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# Genetic Variability of Sorghum [Sorghum Bicolor (L) Moench] Inbreed Lines for Transpiration Efficiency

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## **BAHIR DAR UNIVERSITY**

# COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

# **GRADUATE PROGRAM IN PLANT BREEDING**

GENETIC VARIABILITY OF SORGHUM [Sorghum bicolor (L) MOENCH] INBREED LINES FOR TRANSPIRATION EFFICIENCY

> M.Sc Thesis By Meron Bogale Agajie

> > November 2022 Bahir Dar, Ethiopia



# BAHIR DAR UNIVERSITY COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES GRADUATE PROGRAM IN PLANT BREEDING

# GENETIC VARIABILITY OF SORGHUM [Sorghum bicolor (L) MOENCH] INBREED LINES FOR TRANSPIRATION EFFICIENCY

# M.Sc Thesis By Meron Bogale Agajie

# SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (M.SC.) IN PLANT BREEDING

November 2022 Bahir Dar, Ethiopia

### THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by Miss Meron Bogale Agajie entitled **GENETIC VARIABILITY OF SORGHUM** [*Sorghum bicolor* (L) **MOENCH**] **INBREED LINES FOR TRANSPIRATION EFFICIENCY**. We hereby certify that, the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (M.Sc.) in Plant Breeding.

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Date

#### DECLARATION

This is to certify that this thesis entitled GENETIC VARIABILITY OF SORGHUM [Sorghambicolor (L) MOENCH] INBREED LINES FOR TRANSPIRATION EFFICIENCY submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Plant Breeding to the Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dur University by Miss Meron Bogale (ID. No. 1300268) is an authentic work carried out by her under our guidance. The matter embodied in this project work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

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## **DEDICATION**

This thesis is dedicated to my mother Tiruye Beyene. She had a hope to see my success.

### **GENETIC VARIABILITY OF SORGHUM** [Sorghum bicolor (L) MOENCH] **INBREED LINES FOR TRANSPIRATION EFFICIENCY**

By: Meron Bogale,

Advisors: Dr. Alemu Abate<sup>1</sup> and Dr. Taye Tadesse<sup>2</sup>

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#### Abstract

Sorghum [Sorghum bicolor (L.) Moench] is the king of cereal crops in the arid and semiarid tropics, where drought is a recurrent problem affecting crop production because of erratic amount and distribution of rainfall. Although sorghum has a genetic potential to withstand the effect of drought, it is being affected by the current climate change scenarios. The present experiment was conducted with the view of assessing sorghum variability to transpiration efficiency, and association among traits using 101 sorghum genotypes in greenhouse facility at Melkassa Agricultural Research Center during 2021/22. The design of the experiment was RCBD with two replications. Data analyses were computed using SAS 9.4. The analysis of variance showed significant difference among genotypes for all traits considered, indicating the presence of considerable genetic variability among tested genotypes. High heritability coupled with high genetic advance as percentage of the mean (GAM) was recorded for plant transpiration efficiency and shoot transpiration efficiency. Moderate heritability coupled with high GAM were recorded for total dry biomass, shoot dry biomass, root dry biomass, shoot fresh biomass, water use, and leaf area, suggesting the possibility of improving these traits through direct selection. Correlation analysis revealed that plant transpiration efficiency had positively significant genotypic and phenotypic correlations with shoot transpiration efficiency, total dry biomass, shoot fresh biomass, root dry biomass, shoot fresh biomass and leaf chlorophyll content. Path coefficient analysis showed that total dry biomass was directly affected by plant transpiration efficiency, shoot transpiration efficiency, shoot fresh biomass, leaf chlorophyll content, leaf area, water use and leaf number. Cluster analysis grouped genotypes into six clusters. The maximum inter-cluster distance was between cluster II and cluster V, suggesting the possibility of improving genotypes through hybridization. The four principal components with eigenvalues greater than one accounted for about 79% of the total variation among genotypes, indicating that the traits considered were appropriate to detect variation among tested genotypes. Overall, the present study indicates the presence of considerable genetic variability to improve transpiration efficiency of sorghum and to develop adaptable and heigh yielder sorghum varieties for drought stress environment.

*Keywords:* - adaptation to drought; path coefficient analysis; principal component analysis; variability

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# LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance		
CSA	Central Statics Agency		
CV	Coefficient of Variation		
DUL	Drained Upper Limit		
EIAR	Ethiopian Institute of Agricultural Research		
FAO	Food and Agricultural Organization		
GA	Genetic Advance		
GAM	Genetic Advance as Percentage of the Mean		
GCV	Genotypic Coefficient of Variation		
H <sup>2</sup> b	Broad Sense Heritability		
MARC	Melkassa Agricultural Research Center		
PCA	Principal Component Analysis		
PCV	Phenotypic Coefficient of Variation		
TE	Transpiration efficiency		
VPD	Vapor Pressure Deficit		

#### **Chapter 1. INTRODUCTION**

#### **1.1.Background and Justification**

Sorghum [Sorghum bicolor (L.) Moench], grouped as C4 plants and it is the world's fifth most important cereal crop after wheat, maize, rice, and barley in terms of production (FAO, 2021). Sorghum is widely adaptable in the semi-arid tropics; it is one of the major dry land crops in areas where drought stress is the major impediment to crop production (Vadez, 2016). Sorghum is a multi-purpose crop grown in diverse agro-ecologies; it serves as a dietary staple crop for millions of people, especially in arid and semi-arid farming systems. It requires less water than most cereals; hence it offers great potential for supplementing food and feed resources, especially in dry lands. Additionally, sorghum grain is used as livestock feed and the production of local beverages, while the stalk is used for animal feed, firewood, and as a construction material (McGuire, 2000).

In Ethiopia, sorghum is a major staple food crop, in area coverage sorghum ranked third next to the teff and maize, as to production ranked fourth after teff, wheat and maize (CSA 2021). It is cultivated over a wide range of altitudes (400 to 2500 m.a.s.l) and rainfall conditions (Nawaz, 2014). It is used as a food and feed crop in dry lowland areas, where moisture is limiting, and it is often the only crop grown because of its vast flexibility and tolerance to unfavorable circumstances (Huang, 2018). In such drought-prone environments, it is essential to use the limited available water efficiently to stabilize or increase yield (Sinclair *et al.*, 2005).

Sorghum production is constrained by several biotic, abiotic, and socio-economic factors. Amongst the most important abiotic constraints, drought is the most important. It is the major cause of poor crop performance and reduction of yield, and sometimes it causes total crop failure (Yared Assefa *et al.*, 2010). Drought can occur at any stage of crop development. In the arid and semi-arid tropics, the probability of drought is the highest at the start and end of the growing season. Drought stress at the beginning of the growing season affects severely plant establishment. The occurrence of drought at flowering or grain filling stage may result in yield

reduction or complete yield loss (Blum, 1996). Drought stress in Ethiopia has been a major concern and affected millions of farmers since the early 1970s.

The dry land areas of Ethiopia covered around 66% of the total area of crop production is mainly dependent on rainfall, which is low and erratic throughout the cropping season (Geremew Gebeyehu *et al.*, 2004). The 2015 drought in Ethiopia, aggravated by El Nino, more than 10 million farmers were affected due to the failure of the crop (FAO, 2018). Until now, the problem of drought is the major production constraint in the major sorghum growing areas of Ethiopia.

Transpiration efficiency (TE) is an indispensable phenomenon associated with plant drought tolerance (Mian *et al.*, 1998). It is referring to the amount of aboveground biomass (kg dry matter) per unit of water transpired (Kemanian*et al.* 2005). Understanding crop transpiration efficiency of crop in a climate change environment could have dual benefits. Firstly, it helps to identify genotypes that have high assimilation rates under temperature and water-deficit stresses. Secondly, it helps to realize a range of management practices and adopt to reduce soil water evaporation though limiting the exposure of the plant to water-deficit stress thereby maintaining or increasing crop productivity (Hatfield and Dold, 2019).

Enhancing TE, especially in dry land environments, is likely to have a large impact on improving grain yield. This is because a higher TE trait would either enable plants to delay water stress symptoms or produce more biomass from the same amount of available soil moisture or a combination of both (Xin *et al.*, 2008). A key to increased grain yield under drought stress is to maximize water availability during grain filling (Turner, 2004). This can be achieved through several pathways (van Oosterom*et al.*, 2011), including restriction of pre-anthesis water use through early flowering or reduced canopy size (Borrell *et al.*, 2014), increased access to water through changes in root architecture (Singh *et al.*, 2012), or increased efficiency in the amount of biomass produced per unit of water transpired by the crop (Hatfield and Dold, 2019).

#### 1.2. Statement of problem

As sorghum is a major food and feed crop for arid and semi-arid areas of Ethiopia, its production becomes low because of various constraints (CSA, 2021). Water stress is one of the major constraints for the low productivity of this crop. Therefore, sorghum genotypes that make efficient use of water are needed for growth under those drought stress environments. Under dry land conditions, plants with higher transpiration efficiency were reported to produce higher grain yield than plants with lower transpiration efficiency (Passioura 1977). Muchow et al. (1991) and Hammer et al. (1996) suggested that even a small increase in transpiration efficiency (i.e., 10%) may have a great impact on sorghum yield. Hence, the potential advantages make pursuing transpiration efficiency worthwhile. This makes that transpiration efficiency is a good selection criterion for sorghum in drought prone areas. In our country, the use of transpiration efficiency as a selection criterion in breeding programs is not common. Hence, there is an opportunity to further investigate in key Ethiopian sorghum lines to generate potential options for plant breeding. However, adequate research has not been conducted on genetic variability of sorghum genotypes to transpiration efficiency. Therefore, the present study was conducted in the view of assessing genetic variability of sorghum genotypes to transpiration efficiency to develop adaptable and heigh yielder sorghum varieties for arid and semi-arid areas.

### 1.3. Objective of the Study

- 1.3.1. General objective
  - ✤ To characterize the transpiration efficiency and performance of sorghum inbred lines
- 1.3.2. Specific objectives
  - ✤ To quantify variation among sorghum genotypes for transpiration efficiency;
  - ✤ To estimate heritability and genetic advance of traits;
  - ✤ To determine association among traits;
  - To determine genetic relationship among sorghum genotypes using the traits measured; and
  - To identify sorghum inbred lines with better transpiration efficiency for a future breeding program.

#### **Chapter 2. LITERATURE REVIEW**

#### 2.1. Botany of Sorghum

The sorghum plant's scientific name is Sorghum bicolor (L.) Moench, and it is a member of the Poaceae family. Sorghum originated in Africa, where it is a major food crop and has numerous varieties including grain sorghums used for food, grass sorghums grown for hay and fodder and broomcorn used in making brooms and brushes. Now a day it is grown in all parts of the world specially in Africa, Asia and Central America, because it is valued in hot and arid regions for its resistance to drought and heat. Sorghum is a perennial crop by nature and hence, a very suitable forage crop but it grows as an annual crop for the use of grain (Poehlman and Sleper, 1995). It is a vigorous grass with up to 6 m height (Dicko *et al.*, 2006), stalks and leaves are coated with a white wax and the pith or central portion of the stalks of certain varieties is juicy and sweet.

The tiny flowers are produced in panicles that range from loose to dense; each flower cluster bears 800–3000 kernels. Sorghum is naturally self-pollinating, but it can outcross up to 30% depending on the type of panicle (Poehlman and Sleper, 1995); its flowers open during the night or early morning. The opening of a flower starts from the top of the panicle and finishes the entire panicle within six to nine days (Laidlaw and Godwin, 2009). The seeds vary widely among different types in color, shape and size, but they are smaller than those of wheat. Sorghum has a lower feed quality than corn (maize). However, it is high in carbohydrates with 10 percent protein, 3.4 percent fat and contains calcium and small amounts of iron, vitamin  $B_1$  and niacin. For human consumption, the gluten-free grain is usually ground into a meal that is made into porridge, flatbreads and cakes. The characteristic strong flavor can be reduced by processing.

Sorghum, grain forage or sugar crop is among the most efficient crops in conversion of solar energy and use of water. Sorghum is known as a high-energy, drought tolerant crop. Because of its wide uses and adaptation, sorghum is one of the really indispensable crops required for the survival of humankind.

#### 2.2. Origin and Domestication of Sorghum

Sorghum [*Sorghum bicolor* (L.) Moench] was first domesticated in north-eastern Africa. Vavilov (1951) suggested Ethiopia as a center of origin of sorghum due to the wide variation of the crop. Some researchers argue for multiple centers of origin for the crop. Some authors also suggest the origin of sorghum to be India (Meadow, 1996), while others have proposed the origin and domestication of sorghum as southern China (Qiao and Zhenshan, 1970) and northern China (Kimber, 2000). All theories concerning the origin and domestication of sorghum were based on archaeological evidence. Therefore, archeological evidence identified regions in Sudan, Ethiopia and West Africa as center of origin of sorghum, with evidence for more than one domestication event (Ananda *et al.*, 2020).

Doggett (1988), on the other hand, reported that sorghum originated in northeastern parts of Africa comprising Ethiopia, Sudan, and East Africa. These regions contain the maximum diversity of both wild and cultivated species. Early domestication and selection of the crop in response to environmental factors and human needs resulted in wider variability. The environmental factors included day length, altitude, temperature, rainfall, and soil characteristic. Human needs usually reflect bigger panicle, non-shattering habits, large grain, tall plant height, and early crop duration. The greater diversity is, therefore, partly due to the diverse physical environment (Rao *et al.*, 2002). As a result, new and stable sorghum biotypes emerged and attributed to the selection, adaptation, intercrossing, and movement of plant material from place to place.

The introduction of new biotypes evolved in other places and intercrossing with the native biotypes resulted in the development of new biotypes. This movement and evolution of biotypes gave rise to five sorghum races: bicolor, caudatum, guinea, kafir, and durra (Rooney, 2000). Sorghum is a cultivated tropical cereal grass. It is quantitatively the world's fifth largest and most important cereal grain after wheat, maize, rice and barley (FAO, 2021). In Africa, sorghum is still largely a subsistence food crop.

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#### 2.3. Taxonomy of Sorghum

In the genus Sorghum, 25 species are recognized and are taxonomically grouped into five subgenera: Eusorghum, Chaetosorghum, Heterosorghum, Parasorghum, and Stiposorghum (Garber, 1950). From those groups, Eusorghum includes cultivated sorghum, *Sorghum bicolour* (L.) Moench (2n=20) and its subspecies Drummondii and Arundinaceum, the wild species *S. almum Parodi*, *S. propinquum* (Kunth) Hitch and *S. halepense* (L.) Pers (de Wet, 1978). Sorghum bicolor is a scientific name for grain sorghum which belongs to the Poaceae (grass) family of the genus Sorghum (Smith and Frederickson, 2000).

Sorghum is subdivided into four groups; grain sorghums, grass sorghums (for pasture and hay), sweet sorghums (used to produce sorghum syrups), and broom corn (for brooms and brushes) (Liang, 1988). In Africa, there are two basic types of sorghums: white sorghum and red sorghum; white sorghum is sweeter and used as a grain crop while red sorghum is less tasty to eat but is used to make beer; red sorghum has a high content of tannin due to this the attack of bird is less. Large, erect stem (some 50 cm to 6 m tall), and semi-compact or compact head is the most used types of sorghum species (EIAR, 2010).

#### 2.4. Ecological Requirements for Sorghum Production

Sorghum is adapted to a wide range of ecological conditions, surviving in the tropical, subtropical and temperate regions of the world (Volker and Wolfgang, 2005). It is planted in areas considered to be too dry and hot for other cereals to survive because of its tolerance to drought and heat stress (Atif *et al.*, 2015). Sorghum is one of the most resilient crops which grows on up to 2000 meters above sea level (Kimenye, 2014). Sorghum grows well in a wide range of soils except in waterlogged places. It grows best on medium-textured and light-textured soils and less satisfactorily on heavy textured (clay) soils. It also tolerates a pH ranging from 5.5 to 8.5 and some degree of salinity, alkalinity, and poor drainage (Asfaw Adugna *et al.*, 2005). Sorghum is a warm-weather crop, which requires high temperatures for good germination and growth. A temperature of 27 to 30°C is required for optimum growth and development through the crop, it can survive below 21 °C without a dramatic effect on growth and yield. Sorghum is a short-day plant requiring long night hours before the reproductive stage. Sorghum is known to be drought tolerant and can do well in areas with little rainfall but performs better in conditions where water is available. In Africa sorghum growing where drought stress and loss of soil moisture are the major constraints. Sorghum performs well under optimum conditions of deep and well-drained fertile soils, moderate to high relatively stable rainfall distribution most of which should be received during the vegetative phase and temperate to warm weather (20-30°C). However, it is mostly grown in semi-arid or sub-tropical regions due to its resistance to harsh weather conditions where other crops can't survive.

#### 2.5. Importance of sorghum

Sorghum is the most preferable cereal crop for semi-arid regions of Eastern Africa and South Asia, while it is used as animal feed in these areas of the world (Reddy *et al.*, 2004; Maulana *et al.*, 2017). The grain is used for making injera (a fermented flatbread for which tef is preferred over sorghum) and porridge, or maybe popped, or boiled whole. The sweet stems of some varieties are used as a confection. In the dry lowland areas of Ethiopia, which cover 66% of the total area and where rainfall is erratic and crop failure is common, sorghum plays a significant role both as food and feed in the mixed crop livestock farming system (Yilma Kebede and Abebe Menkir, 1986; Geremew *et al.*, 2004). The grain is also used in making edible oil, starch, dextrose (a sugar), paste, and alcoholic beverages. The stalks are used as fodder and building materials. Sweet sorghums, or sorgos, are grown mainly in the United States and southern Africa for forage and for syrup manufacture and are sometimes used in the production of ethyl alcohol for biofuel.

#### 2.6. Production and Productivity of Sorghum

Globally Sorghum [*Sorghum bicolor* (L.) Moench] ranks fifth in production (63.9 million MT) among crops (FAO, 2021). It covers over 42 million hectares as a rainfed crop mostly by subsistence farmers in the semiarid tropics of Africa, Asia, and Latin America (Reddy *et al.*, 2004). Ethiopia is the world's sixth-largest sorghum producer and Africa's third-largest sorghum producer, after Nigeria and Sudan. Next to tef and maize sorghum is largely considered as the

most important staple food and feed crop in Ethiopia (Taye Tadesse, 2016; Chala Gebeyehu, 2019).

Cereals contributed about 88.36% of total grain production and cover 81.19% of the total area allocated for grains crops. Sorghum contributes 13.22% of total cereal production and 12.94% of the total area allocated for cereals (CSA, 2021). Sorghum is cultivated over a wide range of elevation and rainfall conditions in Ethiopia. It is a particularly important food and feed crop in dry lowland areas, where moisture is limited and it is often the only crop grown (Birhane Gebrekidan and Yilma Kebede, 1978; Damon, 1962).

#### 2.7. Production Constraints of Sorghum

The productivity of sorghum in Ethiopia is low owing to various biotic and abiotic production constraints. Drought, low soil fertility (nutrient deficiency), stem borers, shoot fly, quelea birds, *Striga hermonthica*, and other weeds are recognized as major production constraints in Eastern Africa (Wortmann *et al.*, 2006). Although these constraints cause significant grain yield losses, the relative importance varies from region to region within and among the countries. In Ethiopia, drought and Striga were found to be very important in the north and northeastern parts of the country (Wortmann *et al.*, 2006). Research has also shown that moisture deficit during grain filling is most important for Ethiopia and Mozambique. Although mid-season stress is relatively less important as compared to other growth stages in Ethiopia, it is said to be very important in other countries (Wortmann *et al.*, 2006; Dejene K. Mengistu, 2009). Moisture stress contributes to poor crop performance and yield. In Ethiopia, where more than 50% of the total area is semi-arid (Gamachu Daniel, 1977), insufficient, unevenly distributed, and unpredictable rainfall is usually experienced in drier parts of the country.

At one point rain may be abundant and perhaps wasted through runoff; in some years much, rain may fall completely outside the growing season. In other years the amount of rain may be low after the crops have germinated and soil moisture may be severely depleted. Consequently, in almost all lowland areas crops are prone to periodic moisture stress in one way or another because of the aforementioned realities (EARO, 2001). The effect of moisture stress on crop

yield is dependent on the stage of plant development. Anthesis and grain filling stages appear to be more vulnerable; the occurrence of drought at these stages may result in reduced yield and/or complete crop failure (Younesi and Moradi, 2009). Although drought stress at the beginning of the growing season severely affects plant establishment, plants tend to recover soon when the rain falls late (Ramu*et al.*, 2008).

#### 2.8. Effects of Drought on Growth and Development of Sorghum

As with all crops, sorghum grain yield is dependent on water supply (soil water at planting and in-season precipitation). In the semi-arid tropics where dry land farming is practiced, drought is a common phenomenon that occurs at different periods during the growing season. There is also a high season-to-season variability of rainfall, temperature, and radiation in the tropics. Agricultural conditions greatly vary in topography, soil, existing agricultural practices, and other associated biotic stress factors (Chapman *et al.*, 2000). Grain yield is more dependent on rainfall or irrigation well distributed over the growing season depending on demand at each stage than on total water available through the growing season (Yared Assefa *et al.*, 2010).

Water stress can have major consequences on the growth, development, and yield of sorghum by affecting several physiological, morphological, and biochemical processes. It is the major cause of poor crop performance and low yields, and sometimes it causes total crop failure (Yared Assefa *et al.*, 2010). The occurrence of water stress at the pre-flowering and post-flowering stages of development has the most adverse effect on yield (Xiong *et al.*, 2006). Also, stress at the seedling stage of development severely affects plant establishment. If it occurs at flowering, or in the grain filling stages, it may cause reduced yields or complete crop failure. Researchers have classified drought as either pre- or post-flowering stress. The reactions of genotypes to these stresses are variable and controlled by different genetic mechanisms.

#### **2.9. Transpiration Efficiency**

Almost a century ago, Briggs and Shantz (1913) showed that crop species differ in their transpiration efficiency. Since then, the C3 and C4 photosynthetic pathways have been

elucidated, and differences in transpiration efficiency have been related to them. Plants with the C4 type of photosynthesis have transpiration efficiencies that are about twice those of C3 plants (Turner, 1993). Differences in transpiration efficiency (TE) have been linked to traits that affect photosynthetic capacity thus biomass production and transpiration rate (Vadez *et al.*, 2011a). Photosynthesis is closely associated with transpiration rates through stomatal conductance, which determines the rate of CO<sub>2</sub> uptake into the leaves. Because CO<sub>2</sub> diffuses at a slower rate through the stomata than water vapor (Caemmerer and Farquhar, 1981), reduced conductance tends to result in increased TE. Although this is advantageous under drought stress, the associated reduction in CO<sub>2</sub> uptake is likely to cause a yield penalty under well-watered conditions, where biomass accumulation is radiation limited. Simulation studies in both sorghum (Sinclair *et al.*, 2005) and maize (Messina *et al.*, 2015) have shown that the associated saving of water before flowering can routinely increase grain yield by amounts exceeding 20% in water limited environments. However, within each species, differences in transpiration efficiency occur, including the C4 plant sorghum (Hammer *et al.*, 1997; Mortlock and Hammer, 1999).

Transpiration efficiency (TE) at the plant level can be defined as the amount of biomass accumulated per unit of water transpired and is a preferred measure for examining potential genetic variation in crop transpiration efficiency (Mortlock and Hammer, 1999; Vadez, 2016). At a plot level it can be defined as water-use efficiency (WUE) = grain yield/water received or as WUE = total biomass/evapotranspiration (Vadez *et al.*, 2014). The relevance of TE to crop growth in water limited environments has been indicated in previous studies (Donatelli *et al.*, 1992; Beggi *et al.*, 2015; Vadez, 2016). Xin *et al.* (2009), discussed the benefits of increased TE to sorghum production in water limited environments in two ways. Firstly, high TE allows the sorghum plant to accumulate more biomass and possibly more yield if the harvest index (HI) remains the same, from the same or a given amount of water available to the plant throughout the growing period. Secondly, high TE may allow the sorghum plant to complete the crop life cycle with the same limited amount of soil water, by delaying the onset of severe stress before the next rain. Hence, improvement in TE through breeding may result in a considerable yield increase in sorghum in water limited environments (Xin *et al.*, 2009; Mortlock and Hammer, 1997).

#### 2.10. Variability for Transpiration Efficiency

Genetic variability is a base for crop improvement as it provides raw material to plant breeders to recombine the genes of different characters in the same plant for the development of a desirable variety. Assessment of the genetic diversity within crop germplasm is fundamental for the breeding and conservation of genetic resources and is particularly useful as a general guide in the choice of parents for breeding hybrids (Talebi *et al.*, 2008). The conservation and use of diverse collections of plant genetic resources are the backbones of plant breeding programs, so this genetic variability is the raw material for the crop breeding industry on which selection acts to evolve superior genotypes. The base of genetic variability is the geneti

The crop genetic variability not only helps varieties to adapt to diverse environments, to enhance tolerance to unfavorable conditions but also to produce diversity and to get better yield and quality of products to serve the needs of the people. Genetic variability is a measure of the tendency of individual genotypes in a population to vary from one another. Sorghum studies on a range of sorghum lines revealed that in addition to yield variation observed due to harvest index (HI), TE also played a significant role in the observed variation under terminal stress (Vadez *et al.*, 2011b). Different studies have revealed significant genetic variation in TE among diverse sorghum germplasm and in different environmental conditions (Donatelli *et al.*, 1992; Vadez *et al.*, 2011b; Vadez, 2016).

#### 2.11. Heritability and Genetic Advance

#### 2.11.1. Heritability

According to Falconer and Mackay (1996), heritability is defined as the measure of the correspondence between breeding values and phenotypic values. Heritability is classified into broad and narrow senses (Acquaah, 2012). Heritability in the broad sense is defined as the proportion of phenotypic variance that is attributable to an effect for the whole genotype, comprising the sum of additive, dominance, and epistatic effects (Nyquist, 1991; Falconer and Mackay 1996). Moreover, it is the relative magnitude of genotypic and phenotypic variance for

the traits, and it gives an idea of the total variation accounted for genotypic effect. This gives an idea of the total variation ascribable to genotypic effects, which are an exploitable portion of variation. On the other hand, narrow sense heritability is the ratio of additive genetic variance to the total phenotypic variance, and it gives the best estimate of heritable variance which can be fixed by selection (Falconer and Mackay, 1996; Sleper and Poehlman, 2006; Piepho and Mohring, 2007).

Heritability is often used by plant breeders to quantify the precision of single field trials or series of field trials and is a key parameter in quantitative genetics because it determines the response to selection. Thus, heritability plays a predictive role in breeding, expressing the reliability of phenotype as a guide to its breeding value. It is the breeding value that determines how much of the phenotype would be passed onto the next generation (Tazeen *et al.*, 2009). There is a direct relationship between heritability and response to selection, which is referred to as genetic advance. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone (Larik *et al.*, 2000; Bisne *et al.*, 2009). Kalpande *et al.* (2014), reported low heritability values for plant height. A high estimate of heritability together with high genetic advance as percent of mean was recorded for grain yield and total biomass. Kamatar *et al.* (2015), observed high heritability estimates for plant height. Lakshmi *et al.* (2020), reported high heritability values for transpiration efficiency of biomass, transpiration efficiency of seed and total biomass among maize genotypes.

#### 2.11.2. Genetic advance (GA)

Genetic advance measures the expected genetic progress that would result from selecting the best performing genotypes for a character being evaluated (Allard, 1999). The estimate of genetic advance as percent of mean provides more reliable information regarding the effectiveness of selection in improving the traits. Genetic advance denotes the improvement in the genotypic value of the new population over the original population (Ghosh and Sharma, 2012). Moreover, genetic advance provides information on expected genetic gain resulting from the selection of superior individuals (Satheesh and Saravanan, 2012).

According to Allard (2000), genetic advance under selection is a genotypic value, which depends on three things such as genetic variability, heritability, or masking effect of non-genetic variability on the genetic variability and the selection intensity applied. Genetic progress would increase with an increase in the variance. Therefore, the utility of estimates of heritability is increased when they are used in conjunction with the selection differential, the amount by which the mean of the selected lines exceeds the mean of the entire group (Johnson *et al.*, 1955). Generally, genetic advance gives a clear picture and precise view of segregating generations for possible selection. Higher estimates of heritability coupled with better genetic advance confirm the scope of selection in developing new genotypes with desirable characteristics (Ajmal *et al.*, 2009). Coupling of high genetic advance with high heritability for total biomass, transpiration efficiency of biomass, transpiration of seed and total biomass was reported by (Rahman *et al.*, 2015).

#### 2.12. Association of Traits

#### 2.12.1. Correlation of Traits

As mentioned on Gomez and Gomez (1984), the degree of association among two or more traits could be expressed through correlation coefficient analysis. It measures the extent to which two variables are related. Therefore, to improve targeted trait breeding program should understand the relationship of different traits. Correlation analysis measures the mutual association of any two plant characters and determines the trait on which selection should be done. Basic knowledge on correlation which exists between traits serves as the basis for planning efficient breeding program for crop improvement (Johanson *et al.*, 1955; Bhatt, 1973).

Correlation classified as phenotypic, genotypic, and environmental. Phenotypic correlation is association between two observed characters of plant. Environmental correlation is relation of trait due to environmental influence (Singh, 2001). Genetic correlation is relationship between two traits due to gene effect, it may be reflected from pleiotropic action of genes or correlation between causal loci in two traits (Allard, 1960). Pleiotropism is genetic effect of single gene on multiple phenotypic traits. According to Fikru Mekonnen *et al.* (2014), significant strong positive phenotypic and genotype correlations were observed between seed yield and biomass in

lentil. Tyagi and Khan (2011), reported biological yield/plant and number of primary branches/plants showed positive and significant correlations with seed yield/plant. positive and highly significant correlation of TE biomass with total biomass and seed yield was reported by (Lakshmi *et al.*, 2020).

#### 2.12.2. Path Coefficient Analysis

According to Gomez and Gomez (1984), correlation does not imply or assume any cause-andeffect relationship between variables. In fact, one variable could influence the other or *vice versa*, or both could be influenced by some third variable. Path coefficient analysis permits the separation of the correlation coefficient into a component of direct and indirect effects and measures the relative importance of each (Sharma, 1998).

Path coefficient analysis is simply a standardized partial regression coefficient and as such measures the direct and indirect effect for one variable upon another and permits the separation of the correlation coefficient into components of direct and indirect effect (Dewey and Lu, 1959). Moreover, using path coefficient analysis, it is easy to determine which trait is influencing the dependent trait substantially. The information obtained by this technique helps in indirect selection for genetic improvement of dependent trait and measures the relative importance of each trait (Ariyo *et al.*, 1987).

Path analysis provides clear picture of character associations for formulating efficient selection strategy. Since the correlation coefficient alone is inadequate to interpret the cause and effect of relationships among the traits. Because path coefficient analysis furnishes information of influence of each contributing traits directly as well as indirectly and enables breeders to rank the genetic attributes according to their contribution (Cyprien and Kumar, 2011). As the yield is polygenically controlled and influenced by its component characters, direct selection for yield is often misleading. Path analysis has been used by plant breeders to assist in identifying traits that are useful as selection criteria for improvement (Milligan *et al.*, 1990).

Generally, path coefficient analysis is a statistical technique of partitioning the correlation coefficients into its direct and indirect effects, so that the contribution of each character to yield

could be estimated. It is used in plant breeding programs to determine the nature of the relationships between dependent trait and other traits that are useful as selection criteria to improvement (Mohamed *et al.*, 2012). Tyagi and Khan (2011) reported biological yield/plant and number of primary branches/plants was exerted positive and high direct effects on seed yield/plant.

#### 2.13. Genetic Distance and Clustering

Genetic distances are measures of the average genetic divergence between cultivars or populations (Souza and Sorrells, 1991). Moll *et al.* (1965), defined genetic divergence of two cultivars as a function of their ancestry, geographic separation, and adaptations to differing environments. Genetic distance is the extent of gene differences between cultivars as measured by allele frequencies at sample loci. Genetic similarity is the converse of genetic distances, and it refers to the extent of gene similarities among cultivars (Smith, 1984).

To develop a sound hybridization program, it is necessary that the varieties should be genetically divergent especially for quantitative characters that contribute towards yield (Singh, 1983). Thus, crosses between groups with maximum genetic divergence would be more responsive to improvement since they are likely to produce desirable recombination and segregation in their progenies after hybridization. In any breeding program, therefore, genetic diversity must be introduced periodically into the population to provide new recombination and selection potential (Welsh, 1981). The D<sup>2</sup> values represent the index of genetic divergence among the genotypes both at intra-cluster and inter-cluster levels. It would, therefore, be logical to effect crosses between genotypes belonging to the clusters which are separated by greatest generalized distance and show maximum divergence (Singh, 1983). Besides assisting in the selection of divergent parents for a breeding program, D<sup>2</sup> statistic is useful to determine the relative contribution of each component character to the total divergence. The criterion used in clustering is that any two genotypes belonging to the same cluster show, at least on the average, smaller D<sup>2</sup> value than those belonging to two different clusters (Bhatt, 1973).

#### 2.14. Principal component analysis

Principal component analysis is a powerful tool for investigating and summarizing underling trends in complex data structures (Legendre and Legendre, 1998). According to Ogunbodede (1997), it helps to identify plant characters that contribute most to the variation within a group of entries. It is also a common ordination numerical technique, which reduces the dimensions of multivariate data by removing inter-correlation among variables (characters on which units are to be compared) and enables multi-dimensional relationship to be plotted on two or three principal axes. PCA chooses independent or orthogonal axes, which are minimally correlated and represents linear combination of the original characters (Clifford and Stephenson, 1975).

In addition, the relative discriminating power for axes and their associated characters are measured by eigen values and factor scores, respectively. Many variables are often measured by plant breeders, some of which may not be of sufficient discriminatory power for germplasm evaluation, characterization, and management. In such case, principal component analysis may be used to reveal patterns and eliminate redundancy in data sets as morphological and physiological variations routinely occur in crop species. Therefore, knowledge of the nature, extent and organization of this variation could be useful for genetic improvement of crop species. Until a collection has been properly evaluated and its attributes become known to breeders, it has little practical use. Germplasm evaluation in the broad sense and in the context of genetic resources is the description of the material in a collection that covers the entire range of activities starting from the receipt of the new samples by the curator and growing of these for seed increase, characterization, and preliminary evaluation and for further evaluation.

According to Sharman (1998) and Chahal and Gosal (2002), characters with largest absolute value closer to unity within the first principal component influence the clustering more than those with lower absolute value closer to zero. The positive and negative loading shows the presence of positive and negative correlation trends between the components and the variables. The characters, which load high positively or negatively, contributed more to the diversity and they were the ones that most differentiated the clusters.

#### **Chapter 3. MATERIALS AND METHODS**

#### **3.1. Experiment Site**

The experiment was conducted at Melkassa Agricultural Research Center (MARC) greenhouse in 2021/22. The center is located at 8°24'North and 39°21'East in the Oromia National Regional State. Melkasa is found 107 km away from Addis Ababa and 17 km from Adama. It is located at an altitude of 1550 m.a.s.l and receives a mean annual rainfall of 763 mm with average minimum and maximum temperatures of 14°C and 28.4 °C, respectively. The areas typically represent the dry lowland areas where drought and heat stress are the major challenges for crop production.

#### **3.2. Experimental Materials**

In the present study, 99 sorghum genotypes sampled from the preliminary variety trial (one of the variety releases stages which the genotypes developed through crossing are preliminarily tested at different location), developed through crossing targeted to the dry lowland areas. The test genotypes were derived through pedigree selection based on flowering time and adaptation to drought stress environments. These genotypes were evaluated for their performance in transpiration efficiency to develop adaptable and heigh yielder sorghum varieties for arid and semi-arid areas. Along the test genotypes, the prominent improved sorghum varieties, Melkam and Argity, for the dry lowland areas were used as a check. The description of the genotypes that were evaluated in this experiment is summarized in Appendix Table 1.

#### 3.3. Experimental Design and Procedure

The experiment was conducted in a greenhouse facility at Melkasa Agricultural Research Center. Whole plant level TE was determined by using 16-liter buckets filled with silt clay loam soil (pH of 7.9), which was taken from the trial field of MARC. In total 204 lysimeters including two additional lysimeters without plants were used for this experiment. Due to the presence of shade effect on greenhouse the experiment was laid out in a Completely Randomized Block Design with two replications. Each replication had a similarly treated pot with no plant to measure the extent of water loss through evaporation. In addition, lysimeters without plant were used as a reference to know the drained upper limit. All lysimeters including reference lysimeters were filled with an equal amount of soil. Before the experiment, the reference lysimeters were drilled in to bottom and watered carefully until they started to drain, and they were sealed by plastic to prevent evaporation, when they stopped the draining their average weight was taken as the DUL (drained upper limit) for the experimental lysimeters. After watering all pots with an equal amount of water five seeds were sown per lysimeter and thinned to a single plant at 14 days after sowing. Fertilizer was applied based on recommended dose (40 kg/ha). When most of the plants had four leaves, the lysimeters were covered by plastic to prevent the evaporation of water from the soil surface. The initial weight of each lysimeter was recorded immediately after covering. The water use of plants was recorded by weighing each lysimeter three times a week until harvest. Additional water was replenished by carefully adding it under the plastic cover through small openings placed in the plastic to allow plant growth. When the plants had fully emerged flag leaf, they were harvested at base of the plant that soil level. The final weight of each lysimeter was recorded at the time of harvesting. Each plant was partitioned into leaves and stems and then dried in an oven at 70 °C for 72 hours. When biomass attained constant weight, the dry biomass of each plant was recorded. The root of each plant was recovered from the soil by washing carefully and dry biomass was recorded similar to shoot biomass.

#### 3.4. Data collection

Data on the following quantitative traits were collected:

- 1. Initial weight (kg): All lysimeters were weighed directly after covering of soil surface to determine their initial weight.
- 2. Final weight (kg): All lysimeters were weighed prior to harvest to determine their final weight.
- 3. Shoot fresh biomass (g): shoot fresh weight was measured immediately after harvest.
- Shoot dry biomass (g): Shoots were cut up and dry weight was measured after drying it for at least 72 hours at a temperature of 70 °C.

- 5. Root dry biomass (g): The root was recovered from the soil by carefully washing out the soil, and root dry weight was measured like a shoot dry biomass.
- 6. Total water use (L): Total water use was determined as the sum of difference between the initial and final weight of each lysimeter, any water added during the experiment and the average water loss from the control lysimeters.
- 7. Plant height (cm): The height was measured in centimeter from the ground level to the tip of the plants at flag life stage.
- 8. Chlorophyll content of leaves: Leaf chlorophyll content was recorded from the third leaves of a plant using a chlorophyll content meter (SPAD).
- 9. Number of leaves (count): It was recorded by counting the leaves from an individual plant at the harvesting stage.
- Leaf area (cm<sup>2</sup>): It was measured by passing all leaves through an electronic leaf area meter (LI-3100C)
- 11. Transpiration efficiency (TE) (g kg<sup>-1</sup>) was calculated as the ratio of dry biomass (shoot and root) per unit of water transpired.

#### **3.5. Data Analysis**

3.5.1. Analysis of Variance

The data were tested for their normal distribution before moving to an analysis by using Minitab software (MINITAB Inc. USA, 2016). The data of each trait were subjected to analysis of variance for RCBD design. ANOVA was done based on the procedures outlined by Gomez and Gomez (1984) with the help of SAS software version 9.4. The mean comparison was done by using Duncan's multiple range test at a 5% level of significance.

The linear general model for analysis of variance of an experiment conducted in one location using RCBD design is:  $-x_{ij} = \mu + t_i + r_j + \epsilon_{ij}$  where; $\mu =$  the overall mean, $t_i =$  the i<sup>th</sup>treatment effect,  $r_j =$  the j<sup>th</sup> replication effect, and  $\epsilon_{ij} =$  the error term.

Source	Df	SS	MS
Replication	r-1	RSS	RMS = RSS/r-1
Treatment	t-1	TSS	TMS = TSS/t-1
Error	(r-1) (t-1)	ESS	EMS = ESS/(r-1) (t-1)

Table 1: Analysis of variance (ANOVA) Model for RCB design

Where: r= number of replications; t = number of treatments; Df = degree of freedom; SS = Sum of squares; MS = mean squares; RSS and RMS are sums of squares and mean of replication, respectively; TSS and TMS are sums of squares and mean of treatment, respectively, and EMS is mean of squares of error.

#### 3.5.2. Estimation of Variance Components

The genotypic and phenotypic variance and phenotypic and genotypic coefficient of variation was estimated using the following formulas as suggested by Syukur *et al.* (2010).

Genotypic variance:  $(\sigma^2 g) = \frac{MSg - MSe}{r}$ 

Where: MSg = mean squares of genotypes; MSe= mean squares of experimental error; and r= number of replications.

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

Where:  $\sigma^2 p$  = Phenotypic variance;  $\sigma^2 g$  = Genotypic variance;  $\sigma^2 e$  = Error variance.

Coefficients of variations at phenotypic and genotypic levels were estimated based on the method suggested by Burton and Devane (1953) and Deshmukh *et al.* (1986) using the following formula:

$$PCV = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$$

Where: PCV= Phenotypic coefficient of variation;  $\sigma^2 p$ = the phenotypic variance; and  $\overline{X}$  = the grand mean for the trait considered.

$$\text{GCV} = \frac{\sqrt{\sigma^2 g}}{\bar{\text{X}}} \times 100$$

Where: GCV= genotypic coefficient of variation;  $\sigma^2 g$ = the genotypic variance; and  $\overline{X}$  = the grand mean for the trait considered. According to Deshmukh *et al.* (1986), PCV and GCV value greater
than 20% are regarded as high, whereas values less than 10% are considered low and values between 10% and 20% are classified as moderate.

## 3.5.3. Broad Sense Heritability

Heritability specifies the proportion of the total variability that is due to a genetic cause. Information on heritability provides the relative practicability of selection for a particular trait. Different traits have different levels of heritability that can contribute to the improvement of a trait in breeding programs. The proportion of phenotypic variance that is attributable to an overall genetic variance for the genotype was estimated using broad sense heritability according to the formula from Falconer and Mackay (1996) as follow: -

$$H^2b = \left(\frac{\sigma^2 g}{\sigma^2 p}\right) x \ 100$$

Where,  $H^2b$  = heritability in broad sense;  $\sigma^2 p$  = phenotypic variance; and  $\sigma^2 g$  = Genotypic variance.

According to Johnson *et al.* (1955), heritability was classified as low (<30%), medium (30-60%), and high (> 60%).

### 3.5.4. Genetic advance and Genetic advance as percent of mean (GAM)

Genetic advance in the absolute unit (GA) and Genetic advance as percent of the mean (GAM), assuming selection of superior 5% of the genotypes were estimated in accordance with the methods illustrated by Johnson *et al.* (1955) as:

$$GA = K * \sigma P * H^2 b$$

Where: GA = Genetic advance;  $SDp = Phenotypic standard deviation on mean basis; <math>H^2b =$  Heritability in the broad sense; and K = the standardized selection differential at 5% selection intensity (K=2.06).

Genetic advance as percent of mean (GAM)

Genetic advance as percent of mean was calculated to compare the extent of predicted advance of different traits under selection, using the following formula.

$$GAM = \frac{GA}{\bar{x}} \times 100$$

Where: GAM = Genetic advance as percent of mean; GA = Genetic advance; and  $\bar{x}$ =Mean of the population in which selection employed. Genetic advance as percent of the mean is categorized as low (0-10%), moderate (10-20%) and high (>20%) as given by Johnson *et al.* (1955).

## 3.5.5. Phenotypic and genotypic correlation analysis

Simple linear (Pearson) correlation coefficient was performed to understand the relationship among the traits studied in the experiment (Schober *et al.*, 2018). The degree of association between transpiration efficiency with plant biomass and other traits was measured. Phenotypic and genotypic correlations were computed using the formula given in the Miller *et al.* (1958). Phenotypic correlation between traits x and y.

$$rPxy = \frac{Covpxy}{\sqrt{\sigma^2 Px * \sigma^2 Py}}$$

Where: Covpxy-phenotypic covariance between the two traits;

 $\sigma^2 P x$ - phenotyic variance x;

 $\sigma^2 P$ y-Penotypic variance of y;

Genotypic correlation between traits x and y

$$rgxy = \frac{Covgxy}{\sqrt{\sigma^2 gx * \sigma^2 gy}}$$

Where: Covgxy - Genotypic covariance between character x and y,

 $\sigma^2 g_x$  - Genotypic variance of x,

 $\sigma^2 g_y$  - Genotypic variance of y

Phenotypic correlation coefficient was tested for their significanceusing the following formula

$$t = \underline{r}$$

Where: rp= phenotypic corelation; SE ( $r_p$ )= standard error of phenotypic correlation

 $SE(rp) = \frac{\sqrt{1 - r2p}}{n - 2}$ 

where; n=numberof genotypes tested;  $r_p$  is phenotypic correlation coefficient Genotypic correlation coefficient was tested with the following formula

$$t = \frac{rgxy}{SErgxy}$$

Where:- SErgxy =  $\frac{\sqrt{1 - r^2 gxy}}{2H x * Hy}$ 

SErgxy = Standard error of genotypic correlation coefficient between character X and Y; rgxy= genotypic correlation coefficient between character X and Y; Hx = heritability for character x; and Hy = heritability for character y. The calculated absolute t value was tested against the tabulated t- value at g-2 degree of freedom for both phenotypic and genotypic correlations.

# 3.5.6. Path coefficient analysis

Path analysis was developed as a method of decomposing correlations into different pieces for interpretation of effects. It determines the direct and indirect effects of traits on other traits. In this experiment, it was used to estimate the effect of other traits on biomass of plant. It estimated by simultaneous equation using the formula as applied by Dewey and Lu (1959):

rij = Pij + 
$$\Sigma$$
 rik Pkj

Where: rij = Mutual association between the independent trait (i) and dependent trait (j) as measured by the genotypic and phenotypic correlation coefficient; Pij = components of direct effects of the independent trait (i) on the dependent variable (j) as measured by the path coefficients and  $\Sigma$  rikpkj = summation of components of indirect effects of a given independent trait (i) on a given dependent trait (j) via all other independent trait (k).

The contribution of the remaining unknown factor was measured as the residual factor (PR), which is calculated as: pr = 1 - rijPij – where: pr is the residual factor; Pij the direct effect and rij is the correlation. The magnitude of PR indicates how best the causal factors account for the variability of the dependent trait.

## 3.5.7. Genetic distance and clustering analysis

Cluster analysis has a power to tell us how genotypes are genetically like each other or different from each other by grouping them into clusters. For cluster analysis, the mean data of the 101 sorghum genotypes for the 11 traits were first standardized to a mean of zero and a variance of unity to avoid differences in scales used for recording data.

The Mahalanobis generalized distance (D<sup>2</sup> statistics) was used to estimate the distance between and within clusters (divergence) using SAS software as per the following formula:

$$D^2 ij = (Xi - Xj) S - 1(Xi - Xj)$$

Where:  $D^2 ij$  = the distance between any two groups i and j, Xi and Xj = vector means of the traits for the ith and jth groups, respectively, and S-1= the inverse of the pooled covariance matrix.

Based on the squared distances (D<sup>2</sup>) values, clustering of genotypes was done using Tocher's method as described by Singh and Chaudhary (1999). The significance level of genetic distance between and within clusters was tested at 5% level of probability using chi-square test. The  $D^2$  values obtained for pairs of clusters using SAS statistical package were considered as the calculated values of Chi-square ( $\chi^2$ ) and tested for P degree of freedom, where P is the number of traits considered (Singh and Chaudhary, 1985). The cubic clustering criterion (CCC), pseudo-F (PSF) statistic and the pseudo T<sup>2</sup> (PST<sup>2</sup>) statistic were examined by using SAS version 9.4 PROC clustering procedure to decide the numbers of clusters.

# 3.5.8. Principal component analysis

Principal component analysis (PCA) was computed to find out the traits, which contributed more to the total variation. The data was standardized to mean zero and variance of one before computing principal component analysis. The PCs with eigen value greater than one were used as mentioned by Jeffers (1967). Correlations between the original traits and the respective PCs were calculated.

The principal component analysis was computed using the following equation:

PC1=b11(x1) +b12 +b1p=(XP) - Where: pc1= the subjects score on pc1 (the first component extracted), b1p=the regression coefficient (weight) for observed variable p, as used in creating principal component 1 and xp=the subjects score on observed variable p.

# **Chapter 4. RESULTS AND DISCUSSION**

## 4.1. Analysis of Variance

The mean squares for genotypes from the analysis of variance revealed highly significant difference (P<0.001) among genotypes for all traits considered in the present study (Table 2); plant transpiration efficiency, shoot transpiration efficiency, chlorophyll content of leaf, leaf number, plant height, shoot dry biomass, shoot fresh biomass, total dry biomass, water use, root dry biomass and leaf area.

These results showed that presence of adequate genetic variability for transpiration efficiency among sorghum genotypes considered in the study and the possibility of improving drought tolerance through strong selection for transpiration efficiency traits. The presence of genotypic variability in transpiration efficiency of sorghum genotypes was confirmed by different previous reports (Henderson *et al.*, 1998; Mortlock & Hammer, 1999; Xin *et al.*, 2009). In addition, the existence of significant difference among sorghum genotypes for transpiration efficiency, total dry biomass, water use, and leaf area had been reported by Hammer *et al.* (1997). Also, the existence of significant genotypic differences among sorghum genotypes for root biomass mass and shoot biomass had been reported by Kulathunga *et al.* (2021). In Ethiopia, a single study conducted on the variability of local sorghum genotypes for transpiration efficiency revealed the presence of considerable genetic variability among sorghum genotypes for transpiration efficiency revealed the presence of considerable genetic variability among sorghum genotypes for transpiration efficiency revealed the presence of considerable genetic variability among sorghum genotypes for transpiration efficiency revealed the presence of considerable genetic variability among sorghum genotypes for transpiration efficiency revealed the presence of considerable genetic variability among sorghum genotypes for transpiration efficiency for transpiration efficiency for transpiration efficiency (Alemu Tirfessa 2018).

							CV	
Traits	MSg	MSb	Mse	Mean	Max	Min	(%)	<b>R</b> <sup>2</sup>
PTE(gkg- <sup>1</sup> )	2.16**	6.494497**	0.33	4.65	6.99	2.68	12.29	0.87
STE(gkg- <sup>1</sup> )	1.13**	4.5270178**	0.20	3.26	5.28	1.99	13.74	0.85
LCC	30.77**	$0.22^{**}$	18.18	46.52	56.85	38.2	9.17	0.63
LA(cm <sup>2</sup> )	11521.894**	19821.21 <sup>ns</sup>	5384.771	283.28	522	126.9	25.90	0.69
LN(cou)	3.49**	48.52**	2.18	11.16	15.5	8	13.23	0.65
PH(cm)	325.46**	1896.84**	129.40	68.78	106	43	16.54	0.73
TDB(g)	278.54069**	2.39604 <sup>ns</sup>	76.47	68.42	92	46	12.78	0.78
WU(L)	16495929**	80180100**	6336003.00	15251.96	23150	10675	16.50	0.73
SDB(g)	153.82069**	29.35149 <sup>ns</sup>	46.64	48.08	66	33	14.20	0.77
RDB(g)	44.042772**	14.975248 <sup>ns</sup>	15.58	20.33	31.5	10	19.41	0.74
SFB(g)	4076.7385**	32.4802 <sup>ns</sup>	1659.55	217.69	311	162.5	18.71	0.71

Table 2: Mean squares and simple statistics for 11 traits of sorghum genotypes tested at MARC in 2021/22

**Note** \*, \*\* significant at 0.05 and 0.01 probability levels respectively; NS =non-significant, MSg = mean squares of genotypes; MSb= mean squares of block; MSe = mean squares of error; Max= maximum mean value; Min= minimum mean value;  $CV = coefficient of variation; R^2 = coefficient of determination; PTE = plant transpiration efficiency; STE = shoot transpiration efficiency; LA = leaf area; LCC = leaf chlorophyll content; PH= plant height; LN= leaf number; SDB= shoot dry biomass; SFB= shoot fresh biomass; TDB= total dry biomass; WU= total water use;RDB=rootdrybiomass;$ 

# 4.2. Mean and Range of Traits

The mean values and ranges of transpiration efficiency traits showed considerable variation among sorghum genotypes included in the present study (Table 2). Results from mean separation analysis showed a significant difference. There was a wide range in plant transpiration efficiency for the 101 genotypes with a mean value of 4.65 g kg<sup>-1</sup>. The maximum plant transpiration efficiency (6.99 g kg<sup>-1</sup>) was observed from ETSC17300-4-1, followed by ETSC17194-3-1 (6.75 g kg<sup>-1</sup>); and the minimum PTE value (2.68 g kg<sup>-1</sup>) was recorded from ETSC17252-19-2, followed by ETSC17045-12-2 (2.74 g kg<sup>-1</sup>). Shoot transpiration efficiency ranged from 1.99 g kg<sup>-1</sup> to 5.28 g kg<sup>-1</sup> with a mean value of 3.26 g kg<sup>-1</sup>. The maximum value was recorded from ETSC17300-4-1 and the minimum one was from ETSC17045-12-2. The minimum (38.2) and maximum (56.85) chlorophyll content of the leaf was observed on ETSC17328-11-1and ETSC17245-3-3, respectively, with the mean value of 46.52. The number of leaves ranged from 8 to 15.5 leaves per plant with a mean of 11.16. The minimum number of leaves was recorded from ETSC17129-12-1 and ETSC17172-2-3, while the maximum number was recorded from ETSC17074-10-1.

Plant height also showed highly significant variation with the range of 43 to 106 cm among genotypes with a mean of 68.78 cm. The highest value of (106cm) plant height was recorded for genotype ETSC17074-10-1, followed by ETSC14252-3-2 (102 cm); while the minimum value (43 cm) was recorded for genotype ETSC17300-2-1. In terms of leaf area genotypes vary from 126cm<sup>2</sup> to 522cm<sup>2</sup> with a mean value of 283.3cm. The maximum leaf area was recorded from ETSC17155-2-1 and the minimum one was recorded from ETSC17304-7-2. In terms of total dry biomass, it ranged from 46 g plant<sup>-1</sup> to 92 g plant<sup>-1</sup> with a mean of 68.42 g plant<sup>-1</sup>. The maximum value of total dry biomass was recorded from Argiti while the minimum value was recorded for ETSC17276-5-1. Shoot dry biomass also shows significant variation with a minimum value of 33 g plant<sup>-1</sup> for ETSC17272-1-1 and a maximum value of 66 g plant<sup>-1</sup> for ETSC17262-2-2 to 311g plant<sup>-1</sup> for ETSC17155-2-1 with a mean of 228.03g plant<sup>-1</sup>.

Significant variation among genotypes was shown in terms of water use; it was ranging from 10675ml to 23150ml with a mean of 15251.96.5ml. The maximum water was used by ETSC17112-10-1and the minimum one was by ETSC17272-8-1. Also, genotypes significantly differed in their root dry biomass with the range of 10 g plant<sup>-1</sup> for ETSC17109-10-2 to 31.5 g plant<sup>-1</sup> for ETSC17295-8-1 with the mean of 20.33 g plant<sup>-1</sup>. These results showed the existence of wide ranges of variations among mean values of sorghum genotypes for all the traits, suggesting the presence of considerable genetic variability among sorghum genotypes; therefore, this indicates the possibility of selection for the development of drought tolerant variety. Hammer *et al.* (1997), has reported the presence of genetic variability in wider range for plant transpiration efficiency (4.4 to 7.7 g kg<sup>-1</sup>) and total biomass (13 to 161 g). Also, Vadez *et al.* (2011b), has reported the presence of genetic variability in wider range for plant transpiration efficiency (3.21 to 6.09 g kg<sup>-1</sup>). R<sup>2</sup> (coefficient of variation) indicates the fitness of model and percentage of variation in a response variable explained by its relationship with one or more predictor variables.

## **4.3. Estimation of Variance Components**

The phenotypic variance, genotypic variance, environmental variances, genotypic coefficient of variation, phenotypic coefficients of variation, broad-sense heritability, genetic advance, and genetic advance as percent of the mean are presented in Table 3.

The phenotypic coefficient of variation ranged from 10.63% to 32.46% and the genotypic coefficient of variation ranged from 5.39% to 20.91%. A high phenotypic coefficient of variation was observed for leaf area and a high genotypic coefficient of variation was observed for shoot transpiration efficiency, while low phenotypic and genotypic coefficient of variation was observed for leaf chlorophyll content. According to Deshmukh *et al.* (1986), PCV and GCV value greater than 20% are regarded as high, whereas values less than 10% are considered as low and values between 10% and 20% as moderate. Therefore, in the present study, a high phenotypic coefficient of variation was noticed for plant transpiration efficiency (23.99%), shoot transpiration efficiency (25.02%), water use (22.15%), leaf area (32.46%), shoot dry biomass (20.82%), root dry biomass (26.85%), shoot fresh biomass (25.6%) and plant height (21.93).

Moderate PCV value was observed for total dry biomass (19.47%) and leaf number (15.08%). PCV was low for leaf chlorophyll content (10.63%).

In terms of genotypic coefficient of variation, high value was recorded for plant transpiration efficiency (20.61%) and shoot transpiration efficiency (20.91%). Moderate GCV was recorded for water use (14.78 %), leaf area (19.55%), plant height (14.4%), shoot dry biomass (15.22%), shoot fresh biomass (15.97%), total dry biomass (14.69%) and root dry biomass (18.56%). Low GCV was observed for leaf chlorophyll content (5.39%) and leaf number (7.25%). Both plant transpiration efficiency and shoot transpiration efficiency recorded high values of GCV and PCV; revealed that presence of high variability for these traits among the sorghum genotypes and there is a great scope for the improvement of these traits by direct selection among the genotypes. Water use, leaf area, shoot dry biomass, root dry biomass, shoot fresh biomass and plant height were recorded as high values of PCV but had a medium value of GCV, indicating that the apparent due to genetic factors but also due to the environmental condition. Generally, the presence of high and moderate variation in GCV indicates that genotypes had substantial broad based genetic variability for selection, implying that acceptable improvement of transpiration efficiency could be obtained through the selection of these traits. High PCV and GCV values of plant height from sorghum genotypes have been reported by Bello et al. (2007) and Kassahun et al. (2015).

Generally, in the present study the result indicated the presence of smaller differences between the values of GCV and PCV, suggesting a lower effect of environment and higher contribution of genetic factors for the variability of traits among sorghum genotypes. Plant transpiration efficiency, shoot transpiration efficiency and total dry biomass showed relatively smaller difference between their PCV and GCV values than other traits, suggesting the variability of sorghum genotypes for these traits was due to the effect of genetic components.

# 4.4. Heritability and Genetic Advance

According to Johnson *et al.*, (1955) heritability was classified as low (<30%), medium (30-60%), and high (> 60%). Based on this classification, in the present study, high estimates of heritability

were recorded for plant transpiration efficiency (73.77%) and shoot transpiration efficiency (69.86), indicating relatively little influence of environmental conditions on these traits and improvement through selection based on phenotypic performance would be effective for these traits. The higher values of heritability of traits are indicative that the selection can be made based on these traits (Ali *et al.*, 2012). Moderate heritability was observed for total dry biomass (56.92%), shoot dry biomass (53.47%), root dry biomass (47.75%), shoot fresh biomass (42.14%), water use (44.5), plant height (43.1%) and leaf area (36.3%). These results suggest the presence of an adequate heritable portion of variation that can be exploited via the direct selection of superior genotypes based on their phenotypic performance. Similar to the present study, Yemata Beze (2019) reported moderate heritability for above ground biomass of sorghum genotypes. Low heritability estimate was recorded for leaf number (23.11%) and leaf chlorophyll content of plant (25.72%).

Genetic advance as percent of mean ranged from 5.63% for leaf chlorophyll content to 36.46% for plant transpiration efficiency. The highest genetic advance as percent of mean (>20%) was recorded for plant transpiration efficiency (36.46%), shoot transpiration efficiency (36.01%), total dry biomass (22.83%), shoot dry biomass (22.93%), root dry biomass (26.41%), shoot fresh biomass (21.36%) leaf area (24.27%) and water use (20.31%), suggesting that expression of these traits is influenced by genetic factors and expected to give greater response for selection. Moderate genetic advance as percent of mean (10-20%) was obtained for plant height (19.47%). Moreover, Udeh and Ogbu (2011) and Johnson et al. (1955) suggested that only a high estimate of heritability doesn't always provide a high prediction of genetic gain to ensure effective selection for improvement rather it is better to coupling high heritability with high genetic advance as percent of mean to give greater response for selection. High heritability coupled with high genetic advance as percentage of the mean (>60%, >20%) were obtained for plant transpiration efficiency (73.77%, 36.46%) and for shoot transpiration efficiency (69.86%, 36.01%), suggesting that phenotypic selection based on these traits would be effective for improvement of sorghum genotypes for transpiration efficiency. Coupling of high genetic advance with high heritability for transpiration efficiency on maize was reported by (Lakshmi et al., 2020).

Moderate heritability coupled with high genetic advance as percent of mean (30-60%, >20%) were recorded for total dry biomass (56.92%, 22.83%), shoot dry biomass (53.47%, 22.93%), root dry biomass (47.75%, 26.41%), shoot fresh biomass (42.14%, 21.36%), water use (44.5%, 20.31%) and leaf area (36.3%, 24.47%). Moderate heritability coupled with moderate genetic advance as percentage of mean (30-60%, 10-20%) was obtained for plant height (43.1%, 19.47%). Low estimate of heritability coupled with low genetic advance as percentage of mean (30-60%, <10) were recorded for number of leaf (23.11%, 7.18%) and leaf chlorophyll content (25.72%, 5.63%). This result indicated that number of leaf and leaf chlorophyll content was highly influenced by environment.

Table 3: Estimates of variances, heritability, and genetic advance for 11 traits of sorghum genotypes tested at MARC in 2021/22

Traits	σ <sup>2</sup> g	σ²p	σ²e	GCV (%)	PCV (%)	H <sup>2</sup> b (%)	GA	GAM (%)
РТЕ	0.92	1.24	0.33	20.61	23.99	73.77	1.69	36.46
STE	0.47	0.67	0.20	20.91	25.02	69.86	1.17	36.01
TDB	101.04	177.50	76.47	14.69	19.47	56.92	15.62	22.83
SDB	53.59	100.23	46.64	15.22	20.82	53.47	11.03	22.93
RDB	14.23	29.81	15.58	18.56	26.85	47.75	5.37	26.41
SFB	1208.59	2868.14	1659.55	15.97	24.60	42.14	46.49	21.36
WU	5079963.00	11415966	6336003.00	14.78	22.15	44.50	3097.22	20.31
LA	3068.56	8453.33	5384.77	19.55	32.46	36.30	68.75	24.27
LCC	6.29	24.48	18.18	5.39	10.63	25.72	2.62	5.63
LN	0.66	2.83	2.18	7.25	15.08	23.11	0.80	7.18
PH	98.03	227.43	129.40	14.40	21.93	43.10	13.39	19.47

**Note**:  $\sigma^2 p$  = Phenotypic variation;  $\sigma^2 g$  = Genotypic variation;  $\sigma^2 e$  = Environmental variance; GCV (%) = Genotypic coefficient of variation, PCV (%) = Phenotypic coefficient of variation, H <sup>2</sup>b (%) =Broad sense heritability; GA (5%) = genetic advance at 5% selection intensity; GAM =Genetic advance as percent of mean; PTE = plant transpiration efficiency; STE = shoot transpiration efficiency; LA = leaf area; LCC = leaf chlorophyll content; PH= plant height; LN= leaf number; SDB= shoot dry biomass; SFB= shoot fresh biomass; TDB= total dry biomass; WU= water use; and RDB= root dry biomass.

#### **4.5.**Correlation of Traits

4.5.1. Phenotypic correlation of traits

The results of phenotypic correlation coefficient analysis showed that plant transpiration efficiency had highly significant and positive correlation with shoot transpiration efficiency (0.95), total dry biomass (0.63), shoot dry biomass (0.58), root dry biomass (0.49), shoot fresh biomass (0.33) and had a significant and positive correlation with leaf chlorophyll content of the plant. Similar to the present results, positive correlation between plant transpiration efficiency and total dry biomass had been reported by Vadez *et al.* (2011b). Also, positive correlation of plant transpiration efficiency with shoot dry biomass and total dry biomass had been reported by Xin *et al.* (2008). Further plant transpiration efficiency showed negative phenotypic correlation with leaf number (-0.34) and water use (-0.63). In agreement with these results, plant transpiration efficiency was negatively correlated with water use (Blum 2005).

Similarly, shoot transpiration efficiency shows positive and highly significant correlation with plant transpiration efficiency (0.95), shoot dry biomass (0.67), shoot fresh biomass (0.40), total dry biomass (0.61), and root dry biomass (0.26); whereas shoot transpiration efficiency had showed significantly negative correlation with leaf number (-0.3) and water use (-0.59). A favorable correlation between shoot transpiration efficiency and plant transpiration efficiency and with biomass production revealed the possibility of improving plant transpiration efficiency and shoot transpiration efficiency simultaneously.

Total dry biomass had positive and highly significant phenotypic correlation with plant transpiration efficiency (0.63), shoot transpiration efficiency (0.61), root dry biomass (0.73), shoot dry biomass (0.93), shoot fresh biomass (0.70), leaf area (0.48) and had significant and positive phenotypic correlation with leaf number (0.15) and water use (0.14). Shoot dry biomass showed positive and highly significant correlation with shoot fresh biomass (0.73), total dry biomass (0.93), root dry biomass (0.43), shoot transpiration efficiency (0.67), plant transpiration efficiency (0.58), leaf area (0.50) and had positive significant correlation with water use (0.15), and leaf number (0.18). Similarly, Xin *et al.* (2008), reported that both shoot biomass and total

biomass were positively correlated with plant transpiration efficiency. Root dry biomass showed positive and significant phenotypic correlation with shoot fresh biomass (0.35), shoot dry biomass (0.43), shoot transpiration efficiency (0.26), plant transpiration efficiency (0. 49), total dry biomass (0.73) and leaf area (0.25). Shoot fresh biomass shows positively high significant phenotypic correlation with total dry biomass (0.70), root dry biomass (0.35), shoot dry biomass (0.73), shoot transpiration efficiency (0.40), plant transpiration efficiency (0.33), leaf area (0.46), leaf number (0.21) and water use (0.17). Similar to present study Van Oosterom *et al.* (2021), reported the positive association of biomass accumulation with water use.

Water use had positive and significant correlation with shoot fresh biomass (0.17), total dry biomass (0.14), shoot dry biomass (0.15), leaf area (0.57) and leaf number (0.58). Leaf area showed positive and highly significant phenotypic correlation with shoot fresh biomass (0.46), total dry biomass (0.48), root dry biomass (0.25), shoot dry biomass (0.50), water use (0.57), leaf number (0.49) and had negative correlation with leaf chlorophyll content (-0.39). Leaf chlorophyll content showed positive and significant phenotypic correlation with plant transpiration efficiency (0.16) and had negative correlation with water use (-0.36), leaf area (-0.39) and shoot fresh biomass (-0.16). Leaf number had positive and highly significant phenotypic correlation with shoot fresh biomass (0.21), water use (0.58), leaf area (0.49) and had significant positive correlation with total dry biomass (0.15) and shoot dry biomass (0.18). Plant height had shown non-significant correlation with plant transpiration efficiency, shoot transpiration efficiency, shoot fresh biomass, total dry biomass, water use and leaf number. Similarly, Kulathunga et al, (2021), reported that the absence of association between plant height and shoot transpiration efficiency. Therefore, positively significant correlation of traits indicates the possibility of simultaneous improvement through selection.

## 4.5.2. Genotypic correlation of traits

In the present study, plant transpiration efficiency had highly significant and positive genotypic correlation with shoot transpiration efficiency (0.96), total dry biomass (0.69), shoot dry biomass (0.63), root dry biomass (0.56), shoot fresh biomass (0.37) and leaf chlorophyll content (0.18), indicating that increase in transpiration efficiency of the plant is because of increment of one or

more of those characters since they can be improved simultaneously. Similar to the present result, positive correlation between TE and total biomass had been reported by Xin *et al.* (2009). However, plant transpiration efficiency had negative genotypic correlations with leaf number (-0.44) and water use (-0.66). In agreement with present result, Blum (2005), reported negative correlation of transpiration efficiency with water use. On the other side Vadez *et al.* (2011b) and Xin *et al.* (2008), reported the absence of a relationship between TE and total water use. But in contrast to present result, Peng and Krieg (1992), was reported presence of positive correlation between water use and transpiration efficiency.

Shoot transpiration efficiency had highly significant and positive correlation with plant transpiration efficiency (0.96), shoot dry biomass (0.71), shoot fresh biomass (0.43), total dry biomass (0.67), root dry biomass (0.36) and leaf chlorophyll content (0.16). Whereas shoot transpiration efficiency showed genotypically negative correlation with leaf number (-0.38) and water use (-0.62). Total dry biomass showed positive and highly significant correlation with plant transpiration efficiency (0.69), shoot transpiration efficiency (0.67), shoot dry biomass (0.94), shoot fresh biomass (0.72), root dry biomass (0.76) and leaf area (0.47). Shoot dry biomass showed positive and highly significant correlation with shoot fresh biomass (0.76), total dry biomass (0.94), root dry biomass (0.49), shoot transpiration efficiency (0.67), plant transpiration efficiency (0.63) and leaf area (0.53). Strong correlation of transpiration efficiency and biomass production indicates genotypes with high transpiration efficiency could produce high amount of biomass, this leads to increment of grain production (Peng et al., 1991).

Root dry biomass showed positive and highly significant genotypic correlation with shoot fresh biomass (0.38), shoot dry biomass (0.49), shoot transpiration efficiency (0.36), plant transpiration efficiency (0.56), total dry biomass (0.76) and leaf area (0.29). Shoot fresh biomass shows positive and highly significant genotypic correlation with total dry biomass (0.72), root dry biomass (0.38), shoot dry biomass (0.76), shoot transpiration efficiency (0.43), plant transpiration efficiency (0.37), leaf area (0.53). Whereas shoot fresh biomass had negative genotypic correlation with leaf chlorophyll content (-0.24). Genotypically water use had positive correlation with leaf area (0.60) and leaf number (0.66). Leaf area showed positive and highly significant genotypic correlation with shoot fresh biomass (0.53), total dry biomass (0.47), root

dry biomass (0.29), shoot dry biomass (0.47), water use (0.60) and leaf number (0.51). Leaf number had positive and highly genotypic correlation with water use (0.66) and leaf area (0.51). Plant height had shown non-significant correlation with plant transpiration efficiency. Similarly, Kulathunga *et al.* (2021), report the absence of correlation between plant transpiration efficiency of sorghum with plant height.

Overall, plant transpiration efficiency had significant and positive genotypic and phenotypic correlations with shoot transpiration efficiency, total dry biomass, shoot fresh biomass, root dry biomass, shoot fresh biomass and leaf chlorophyll content. The close correspondence of genotypic and phenotypic correlation coefficients and large genotypic correlation coefficient indicated the manifestation of great genetic correlations and the witnesses that the phenotypic correlations between plant transpiration efficiency and other traits were highly determined by correlations due to the genetic effect.

Variable	SFB	TDB	RDB	SDB	STE	PTE	WU	LA	LCC	LN	PH
SFB	1	0.72**	0.38**	0.76**	0.43**	0.37**	0.15 <sup>ns</sup>	0.53**	-0.24*	0.17 <sup>ns</sup>	0.05 <sup>ns</sup>
TDB	$0.70^{**}$	1	0.76**	0.94**	0.67**	0.69**	0.05 <sup>ns</sup>	0.47**	-0.10 <sup>ns</sup>	0.05 <sup>ns</sup>	-0.12 <sup>ns</sup>
RDB	0.35**	0.73**	1	0.49**	0.36**	0.56**	-0.01 <sup>ns</sup>	0.29**	-0.02 <sup>ns</sup>	-0.05 <sup>ns</sup>	-0.17 <sup>ns</sup>
SDB	0.73**	0.93**	0.43**	1	0.71**	0.63**	0.08 <sup>ns</sup>	0.47**	-0.13 <sup>ns</sup>	0.09 <sup>ns</sup>	-0.07 <sup>ns</sup>
STE	0.40**	0.61**	0.26**	0.67**	1	0.96**	-0.62**	-0.07 <sup>ns</sup>	0.16*	-0.38**	-0.08 <sup>ns</sup>
РТЕ	0.33**	0.63**	0.49**	$0.58^{**}$	0.95**	1	-0.66**	-0.10 <sup>ns</sup>	$0.18^{*}$	-0.44**	-0.12 <sup>ns</sup>
WU	$0.17^{*}$	0.14*	0.06 <sup>ns</sup>	$0.15^{*}$	-0.59**	-0.63**	1	0.60**	-0.39**	0.66**	0.06 <sup>ns</sup>
LA	0.46**	0.48**	0.25**	0.50**	-0.03 <sup>ns</sup>	-0.08 <sup>ns</sup>	0.57**	1	-0.45**	0.51**	-0.06 <sup>ns</sup>
LCC	-0.16*	-0.11 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.14	0.13 <sup>ns</sup>	0.16*	-0.36**	-0.39**	1	-0.47**	-0.18 <sup>ns</sup>
LN	0.21**	0.15*	0.04 <sup>ns</sup>	$0.18^{*}$	-0.30**	-0.34**	0.58**	0.49**	-0.38**	1	0.14 <sup>ns</sup>
РН	0.04 <sup>ns</sup>	-0.11 <sup>ns</sup>	-0.16*	-0.06*	0.02 <sup>ns</sup>	-0.02 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.19**	-0.09 <sup>ns</sup>	0.01 <sup>ns</sup>	1

Table 4: Genotypic (above diagonal) and Phenotypic (below diagonal) correlation of traits for tested sorghum genotypes

**Note:** \*, significance at 0.05 and \*\* significance at 0.01 probability levels. NS =Non-Significant; PTE = plant transpiration efficiency; STE = shoot transpiration efficiency; LA = leaf area; LCC = leaf chlorophyll content; PH= plant height; LN= leaf number; SDB= shoot dry biomass; SFB= shoot fresh biomass; TDB= total dry biomass; WU= water use; and RDB= root dry biomass.

# 4.6. Path coefficient analysis

## 4.6.1. Phenotypic direct and indirect effects of traits on total dry biomass

In the present study, the path analysis was made on phenotypic and genotypic correlation coefficients using total dry biomass as a dependent variable and other traits as independent variables because the ultimate goal of selection of genotypes with high transpiration efficiency is to increase biomass production; genotypes with greater biomass producing ability have greater grain production ability (Peng et al., 1991). Phenotypic path coefficient analysis revealed that total dry biomass was positively and directly affected by plant transpiration efficiency (1.23), shoot transpiration efficiency (0.22), shoot fresh biomass (0.24), water use (0.71), leaf area (0.05), leaf chlorophyll content (0.04) and leaf number (0.04). Direct effects of these traits on the transpiration efficiency of plant ensured such a truthful relationship should be exploited in improving the biomass of sorghum. However, phenotypic path coefficient analysis shows the presence of direct negative effect of plant height on biomass of sorghum crop (-0.023).

Selection based on correlation alone may be misleading because it measures only the mutual association between two characters (Izge*et al.*, 2012). However, path coefficient analysis specifically measures the relative contribution of different traits to plant transpiration efficiency. The manifestation of considerable direct effect, as well as higher order phenotypic correlation between biomass and other traits revealed that biomass production of sorghum can be improved through direct selection these traits.

A highly significant phenotypic correlation of plant transpiration efficiency with dry biomass was due to its high positive direct effect (1.23) on dry biomass. The presence of a highly significant phenotypic correlation of shoot transpiration efficiency with total dry biomass was due to the high positive indirect effect of plant transpiration efficiency (1.17) and positive direct effect of shoot transpiration efficiency (0.22). Positive and highly significant phenotypic correlation of shoot fresh biomass was due to positive direct effects shoot fresh biomass with dry biomass was due to positive direct effects shoot fresh biomass (0.24) and positive indirect effect of plant transpiration efficiency (0.41) and water use (0.12). Significant phenotypic correlation of water use with dry biomass was due to its positive direct effect (0.71) and positive indirect effect of shoot transpiration efficiency (0.13). Highly

significant and positive phenotypic correlation of leaf area with dry biomass was due to positive indirect effect of water use (0.40) and shoot fresh biomass (0.11). A significant phenotypic correlation of leaf number with dry biomass was due to the positive indirect effect of water use (0.41).

Plant transpiration had positive indirect effect on total dry biomass through shoot fresh biomass (0.41), shoot transpiration efficiency (1.17) and leaf chlorophyll content (0.20). However, plant transpiration efficiency had negative indirect effect on total dry biomass through water use (-0.78) and leaf number (-0.42). Water use had positive indirect effect on dry biomass through leaf number (0.41), leaf area (0.40) and shoot fresh biomass (0.12). But water use had negative indirect effect on dry biomass through shoot transpiration efficiency (-0.45) and leaf chlorophyl content (-0.26). Leaf area had positive indirect effect on dry biomass through shoot fresh biomass (0.02), water use (0.03) and leaf number (0.02). Plant height had positive indirect effect on dry biomass through shoot fresh biomass through plant transpiration efficiency, water use, leaf area and leaf chlorophyl content.

Phenotypic direct and indirect path coefficient analysis revealed that sorghum genotypes with high transpiration efficiency could produce high amount of biomass. The results of phenotypic residual effects (R=0.06) revealed that 94% of the biomass was contributed by the traits studied in this experiment. The remaining 6% was contributed by other factors.

Traits	SFB	STE	РТЕ	WU	LA	LCC	LN	РН	rp
SFB	0.2382	-0.0889	0.4061	0.1175	0.0227	-0.0073	0.0092	-0.0011	$0.7^{**}$
STE	0.0941	0.2248	1.1661	-0.4189	-0.0014	0.0059	-0.0129	-0.0005	0.61**
РТЕ	0.0787	-0.2133	1.2291	-0.4493	-0.0039	0.0074	-0.015	0.0005	0.63**
WU	0.0395	0.133	-0.7799	0.7082	0.0281	-0.0164	0.0251	0.0018	0.14*
LA	0.1097	0.0065	-0.0961	0.4047	0.0492	-0.0175	0.0212	0.0044	$0.48^{**}$
LCC	-0.0382	-0.0294	0.2009	-0.2562	-0.019	0.0454	-0.0164	0.0022	-0.11 <sup>ns</sup>
LN	0.0506	0.0664	-0.424	0.4087	0.024	-0.0172	0.0435	-0.0003	0.15*
РН	0.0107	-0.0045	-0.0253	-0.055	-0.0091	-0.0041	0.0005	-0.0237	0.11 <sup>ns</sup>
Residual									0.06

Table 5: Phenotypic direct (bold diagonal) and indirect (off-diagonal) effects of other traits on total dry biomass of 101 sorghum genotypes

**Note:** \*, significance at 0.05 and \*\* significance at 0.01 probability levels. NS =Non-Significant; PTE = plant transpiration efficiency; STE = shoot transpiration efficiency; LA = leaf area; LCC = leaf chlorophyll content; PH= plant height; LN= leaf number; SFB= shoot fresh biomass; TDB= total dry biomass; WU= water use; and rp= phenotypic correlation.

# 4.6.2. Genotypic direct and indirect effects of traits on total dry biomass

The genotypic path coefficient analysis revealed that plant transpiration efficiency exerted the maximum positive direct effect (1.28) on total dry biomass, followed by water use (0.71) and shoot fresh biomass (0.22) indicating that the selection for these traits was likely to bring about an overall improvement in biomass production directly. The lowest positive direct effects were contributed by leaf area (0.04), leaf chlorophyll content (0.04) and leaf number (0.02) on total dry biomass, indicating that indirect effects would be the cause of correlation. However, genotypic path coefficient analysis shows the presence of direct negative effect of plant height (-0.023) and shoot transpiration efficiency (-0.21) on dry biomass of sorghum.

The presence of a highly significant genotypic correlation of shoot transpiration efficiency with total dry biomass was due to the high positive indirect effect of plant transpiration efficiency (1.22). A highly significant genotypic correlation of plant transpiration efficiency with dry biomass was due to its high positive direct effect (1.28) on dry biomass. Positive and highly significant genotypic correlation of shoot fresh biomass with dry biomass was due to positive direct effects shoot fresh biomass (0.22) and positive indirect effect of plant transpiration efficiency (0.47) and water use (0.10). Highly significant and positive genotypic correlation of leaf area with dry biomass was attributed by positive indirect effect of water use (0.43) and shoot fresh biomass (0.12).

Plant transpiration efficiency had positive indirect effect on dry biomass through shoot fresh biomass (0.46), shoot transpiration efficiency (1.22) and leaf chlorophyll content (0.23). However, plant transpiration efficiency had negative indirect effect on total dry biomass through water use (-0.84), leaf number (-0.55) and leaf area (0.12). Water use had positive indirect effect on dry biomass through leaf number (0.47), leaf area (0.43), shoot fresh biomass (0.11) and plant height (0.04). But water use had negative indirect effect on dry biomass through plant transpiration efficiency (-0.47), shoot transpiration efficiency (-0.44) and leaf chlorophyl content (-0.28). Leaf area had positive indirect effect on dry biomass through shoot fresh biomass (0.02), water use (0.02) and leaf number (0.02). Plant height had positive indirect effect on dry biomass through plant transpiration efficiency, shoot transpiration efficiency, leaf area and leaf

chlorophyll content. Leaf number had positive indirect effect on dry biomass through shoot fresh biomass, leaf area, water use and plant height. However, it had negative indirect effect on total dry biomass through plant transpiration efficiency, shoot transpiration efficiency and leaf chlorophyll content.

Generally, genotypic, and phenotypic direct and indirect path coefficient analysis revealed that sorghum improvement towards increasing biomass production of sorghum would be due to the increment of shoot fresh biomass, plant transpiration efficiency, leaf area, leaf number and leaf chlorophyll content. The results of genotypic residual effects (R=0.05) revealed that 95% of the total dry biomass was contributed by the traits studied in this experiment. The remaining 0.05% was contributed by other factors.

Traits	SFB	STE	РТЕ	WU	LA	LCC	LN	РН	rp
SFB	0.2215	-0.0901	0.4663	0.1088	0.0198	-0.0104	0.0039	-0.0019	0.72**
STE	0.0949	-0.2103	1.2245	-0.4412	-0.0025	0.0069	-0.0087	0.0029	0.67**
РТЕ	0.0809	-0.2017	1.2769	-0.4678	-0.0037	0.008	-0.0098	0.0043	0.69**
WU	0.0339	0.1305	-0.8401	0.7111	0.0226	-0.0171	0.0148	-0.0023	0.05 <sup>ns</sup>
LA	0.1169	0.0141	-0.125	0.4281	0.0376	-0.0197	0.0116	0.0024	0.47**
LCC	-0.052	-0.0331	0.2331	-0.2758	-0.0168	0.044	-0.0106	0.0066	-0.1 <sup>ns</sup>
LN	0.0387	0.0808	-0.5573	0.4668	0.0193	-0.0206	0.0225	-0.0051	0.05 <sup>ns</sup>
РН	0.0112	0.0165	-0.147	0.0445	-0.0024	-0.0078	0.0031	-0.0372	-0.12 <sup>ns</sup>
Residual									0.05

Table 6: Genotypic direct (bold diagonal) and indirect (off-diagonal) effects of other traits on total dry biomass of 101 sorghum genotypes

**Note:** \*, significance at 0.05 and \*\* significance at 0.01 probability levels. NS =Non-Significant; PTE = plant transpiration efficiency; STE = shoot transpiration efficiency; LA = leaf area; LCC = leaf chlorophyll content; PH= plant height; LN= leaf number; SFB= shoot fresh biomass; TDB= total dry biomass; WU= water use; and rp= genotypic correlation.

## 4.7. Clustering of Genotypes

In the present study, cluster analysis based on 11 traits grouped the 101 sorghum genotypes into 6 different clusters as shown in Table 7. The mean value of all traits in each cluster is also presented in Table 8. Cluster I comprised 31 genotypes with unique characteristics including moderate plant transpiration efficiency (4.81), shoot transpiration efficiency (3.3), leaf chlorophyll content (46.26), leaf number (11.18), plant height (73.03), leaf area (229.71), shoot dry biomass (42.58), total dry biomass (62), water use (13043.55), root dry biomass (19.42) and low shoot fresh biomass (206.44). Genotypes grouped in cluster I are almost moderate for all traits.

The second cluster consisted of 16 genotypes, which comprised genotypes with low transpiration efficiency of plants (3.34), low shoot transpiration efficiency (2.44), high chlorophyll content of leaf (48.47), low number of leaves (10.59), a dwarf in plant height (61.25), low in shoot dry biomass (39.13), moderate value of leaf area(249.55), low in shoot fresh biomass (203.25), low total dry biomass (53.69), moderate water use (16400) and low in root dry biomass (14.56), indicating genotypes which grouped in this cluster had poor performance for most of traits.

Cluster III consisted of 36 genotypes with the following feature: high plant transpiration efficiency (5.34), moderate shoot transpiration efficiency (3.77), medium chlorophyll content of leaf (45.28), moderate number of leaf (11.43), dwarf in plant height (63.79), high leaf area (304.57), high shoot dry biomass (55.18), high shoot fresh biomass (250.29), high total dry biomass (78.24), medium water use (14907.31) and high in root dry biomass (23.06). Genotypes in cluster III had better performance next to cluster V. Cluster IV contained 12 genotypes, which is characterized by low plant transpiration efficiency (3.39) and shoot transpiration efficiency (2.43), high leaf chlorophyll content (48.16), plant height (72.08) leaf area (357.96) and water use (20498.33), moderate number of leaf (10.88), shoot dry biomass (47.79), shoot fresh biomass (229.42), root dry biomass (18.83) and total dry biomass (66.63), indicates genotypes in this cluster were transpire water highly but they couldn't produce sufficient biomass as their water usage.

The fifth cluster consists of five genotypes, which comprised genotypes with high plant transpiration efficiency (5.9), shoot transpiration efficiency (4.08), shoot fresh biomass (282.3), total dry biomass (90.4), root dry biomass (27.9), shoot dry biomass (62.5), leaf area (402.6), chlorophyll content of leaf (48.25), plant height (87) and moderate in water use (15436) and leaf number (10.80). Cluster V had comprised of elite genotypes because the performs better for all traits. Cluster six had a single genotype with a characteristics of moderate plant transpiration efficiency (4.42), low shoot transpiration efficiency (2.76) and leaf chlorophyll content (39), a high number of leaves (15.5) and plant height (106), moderate area of leaf (224.18), low shoot dry biomass (28), medium shoot fresh biomass (205), total dry biomass (61) and water use (13875) and high in root dry biomass (23).

Overall, the present cluster analysis revealed considerable divergence among sorghum genotypes, suggesting the possibility of improving sorghum genotypes for transpiration efficiency through hybridization by selecting appropriate parental sorghum genotypes. Crosses between genotypes from clusters II and V, and cluster IV and V are important because they compromised genetically distant genotypes Therefore, promising sorghum genotypes with desirable traits could be selected and used for hybridization for further sorghum improvement. According to Rahim *et al.* (2010) and Ali *et al.* (2008), described that the cross between genotypes with maximum genetic distance would bring maximum heterosis. Also, it is possible to directly select genotypes with better performance for high transpiration efficiency and biomass production from cluster V and III.



Figure 1: Dendrogram of 101 sorghum genotypes for 11 traits with average linkage clustering strategy. Codes (ID) of genotypes is as indicated in Appendix Table 1.

Table 7. Distribution and grouping of 101 sorghum genotypes in six clusters

Cluster	N <u>o</u>	<b>Proportion %</b>	Genotypes
	Genotypes		
Cl	31	30.69	ETSC17258-1-2, ETSC17262-2-2, ETSC17044-17-2, ETSC15363-1-2, ETSC17253-9-3, ETSC17023-
			14-1, ETSC17295-5-3, ETSC17023-3-1, ETSC17310-2-3, ETSC17322-4-1, ETSC17295-2-1,
			ETSC17323-21-2,, ETSC17360-5-1, ETSC17032-8-1, ETSC17349-33-1, ETSC17272-11-1,
			ETSC17272-6-1, ETSC17195-2-1, ETSC17354-9-1, ETSC17272-8-1, ETSC300386, ETSC17072-1-1,
			ETSC17112-3-2, ETSC17073-6-2, ETSC16010-2-1, ETSC17269-6-1, ETSC17269-3-1, ETSC17361-8-
			3, ETSC17037-6-1, ETSC17177-2-1, ETSC300354
C2	16	15.84	ETSC17109-10-2, ETSC17285-14-1, ETSC17297-10-1, ETSC17252-19-2, ETSC17045-12-2,
			ETSC17296-14-1, ETSC17296-5-1, ETSC17360-5-2, ETSC17172-4-2, ETSC17045-7-1, ETSC17272-
			1-1, ETSC17258-13-1, ETSC17276-5-1, ETSC17300-9-3, ETSC14020-1-1, ETSC17172-2-3
C3	36	35.64	ETSC17268-3-1, ETSC17065-2-1, ETSC17300-2-1, ETSC15438-4-1, Argiti, ETSC17112-13-2,
			ETSC17129-12-1, ETSC17007-9-1,ETSC17301-5-2, ETSC17276-4-2, ETSC17240-8-1, ETSC17129-
			6-1, ETSC17140-9-1, ETSC17137-4-2, ETSC17304-7-2, ETSC17194-3-1, ETSC17277-3-1,
			ETSC17268-8-1, ETSC17300-4-1, ETSC17064-6-1, ETSC17137-4-3, ETSC17131-1-1, ETSC17130-2-
			1, ETSC17276-9-1, ETSC17258-5-2, ETSC17328-11-1, ETSC17201-1-2, ETSC17135-12-1,
			ETSC17285-5-2, ETSC15312-3-1, ETSC17172-8-1, ETSC17300-1-2, ETSC14325-4-1, ETSC17122-5-
~ .			1, ETSC17093-6-1, Melkam
C4	12	11.88	ETSC17164-1-2, ETSC17201-7-3, ETSC17004-11-3, ETSC17268-7-1, ETSC17353-27-2,
			ETSC17245-3-3, ETSC17198-8-1, ETSC17083-6-1, ETSC17280-3-1, ETSC17068-6-1, ETSC17112-
~-			10-1, ETSC17300-1-1
C5	5	4.95	ETSC14252-3-2, ETSC17155-2-1, ETSC17152-1-1, ETSC17295-8-1, ETSC17153-6-3
C6	1	0.99	ETSC17074-10-1

	Cluster					
	Ι	II	III	IV	V	VI
РТЕ	4.81	3.34*	5.34	3.39	5.90**	4.42
STE	3.30	$2.44^{*}$	3.77	2.43	$4.08^{**}$	2.76
TDB	62.00	53.69 <sup>*</sup>	78.24	66.63	90.40**	61.00
SDB	42.58	39.13	55.18	47.79	62.50**	$38.00^{*}$
RDB	19.42	14.56*	23.06	18.83	$27.90^{**}$	23.00
SFB	206.44	$203.25^{*}$	250.29	229.42	282.30**	205.00
WU	$13043.55^{*}$	16400.00	14907.31	20498.33**	15436.00	13875.00
LA	229.71	249.55	304.57	357.96	402.60**	$224.18^{*}$
LCC	46.26	$48.47^{**}$	45.28	48.16	48.25	39.60
LN	11.18	10.59*	11.43	10.88	10.80	15.50**
РН	73.03	61.25*	63.79	72.08	87.00	106.00**

Table 8: Cluster means for 11 traits of 101 sorghum genotypes

Note:\* and \*\* are the lower and highest cluster mean; PTE = plant transpiration efficiency; STE = shoot transpiration efficiency; LA = leaf area; LCC = leaf chlorophyll content; PH= plant height; LN= leaf number; SDB= shoot dry biomass; SFB= shoot fresh biomass; TDB= total dry biomass; WU= water use; and RDB= root dry biomass;

Regarding to the inter-cluster distance, the maximum distance was found between cluster II and cluster V (78.66), followed by cluster V and cluster VI (59.61), and cluster IV and V (55.97). The minimum distance was found between cluster I and II (12.08), followed by cluster I and III (12.57), cluster II and IV (16.25) and cluster III and V (16.52). Cluster I and cluster II are the closest clusters and, Cluster II and cluster V are the most distant clusters than other. Cluster V is relatively most divergent cluster for the rest of other clusters while cluster I is the closest clusters for the remaining clusters. These higher and significant inter- cluster distance between clusters indicates the presence of wider genetic diversity among sorghum genotypes for the studied traits. The extent of diversity present in the studied sorghum genotypes implied an opportunity for

sorghum improvement through selection or targeted crossing to increase the transpiration efficiency of sorghum to water limited environments.

The intra-cluster values were lower than the inter-cluster values, indicating that the genotypes within the cluster were more related than between clusters. In general, it is advised that genotypes chosen based on large cluster distances could lead to a broad spectrum of beneficial genetic diversity for sorghum improvement.

Table 9: Intra-cluster (bold diagonal) and inter-cluster (off-diagonal) distance matrix between six clusters

Cluster	Ι	II	III	IV	V	VI
I	3.7286	12.08466	12.56616	25.12656	41.28427	22.94068
II		3.3678	32.93396	16.25179	78.65616	43.49482
III			2.7192	31.07045	16.51911	41.00451
IV				2.7308	55.97109	45.57502
V					3.0054	59.61531
VI						2.9579

 $\chi 2=24.72$  at 1% probability level and  $\chi 2=19.67$  at 5% probability level

# 4.8. Principal Component Analysis

The principal component analysis used to reduce the number variables into important component and identify most important traits which contributes to source of vacation (Ahmadizadeh and Felenji, 2011). In the present study, four PCs out of ten have selected based on their eigenvalue of greater than one. These PCs accounted for 79% of the total variation, from which PC1 contributed 37% of the variation, PC2 20%, PC3 13%, and PC4 contributes 9% of the variation among 101 sorghum genotypes. The four principal components had eigenvalues of 4.4, 2.36,

1.59, and 1.1, respectively. This indicates that the identified traits within this axis showed great influence on the phenotype of the genotypes and could effectively be used in selection criteria.

Traits such as plant transpiration efficiency, shoot transpiration efficiency, total dry biomass, root dry biomass, shoot dry biomass, shoot fresh biomass and leaf area had high contribution to the first PC. These indicated that these traits had higher relative contribution to the total diversity. The second contributor of explained variation was PC2 it was associated with PC loading scores of plant transpiration efficiency, shoot transpiration efficiency, leaf area, shoot fresh biomass and water use. PC3 was associated with PC loading scores of leaf chlorophyll content, leaf number plant height and shoot transpiration efficiency. The fourth PC was associated with PC loading scores of plant height and shoot transpiration efficiency. The fourth PC was associated with PC loading scores of plant height, leaf chlorophyll content and leaf number. The higher eigenvectors value in each principal component indicated that greater contribution of those traits to the discrimination of the populations into clusters. Principal component analysis could explain high level of diversity among the studied genotypes. Therefore, selection efforts based on these traits might be more effective in sorghum improvement program for improving the genotypic value of the new populations.

	Eigenvecto	r values		
Traits	PC1	PC2	PC3	PC4
РТЕ	0.40	0.34	0.10	0.02
STE	0.39	0.31	0.17	0.02
TDB	0.46	-0.14	0.04	-0.06
SDB	0.43	-0.15	0.12	-0.05
RDB	0.34	-0.06	-0.12	-0.06
SFB	0.35	-0.26	0.01	0.11
WU	-0.09	-0.60	-0.07	-0.10
LA	0.17	-0.53	-0.02	-0.08
LCC	-0.10	-0.13	0.57	0.30
LN	0.07	0.10	-0.55	-0.47
РН	0.07	0.05	-0.41	0.51
Eigenvalue	4.40	2.36	1.59	1.10
proportion (%)	37	20	13	9
Cumulative (%)	37	56	70	79

Table 10: Eigenvalues, percent of cumulative variance, and eigenvectors for 11 characters studied in 101 genotypes

Note: PTE = plant transpiration efficiency; STE = shoot transpiration efficiency; LA = leaf area; LCC = leaf chlorophyll content; PH= plant height; LN= leaf number; SDB= shoot dry biomass; SFB= shoot fresh biomass; TDB= total dry biomass; WU= water use; RDB= root dry biomass. The first two principal components which accounted for 61.31% of the variance were plotted to observe relationships between the measured traits of 101 sorghum genotypes (Figure 2). Genotypes that are closely located on biplot perceived as similar when rated on the given attributes. The genotypes far from the point of origin are more diverse from the others. According to Dehghani *et al.* (2008), the correlation between any two traits is approximated by the cosine of the angle between their vectors. The relationship shown a positive association between total dry biomass, shoot dry biomass, root dry biomass, shoot fresh biomass, plant transpiration efficiency and shoot transpiration efficiency.

The biplot gave more opportunity to assess which genotypes were good for which traits that would help as baseline information for improvement. Based on the results genotypes (G5, G45,G65,G33,G46,G6,G82,G96,G67,G62,G87) could be directly selected for their high plant transpiration and shoot transpiration efficiency. The selection of genotypes based on their value of transpiration efficiency would improve biomass production of the genotype, this leads to improvement of grain yield production (Peng *et al.*, 1991).



Figure 2. Principal component analysis biplot describing the relative position of 101 sorghum genotypes and 11 traits

# **Chapter 5. CONCLUSION AND RECOMMENDATIONS**

# 5.1. Conclusion

Sorghum is a multi-purpose crop grown in diverse agro-ecologies for millions of people, especially in arid and semi-arid areas. However, its production is affected by drought. Transpiration efficiency is a trait associated with plant drought tolerance. Hence, in the present study, a total of 99 inbreed lines with two checks were used to evaluate for their variability for transpiration efficiency.

In the present study, sorghum genotypes showed genetic variability for plant transpiration efficiency and association of traits, indicating presence of considerable genetic potential for the improvement of sorghum transpiration efficiency through direct selection of genotypes. The presence of genetic variability in sorghum ensures the possibility to achieve crop improvement goals through selection and hybridization. Analysis of trait association also showed that transpiration efficiency of sorghum can be improved through indirect selection of related traits such as shoot transpiration efficiency, total dry biomass, shoot dry biomass, root dry biomass, and shoot fresh biomass. From, the cluster analysis, Clusters II and V consisted of distantly related genotypes, indicating the possibility of improving sorghum through hybridization for the maximum heterosis. Based on the cluster mean genotypes incorporate in cluster three and five may be the most important donor parent to improve the transpiration efficiency of sorghum.

Thus, principal component analysis showed that plant transpiration efficiency, shoot transpiration efficiency, total dry biomass, shoot dry biomass, root dry biomass, and shoot fresh biomass were the most contributors to explained genetic variability of the tested genotypes. This revealed that these traits are the most determinate in genetic variability of the targeted genotypes selection of sorghum genotypes based on these characters could give better chance in selecting genotypes for improvement. In general, presence of variability for transpiration efficiency among inbreed lines indicates that there is great scope for development of adaptable and high yielder sorghum variety for those arid and semi-arid areas.

# 5.2. Recommendation

Results from the present study revealed that, ETSC17194-3-1, ETSC17194-3-1, ETSC17153-6-3, ETSC15312-3-1, ETSC17304-7-2, ETSC17240-8-1, ETSC17130-2-1, ETSC17300-2-1, ETSC17272-8-1, ETSC17258-5-2, ETSC17295-8-1 are most promising genotypes better than the check, which are to be considered in future sorghum breeding program for the improvement of sorghum transpiration efficiency. Whereas experiment should be verified in the actual field to test genotypes adaptation in semi-arid environments. However there has been limited research in exploiting the sorghum potential for water limited environments. As sorghum is the dominant crop in the dry lowlands of Ethiopia improving its transpiration efficiency needs to get attention in the breeding program.
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APPENDICES

No	Genotype	Pedigree
1	ETSC17164-1-2	ICSR24010/B35/ETSL100307
2	ETSC17268-3-1	MR812/B35/Gambella1107
3	ETSC17258-1-2	ICSR24010/B35/SRN39
4	ETSC17262-2-2	ICSR24010/B35/KariMatama1
5	ETSC17065-2-1	PGRCE69420/IS10892/ETSL101853
6	ETSC17300-2-1	PGRCE6940/SAR24/SRN39
7	ETSC17044-17-2	PGRCE69420/87PW3173/Melkam
8	ETSC15438-4-1	14MILSDT7086/Meko-1
9	ETSC17109-10-2	WSV387/P9403/E-36-1/ICSV-93046
10	ETSC15363-1-2	S35/Gambella1107
11	ETSC17253-9-3	ICSR24010/B35/M-204
12	ETSC17023-14-1	90BK4184/85MW5552/NTJ2
13	ETSC17285-14-1	PGRCE69420/87PW3173/SRN39
14	Argiti	WSV387/P9504
15	ETSC17295-5-3	PGRCE6940/SAR24/M-204
16	ETSC17201-7-3	CR:35:5/ICSV-1005/76T1#23/Gambella1107
17	ETSC17112-13-2	WSV387/P9403/E-36-1/KariMatama1
18	ETSC17129-12-1	SDSL2690-2/76T1#23/NTJ2
19	ETSC17023-3-1	90BK4184/85MW5552/NTJ2
20	ETSC17297-10-1	PGRCE6940/SAR24/Melkam
21	ETSC17310-2-3	((148/E-35-1)-4/CS3541derive5-4-2-1)/P9401/M-204
22	ETSC17007-9-1	PGRCE6940/SAR24/Framida
23	ETSC17252-19-2	(ICSV111/B35)/ICSV111/ETSL102496
24	ETSC17322-4-1	((148/E-35-1)-4/CS3541derive5-4-2-1)/P9401/ETSL102496
25	ETSC17004-11-3	PGRCE6940/SAR24/ETSL101848
26	ETSC14252-3-2	ETSL101866/S35
27	ETSC17301-5-2	PGRCE6940/SAR24/B35
28	ETSC17045-12-2	PGRCE69420/87PW3173/ETSL101857
29	ETSC17296-14-1	PGRCE6940/SAR24/Gambella1107
30	ETSC17276-4-2	MR812/B35/KariMatama1
31	ETSC17296-5-1	PGRCE6940/SAR24/Gambella1107
32	ETSC17360-5-2	WSV387/P-9403/ETSL101853
33	ETSC17240-8-1	(ICSV111/B35)/ICSV111/Gambella1107
34	ETSC17268-7-1	MR812/B35/Gambella1107
35	ETSC17295-2-1	PGRCE6940/SAR24/M-204
36	ETSC17323-21-2	90BK4184/85MW5552/M-204
37	ETSC17129-6-1	SDSL2690-2/76T1#23/NTJ2
38	ETSC17140-9-1	WSV387/P9403/B35/KariMatama1
39	ETSC17353-27-2	WSV387/P-9403/ETSL101848
40	ETSC17360-5-1	WSV387/P-9403/ETSL101853
41	ETSC17137-4-2	WSV387/P9403/B35/SRN39

Appendix Table 2:Sorghum genotypes and their pedigree

42	ETSC17245-3-3	(ICSV111/B35)/ICSV111/Framida
43	ETSC17172-4-2	MR812/B35/NTJ2
44	ETSC17032-8-1	90BK4236/87PW3173/ETSL101857
45	ETSC17304-7-2	PGRCE6940/SAR24/NTJ2
46	ETSC17194-3-1	LocalBulk(White)/SRN39/76T1#23/NTJ2
47	ETSC17277-3-1	MR812/B35/ETSL101853
48	ETSC17198-8-1	LocalBulk(White)/SRN39/76T1#23/ETSL101865
49	ETSC17268-8-1	MR812/B35/Gambella1107
50	ETSC17155-2-1	MR812/76T1#23/ETSL100307
51	ETSC17300-4-1	PGRCE6940/SAR24/SRN39
52	ETSC17083-6-1	KEY8561/Korkora/ETSL101857
53	ETSC17349-33-1	Macia/76T1#23/ETSL102496
54	ETSC17064-6-1	PGRCE69420/IS10892/KariMatama1
55	ETSC17272-11-1	MR812/B35/SRN39
56	ETSC17272-6-1	MR812/B35/SRN39
57	ETSC17280-3-1	MR812/B35/ETSL102496
58	ETSC17137-4-3	WSV387/P9403/B35/SRN39
59	ETSC17195-2-1	LocalBulk(White)/SRN39/76T1#23/KariMatama1
60	ETSC17354-9-1	WSV387/P-9403/ETSL101857
61	ETSC17272-8-1	MR812/B35/SRN39
62	ETSC17131-1-1	SDSL2690-2/76T1#23/ETSL101853
63	ETSC300386	(ICSV111/B35)/ICSV111
64	ETSC17045-7-1	PGRCE69420/87PW3173/ETSL101857
65	ETSC17130-2-1	SDSL2690-2/76T1#23/KariMatama1
66	ETSC17276-9-1	MR812/B35/KariMatama1
67	ETSC17258-5-2	ICSR24010/B35/SRN39
68	ETSC17072-1-1	((148/E-35-1)-4/CS3541derive5-4-2-1)/P9401/ETSL101857
69	ETSC17112-3-2	WSV387/P9403/E-36-1/KariMatama1
70	ETSC17272-1-1	MR812/B35/SRN39
71	ETSC17328-11-1	90BK4184/85MW5552/SRN39
72	ETSC17073-6-2	((148/E-35-1)-4/CS3541derive5-4-2-1)/P9401/SRN39
73	ETSC17152-1-1	MR812/76T1#23/NTJ2
74	ETSC17201-1-2	CR:35:5/ICSV-1005/76T1#23/Gambella1107
75	ETSC17258-13-1	ICSR24010/B35/SRN39
76	ETSC16010-2-1	14MWLSDT7324/M-204
77	ETSC17068-6-1	((148/E-35-1)-4/CS3541derive5-4-2-1)/P9401/M-204
78	ETSC17269-6-1	Dekeba/Framida/Dekeba//Dekeba/Framida/Dekeba
79	ETSC17135-12-1	WSV387/P9403/B35/Melkam
80	ETSC17112-10-1	WSV387/P9403/E-36-1/KariMatama1
81	ETSC17285-5-2	PGRCE69420/87PW3173/SRN39
82	ETSC15312-3-1	Debir/(Hodem/Gobiye)
83	ETSC17172-8-1	MR812/B35/NTJ2
84	ETSC17269-3-1	Dekeba/Framida/Dekeba//Dekeba/Framida/Dekeba
85	ETSC17295-8-1	PGRCE6940/SAR24/M-204

86	ETSC17276-5-1	MR812/B35/KariMatama1
87	ETSC17300-1-2	PGRCE6940/SAR24/SRN39
88	ETSC17361-8-3	WSV387/P-9403/ETSL100307
89	ETSC17037-6-1	90BK4236/87PW3173/KariMatama1
90	ETSC17177-2-1	LocalBulk(White)/SRN39/E36-1/Melkam
91	ETSC14325-4-1	Macia/S35
92	ETSC17122-5-1	SDSL2690-2/76T1#23/ETSL101848
93	ETSC17074-10-1	((148/E-35-1)-4/CS3541derive5-4-2-1)/P9401/Framida
94	ETSC17300-1-1	PGRCE6940/SAR24/SRN39
95	ETSC17300-9-3	PGRCE6940/SAR24/SRN39
96	ETSC17153-6-3	MR812/76T1#23/KariMatama1
97	ETSC14020-1-1	Gambella1107/SRN39
98	ETSC300354	(\$35/B35)/\$35
99	ETSC17093-6-1	WSV387/76T1#23/Gambella1107
100	ETSC17172-2-3	MR812/B35/NTJ2
101	Melkam	WSV387

Genotype	РТЕ	STE	TDB	SDB	RDB	SFB	WU	LA	LCC	LN	РН
ETSC17164-1-2	3.85	2.98	63.5	49	14.5	260.5	16475	282.57	43.40	12	91
ETSC17268-3-1	4.08	3.135	60	46	14	227	14650	348.28	46.55	11.5	47
ETSC17258-1-2	5.27	3.715	62.5	44	18.5	211.5	11875	251.97	47.65	11	73
ETSC17262-2-2	4.735	3.135	53.5	35.5	18	162.5	11450	161.02	40.75	11.5	63
ETSC17065-2-1	4.51	2.97	82	53.5	28.5	244.5	18550	321.69	44.40	11.5	77
ETSC17300-2-1	6.305	4.8	83.5	63.5	20	235	13750	279.79	45.50	9.5	43
ETSC17044-17-2	5.005	3.09	63	39	24	178	12675	224.16	45.15	13	83
ETSC15438-4-1	5.805	4.08	82.5	58	24.5	295	14325	266.81	52.10	11	66
ETSC17109-10-2	3.545	2.84	51	41	10	196	14325	183.85	53.60	11.5	55
ETSC15363-1-2	5.32	4.195	71	56	15	212.5	13375	263.27	43.25	13.5	70
ETSC17253-9-3	4.635	3.395	60.5	44.5	16	221.5	13050	229.07	41.95	10.5	60
ETSC17023-14-1	4.81	2.955	63.5	39	24.5	189.5	13200	206.44	50.30	9.5	81
ETSC17285-14-1	3.69	2.615	51	36	15	207.5	13800	245.49	46.60	11	52
Argiti	6.175	4.43	92	66	26	236	14888	415	46.80	11	51
ETSC17295-5-3	4.55	3.18	51.5	36	15.5	188.5	11325	216.06	47.35	11	80
ETSC17201-7-3	3.93	2.97	73.5	55.5	18	220	18850	327.26	50.15	10	86
ETSC17112-13-2	4.24	3.04	69	49.5	19.5	251	16250	305	43.00	12	56
ETSC17129-12-1	5	3.56	83	59	24	247.5	16575	342.09	49.70	8	64
ETSC17023-3-1	5.275	3.795	68	50	18	216.5	12875	167.58	48.40	13	72
ETSC17297-10-1	3.29	2.275	55	38	17	194	16750	235.7	49.95	10	56
ETSC17310-2-3	4.43	2.82	67.5	43	24.5	185	15375	278.03	51.75	10.5	59
ETSC17007-9-1	4.65	2.93	66	41.5	24.5	234.5	14205	261.69	46.50	11	49
ETSC17252-19-2	2.68	2.07	51	39.5	11.5	188.5	19050	340.46	48.65	10	87
ETSC17322-4-1	5.535	3.95	59.5	42.5	17	211.5	10775	224.12	50.60	9.5	61

Appendix Table 3: Mean performance of 101 sorghum genotypes for 11quantitative traits

ETSC17004-11-3	3.13	2.17	68.5	47.5	21	206.5	21825	222.42	44.20	9	64
ETSC14252-3-2	5.87	4.1	89.5	62.5	27	275	15250	259.98	52.55	11	102
ETSC17301-5-2	5.29	3.52	73	48.5	24.5	233	13850	337.84	44.75	11.5	57
ETSC17045-12-2	2.735	1.985	47.5	34.5	13	164	17350	241.94	48.55	11	49
ETSC17296-14-1	4.17	3.005	61	43.5	17.5	230	14725	233.52	52.05	10.5	56
ETSC17276-4-2	5.07	3.755	77.5	57.5	20	227.5	15445	252.52	46.75	12.5	60
ETSC17296-5-1	2.92	2.015	48.5	33.5	15	177.5	16625	202.45	44.90	13	62
ETSC17360-5-2	3.575	2.565	59	42.5	16.5	224.5	16700	290.48	44.70	10	68
ETSC17240-8-1	6.345	4.57	75.5	54.5	21	224	11850	241.57	42.30	11.5	59
ETSC17268-7-1	3.615	2.435	70	47	23	198.5	22425	378.05	43.75	10.5	66
ETSC17295-2-1	4.64	2.98	71.5	45.5	26	178	15875	221.55	43.85	11	83
ETSC17323-21-2	4.525	2.695	68.5	41	27.5	204	15125	297.14	50.40	12	66
ETSC17129-6-1	5.465	3.94	78.5	56.5	22	231	14500	373.54	47.00	13.5	59
ETSC17140-9-1	4.8	3.4	76	54	22	271.5	15875	286.19	51.20	9.5	71
ETSC17353-27-2	2.925	2.19	57.5	43	14.5	232	21900	431.48	51.90	11	66
ETSC17360-5-1	4.155	3.275	54.5	43	11.5	233	13075	285.86	44.55	10	71
ETSC17137-4-2	4.02	2.565	74.5	47.5	27	239	18525	376.01	47.80	12.5	52
ETSC17245-3-3	3.485	2.6	66.5	49.5	17	246	19195	349.25	56.85	13.5	65
ETSC17172-4-2	3.375	2.33	62	43.5	18.5	226	19475	327.73	45.20	11.5	55
ETSC17032-8-1	5.015	3.27	56	36.5	19.5	183.5	11050	155.9	45.00	11	79
ETSC17304-7-2	6.42	4.525	81	57	24	249	12575	126.93	48.90	11	54
ETSC17194-3-1	6.75	4.87	81.5	59	22.5	253.5	12050	267.04	44.35	11	59
ETSC17277-3-1	5.255	3.825	80	58.5	21.5	298	15175	340.4	40.95	11.5	82
ETSC17198-8-1	3.065	2.18	71	50.5	20.5	230.5	23150	414.34	44.85	11	62
ETSC17268-8-1	5.26	3.965	81.5	61.5	20	248	15500	321.83	51.30	11.5	60
ETSC17155-2-1	5.18	3.585	90.5	62.5	28	311	17450	521.96	49.40	10	86
ETSC17300-4-1	6.99	5.275	79.5	60	19.5	229	11350	210.84	46.65	9.5	63
ETSC17083-6-1	2.975	2.065	65.5	45.5	20	220.5	21985	479.73	52.90	11.5	59
ETSC17349-33-1	5.19	3.935	59	44	15	185.5	11425	267.29	49.80	10.5	56

ETSC17064-6-1	4.935	3.35	76	51	25	275.5	15375	339.5	43.75	13.5	56
ETSC17272-11-1	4.83	3.055	65	40.5	24.5	198	13450	256.27	47.30	11.5	86
ETSC17272-6-1	4.735	3.015	71.5	45.5	26	204	15525	236.08	44.60	11	66
ETSC17280-3-1	3.27	2.25	64	44	20	264	19525	353.03	45.25	11.5	87
ETSC17137-4-3	4.515	3.185	76.5	54	22.5	256.5	16950	367.07	54.00	9.5	50
ETSC17195-2-1	4.78	3.325	62.5	43.5	19	206	13050	169.21	41.75	12	74
ETSC17354-9-1	4.18	2.965	51.5	36.5	15	196	12275	214.78	46.30	10	63
ETSC17272-8-1	6.3	4.11	67.5	44	23.5	200	10675	196.72	44.85	11.5	61
ETSC17131-1-1	5.43	3.885	89.5	64	25.5	271.5	16325	257.47	42.05	12.5	67
ETSC300386	4.31	3	63	44	19	221.5	14800	287.92	47.60	11.5	69
ETSC17045-7-1	3.39	2.425	55	39.5	15.5	201	16875	251.57	46.25	11.5	61
ETSC17130-2-1	6.34	4.02	77	48.5	28.5	227.5	12450	251.94	39.60	11.5	73
ETSC17276-9-1	5.3	3.625	86	60.5	25.5	273	16275	377.89	38.80	14	84
ETSC17258-5-2	6.225	4.545	83.5	61	22.5	274	13425	341.56	50.00	9	78
ETSC17072-1-1	5.475	3.52	60.5	39	21.5	173	11075	172.29	49.25	10.5	58
ETSC17112-3-2	5.01	3.52	72.5	51	21.5	235	14475	279.48	47.70	11.5	84
ETSC17272-1-1	3.175	2.095	49.5	33	16.5	171.5	15900	248.64	45.40	12	60
ETSC17328-11-1	5.625	3.79	70.5	47.5	23	211	12500	236.49	38.20	14	73
ETSC17073-6-2	4.385	3.155	51.5	37	14.5	213.5	11700	201.69	49.00	11	79
ETSC17152-1-1	5.59	3.95	90.5	64	26.5	307	16175	483.57	42.05	11.5	63
ETSC17201-1-2	5.085	3.775	85.5	63.5	22	267	16825	372.61	44.00	12	68
ETSC17258-13-1	3.78	3.065	52.5	42.5	10	234.5	13975	201.54	50.35	10.5	59
ETSC16010-2-1	4.885	3.035	62	38.5	23.5	194	12675	196.84	50.85	9.5	87
ETSC17068-6-1	4.205	2.775	66	43.5	22.5	217	15700	326.78	50.90	10	84
ETSC17269-6-1	4	2.59	52	33.5	18.5	186	12975	259.77	44.35	11	94
ETSC17135-12-1	4.335	2.955	71.5	49	22.5	263	16525	297.9	47.55	11	53
ETSC17112-10-1	3.095	2.23	66.5	48	18.5	213	23150	356.72	45.95	10.5	69
ETSC17285-5-2	5.67	4.045	75	53.5	21.5	251.5	13425	313.48	47.60	12	66.5
ETSC15312-3-1	6.455	4.795	85.5	63.5	22	235	13250	331.95	45.25	11.5	79

ETSC17172-8-1	4.665	3.06	75.5	49.5	26	262	16175	302.89	41.40	11.5	71
ETSC17269-3-1	4.58	3.37	58.5	43	15.5	250.5	12775	252.27	39.00	11	76
ETSC17295-8-1	6.185	4.055	91.5	60	31.5	252	14775	374.94	50.85	11	90
ETSC17276-5-1	2.845	2.07	46	33.5	12.5	179	16125	203.36	50.70	10	78
ETSC17300-1-2	5.4	3.755	80	55.5	24.5	267	14875	216.64	45.85	9.5	72
ETSC17361-8-3	4.195	3.14	62	46.5	15.5	261	14825	334.32	46.85	10.5	77
ETSC17037-6-1	5.185	3.325	65.5	42	23.5	236	12625	196.71	50.75	11	69
ETSC17177-2-1	4.15	3.01	64.5	47.5	17	219	16400	224.5	39.60	13.5	80
ETSC14325-4-1	4.685	3.125	70.5	47	23.5	220	15050	339.71	39.30	13	66
ETSC17122-5-1	4.685	3.17	77	52	25	264	16350	346.65	42.00	12	82
ETSC17074-10-1	4.415	2.76	61	38	23	205	13875	224.18	39.60	15.5	106
ETSC17300-1-1	3.085	2.315	67	50.5	16.5	244.5	21800	373.9	47.80	10	66
ETSC17300-9-3	4.34	3.415	61	48	13	228	14000	187.2	53.20	9.5	66
ETSC17153-6-3	6.655	4.705	90	63.5	26.5	266.5	13530	372.53	46.40	10.5	94
ETSC14020-1-1	2.93	2.05	55	38.5	16.5	202	18775	300.46	45.95	9.5	63
ETSC300354	4.94	3.855	62	48.5	13.5	245	12525	192.6	43.50	12	84
ETSC17093-6-1	5.205	3.905	83	62.5	20.5	262	16075	338.22	44.95	10	65
ETSC17172-2-3	3.035	2.195	54	39	15	228	17950	298.44	49.50	8	53
Melkam	5.195	3.52	77.5	52.5	25	256	14925	257.62	39.45	14	64

## **BIOGRAPHICAL SKETCH**

The author was born in January 1989 in Amhara, Ethiopia. She attended her elementary education at Trgi Junior school and secondary education at Gisa secondary school. Then she attended her preparatory education at Bahir Dar Tana Hike secondary and preparatory school. Then after, she joined Assosa University in 2016 and honored her bachelor degree in plant science on June 2018. After graduation she joined Ethiopian Institute of Agricultural Research (EIAR) in October 2019, and she has been working at Melkassa Agricultural Research Center (MARC) in sorghum and millet research program.