	☐
		Chalky white grain	☐
		Yellow grain	☐
20.	How do you store your grain sorghum?	In traditional store *Kofo*	☐
		In polyethylene / Sisal sacks	☐
		In Under ground structure	☐
		On floor	☐
		Above ground raised metal beam	☐
		Others	☐

21.	What post harvest problems do you face in your sorghum produces? Please use the following ranking 1 = Most important, 2 = Somewhat important, and 3 = Not important		1	2	3
		Infestation by storage pests, birds & rodents.	☐	☐	☐
		Poor threshing technologies	☐	☐	☐
		High moisture content at harvest	☐	☐	☐
		Grain mould due to poor handling	☐	☐	☐
		Cost of transportation & Marketing	☐	☐	☐
22.	What type of seed treatment do you practice for sorghum if any?	Traditional (using ashes or cow urine)		☐	
		Using modern (chemical application)		☐	
23.	How long do you store your sorghum produce?	(1-3) Months		☐	
		6 months		☐	
		One year		☐	
		More than a year		☐	
24.	Which type of sorghum landraces can be stored for longer period without any post harvest problems?	Yellow grain		☐	
		White grain sorghum		☐	
		Mixed Grains		☐	
		Chalky white grain		☐	
		Red grain sorghum		☐	
26	How do you regard as characteristics of a good variety of sorghum for drought tolerance? Please use the following ranking 1 = Most important, 2 = Somewhat important, and 3 = Not important		1	2	3
		Give reasonable yield during Drought	☐	☐	☐
		Better adaptation to the area	☐	☐	☐
		Early maturing	☐	☐	☐

27	Name the improved sorghum varieties you cultivate in the previous three years if any.	1._____ 2. _____ 3. _____		
	Describe the most grown landraces of sorghum in your field.	1. 2. 3.		
28	Have you ever heard about improved sorghum varieties?		Y/N	Yes ☐ No ☐
29	If Yes, are the improved varieties better than the local ones in relation to drought tolerance?		Y/N	Yes ☐ No ☐
30	During moisture stress (drought) season what type sorghum do you grow?	Improved varieties Landraces Shift to other crops types such as pearl millet		1 2 3 ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
31	What are the sources of your seed of sorghum varieties?	Close relatives Neighbour farmer Traders Farmers association Extension service Seed depot/company Others, specify		☐ ☐ ☐ ☐ ☐ ☐ ☐
32	What would you regard as characteristics of good seed of sorghum? Please use the following ranking 1 = Most important, 2 = Somewhat important, and 3 = Not important	High germination Large grain size No admixture with other seeds, etc Chemical treatment applied Good packaging Other (specify)		1 2 3 ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
33	Have you ever been unable to keep your own sorghum seed from one year to next?	Y/N		Yes ☐ No ☐

34	What was the reason for loss of seed over the past 5 years if it happens?	Drought Striga infestation No harvest Insects Eaten-up Other (specify)	1 2 3 ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
35.	Comment on improved seed availability and distribution?	Adequate Inadequate Poor I don't know the existence of improved seed	☐ ☐ ☐ ☐

DROUGHT MANAGEMENT PRACTICES

36	Have ever encountered drought/ moisture in your field of sorghum	Y/N	Yes ☐ No ☐
37	If yes how often did you encountered drought the past 6 yrs	Every 1year ☐ Every 2 years ☐ Every 3 years	
38	Specify the growth stage in which the crop is affected by drought	Seedling ☐ Flowering ☐ Post flowering ☐	
39	If you lost your sorghum seed due to shortage of rainfall what are the sources of seed?	Self saved seed from previous harvest Acquired seed Purchased seed From relatives	Kg Kg Kg Kg
40	Specify the type of control options for the drought stress if it happens	1. 2.	

41	Do you perform the following crop management practices?	Weeding	Y/N	Yes ☐	No ☐
		How many times	1. ☐	2. ☐	3. ☐
		Cultivation (Gusia)	Y/N	Yes ☐	No ☐
		Fertilizer applic.	Y/N	Yes ☐	No ☐
		- If yes, the type		DAP ☐	Urea ☐
		Intercropping	Y/N	Yes ☐	No ☐
		Crop rotation	Y/N	Yes ☐	No ☐
	If yes what are the advantage and disadvantage of such practices?	1. 2.			
42	Do you practice water harvesting system in your field	Y/N		Yes ☐	No ☐
	If yes, what are the advantages from such practice	1. 2.			
43	Seed selection while the crop is in the field?	Y/N		Yes ☐	No ☐
44	If yes what are the base criteria of your selection	Panicle size ☐ Seed size ☐ Seed colour ☐ Plant uniformity ☐ Early maturing ☐			

Check list for Participatory Rural Appraisal

1. How important is farming for the household in your village?
2. How important is sorghum in your village?
3. Is the area under sorghum is increasing or decreasing? Why?
4. What is the average yield of sorghum in good season (adequate rainfall and less pest infestation) and poor seasons (drought and high pest infestation)?

5. Which months do you have adequate amount of sorghum grain for food and which months in shortage? What measures do you take in shortage of sorghum grain?
6. What are the uses of sorghum biomass?
7. What is the land tenure system in your village? How do people get land for farming?
8. What is the trend of young people in the village taking up farming?
9. What are the major sorghum landraces grown in your area?
10. Are there any sorghum landraces disappeared from you village?
11. If yes, what could be the main reason for extinction of the landraces?
12. How is the trend of using improved sorghum varieties from Ministry of Agriculture?
13. What are the cropping calendars, types of weeds, diseases and insects of sorghum in your area?
14. Frequencies of drought (moisture stress) occurrences in your village/ sub region.
15. How do you manage if drought occur in your village in relation to:
    a. Decide which crop to be sown?
    b. The type of sorghum to be grown?
16. What are the drought control options if it occurs?
17. Sorghum post harvest handling
    a. Storage?
    b. Post harvest problems?
    c. Management of post harvest problems?
    d. Marketing problem?

Secondary Data

1. Population of selected sub region? Number of households & average family size? (from Local administration).
2. Land tenure system of the village (from local administration and MoA).
3. What are climatic requirements of sorghum (rainfall, temperature and soil)?

4. Proportion of sorghum grown in the sub region comparing with other cereals (from MoA)
5. Total area (ha) and production (tons) and productivity (t ha^{-1}) of sorghum in the surveyed sub region (from MoA).
6. Area cultivated under improved seed varieties and landraces of sorghum in the previous three years in the specific sub region. (from MoA)
7. Source of seed for supplying farmers in case of emergency. (from local admin and MoA)

Appendix 2. Sorghum accessions from Eritrean gene bank used in the study of genetic diversity analysis

No.	Accession Identifier	Local name	Collection site/region	Characteristics of the accessions				
				Biological status	Adaptation	maturity	Seed colour	Panicle orientation
1	EG 469	Tsaeda Baznay	Gash Barka	Landrace	Lowland	Medium	White	Semi-compact
2	EG 473	Wediferej	Gash Barka	Landrace	Lowland	Medium	Red	Compact and bent
3	EG 480	Koden GB1	Gash Barka	Landrace	Lowland	Medium	White	Compact and
4	EG 481	Wedi susa†	Anseba	Landrace	Midland	Early	White	Compact
5	EG 494	Tsaeda bariyay	Gash Barka	Landrace	Lowland	Early	White	Semi-compact
6	EG 497	Hile An1†	Anseba	Landrace	Midland	Early	White	Compact
7	EG 519	Wedi Mehari	Gash Barka	Landrace	Lowland	Late	White	Semi-compact
8	EG 520	Gumbilu So1	South	Landrace	Highland	Late	Brown	Semi-compact
9	EG 526	Wedi Aker Ans	Anseba	Landrace	Midland	Early	Chalky white	Compact and
10	EG 532	Bicha meshela	South	Landrace	Highland	Late	Yellow	Loose erect
11	EG 537	Anseba	South	Landrace	Highland	Late	Golden white	Semi-compact
12	EG 538	Daguya†	South	Landrace	Highland	Very late	Brown	Open loose
13	EG 540	Tsaeda hile	Gash Barka	Landrace	Lowland	Medium	White	Compact erect
14	EG 544	Koden So1†	South	Landrace	Highland	Late	White	Compact erect
15	EG 546	Embulbul An1	Anseba	Landrace	Midland	Early	Brown	Compact and bent
16	EG 547	Koden GB2	Gash Barka	Landrace	lowland	Medium	White	Compact erect
17	EG 551	Tseda	South	Landrace	Highland	Late	White	Semi-compact
18	EG 554	Gumbilu So2†	South	Landrace	Highland	Late	Brown	Semi-compact
19	EG 555	Wedi keiho	South	Landrace	Highland	Late	Red	Open loose and
20	EG 557	Ganseber†	South	Landrace	Highland	Very late	Golden white	Open semi-
21	EG 584	Wedi Ferej	Gash Barka	Landrace	Lowland	Medium	White	Semi-compact
22	EG 711	Embulbul An2	Anseba	Landrace	Midland	Very early	Brown	Compact and bent
23	EG 717	Letemhret†	Gash Barka	Landrace	Lowland	Medium	Red spotted white	Semi-compact
24	EG 723	Hile An2	Anseba	Landrace	Midland	Early	Red	Compact and bent
25	EG 724	Koden zerzer	South	Landrace	Highland	Late	White	Open-loose
26	EG 726	Koden tsaeda	South	Landrace	Highland	Very late	White	Semi-compact
27	EG 731	Amal 1†	South	Landrace	Highland	Very late	Yellow	Compact erect
28	EG 732	Hile GB1	Gash Barka	Landrace	Lowland	Medium	White	Compact erect
29	EG 735	Koden GB3	Gash Barka	Landrace	Lowland	Medium	White	Semi-compact
30	EG 736	Senadr	Anseba	Landrace	Midland	Medium	Red	Semi-compact

Appendix 2. Continued

No.	Accession Identifier	Local name	Collection site/region	Characteristics of the accessions				
				Biological status	Adaptation	maturity	Seed colour	Panicle orientation
31	EG 745	Koden So2	South	Landrace	Highland	Late	White	Compact erect
32	EG 746	Tsaeda Mashela	South	Landrace	Highland	Late	White	Semi-compact
33	EG 750	Gumbilu So3	South	Landrace	Highland	Late	Yellow	Semi-compact
34	EG 756	Tsaeda meshela	Anseba	Landrace	Midland	Medium	White	Compact bent
35	Kibra	Kibra	Anseba	Landrace	Midland	Early	White	Compact bent
36	EG 772	Tsaeda koden	South	Landrace	Highland	Very late	White	Compact erect
37	EG 775	Zengada†	South	Landrace	Highland	Very late	Dark red	Loose drooping
38	EG 779	Gumbilu So4	South	Landrace	Highland	Late	Brown	Semi-compact
39	EG 782	Tsaeda hile†	Gash Barka	Landrace	Lowland	Medium	White	Compact
40	EG 783	Aklamoy	Gash Barka	Landrace	Lowland	Late	Light red	Semi-compact
41	EG 786	Embulbul GB	Gash Barka	Landrace	Lowland	Early	Light red	Compact bent
42	EG 787	Duruta 1†	Gash Barka	Landrace	Lowland	Medium	Light red	Compact bent
43	EG 789	Ajebsidu	Gash Barka	Landrace	Lowland	Late	Chalky white	Compact erect
44	EG 791	Korekora†	Gash Barka	Landrace	Lowland	Medium	Chalky white	Compact erect
45	EG 794	Wedi Ferej†	Gash Barka	Landrace	Lowland	Medium	Brown	Compact bent
46	EG 797	Wedi Aker GB	Gash Barka	Landrace	Lowland	Early	Chalky white	Compact erect
47	EG 801	Wedi Halibay	Anseba	Landrace	Midland	Medium	White	Semi –compact
48	EG 802	Segurtay	Anseba	Landrace	Midland	Medium	White	Compact bent
49	EG 806	Hiriray GB1	Gash Barka	Landrace	Lowland	Early	Red	Semi-compact
50	EG 812	Senadr keih	Anseba	Landrace	Midland	Early	Red	Semi-compact
51	EG 813	Wedi Ferej	Anseba	Landrace	Midland	Early	Red	Compact bent
52	EG 815	Estif	Gash Barka	Landrace	Lowland	Medium	Chalky white	Oval erect
53	EG 830	Wedi Arbaa†	Gash Barka	Landrace	Lowland	Early	White	Compact erect
54	EG 836	Hugurtay An†	Anseba	Landrace	Midland	Medium	White	Compact bent
55	EG 843	Koden GB4	Gash Barka	Landrace	Lowland	Medium	White	Semi-compact
56	EG 845	Hiriray GB1	Gash Barka	Landrace	Lowland	Early	Red	Compact bent
57	EG 846	Habarat	Gash Barka	Landrace	Lowland	Medium	White	Loose bent
58	EG 849	Hugurtay GB	Gash Barka	Landrace	Lowland	Medium	White	Compact bent
59	EG 850	Koden So3	South	Landrace	Highland	Late	White	Semi-compact
60	EG 855	Gumbilu †	Gash Barka	Landrace	Lowland	Late	Brown	Semi-compact

Appendix 2. Continued

No.	Accession Identifier	Local name	Collection site/region	Characteristics of the accessions				
				Biological status	Adaptation	maturity	Seed colour	Panicle orientation
61	EG 857	Wedi Ferej GB	Gash Barka	Landrace	Lowland	Medium	Red	Compact bent
62	EG 858	Tsaeda Hile†	South	Landrace	Highland	Medium	White	Open loose
63	EG 859	Amal 2	South	Landrace	Highland	Very late	Yellow	Compact erect
64	EG 864	Chimro†	Gash Barka	Landrace	Lowland	Early	Yellow	Compact bent
65	EG 870	Agebsidu	Gash Barka	Landrace	Lowland	Medium	Chalky white	Compact erect
66	EG 873	Wedi Ferej GB	Gash Barka	Landrace	Lowland	Medium	Red spotted white	Compact bent
67	EG 875	Korokora	Gash Barka	Landrace	Lowland	Medium	Chalky white	Semi-compact
68	EG 881	Tsaeda	Gash Barka	Landrace	Lowland	Early	White	Open loose
69	EG 883	Kinabiba 1†	Gash Barka	Landrace	Lowland	Medium	Brown	Loose drooping
70	EG 885	Duruta 2†	Gash Barka	Landrace	Lowland	Medium	White	Semi-loose erect
71	EG 889	Kileaentu	Gash Barka	Landrace	Lowland	Medium	White	Semi-compact
72	EG 890	Kinadirga†	Gash Barka	Landrace	Lowland	Medium	White	Loose drooping
73	EG 893	Kinibiba 2†	Gash Barka	Landrace	Lowland	Medium	Red	Loose drooping
74	EG 896	Kinadirga	Gash Barka	Landrace	Lowland	Medium	Yellow	Semi-loose bent
75	EG 898	Koden GB 5	Gash Barka	Landrace	Lowland	Medium	White	Semi-compact
76	Hamel	Hamelmalo	National	Improved	Mid &	Early	Brown	Semi-compact
77	EG 1075	Hartsetsa	N.Red Sea Red S	Landrace	Lowland	Medium	Light red	Compact bent
78	EG 1076	Letemhret	Anseba	Landrace	Midland	Late	Brown	Semi-loose
79	EG 1157	Hijeri keih	N.Red Sea	Landrace	Lowland	Early	Brown	Semi-compact
80	EG 1168	Hijeri hatsir	N.Red Sea	Landrace	Lowland	Medium	White	Compact erect
81	EG 1172	Hijeri newih	N.Red Sea	Landrace	Lowland	Late	White	Semi-loose erect
82	ICSV 111	ICSV 111 IN	ICRISAT	Improved	Mid &	Medium	White	Semi-loose erect
83	EG 1208	Fetereta	N.Red Sea	Landrace	Lowland	Medium	White	Semi-compact
84	EG 1224	Mahajen	Gash Barka	Landrace	Lowland	Late	Chalky white	Compact erect
85	EG 1233	Wedi Aker	Gash Barka	Landrace	Lowland	Early	Chalky white	Compact erect
86	EG 1235	Gedam hamam	Gash Barka	Landrace	Lowland	Medium	Chalky white	Semi-compact
87	EG 1237	Amal †	South	Landrace	Highland	Late	Brown	Semi-compact
88	EG 1239	Alhiya 1	N.Red Sea Red	Landrace	Lowland	Medium	White spotted Red	Semi-loose bent
89	EG 1246	Tseda meshela	South	Landrace	Highland	Late	White	Semi-compact
90	EG 1256	Kinabiba keih	Gash Barka	Landrace	Lowland	Medium	Red	Loose erect
91	EG 1257	Gedem hamam	Gash Barka	Landrace	Lowland	Medium	Chalky white	Semi-compact

Appendix 2. Continued

92	EG 1258	Feterita eriana	Gash Barka	Landrace	Lowland	Medium	White	Semi-loose erect
93	EG 1259	Tetron	Gash Barka	Landrace	Lowland	Very late	White	Semi-loose erect
94	EG 1261	Hameray	Gash Barka	Landrace	Lowland	Medium	Red	Loose erect
95	EG 2161	Hijeri	N.Red Sea	Landrace	Lowland	Medium	White	Semi-compact
96	EG 2453	Bariyay 1	N.Red Sea	Landrace	Lowland	Medium	Red	Semi-loose bent
97	EG 2456	Alhiya 2	N.Red Sea	Landrace	Lowland	Medium	White spotted Red	Semi-loose bent
98	EG 2457	Bariyay 2	N.Red Sea	Landrace	Lowland	Medium	Red	Semi-loose bent

† These 22 accessions were also used in the genetic diversity study by Ghebru, Schmidt, & Bennetzen, 2002.; **AI**. Accession identifier

- **Maturity intervals**: Early = 80-105 days, Medium = 105-130 days, Late = above 130 days
- **Adaptation category:** Lowland = below 900m, midland = 900m-1500m, Highland= above 1500 m above sea level
- GB = Gash Barka, An = Anseba, So = south regions

Appendix 3. Forty two (42) regional reference sets of sorghum genotypes obtained from Kiboko, Kenya, ICRISAT used for the genetic diversity study

S.N	Local Name	Origin	S.N	Local	Origin
99	Gambella 1107	Ethiopia	120	Jeri	South Sudan
100	89MW5003	Ethiopia	121	Medenge	South Sudan
101	M36121	Ethiopia	122	Okabir	South Sudan
102	IS 11758	Ethiopia	123	Deri	South Sudan
103	ZZ #308	Ethiopia	124	Kodu kine	South Sudan
104	KARI Mtama 1	Kenya	125	Oderi	South Sudan
105	KARI Mtama 3	Kenya	126	Framida	Sudan
106	Ochuti	Kenya	127	Gadam	Sudan
107	E 6518	Kenya	128	SRN 39	Sudan
108	Ofunjo	Kenya	129	Tabat	Sudan
109	Nakhadabo	Kenya	130	AG 8	Sudan
110	Makueni local	Kenya	131	AG 3	Sudan
111	Kiboko local 2	Kenya	132	Tegemeo	Tanzania
112	Teso#17(Etoroit)	Kenya	133	Pato	Tanzania
113	Siaya#66-2(Gopari)	Kenya	134	Macia	Tanzania
114	Siaya#50-3	Kenya	135	Wagita	Tanzania
115	Siaya# 78	Kenya	136	Ex Tanzania	Tanzania
116	Asinge Local	Kenya	137	IS 8193	Uganda
117	Siaya#42	Kenya	138	Serena	Uganda
118	Siaya#46-2	Kenya	139	Seredo	Uganda
119	Siaya#82-2	Kenya	140	5DX 160	Uganda