CHARACTERIZATION AND ASSOCIATION MAPPING FOR DROUGHT ADAPTATION IN ETHIOPIAN SORGHUM [*Sorghum bicolor* (L.) Moench] GERMPLASM

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Characterization and Association Mapping for Drought Adaptation in Ethiopian Sorghum [*Sorghum bicolor* (L.) Moench] Germplasm

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DEDICATION

This thesis was dedicated in memory of my late father.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

Alemuwas born on 30 May 1990 at Libbo-kemkem Woreda, South Gondar Zone in Amhara Region. He attended his elementary school from 1997 to 2000 at Kura seckel elementary school, from 2001 to 2004 at Ambomeda elementary schooland then joined to Addis-Zemen junior secondary schools from 2005 to 2008. Following the completion of his secondary education, he joined to Gondar University Faculty of Natural and Computational Science in 2009 and graduated with Bachelor of Science degree in Biotechnology in 2011. In October 2014, he joined College of Agriculture and Veterinary Medicine, Jimma University for his M.Sc. study in Agriculture (Plant Biotechnology).

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ACRONYMS AND ABBREVIATIONS

AM	Association Mapping
CTAB	Cetyltrimethyl-Ammonium Bromide
D’	The standardized linkage disequilibrium coefficient
EDTA	Ethylene Di Amine Tetra Acetic Acid
FAOSTAT	Food and Agriculture Organization *Statistics* Division
GLM	General Linear Model
IBC	Institute of Biodiversity Conservation
LD	Linkage Disequilibrium
LE	Linkage Equilibrium
MAS	Marker Assisted Selection
Mb	Mega Base Pair
MCMC	Markov Chain Monte Carlo Algorithm
MLM	Mixed Linear Model
QTL	Quantitative Trait Loci
R^2	The square of the correlation coefficient between alleles at two loci
RIL	Recombinant Inbred Line
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats

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ABSTRACT

Ethiopia is frequently affected by drought. As a result, drought is the major factor that affects sorghum production in Ethiopia. Knowledge of drought tolerance related traits and their mechanisms are the key component in selecting genotypes that withstand the effects of drought. The objectives of this study were to assess genetic variability among sorghum germplasm for root and shoot traits, to map chromosomal regions (QTL) associated with root and shoot traits related to drought adaptation and to identify SSR markers associated with drought adaptation traits.The experiment was carried out at Jimma University, College of Agriculture and Veterinary Medicine under greenhouse condition. One hundred thirty-six sorghum genotypes were characterized for twelve traits using a high throughput root phenotyping platform in a randomized complete block design with three replications. Among these genotypes, 108 of them were used for studying population structure and trait-marker association analysis using 39 SSR markers. The analysis of variance indicated that highly significant difference ($P<0.01$) were observed among the genotypes for all thestudied traits. The coefficients of correlation among different drought related traits showed that there was a significant positive and negative association among different drought related traits. The first three principal components (PCs) with eigenvalues greater than one accounted for 56.4% of the total genotype variation, the remaining 43.60% accounted for the last nine principal components.Moderate genotypic variation was exhibited for leaf area, shoot fresh weight, shoot dry weight, root to shoot ratio and root angle.All 136 genotype were grouped into four clusters whereby adifferent member within a cluster being assumed to be more closely related in terms of the trait under consideration with each other than that member in different clusters is. While highest phenotypic variation was exhibited for leaf area, shoot fresh weight, shoot dry weight, root dry weight and root to shoot ratio. Broad sense heritability ranged from 19.35% for root length to 71.08% for shoot fresh weight at seedling stage and high heritability was recorded for shoot fresh weight (71.08%), leaf area (70.22%) and root angle (66.22%). High heritability combined with high genetic advance was observed for shoot fresh weight, root angle, and leaf area. The 108 genotypes were grouped into three distinct subgroups. The plots of LD (r^2) for pairs of loci, versus the genetic distance in cM, showed a clear trend on linkage disequilibrium decay in the studied genotypes and based on trend line it is around 15-20 cM. A total of 25 significant marker-trait associations/QTLs ($P \leq 0.05$) were detected with 14 SSR markers and these markers were localized with previously identified QTL. As a future line of work, genotypes that showed desirable phenotypes such as narrow root angle need to be evaluated under field condition to verify their performance and thereby they can be used in the breeding programs. As this study is the first in Ethiopia, the identified QTLs need to be validated through repeated phenotypic measurement in independent or related populations. The SSR markers found to be associated with traits need to be validated before their use in marker-assisted selection.

Keywords:*Association mapping, Drought, imaging, phenotyping, QTLs, Root angle & SSR*

1. INTRODUCTION

Sorghum (*S. bicolor*) is an annual crop andbelongs to the genus Sorghumin the Poaceae family (Clayton and Renvoize, 1986). It is a diploid (*2n=2x=20)*, principally self-pollinating and relatively easy to cross and self-fertilize (Mullet *et al.*, 2014).

The world sorghum Production in 2015/2016 cropping season was 60.16 million tons. In 2016/2017 cropping season, 64.19 million tons were estimated and represent an increase of 4.03 million tons or a 6.7% in sorghum production around the globe (USDA, 2016). According to this report, Ethiopia is ranked seventh in the world and third in Africa next to Nigeria and Sudan by producing 3,700,000 metric tons. In Ethiopia, out of 12.8 million hectares covered under cereals, sorghum took up 1.8 million hectares of the total crop area in 2013/2014. Sorghum was the third largest cereal crop after tef and maize in terms of area coverage and total production (CSA, 2015). The main use of sorghum in Ethiopia is for human consumption in the form of 'injera', bread, porridge, local beer and other products. The stalk is used for animal feed, firewood, and broom making and construction rural houses.

The great advantage of sorghum is that it can grow or become dormant under adverse conditions and resume growth after relatively severe drought in arid regions where droughts cause other crops to fail. This isdue to its C4 photosynthetic nature, extensive root system and the presence of waxy leaves (Muui*et al*., 2013). Its efficient use of water also makes it the crop of choice to boost food security in drought-stricken regions (Ediage *et al.*, 2015). Moreover, researchers have indicated that it has inherent climate resilience that is likely to become more important under harsh environmental conditions (Paterson, 2014).Having a small genome (~730Mb) (Patterson*et al.*, 2009) is important for the study and development of molecular markers which can be used to assess genetic diversity and marker assisted selection (Sun *et al.*, 2011, Besufekad and Bantte, 2013).

Biotic and abiotic factors such as pests, insects, diseases, weeds, temperature, the wind, and droughts are the major constraints in sorghum production (Worthmann*et al.*, 2009). Among the different abiotic stresses, the lack of water, drought, is one of the major constraints that limit crop production and quality and it is by far the most complex and devastating on a global scale (Pennisi, 2008). "Now a day's drought is the major environmental factor in the world that limits the productivity of crops. It is the most important natural hazard facing our world today. Drought and desertification, threaten the livelihood of over one billion people in

more than 110 countries around the world and each year causes an estimated US $ 42 billion in lost agricultural productivity" (Annan, 2005).Therefore to meet the food needs of the human population, determining the genetic basis of complex traits which is responsible for drought adaptation is a major goal. Association mapping has been a key tool for identifying the genetic basis of quantitative traits in plants. Climate change is favoring sorghum production in Ethiopia due to frequents even in areas that were originally favorable for crop production.

Recent research at the University of Queensland in Australia has identified significant QTL for root angle that co-locates with QTL for stay-green (Mace *et al*., 2012). This makes root angle a potentially valuable trait in developing drought tolerant sorghum varieties. Hence, knowledge of genetic variability for root and shoot traits is the key component in selecting better parents that withstand drought for the future breeding program. As water resources for agricultural uses become more limiting and limiting, the development of drought-tolerant lines will become increasingly important.Singh *et al.* (2011) reported genetic variation in the structure and function of sorghum root systems (nodal root angle) and indicated that genotypic differences in nodal root angle in seedling stage may result in differences in water extraction patterns of mature plants. Hamada *et al. (*2012) identified novel quantitative trait loci (QTL) controlling the gravitropic and hydrotropic responses of wheat roots. Moreover, they identified one QTL for seminal root number per seedling on chromosome 5 and two QTL for seminal root elongation rate on chromosomes 5 and 7. They concluded that gravitropic and hydrotropic responses of wheat roots, which play a significant role in establishing root system architecture, are controlled by independent genetic factors. In support of this, Nakamoto and Oyanagi, (1994) also reported that the growth angle of nodal roots was positively correlated with the growth angle of the seminal roots of seedling stages of the crop.Borrel (2000).identified sources of "stay-green" drought tolerance derived from sorghum lines native to Ethiopia. .As a center of origin and diversity for domesticated sorghums, Ethiopia may harbor unique wild germplasm that is worthy of further conservation efforts.Besufekad &Bantte (2013) worked on drought Tolerance and identified four SSR markers associated with days to 50% flowering, panicle exertion and grain weight per panicle.

Ethiopia, where sorghum is believed to be first domesticated and where the greatest genetic variation for both cultivated and wild sorghum is found, is a rich source of sorghum

landraces, which are valuable sources of desirable genes such as for drought tolerance (Amsalu *et al*., 2000). Around 10,000 sorghum germplasms have been collected by the Ethiopian Biodiversity Institute. But these germplasm collections have not yet been systematically characterized for drought related traits, including root and shoots. Its productivity has not kept pace with increasing demand hence, their potential as sources of useful genetic variation has not been exploited,This is due to lag of germplasm improvement efforts, relative to other cereals, and the extreme environmental conditions(drought, temperat ure, etc.) present in the country. Studies of sorghum root and shoots architectures at seedling stage through high-throughput phenotypic platform also have been less common despite the fact that plant architecture particularly root angle and leaf area have a direct impact on drought adaptation There also limited research work on the screening pattern of the germplasm collection for drought tolerance and identification of chromosomal regions associated with this trait. Root and shoot phenotypic data, together with genotyping by SSRs will enable an association genetics approach in identifying chromosomal regions associated with root and shoot traits. Therefore, in light of the above points the present study was conducted with the following specific objectives:

1. To assess genetic variability among sorghum germplasms for root and shoot traits
2. To map chromosomal regions (QTL) controlling root and shoot for drought adaptation
3. To identify SSR markers significantly associated with drought adaptation traits

2. LITERATURE REVIEW

2.1. Origin and Botanical classification of sorghum

*[Sorghum bicolor (*L*) Moench]* is a genus of plants in the grass family. It belongs to the Poaceae family and tribe Andropogoneae (Clayton and Renvoize, 1986). It is representing all annual cultivated, Wild and weedy sorghums along with two rhizomatous taxa, S. Halepense and S. Propinquum (Wet, 1978). Having a small genome (~ 730Mb) (Paterson,*et al.*, 2009) makes a model crop in the grass family.

The previous study shows that Sorghum was domesticated in Ethiopia some 3000 or more years ago from the wild sorghum species (*Sorghum arundinaceum*) by disruptive (uncontrollable) selection, and from there it spread to other parts of the world (Doggett, 1965). Ramu*et al.*, (2013) also reported as Ethiopia is the center of origin for this crop and yet little is known about the genetic structure of even the present wild populations. However, most evidence point to the northeast quadrant of Africa, which includes Sudan, Eretria, and Ethiopia as the center of origin of sorghum, where the greatest variabilities are found (Purseglove, 1975).

2.2. Diversity and ecological adaptation of sorghum

One of the world centers of crop evolution and origin, Ethiopia has long been recognized as an important area of diversity for several major and various minor crops (Kebede, 1991).The amount of genetic variability available in sorghum (*Sorghum bicolor (*L.) Moench) is vast and much of the genetic variability is available in areas of the first domestication of the crop (Africa) and regions of early introduction (Asia) (Amsalu *et al*., 2000). In Africa, the genetic variability is available in both cultivated species and wild progenitors of the crop (Gebrekidan, 1982). Germplasm is evaluated for growth traits, agronomic performance, yield and responses to biotic and abiotic stresses using visual observations and measurement on standard scales. Soil, water (rainfall) and solar energy constitute the natural resources of semi-arid crop production systems with a well-established relationship of subsistence adaptation.

The Earth is a water-scarce planet. Feeding more people with less water is a major challenge facing humanity (Foley *et al.*, 2011). With the projections of global climate change and rapid population growth, the study is highly aimed at understanding abiotic stress tolerance and

adaptation, with a particular emphasis on maintaining yield under hot and dry environmental conditions. Because of the innate nature of sorghum's tolerance to drought and moderate tolerance to salinity stresses, sorghums believe to be logical model crop in such studies amongst cereals (Ngara and Ndimba, 2014). Sorghum populations adapted to limited water conditions can maintain a higher relative growth rate under water restriction as compared to those adapted to more humid conditions (Leguizamón and Acciaresi, 2014).

2.3. Production, Utilization, and constraint associated with sorghum

According to the U.S Grains Council, 2012, world sorghum production has risen slightly from 60 million metric tons to 65 million metric tons over the past decade. In 2009, 82% of the harvested area of sorghum was from Africa and Asia, where average yields were 904 and 1,096 kg ha-1, respectively. The United States harvested 2.2 million ha, while Europe harvested 151,526 ha and produced 4,355 and 4,451 kg ha-1, respectively (FAO, 2011). In Ethiopia, according to the Ethiopian CSA data report within 2014/15 (2007E.C.) of Belg season, 36917.84 hectares of land were covered by sorghum and 168371.17 quintals obtained.

Sorghum was mainly used for the different industrial sectors such as animal feed, alcohol distilleries, and starch industries. The grains used more recently in the United States as a feedstock for the renewable fuels industry and into the gluten-free food market. In Africa and India, it is an important part of the diet in the form of unleavened bread, boiled porridge or gruel, and specialty foods such as popped grain and beer. Grain sorghum is becoming a potential field crop in Europe for cattle feed (Benji and Dahlberg, 2004).

The continuing demand for sorghum is reflected in the trend for increasing area under sorghum in Africa over the last fifty years. Unfortunately, however, crop productivity has not kept pace with increasing demand, due mainly to a lag in crop improvement efforts in sorghum and millets, relative to other cereals, and the extreme environmental conditions and resource constrained, low-input farming systems where these crops are grown. Furthermore, in such dry land environments, the issues of climate variability, change, and land degradation is acute with a lack of progress the result of neglect, remoteness, and weak national institutions. Despite these factors, there is a strong case for stepping up the efforts towards the development of technologies (germplasm improvement, agronomic management),

markets and institutions to advance the case for sorghum and millets in the dryland tropics of Africa.

There are biotic and abiotic factors which aresignificant constraints for sorghum production. The combination of soil nutrients and water deficits during crop establishment, early growth and grain fill period are also the most important constraint to this problem. Plant diseases especially fungal diseases such as Striga, Smut, Rust, anthracnose and Grain mold as well as insects such as stem borer complex Shoofly and the grasshopper/locust complex can result in complete crop loss (Wortmann*et al.*, 2009).

2.4. Screening for drought adaptation in sorghum

Among the different abiotic stresses, drought is the major factor limiting yields in most of the sorghum-growing areas of the world and it is by far the most complex and devastating on a global scale (Pennisi, 2008). “Now a day’s drought is the major environmental factor in the world that limits the productivity of crops. It is the most important natural hazard facing our world today. Drought and desertification, threaten the livelihood of over one billion people in more than 110 countries around the world and each year causes an estimated US $ 42 billion in lost agricultural productivity” (Annan, 2005). Drought stress in sorghum depends on some factors which will include the duration of exposure to either high or low temperature, the activity or stage of growth of the exposed tissue and finally the thermal adaptation of the particular sorghum cultivar(Peacock, 1982). Rapid population growth and environmental variability over the next century are expected to threaten global food security. In the face of these challenges, crop yield for food and fuel must be maintained and improved using fewer input resources. Therefore, the future research program should be focused on developing of crops and plants with enhanced tolerance to drought (Mittler, 2006). And there are genetic loci that ensure productivity in drought condition by activating specific molecular and physiological changes to minimize damage within the germplasm of sorghum, their wild relatives, and species that are adapted to extreme environments. These loci control the growth pattern of root and shoot traits. Good characteristics of the plant having these loci can minimize the amount of water loss through evaporation and have roots interring deep to the soil and extract easily underground water. Several methods in both field and controlled-environment facilities are commonly being used for screening drought tolerance. In this document, the focus will be on the architecture of root and shoot traits:-(drought tolerance) screening tools.

2.5. Mechanisms of Drought adaptation

Drought tolerance in crops is the ability to grow, flower and display economic yield with minimum loss in a water deficit environment relative to a water-constraint free production environment (Farooq *et al*., 2009). To improve crop productivity, it is necessary to understand the mechanisms of plant responses to drought conditions with the ultimate goal of improving crop performance in areas where rainfall is limiting (Tuinstra*et al*., 1996). Plants have developed numerous strategies to control water status and to survive under drought which includes escape, avoidance and tolerance strategies.

1) Drought escape is the ability of a plant to complete its developmental stage before serious soil and plant water deficits develop. Plants that escape drought exhibit a rapid phenological development and ahigh degree of developmental plasticity, being able to complete their life cycle before physiological water deficit occurs. Escape strategies rely on successful reproduction before the severe stress is perceived. In environments with terminal drought stress and where physical or chemical barriers inhibit root growth, drought escape through early flowering and/or short growth duration is advantageous (Farooq *et al*., 2009). On the other hand, later flowering can be beneficial in escaping early season drought if drought is followed by rains (Ludlow and Muchow, 1990). Under non-stress conditions, however, late-flowering varieties tend to yield higher than the early flowering ones (Turner, 1986; Ludlow and Muchow, 1990). This is because the early flowering varieties are likely to leave the yield potential unutilized (Ludlow and Muchow, 1990).

2.) Drought (or dehydration) avoidance is the plant's ability to retain a relatively higher level of hydration under conditions of soil or atmospheric water stress (Hussain, 2006). Plants that avoid dehydration employ either reduced transpiration (water savers) or develop means other than reduced transpiration (water spenders) such as special root features to increase water uptake, leaf and stomata characteristics to reduce water loss and osmotic adjustment to lower the osmotic potential or in combination of all (Farooq *et al*. 2009). Osmotic adjustment helps some plants to maintain turgor pressure through the active accumulation of solutes (osmoprotectants, or compatible solutes). These molecules, which act as osmotic balancing agents, are accumulated in plant cells in response to drought stress and are subsequently degraded after the alleviation of the stress (Ludlow and Muchow, 1990). Osmo.protectants include amino acids, sugar alcohols, polyols and quaternary ammonium and tertiary sulfonium compounds and help in protecting cell components from the adverse effect of

water loss through theexpression of cell rescue mechanisms and through increased capacity of plants to recover after stress (Ludlow and Muchow, 1990).

3) Dehydration tolerance describes the ability of plants to continue metabolizing and growth at low leaf water potential and to maintain growth despite dehydration of the tissue or to recover after release from stress conditions (Hussain, 2006). Translocation is considered as one of the most dehydration-tolerant processes in plants which can proceed functioning at levels of water deficit sufficient to inhibit photosynthesis.

2.5.1. Drought resistance traits

Over the years many physiological, morphological, and developmental traits have been suggested to be useful in improving drought resistance. These traits can be broadly categorized according to whether they are either constitutive (i.e. always expressed by the plant) or induced (i.e. only expressed in response to drought conditions), or in some cases a combination of the two. Most commonly 12 drought resistant traits are *Phenology,Seedling vigor, Growth habit and plant architecture,Leaf xeromorphy, Root distribution and anatomy,Osmotic adjustment,Current assimilate redistribution, Remobilization of assimilates, Leaf senescence, C13 discrimination,Excised leaf water loss andCombinations of thetrait.*

2.6. Phenotyping of traits for association mapping

In recent years, genetic tools for profiling crop germplasm has benefited from rapid advances in DNA sequencing, and now similar advances are needed to improve the throughput of plant phenotyping. Plant phenotyping is any procedure of measuring plant characteristics that can be expressed quantitatively or qualitatively, at the level ranging from single cells, through whole plants (Dhondt *et al*., 2013), to field plots. The goal of plant phenotyping and analysis is to measure the physiological, growth, development, and other phenotypic properties of plants through automated processes. For crop improvement efforts, to meet the expected requirement for increased crop yield potential in the coming decades (Bruinsma, 2009 and Tillman *et al.*, 2011), crop scientists and breeders will need to connect phenotype to genotype with high efficiency. This connection has been partly facilitated through tremendous gains in biotechnology, including marker-assisted selection, association mapping and the increasing availability of low-cost DNA sequence information (Ingvarson and Street, 2011). Association

mapping studies often are long-term projects, with phenotyping being conducted over the years in multiple locations (Flint-Garcia*et al*., 2005).

However, the biotechnology advances have not been matched by complementary methods to effectively and efficiently phenotype at the crop scale due to the limited availability of field-based high-throughput phenotyping methods (Tuberosa, 2014 and Cobb*et al.*, 2013); the large variability of phenotyping protocols; the multitude of phenotypic traits that are measured; and the dependence of these traits on the environment (Krajewski *et al., 2015).*Since the collection of high-quality phenotypic data is essential for association mapping, each researcher should assess the quality of the experiment for which they are responsible.

2.7. QTLs discovery for drought adaptation

The plant architectures are considered highly dynamic and respond to changes in environmental parameters, including stresses such as drought, nutrient deficiencies, waterlogging, and salinity. Considerable understanding of growth and development for root and shoots both at the whole plant level and at the molecular level is achieved. Therefore the next step is to devise strategies for identification of important genes/QTLs associated with various root and shoot traits followed by their validation and subsequently introgression into crops through molecular breeding approaches (Varshney*et al*. 2011b). Regarding genetic control, root and shoot traits are believed to be complex controlled by some genes/QTLs. QTLs are a segment of DNA that affects a quantitative trait or the region within the genome that contains genes associated with a particular quantitative trait. Therefore, understanding the genetic control of root development and functions of root trait components are considered inherently important for breeding improved cultivars for root traits that are well adapted to variable climates. Genetic linkage map constructions have been recognized as an essential tool for molecular plant breeding using DNA markers because they are neutral, lack epistasis and are simply inherited in a Mendelian nature (Stuber *et al*, 1992).

QTLs can be categorized according to the stability of their effects across environmental conditions. A ‘‘constitutive’’ QTL is consistently detected across most environments, while an ‘‘adaptive’’ QTL is detected only in specific environmental conditions or increases in expression with the level of an environmental factor (Vargas *et al*., 2006). The sensitivity to environmental conditions may be due to the responsiveness of regulation (e.g. transcription)

of the QTL gene to an environmental cue. Alternatively, differences in response may have an indirect cause (e.g., genotypes with larger root systems will be less affected by water shortage or nutrient deficit, so genes controlling root development may underpin QTLs defined by abscisic acid [ABA] content, stomatal conductance or biomass accumulation). Additionally, QTLs that alter flowering time often influence yield under water or nutrient deficit because the duration of the crop life cycle affects the timing and intensity of the stress experienced by the plants (Reynolds and Tuberosa, 2008).

However, one of the limiting factors in the genomic analysis of many plant species, including sorghum, is that many of QTLs reported from experimental populations developed from a bi-parental cross often turn out to be unique to a specific genetic background, and there has been limited success in applying the results across breeding populations. Linkage analysis in plants typically assigns QTLs to 10 to 20 cM intervals because of the limited number of recombination events that occur during the construction of mapping populations (Holland, 2007). While hundreds of linkage analysis studies have been conducted in various plant species over the past two decades, only a limited number of identified QTLs were cloned or tagged at the gene level (Price, 2006).

Therefore, association analysis, whereby genes and QTL are detected in a random set of genotypes from a mixed genetic background that has accumulated a much larger number of crossing-over events since their last common progenitor, is a viable solution to this problem (Breseghello and Sorrells, 2006). Such association studies have proved useful for the identification of candidate loci associated with numerous traits in theanimal as well as plant species (Appels *et al*., 2013; Korte and Farlow, 2013).

2.8. Association mapping

Association mapping is a method of mapping quantitative trait loci that takes advantage of historic linkage disequilibrium to link phenotypes to genotypes. It is based on the idea that traits that have entered to a population only recently will still be linked to the surrounding genetic sequence of the original evolutionary ancestor. It harnesses the genetic diversity of natural populations to potentially resolve complex trait variation to single genes or individual nucleotides.

As a new alternative to traditional QTL-mapping, in biparental crosses, population-based association studies,have the following advantages. It has broader genetic variations within the

wider background for marker-trait correlations. because of the utilization of recombination events from larger number of meiosis throughout the germplasm developmental history, the likelihood for a higher resolution mapping. It has the possibility of exploiting historically measured trait data for the association and no need for the development of expensive and tedious biparental populations makes the approach time-saving and cost-effective (Yu and Buckler, 2006).

Based on the scale and focus of a particular study,generally,the two approaches of commonly applied association mapping techniques are whole genome scans (Kraakman *et al.,* 2004) and candidate gene approach (Wilson *et al.,* 2004). Whole genome scans focus on identification of genomic regions on all chromosomes related to the trait of interest. It surveys genetic variation in the whole genome to find signals of association for various complex traits (Risch and Merikangas, 1996). It is a compressive approach to search the genome systematically for casual genetic variation. A large number of markers were tested for association with various complex traits, and prior information regarding candidate genes is not required. Success and resolution of genome scans are dependent on the extent of LD. For example, increased LD decay, often represented by plotting LD versus genetic distance, requires a large number of closely linked markers, rendering the use of genome scans more laborious.

Candidate geneassociation mapping relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits. Where a candidate gene for a trait has been identified, polymorphisms within the gene can be correlated with phenotypic variation (Thornberry *et al.,* 2001) and are most useful when LD decays rapidly with increasing physical distance. Candidate genes are selected based on prior knowledge from the mutational analysis, biochemical pathway, or linkage analysis of the traits of interest. An independent set of random marker needs to be scored to infer genetic relationships. It is low-cost, hypothesis - driven and trait specific approach but will miss other unknown loci.

Although association analysis shows great promise as an efficient and valuable tool for gene discovery, the disadvantages of this approach are mainly Type I errors; associations could be caused by population structure, and there would be a lack of linkage information among the markers identified for significant associations. All these can be attributed to population stratification caused by gene drift, founder effects or selection (Pritchard *et al*., 2000). Therefore, the analysis of marker-trait associations must account for the presence of

population structure. Failure to do so can cause the detection of spurious associations between traits and unlinked markers.

In AM studies, several methods have been proposed for estimating population structure and modeling, including distance and model-based methods (Pritchard *et al.,* 2000; Peleg *et al.,* 2008). Distance based estimates of population structure are based on clustering of individuals with pair-wise genetic distance estimates between individuals (Nei, 1978). In contrast, model-based methods assign individuals probabilistically to one or more subpopulation. The most common model-based approach is Bayesian modeling where allele frequencies are used to estimate the likelihood of an individual belonging to a particular subpopulation. This approach allows assign of individuals to respective populations that can be integrated into statistical models to account for population structure in AM studies. The software STRUCTURE (Pritchard *et al.,* 2000) has been developed to account for population structure and has been implemented in AM studies in some crop species.

In maize, a major QTL originally reported for leaf ABA concentration (Tuberosa *et al*., 1998) was later shown to affect root size and architecture (Giuliani *et al*., 2005b) and grain yield (Landi *et al*., 2007).Recently, Uga *et al*., 2011, 2013 used genetic mapping to identify a major root angle QTL in rice, (DRO1–for Deeper rooting 1) and then cloned the DRO1 gene responsible for this QTL via fine-scale mapping. For the first time, Mace *et al., 2012* were reported *four* QTL for nodal root angle (*QRA*). The author also reported three QTL for root dry weight, two for shoot dry weight, and three for plant leaf area in sorghum. Besufekad and Kassahun, 2013 reported that four SSR markers consistently associated with days to 50% flowering, panicle excretion and grain weight per panicle.

2.9. Linkage disequilibrium mapping

Genetic linkages are the coinheritance of different loci within a genetic distance on the chromosome. Linkage equilibrium occurs when the genotype present at one locus is independent of the genotype at a second locus. Linkage disequilibrium occurs when genotypes at the two loci are not independent of another. The term linkage disequilibrium is misleading for two reasons. First, non-random associations of alleles at two loci can occur even if the two genes are unlinked. Second, just because two loci are linked this does not mean that they will be in linkage disequilibrium. Usually, there is significant LD between

sites that are more distant or sites located on different chromosomes, caused by some specific genetic factors (Stich *et al., 2006).*

*D*_ and *r*2 are the most commonly used measures of LD (Gupta *et al*., 2005). The r^2, the square of the correlation coefficient between the two loci have more reliable sampling properties than *D*_ with the cases of low allele frequencies. Considering the objective, the most appropriate LD quantification measure is r^2 that is also an indicative of marker-trait correlations (Oraguzie*et al.*, 2007). The r^2 value varies from 0 to 1, and it will be equal to 1 when only two haplotypes are present. The r^2 value of equal to 0.1 (10%) or above considered the significant threshold for the rough estimates of LD to reveal an association between pairs of loci (Whitt and Buckler, 2003).

LD can be calculated using available haplotyping algorithms (Oraguzie*et al.*, 2007). Several computer software packages are available and can be utilized for calculation of LD using variety type of molecular markers.

Graphical display of pair-wise LD between two loci is very useful to estimate the LD patterns measured using a large number of molecular markers. The large red blocks of haplotypes along the diagonal of the triangle plot indicate the high level of LD between the loci in the blocks, meaning that there has been a limited or no recombination since LD block formations. Some of the software packages measuring LD such as "Trait Analysis by association, Evolution, and Linkage" (TASSEL) (Whitt and Buckler, 2003) and Power Marker (Liu and Muse, 2005.) have LD graphical display features. The strong block-like LD structures are of a great interest in association mapping which simplifies LD mapping efforts of complex traits (Zhang *et al.*,2002) LD blocks are very useful in association mapping when sizes are calculated, which suggest the needs for the minimum number of markers to efficiently cover the genome-wide haplotype blocks in association mapping.

3. MATERIALS AND METHODS

3.1. Genetic material

The genetic materialsconsisted of 136 germplasms (127 landraces and 9 released varieties). Landraces were collected from all sorghum growing woredas of the country by IBC of Ethiopia and released varieties were provided by Melkasa and Sirinka Agricultural Research Centers. From 136 germplasm, only 108 germplasms (101 landraces and 7 released varieties) were used for molecular evaluation due to the unavailability of molecular data for the remaining 28 germplasms.

Table.1. List of sorghum landraces used in the study

Entries.	Germplasm	Region	Zone	Wereda	Altitude
1	69046	Oromia	Bale	Ginir	1630
2	69057	Oromia	Bale	Ginir	1630
3	69092	Oromia	NA	Na	
4	69094	SNNP	Gamogofa	Bako gazer	1410
5	69105	SNNP	Gamogofa	Hamer bena	500
6	69183	Oromia	Mirab Meyiso	Mieso	1460
7	69192	Oromia	Harerge	Mieso	1530
8	69210	Amhara	Wello	Bati	1640
9	69236	Oromia	Harerge	Doba	1800
10	69238	Oromia	Harerge	Doba	1800
11	69286	Amhara	D/Wello	Sayint	NA
12	69306	Amhara	S/Gondar	Debark	1470
13	69371	Gambelia	Illubabor	Itang	550
14	69391	Gambelia	Illubabor	Itang	550
15	69392	Gambelia	Illubabor	Itang	550
16	69468	Gamella	Illubabor	Abobo	530
17	69492	SNNP	Gamogofa	Bonke	1150
18	69494	SNNP	Gamogofa	Bonke	1150
19	70068	Oromia	Shewa	Boset	1450
20	70075	Oromia	Shewa	Adama	1600
21	70301	Tigray	Mehakeleay	Mychew	NA
22	70306	Oromia	Harerge	Bale	1720
23	70537	SNNP	GuraGe	Cheha	NA
24	71021	Oromia	Harerge	Meta	NA
25	71370	Oromia	Harerge	Kersa	NA
26	71418	Tigray	Mirabawi	Kafta humera	710
27	71421	Tigray	Mirabawi	Kafta humera	710
28	71422	Tigray	Mirabawi	Kafta humera	710

Table.1.(Continued …)

Entries.	Germplasm	Region	Zone	Wereda	Altitude
29	71425	Tigray	Mirabawi	Kafta humera	710
30	71477*	Tigray	Debubawi	Enderta	NA
31	71522	Oromia	Mirab Harerge	Habro	NA
32	71536	Oromia	Mirab Harerge	Habro	NA
33	71539	Oromia	Mirab Harerge	Habro	NA
34	71570	Gambella	Illubabor	Gambella	630
35	71590	Tigray	Mehakeleay		NA
36	71621*	Gambella	Illubabor	Gambella	
37	71657	Gambella	Illubabor	Gambella	630
38	71744*	Amhara	Semen Gondar	Chilga	
39	71748	Amhara	Semen Gondar	Chilga	1000
40	71788	Amhara	Misirak Gojam	Guzamn	NA
41	71795	Amhara	Misirak Gojam	Guzamn	NA
42	71810	Oromia	Na	Na	NA
43	72451*	Amhara	Debub Wello	Ambassel	NA
44	72588	Amhara	Debub Wello	Kalu	NA
45	72997	Affar	Zone 1	Ayisaita	1450
46	73067	Amhara	Semen Wello	Weldiya	NA
47	73068	Amhara	Semen Wello	Weldiya	NA
48	73096	Amhara	Debub Wello	Kalu	NA
49	73341	Tigray	Mehakeleay	Adwa	1400
50	73358*	Tigray	Mehakeleay	Adwa	NA
51	73636*	Amhara	Misirak Gojam	Enemay	NA
52	73637	Amhara	Misirak Gojam	Enemay	NA
53	73641	Affar	Wello	Ayisaita	NA
54	73646	Amhara	Misirak Gojam	Enemay	NA
55	7 3762	Tigray	Debubawi	Enderta	NA
56	73797	Tigray	Debubawi	Enderta	NA
57	73992*	Tigray	Mirabawi	Tahtaykoraro	NA
58	74097	Amhara	Semen Wello	Gubalafto	1470
59	74108	Tigray	Debubawi	Rayazebo	NA
60	74115	Tigray	Debubawi	Rayazebo	NA
61	74262	Amhara	semen Shewa	Efratana gidim	1420
62	74268	Amhara	Semen Shewa	Gerar jarso	
63	74703	SNNP	Benchi Maji	Tahtaykoraro	NA
64	74761*	NA	NA	NA	NA
65	74766	Oromia	Semen Shewa	Ejerie	1600
66	74935	Tigray	Mirabawi	NA	NA
67	74999	Oromia	Mirab Sewa		NA
68	75000	Oromia	Mirab Shewa		NA
69	75030*	Amhara	Semen Gondar	Gondar Zuriya	NA

Table.1. (Continued……)

Entries.	Germplasm	Region	Zone	Wereda	Altitude
70	75066	Oromia	Arssi	Merti	100
71	75129	Oromia	Jimma	Mana	NA
72	75140	Oromia	Jimma	Mana	NA
73	75154*	Oromia	Misirak Showa	Adama	NA
74	75216	Amhara	Semen Shewa	Debrebirhan	NA
75	75220	Amhara	Semen Shewa	Debrebirhan	NA
76	75353	Amhara	Misirak Gojam	Huletejenese	NA
77	75448	Amhara	Semen Wello	Bugna	NA
78	201318	Amhara	Semen Wello	Gubalafto	1470
79	201349*	SNNP	Bench Maji	Konso special	1100
80	201501*	Oromia	Mirab Harerge	Tulo	NA
81	202505	Amhara	Oromia	Chefe dewar	NA
82	204776	Eritrea			1426
83	206921*	Amhara	Semen Gondar	Wegera	NA
84	206935*	Amhara	Semen Gondar	Belesa	NA
85	206936	Amhara	Semen Gondar	Belesa	NA
86	206937*	Amhara	Semen Gondar	Wegera	NA
87	206943	Amhara	Semen Gondar	Wegera	NA
88	206952	Amhara	Semen Gondar	Wegera	NA
89	206960	Amhara	Debub Gondar	Tachgaynt	NA
90	206962	Amhara	Debub Gondar	Simada	NA
91	214046	SNNP	Benchmaji	Sheko	NA
92	214064*	SNNP	Benchmaji	Sheko	NA
93	215053*	Oromia	Borena	Teltele	NA
94	215054	Oromia	Borena	Teltele	NA
95	215330	Amhara	Misirak Gojam	Hulet ejienese	NA
96	216739	Gambella	Zone1	Itang	550
97	217685	SNNP	Debub Omo	Bako gazer	1410
98	220240*	Eritrea			NA
99	220251	Eritrea			1426
100	220267	Eritrea			1426
101	222882	Gambella	Illubabor		530
102	226080*	Amhara	Semen Gondar	Dembiya	1760
103	229232	Amhara	Semen Shewa	Lay Bet ena Tach bet	NA
104	229238	Amhara	Semen Shewa	Lay Bet ena Tach bet	NA
105	229844	Amhara	Misirak Gojam	Bibugn	1850
95	215330	Amhara	Misirak Gojam	Bibugn	NA
106	230780	Oromia	Borena	Moyale	NA
107	231230*	Oromia	Arssi	Sherka	1740
108	235459	Tigray	Mehakelay	Kola Tembel	1750
109	235466*	Tigray	Mehakelay	Kola Tembel	NA
110	235467*	Tigray	Mehakelay	Kola Tembel	NA
111	235469	Tigray	Mehakelay	Kola Tembel	1700

Table.1. (Continued ….)

Entries.	Germplasm	Region	Zone	Wereda	Altitude
112	235761	Amhara	Semen Gondar	Chilga	NA
113	235913	Amhara	Semen Gondar	Addi arkay	1640
114	235922	Amhara	Semen Gondar	Lay armachi	1000
115	235924	Tigray	Mirabawi	Tsegedie	NA
116	237256	Amhara	Semen Gondar	Azezo	NA
117	237274	Tigray	Mehakelay	Aberegele	1450
118	237287*	Tigray	Mehakelay	Merebelehe	1420
119	237289*	Tigray	Mehakelay	Tahitayadihabo	1350
120	238427	Tigray	Mirabawi	Kafta humera	14-06-00-N
121	238447	Tigray	Mirabawi	Kafta humera	14-02-00-N
122	239137	Diredawa	Diredawa	Diredawa	09-37-97-N
123	241183	Oromia	Mirab Harerge	Mieso	1320
124	241227	Oromia	Mirab Harerge	Mesela	1440
125	241236	Diredawa	Diredawa	Dire dawa	09-30-85-N
126	241728*	SNNP	Benchi Maji	Konso	1600
127	243681*	Tigray	Mirabawi	Tselemt	1200

Table.2. List of the released varieties used

Entry	Released Varieties	Releasing Center	Year of Release	Pedigree/ Source	Adaptation	Special merit
128	B 35	NA	NA	ICRISAT	Lowland	stay Green
129	Baji	MARC/EIAR	1996	85MW5334	Mid-altitude with high rain	High yield
130	Birmash	MARC/EIAR	1989	80LPYT-1	Mid.altitude with high rain	High yield
131	E36-1	NA	NA	ICRISAT	Lowland	Stay Green
132	Gambella	NA	NA		Lowland	Medium to early maturing
133	Gobye	NA	NA	NA	NA	NA
134	Teshale	SRARC-ARARI	2002	ICRISAT	Lowland	Early maturing
135	ICSV745#3)*	MARC/EIAR				
136	ISV745(#5)*	MARC/EIAR				

RV= Released varieties, MARC=Melkasa agricultural research center, EIAR=Ethiopian Institute of Agricultural research, SARC= Sirinka Agricultural research center, ARARI=Amhara regional agricultural research institute, SNNP, Southern nations and nationalities people, NA=Information not available and * indicate germplasm which doesn't use for molecular data analysis

3.2. Experimental Design and Trial Management

Plants were grown in specially designed root observation chambers. Each chamber had 40 cm height, 35 cm width and 3 mm thickness(Fig.1).Chambers were constructed with two transparent Perspex sheets (glass plastic) held in place by folding back metal frame and separated on three sides by 3 mm thick rubber to allow easy removal at harvest.

Figure 1.Size of chambers

Each chamber was filled up with red soil until reached the top edge and it was stacked vertically in the tub containing 50 slots each. Chambers in each slot were once watered to field capacitybefore planting; three Seeds planted in the center of each chamber and thinned to one plant after fully germinated. Each chamber was covered with ablackrubber cover to prevent exposure of the roots to light. Individual plants were grown in a uniform condition until it reaches the six leaves stage (at this stage nodal roots were believed to be visible through the Perspex). Thus, experiments were laid out by using randomized complete block design with three replicationssince there was a problem on the distribution of light intensity in the greenhouseand the standard linear model for an RCBD with both the block and treatment effects fixed and without interaction effects as follows:

$$Y_{ij}=\mu+\beta_{j}+\tau_{i}+\varepsilon_{ij}$$

Yij denotes the response for the experimental unit with the ith treatment in the jth block, μ is the overall mean, β_j is the treatment effect, τ_I is the block effect, and ε_{ij} is the error with i =1,…,t and j = 1,…,b. Because of all germplasms, occur in all blocks, the replicate or complete-block effects do not contribute to the standard error of the mean.

3.3. Data collected

After five weeks, numbers of fully expanded leaves which have a direct relation with photosynthesis activity of the plant were scored through visual observation.The length of shootswas measured starting from the base of the stem to the uppermost leaves with measuring tape whereas stem diameter was measuredwith a digital caliper. Leaf area was taken through in situ measurement of five leaves for each plant by measuring the length and width of leaves with the help of measuring tape and multiplied this parameter with a shape factor 0.69(Lafarge and Hammer 2002). Then, the shoot of each plant was cut off at the base

of the stem and shoot fresh weight were taken with sensitive balance, and shoot dry weight were determined after oven drying at 60^0C for three days.

After removing the shoot, the root system that is visible through the Perspex was captured on both sides of each chamber using CANON SX610 HS digital camera. The image was used to determine the encompassing angle, relative to the vertical plane, of the first flush of nodal roots at a distance of 2 cm from the base of the plant through photo gel software. Root angle for a plant is the mean of four observations (two angles on each of two sides per chamber). After imaging the chamber, root number, and root length were taken, and roots were washed from the soil to determine root fresh weight and dry weight. Root, dry weight was determined by drying roots at 60^0C for three days through theoven and then root to shoot ratio were computed by dividing root dry weight to shoot dry weight.

3.4. DNA isolation and marker genotyping

Seedlings were raised in the greenhouse and fresh leaves from 14 days old seedlings were harvested and dried with silica gel in zipping locked plastic bag. Genomic DNA was extracted from young leaves following a modified CTAB (Cetyl Trimethyl Ammonium Bromide) extraction protocol (Mace*et al*., 2003).The quality and quantity of the extracted DNA were determined by comparing the fluorescence of aliquots of DNA samples with a known concentration of λ-DNA after running them on 0.8% ((0.8g agarose dissolved in 100ml 1xTBE (Tris-Boric Acid-EDTA) buffer)) agarose gel that contained 0.3 µg/ml ethidium bromide solution. At the end of electrophoresis, the gel was visualized using UV light and photographed using a video capture (Flowgen IS 1000). All samples were normalized to the same concentration level (50ng) and used for PCR.

Thirty-nine Simple Sequence Repeat (SSRs) markers, including 22 di, nine tri, and 4-tetra nucleotide or longer motifs, and 4 compound repeats were used. These SSR markers were selected based on their uniform distribution in the sorghum genome. From this marker, four of them were found from chromosome SBI-01, five from chromosome SBI-02, four from chromosome SBI-03, two from chromosome SBI-04, four from chromosome SBI-05 and chromosome SBI-06 each, five from chromosome SBI- 07 and chromosome SBI- 08 each and three from chromosome SBI-09 and chromosome SBI-10 each. These are the same set of markers that are selected and being used by the Generation Challenge Program for genetic diversity assessment of global sorghum germplasm. The list of the SSRs markers, including primer sequences, information on repeat motif and length are given in Table.3.

Table.3. List of sorghum SSR markers used for the study

Marker	Forward primer sequences	Reverse primer sequences	Motif type	AT
gpsb067	TAGTCCATACACCTTTCA	TCTCTCACACACATTCTTC	(GT)10	49
gpsb123	ATAGATGTTGACGAAGCA	GTGGTATGGGACTGGA	(CA)7+(GA)5	50
mSbCIR223	CGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT	(AC)6	55
mSbCIR238	AGAAGAAAAGGGGTAAGAGC	CGAGAAACAATTACATGAACC	(AC)26	55
mSbCIR240	GTTCTTGGCCCTACTGAAT	TCACCTGTAACCCTGTCTTC	(TG)9	55
mSbCIR246	TTTTGTTGCACTTTTGAGC	GATGATAGCGACCACAAATC	(CA)7.5	55
mSbCIR248	GTTGGTCAGTGGTGGATAAA	ACTCCCATGTGCTGAATCT	(GT)7.5	56
mSbCIR262	GCACCAAAATCAGCGTCT	CCATTTACCCGTGGATTAGT	(CATG)3.25	57
mSbCIR276	CCCCAATCTAACTATTTGGT	GAGGCTGAGATGCTCTGT	(AC)9	53
mSbCIR283	TCCCTTCTGAGCTTGTAAAT	CAAGTCACTACCAAATGCAC	(CT)8 (GT)8.5	54
mSbCIR286	GCTTCTATACTCCCCTCCAC	TTTATGGTAGGATGCTCTGC	(AC)9	55
mSbCIR300	TTGAGAGCGGCGAGGTAA	AAAAGCCCAAGTCTCAGTGCTA	(GT)9	61
mSbCIR306	ATACTCTCGTACTCGGCTCA	GCCACTCTTTACTTTTCTTCTG	(GT)7	56
mSbCIR329	GCAGAACATCACTCAAAGAA	TACCTAAGGCAGGGATTG	(AC)8.5	55
SbAGB02	CTCTGATATGTCGTTGTGCT	ATAGAGAGGATAGCTTATAGCTCA	(AG)35	55
Xcup02	GACGCAGCTTTGCTCCTATC	GTCCAACCAACCCACGTATC	(GCA)6	54
Xcup14	TACATCACAGCAGGGACAGG	CTGGAAAGCCGAGCAGTATG	(AG)10	54
Xcup53	GCAGGAGTATAGGCAGAGGC	CGACATGACAAGCTCAAACG	(TTTA)5	54
Xcup61	TTAGCATGTCCACCACAACC	AAAGCAACTCGTCTGATCCC	(CAG)7	54
Xcup63	GTAAAGGGCAAGGCAACAAG	GCCCTACAAAATCTGCAAGC	(GGATGC)4	54
Xgap72	TGCCACCACTCTGGAAAAGGCTA	CTGAGGACTGCCCCAAATGTAGG	(AG)16	55
Xgap206	ATTCATCATCCTCATCCTCGTAGAA	AAAAACCAACCCGACCCACTC	(AC)13/(AG)20	55
Xgap84	CGCTCTCGGGATGAATGA	TAACGGACCACTAACAAATGATT	(AG)14	55
Xisep0310	TGCCTTGTGCCTTGTTTATCT	GGATCGATGCCTATCTCGTC	(CCAAT)4	60
Xtxp010	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC	(CT)14	50
Xtxp012	AGATCTGGCGGCAACG	AGTCACCCATCGATCATC	(CT)22	55
Xtxp015	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	(TC)16	55
Xtxp021	GAGCTGCCATAGATTTGGTCG	ACCTCGTCCCACCTTTGTTG	(AG)18	60
Xtxp040	CAGCAACTTGCACTTGTC	GGGAGCAATTTGGCACTAG	(GGA)7	55
Xtxp057	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	(GT)21	55
Xtxp114	CGTCTTCTACCGCGTCCT	CATAATCCCACTCAACAATCC	(AGG)8	50
Xtxp136	GCGAATAGCATCTTACAACA	ACTGATCATTGGCAGGAC	(GCA)5	55
Xtxp141	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	(GA)23	55
Xtxp145	GTTCCTCCTGCCATTACT	CTTCCGCACATCCAC	(AG)22	55
Xtxp265	GTCTACAGGCGTGCAAATAAAA	TTACCATGCTACCCCTAAAAGTGG	(GAA)19	55
Xtxp273	GTACCCATTTAAATTGTTTGCAGTAG	CAGAGGAGGAGGAAGAGAAGG	(TTG)20	55
Xtxp278	GGGTTTCAACTCTAGCCTACCGAACTTCCT	ATGCCTCATCATGGTTCGTTTTGCTT	(TTG)12	50
Xtxp320	TAAACTAGACCATATACTGCCATGATAA	GTGCAAATAAGGGCTAGAGTGTT	(AAG)20	54
Xtxp321	TAACCCAAGCCTGAGCATAAGA	CCCATTCACACATGAGACGAG	(GT)4+(AT)6+(CT)2	55

The PCR amplification was performed using Gene-Amp PCR system 9600(PE-Applied-Biosystems) in 96-well plates in a total reaction volume of 10µl reaction mixture containing 1µl DNA template (50ng), 1µl 10x PCR buffer, 2µl MgCl2, 1µl of reverse primer, 1.0 µl forward primer directly labeled with 6-FAM (VIC, NED, PET fluoresce dyes), 0.5µl of each dNTP, 0.04 µl Taq DNA polymerase and 3.46 µl H2O. The amplification profile consisted of an initial denaturation of the template DNA at 95^{o}C for 3 minutes, followed by 35 cycles, each for 30 sec at 95^{o}C (denaturation), 1min at 56^{o}C (annealing), and 1 min at 72^{o}C (extension), and a final extension at 72^{o}C for three mins.

3.5. Data analysis

3.5.1. Phenotypic data analysis

All phenotypic data were analyzed with univariate methods in SAS V.9.2 package. Variance analysis was used to evaluate the differences of thetrait among the germplasm. Following the analysis of variance with PROC GLM procedure, protected LSD 0.05 was calculated among the germplasm. The error estimate and person's correlation were computed using variance within the germplasms.Principal component analyses were carried out using the PRIN-COMP function of SAS to identify pair-wise correlations for the traits and PROC CLUSTER was used for clustering analysis.

For statistical analysis of genetic parameters, the analysis of variance of each mean value, phenotypic and genotypic variances, the phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability and genetic advance were calculated.

The genotypic and phenotypic coefficients of variation (GCV and PCV) were computed according to the method advocated by Burton and Devane (1953).

Environmental variance ($\sigma^2 e$) =MSE . Where, MSE= mean square error

Genotypic variance ($\sigma^2 g$) = $\frac{MSG - MSE}{r}$ Where, MSG= mean squarer genotype, (r) = number of replication.

Phenotypic variance ($\sigma^2 p$) = $\sigma^2 g + \sigma^2 e$.

Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\sigma^2 p}}{\bar{x}} * 100$

Genotypic coefficient of variation (GCV) = $\frac{\sqrt{\sigma^2 g}}{\bar{x}} * 100$Where: $\bar{x}$ = Grand mean of the characters under study.GCV and PCV values were categorized as low (0-10%), moderate (11-20%) and high (21% and above) values as indicated by Deshmukh *et al*., (1986).

The repeatability of variety trials is the proportion of the variation among line means that is due to the variation in genotype effects. This statistic also denoted *broad-sense heritability (H)* and was calculated with the formula suggested by Allard (1999).

$H^{2=}\frac{\sigma^2 g}{\sigma 2 p} * 100$ Where, H^2= heritability in the broad sense

Heritability percentage is also categorized as low (0-30 %), moderate (31-60 %) and high (61% and above) as given by Robinson *et al.* (1949).

The expected genetic advance (GA) of the genotypes and its percent of mean at 5% intensity of selection pressurewere calculated according to Lush (1949) and Johnson *et al*. (1955).

$GA\ (\%) = K.H^2 \times \sigma p,$

Where, H^2= Heritability in broad sense,σp = Phenotypic standard deviation, GA= Expected genetic advance and k = the standardize selection differential at 5% selection intensity (K = 2.063)

Genetic advance as percent of the mean (GAM) was computed using the formula given below to compare the extent of predicted genetic advance of different traits under selection:

$$\text{Genetic advance in percentage of mean} = \frac{\text{genetic advance} * 100}{\text{population mean}} = \text{GAM} = \frac{GA * 100}{\bar{x}}$$

GAM was categorized as low (0-10%), moderate (11-20%) and high (Above 20%) following Johnson *et al.* (1955).

3.5.2. Molecular data analysis

Population structure among the 108 sorghum germplasm (101 landraces and seven released varieties) was conducted using the model-based Bayesian clustering algorism software package *"STRUCTURE"* version 2.3.4 (Prichard *et al.*,2000a). By setting the number of k levels from, one to nine, with eight times repetition for each k, nine independent structure runs were performed with 10,000 burn-in time and 100,000 iterations of Markov chain convergence for each run.

The plot of the average *log likelihood* values over eight runs for each *K* ranging the k-values from one to nine showed that the *log likelihood* estimates increase progressively as *K* increases. The structured output was imported to structure harvester (a website and program for visualizing STRUCTURE output and implementing the Evanno method)to find the trueKand the ad-hoc criterion described by(Evanno *et al.*,2005)were used to detect the most probable number of subpopulations reliably(Earl, 2012).The number of

subpopulationswasfound to be at k=three and on this basis, three sub-populations were assumed in the association mapping.

Thus, the population structure for K equal threewas selected to be assigned as the proportion membership for each germplasm and used by GLM in the TASSEL software forthe structure-based association analysis. All STRUCTURE runs were performed using the admixture model with the option of correlated allele frequency between populations.

LD values (r^2 and p-value) between marker fragments were calculated using TASSEL 4 standalone software. The genetic distances between marker pairs were calculated based on the position of these markers on the genetic map. Minor loci with a frequency < 0.05 were filtered out to reduce problematic and biased LD estimations between pairs of loci. The r^2 valueswere plotted as a function of map distances, and LD decay (r^2< 0.1) was estimated using the average distances of marker pairs showing LD values lower than 0.1(Shi *et al.*, 2010).

Mean values of the 12 drought related traitswere subjected to association analysis with SSR loci, based on the whole 108 germplasm used in this study. Association analysis between the markers and drought related traits were performed based on the general linear model by using the software TASSEL 4 standalone. The significance of associations between loci and traits was based on an F-test with P values calculated by TASSEL at 5% significant level (Wang *et al*., 2011).

4. RESULT ANDDISCUSSION

4.1. Analysis of variance

For shoot traits, the analysis of variance indicated that highly significant differences ($P<0.01$) existed among germplasms (Table 4).This broad range of variation present among germplasms is important for thebreeder to select genotype which can adapt to thedifferentagro-ecological condition.

Leaf area showed a mean of 13.92cm^2 and among 136 germplasms, 58 of them were scored above the mean whereas the remaining 78 were scored below the mean value. Minimum (7.51cm^2)and maximum(30.21cm^2)leaf areawas observed in germplasms 206935 and 71539, respectively.Crop water use can be reduced by decreasing leaf area and/or transpiration per unit leaf area. Leaf area can be constrained by reducing tillering (Kim *et al.*, 2010), leaf number per culm, and/ or individual leaf size (Borrell*et al.*, 2000a). Reduced canopy size also has been linked to increased grain yield under post-anthesis drought stress (Oosterom *et al.*, 2011; Borrell*et al.*, 2014). Because more leaf area might cause more water loss due to more evapotranspiration from the surface and small leaf area may not as such productive, optimum leaf area is required for carrying out enough photosynthesis to run the essential processes of the plant (Ali *et al*., 2009; Khaliq *et al*., 2008). Narrow and erect leaves will have lower LA, which helps to reduce the depletion of soil moisture due to transpiration. But low productivity in rainfed sorghum is due to slower LA development and faster leaf senescence.

The number of fully expanded leavesdetermines the time at which nodal root angle is visible to perspex for each germplasm and ranged from 4.93 (235924) to 7.1 (230780) with an average of 5.68. Among the genotypes, 65 scored above the mean whereas 71 germplasms scored below the mean. Reduced growth duration is associated with reduced leaf numbers (Blum, 2004).

Shoot-length ranged from 15cm (69301 and 31230) to 31cm (206937) with an average length of 22.02cm. Among germplasms, 61 were scored above the mean and 75 germplasms were scored below this mean value. The stem of sorghum consists of many alternating nodes and internodes.Since each leafappear at the node of the stem, stem length determines the number of leaves present in the plant. Therefore plants that are genetically short have small

numbers of leaves. This minimizes the amount of water loss due to evapotranspiration and important for drought adaptation.

Stem diameter which has a direct relation with yield by determining the size of the panicle at afinal growth stage (particularly the diameter of uppermost internode) ranged from 3.05mm (231230) to 5.81 mm (238447) with an average of 4.30mm. Out of 136 germplasm, 58 germplasms were above the mean and 78 of were below the mean.

Shoot fresh weight ranged from 2.86g (69094) to 11.36g (71539) with a mean value of 4.86g. Among germplasms under this investigation, 64 were scored above the mean and 72 germplasms were scored below the mean. Shoot dry weight ranges from 0.54g (E36-1) to 2.24 g (71539) with a mean of 0.92g out of which 56 germplasms and 72 germplasms were scored above and below the mean value, respectively.Higher relative dry weight values are supposed to be leading to vigorous accumulation of osmotica resulting inability of plants to withstand against severe drought conditions (Jones *et al*., 1980).

Nodal root angle, which has an implication in water extraction pattern of the plant from the soil, ranged from14.5^{o} (71539) to 32.5^{o} (72451) (Fig.2) with a mean value of 23.56^{0}. Among germplasms, 67 of them had a score of above the mean and 69 had below the mean. In agreement with this findingSingh *et al* (2010) reported nodal root angle ranging from about 15^{o} to 50^{o} for 44 sorghum inbred lines. Singh *et al* (2010) and Mace *et al*.(2012) reported the associations between variations in nodal root angle and water capture and yield in sorghum. The spatial distribution and morphology of roots affect the ability of plants to access nutrients. Narrow root angles are important for plants grown in drought prone areas to extract water deep from the soil whereas wide root angles are important for plants grown during the raining season where water shortage is not a problem.

Fig.2.Image for narrow and wide root angle

Rootlength ranged from 41 cm (75030) to 60 cm (73068) and (71570) with a mean of 48.67cm, in which, 51, germplasm had a score of above the mean and 85 germplasm had a score below the mean. Root number ranged from 4 (206943) and (230780) to 10 (71570) with a mean of 6.47 in which 71 germplasms scored above the mean and 65 germplasms scored below the mean. Among the various traits, root length is the most important trait for evaluating the genotypes for drought tolerance. Deep-rooted plants yield more under moisture stress conditions by developing roots over long distances and accesses deep water from the soil.(Mambani and Lal, 1983).

Root fresh weight ranged from 1.77g (206962) to 5.78g (69238) with a mean of 3.75g, having 66 germplasms above that score and 70 germplasms below the score. Root dry weight ranged from 0.26g (2372287) to 1.03g (71539) with a mean of 0.58g, in which 71 germplasms scored above the mean, and 61 germplasms scored below the mean. Drought resistance plants had higher values of root dry weight than those of drought susceptible ones. Therefore in order to improve germplasm for drought adaptation breeder must focus on these traits

Root to shoot ratio ranged from 0.2 (206962) to 1.08 (Gambella) showed a mean value of 0.68 from that (81) germplasm had a score of below the mean and the rest (55) germplasms were above the mean. The maximum root to shoot ratio were observed from four released varieties namely: Gambella (1.08), ICSV745 (#5) (1.03), E36-1 (1.01), Gobye (1) and three local landraces 237289 (1.13), 235924 (1.13) and 73358 (1.01). Due to the drought resistant nature of the released varieties, most of thereleased varieties with some local landraces have larger root to shoot ratio.

Table.4. Means performance of 136 germplasm for 12 drought related traits

Acc.	LA	SHL	LN	STD	SHFW	SHDW	RL	RN	RFW	RDW	RSHR	RA
69046	15.74	18.00	5.80	3.67	3.51	0.63	50.00	6.00	3.42	0.44	0.70	25.00
69057	14.85	24.00	5.10	3.30	3.33	0.69	48.00	8.00	2.30	0.39	0.57	19.25
69092	13.90	20.67	5.67	3.92	4.85	0.98	51.67	4.33	4.43	0.54	0.55	26.17
69094	11.04	26.00	5.30	4.58	2.86	0.59	45.00	6.00	2.88	0.47	0.80	16.50
69105	12.60	20.00	5.75	4.01	4.31	0.92	46.00	6.00	3.97	0.47	0.51	25.00
69183	8.23	24.67	6.13	5.05	7.37	1.35	49.33	6.67	3.23	0.68	0.50	25.25
69192	10.57	20.00	5.73	5.02	5.43	0.96	49.33	7.67	4.91	0.66	0.69	22.17
69210	19.31	21.00	5.63	5.40	5.70	1.07	48.67	6.00	4.74	0.61	0.57	22.58
69236	13.43	20.00	5.45	4.07	4.54	0.79	49.00	5.50	2.81	0.52	0.66	21.63
69238	19.58	23.67	5.60	4.39	7.22	1.12	51.33	8.00	5.78	0.76	0.68	23.33
69286	12.31	24.33	5.97	5.17	5.50	1.07	50.00	7.33	4.93	0.60	0.56	23.75
69306	16.30	25.00	6.10	4.61	3.89	0.73	47.00	7.00	3.44	0.47	0.64	25.75
69371	17.90	21.50	5.40	4.59	4.22	0.70	47.00	7.50	3.69	0.57	0.81	16.38
69391	20.13	15.00	5.60	3.58	5.51	0.79	48.00	7.00	5.20	0.69	0.87	23.38
69392	12.93	19.00	5.37	4.09	5.70	0.95	46.33	7.33	3.88	0.38	0.40	19.92
69468	13.21	23.00	5.77	4.24	5.30	0.89	46.67	6.33	3.72	0.58	0.65	22.92
69492	7.78	24.00	6.20	4.68	3.15	0.74	48.00	7.00	3.58	0.62	0.84	24.75
69494	23.93	21.00	5.65	4.04	6.31	1.23	44.00	6.50	4.32	0.53	0.43	23.25
70068	18.09	21.33	5.60	4.71	5.62	0.98	47.67	5.00	3.16	0.49	0.50	21.92
70075	7.87	26.50	6.45	4.81	5.54	1.20	53.50	5.50	3.58	0.55	0.46	25.38
70301	15.13	20.33	5.80	4.11	6.25	0.98	51.33	6.67	3.50	0.55	0.56	24.92
70306	13.23	22.67	5.67	4.40	4.86	0.89	49.00	6.33	3.80	0.67	0.75	25.08
70537	9.29	22.00	5.55	3.66	2.89	0.57	52.50	6.50	2.44	0.37	0.65	24.50
71021	11.82	25.50	5.10	3.93	3.46	0.63	48.00	5.50	2.50	0.37	0.59	21.38
71370	14.53	22.67	5.80	5.03	5.33	1.05	53.33	8.00	4.63	0.77	0.73	25.08
71418	12.44	18.67	5.73	3.93	4.86	0.89	48.00	5.33	3.33	0.55	0.62	24.75
71421	12.59	22.00	5.25	4.54	5.21	0.91	53.00	7.50	3.17	0.61	0.67	22.63
71422	13.18	25.00	5.47	4.68	4.38	0.99	48.67	7.33	3.67	0.68	0.69	24.00
71425	12.22	18.00	5.27	3.52	4.12	0.69	49.67	5.67	3.05	0.50	0.72	16.00
71477	17.63	25.33	5.53	4.55	6.10	1.04	52.00	7.67	4.19	0.60	0.58	25.83
71522	14.91	21.50	5.95	3.71	4.35	0.82	49.00	7.50	3.43	0.60	0.73	24.13
71536	9.77	21.33	5.50	3.50	3.62	0.67	53.67	5.67	3.37	0.53	0.79	22.67
71539	30.21	25.00	6.90	5.17	11.36	2.24	50.00	6.00	5.23	1.03	0.46	14.50
71570	10.76	22.00	5.60	4.00	4.97	0.79	60.00	10.00	3.38	0.43	0.54	23.25
71590	12.85	22.67	5.50	3.93	4.69	0.87	42.33	7.00	3.70	0.63	0.72	22.33
71621	14.29	21.50	5.95	5.69	5.33	1.01	52.50	8.00	4.79	0.66	0.65	26.38
71657	10.88	23.33	5.73	4.24	4.48	0.83	51.67	7.67	3.40	0.52	0.63	27.58
71744	11.90	19.67	5.63	4.31	4.50	0.95	49.00	6.33	4.50	0.71	0.75	27.75
71748	10.59	23.50	5.35	3.97	3.57	0.77	52.00	5.50	2.97	0.52	0.68	24.13
71788	11.62	18.33	5.70	3.75	4.48	0.79	50.67	6.67	3.23	0.66	0.84	28.25
71795	14.85	26.00	5.00	4.26	3.47	0.70	50.00	5.00	2.82	0.51	0.73	24.75
71810	12.74	25.00	5.63	4.24	4.21	0.77	50.00	7.00	3.54	0.62	0.81	25.50

Table.4. (Continued….)

Acc.	LA	SHL	LN	STD	SHFW	SHDW	RL	RN	RFW	RDW	RSHR	RA
72451	11.87	22.00	5.60	4.94	6.63	0.90	47.67	5.00	3.38	0.48	0.53	32.50
72588	14.22	18.00	6.25	3.92	3.97	0.92	45.50	8.00	4.87	0.60	0.65	22.13
72997	10.34	20.50	5.70	3.24	3.79	0.64	49.50	6.50	3.16	0.42	0.66	22.63
73067	13.20	19.00	5.50	3.88	5.42	0.73	49.33	5.00	3.63	0.37	0.51	26.58
73068	8.72	23.00	5.00	3.67	4.15	0.65	60.00	6.00	3.68	0.34	0.52	24.00
73096	18.32	23.00	5.40	4.10	6.57	1.12	50.00	8.00	4.23	0.58	0.52	25.75
73341	10.27	29.00	5.85	4.05	5.59	1.05	45.00	6.50	3.21	0.63	0.60	24.88
73358	11.13	23.67	5.73	3.85	4.65	0.75	51.33	5.67	4.50	0.76	1.01	28.92
73636	17.83	23.00	6.50	4.91	3.85	0.84	48.00	7.00	3.68	0.64	0.76	26.75
73637	12.42	20.73	6.23	3.93	4.97	0.92	48.00	7.67	3.78	0.66	0.72	20.58
73641	14.24	18.00	5.85	3.67	3.50	0.80	49.50	7.00	3.84	0.55	0.69	23.63
73646	16.98	20.00	5.60	4.62	9.20	1.14	48.00	6.00	4.95	0.60	0.53	24.50
7 3762	18.42	26.00	5.85	3.84	4.65	0.76	46.00	5.50	2.86	0.39	0.51	23.75
73797	11.30	21.60	5.90	4.87	6.01	1.20	51.00	8.67	3.79	0.59	0.49	23.75
73992	12.98	22.00	5.73	3.99	5.20	1.08	47.00	6.67	3.91	0.56	0.52	23.42
74097	15.53	24.00	5.80	4.16	5.71	1.09	51.67	6.67	4.57	0.75	0.69	24.00
74108	13.34	18.40	5.10	3.30	4.24	0.66	46.00	6.00	4.87	0.60	0.91	21.00
74115	15.94	23.67	5.67	5.55	7.51	1.76	51.00	8.00	5.14	0.81	0.46	23.67
74262	14.59	22.33	5.40	4.08	5.44	0.90	52.67	5.67	3.94	0.56	0.62	23.50
74268	20.80	27.00	6.00	4.19	5.64	1.05	46.67	6.67	4.51	0.80	0.76	27.17
74703	14.81	16.67	6.07	4.29	5.16	0.91	45.33	7.67	4.64	0.53	0.58	24.75
74761	15.48	20.33	5.80	4.08	4.67	0.99	49.33	6.67	3.89	0.63	0.64	25.75
74766	19.18	21.00	6.20	4.33	4.85	0.89	48.00	6.00	3.58	0.57	0.64	31.25
74935	10.29	24.00	5.70	4.28	3.54	1.15	46.00	6.00	3.07	0.45	0.39	20.50
74999	14.53	23.67	5.20	4.27	4.92	0.89	52.67	5.33	3.65	0.80	0.90	28.17
75000	9.63	28.50	6.50	5.51	5.30	1.21	49.00	7.00	4.76	0.75	0.62	23.25
75030	21.11	19.00	5.40	5.66	6.62	1.06	41.00	6.00	5.60	0.68	0.64	19.00
75066	20.15	16.00	5.10	3.95	3.32	0.61	45.00	5.00	3.84	0.43	0.70	25.75
75129	17.40	23.00	5.40	3.93	4.11	0.87	50.00	6.00	4.22	0.56	0.64	25.00
75140	12.85	19.50	5.55	4.16	5.32	0.87	47.50	5.00	4.44	0.65	0.75	25.63
75154	9.77	22.00	5.90	4.72	5.99	1.58	47.00	7.00	4.30	0.58	0.37	22.50
75216	12.42	23.00	5.60	4.27	5.85	1.05	54.00	7.00	3.96	0.86	0.82	25.50
75220	11.67	23.67	5.73	4.88	5.55	0.91	49.67	4.67	3.85	0.60	0.66	24.50
75353	14.36	22.33	5.17	3.80	5.14	1.10	50.67	6.00	3.28	0.50	0.45	26.00
75448	14.26	22.53	5.93	4.94	5.31	1.19	45.67	7.00	3.60	0.61	0.51	23.50
201318	10.73	27.33	5.97	4.40	5.23	1.03	46.67	6.33	3.75	0.55	0.53	22.33
201349	16.67	20.50	5.65	4.04	4.82	0.98	48.50	6.00	3.23	0.55	0.56	22.13
201501	11.26	22.00	5.77	4.01	4.74	0.77	46.67	5.33	2.84	0.43	0.56	30.25
202505	9.12	21.00	5.50	4.36	5.13	0.65	49.00	6.00	2.83	0.32	0.49	23.00
204776	12.10	19.50	5.90	4.03	4.43	0.75	51.00	6.50	2.56	0.37	0.49	24.88
206921	14.35	23.33	5.97	4.29	3.56	0.86	48.00	6.00	3.37	0.61	0.71	28.67
206935	7.51	20.00	5.70	4.35	3.72	0.65	46.00	6.00	2.79	0.36	0.55	19.00
206936	12.23	23.00	5.70	4.05	5.07	0.92	51.33	5.33	3.93	0.56	0.61	28.00

Table.4. (Continued….)

Acc.	LA	SHL	LN	STD	SHFW	SHDW	RL	RN	RFW	RDW	RSHR	RA
206937	22.05	31.00	5.70	4.84	5.34	1.09	48.00	6.00	3.10	0.62	0.57	26.00
206943	11.07	26.00	6.00	3.75	3.59	0.81	44.00	4.00	3.25	0.55	0.68	20.00
206952	21.41	24.00	6.20	5.43	5.49	0.94	53.50	7.00	4.06	0.56	0.60	27.00
206960	13.67	24.00	5.57	3.78	4.14	0.80	50.67	6.00	3.08	0.50	0.63	22.42
206962	11.75	23.60	5.50	4.70	5.55	1.34	45.00	5.00	1.77	0.27	0.20	22.25
214046	15.68	21.33	5.07	4.31	4.07	0.88	46.00	6.67	2.79	0.41	0.47	21.17
214064	14.66	28.00	6.40	4.47	4.68	1.04	48.00	6.00	4.03	0.87	0.84	18.50
215053	21.31	22.50	5.75	5.15	5.72	1.12	49.50	7.00	4.43	0.74	0.66	23.00
215054	19.43	20.67	6.20	4.99	5.99	0.86	44.67	7.33	5.14	0.77	0.90	22.25
215330	13.96	21.33	5.77	4.55	4.75	0.94	46.33	6.67	3.83	0.61	0.65	19.08
216739	12.33	25.00	6.10	3.50	3.70	0.57	42.00	6.00	2.28	0.30	0.53	22.75
217685	11.18	23.00	6.70	3.85	3.71	1.21	47.00	6.00	3.93	0.61	0.50	20.75
220240	19.60	18.50	6.10	4.68	4.11	0.90	49.50	6.50	3.01	0.50	0.56	20.75
220251	18.72	23.33	5.93	4.95	6.17	1.09	48.33	7.00	4.11	0.55	0.50	23.42
220267	17.78	19.00	5.07	3.93	3.29	0.67	49.00	6.00	2.24	0.49	0.73	23.50
222882	16.09	19.00	5.10	4.79	5.97	1.26	43.00	8.33	4.50	0.68	0.54	17.42
226080	8.94	22.00	5.90	5.31	5.29	0.87	47.00	7.00	3.89	0.58	0.67	30.75
229232	20.53	29.00	5.50	4.29	5.27	1.10	48.00	6.00	4.10	0.57	0.52	19.00
229238	16.48	17.50	5.60	4.01	5.03	0.86	43.00	6.00	3.23	0.50	0.58	16.38
229844	13.09	21.67	5.73	3.96	4.15	0.80	49.67	5.33	3.79	0.64	0.80	27.92
230780	7.57	20.00	7.10	5.49	6.81	1.45	45.00	4.00	3.74	0.63	0.43	24.50
231230	8.33	15.00	6.20	3.05	3.35	0.56	43.00	6.00	3.41	0.33	0.59	21.50
235459	11.82	23.00	5.45	3.96	4.54	0.99	48.00	7.00	3.80	0.54	0.55	24.63
235466	10.29	20.40	5.70	4.04	3.65	0.84	50.00	6.33	3.01	0.71	0.85	20.67
235467	9.69	20.50	5.15	3.92	4.71	0.84	49.50	5.50	3.58	0.47	0.56	27.75
235469	13.30	22.33	5.33	4.51	4.99	0.88	50.33	7.33	5.06	0.77	0.88	20.08
235761	13.55	17.00	5.50	4.70	5.39	0.91	43.00	7.00	4.57	0.62	0.68	21.75
235913	10.77	22.50	5.25	3.77	4.29	0.83	48.50	5.50	2.53	0.74	0.89	20.00
235922	11.77	20.33	6.13	4.55	4.58	0.86	49.67	8.00	3.58	0.60	0.70	21.58
235924	14.45	18.67	4.93	3.89	3.51	0.62	50.00	7.33	3.72	0.70	1.13	25.08
237256	13.63	21.00	5.60	5.05	4.88	0.79	47.50	6.50	4.54	0.70	0.89	21.50
237274	12.29	30.00	5.90	4.24	5.25	1.72	45.00	8.00	3.28	0.67	0.39	26.00
237287	10.13	24.00	5.00	3.25	3.91	0.66	46.00	5.00	1.99	0.26	0.39	29.50
237289	11.35	21.00	5.60	3.97	4.62	0.71	43.50	8.00	4.80	0.80	1.13	18.50
238427	8.20	18.50	5.50	3.46	4.75	0.57	48.00	6.00	3.15	0.43	0.75	22.63
238447	9.18	20.00	5.30	5.81	5.24	0.85	50.00	8.50	3.81	0.59	0.69	23.00
239137	13.17	21.50	5.25	4.03	4.08	0.63	48.00	6.50	3.68	0.57	0.90	21.25
241183	12.19	20.33	5.23	4.80	5.39	0.93	58.67	7.67	4.65	0.91	0.98	30.67
241227	13.12	28.33	5.53	3.64	4.15	0.91	49.67	5.67	2.87	0.59	0.65	23.17
241236	14.62	30.07	5.50	3.23	3.87	0.82	52.67	5.67	3.28	0.54	0.66	26.00
241728	13.88	21.00	5.70	4.27	5.73	1.00	50.50	7.50	4.87	0.68	0.68	20.75
243681	10.18	19.67	5.23	4.67	3.91	0.67	48.00	7.00	2.87	0.44	0.66	22.00

Table.4. (Continued…)

Acc.	LA	SHL	LN	STD	SHFW	SHDW	RL	RN	RFW	RDW	RSHR	RA
B35	9.18	20.67	5.70	4.50	5.34	0.92	52.00	7.00	4.29	0.52	0.57	25.58
Baji	12.16	18.90	6.20	4.85	4.75	0.89	50.50	6.50	3.92	0.54	0.61	25.50
Birmash	23.71	18.50	5.45	4.71	4.42	0.95	47.50	6.50	4.72	0.68	0.72	22.63
E36-1	20.28	19.50	5.00	3.09	3.88	0.54	45.50	6.50	4.14	0.55	1.02	21.50
Gambella	14.32	24.00	5.60	3.99	3.69	0.74	46.00	5.00	3.98	0.80	1.08	25.75
Gobye	14.64	24.00	5.33	4.52	4.52	0.87	49.67	7.33	4.43	0.87	1.00	23.25
Teshale	12.14	19.00	5.20	3.88	3.81	0.77	45.00	7.00	3.84	0.67	0.87	21.25
ICSV745(#3)	15.31	18.33	5.43	4.75	3.58	0.60	51.67	5.33	3.23	0.62	1.03	16.92
ISV745(#5)	17.18	19.00	6.53	4.17	4.54	0.99	50.00	5.33	3.62	0.58	0.59	23.67
Min	7.51	15	4.93	3.05	2.86	0.57	42	4.33	1.77	0.26	0.20	14.5
Mean	13.92	22.02	5.68	4.30	4.86	0.92	48.67	6.47	3.75	0.58	0.68	23.56
Max	30.21	30.7	6.9	5.81	11.36	2.24	60	10	5.78	1.03	1.13	30.75
CV (%)	9.65	6.39	0.36	7.74	11.82	13.85	3.67	9.37	11.98	12.81	27.87	3.59
LSD (5%)	5.36	6.05	1.03	1.42	2.44	6.85	8.99	2.59	1.88	2.76	2.92	15.05

Where, CV= coefficient of variation, LSD= least significant difference, LA= average Leaf area; LN= Number of fully expanded leaves; RA= Nodal root angle; RDW= Root dry weight; RFW= Root fresh weight; RL= Root length; RN= Number of promising root; SHDW= Shoot dry weight; SHFW= Shoot fresh weight; SHL= Shoot length; STD=Stem diameter and RSHR=root to shoot ratio

4.2. Correlation among traits

Coefficients of correlation (Table.5) showed that there was a significant positive and negative relation among traits. From this study, leaf area showed a significant positivecorrelation with all traits except root length and root angle. But it had a significant negative correlation with root length and root angle. Therefore in order to increase shoot fresh and dry weight which have a direct relation with drought tolerance and yield, breeders must select germplasm having narrow root angle.Since roots are most important for growth and development, germplasms possessing good rooting system at seedling stage under drought conditions could be selected for drought tolerance.

Table.5.Correlation among 12 drought-related traits in the studied germplasm

	LA	SHL	LN	STD	SHFW	SHDW	RL	RN	RFW	RDW	RSHR
SHL	-0.018	1									
LN	0.033	0.108	1								
STD	0.152**	0.149**	0.406**	1							
SHFW	0..275**	0.059	0.328**	0.553**	1						
SHDW	0.265**	0.209**	0.410**	0.548**	0.780**	1					
RL	-0..121*	0.058	-0.103	-0.064	-0.067	-0.067	1				
RN	0.102	0.002	0.165**	0.297**	0.248**	0.247**	.008	1			
RFW	0.352**	0.024	0.288**	0.452**	0.591**	0.522**	0.00	0.348**	1		
RDW	0.255**	0.187**	0.252**	0.401**	0.473**	0.514**	0.041	0.313**	0.670**	1	
RSHR	-0.002	-0.060	-0.199**	-0.159**	-0.304**	-0.459**	0.020	0.040	0.116*	0.369**	1
RA	-0.123*	0.129*	0.000	-0.064	-0.010	-0.020	0.245**	-0.131*	-0.078	-0.105	-0.08

*Where * and **, significant at P>0.05 and P>0.01, respectively, LA= average Leaf area; LN= Number of fully expanded leaves; RA= Nodal root angle; RDW= Root dry weight; RFW= Root fresh weight; RL= Root length; RN= Number of root; SHDW= Shoot dry weight; SHFW= Shoot fresh weight; SHL= Shoot length; STD=Stem diameter.*

The stem diameters shoot dry weight and root dry weight, all of them had a significant positive association for all traits under studied except for root angle and root length. Root lengths had a significant negative association with leaf area and plants having long roots with small leaves area are appropriate in drought prone area to extract water deep from the soil and maintain water loss through evaporation. Root number had a significant positive association with number of fully expanded leaves, stem diameter, shoot fresh weight, shoot dry weight, and root fresh weight and root dry weight. However, this trait had a negative correlation with root angle. In other cases, root angle had asignificant positive association with shoot length and significant negative association with leaf area and root number.

Bocev (1963) reported that seedlings showing well developed rooting system also show well developed rooting system at final stage, thus providing evidence that the plant types showing drought tolerance at seedling stage will also show tolerance at later growth stages. In water stress conditions, root branches spread to explore water and root system became very strong and supportive by further branching under water deficit conditions and in normal conditions water easily available and roots find water nearby level so no need of extensive branching.

4.3. Principal components

In order to assess the pattern of variation, principal component analysis was done by considering all 12 traits simultaneously. The first three principal components (PCs) with

eigenvalues greater than one accounted for 56.4% of the total genotypicvariation (Fig.3), the remaining 43.6% contributed by the othernine principal components.

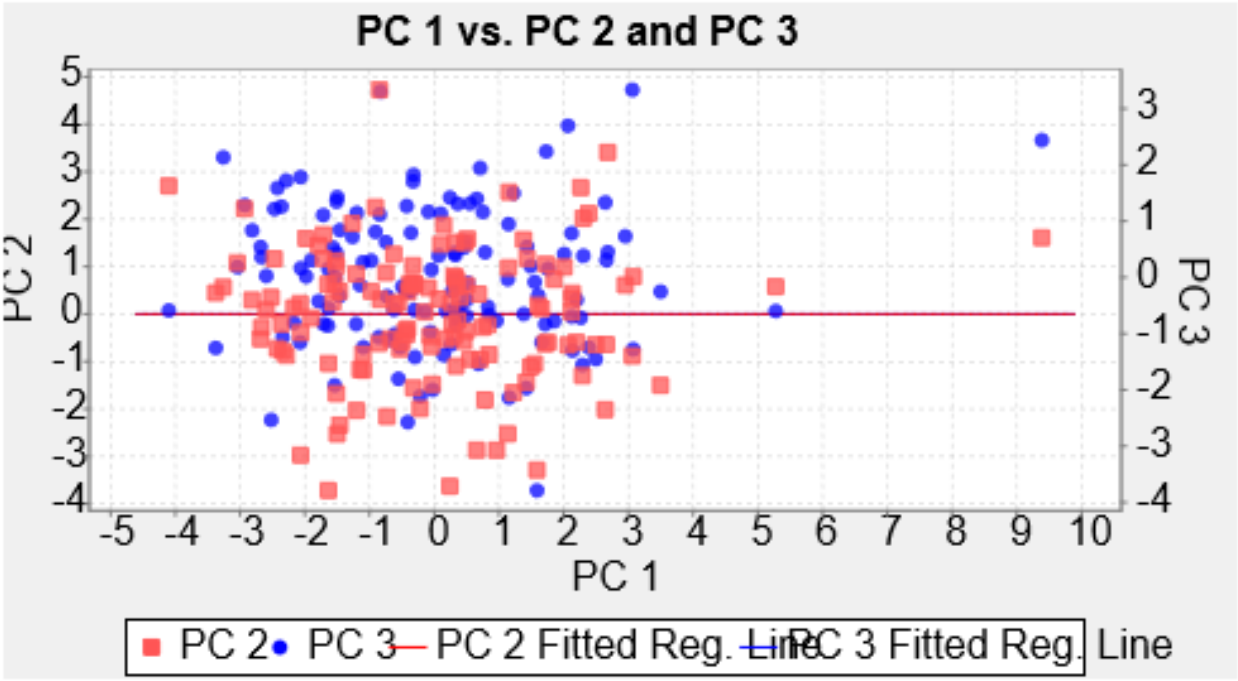

Figure.3. Principal component analyses (PC1, 31.67% Vs PC2, 11.12% and PC3, 13.61%) of phenotypic data showing 56.4% of the total genotypic variation among136 germplasm

The first two PCs accounted for 45.28% of the total variation. While the first principal component axis (PCA1) accounts for 31.67% of the total genotypic variation with the large loadings (Table. 6) on shoot dry weight, shoot fresh weight, root fresh weight, root dry weight, stem diameter, indicating the greatest variation towards the divergence. That means the highest and the lowest shoot dry weight, shoot fresh weight, root fresh weight, root dry weight, stem diameter, root number, thenumber of fully expanded leaves and average leaf area were differentiated by the PC1 loading values.The second PCwas depended on root dry weight,root fresh weight,root number, and leaf area.Whereas the third PC had large loading effects on root angle, root length,shoot length, root dry weight and root to shoot ratio which shows clear differentiation between germplasms discussed in the ANOVA with increased and decreased root angle, root length,shoot length and root to shoot ratio. In general maximum contribution towards the variability of germplasm was due to root angle, root dry weight, root fresh weight, root length, shoot dry weight, shoot fresh weight, stem diameter and shoot length.

Table.6: Principal component axis

	Prin 1	Prin 2	Prin 3
LA	0.202665	0.187266	-0.232030
SHL	0.097154	-0.162292	0.398843
LN	0.270577	-0.208190	-0.027753
STD	0.374577	-0.095203	0.003012
SHFW	0.427604	-0.150702	-0.045467
SHDW	0.437313	-0.255395	-0.022512
RL	-0.045245	-0.024162	0.615533
RN	0.231744	0.204468	0.006009
RFW	0.400591	0.259878	0.081086
RDW	0.366448	0.366448	0.238120
RSHR	-0.091511	-0.091511	0.213897
RA	-0.054734	-0.282778	0.543863
Eigen value	3.80029062	1.633383	1.334333
Difference	2.16690747	0.29905	0.337438
Percent proportion	31.67	11.12	13.61
Cumulative	0.3167	0.4528	0.564

Where, LA= average Leaf area; LN= Number of fully expanded leaves; RA= Nodal root angle; RDW= Root dry weight; RFW= Root fresh weight; RL= Root length; RN= Number of promising root; SHDW= Shoot dry weight; SHFW= Shoot fresh weight; SHL= Shoot length; STD=Stem diameter; RSHR=root to shoot ratio.

Consistent withMace *et al.*, (2012), traits associated with drought adaptation at the six-leaf stage (root dry and fresh weight, shoot dry and fresh weight, total leaf area, leaf number, root number and root length) tended to group together, indicating a high correlation among them. In contrast, nodal root angle at the six-leaf stage grouped separately and was generally independent of other traits as indicated in fig.4.

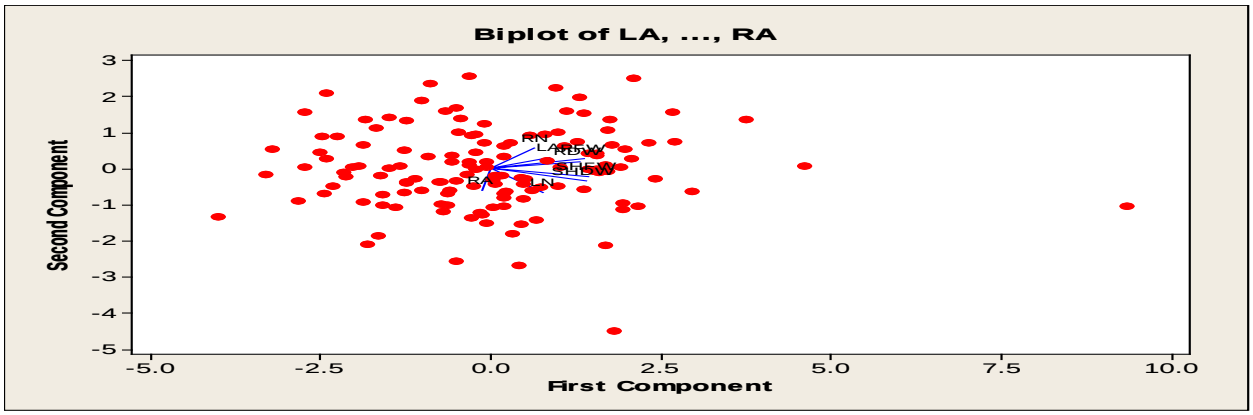

Figure.4.Biplot generated from principal components analysis of traits measured for 136 germplasms of sorghum. Directional vectors represent root and shoot traits, and the points are genotype value.

4.4. Clustering of Genotypes and divergence analysis

All 136 genotype were grouped into four clusters (fig.5) by examining the Cubic clustering criterion (CCC), pseudo F (PSF) statistic and the pseudo T^2 (PST^2) statistic using PROC clustering strategy to decide the numbers of clusters. Different member within a cluster assumed to be more closely related in terms of the trait under consideration with each other than those members of the different cluster.Cluster one consisted of 23 germasms, which characterized by a maximum in root length and intermediate for all other traits. Cluster two consisted of 38, genotype characterized by highest root to shoot ratio and smallest root length, root number and root angle. Cluster three consisted of 52the genotype with maximum averages for a number of fully expanded leaves and root number. This group also consisted low average for leaf area, shoot length, stem diameter, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. Cluster four consisted of 23 germplasms genotype, with amaximumin leaf area, shoot length, stem diameter, shoot fresh weight, shoot dry weight, root fresh weightand root dry weight.

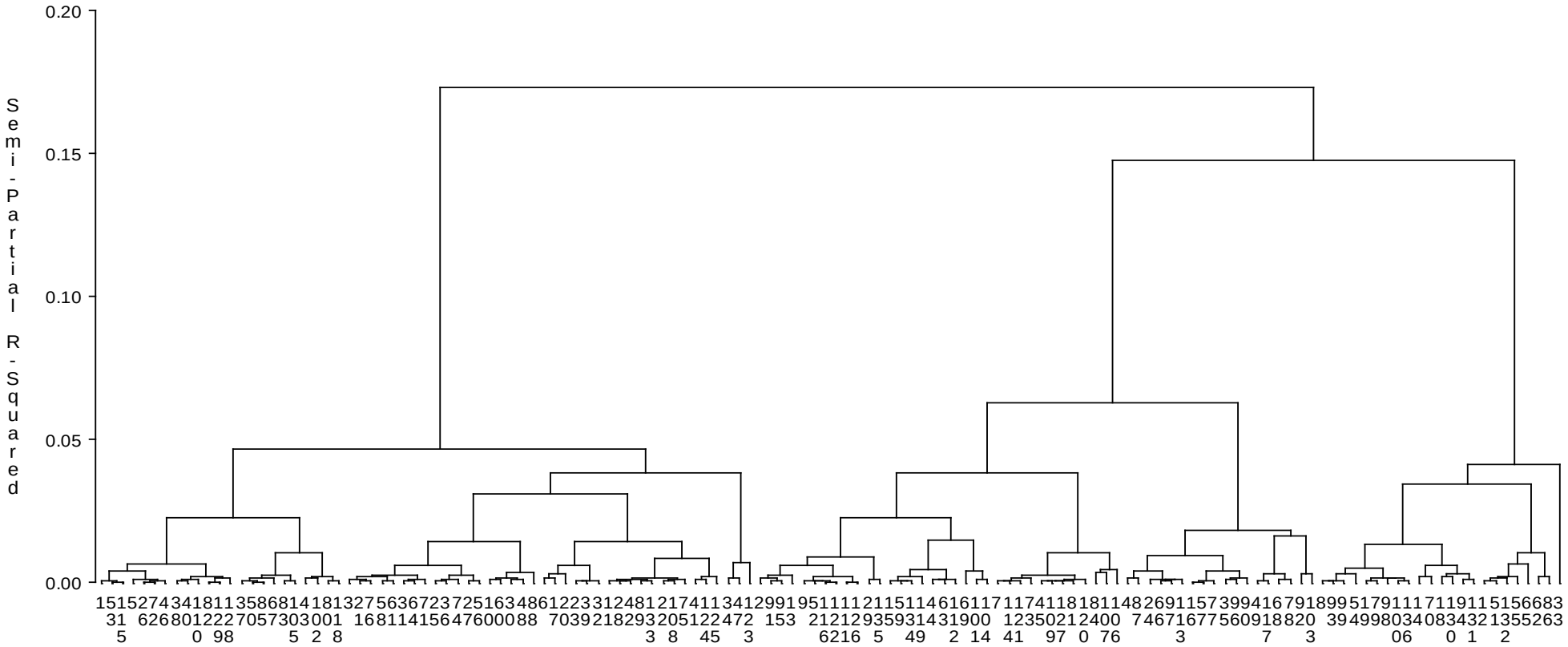

Figure 5. Dendrogram based on genetic relationships of 136-sorghum genotype

4.5. Estimation of genotypic and phenotypic variance

Moderate genotypic variation was exhibited for leaf area, shoot fresh weight, shoot dry weight, root to shoot ratioand root angle. While the highest phenotypic variation was exhibited for leaf area, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight,and root to shoot ratio. Lower values of genotypic variance were noticed in shoot length, stem diameter, the number of fully expanded leaves, root number, root length, root fresh weight and root dry weight. While the lower value of phenotypic variance was exhibited for a number of fully expanded leaves and root length, which is indicative of the stable nature of these characters.

Breeder success in selecting germplasms possessing higher yield and growth traits depends largely on the existence and exploitation of genetic variability of the fullest extent. In general, the phenotypic coefficient of variation (PCV) was higher than its genotypic counterpart (GCV) for all the characters studied (Table. 7).The GCV provides a measure for comparing genetic variability in various traits whereas PCV measures total relative variation. High values of GCV suggested better improvement for selection of traits. However, the estimation of heritable variation with the help of genetic coefficient of variation alone may be misleading. Burton (1962) suggested that the genetic coefficient of variation together with heritability estimates gave a better picture of the extent of heritable variation.

Broad sense heritability ranged from 19.35% for root length to 71.08% for shoot fresh weight at seedling. High heritability was recorded for leaf area (70.22%), shoot fresh weight (71.08%) and root angle (66.22%). Moderate values were obtained for shootlength (54.44%), stem diameter (36.29%), shoot dry weight (35.36%), root number (34.4%), root fresh weight (37.57%), root dry weight (37.81%) and root to shoot ratio (44.98%). The lowest heritability values were obtained for leaf number (27.7%) and root length (19.55%).

High heritability combined with high genetic advancewas observed for shoot fresh weight, root angle, and leaf area. It indicates less influence of environment on the expression of these characters; and prevalence of additive gene action in their inheritance (Panse, 1957). Hence, these droughts related traits require simple selection in breeding programs. Moderate heritability with high genetic advance was recorded for shoot dry weight, root fresh weight, root dry weight and root to shoot ratio. Moderate heritability with moderate genetic advance was recorded for root number, shoot length and stem diameter. The low value of expected

genetic advance was recorded for root length and number of fully expanded leaves due to low variability for the trait indicated by the low phenotypic and genotypic variance values. This indicates that the importance of genetic variability in improvement through selection.

Knowledge on the heritability of traits is helpful in deciding the selection procedure to improve the trait given. Higher estimates of heritability with genetic advance as percent of the mean was observed for leaf area, Root angle and shoot dry weight indicating the presence of additive gene action, and so selection can be easily made for these traits. The trait, which expressed high heritability and low genetic advance, showed non-additive gene interaction. Hence, heterosis breeding would be recommended for that trait.

Table.7.Genotypic and phenotypic variance of the mean for traits

Characters	Range	σ2g	σ2p	σ2e	GCV (%)	PCV (%)	H2 (%)	GA	GAM (%)
LA	7.51_30.21	6.49	13.15	6.65	18.32	26.09	70.22	5.75	41.32
SHL	15_30.7	3.21	10.80	7.59	8.16	14.99	54.44	4.034	18.4
LN	4.93_6.9	0.02	0.26	0.24	2.49	8.99	27.7	0.29	5.23
STD	3.05_5.81	0.07	0.53	0.46	6.13	16.89	36.29	0.56	12.90
SHFW	2.86_11.36	0.26	1.61	1.35	18.41	25.9	71.08	2.14	43.63
SHDW	0.57_2.24	0.01	0.08	0.07	10.87	30.74	35.36	0.22	23.75
RL	42-60	0.50	13.28	12.78	1.44	7.44	19.35	1.6	3.25
RN	4.33_10	0.20	1.69	1.49	6.87	19.97	34.4	0.95	14.59
RFW	1.77_5.78	0.13	0.92	0.79	9.51	25.31	37.57	0.77	20.21
RDW	0.26_1.03	$3.3*10^{-3}$	$2.3*10^{-2}$	0.02	9.79	25.89	37.81	0.13	22.44
RSHR	0.20-1.13	0.01	0.05	0.04	14.79	32.88	44.98	0.21	31.34
RA	14.5_30.75	6.30	14.25	7.95	10.65	.16.01	66.52	5.07	36.58

σ2g= Genotypic variance, σ2p =phenotypic variance, σ2e = environmental variance, GCV= genotypic coefficient of variation, PCV=phenotypic coefficient of variation, H2 = heritability in broad sense, GA = expected genetic advance, GAM (%) = genetic advance as percent of the mean.

4.6. Population structure

The present study showed that 108 germplasms studied were grouped into three distinct classes as shown in Fig. 5 and 6.The plot of the average log likelihood values over eight runs for each K (k-values ranging from 1 to 9) showed that the log likelihood estimates increase progressively as K increases (Fig. 6) and did not show a clear peak to determine the true K (number of subgroups). The ad hoc criterion described by Evanno *et al., (*2005) was used to detect the most probable number of sub-populations andwas found to be 3 (Fig.7).

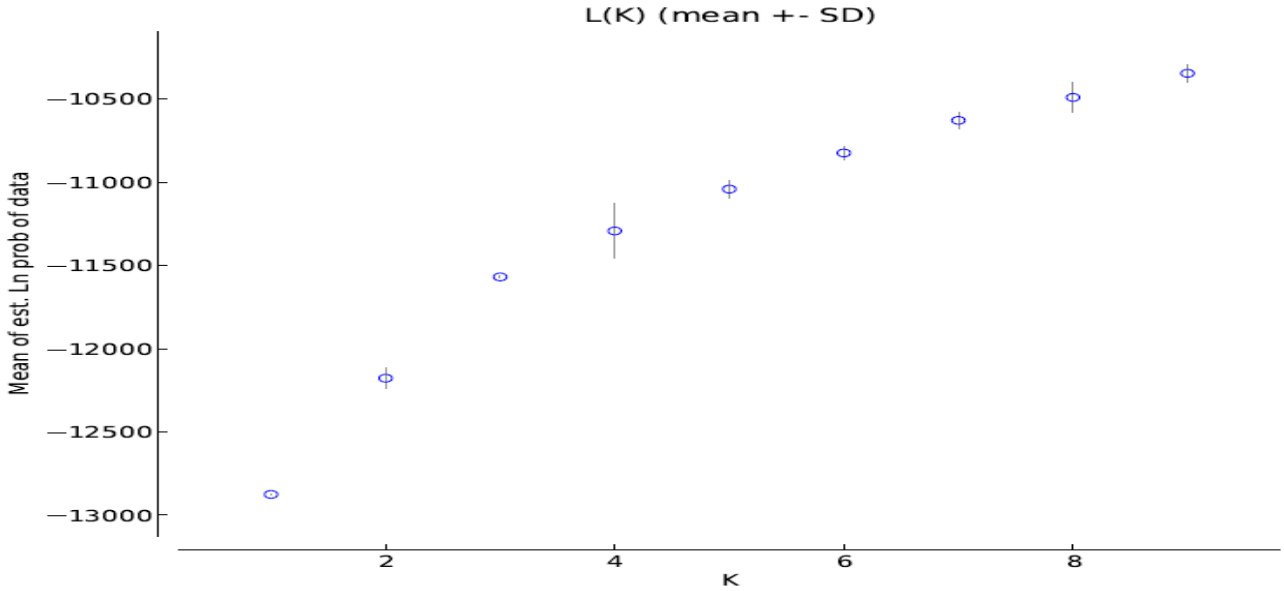

Figure 6. Posterior probability, ln $P(D)$, of the data as a function of the number of subpopulations (k), where k was allowed to range from 1 to 9.

Circles represent the average of the eight independent runs for each value of k.

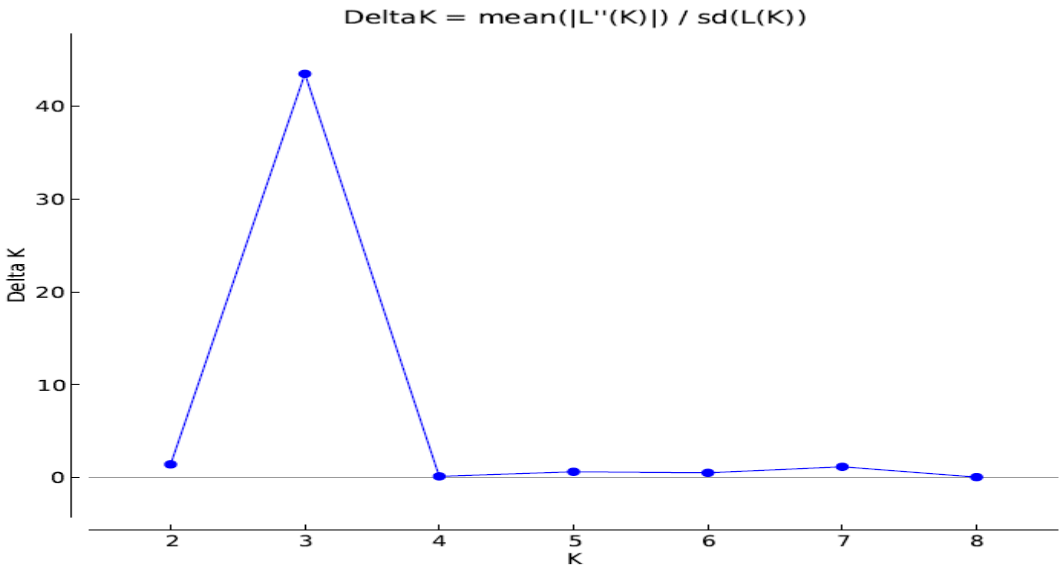

Figure 7.Values of K (x-axis) with modal values used to detect the true K (y-axis) of three groups (K =3).

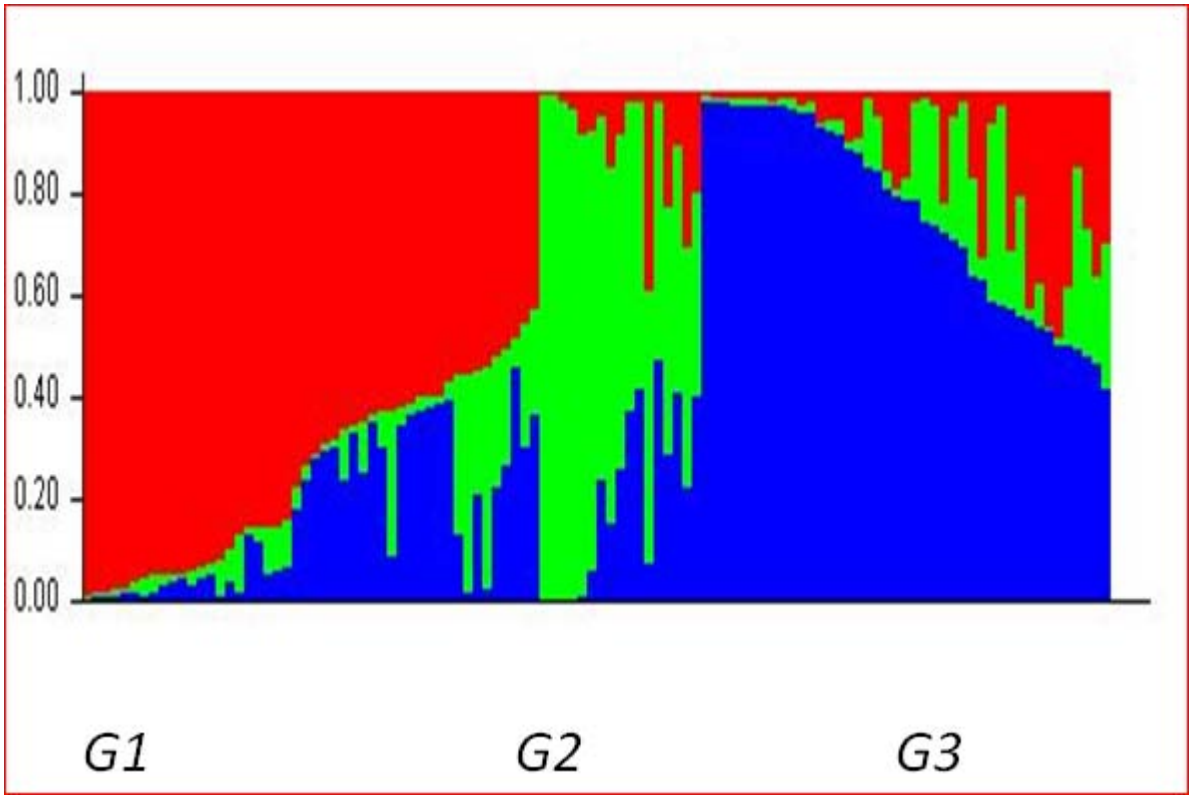

Figure 8.Population structure in the studied population

The subpopulations (K= 3) are represented by different colors as indicated at above (Group-1=red, Group-2=green, and Group- 3=Blue).

As indicated in Fig 8, the first group, G-1 (red) consisted of 47 germplasms of which 11 were from Amhara, eight from Oromia, six from SNNP, one from Afar, five from Tigray, seven from Gambelia and six released varieties. The second groupG-2 (green)which is the smallest of the three subpopulations consisted of 16 germplasms: 6 were from Oromia, three from Tigray, five from Amhara, and one from Dire Dawa and one from SNNP. The third group, G-3(blue) consisted of 45 germplasms of which, 18 were from Amhara, 14 from Oromia, seven from Tigray, three from Eritrea, one from Afar,one from Dire Dawa and one released variety. The distribution of germplasms into the three groups are without reflecting their region of origin and this might be an indication of the presence of wide variations among germplasms within the regions as well as thelack of strong regional differentiation, which might be due to gene flow between the regions. This result is in agreement with Atnafu (2010); Nemera *et al*.(2006), Perumal *et al*.(2007) andAlemu (2009).

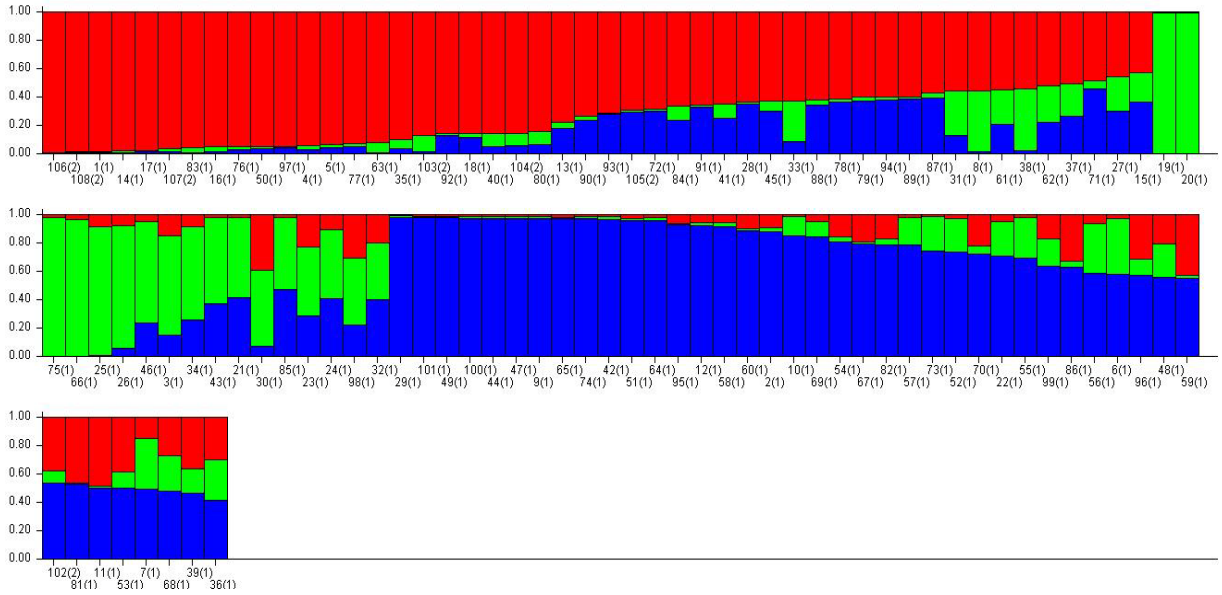

Figure 9.Proportional membership of the studied sorghum germplasms.

A single vertical line is broken into one to three- colored segments (blue, green, and red) with lengths proportional to each of the three inferred population subgroups represents each individual sample. The numbers outside the brackets are entry numbers for individuals examined and the number inside the bracket (1 and 2) represents landrace (1) and improved variety (2)(fig.9).

4.7. Linkage disequilibrium

There were 325 pairwise locus comparisons for all germplasms and the majority of loci pairs (67.38%) were independent loci (non-significant). In all germplasms, 106 loci pairs (32.62%) had a significant ($p < 0.05$) mean LD of 0.14, with an $R^2 > 0.02$ for 33 evaluated loci pairs.

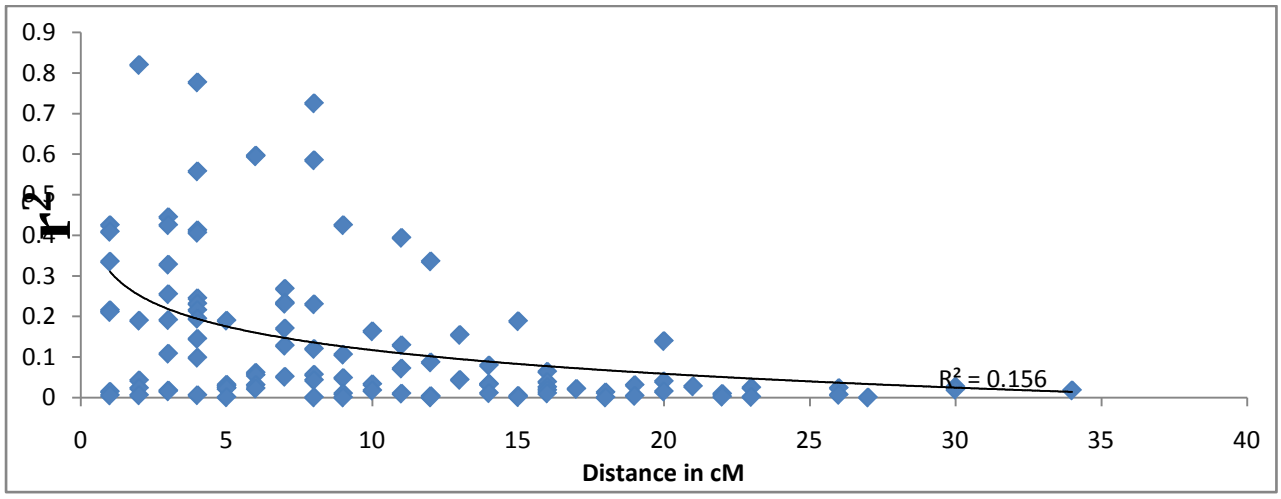

Figure 100. Linkage disequilibrium decay plot generated by 39 SSR markers

The plots of LD (r^2) for pairs of lociversus the genetic distance in cM, betweenloci in the pairswere drawn from r^2 values calculated by TASSEL and showed a clear trend on linkage disequilibrium decay in the studied germplasm. As shown above (Fig 10), LD decay is considered when is r^2below 0.1 thresholds and based on trend line the average linkage disequilibrium (LD) decay distances of15-20 cM.As LD is broken down by recombination, and recombination is not distributed homogeneously across the genome, blocks of LD are expected. Inter-chromosomal LD variation has been reported in barley (*Hordeum vulgare*) (Malysheva *et al.*, 2006), maize (*Zea mays*) (Yan *et al*., 2009), tomato (*Solanum lycopersicum*) (Robbins *et al*., 2011) and bread wheat (*Triticum aestivum*) (Zhang *et al*., 2010) where it varied between less than 1 cM to more than 30 cM ($r2 > 0.1$).

4.8. Association among traits and SSR marker

Table.8. Marker-trait associations

Trait	Marker	Marker-F	Marker-p	markerR2	Chrs	Position	Allele	Obs	Estimate
LN	mSbCIR262	5.48696	0.02244	0.08235	7	88.8	218:218	64	-0.859
LN	mSbCIR300	5.48696	0.02244	0.08235	7	58.3	106:106	64	-0.859
RFW	Xtxp273	3.85466	0.00547	0.06636	8	0	195:211	49	-0.567
RN	Xcup02	8.47263	0.00508	0.11928	9	71.9	200:200	46	-1.07
RN	Xcup02	8.47263	0.00508	0.11928	9	71.9	197:197	17	0
SHFW	mSbCIR283	10.06503	0.00242	0.14236	10	18.1	112:112	39	-0.26
SHFW	mSbCIR283	10.06503	0.00242	0.14236	10	18.1	112:120	23	0
SHL	mSbCIR262	4.5348	0.03726	0.06653	7	88.8	218:218	64	-0.94
SHL	mSbCIR300	4.5348	0.03726	0.06653	7	58.3	106:106	64	-0.94
STD	mSbCIR262	5.87968	0.01829	0.08771	7	88.8	218:218	64	-0.29
STD	mSbCIR300	5.87968	0.01829	0.08771	7	58.3	106:106	64	-0.29
LA	Xcup02	4.40927	0.04262	0.10525	9	71.9	197:197	37	-0.51
LN	Xcup63	4.61483	0.03831	0.11064	2	51.6	146:146	16	-0.293
LN	Xcup63	4.61483	0.03831	0.11064	2	51.6	140:146	25	0
RA	Xtxp015	6.88924	0.01265	0.13773	5	42.1	211:211	37	5.54758
RA	Xtxp265	4.93643	0.03286	0.10348	6	51.17	184:201	33	2.85348
RA	Xtxp321	8.01541	0.00774	0.16401	8	68.1	192:198	36	5.76615
RDW	Xtxp145	5.30097	0.02819	0.12383	6	64.9	214:214	33	0.27924
RDW	Xtxp273	5.62511	0.02369	0.12331	8	0	195:211	35	0.26818
RL	Xtxp136	4.38885	0.04308	0.08879	5	57.6	237:240	35	0
RL	Xtxp145	5.31024	0.02806	0.1222	6	64.9	214:214	33	6.11076
SHDW	Xtxp265	5.17369	0.02917	0.12817	6	51.17	184:201	33	-0.229
SHFW	mSbCIR329	4.90778	0.03297	0.10924	10	0.3	113:113	20	-0.557
SHFW	mSbCIR329	4.90778	0.03297	0.10924	10	0.3	111:111	21	0
SHFW	Xtxp265	4.3938	0.04337	0.10852	6	51.17	184:201	33	-0.734

Table 8. Contented

STD	Xtxp145	5.42482	0.02654	0.13481	6	64.9	214:214	33	1.0799
RSHR	Xcup02	3.03013	0.00904	0.06256	9	71.9	197:197	37	0.19283
RSHR	Xtxp021	3.79914	0.00593	0.08047	4	32.6	171:171	30	0.15111
RSHR	Xtxp265	3.93642	0.00514	0.09172	6	51.17	184:201	33	0.17187
RSHR	Xtxp320	4.15743	0.04906	0.08712	10	62.8	260:275	33	0.18489

Where,LA=average Leaf area; LN=Number of fully expanded leaves; RA= Nodal root angle; RDW= Root dry weight; RFW= Root fresh weight; RL= Root length; RN= Number of promising root; SHFW= Shoot fresh weight; SHL= Shoot length; STD=Stem diameter

A total of 25 significant marker-trait associations ($P \leq 0.05$) were detected. The phenotypic effect of SSR marker alleles on the associated characters and the number of germplasms carrying each significantly associated marker allele were identified (Table.8). The 25 significant marker-trait associations were identified using 14 different SSR markers for 12 traits, with R^2 ranging from 6.636% to 16.4%.

Nodal root angle: Three SSR markers (Xtxp015, Xtxp265, and Xtxp321) having a significant association ($P \leq 0.05$) with QTLs for nodal root angle were detected on chromosome 5, 6and 8, with effects of 5.55%, 2.85% and 5.77% of the total phenotypic variation, respectively. SSR markers linked to nodal root angle in sorghum were previously reported on chromosome five and nine by Singh, *et al.* (2010) using association mapping. For nodal root angle, two significant QTL on chromosome5, and two suggestive QTL on chromosome 8 and chromosome10 were also previously detected by Mace*et al*. (2012) using single marker analysis.

Leaf area: OneSSR marker (Xcup02) with a significant association ($P \leq 0.05$) with leaf area was detected on chromosome 9. This marker had an effect of -0.51 %, explaining of the total phenotypic variation. A QTL controlling total leaf area was previously reported on chromosome eight by Mace, *et al*. 2012 using single marker analysis.

Number of fully expanded leaves: Threemarkers (Xcup63, MSBIR 262 and MSBIR 300) with a significant association ($P \leq 0.05$) to thetotal number of fully expanded leaves were detected on chromosomes 2and 7 (Xcup63 on 2 and MSBIR 262 and MSBIR 300 on 7). Marker MSBIR 262 and MSBIR 300 each had an effect of -0.859% of the total phenotypic variation whereas for Xcup63 two genotypes (146:146 and 140:146) were significantly associated with the number of fully expanded leaves (LN). At this marker locus, there were 16 germplasms with genotype 146:146 and 25 germplasms with genotype 140:146. For the trait LN, the difference between the two genotypes (146:146 and 140:146) was 0.293. In

other words, the presence of allele 146 in its homozygous state (146:1146) decreased LN by 0.293 in 16 germplasms compared to its heterozygous state (140:146) in 25 germplasms.

Shoot length: Two loci (MSBIR 262 and MSBIR 300) on chromosome 7 showed significant association ($P \leq 0.05$) with shootlength.Both MSBIR 262 and MSBIR 300 had an effect of -0.94 % of the total phenotypic variation.

Stem diameter: Three loci, MSBIR 262 and MSBIR 300 on chromosome 7 and XtxP 145 on chromosome 6, showed significant association ($P \leq 0.05$) with stem diameter. MSBIR 262 and MSBIR 300 had an effect of -0.29 % each of the total phenotypic variation, whereas XtxP 145 had an effect of explaining 1.08 % of the total phenotypic variation.

Shoot fresh weight: Three loci, MSBIR 283 and MSBIR 329 on chromosome 10 and XtxP 265 on chromosome 6, showed significant association ($P \leq 0.05$) with shoot fresh weight. MSBIR 283, MSBIR 329 and XtxP265 had an effect of -1.54 %,-0.56 % and -0.73% respectively of the total phenotypic variation. For MSBIR283 two genotypes (112:112 and 112:120) were significantly associated to shoot fresh weight (SHFW). At this marker locus, there were 39 germplasms with genotype 112:112 and 23 germplasms with genotype 112:120. For the trait SHFW, the difference between the two genotypes (112:112 and 112:120) was 0.24. In other words, the presence of allele 112 in its homozygous state (112:112) decreased SHFW by 0.24 in 39 germplasms compared to its heterozygous state (112:120) in 23 germplasms. For mSbCIR329 on chromosome 10 at position 0.3 Mb that also linked to SHFW, there were two genotypes (113:113 and 111:111). For this marker, there were 20 germplasms with genotype 113:113 and 21 germplasms with genotype 111:111. For this trait (SHFW), the difference between the two genotypes ((113:113 and 111:111) on expressing therate of its effect on this trait was 0.557.

Shoot dry weight: One locus (XtxP 265) with a significant association ($P \leq 0.05$) with shoot dry weight was detected on chromosome 6. Ithad an effect of -0.229 % of the total phenotypic variation. QTL controlling shoot dry weights were also previously reported on chromosome one and five byMace*et al*. (2012) using single marker analysis.

Root fresh weight: One locus (XtxP 273) with a significant association ($P \leq 0.05$) with rootfresh weight was detected on chromosome 8. This markerhad an effect of -0.57 % of the total phenotypic variation.

Root dry weight: Two loci (XtxP 145 and XtxP 273) on chromosome 6 and eight respectively showed significant association (P≤0.05) with root dry weight. XtxP 145 had an effect of 0.28 % to the total phenotypic variation whereas XtxP 273 had an effect of 0.27 % onthe total phenotypic variation. One significant QTL and two suggestive QTL controlling root dry weights were also previously identified on chromosome-5, chromosome-2, and chromosome-8, respectively by Mace*et al*. (2012) using single marker analysis.

Root number: One locus (Xcup02) with a significant association (P≤0.05) with root number was detected on chromosome 9. Marker Xcup02 had an effect of -1.07 % of the total phenotypic variation. There were two genotypes for this locus (200:200 and 197:197) having 46 germplasms with genotype 200:200 and 17 germplasms with genotype 197:197. For this trait (RN), the difference between the two genotypes (200:200 and 197:197) on expressing therate of its effect on this trait was 1.07.

Root length: Two loci (XtxP 136 and XtxP 145) on chromosome 5 and 6 respectively showed significant association (P≤0.05) with root length. XtxP 136 had no effect whereas XtxP 145 had an effect on 6.11 % of the total phenotypic variation.

Root to shoot ratio: Four loci (Xtxp021, Xtxp265, Xcup02,and Xtxp320)with a significant association (P≤0.05) with root to shoot ratio were detected on chromosome 4, 6, 9 and10 respectively. The effect of these markers on the phenotypic variation were0.15%, 0.177%, 0.19 % and0.18%, respectively.

Both locus MSBIR 262 and MSBIR 300 showed significant simultaneous associations (P≤0.05) with three characters, namely number of fully expanded leaves, shoot length and stem diameter on chromosome 7 and had an effect of explaining -0.86 %, -0.94 %, and -0.29% respectively of the total phenotypic variation. Locus XtxP 145 showed significant simultaneous associations (P≤0.05) with three characters, namely root dry weight, root length, and stem diameter on chromosome 6 and had an effect of explaining 0.28 %, 6.11 %, and 1.08 % respectively of the total phenotypic variation. Locus XtxP 265 showed significant simultaneous associations (P≤0.05) with three characters, namely, root angle, shoot dry weight and shoot fresh weight on chromosome 6 and had an effect of explaining 2.85 %, -0.23 %, and -0.73 %,respectively of the total phenotypic variation. Locus Xcup02 showed significant simultaneous associations (P≤0.05) with two characters, namely root number and leaf area on chromosome 6 and had an effect of explaining -1.07 %, and -0.51 % respectively

of the total phenotypic variation. Locus Xtxp273 showed significant simultaneous associations (P≤0.05) with two characters, namely, root fresh weight and root dry weight on chromosome 8 and had an effect of explaining –0.57 %, and 0.27 % respectively of the total phenotypic variation.

For all twelve traits, 14 associated markers and out of these six markers were detected in more than one trait. As shown in Table.8, some QTL showed positive effects whereas others had negative effects; and some had null allelic effects. This was due to the presence of different alternative alleles expressing their effect differently to under environmental condition

In order to identify overlaps between QTLs found in this study and QTL found in other studies, markers that showed association in this study were projected with markers showing association for traits in previous studies(Fig.11). Doing that, the 14 associated markers identified were near the QTL-linked/associated markers controlling the same or different traits identified in other reports on the reference map. Considering the different markers used in the prior studies and the precision of QTL detection, these nearby marker pairs should be linked to the same QTLs reported.

The linked markers highlighted in red are the present findings whereas those in black are previously identified ones. Genetic position in centiMorgan(cM) are indicated on the left of the map, and the corresponding marker names are indicated on the right

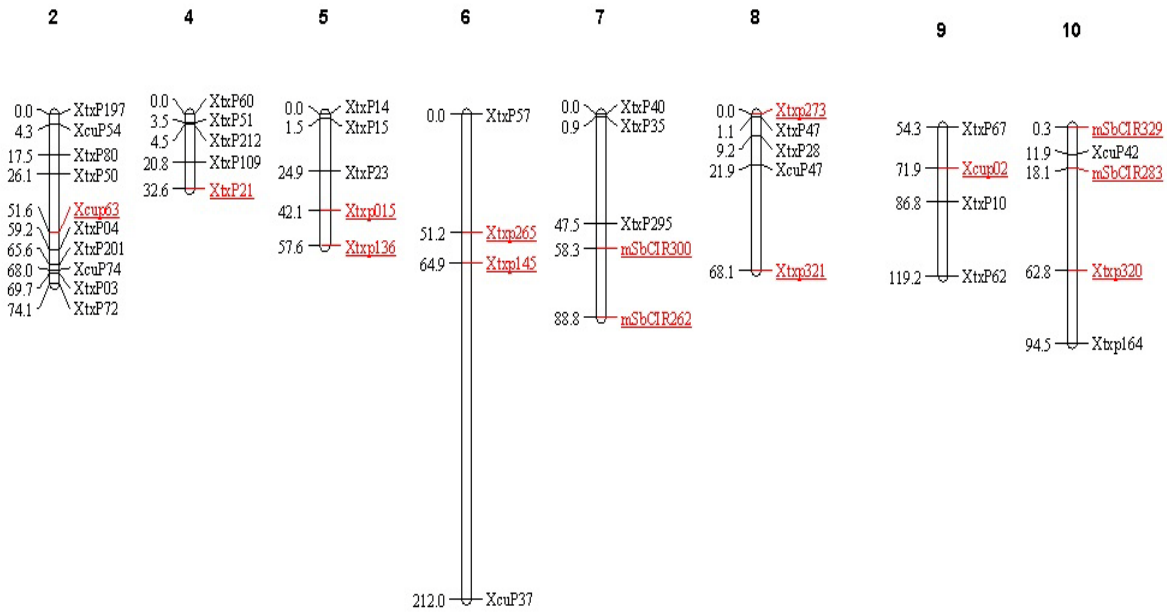

Figure 111. Locations of QTL for different traits

Where: chromosome number 2: number of fully expanded leaves,4: root to shoot ratio,5: root angle and root length, 6:root angle, root length, shoot fresh weight ,shoot dry weight, root to shoot ratio, stem diameter and root dry weight, 7:stem diameter, shoot length and number of fully expanded leaves, 8: root angle, root fresh weight,and root dry weight, 9: root number, root to shoot ratio and leaf area, 10: root to shoot ratio and shoot fresh weight(Fig.11).

5. SUMMERY AND CONCLUSION

Although sorghum is relatively a drought tolerant crop compared to other cereals,droughtis still the major constraint for its production. Therefore, knowledge of genetic variability for drought related traits is the key component in selecting germplasms that withstand drought for the future breeding program. Although Ethiopia is centers of diversity of sorghum species, there has been limited research work so far on screening pattern of drought resistant variety through high throughput phenotyping technology and on the issue of marker-trait association for drought adaptation. All these existing facts jointly necessitate screening drought adaptable varieties of sorghum.

To overcome the limitation of traditional QTL mapping such as restricted number of allelic variation in each cross, small number of recombination events per chromosome and low number of linkage disequilibrium for identified QTL in a cross consisting of a few hundred offspring, association mapping can be used by molecular breeders to map QTLs associated with desired traits. The precision of QTL mapping largely depends on the genetic variation in the mapping population, the size of a mapping population, and a number of marker loci used. By taking into account the importance of association mapping for dissecting the complex quantitative traits, the present study was notably conducted to characterize andmap of QTLs associated with drought adaptation on sorghum seedling. With its detail task, assessment of genetic variability among sorghum germplasms for traits associated with drought adaptation on shoot and root, map QTLs associated with drought adaptation on this trait, and identification of SSR markers tightly linked to this characters have also been worked out.

In this study, one hundred thirty-six sorghum germplasms (127 landraces and nine released varieties) were used. These populations were evaluated in custom-made special design root observation chamber at JUCAVM greenhouse using randomized complete block design replicated three times. The phenotypic data were collected including root angle, root length, root number, root fresh weight, root dry weight, leaf area, thenumber of fully expanded leaves, shoot length, stem diameter, shoot fresh weight, shoot dry weight and root to shoot ratio.

The analysis of variance indicated that there is a highly significant difference ($P<0.01$) among germplasm for all studied trait. The coefficients of correlation among the traits understudied (Table.5) also showed that most of the trait revealed a significant positive and

negativeassociation with each other. For the principal component analysis of traits, the first three principal components (PCs) with eigenvalues greater than one are accounted for 56.4% of the total genotypic variation, leaving the remaining 43.6% in the last nine principal components. Shoot dry weight, shoot fresh weight, root fresh weight, root dry weight, stem diameter, root angle, root length and shoot length and leaf area were the considered characters indicating the lion share of these traits towards the divergence. This technique could be used as an efficient tool for selecting germplasms based on desired traits in the early stages of the breeding process. Therefore, it may be possible to conclude that these traits should be taken into consideration by breeders while selecting the genetically diverse parents for further utilization.

In this study, the phenotypic coefficient of variation (PCV) was higher than its genotypic counterpart (GCV) for all the characters studied. Higher estimates of heritability with genetic advance as percent of the mean was observed for leaf area, root angle and shoot dry weight indicating the presence of additive gene action, and so selection can be easily made for these traits. Moderate heritability with high genetic advance was recorded for shoot dry weight, root fresh weight, root dry weight and root to shoot ratio.

For association mapping, the 108-sorghum germplasm contained three distinct subgroups and to avoid false positive association results or to maintain low false positive results, different significance tests (population structure and permutation testing) were employed. Generally, 25 significant marker-trait associations ($P \leq 0.05$) were detected using 14 different SSR markers. For all twelve traits, the GLM ($p < 0.05$) detected 14 associated markers and six markers were detected in more than one trait. When we employed the reference map as a bridge to compare the results of the present study with those from previous studies, the 14 associated markers identified were near the QTL-linked/associated markers controlling the same traits identified in other reports, at distances of less than 1–2 LD decay on the reference map. Considering the different markers used in the prior studies and the precision of QTL detection, these nearby marker pairs should be linked to the same QTLs reported.Although a large number of molecular markers are necessary for the genome-wide association study, this study can serve as an initial effort for the association mapping studies in sorghum, particularly in our country.Based on this study, the following recommendations were suggested:

- Germplasms that showed desirable phenotypes such as narrow root angle need to be evaluated under field condition to verify their performance and thereby they can be used in the breeding programs
- As this study is the first in Ethiopia, the identified QTLs need to be validated through repeated phenotypic measurement in independent or related populations
- The SSR markers found to be associated with traits need to be validated before their use in marker-assisted selection.

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7. APPENDIX

Appendix Table.1: Analysis of variance for the twelve drought-related traits in 136 SorghumGermplasms evaluated for drought adaptation

variation	LA	SHL	LN	STD	SHFW	SHDW	RL	RN	RFW	RDW	RSHR	RA
Rep	27.83	89.35	1.38	2.3	50.91	1.21	27.71	3.31	3.57	0.18	0.102	3.92
P-value	**	**	**	**	**	**	NS	NS	*	**	NS	NS
Genotype	26.15	17.23	0.29	0.67	2.13	0.1	18.98	2.1	1.18	0.034	0.07	23.7
P-value	**	**	*	**	**	**	**	**	**	**	**	**
CV	9.65	6.39	0.36	7.74	11.82	13.85	3.67	9.37	11.98	12.81	27.87	3.59
MSE	6.65	7.59	0.24	0.46	1.35	0.07	12.78	1.49	0.79	0.02	0.04	7.94

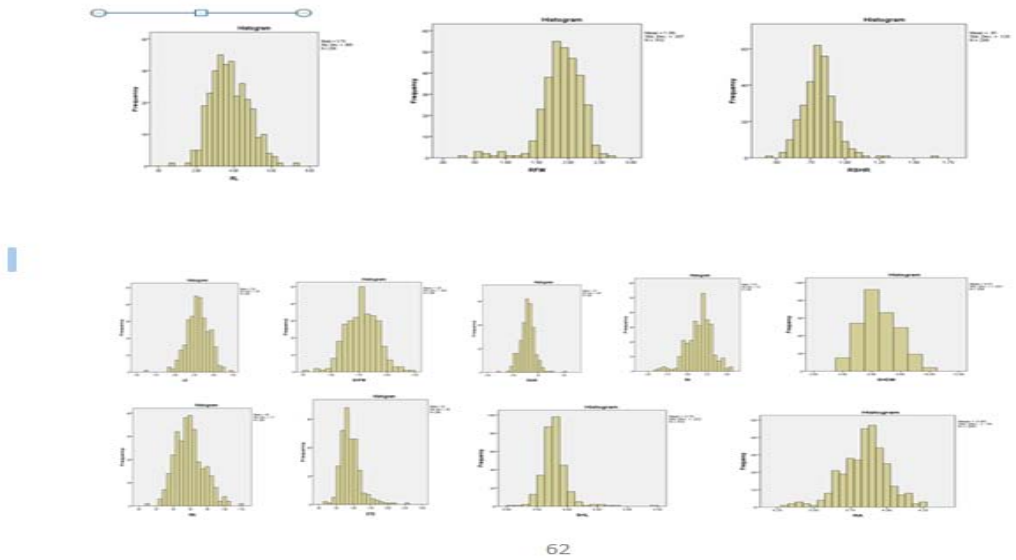

Figure 1.Frequency distribution of phenotypes for 12drought-related traits in 136-sorghum germplasm