



The study of morpho-physiological characters in sorghum (*Sorghum bicolor* (L.) Moench) introgression lines under post-flowering drought stress.

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ABSTRACT

The study of morpho-physiological characters in sorghum (*Sorghum bicolor* (L.) Moench) introgression lines under post-flowering drought stress.

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Drought is a serious problem in rainfed areas due to rapid change in climatic conditions. Among prevailing a biotic stresses, it is the most significant and severe factor inhibiting plant growth and production through impairing normal growth, disturbance of water relations, reduction of water use efficiency and yield performance. The objective of this study was to evaluate morphological, physiological and yield performance of sorghum introgression lines under post flowering stress. The field experiment was conducted on seven stay-green QTLs introgression lines (marker assisted backcrossing derivatives), two stay-green donor parents and three recurrent parents obtained from Melkassa Agricultural Research Center. The experimental materials were tested in split plot design under well watered (WW) and drought stress (DS) growing conditions at Melka Werer field site during the post-rainy cropping season of 2014. The combined ANOVA revealed that effect due to moisture regimes (MR) was highly significant ($P < 0.05$) for all traits. Differences among the genotypes were also highly significant ($P < 0.05$) for all traits considered. Post-flowering drought stress reduced morphological, physiological and yield related traits relative to the well watered condition.

Drought induction reduced average leaf area, green leaf number, chlorophyll content, relative water content, CO₂ assimilation, transpiration, water use efficiency, root length, root dry weight, grain yield, hundred kernel weight and panicle weight. B35, E36-1, Meko x B35-120, Meko x B35-116, Teshale x B35-2011 and Teshale x E36-1 showed better drought stress tolerance and stay-green property. Meko x B35-120, Meko x B35-116, Teshale x B35-2011 and Teshale x E36-1 was selected for maximum grain yield under post-flowering drought condition. Correlation analysis revealed that chlorophyll content, green leaf area, assimilation rate, water use efficiency, lower rate of leaf senescence, root length, root dry weight and grain yield have been found to be morpho-physiological markers for drought tolerance and for stay-green property during post-flowering stage.

Keywords: Drought, Moisture regimes, Interogressed lines, Physiological and morphological performances, Stay-green and stress tolerance.

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LIST OF ACRONOMYS

A: Rate of photosynthesis

ATP: Adenosine tri phosphate

ANOVA: Analysis of variance

CCM: Chlorophyll content meter

DF: Days of Emergency

DAF: Days after flowering

DAP: Diammonium phosphate

DTM: Days to maturity

DS: Drought stress

DW: Dry weight

E: Rate of transpiration

EIAR: Ethiopian Institute of Agricultural Research

FW: Fresh weight

GLN: Green leaf number

GLA: Green leaf area

GY: Grain yield

HGT: Hundred grain weight

ICRISAT: International Crop Research Institute for Semi- Arid Tropics

MR: Moisture regimes

N: Nitrogen

NG: Number of green leaves

NSL: Number of senesced leaves

PAR: Photosynthetic active radiation

PH: Plant height

PWT: Panicle weight

RD: Root dry weight

RL: Root length

Rubisco: Ribulose - 1, 5 - bisphosphate carboxylase/oxygenase

RuBP: Ribulose bisphosphate

RWC: Relative water content

SE: Standard error

SG: Stay green

SGS: Stay green score

TW: Turgid weight

WUE: Water use efficiency

CHAPTER ONE

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal crops that belongs to the grass family, it predominantly grows in arid and semi-arid parts of the world (Techale Birhan *et al.*, 2014). The crop evolved in semi-arid tropical part of Africa where it is still used as a major food grain (Kapanigowda *et al.*, 2013; Firew Mekbib, 2007). Globally, it is the fifth major cereal crop in terms of production after maize (*Zea mays* L. ssp. *mays*), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and barely (*Hordeum vulgare* L.) and in Ethiopia it is the fourth staple crop both in cultivated area and in total grain production among the major five cereal crops produced, preceded by teff (*Eragrostis tef* (Zucc.) Trott.), maize, wheat and followed by barley (Asfaw Adugna, 2007).

Sorghum is cultivated as a major crop in areas with poor soil nutrient content, erratic rainfall and other harsh environmental conditions (Bibi *et al.*, 2012; Techale Birhan *et al.*, 2014). It is also considered as the most tolerant crop to many stresses, including heat, drought, salinity and flooding as compared to other cereal crops (Bibi *et al.*, 2012). It is used as a representative crop for studying physiological and genetic mechanisms of drought tolerance of crops, Sub-Saharan Africa region area (Kapanigowda *et al.*, 2013). It is recognized as drought tolerant crop typically grown under rainfed conditions in regions where water is the main limiting factor for yield reduction (Xoconostle-Cazares *et al.*, 2011).

World widely drought stress is considered as a major problem for crops productivity, it is a complex phenomenon which is controlled by many genes, and its effect depending up on timing, duration and severity drought (Sakhi *et al.*, 2014). Throughout the world moisture deficit causes

a wide effect on crops starting from germination to grain filling by disturbing biochemical, physiological and morphological processes of crops (Kumar *et al.*, 2011).

Pre-flowering and post-flowering stages has been identified as responses of sorghum to drought. Pre-flowering drought stress responses expressed when drought stress occur during panicle differentiation prior to flowering; with symptoms that includes delayed flowering; poor panicle exertion; panicle blasting and floret abortion; and reduced panicle size (Malala, 2010). Since, the panicle is directly affected, severe pre-flowering stress can result in drastic reductions in grain yield (Kumar *et al.*, 2011). Post-flowering drought stress occurs during the grain filling stage with symptoms that includes pre-mature plant (leaf and stem) death or plant senescence, stalk collapse and lodging, and charcoal rot, along with a significant reduction in seed size, particularly at the base of the panicle (Kumar *et al.* , 2011). Stay green trait is considered as post flowering drought tolerance which enables plants to remain green and fill grain normally. Such green stalks also have good resistance to stalk lodging and to charcoal rot (Borrell *et al.*, 2001).

International crops research institute for the semi-arid tropics (ICRISAT) has been the primary and the most important source of sorghum germplasm introduction for Ethiopia, in terms of introduction of genotypes with drought tolerance mechanisms (Asfaw Adugna, 2007). In Ethiopia researchers have been engaged in caring out researches that conforms the stay green trait drought tolerance through investigations of which mainly focused on morphological characteristics (Techale Birhan *et al.*, 2014; Asfaw Adugna, 2007). For instance Asfaw Adugna and Alemu Tirfessa, 2014 has reported the presence of variation in SG property among eight sorghum introgression lines evaluated. Since the genotypes were evaluated using few morphological parameters, therefore there is a need to use physiological parameters to analyze drought tolerance in SG traits. Moreover, correlating various morpho-physiological and yield

parameters would give more reliable and used as a selection criteria for further breeding program (Yared Assefa *et al.*, 2010).

Evaluating introgression lines with SG trait would play a role in crop improvement programs due to the fact that introgression lines have stay green trait which is inherited from donor stay green parents. Despite the fact that, researches has been conducted for the evaluation of SG traits there is still a gap in studying new released introgression lines with morpho-physiological and yield related traits.

In the present field experiment, responses of sorghum genotypes to post flowering drought were evaluated in terms of morphological, physiological, root and yield parameters.

Furthermore, research conducted for SG genotype with in root traits were few. Therefore, it is crucial to screen sorghum genotypes for SG trait under field condition.

1.1. OBJECTIVES

1. 1.1.General objective

The general objective of this study is to evaluate the morphological, physiological and yield performance of sorghum introgression lines under post flowering drought stress

1.1.2. Specific objectives

- To evaluate variations among stay-green sorghum introgression lines and their parents for morpho-physiological traits under post-flowering drought stress.
- To measure the gas exchange parameters of sorghum genotypes under post flowering drought stress.
- To quantify the chlorophyll content of sorghum genotypes.
- To compare the performance of sorghum introgression lines among themselves and with their parents.
- To identify genotypes that could be used in areas prone to post flowering drought stress.
- To evaluate morphological and physiological adaptive traits of sorghum genotypes.

CHAPTER TWO

1. LITRETURE REVIEW

1.1. Origin, distribution, Botanical description and Ecological adaptation of *S. bicolor*

Sorghum is originated in the northeastern quadrant of Africa, where the greatest variability in wild and cultivated species is found, the area is characterized by erratic and unpredictable rain fall (Walker, 1999; Malala, 2010; Chikuta, 2010; Pray and Nagarajan, 2009). It is an indigenous crop to Ethiopia, beginning from 4000-3000B.C. It is domesticated to the surrounding countries as Ethiopia is origins of its domestication (Getahun Yemata *et al.*, 2014).

Sorghum belongs to the family Poaceae, tribe Andopogoneae, Sub-tribe Sorghinae, Genus Sorghum Moench (Parasad and Staggenborg, 2010). All cultivated sorghum belongs to *Sorghum bicolor* subsp. *bicolor*. Morphological characteristics of sorghum depend on growing environment and sorghum varieties. By nature sorghum is perennial crop however, depending on the purpose of the end product harvest season, grain sorghum is an annual crop and for forage it is perennial (Parasad and Staggenborg, 2010).

In Africa Ethiopia is second largest producer of sorghum, after the Sudan. Sorghum cultivation area in Ethiopia is mainly domain in eastern and northwest part of the country, where the area is characterized with dry and low soil fertility (Demeke Mekonnen and Di –Marcantonio, 2013; Tekle Yoseph and Zemach Sorsa, 2014). It is used for making *injera* (the traditional bread, made from *teff* in more productive areas of the country), feed and for local beer production, with the remainder held for seed. It is used as drought tolerant crops in areas which are affected by adverse climate condition (Demeke Mekonnen and Di –Marcantonio, 2013). In comparison

with world's production Ethiopia's sorghum production has lower rate, this is attributed by biotic and edaphic (Tekle Yoseph and Zemach Sorsa, 2014).

S. bicolor is an erect plant with a solid stem, sweet, dry or juicy and grooved the length ranged from 0.8m-5m (Chikuta, 2010). Under favorable conditions the stem gets longer and more internodes which are covered with a thick waxy layer, which gives its blue color (Taylor, 2003; Parasad and Staggenborg, 2010). The leaf has a prominent mid-rib; typical leaf blades are on average 8-12 cm wide and 50-90 cm long (Parasad and Staggenborg, 2010), which are arranged in alternate of two ranks and the leaf sheaths are 15-55 cm long and encircle the stem (Chikuta, 2010). Depending upon the environmental conditions the number of leaves differs from 8 to 22 per plant (Parasad and Staggenborg, 2010). The leaf sheath and stem are characterized by a waxy bloom. Tillering depends upon seed source (variety) and environmental condition (Parasad and staggenborg, 2010). Inflorescence of *S. bicoloris* is loose to dense panicle, usually erect and has many primary branches bearing spikelet (Chikuta, 2010; Parasad and staggenborg, 2010).

Panicle shape and the color of panicle differ depending on the race, kernel numbers range from 800-3000 per panicle, the seeds are roundish, ovoid to flat, and can be white, pink, red, yellow or brownish, it consists of testa, embryo and endosperm (Human *et al.* , 2006). Mature seeds have a black spot near the base (Chikuta, 2010). Furthermore, the grain has tannin in the seed coat testa and the pericarp which gives bitter taste and protect the seed from predation of insect and fungal attack (Taylor, 2003).

Root system of *S. bicolor* is divided into two; primary root which grows with seedling emergence, provide water and nutrient these functions will be replaced by secondary root which develop later after the growing of the crop and the growth of the primary root is limited while the secondary root system develops up to 1 m laterally and downward up to 2m (Parasad and Staggenborg,

2010). Sorghum grows in a wide range of agro-ecologies most importantly in the moisture stressed condition where other crops can least survive (Asfaw Adugna, 2007). Moreover, it grows in low fertility, moderately acidic and highly alkaline soils, but it is best adapted to fertile, well drained soils at a pH between 6.0–6.5, It is also known to have wide adaptability, ranging from lowland, medium and highland altitude (Dial *et al.* , 2012).

1.2. Importance of Sorghum

Sorghum is an exceptional crop among the foremost crops which are used as a source of food source, mostly because of its adaptability to different harsh environment (Techale Birhan *et al.*, 2014). Nutritional uses of sorghum are widely described in different scientific papers based on *in vivo* and *in vitro* experiments (Wilhelm *et al.*, 2004; Leder, 2004). Starch takes high percent from carbohydrate; the insoluble dietary fiber of sorghum is used as a source of fiber which prevents gastrointestinal problems (Leder, 2004). The composition of protein content varies due to genotypes and water availability, temperature, soil fertility and grain development environmental conditions (Leder, 2004). Crude fat content of sorghum averages about 3%, which is higher than that of wheat and rice (Henley, 2010). Sorghum grain also contains vitamins which is important to human physiology apart from maize and durum wheat (Pray and Nagarajan, 2009). Moreover, sorghum is considered as good source minerals (Pray and Nagarajan, 2009) specifically it is a source of iron and zinc, which plays a major role for energy metabolism (Henley, 2010).

In Asia and Africa, sorghum is used for human consumption and for animal feed (Taylor, 2003). In recent years it is used as source for bioethanol production specially with sweet sorghum varieties which have high sugar content on their stalk (Parasad and Staggenborg, 2010). Sorghum is the fourth most crop in terms of production level for bio-fuel production after corn (*Zea mays*

L), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) (Wilhelm *et al.*, 2004). Using sorghum as a source of bio-fuel will decrease the cost of enzymatic conversion of starch to sugar because sorghum cultivars possess readily available fermentable sugars within the culm (Wilhelm *et al.*, 2004; Taylor *et al.*, 2003). In comparison to other crops which are used for bioethanol production like sugarcane and maize, sorghum is tolerant to drought and salt stress these makes sorghum preferable to bio-fuel production in industries (Taylor, 2003). Besides it is the only plant that all parts of the plant used including grain, stalk and leaves (Almodares and Sharif *et al.*, 2007).

2.3. Utilization

Sorghum grain of the century in Africa (Taylor, 2003), it is a multipurpose crop grown for food, animal feed and industrial purposes (Bibi *et al.*, 2012). Most of the parts except root are used including grain, stalks and leaves (Henley, 2010). This is processed in different forms, with a wide variety of traditional foods, such as semi-leavened bread, dumpling and fermented and non-fermented porridges gruel (Dahlberg *et al.*, 2011). In Africa it is an important part of diet in the form of unleavened bread, boiled porridge or gruel (Dahlberg *et al.*, 2011). In Ethiopia a staple food which it is used for fermented foods and beverages, *Injera* a large circular, fermented pancake-like bread. Industrially used for beverage production such as beer production (Taylor, 2003; Getahun Yemata *et al.*, 2014).

1.3. Drought

Under natural and agricultural conditions plants are often exposed to various environmental stresses, biotic and abiotic stress factors are major constraints which seriously reduce productivity of crops (Rejeb *et al.*, 2014; Bibi *et al.*, 2012). In response to these plants developed different adaptation mechanisms which are considered as an advantage for critical period of time

(Rejeb, *et al.*, 2014, Zlatev and Lidon, 2012). Stress is any environmental factor that affects plant physiology, morphology and biochemical processes (Rejeb *et al.*, 2014). It also decreases the potential growth and development of plants by damaging plant system from cellular to system level (Mahajan and Tuteja, 2005; Zlatev and Lidon, 2012). Drought stress (water deficit) can be defined as a situation in which plant water potential and turgor are reduced enough to interfere with normal functions, tissue or cell water content is below the highest water content exhibited at the most hydrated state (Taiz and Zeiger, 2006; Oliveira *et al.*, 2013). The global agricultural land area is rainfed and adversely affected by drought. Due to this fact, drought reduces crop production and risks the well being of livelihood as well as food security (Dial, 2012). The effect of drought depends up on timing of occurrence during season, duration and intensity of the drought (Majid *et al.* , 2007).

Water deficit stress disturbs the physiology of plants by reduction of water content, turgor loss, decrease total water potential, wilting, closure of stomata, decrease cell enlargement and growth (Taiz and Zeiger, 2006). Plants respond to water deficits by closure of stomata, limitation of gas exchange, disruption of metabolism, destruction of cell structure, decreasing of photosynthesis activity, disturbance of metabolism, and finally death (Cramer *et al.*, 2003; Oliveira *et al.*,2013).

Generally, research shows that sorghum has a potential to alleviate chronic drought and food insecurity in drought prone areas of the world due to the fact that sorghum is the most drought tolerant crop which grows under small moisture content (Taylor *et al.*, 2003). In comparison with maize and wheat, sorghum is well adapted to semiarid and sub-tropical agronomic conditions of Africa these is because it is originated in these area and these adaptation assures food security in these area (Taylor, 2003).

Despite one third of world's sorghum production is produced by Africa which is 20 million tons per annum; environmental stress such as drought is a major constraint for crop production in this region (Taylor, 2003). From all of environmental factors that reduce crop production receding of soil moisture is considered as the major problem which further affects crops production in terms of impediment or avoidance of crop establishment with distortion of seed germination, destruction of established crops, disturbance with insect and crop disease, changing in physiological processes with decreasing of photosynthetic ability of the plants and osmotic behavior of cells, morphological and biochemical process of economically important crops (Ulemale *et al.*, 2013). Furthermore, when crops are exposed to frequent and prolonged drought, production and yield capacity of crops decreases as a result of reduction of decreasing in diffusion rate of nutrients from soil to root and restriction of transpiration rates by closure stomatal (Tilahun Amede and Schubert, 2003). Moreover, these impair active transport and membrane permeability (Taiz and Zeiger, 2006). Although sorghum has developed root system mechanism under drought stress condition, the yield under dry land conditions is much less than irrigated condition (Kamara *et al.*, 2014). This shows that drought tolerance in sorghum is not absolute (Yared Assefa *et al.*, 2010). Moreover, moisture deficit at the reproductive stage causes the principal decline in yield as compared to stress happening at any other growth stages (Ali *et al.*, 2011). In monocarpic cereal crops grain filling and leaf assimilation are usually antagonistic to each other; remobilization from the vegetative organ especially from leaf blades to the developing grain, causing leaf senescence and decrease photosynthesis after flowering (Jin-Dong *et al.*, 2009).

The severity and effects of drought is increasing with increasing world population and climate change, to alleviate these problems and to maintain stable crop production (kamran *et al.*, 2014).

Improvement of genotypes which are tolerant to such environmental condition is ideal way for quality production of crops. Consequently, improved genotypes with better performance under drought prone areas are an ultimate solution for those of farmers who are facing drought for major yield reduction (Ulemale *et al.*, 2013). Moreover, for evaluation of newly developed drought tolerant varieties physiological and morphological approaches are a useful technique for understand the response of crops (kamran *et al.*, 2014).

1.4.The Effect of drought on growth and development of plants

Under prolonged drought stress plants are affected at various levels of organization, from cell to organ level due to this many plants suffer from dehydration of cells and tissues which is followed by death (Seyed *et al.*, 2012). According to Farooq *et al.* (2009) growth is an irreversible increase in volume, size, or weight, which comprises the phases of cell division, cell elongation, and differentiation. Lack of soil moisture restricts plant growth, both in terms of the total quantity of tissue produced and the time that the plant tissue is produced, cell division and cell growth are the two primary processes involved in plant growth (Farooq *et al.*, 2009).

Leaf expansion and root elongation are the most water deficit sensitive turgor-dependent activities as a result of cell and cell wall shrinking (Taiz and Zeiger, 2006). Moreover, water deficit results in decreasing leaf expansion and leaf area which results from biophysical effect of turgor pressure (Taiz and Zeiger, 2006; Rostampour, *et al.*, 2012). Continued drought stress can accelerate leaf senescence and lead to death of leaf tissue, resulting in leaf drop, particularly old and mature leaves (Prasad *et al.*, 2008). In addition water stress affects almost every developmental stage of the plant (Bibi *et al.*, 2012). Decreasing leaf area is an advantage for conserving water due to transpiration, these is used as drought tolerance mechanisms (Asafw Adugna and Alemu Tirfessa, 2014). However, reductions of leaf area expansion decrease the

photosynthetic area besides plants grown under drought condition have lower stomatal conductance in order to conserve water. Consequently, CO₂ fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants (Mafakheri *et al.*, 2010). Furthermore, drought decreasing branches growth rate and stem growth (Taiz and Zeiger, 2006).

Depending on the time and intensity of water stress, cereal crops experience different types of drought (Jordan, 2009). Sorghum crop experiences drought stress at two distinct phases; during pre-flowering stress (panicle development stage) which is prior to flowering and between anthesis and grain development (post flowering stress) (Nagarjuna, 2007, Asfaw Adugna and Alemu Tirfessa, 2014). In sorghum cultivars it has been observed that flowering and grain-filling stages are the most critical stages of growth (Malala, 2010; Shamsi *et al.*, 2010).

Drought affects germination, growth of seedling and germination time (Khodarahmpour, 2011). When it occurs at post-flowering stage it leads to failure to fertilization by impairing the function of pollen and ovule these decrease the number of grain produced. Drought stress affects germination of corn (*Zea mays* L.) hybrids, some of the varieties shows decrease in germination percent (Khodarahmpour, 2011).

1.5.The effect of drought on physiology of plants

1.5.1. Relative water content

Drought decreases relative water content and water potential (Kamran *et al.*, 2014). Both under pre and post-flowering dehydration relative water content decreases in accordance with Abuhay Takele and Farrant (2013). Measuring relative water content is useful indicator of plant water balance and water potential parameter under drought stress conditions (Kamran *et al.*, 2014). Since, it conveys the relative amount of water present on the plant tissues (Yamasaki and

Dillenburg, 1999). Under water deficit, cell membrane subjects to unnecessary changes such as cleavage in the membrane and sedimentation of cytoplasm content and in this condition the ability to osmotic adjustment is reduced (Arjenaki *et al.*, 2012 ; Khodadadi 2013 ; Talebi *et al.*, 2013).

Different studies have shown that there is a significant difference among genotypes for relative water content when grown under drought stress condition (Hasheminasab *et al.*, 2012; Abdullah *et al.*, 2011). According to Abdullah *et al.* 2011 drought susceptible cultivars of durum wheat relative water content decreased in response to drought. Highest relative water content was observed at vegetative stage. While, it decreased gradually at the later stage. On the other hand the highest value of relative water content observed in drought tolerant varieties at various growth stages this is due to the ability of the drought tolerant varieties to absorb more water from the soil, maintaining higher concentration of osmolyte, lowering the damage of cell membrane through increasing the sustainability of cell membrane and the ability to control water loss through stomata (Arjenaki *et al.*, 2012; Abdullah *et al.*, 2011; Hasheminasab *et al.*, 2012).

In developing breeding programs to improve drought resistance crops it is necessary to study the physiological mechanisms for quantifying plant water stress response. According to Talebi *et al.* (2013) in chickpea genotypes relative water content is proposed and used as the best screening method for selection of drought tolerant genotypes as there is a significant difference between drought tolerant and susceptible in response to relative water content. Even though, there were other physiological parameters has been used in the study using relative water content is indicated as the reliable, relatively inexpensive, rapid and the most securing method which is used for screening of drought tolerant genotypes (Talebi *et al.*, 2013).

1.5.2. Gas exchange and water use efficiency

As the time, duration and intensity of drought increases photosynthesis and transpiration decreases these is as a result of decrease of stomatal conductance (Farooq *et al.*, 2009). Plants have different characteristics in response to stress factors, in sorghum post-flowering drought stress reduces photosynthesis, due to stomatal and non stomatal limitations (Taiz and Zeiger, 2006; Mafakheri *et al.*, 2010) rapid closure of stomata avoid further transpiration under drought condition result in restriction of CO₂ diffusion into the leaf (Grzesiak *et al.*, 2006). Which is used as drought avoidance mechanism for reduction of water loss (Abuhay Takele and Farrant, 2013). Non-stomatal feature of drought for limiting photosynthesis has been attributed by reduced carboxylation efficiency, reduced ribulose-1,5-biphosphet (RuBp) regeneration, reduced amount of functional rubisco or inhibited functional activity of photosystem II, inhibition or damages in the primary photochemical, and biochemical processes (Zlatev and Yordanov , 2005; Farooq *et al.*, 2009) and change in chlorophyll synthesis and interruption of processing and distribution of assimilates (Grzesiak *et al.*, 2006).

Water use efficiency is the amount of biomass production per unit of water which is consumed through evapotranspiration thus plant with high transpiration rate produce high amount of biomass and increased grain yield production (Thevar *et al.*, 2010). High root biomass contributes for high absorption efficiency, thus these affords the cost of transpiration and increase water use efficiency and transpiration (Thevar *et al.*, 2010). Effective water use also includes filling the transpiration water demand, with minimizing non-stomatal water loss and minimizing soil evaporation (Blum, 2009).

According to Songsri *et al.* (2009) drought tolerant pea nut genotypes had higher dry weight and longer root system which contributes to higher water use efficiency. The input of root trait for high water use efficiency and higher photosynthesis is a useful tool to study how genotypes cope up with drought stress environment (Songsri *et al.*, 2009).

As the biochemistry of photosynthesis cannot be improved genetically, genotypes with high transpiration can fill this gap (Blum, 2009). High transpiration rate is positively correlated with high yield production. In view of the fact that, transpiration contributes for high yield production breeding programs with releasing of genotypes with high transpiration efficiency will contribute for greater yield production under water deficit environments (Blum, 2009).

Depending on the crop variety genotypes are well adapted to drought prone areas through biochemical, physiological and morphological adaptation (Addise Yalew, 2010). Moreover, the adaptation of mechanisms of sorghum to arid environments such as delay in leaf rolling, partial opening of stomata, higher relative water content and higher water use efficiency contributes for tolerance of moderate drought in pre-flowering and post-flowering (Abuhay Takele and Farrant, 2013). According to Kapanigowda *et al.* (2013) in sorghum genotypes which were subjected to drought stress had genetic variation for CO₂ assimilation, in the study it has been suggested that for selecting drought tolerant genotype assimilation efficiency can be used as a criteria (Kapanigowda *et al.*, 2013).

2.5.3 . Chlorophyll content (greenness)

Chlorophyll is light absorbing green pigment; leaf greenness depends on the concentration of chlorophyll (Soo-Cheul *et al.*, 2007). Chlorophyll concentration or leaf greenness is affected by nitrogen status of the plant and external environmental factors (Borrell *et al.*, 2001). Exposure to drought stress leads to a significant decrease in Chlorophyll *a* and Chlorophyll *b* contents (Talebi *et al.*, 2013; Keyvan, 2010). According to Mafakheri *et al.* (2010) pea nut genotypes under pre flowering and post flowering drought, chlorophyll *a*, chlorophyll *b* and total chlorophyll content decreases as a consequence light absorption capacity of a leaf decreases; thus, it reduce photosynthetic capacity (Mafakheri *et al.*, 2010).

In different studies determination of chlorophyll content is used as a screening tool for selection of drought tolerant genotypes (Malala, 2010; Borrell *et al.*, 2000b; Borrell *et al.* 1999). In relation to this Farshadfar *et al.* (2013) shows that drought tolerant lines of wheat have higher chlorophyll content under drought condition the same results has also been found by Arunyanark *et al.* (2008), pea nut and barely drought tolerant genotypes had stable chlorophyll content both under mild and severe drought therefore, therefore, chlorophyll content measurement can be used as a selection criteria for breeding programs (Arunyanark *et al.*, 2008; Rong-hua *et al.*, 2006).

1.6.The Effect of drought on root character

Roots are an important part of plant for extracting water, minerals and organic nutrients (Atta *et al.*, 2013). Drought decreases root length, fresh weight and dry weight (Bibi *et al.*, 2012; Songsri *et al.*, 2009; Techale Birhan *et al.*, 2014). In drought tolerant genotypes root traits are positively correlated with grain yield these is because of high water extracting ability of a plant leads to more photosynthetic activity, thus photo assimilate and partitioning becomes higher and also grain yield, in wheat drought tolerant genotypes about 45 % of yield production were given up

by root trait (Atta *et al.*, 2013). Furthermore, these traits which were brought from the recurrent parents were heritable to the new lines (Atta *et al.*, 2013). For stable crop production in arid and semi-arid environments crops that have high water use efficiency contributes for higher production, these can be enhanced through high water extraction with larger root system which assures that confronting of drought impacts in global food production (Atta *et al.*, 2013) these has been observed in peanut genotypes with higher root dry weight contributes for higher water use efficiency (Songsri *et al.*, 2009) these were observed for drought tolerant genotypes. On the other hand drought significantly reduce root length, and root dry weight for drought susceptible genotypes (Songsri *et al.*, 2009). Additionally, ABA (Abscisic acid) is synthesized in the root and transported to leaf for closure of stomata, which make sure that conservation of water (Bibi *et al.*, 2012; Legesse Negash, 2010).

According to Bibi *et al.* (2012) root length is positively correlated with drought resistance, drought tolerant sorghum genotypes had longer root than sensitive genotypes it contributes about 56.6% for drought tolerance (Bibi *et al.*, 2012). Thus, this root trait can be used as selection criteria for drought tolerant genotypes (Bibi *et al.*, 2012; Songsri *et al.*, 2009; Ulmale *et al.*, 2013).

1.7. Effects of drought on yield and yield components

The basic aim of all research activities in agriculture and crop sciences is to increase grain yield of crops under environmental constraints (Majid *et al.*, 2007; Sharafizad *et al.*, 2013). Post-flowering drought stress significantly decreases grain yield as compared to pre-flowering drought stress due to failure in pollen grain fertility and improper grain filling (Shamsi *et al.*, 2010).

In sorghum cultivars biological yield, 100-grain weight and grain yield showed significant difference between genotypes in irrigated and non-irrigated condition (Vinodhana and Ganesamurthy, 2010; Shamsi *et al.*, 2010; Farshadfar *et al.*, 2013). Grain yield had the highest decrease percent of traits under drought stress condition that it was due to reduction in biological yield, number of seeds under drought stress (Malala, 2010). In sorghum genotypes 100-grain weight reduced by drought stress these were due to decrease in the assimilation rate and lower photo assimilate translocation to physiological sinks and shortening the grain-filling period (Malala, 2010). The reason for this reaction is decrease in competition for gaining photosynthetic substances, where as exerting drought stress at grain filling stage reduces the capacity of transferring photosynthetic substances to grain meaningfully and decreases the weight of 100 grains (Hossain *et al.*., 2010; Zare *et al.*, 2011; Sharafizad *et al.*, 2013; Blum, 2009). According to Rostampour *et al.* (2012) drought affect both relative water content and chlorophyll content as a result dry matter yield is negatively affected these is also correlated with stomatal conductivity and accessibility of plant to carbon dioxide as a consequence dry matter production decreases. Besides the other factors, leaf area during grain filling is considered to be the most important character for high yielding under drought stress condition while low yielding cultivars of grain sorghum have smaller leaf area at flowering and at early grain filling stages (Nagarjuna, 2007; Vinodhana and Ganesamurthy, 2010). In a condition of most favorable water availability, plants fill their grains using a combination of current photosynthesis and translocation of carbohydrates from other parts of the plant of source (Jordan, 2009). However, drought stress occurrence during post-flowering stage (during grain filling) the amount of photosynthesis is reduced in response to low water availability as a result of lower supply of water to the demand of photosynthetic activity (Kapanigowda *et al.*, 2012). The plant responds to this reduction in

photosynthetic capacity by increasing the amount of stem reserves translocated from other parts of the plant such as the stem, roots and leaves. If the assimilate deficit is sufficiently large, the translocation of carbon and nitrogen from leaves will cause leaf senescence. In severe cases, the lack of assimilate to fill the developing grain results in reduced grain size and, ultimately reduced grain yield (Jordan, 2009).

1.8. Drought resistance mechanisms

In both natural and agricultural conditions, plants are frequently exposed to environmental stresses (Taiz and zeiger, 2006). Drought resistance refers to the degree to which a plant is adapted to dry conditions; drought tolerant plant species and genotypes can be determined by different morphological and physiological traits (Keresá *et al.*, 2008; Shattuck and Bradford, 2010). There are a number of drought resistance mechanisms which enables plants to adapt to extreme drought conditions. Thus, the ability of crops to grow under severe water deficits as well as maintaining yield under the water limited environment would be valuable traits to increase crop production (Shattuck and Bradford, 2010).

1.8.1. Drought tolerance

In these mechanism plants develop ability to maintain tissue hydration and ability to function at low water potential (Taiz and Zieger, 2006). Drought tolerance also includes exhibiting metabolic protection induced or constitutive against the damage effect of dehydration and co-developing oxidative stress (Chaves *et al.*, 2003).

It also involves osmotic adjustment and more rigid cell walls (Chaves *et al.*, 2003). Many of the evergreen shrubs and trees in arid or semi-arid regions maintain high solute concentration in their tissue (Chaves *et al.*, 2003). In some legume crop plants partial plant dormancy to survive the dry season is another tolerance strategy by repression of genes encoding photosynthetic proteins

(Chaves *et al.*, 2003). According to Tilahun Amede and Schubert (2003), osmotic adjustment and maintenance of high leaf water potential were used as drought tolerance mechanism, in Common bean and chick pea respectively. Maintenance of high leaf water potential in common bean was as a result of longer root length, higher root dry weight and higher osmotic adjustment (Tilahun Amede and Schubert, 2003).

1.8.2. Drought escape

Plants which grow under residual soil moisture are subjected to accelerated early growth, because of depletion of soil moisture and too little soil moisture for the completion of plants life cycle, under these condition only plants that have some available water for reproduction will produce seeds for the next generation (Taiz and Zeiger, 2006). These could be determined by elasticity which is the ability of a plant being able to complete its life cycle before the occurrence of drought period (Chaves *et al.*, 2003) and preservation for the upcoming generation by maintaining a fruit with better partitioning of assimilates (Chaves *et al.*, 2003; Farooq *et al.*, 2009). However, more time the crop takes for completing life cycle under normal condition is the more yield production, thus shortening the period of that crop life cycle will cost yield reduction (Farooq *et al.*, 2009).

1.8.3. Drought avoidance

Dehydration avoidance is common to both annuals and perennials and is associated with a variety of adaptive trait. These involve minimizing water loss and maximizing water uptake (Chaves *et al.*, 2003). Plants can also avoid drought conditions by avoiding tissue dehydration, while, maintaining tissue water potential as high as possible, or by tolerating low tissue water potential. Stomatal closure used as a mechanism for drought avoidance and retain higher relatively higher water status (Tilahun Amede and Schubert, 2003).

Plants which use drought avoidance as a mechanism of drought resistance maintains high water potential under drought stress condition. These have been shown in cowpeas [*Vigna unguiculata* (L.) Walp.] Moreover, drought avoidance has been maintained by low transpiration rates by reduction of leaf area and reduced transpiration per unit of leaf area (Hall and Schulze, 1980). Drought tolerant genotypes can avoid drought in different ways; by closing stomata that prevents excessive transpiration, but also reduces photosynthesis (mechanism of drought avoidance), or by enhanced accumulation of different osmolytes that maintain plant turgor and enable normal metabolism in water stress conditions (mechanism of drought tolerance) (Keresa *et al.*, 2008).

1.9.Stay green trait

Chlorophyll is the most important pigment which absorbs sunlight and gives greenness to plants. Under natural and environmental constraints the greenness of leaves decrease due to progressive breakdown of chlorophyll (Borrell and Hammer, 2000). Leaf pigments are a complex of proteins and other organic compounds, during leaf senescence synthesis of amino acid come to an end and accessible proteins becomes degraded without being replaced, these results in chlorophyll degradation and yellowing characteristics of leaves (Campanile *et al.*, 2000).

Stay-green is defined as maintenance of green leaf for extended period (delayed leaf senescence) (Borrell, 2010; Borrell, 1999; Campanile *et al.*, 2000). Carbohydrates and nitrogen is assimilated by vegetative organ and used as a source for the supply of assimilates to the sink reproductive organs due to stay green genotypes had character for larger pool of nitrogen in leaves and delayed remobilization of nitrogen from the leaves, sufficient supply of assimilates from the source increase grain yield (Kassahun Bantte *et al.*, 2010; Borrell and Hammer, 2000).

At the final stage of leaf development stay-greenness (delayed senescence) is an important trait which contributes for increased source strength and grain production (Soo-Cheul *et al.*, 2007). According to Borrell *et al.* (1999) stay green trait contributes to grain yield which is determined by grain size and number. Thus, grain size is positively correlated with leaf greenness, during grain filling period by which reducing rate of leaf senescence from 3% to 1% loss of leaf area per day resulted in doubling of grain size from about 15 mg to 30 mg. Green leaf area, increasing water use efficiency, leaf nitrogen and stem competition for assimilate increase yield additionally grain number and grain size determines increasing of yield, thus stay green trait contributes by increasing yield by controlling those traits under drought stress condition (Borrell *et al.*,1999). Moreover, recently Hammer (2006), proposed the idea of water use management positively correlated with stay green trait. It is undeniable that crops which are under terminal drought need to access water resource to compensate the demand for photosynthesis and to increase crop yield (Hammer, 2006). Beside good quality of forage and higher yield production stay-green character confers resistance of crops to charcoal rot and stalk lodging (Borrell *et al.*, 2000a).

Stay green trait expressed in two ways functional and non-functional, the first one is characterized by delays of leaf yellowing, maintaining photosynthetic competence, while in non functional stay-green leaf greenness is maintained without sustaining photosynthetic activity (Soo-Cheul *et al.*, 2007). Thus, functional stay green increase yield by maintaining photosynthetic activity and good seed setting rate (Soo-Cheul *et al.*, 2007) it has also been a target trait in breeding programs for increasing crop production under post-flowering drought, due to the fact that they accumulate more soluble sugar than the senescent type. Stay-green

expression could be affected by the severity of drought during grain filling and the amount of nitrogen in the leaves (Kassahun Bantte *et al.*, 2010).

Despite of stay-green phenotype is superficially similar in all species and genotypes, genetically and physiologically they are diverse according to these difference stay-green classified in to five types, In type A stay-green, senescence is initiated late but proceeds at a normal rate; type B stay-green initiates senescence on schedule, but there after senescence comparatively progress slowly; In type C stay-green behavior, chlorophyll may retained more or less indefinitely pigment retention keeps in the outer appearance of the leaf however, physiological photosynthetic activity is below the artificial greenness shows the decline of greenness, type D shows rapid tissue death but stay-green confers by killing the leaf through drying or freezing ; In type E the photosynthesis capacity of an intensely green genotype follows the normal ontogenic pattern but comparison of absolute pigment content identifies it as a stay-green, type A and B are more functional stay-green types (Thomas and Howarth, 2000).

Although the underlying physiological mechanisms of the expression of a stay-green phenotype is not explained, the trait has been described as the best trait which enables crops to resist post flowering drought and increase crop production under such harsh environment. There are proposed explanation which have made by scientists for the expression of stay-green as a role of nitrogen (N) status of the plants, either balancing between demand and supply of nitrogen, more N to be taken up at post-anthesis (Borrell and Hammer, 2000). Furthermore, it is explained by Borrell and Hammer (2000), in sorghum stay-green hybrids produce 47% more post-anthesis biomass than senescent varieties under post-flowering drought. To pin point the characteristics of stay green trait it could be as a result of nitrogen balance between demand of grain and nitrogen supply during grain filling period. In comparison with senescent type stay green hybrids there is

higher allocation of nitrogen to the leaves which enhance the plant yield production and lodging resistance under terminal drought these is as consequence of increasing in specific leaf nitrogen, radiation use efficiency and transpiration efficiency (Borrell , 2010).

1.10. Determination of stay-green trait by Molecular marker-based QTL (Quantitative trait loci)

For centuries it was difficult to locate molecular map of genes which gives a special character to crops. In most of agronomical cases crops have multifarious inheritance which is controlled by many genes with different location in a given allele (Kassahun Bantte *et al.*, 2010). The complex trait of crops is associate with genetic loci which are called quantitative trait loci (QTLs) (Moull, 2004; Soo-Cheul *et al.*, 2007; Kassahun Bantte *et al.*, 2010)

Improvement in molecular marker technologies and the application for identifying QTL is a powerful approach for focusing on the function of specific loci which are responsible for a particular trait. Molecular marker assisted of QTL determination contributes a better understanding of drought tolerance trait inheritance in sorghum (Moull, 2004).

Introduction of new trait into breeding program which facilitates by molecular assisted back crossing is easier to get control over complex trait such as drought tolerance with increasing the performance of recurrent parents by interogressed lines with a trait of interest. Whereas, significant work has been done on the identification of QTLs of for the transfer of stay green trait in to elite cultivars (Kassahun Bantte *et al.*, 2010). According to Kassahun Bantte *et al.* 2010 senescent type of genotype shows typical leaf senescence patterns which were shown by decline in green leaf area with days after flowering. Where green leaf area drop down as the days to flowering precede these follows the normal pattern of monocarpic leaf senescence. On the other hand, these trends of leaf dying were affected by environmental water content and genotype.

In agreement with Kassahun Bantte *et al.* 2010, leaf area of most of QTL introgression lines were intermediate between the donor (stay-green) and recurrent parents. The rate of declining of green leaf area after flowering varied between genotypes while the onset began after 15 days of flowering in all genotypes. B35 reach maturity with significant percent of green leaf area (40 %) while the senescent type reached maturity with 0% of green leaf area. Even though the interogressed lines reach maturity with no green leaf stay green genotypes shows greater percent of green leaf area during decisive late stage of grain filling period (Kassahun Bantte *et al.*, 2010).

Delay in leaf senescence of B35 than the encounter drought susceptible genotypes could be as a result of a potential to greater access to nitrogen even water availability in the soil decreases, as consequence of accessing soil N under a limited water supply or with superior N pool in the vegetative parts of the plant (Kassahun Bantte *et al.* , 2010). Moreover, Borrell and Hammer (2000), elaborate these idea that stay green genotypes had higher percentage of N in the early time of 27 days after emergence as a consequence they have higher specific leaf N at the next stage anthesis, mid grain filling and also during maturity.

Green leaf area decreased as the stage proceeds to maturity which has shown in stressed environmental condition and also in irrigated environment. On the contrary, stay green donors had a considerable percent of green leaf area from the early stage to maturity unlike the recurrent parents which go down with percent of green leaf area (Kassahun Bantte *et al.* , 2010). Despite of decreasing in percents of green leaf area as maturity reached the stay-green QTL introgression lines were intermediate between the parents and donors, with retaining green leaf area at mid grain filling and at maturity, in general most of the introgression lines had a significant amount of green leaf area than the recurrent parent at mid grain filling period, the introgression lines from the recurrent parents were more stay green than the parental lines these

point toward to transfer of QTL traits from stay green to the recurrent parents which further shows ease of leaf senescence in most of the backcross derivatives thus, intercrossed stay-green QTLs were well expressed phenotypically (Kassahun Bantte *et al.* , 2010). Furthermore, the higher degree of stay-green in B35 could be a result of either an additive effect of multiple QTLs, a direct effect of specific QTLs, or a result of complex interactions between favorable alleles at these QTLs, effects that were not replicated in any of the individual derivatives (Kassahun Bantte *et al.* , 2010).

With the control of genes and environmental interactions drought tolerance is expressed (Reddy *et al.*, 2014).The expression of stay green under drought condition is a result of control of QTL which explains the relationship of QTL and expressed traits like stay greenness. QTL mapping undertaken with new recombinant inbred lines which are crossed with donor parent and recurrent parent which receive new gene (Reddy *et al.*, 2014). Thus, enhancement of crops resistance to drought ensures a better yield production, resistance to lodging and charcoal rot (Reddy *et al.*, 2014).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the study area

The experiment was conducted at Melka werer, Afar Region, Ethiopia, in Werer Agricultural Research Center ($9^{\circ} 22''$ N and $40^{\circ} 11''$ E) at an altitude of 750m above sea level. In January, 2014. The area is recognized as semi-arid drought prone area. Melka Werer site was preferred for the study because the historical weather data showed that there is less rainfall during the study period. Moreover, the area has well organized irrigation facility.

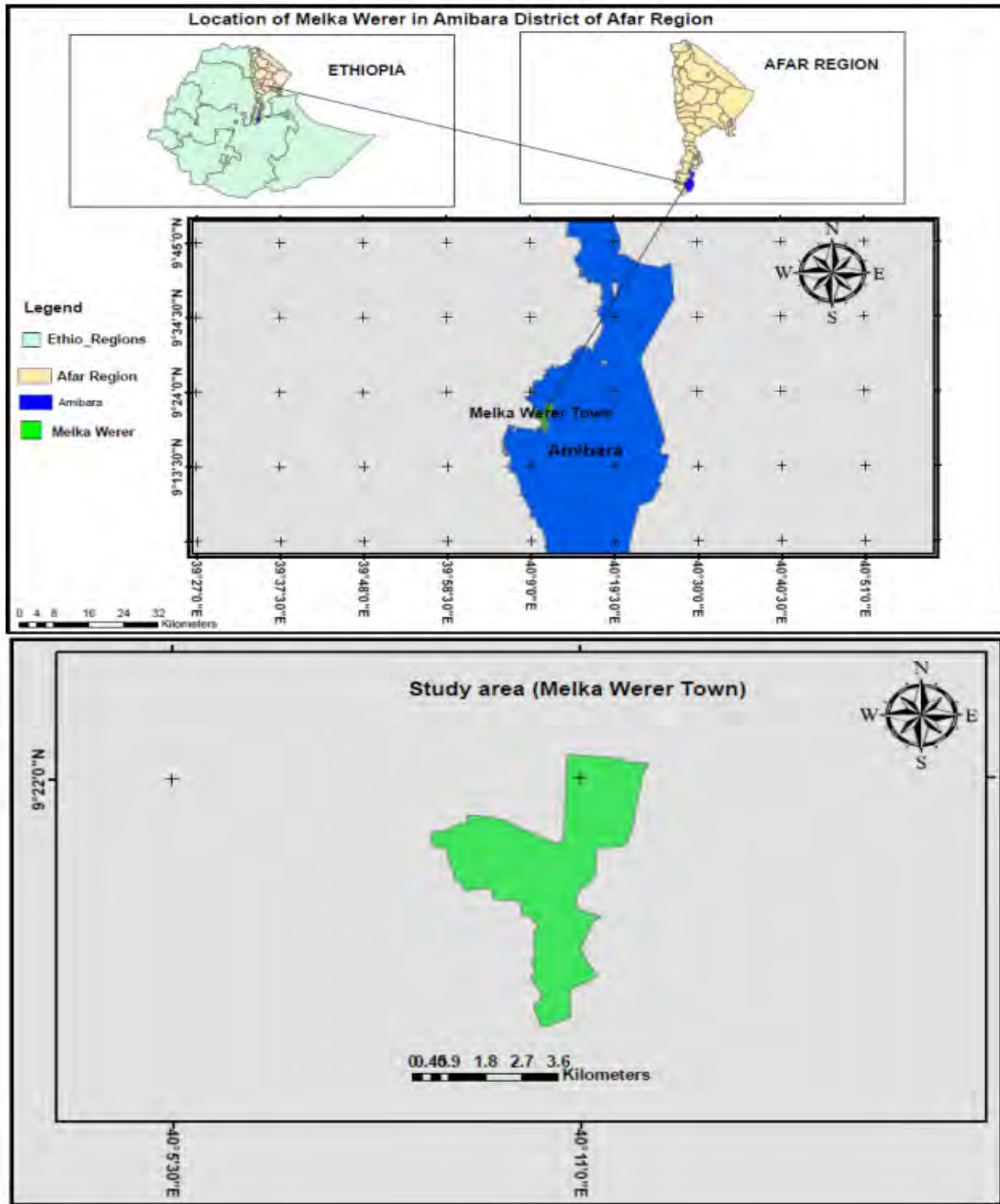


Fig.1. Location of study area

3.2. Climatic condition during the crop growth period

The data were collected from Ethiopian Meteorological Agency during the crop growth period in 2014 (Table 1). The rainfall ranged from 0.0 mm (February and June) to 57.3 mm (March), with 22.3 °C (March) to 33.5 °C (February) minimum and 21.5 °C (February) to 33.9 °C (April) maximum temperature.

Table.1. The monthly mean maximum and minimum temperature (°C) and rainfall (mm) during the study period

	February	March	April	May	June
Rain fall	0.0	57.3	21.3	11.4	0.0
Max. Tempreature	21.5	33.3	33.9	31.2	32.5
Min. Tempreature	33.5	22.3	25.6	27.5	30.87

3.3. Planting material

In the present study, seven stay-green sorghum introgression lines (derived from marker assisted backcrossing), their recurrent parents (Meko, Teshale and Gambella1107) and the stay-green donor parents B35 and E36-1 were evaluated for morphological, physiological, root characters and yield performance under induced post flowering condition (Table 2). The materials were obtained from national sorghum improvement research project of the Ethiopian Institute of Agricultural Research (EIAR) at Melkassa Agricultural Research Center, Melkassa, Ethiopia. The recurrent parents are locally adapted early maturing high yielding varieties released for drought prone lowland environments and senescent varieties which are affected by terminal

drought (Meko, Teshale and Gambella1107) whereas, the source of the stay green donor B35 and E36-1 lines, which are originally obtained from Ethiopia.

Table. 2. List of parents and stay-green sorghum introgression lines used for the study

No.	Pedigree	Identification number
1	Meko	2011MS BS #6
2	Meko x B35- 120	2011MSSB#120
3	Meko x B35-116	2011MSSB#116
4	Meko x B35- 117	2011MSSB#117
5	B35	2011MS SB # 45
6	Teshale	2011MS BS # 5
7	Teshale x E36-1	2010 MW # 12
8	Teshale x B35- 2012	2012MSSB#134
9	Teshale x B35- 2011	2011MSSB#134
10	E36-1	2010 MW # 209
11	Gambella1107	2011MS BS # 4
12	Gambella1107 x B35	2011MSSB#127

Key: the first 4 digit number “2011” show the year, the next two letter code “MS” show the location, which in this case is Melkassa, the next two letter code “SB” shows that the seed was sampled from selfing block and the last number #134 shows the serial number of the line in that year’s selfing block at Melkassa.

3.4. Experimental design and treatments

The field experiment were laid out in split plot design with three replications. The main plot factors were the two soil moisture regimes; well watered and drought stressed growth conditions and the sub-plot factor was the genotypes. The experiment was conducted during the 2014 post-rainy season (February-June) and the two contrasting soil moisture regimes were created through varying frequency of irrigation. The seeds were drilled directly in to three rows of 3.5 meter length with spacing of 75 cm between rows, with 235.5 m² main plot, sub plot 7m² (3.5m x 2 m) size, and 17 plants per row. Two weeks after sowing seedling were thinned to an interplant spacing of 15cm. Fertilizers were applied at the recommended rates of 50 Kg ha⁻¹ urea (46% N) and 100 Kg ha⁻¹ diammonium phosphate (DAP; 18% N and 46% P₂O₅). Half of the urea and all the DAP were applied in to seed row during sowing. The remainder of the urea was top dressed at the six to eight leaf stages.

All recommended management practices (weeding, cultivation, etc) were applied uniformly for both growing conditions. Furrow irrigation method was used for the application of irrigation. For both growing conditions, the field was equally irrigated until flowering. However, after flowering, the drought stressed condition was deprived of irrigation water to induce post-flowering drought. On the contrary, the well water treatments received irrigation water four times after flowering so that no drought stress could occur at any stage.

3.5. Data collection

3.5.1. Morpho-phenological and physiological data

Days to emergence (DE): The number of days from sowing to emergence of 50% of the seedlings in a plot was recorded as days to emergence.

Days to flowering (DF): The number of days from 50% seedling emergence to 50% of plants in each plot flowered.

Days to maturity (DTM): The number of days from DE to when 95% of the plants in a plot formed black layer on kernels.

Plant height (PH): Plant height was recorded from 5 randomly selected and tagged plants it was measured in meters from the ground level to the tip of the head.

Green leaf area (GLA): was measured at maturity by taking the length and breadth of fully opened and extended leaf. Leaf length was measured from the leaf base to the tip and width was measured at the widest region of leaf lamina. The product of leaf length, breadth and the shape factor 0.747 was expressed as leaf area in cm² per plant (Sticker *et al.*, 1961 cited in Nagarjuna, 2007)

$$LA \text{ (cm}^2\text{)} = \text{Breadth} \times \text{Length} \times \text{shape factor (0.747)}$$



Figure.2. Measurement of leaf area

Chlorophyll content of leaves: Leaf chlorophyll content was measured from five randomly selected and tagged plants per plot at flowering. Measurement was taken from two leaves per plant using a chlorophyll content meter (CCM-200 pulse). The chlorophyll meter readings were taken at the base of the leaf lamina of the second and fourth leaves from the top. Chlorophyll meter values provide an indication of the relative amount of total chlorophyll present in plant leaves.

Stay-green score (SGS): Visual stay-green ratings were scored at physiological maturity and it was determined if drought induction causes leaf and plant death in a plot. 1 to 5 scoring were used as scales based on the proportion of leaf area of normal sized leaves that had prematurely senescence and died. A rating 1 indicates completely green normal size leaves (no leaf death), 2 = 25% of the leaves died, 3 =26 to 50% of the leaves died, 4 =51 to 75% are dead, 5 =76 to 100% of the leaves and stem are dead (complete plant death).

3.5.2. Measurement of physiological parameters

Gas exchange parameters: Net photosynthesis and transpiration rate per unit area were measured as gas exchange parameters using portable photosynthesis system Lc pro⁺ photometer under PAR of 1000 $\mu\text{molm}^{-2}\text{s}^{-1}$. The instrument was held on the 2nd and 3rd intact leaves from the top of each plant. Three measurements, a total of sixth, were taken on an individual plant. These measurements were taken from 07:00AM to 10:00AM after two weeks of post-flowering drought induction.

Water use efficiency (WUE): it was determined by taking the ratio of assimilation rate to water lost due to transpiration.



Figure. 3. (A) Measuring leaf gas exchange using Lc pro⁺ photometer (B) Measuring chlorophyll content using chlorophyll content meter (CCM-200 pulse).

Relative water content (RWC): Flag leaf samples were taken randomly from each genotype and 2 cm² discs were excised and fresh weight (FW) was measured. Then, samples were placed in distilled water for 24 h and reweighed to obtain turgid weight (TW). Leaf samples were oven dried at 70°C for 24 hr followed by dry weight (DW) measurement. RWC was calculated using the following formula (Barrs, 1968, cited in Hasheminasab *et al.*, 2012).

$$\text{RWC}(\%) = \frac{(\text{Fresh weight} - \text{dry weight})}{(\text{Turgid weight} - \text{dry weight})} \times 100$$

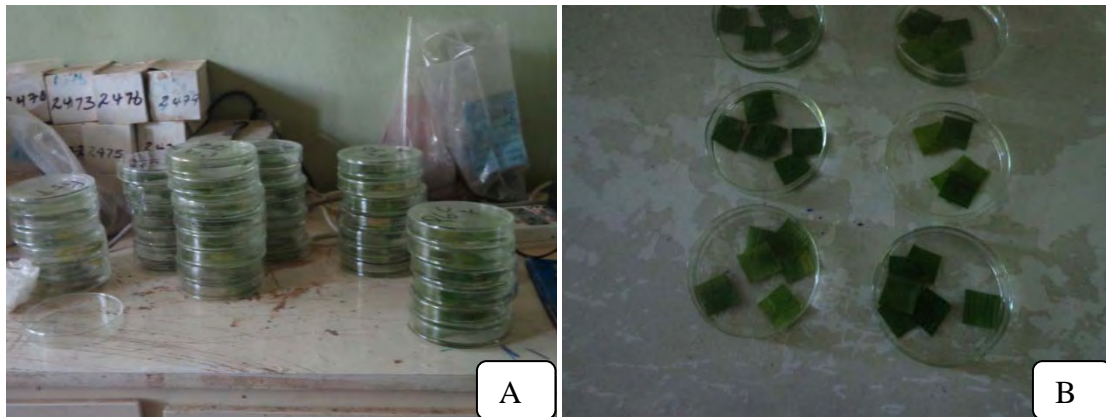


Figure.4. Leaf discs ready for turgid weight determination (A) and Leaf discs floated on water (B).

3.5.3. Determination of root related characters

After harvest observations were recorded on root length and dry weight.

Root length (cm): From each treatment 3 sample plants were taken by separating the shoot and root system. Root length was measured in centimeters from root collar to the tip of the main root (Devi *et al.*, 2013).

Root dry weight (g/plant): From each treatment 3 sample plants were taken and the excavated roots were washed and oven dried at 70°C for 72 hrs and root dry weight (g) was recorded using sensitive electronic balance (Devi *et al.*, 2013).

3.5.4. Determination of yield and yield components

To determine grain yield and its components, all the heads were harvested and oven dried at 70°C for 72 hrs.

Yield/panicle and hundred seed weight: Dried panicles with mature seeds were taken from each plant. After taking dried panicles, panicle weight were measured. Subsequently, the panicles were dried the seeds were threshed mechanically (using hand). Hundred grain weight (Hgw) were counted using seed counter machine. Whereas, grain yield per plot was measured in grams, which was later converted to kilogram per hectare.



Figure.5. Seed counter machine (Contador Pfeuffer, German company) (A), Measuring 100 seed weight (B).

3.6. Statistical analysis

All the collected raw data were subjected to analysis of variance using STATISTICA Software (Version7, STATISTICA Inc., USA) to see variations among sorghum genotypes, between the irrigation levels, and genotypes-by-irrigation interaction. The genotype means were separated using the Tukey's HSD range test at 5% level of significance. The correlations between the studied traits were analyzed using Product Moment and Partial Correlation Test, whereas all the graphs were generated with the Sigma Plot 11.0 (Systats Software, Inc.).

4. RESULTS

The result of the present study showed significant differences for genotypes for all measured traits except days to seedling emergence and days of flowering ($P < 0.05$), which indicates that the studied genotypes were diverse. In response to post-flowering drought stress there were significant differences among the genotypes ($P < 0.05$) for all the traits. Genotype-by-irrigation interaction was significant for all traits ($P < 0.05$).

4.2. Morpho-phenological trait

4.2.1. Morphological traits

Based on analysis of variance, plant height showed significant differences between genotypes under the water regimes and for genotypes between the two water regimes at $p < 0.05$ (Table 3). Under well watered condition it ranged from 0.8m (B35) to 2.65 m (Teshale) while under drought stress condition (B35) were the shortest 0.61m and (Teshale) had the longest height 2.34m. Plant height for Meko x B35- 117 and Meko x B35- 120, were less affected by drought stress. While, Teshale remarkably demonstrated a reduction due to drought induction.

Analysis of variance revealed significant difference in green leaf area between genotypes under the two moisture regimes and for genotypes between the two water regimes at $P < 0.05$ (Table 3). Among the genotypes, Gambella1107 and Meko x B35-116 had higher green leaf area under well watered condition while under drought stress condition Meko x B35-120 and Gambella1107 had higher green leaf area and Teshale had smaller leaf area. Among the introgression lines Teshale derivatives had higher leaf area than their recurrent parent Teshale. Gambella1107 and Meko have higher leaf area than their introgression lines Gambella1107 x B35, Meko x B35-117 and Meko x B35-116.

As time of exposure to drought stress increased plants of DS showed a sharp decline in GLA in comparison with WW plants. DS cause a significant reduction in GLA of Meko x B35-116, Gambella 1107 and Gambella1107 x B35when it compares with WW plants. On the other hand Meko x B35-120 and Teshale x E36-1 shows the lowest reduction in GLA.

Table.3 Mean value \pm S.E. of Leaf area (LA) and plant height (PH) of sorghum under well watered (WW) and drought stressed (DS) growing conditions.

Genotypes	Irrigation	Traits	
		PH	LA
Meko	WW	1.8 \pm 0.14 (ae,g-i)	446.25 \pm 16.68(agij)
	DS	1.6 \pm 0.06 (aehi)	429.5 \pm 6.61(a,efgi)
Meko x B35- 120	WW	1.9 0 \pm .13 (ace,g-i)	491.57 \pm 15.24(a,g-j)
	DS	1.83 \pm 0.00 (ae,g-i)	477.43 \pm 14.35(aghij)
Meko x B35- 116	WW	1.76 \pm 0.04 (aehi)	512.65 \pm 19.23(aefgi)
	DS	1.71 \pm 0.07 (aehi)	352.80 \pm 16.03(bcdfi)
Meko x B35- 117	WW	1.86 \pm 0.07 (ae,g-i)	402.31 \pm 14.82(d-g,i)
	DS	1.77 \pm 0.09 (ae,g-i)	355.79 \pm 6.64(bfi)
B35	WW	0.8 \pm 0.01 (bd)	374.44 \pm 12.60(b-g,i)
	DS	0.61 \pm 0.01 (bd)	357.78 \pm 17.98(bcdfgi)
Teshale	WW	2.65 \pm 0.14(c,f-h)	337.62 \pm 16.39(bcdi)
	DS	2.34 \pm 0.12 (c,e-h)	311.37 \pm 19.96(bi)
Teshale x E36-1	WW	2.24 \pm 0.07 (agi)	435.46 \pm 11.22(afgi)
	DS	2.16 \pm 0.03 (a,f-hi)	427.01 \pm 19.19(afgi)
Teshale x B35-2012	WW	2.24 \pm 0.12 (ac,f-i)	437.56 \pm 5.80(a,e-h)
	DS	2.09 \pm 0.09 (acgh)	421.84 \pm 15.88(i)
Teshale x B35-2011	WW	2.45 \pm 0.12 (fgh)	456.15 \pm 19.89(a,eg,ij)
	DS	2.25 \pm 0.05 (gh)	439.55 \pm 11.24(afgij)

E36-1	WW	1.82± 0.03 (aghi)	457.34 ±13.08(ae,f-j)
	DS	1.75 ± 0.10 (ai)	427.43± 12.83(ae, fi)
Gambella1107	WW	1.93± 0.05(afgh)	527.37±19.66(aj)
	DS	1.87±0.15 (a,f-h)	472.13± 12.84(ac,d-i)
Gambella1107x B35	WW	1.02±0.04 (d)	483.29± 11.59(a-g,ij)
	DS	0.95 ±0.07 (d)	415.35 ±13.99(b-f,gi)

Means followed by a common letter with in a column are not significantly different at P <0.05.

4.2.2. Days to maturity

The ANOVA showed significant difference in days to maturity among the genotypes under the different water regimes and for genotypes between the two regimes at p < 0.05. Under well watered growing condition days to maturity ranged from 94.66 (B35) to 99 (Teshale). Under drought stress condition, DTM ranged from 90 days (Meko x B35-117) to 93.66 (Meko x B35-120 and Teshale x B35-2012) (Figure 6).

Days to maturity was shortened due to post-flowering drought stress in (Teshale), (Gambella 1107 x B35) and (Meko x B35-117) as compared to the well watered condition. While, B35 has shows the lowest reduction in days of maturity.

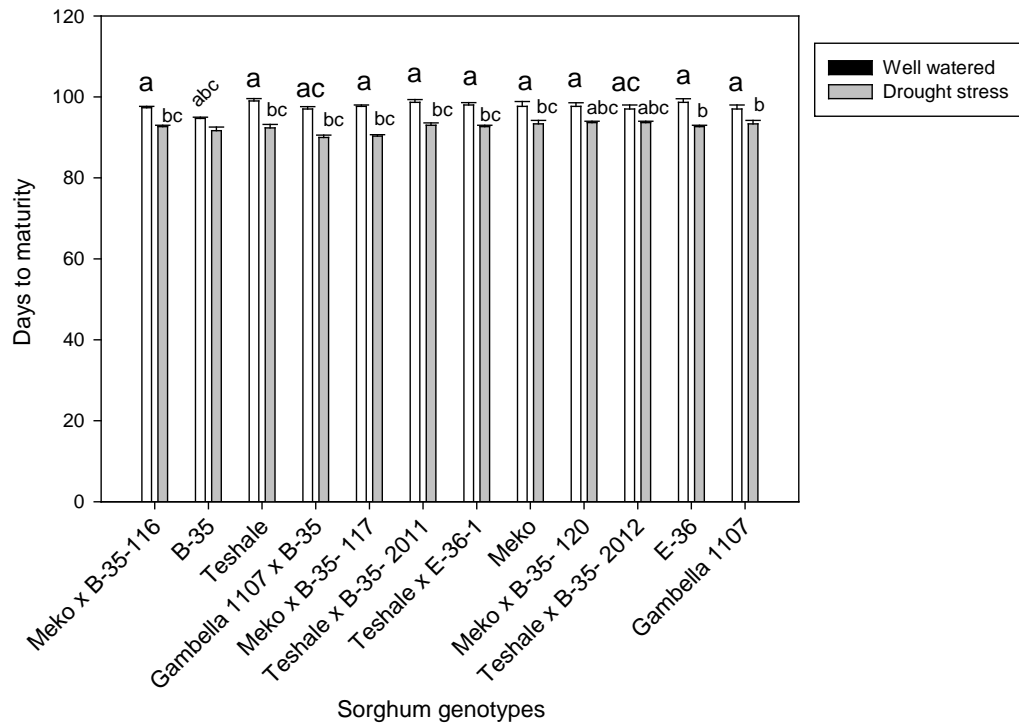


Figure .6. Days to maturity of sorghum genotypes under well watered and drought stress (DS) growing conditions. Bars represented by the same letters are not significantly different at $p < 0.05$.

4.2.3. Leaf senescence at maturity

The result of leaf senescence showed significant variation in green leaf number for genotypes under the two moisture regimes and between the two water regime for genotypes at $p < 0.05$ (Table 4). Under post-flowering drought stress condition (B35), (E36-1), (Meko x B35- 120), and (Teshale x B35- 2011) had more number of green leaves than the rest of the genotypes whereas, under well watered condition the number of green leaves ranged from 4 (Teshale) to 14 (B35, E36-1 and Teshale x B35- 2011). The GLN showed decreasing trend for drought treatment, it was being higher to Meko and Gambella. While, E36-1, B35, Meko x B35- 120 and

Meko x B35- 116 commenced lower rate of decreasing due to drought treatment application for GLN; whereas the remaining genotypes did not show a significant reduction of GLN.

Table.4. Mean value \pm S.E. of Green leaf number (GLN) for sorghum genotypes tested under well watered (WW) and drought stress (DS) growing conditions.

Genotypes	Irrigation	Trait
		GLN
Meko	WW	10 \pm 0.52(f-j,l)
	DS	7 \pm 0.50(aehjl)
Meko x B35- 120	WW	13 \pm 0.23(fbhik)
	DS	11 \pm 0.34(hijl)
Meko x B35- 116	WW	6 \pm 0.2(aci)
	DS	6 \pm 0.23(a,c-f, hl)
Meko x B35- 117	WW	10 \pm 0.52(d,f-ijl)
	DS	7 \pm 0.2(aehjl)
B35	WW	14 \pm 0.30(bik)
	DS	14.13 \pm 0.29(bik)
Teshale	WW	4 \pm 0.52(a)
	DS	4 \pm 0.11(a)
Teshale x E36-1	WW	11 \pm 0.2(cdfhijl)
	DS	8 \pm 0.7(aghjl)
Teshale x B35-2012	WW	12 \pm 0.5(ik)
	DS	9.06 \pm 0.7(jl)
Teshale x B35-2011	WW	14 \pm 0.11(bik)
	DS	10 \pm 0.11(f-jl)
E36-1	WW	14 \pm 0.5(k)
	DS	13.6 \pm 0.52(k)
Gambella1107	WW	9 \pm 0.41(l)
	DS	7 \pm 0.52(acdegh)
Gambella1107x B35	WW	10 \pm 1.02(cd,f-j,l)
	DS	8 \pm 0.41(a,c-g,ijl)

Means followed by a common letter with in a column are not significantly different from each other at $P < 0.05$.

4.2.4. Relative chlorophyll content (Greenness)

The ANOVA indicated that there were significant variations among the genotypes under the two moisture regimes and for genotypes between the two water regime and for genotypes between the two water regimes at $P < 0.05$ (Table 5). However, under drought stress condition, introgression lines and their donor parent's maintained better chlorophyll content until physiological maturity, which was significantly higher than that in recurrent parents. Accordingly, the two donor parents (B35) and (E36-1) maintained the highest chlorophyll content 54.45 (B35) and 52.76 (E36-1). Under well watered condition, genotypes were also significantly different for chlorophyll content and still (B35) and (E36-1) had the highest chlorophyll content.

Comparing the well watered and drought condition recurrent parent (Gambella) drought reduced its chlorophyll content from 36.26 to 23.85, similarly significant ($p < 0.05$) reduction has also showed in (Meko) which reduced from 36.26 to 21.63. Drought stress significantly reduced chlorophyll Meko, Gambella and Teshale while B35, E36-1 and Teshale x B35-2011 were less affected by drought stress.

Induction of drought had significantly hastened leaf senescence compared to well watered condition. The result of stay green scoring showed significant difference among the genotypes, within moisture regimes and the interaction between the two ($P < 0.05$). Those genotypes with stay green trait had lower rate of leaf senescence and parental lines had higher rate of leaf senescence, but the introgression lines had lower rate of leaf senescence. The donor parents B35 and E36-1 had significantly ($P < 0.05$) lower rate of senescence (with stay green score of 1.3) than the other backcrossed and parental line genotypes. A similar trend was also observed under well watered condition where the recurrent parental lines had lower stay green score and the

introgression lines had better scores. The donor lines (B35 and E36-1) scored 1 for greenness which was highly significant ($P < 0.05$).

Table.5. Mean value \pm S.E. of Chlorophyll content meter (CCM) and stay green score (SGS) and for sorghum lines tested under well watered (WW) and drought stress (DS) growing conditions.

Genotypes	Irrigation	Traits	
		CCM	SGS
Meko	WW	36.26 \pm 4.03(a-j)	3.6 \pm 0.33(acdf)
	DS	21.63 \pm 3.27(ag,i)	3.6 \pm 0.33(acdf)
Meko x B35- 120	WW	51.66 \pm 6(a-c,h-j)	2 \pm 0.0(ab,de)
	DS	41.64 \pm 2.13(a-j)	2.3 \pm 0.33(a-e)
Meko x B35- 116	WW	42.22 \pm 1.69(a-j)	2.3 \pm 0.33(a-e)
	DS	31.51 \pm 3.33(a,d-i)	3 \pm 0.33(acdf)
Meko x B35- 117	WW	40.49 \pm 6.62(a-j)	2.3 \pm 0.33(a-e)
	DS	30.61 \pm 3.98(a,d-f,hi)	3 \pm 0.57 (acdf)
B35	WW	57.15 \pm 1.62(a-c,h-j)	1 \pm 0.0(abe)
	DS	54.45 \pm 3.31(acfhij)	1.3 \pm 0.33(abde)
Teshale	WW	28.383 \pm 1.42(a,d-g,i)	2.6 \pm 0.33(a-f)
	DS	18.19 \pm 1.21(efg)	4 \pm 0.33(acdf)
Teshale x E36-1	WW	45.733 \pm 2.14(a-d,f-j)	2 \pm 0.0(ab,de)
	DS	34.480 \pm 7.96(a-j)	2.3 \pm 0.33(a-e)
Teshale x B35-2012	WW	40.65 \pm 3.14(a-d,h-l)	2 \pm 0.0(ab,de)
	DS	28.5 \pm 5.43(ade fg)	2.6 \pm 0.33(a-f)
Teshale x B35-2011	WW	46.98 \pm 2.85(a-d,f-j)	2 \pm 0.0(ab,de)
	DS	38.84 \pm 2.25(a-j)	2.3 \pm 0.33(a-e)
E36-1	WW	55.57 \pm 5.17(abc)	1 \pm 0.33(ae)
	DS	52.76 \pm 1.82(abc)	1.3 \pm 0.33(abe)
Gambella1107	WW	36.26 \pm 4.03(a-l)	3.6 \pm 0.33(acdf)
	DS	23.85 \pm 2.78(a-l)	4.3 \pm 0.66 (b-f)
Gambella1107x B35	WW	36.32 \pm 5.5 (a-j)	2.6 \pm 0.33(a-f)
	DS	25.59 \pm 3.28(a,d-g,i)	3 \pm 0.00(a-d,f)

Means followed by a common letter with in a column are not significantly different at $P < 0.05$.

4.3. Physiological traits

4.3.1. Assimilation rate

The analysis of variance on photosynthetic assimilation rate of the tested genotypes showed significant variation under the two water regimes and for genotypes between the two water regimes ($p < 0.05$) (Table 6). Drought had significantly reduced the assimilation rate of sorghum lines under drought stress condition. B35 and E36-1; the donor parents had high assimilation rate. The introgression line, Meko x B35- 120 had higher assimilation rate followed by Teshale x B35- 2011, Teshale x E36-1' and Meko x B35-116 indicating that the introgression lines had better CO₂ assimilation rate than their recurrent parents. On the other hand, Teshale x B35- 2012, Meko x B35- 117, and Gambella1107 x B35 had lower assimilation rate in that order than their recurrent parents Meko, Teshale and Gambella1107. Under well watered condition, B35 and E36-1 had higher assimilation rate followed by the introgression lines Meko x B35- 120, Teshale x B35- 2011, Teshale x E36-1 in that order.

Drought stress has significantly ($p < 0.05$) reduced CO₂ assimilation rate in comparison to well watered condition for donor parents recurrent parents , and also for the introgression lines but still the introgression lines has got an advantage over their recurrent parent under drought stress.

Each genotypes exhibited significant difference in CO₂ uptake between DS and WW drought treatments. Besides, Teshale, Meko x B35- 117, demonstrated a reduction in assimilation at DS while compared with plants of WW. On the other hand, Meko x B35-116 and Gambella x B35 were no affected by drought stress in CO₂ as such, in comparison with plants of well watered.

4.3.2. Transpiration rate

Transpiration measurements showed significant differences between genotypes under the different water regimes and for genotypes between the two water regimes ($p < 0.05$) (Table 6). The transpiration rate of genotypes under drought condition was significantly lower than the same genotypes tested under well watered condition. The highest transpiration rate was observed in B35 and E36-1. Some of the introgression lines such as Teshale x B35- 2011, Teshale x E36-1, and Meko x B35- 120, had higher transpiration rate than their parents while Gambella1107 x B35 and Meko x B35- 117 had lower transpiration rate than the other introgression lines and recurrent parents.

Transpiration rate declined in each genotype while the stress is induced for Meko, Teshale and Teshale x E36-1. Genotypes response to drought stress revealed that transpiration rate was not affected in Gambella x B35 and Gambella.

Table.6. Mean value \pm S.E. of Assimilation rate (A) and Transpiration rate (E) for sorghum lines tested under well watered (WW) and drought stress (DS) growing conditions.

Genotypes	Irrigation	Traits	
		A	E
Meko	WW	2.0 \pm 0.12(acdgh)	6.51 \pm 0.13(a-e)
	DS	1.36 \pm 0.1(acdfl)	5.61 \pm 0.37(a-e)
Meko x B35- 120	WW	4.39 \pm 0.15(ij)	7.36 \pm 0.02(ace)
	DS	1.66 \pm 0.09(acdl)	6.01 \pm 0.2(a-e)
Meko x B35- 116	WW	1.93 \pm 0.08(acdgh)	6.3 \pm 0.39(ace)
	DS	1.21 \pm 0.04(a-f,jl)	5.57 \pm 0.67 (a-e)
Meko x B35- 117	WW	1.13 \pm 0.09(ade fl)	4.92 \pm 0.03(a-e)
	DS	0.46 \pm 0.02(fl)	4.24 \pm 0.04(bd)
B35	WW	5.13 \pm 0.68(bi)	7.48 \pm 0.29(ace)
	DS	2.39 \pm 0.14(acgh)	6.66 \pm 0.17(a-e)
Teshale	WW	1.68 \pm 0.04(acdghl)	5.87 \pm 0.17(a-e)
	DS	0.75 \pm 0.03 (c-f,l)	5.01 \pm 0.63(a-e)

Teshale x E36-1	WW	2.46 ±0.35(ah)	6.94 ±0.31(abce)
	DS	1.49 ±0.04(acdfl)	6.02 ±0.12(a-e)
Teshale x B35-2012	WW	1.29±0.15(a,c-f,l)	5.51±0.39(a-e)
	DS	0.51± 0.07(jl)	4.55±0.31(ab,de)
Teshale x B35-2011	WW	2.64 ±0.36(agh)	6.98±0.17(ab,ce)
	DS	1.58 ±0.00(acdhl)	6.2±0.46(a-e)
E36-1	WW	4.58 ±0.16(k)	7.43±0.28(ace)
	DS	1.98±0.05(acdef)	6.12±0.04(a-e)
Gambella1107	WW	1.36±0.03(a,cf,jl)	5.2±0.15(a-e)
	DS	0.66 ±0.08(l)	4.99±0.41(a-e)
Gambella1107x B35	WW	0.92±0.02(adefl)	4.27±0.4(bde)
	DS	0.35± 0.02(efl)	3.92±0.26(bd)

Means followed by a common letter with in a column are not significantly different at $P < 0.05$.

4.3.3. Water use efficiency

Analysis of variance showed significant differences in water use efficiency among the genotypes under the two water regime and for genotypes between the two water regimes $P < 0.05$ (Table 7). WUE of genotypes ranged from 0.12 to 0.36 under drought stress condition. Among the backcrossed derivatives Meko x B35- 120, Teshale x B35- 2011, and Teshale x E36-1 had higher WUE than their recurrent parents, Meko and Teshale but the differences were not significant at $p < 0.05$. Under well watered condition, WUE ranged from 0.21 to 0.68.

Each genotype indicate significant difference in WUE to the two water stress supply regimes. Gambella and Teshale shows a significant reduction for WUE in DS than WW plants. While, (Teshale x E36-1), (Tehsale x B35) and (Meko x B35-117) has the lowest reduction in WUE of DS plants in comparison with the WW plants.

4.3.4. Relative water content

Analysis of variance revealed that there were significant differences between genotypes in the two water regime and for genotypes for the two moisture regimes at $p < 0.05$ (Table 7). Under drought stress there were significant differences between backcrossed derivatives and their respective recurrent parents. Accordingly, the introgression line Meko x B35-120 had higher RWC than its recurrent parent Meko but the difference was not statistically significant. The introgression lines Teshale x B35-2011, and Teshale x E36-1 had higher RWC than their recurrent parents, Teshale. In addition, Meko x B35-120 had significantly ($p < 0.05$) higher RWC than Meko x B35-116 and Meko x B35-117. Gambella1107 x B35 had the lowest RWC of all the genotypes. Under well watered condition, the two donor parents, B35 and E36-1 had lower RWC but Gambella1107 x B35 was the introgression line with the lowest value RWC while comparing the genotypes under the two water regimes, (Gambella) followed by (Gambella x B35) and (Meko x B35-117) had lower relative water content in comparison with well watered condition.

All genotypes demonstrated a reduction in RWC with induced drought stress. There was smaller difference in DS plants of E36-1, B35, Meko x B35- 120, Teshale x E36-1 and Teshale x B35-2011 while compared with plants of WW. Besides, Gambella, Gambella x B35 and Meko x B35- 117 had significantly lower RWC in the DS plants than WW plants.

Table.7. Mean value \pm S.E. of Water use efficiency (WUE) and Relative water content (RWC) for sorghum lines tested under well watered and drought stress (DS) growing conditions.

Genotypes	Irrigation	Traits	
		RWC	WUE
Meko	WW	80.59 \pm 5.55(af)	0.32 \pm 0.02(aegi)
	DS	68.84 \pm 8.7(a,e-g)	0.24 \pm 0.01 (ag)
Meko x B35- 120	WW	81.6 \pm 1.39(af)	0.57 \pm 0.04(fh)
	DS	75.83 \pm 3.19(af)	0.27 \pm 0.01(agi)
Meko x B35- 116	WW	77.98 \pm 1.74(acf)	0.3 \pm 0.03(adegei)
	DS	65.34 \pm 3.59(a,c-g)	0.23 \pm 0.02 (acdegei)
Meko x B35- 117	WW	70.58 \pm 1.67(a,e-g)	0.22 \pm 0.02(adegei)
	DS	53.24 \pm 2.37(efg)	0.13 \pm 0.00(daegi)
B35	WW	87.99 \pm 0.12(ab)	0.68 \pm 0.08(bfh)
	DS	80.74 \pm 4.47(af)	0.36 \pm 0.02(ae)
Teshale	WW	76.2 \pm 5.64(af)	0.28 \pm 0.01(acdegei)
	DS	61.26 \pm 2.46(a-g)	0.15 \pm 0.01(cdegei)
Teshale x E36-1	WW	79.05 \pm 5.28(af)	0.36 \pm 0.06(ae)
	DS	70.56 \pm 2.35(a,e-g)	0.25 \pm 0.01(aegi)
Teshale x B35-2012	WW	74.77 \pm 0.75(af)	0.25 \pm 0.03(agi)
	DS	64.21 \pm 4.11(fag)	0.14 \pm 0.03(agi)
Teshale x B35-2011	WW	78.61 \pm 1.29(af)	0.37 \pm 0.04(ae)
	DS	73.92 \pm 4.47(afg)	0.26 \pm 0.01(aegi)
E36-1	WW	85.02 \pm 1.41(a)	0.64 \pm 0.04(h)
	DS	77.34 \pm 1.24(a)	0.3 \pm 0.01(ai)
Gambella1107	WW	76.18 \pm (a)	0.26 \pm 0.01(ai)
	DS	54.53 \pm 1.76(g)	0.14 \pm 0.01(ai)
Gambella1107x B35	WW	68.75 \pm 1.88(a,d-g)	0.21 \pm 0.02(acdegei)
	DS	50.34 \pm 3.38(d-g)	0.12 \pm 0.02(b-e,gi)

Means followed by a common letter with in a column are not significantly different from each other at P <0.05.

4.4. Root related characters

4.4.1. Root length

There were significant differences among the genotypes between moisture regimes and for genotypes between the two water regimes at $p < 0.05$ (Table 8). From Meko introgression lines Meko x B35- 120 and Meko x B35- 116 had longer root length. On the other hand, Teshale crosses; Teshale x E36-1 had longer root length than other derivatives and the parent, Teshale, while Gambella1107 x B35 had almost equal root length with the parent Gambella1107. Under well watered condition, the longest root length was recorded by the stay green introgression line Meko x B35- 120 and the smallest root length was showed by the recurrent parent (Gambella1107).

Genotypes showed reduction in RL while drought stress was induced. Evaluation of the response of genotypes for water deficit stress signified that Teshale showed a significant decline in RL in response to drought stress while E36-1, Gambella, Teshale x B35- 2011 and Gambella x B35 shows the lowest reduction.

4.4.2. Root dry weight

Analysis of variance revealed that significant differences among genotypes were recorded under the two water regimes and for genotypes between the two water regimes $P < 0.05$ (Table 8). Under drought stress, the stay green parents: B35 and E36-1 had significantly higher root dry weight as compared to non stay green parents and introgression lines. Among the backcrossed derivatives; Meko x B35- 120 Teshale x B35- 2011, Teshale x B35- 2012, Teshale x E36-1, Gambella1107 x B35 had higher root dry weight when compared with their recurrent parents. Under well watered condition, B35 and E36-1 produced the highest root dry weight followed by Meko x B35-120, Teshale x B35-2011, Teshale x B35-2012 Teshale x E36-1 whereas, Meko x

B35-116 and Meko x B35-117 produced significantly smaller root dry weight in comparison with their recurrent parents.

For the two water treatments for similar genotypes there were reduction in root length, from recurrent parents (Meko and Teshale) and from introgression lines (Meko x B35-120, Meko x B35-116 and Teshale x B35-2012) had shown reduction in root length under drought condition in comparison with well watered condition.

Genotypes show a significant variation in RDW in response to the water stress levels (Table 8). Reduction in RDW was found in genotypes Meko x B35- 117 and Teshale as drought stress level increased. On the other hand genotypes did not reveal E36-1, Gambella x B35 and Meko x B35-117 shows minimum reduction.

Table.8. Mean value \pm S.E. of Root dry weight (RDW) and Root length (RL) for sorghum lines tested under well watered and drought stress (DS) growing conditions.

Genotypes	Irrigation	Traits	
		RL	RDW
Meko	WW	33.55 \pm 0.98(ac, f-h)	29.61 \pm 0.29(a)
	DS	27.1 \pm 4.36(d,f-h)	23.79 \pm 0.35(abej)
Meko x B35- 120	WW	51.99 \pm 3.71(ae)	37.63 \pm 0.99(hi)
	DS	40.77 \pm 2.74(ag)	31.72 \pm 0.74(ag)
Meko x B35- 116	WW	41.44 \pm 2.05(aeg)	27.94 \pm 0.17(a)
	DS	32 \pm 1.73 (a-d,fgh)	22.76 \pm 1.19(bei)
Meko x B35- 117	WW	34.1 \pm 2.29(acdfgh)	25.59 \pm 1.19(ae)
	DS	25.55 \pm 0.61(cd,f-h)	17.67 \pm 0.02(fij)
B35	WW	37.55 \pm 1.82(acdgh)	41.4 \pm 0.03(chi)
	DS	23.66 \pm 0.66(b-d,fh)	47.78 \pm 0.52(d)
Teshale	WW	39.66 \pm 2(ag)	26.68 \pm 1.33(abe)
	DS	24.77 \pm 0.77(b-d,f-h)	19.86 \pm 0.14(b,df,i)
Teshale x E36-1	WW	41.99 \pm 1.45(aeg)	31.75 \pm 0.05(ag)
	DS	34.44 \pm 3.63(acfgh)	24.32 \pm 0.74(ab, j)
Teshale x B35-2012	WW	32.88 \pm 4.77(adfgh)	32.09 \pm 0.05(agh)
	DS	22.55 \pm 0.48(fgh)	26.46 \pm 0.57(ab)
Teshale x B35-2011	WW	43.66 \pm 2.18(ae)	34.53 \pm 0.58(gh)
	DS	37.11 \pm 1.35(acdgh)	28.87 \pm 0.0(a)

E36-1	WW	36.55± 0.22(afgh)	39.12±0.12(i)
	DS	30.22±0.94(agh)	39.27±0.46(i)
Gambella 1107	WW	32.33±1.66(ach)	27.06± 0.73(a)
	DS	26.22±2.05(h)	20.32± 1.26(j)
Gambella1107x B35	WW	32.88±1.05(ad,fgh)	27.86± 1.58(a)
	DS	26.44± 1.11(cd,f-h)	22.5±1.20(eij)

Means followed by a common letter with in a column were not significantly different at P <0.05.

4.5. Grain yield and yield related traits

Based on the result of the experiment there were significant differences among the genotypes under different moisture regimes and for genotypes between the two water regimes at P < 0.05 (Table 11). Under drought stress condition, Meko x B35-120 attained the highest 100 grain weight and the difference was significant at p < 0.05 than the backcrossed introgression lines of Meko. Moreover, Meko x B35- 117 had a significantly higher 100 grain weight than the stay green donor, B35 although the difference was insignificant, Teshale derivatives Teshale x B35- 2011 and Teshale x E36-1 had higher 100 grain weight than Teshale x B35- 2012 and the parent Teshale. On the other hand, Gambella1107 x B-35 had smaller 100 grain weight than the recurrent parent Gambella1107.

In general, Meko x B35- 120, Meko x B35- 117, Teshale x E36-1, and Meko x B35- 116 had higher 100 seed weight than their respective recurrent parents under well watered condition. The other derivatives, Teshale x B35- 2011, Teshale x B35- 2012 and Gambella1107 x B35 had smaller 100 grain weight.

However, decreasing of 100 seed weight was observed in Meko x B35- 117, Teshale and Gambella x B35 plants while drought stress levels increased. In the contrary, small variation and drought effect were observed for Meko, Teshale x B35- 2012 and Teshale x B35- 2011.

Similarly, significant differences were recorded in panicle weight between genotypes under the different water treatments and for genotypes between the two water regimes at $p < 0.05$ (Table 9). Compare with the other Meko derivatives, Meko x B35- 120 had significantly higher ($p < 0.05$) panicle weight. On the other hand, Meko x B35- 116 had smaller panicle weight than the recurrent parent Meko.

Under drought stress condition, among Teshale backcrossed derivatives Teshale x B35- 2011 and Teshale x E36-1 had higher panicle weight. Among Meko derivatives Meko x B35- 120, Teshale x B35- 2011, and Teshale x E36-1 had higher panicle weight than their respective recurrent parents; Meko and Teshale. While, the other derivatives had smaller panicle weight with average value of 71.70 gm. Under well watered or irrigated condition, significant differences in panicle weight were recorded between backcrossed derivatives and parental lines at $p < 0.05$.

Due to drought treatment a significant reduction for panicle weight were observed in E36-1, Meko x B35- 120 and Teshale x B35- 2011. On the other hand, Gambella1107 and its introgression line Gambella x B35 had shown the lowest reduction in panicle weight for drought treatment.

Table.9. Mean value \pm S.E. of Panicle weight (Pwt) and Hundred grain weight (Hgw) for sorghum lines tested under well watered and drought stress (DS) growing conditions.

Genotypes	Irrigation	Traits	
		Pwt	Hgw
Meko	WW	104.94 \pm 7.71(f-h,i)	3.31 \pm 0.12(adf)
	DS	89.957 \pm 5.96(aefhi)	2.92 \pm 0.21(a-d,f)
Meko x B35- 120	WW	131.33 \pm 4.37(gh)	4.2 \pm 0.33(aef)
	DS	103.11 \pm 4.95(a,e-h)	3.6 \pm 0.37(a,d-f)
Meko x B35- 116	WW	87.53 \pm 9.18(aefhi)	3.36 \pm 0.09 (a,d-f)
	DS	68.31 \pm 5.7(a-e,hi)	2.82 \pm 0.2(a-d,f)
Meko x B35- 117	WW	67.22 \pm 8.26(a-e,hi)	3.69 \pm 0.27(a,d-f)
	DS	50.31 \pm 2.75(di)	2.23 \pm 0.03(a-c)
B35	WW	60.69 \pm 9.4(a-d,i)	2.91 \pm 0.21(a-d, f)
	DS	45.17 \pm 3.97(bcdi)	2.01 \pm 0.15(bc)
Teshale	WW	81.85 \pm 2.84(a,d-f,hi)	3.53 \pm 0.31(adef)
	DS	67.82 \pm 4.41(a-e,hi)	2.46 \pm 0.11(a-c,f)
Teshale x E36-1	WW	116.35 \pm 3.77 (a,f-h)	3.59 \pm 0.3(ab,d-f)
	DS	97.42 \pm 0.99(e-i)	3.02 \pm 0.14(abdf)
Teshale x B35-2012	WW	70.13 \pm 1.3(a-e,hi)	2.87 \pm 0.26(a-f)
	DS	57.5 \pm 2.85(a-e,gi)	2.39 \pm 0.02(a-c,f)
Teshale x B35-2011	WW	121.8 \pm 6.161(a,e-h)	3.44 \pm 0.16(adef)
	DS	98.61 \pm 5.76(a,e-i)	3.13 \pm 0.34(a-c,ef)
E36-1	WW	97.46 \pm 11.42(ahi)	3.53 \pm 00.06(af)
	DS	73.81 \pm 3(a-i)	2.87 \pm 0.15(a-d,f)
Gambella 1107	WW	74.92 \pm 7.13(a-e,fi)	3.31 \pm 0.04(adef)
	DS	60.97 \pm 0.96(a-e)	2.34 \pm 0.13(abcf)
Gambella1107x B35	WW	56.43 \pm 9.59(a-d,i)	2.84 \pm 0.21(a-d,f)
	DS	47.42 \pm 4.28(cdi)	1.77 \pm 0.22(c)

Means followed by a common letter with in a column were not significantly different at P <0.05.

The grain yield of all genotypes subjected to post-flowering drought stress was significantly lower than those under well irrigated growing condition and significant difference has shown for genotypes between the two water regimes at ($p < 0.05$) (Table 11). The average grain yield among the stay-green introgression lines and parents for well watered and drought stress growing conditions were $3979.15 \text{ kg ha}^{-1}$ and $3400.35 \text{ kg ha}^{-1}$, respectively. The grain yield values ranged from 2639 kg ha^{-1} (B35) to $4745.3 \text{ kg ha}^{-1}$ (Meko x B35-120) under well watered and from $2399.5 \text{ kg ha}^{-1}$ (B35) to $4183.1 \text{ kg ha}^{-1}$ (MekoxB35-120) for drought stress growing condition. For well irrigated condition, Meko x B35-116 had higher grain yield than the sister line Meko x B35-117. The donor line B-35 had lower grain yield than its backcrossed derivatives, Teshale x B35- 2011, Meko x B35- 120, and the recurrent parent Meko. Some of the backcrossed lines had higher grain yield than the recurrent parents and the donor lines. These were Meko x B35- 120, Teshale x E36-1, Meko x B35- 116, Teshale x B35- 2011. The lowest grain yield was recorded by B35 and Gambella1107 x B35 followed by Meko x B35- 117.

Under well irrigated condition, Meko x B35- 120 and Teshale x B35- 2011 had higher grain yield. Drought treatment cause quite reductions for grain yield, with comparing genotypes between the two water regimes, specifically in genotypes Teshale and Gambella1107 . On the contrary, for genotypes Meko, Meko x B35- 116, B35 and Gambella1107 x B35 grain yield reduction was no as such significant.

Table.11. Mean value \pm S.E. of Grain yield (GY) for sorghum lines tested under well watered (WW) and drought stress (DS) growing conditions.

Genotypes	Irrigation	Traits
		GY
Meko	WW	4308.9 \pm 9.45 (ac)
	DS	3905.6 \pm 4.33(a,c-e)
Meko x B35- 120	WW	4745.3 \pm 6.23 (ac)
	DS	4183.1 \pm 12.5(ace)
Meko x B35- 116	WW	4364.6 \pm 5.06(ac)
	DS	4094.1 \pm 11.12(ace)
Meko x B35- 117	WW	3442.4 \pm 12.8(a-e)
	DS	2694.0 \pm 2.41(b-e)
B35	WW	2639.0 \pm 5.16(b-e)
	DS	2399.5 \pm 10.2(bde)
Teshale	WW	4347.8 \pm 6.01(ac)
	DS	3264.4 \pm 9.25(ab,de)
Teshale x E36-1	WW	4584.0 \pm 8.37 (ac)
	DS	4133.4 \pm 1.42(ace)
Teshale x B35-2012	WW	3746.4 \pm 6.71(a,c-e)
	DS	2762.8 \pm 10.2 (de)
Teshale x B35-2011	WW	4566.7 \pm 1.73(ac)
	DS	4085.9 \pm 2.44(ace)
E36-1	WW	4095.8 \pm 8.63(ae)
	DS	3602.6 \pm 5.33(a-e)
Gambella 1107	WW	4169.8 \pm 8.8(ae)
	DS	3065.3 \pm 5.06(e)
Gambella1107x B35	WW	2739.2 \pm 5.92(b-e)
	DS	2613.5 \pm 8.3(b-e)

Means followed by a common letter with in a column were not significantly at P <0.05.

4.4.4 Correlations among traits

Under post-flowering drought stress condition, days to maturity was negatively correlated with chlorophyll content ($r^2 = -0.07$). Chlorophyll content had significant and positive correlation with relative water content ($r^2 = 0.62$), green leaf number ($r^2 = 0.80$), root dry weight ($r^2 = 0.80$) and root length ($r^2 = 0.28$). CO_2 assimilation was significantly and positively correlated with transpiration ($r^2 = 0.80$), water use efficiency ($r^2 = 0.95$), relative water content ($r^2 = 0.78$), root dry weight ($r^2 = 0.83$), root length ($r^2 = 0.34$) and green leaf number ($r^2 = 0.72$). The correlation of other traits with grain yield is presented in Table 12.

Table.12. Correlation among different morphological, physiological, root related and yield components

	DM	PH	GLA	CCM	SGS	A	E	WUE	RWC	RDW	RL	GY	HGW	PWT	GLN
DM		<u>0.38</u>	0.31	-0.07	0.09	0.21	<u>0.35</u>	0.16	0.30	0.11	<u>0.36</u>	0.26	<u>0.49</u>	0.31	0.05
PH			0.03	<u>-0.42</u>	<u>0.35</u>	-0.26	-0.05	-0.27	-0.07	<u>-0.51</u>	0.20	0.28	<u>0.40</u>	<u>0.45</u>	<u>-0.48</u>
GLA				0.04	-0.20	0.06	0.08	0.09	0.10	0.03	0.25	0.16	0.30	0.13	0.24
CCM					<u>-0.64</u>	<u>0.73</u>	<u>0.48</u>	<u>0.71</u>	<u>0.62</u>	<u>0.80</u>	0.28	0.05	0.17	0.18	<u>0.80</u>
SGS						<u>-0.63</u>	<u>-0.50</u>	<u>-0.59</u>	<u>-0.47</u>	<u>-0.69</u>	-0.02	0.10	-0.11	-0.18	<u>-0.73</u>
A							<u>0.81</u>	<u>0.95</u>	<u>0.79</u>	<u>0.83</u>	<u>0.34</u>	0.18	<u>0.36</u>	<u>0.44</u>	<u>0.72</u>
E								<u>0.65</u>	<u>0.67</u>	<u>0.61</u>	<u>0.35</u>	0.24	<u>0.38</u>	<u>0.46</u>	<u>0.51</u>
WUE									<u>0.73</u>	<u>0.77</u>	0.31	0.18	<u>0.36</u>	<u>0.42</u>	<u>0.68</u>
RWC										<u>0.69</u>	0.28	0.16	<u>0.47</u>	<u>0.49</u>	<u>0.60</u>
RDW											0.09	0.00	0.05	0.09	<u>0.90</u>
RL												<u>0.56</u>	<u>0.60</u>	<u>0.55</u>	0.10
GY													0.26	<u>0.34</u>	-0.09
HGW														<u>0.73</u>	0.11
PWT															0.11
GLN															

Key: Where, underlined and bold numbers were significant at $p=0.05$; DM= Days of maturity, PH= Plant height, GLA= Green leaf area, CCM=Chlorophyll content meter, SGS= Stay green score, A=Rate of photosynthesis, E=Rate of transpiration, WUE=Water use efficiency, RWC=Relative water content, RDW=Root dry weight, RL=Root length, HGW= Hundred grain weight, PWT= Panicle weight, GLN= green leaf number and GY=grain yield

CHAPTER FIVE

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

5.1.1. Morphological traits

In the present study there were significant differences in plant height due to drought stress, this finding is in agreement with Ali *et al.* (1999) and Emam *et al.* (2010) who reported on decreasing of plant height as a result of severe drought factor. In relation to this Ali *et al.* (1999) pin pointed that, under drought condition maize genotypes plant height decreased as compared to their corresponding control plants. The decrease in plant height might be due to the reduction in cell division, cell elongation and cell enlargement (Ali *et al.*, 1999).

The correlation analysis indicated that plant height has positive correlation with GY. Mohammadi *et al.* (2012) has also found a positive relation between plant height, dry matter production and grain yield which is due to increased translocation of stored photosynthates from the stem reserves.

Post flowering drought has found to decrease the leaf area of genotype (Teshale). Similarly, Asfaw Adugna and Alemu Tirfessa (2014) found reduction of full sized leaves due to drought stress induction which is because of degradation of leaf proteins that are bound to chloroplast Borell *et al.* (2001).

Gambella1107 and Meko have higher leaf area than their introgression lines Gambella1107 x B35, Meko x B35- 117 and Meko x B35-116. This might be due to the fact that the introgression lines used reduction of leaf area as drought tolerant mechanism (Asfaw Adugna and Alemu Tirfessa, 2014).

From the backcrossed lines under DS, Meko x B35-120 has significantly higher leaf area than Meko x B35- 116, which might be due to the difference in evaporative demand (Addisie Yalew and Yemane Gebre-Egziabher, 2011). In comparison with the recurrent parents (Meko and Teshale), the introgression lines (Meko x B35, Teshale x E36-1, Teshale x B35-2011 and Teshale x B35-2012) have higher leaf area. This is in agreement with Surwenshi *et al.* (2007) most of sorghum genotypes had greater leaf area and longer active leaf area duration under post flowering drought stress. This is due to the stay green character retains chlorophyll for long time, absorption of more nitrogen and delay in chloroplast protein degradation (Kassahun Bantte *et al.*, 2010; Kamran *et al.*, 2014).

Post flowering drought stress has resulted in declining of green leaves in recurrent parents (Meko, Teshale and Gambella1107), while the stay green donor genotypes (E36-1) and (B35) and some of the introgression lines also have higher number of green leaves similar results has also been found by Kassahun Bantte *et al.* (2010) and Borrell and Hammer (2000). These is due to stay green contributes to longevity of leaves and even maintenance of greenness under drought stress condition (Borrell *et al.*, 2000b; Soo-Cheul *et al.*, 2007). Moreover, stay green genotypes maintain chlorophyll concentration under drought condition, high relative water content (Razakou *et al.*, 2013), maintain photosynthesis activity (Soo-Cheul *et al.*, 2007) and root system (Parameshwarappa *et al.*, 2012).

On the contrary, drought decrease the number of green leaves Meko & Gambella this is due to serious of biochemical and physiological process which further degrade photosynthetic apparatus (Campanile *et al.*, 2000). It could also be due to break down of chloroplasts which are the first organelles to be targeted under drought stress condition (Homayoun *et al.*, 2011).

5.1.2. Agronomic traits

Drought stress has shortened days to maturity in (Teshale), (Gambella 1107 x B35) and (Meko x B35-117). In relation to this, Aemiro Bezabih (2012) found similar results that exposure of sorghum lines for post-flowering drought stress resulted in shortening of days to maturity by 5% as compared to the well irrigated condition. This may be because decreasing of soil moisture speeds up the time of maturity before it reaches to its normal maturity period Aemiro Bezabih (2012). DM has a positive correlation with GY, similarly Almodares *et al.* (2013) found that biomass was highest in sorghum cultivar which took longer period to physiological maturity and lowest were for cultivars with reduced days after flowering.

5.1.3. Physiological traits

Drought stress decreased relative water content (RWC) in (Gambella), (Gambella x B35) and (Meko x B35-117) which is similar to previous reports (Ulmale *et al.*, 2013; Addisie Yalew and Yemane Gebre-Egziabher, 2011) these is due to lower absorption of water from the soil and loss through the stomatal opening. It may also be due to lower accumulation of osmolytes to maintain tissue turgor (Abdullah *et al.*, 2011).

However, some stay green introgression lines such as Meko x B35-116, Teshale x B35- 2011, Teshale x E36-1, and Meko x B35-120 have higher water content that may show their drought tolerance. Related to this Keyvan (2010) reported similar results in bread wheat cultivars and

found the same result. He reported that drought tolerant cultivars have high relative water content due to more absorption of water and reduction of water loss through stomatal opening. In the present study it has been found that RL and RWC had a positive correlation, like wise Bibi *et al.* (2012) found that extensive root system plays a major role for maintenance of high leaf water content.

5.1.3.1. Gas exchange parameters and water use efficiency

The present study showed that drought stress significantly reduces photosynthesis in (Teshale x B35- 2012, Meko x B35- 117, and Gambella1107 x B35). This finding goes with the report of Khakwani *et al.* (2012). This could be due to stomatal closure (Khakwani *et al.*, 2012) as a consequence of lower water content or hormonal (ABA) signals transferred to the leaves since it leads to closure of stomata to resist CO₂ assimilation. Moreover, Tilahun Amede and Schubert (2003) found photosynthesis reduction in common bean as stomata are closed in response to drought stress. Non-stomatal influence could also be reason to reduce photosynthesis. These include decrease RuBp regeneration, ATPase production, and Rubisco activity (Khakwani *et al.*, 2012).

Decrease in photosynthetic efficiency in the recurrent parents could also be due to reduction in potential quantum yield with the photo-inhibition of PS II, and those which were not decreased in response to drought had maximum quantum yield and high efficiency of using photochemistry and carbon assimilation (Addisie Yalew and Yemane Gebre-Egziabher, 2011).

Photosynthesis is highly and positively correlated with transpiration ($r^2=0.81$). This is due to stomatal opening resulting in high transpiration rate as a consequence of more higher CO₂ assimilation.

Genotypes, B35, E36-1 and introgression lines showed less sensitivity in terms of CO₂ assimilation. This indicates that stay green trait contribute for better assimilation rate under severe water stress conditions these is associated with higher RWC, chlorophyll content and longer root system. Moreover, the increasing of assimilation could be attributed by higher transpiration rate (Taiz and Zeiger, 2006).

Green leaf area was positively correlated with photosynthesis ($r^2=0.11$). Genotypes (Meko x B35- 120, Teshale x B35- 2011 and Teshale x E36-1) has higher green leaf area and also have better CO₂ assimilation rate. Addisie Yalew (2010) has also found a positive and significant correlation between the two traits. Conversely, genotypes which have smaller green leave area also have lower photosynthesis.

In the present study drought has decreased transpiration rate in Meko, Teshale and Teshale x E36-1. This is due to biochemical changes in the root system induced by water deficit stress and closure of stomata (Liu *et al.*, 2002; Siddique *et al.*, 2000). Likewise Liu *et al.* (2002) reported similar results in cotton cultivars under drought stress.

Higher transpiration rate under drought condition were achieved by Meko x B35-120 and Teshale x E36-1, which also had longer root length. Thus, this shows that longer root length increase absorption of underground water to compensate the evaporative cost. Similar relation has also been found by Tilahun Amede and Schubert (2003) in chick pea genotypes.

Some of the stay green genotypes in this study have higher water use efficiency than their recurrent parents which is in agreement with the finding of Thevar *et al.* (2010); Razakou *et al.*(2013) and Songsri *et al.* (2009). This is due to increased CO₂ assimilation and transpiration rate as WUE is a function of the ratio of whole-plant biomass to cumulative transpiration

(Kapanigowda *et al.*, 2012). It could also be the result of higher root dry weight and larger root system (Songsri *et al.*, 2009) which increase water absorption (Thevar *et al.*, 2010; Songsri *et al.*, 2009). On the other hand, Gambella and Teshale showed a reduction in WUE as it is similarly reported by Addise Yalew (2010) which could be as a result of lower RWC, CO₂ assimilation and transpiration rate.

Water use efficiency has also been found to have a positive correlation with root length ($r^2=0.31$). In relation to this Thevar *et al.* (2010) found that sorghum genotypes grown under drought condition have longer root length and better water use efficiency. This is because longer roots enables crops to pay the cost of transpiration demand. Moreover, WUE has got a positive correlation with GY ($r^2=0.38$). This is perhaps due to increased biomass production to cumulative transpiration which further increase assimilates allocation to the grain (Kapanigowda *et al.*, 2012).

5.1.3.2. Chlorophyll content

Drought has significantly reduced chlorophyll content in non stay green parents Teshale, Meko and Gambella1107. Likewise Li *et al.* (2012) found that chlorophyll content has decreased in cotton cultivars. These may be due to the degradation of leaf protein which is bound in to chlorophyll pigment-protein complex and lower N absorption during the grain filling period (Borrell and Hammer, 2000; Borrell *et al.*, 2001).

Genotypes (B35, E36-1) and introgression lines have also higher chlorophyll content than all the other genotypes. Similar results were found in sorghum (Kassahun Bante *et al.*, 2010; Borrell and Hammer, 2000) which can be the result of a increased nitrogen absorption from the soil.

Scoring of stay green rating under exposure to post flowering drought shows that some of the recurrent parents and the introgression lines have higher rate of leaf senescence. Borrell *et al.* (2000) and Soo-Cheul *et al.* (2007) reported similar results where the leaf becomes senescent as plants are exposed to post flowering drought. The donor parents, E36-1 and B35 have significantly lower scoring (more greenness) than the recurrent parents and backcrossed lines. This is due to SG trait that maintains current photosynthesis and delay in chlorophyll degradation (Borrell *et al.*, 2001). This further indicates that they possess functional stay green type (Thomas and Howarth, 2000).

5.1.4. Root traits

5.1.4.1. Root length

In the present study introgression lines (Teshale x B35- 2011 and Meko x B35- 120) had higher root length. This might be because the traits are inherited from the donor parents as the donor parent (B35) has longer root length (Nahar and Gretzmacher, 2011). Similar research output has found by Liu *et al.* (2005) who indicated spring wheat root length has decreased as a result of soil drying which were due to increasing in allocation of assimilates to below ground roots.

On the other hand, Teshale and Teshale x B35-2012 decreases root length under DS. Similar result has found in sorghum (Bibi *et al.*, 2012) and in maize (Nejad *et al.*, 2010) which might be due to lower allocation of assimilates from shoot to root. Consequently, the root might not get adequate strength to penetrate through dry soil and to extend further (Bibi *et al.*, 2012).

5.1.4.2. Root dry weight

In this study, some of the introgression lines (Meko x B35- 120 and Teshale x B35- 2011) have higher root dry weight than other lines and parents. Likewise sorghum seedlings exposed to

drought stress develop extensive root system (Bibi *et al.*, 2012). This is because when the roots elongate in search of water under water limited environments, and accumulation of different solutes all contributes to increasing of root dry weight. On the contrary, root dry weight decreases in some of the lines (Meko x B35- 117, Teshale x B35- 2011 and Gambella1107) which might be due to leaf area reduction which in turn reduces assimilate accumulation (Bibi *et al.*, 2012).

The present study found a positive correlation between RDW and GY. Accordingly Blum (2005) has also found high root biomass ensures better absorption of water, which further contributes to high yield production. Similarly, Viodhana and Ganesamurthy (2010) reported genotypes with longer root length and dry weight have better performance and yield production under drought condition.

5.1.5. Yield and yield components

The present study has showed that drought significantly reduced grain yield in some of introgression lines (Meko x B35-117, Teshale x B35- 2012 and Gambella1107 x B35). Similarly, Asfaw Adugna and Alemu Tirfessa (2014) observed reduction in grain yield in a month of exposure to post flowering drought stress. This can be due to the fact that drought decreases substomatal CO₂ concentration, water content and chlorophyll concentration, which further reduce the photosynthetic activity and translocation of assimilates (Mafakheri *et al.*, 2010).

Moreover, some of the genotypes (Meko x B35-120, Teshale x B35-2011 and Teshale x E36-1) which have high chlorophyll content value also have higher grain yield. Similarly, Kamara *et al.*, 2014 and Ulmale *et al.* (2013) reported chlorophyll content to have positive correlation with grain yield.

Stay green genotypes (Meko x B35- 120), (Meko x B35- 116), (Teshale x B35- 2011) and (Teshale x E36-1) were observed to have higher yield under drought condition due to high GLA ratio at maturity and GLN (Borrell and Hammer, 2000; Soo-cheul *et al.*, 2007, Renuka and Chimmad, 2006).

Panicle weight and 100 grain weight have significant positive correlation with grain yield which is in agreement with the result of Beheshti and Fard (2010), Surwenshi *et al.* (2007) and Malala (2010). With these facts in the present study genotypes with higher panicle weight (Meko x B35- 120, Meko x B35-116, Teshale x E36-1 and Teshale x B35-2011) has higher GY due to the continuous photosynthesis and dry matter allocation to the head. On the contrary, drought has decreased 100 grain weight. Similarly Gholinezhad *et al.* (2009) has found that sunflower 1000-grain weight decreased due to severe drought stress which could be related to the lack of stored carbohydrates and decreasing durability of leaf area. Likewise, Addise Yalew and Yemane Gebre-Egziabher (2011) put forwarded that the decrease in grain yield under post flowering drought in pearl millet is due to reduction of seed size and seed weight per panicle.

5.2. Conclusion

Recently, plant breeders have been using the stay green trait as a means of terminal drought tolerance. In the present study, the performance of seven stay green introgression lines and their parents were studied for their, morphological, physiological, root and yield components under induced post-flowering drought stress. The study has revealed that sorghum genotypes showed different responses under post-flowering drought. There was also interaction between genotypes and the applied moisture regimes for most of the studied characters. Out of the introgression lines Meko x B35- 120, Meko x B35-116, Teshale x B35-2011 and Teshale x E36-1 showed better performance in terms of gas exchange parameters, leaf area, chlorophyll content, root traits and yield components.

Among the measurements chlorophyll content, transpiration, assimilation, water use efficiency, relative water content, root length and root dry weight have a positive contribution to drought tolerance of the genotypes due to these fact most of genotypes with those trait has also better yield under post flowering drought stress.

5.3. Recommendation

Even though positive results were found using morphological and physiological characters, it is recommended that further biochemical studies be done to have a comprehensive understanding of the mechanisms associated with drought tolerance in sorghum. The varieties Meko and Teshale showed better performance for drought tolerance using combination breeding and thus the introgression lines Meko x B35-120, Meko x B35-116, Teshale x B35- 2011 and Teshale x E36-1 were identified for production and further breeding work.

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APPENDICES

Appendix 1. The mean value of phenological periods

Days of 50% emergency							Days of 50% flowering					
Well watered				Drought stress			Well watered			Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	5	6	6	5	6	6	61	62	63	62	60	67
Meko x B35 120	6	6	6	5	6	6	67	65	62	67	62	71
Meko x B35 116	5	5	5	5	5	5	65	63	65	66	63	67
Meko x B35 117	6	6	5	6	6	5	64	62	65	63	62	70
B35	6	6	5	5	6	5	65	60	60	60	60	65
Teshale	6	7	5	6	7	5	69	61	62	67	62	69
Teshale x E36-1	5	7	6	5	7	6	62	62	62	65	60	68
Teshale x B352012	5	6	7	6	6	7	67	63	68	64	62	71
Teshalex B35-2011	5	6	6	6	6	6	68	63	69	67	63	75
E36-1	5	6	5	5	6	5	65	63	65	60	63	68
Gambella	7	6	5	5	6	5	65	65	66	64	62	69
Gambella x B35	6	5	7	6	5	7	71	69	66	69	68	73

Appendix 2. The mean value of phenological period

Days to maturity						
Well watered				Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	97	96	100	95	93	92
Meko x B35 120	98	96	99	94	94	93
Meko x B35 116	97	97	98	93	92	93
Meko x B35 117	98	97	98	90	91	90
B35	95	94	95	90	92	93
Teshale	100	99	98	91	92	94
Teshale x E36-1	98	97	99	93	92	93
Teshale x B35-2012	98	95	98	93	94	94
Teshalex B35-2011	98	98	100	94	92	93
E36-1	99	97	100	93	92	93
Gambella	98	95	98	95	93	92
Gambella x B35	96	97	98	89	90	91

Appendix3.Mean value of Morphological traits

Plant height							Leaf area					
Well watered				Drought stress			Well watered			Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	1.53	1.99	1.9	1.47	1.69	1.65	447.35	460.94	433.24	414	461.02	413.49
Meko x B35-120	1.93	2.12	1.66	1.83	1.83	1.84	489.2	465.98	523.97	419.30	538.23	474.76
Meko x B35-116	1.83	1.76	1.69	1.74	1.83	1.57	501.52	517.88	518.42	353.45	380.71	326.25
Meko x B35-117	1.79	2.01	1.78	1.68	1.68	1.95	359.38	445.38	402.17	344.59	367	355.79
B35	0.78	0.8	0.84	0.63	0.58	0.62	353.48	395.01	374.83	342.2	337.49	393.46
Teshale	2.37	2.86	2.72	2.16	2.57	2.3	345.70	315.22	351.83	360.59	262.14	311.37
Teshale x E36-1	2.22	2.37	2.13	2.35	1.98	2.16	477.60	418.74	409.99	451.51	389.17	440.34
Teshale x B35-2012	2.1	2.47	2.21	1.78	2.23	2.27	428.00	448.05	436.63	370.06	447.82	447.63
Teshale x B35-2011	2.22	2.63	2.51	2.16	2.26	2.34	487.78	419.43	461.23	460.22	421.54	436.88
E36-1	1.81	1.89	1.76	1.68	1.96	1.63	483.45	477.27	411.31	427.65	432.02	424.08
Gambella	1.83	2.02	1.95	1.61	1.87	2.13	561.44	493.31	527.37	469.97	468.3	478.11
Gambella x B35	0.96	0.97	1.11	0.88	0.92	1.05	491.08	474.57	484.24	399.63	423.13	423.3

Appendix 4. Mean value of Morphological trait

Green leaf number						
Well watered				Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	9.8	9.2	11	6	7.6	7.4
Meko x B35 120	13.4	13	12.6	10.4	11.6	11
Meko x B35 116	6.2	5.6	6.2	6.4	5.6	6
Meko x B35 117	9	10.2	10.8	7.2	6.6	7.2
B35	13.8	14.6	13.6	14.2	13.6	14.6
Teshale	5	3.25	3.75	13.8	4.2	4
Teshale x E36-1	11.2	11.2	10.6	6.6	8.8	8.6
Teshale x B35-2012	12.4	11	12.6	8	8.8	10.4
Teshalex B35-2011	13.8	14	14.2	10.2	10	9.8
E36-1	14.6	14.4	13	13.8	12.6	14.4
Gambella	8.4	8.8	9.8	6.8	6.2	8
Gambella x B35	8.6	12	9.4	7.2	8.6	8.2

Appendix 5. Mean value of morphological-physiological trait

Chlorophyll content							Stay green score					
Well watered				Drought stress			Well watered			Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	29.13	36.58	43.09	20.69	16.48	27.72	2	4	5	3	4	4
Meko x B35-120	45.65	45.67	63.66	42.38	37.64	44.91	2	2	2	3	2	2
Meko x B35-116	43.46	38.87	44.35	38.18	27.95	28.42	2	2	3	3	3	3
Meko x B35-117	37.76	31.27	52.44	31.72	23.22	36.9	3	2	2	2	3	4
B35	60.29	56.29	54.87	55.88	59.35	48.12	1	1	1	1	2	1
Teshale	29.94	29.68	25.53	20.43	17.87	16.27	2	3	3	5	4	3
Teshale x E36-1	50.02	43.57	43.61	23.7	29.71	50.03	2	2	2	2	3	2
Teshale x B35-2012	34.42	43.13	44.42	38.9	26.03	20.57	2	2	2	2	3	3
Teshale x B35-2011	49.33	41.3	50.32	35.06	42.86	38.62	2	2	2	3	2	2
E36-1	664.65	55.35	46.73	49.31	53.47	55.52	1	1	1	1	2	1
Gambella	29.13	36.58	43.09	22.7	29.14	19.71	3	4	4	4	4	5
Gambella x B35	26.01	38.11	44.84	31.73	20.47	24.57	2	3	3	3	3	3

Appendix 6. Mean value of physiological traits

CO ₂ Assimilation							Transpiration rate					
Well watered				Drought stress			Well watered			Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	2.18	1.76	2.08	1.17	1.39	1.53	6.57	6.72	6.25	4.88	6.12	5.83
Meko x B35-120	4.19	4.71	4.29	1.66	1.83	1.49	7.32	7.41	7.37	5.61	6.29	6.14
Meko x B35-116	2.09	1.92	1.78	1.17	1.18	1.3	5.65	7.01	6.25	5.07	4.75	6.91
Meko x B35-117	0.96	1.28	1.15	0.44	0.44	0.51	4.86	4.93	4.97	4.16	4.25	4.33
B35	5.88	5.76	3.77	2.65	2.14	2.38	7.31	8.05	7.09	6.45	7	6.54
Teshale	1.77	1.62	1.67	0.78	0.69	0.78	6.07	6.01	5.53	4.64	4.15	6.26
Teshale x E36-1	2.95	2.67	1.76	1.141	1.52	1.54	6.37	7.44	7.03	6.12	5.78	6.18
Teshale x B35-2012	1.57	1.26	1.04	0.57	0.37	0.61	6.24	5.41	4.89	4	4.57	5.1
Teshale x B35-2011	2.95	3.07	1.92	1.6	1.58	1.58	7.2	7.11	6.64	6.7	5.27	6.65
E36-1	4.38	4.9	4.47	2.09	1.98	1.89	7.08	7.24	7.99	6.07	6.22	6.08
Gambella	1.43	1.31	1.34	0.68	0.66	0.65	5.07	5.04	5.51	4.37	4.84	5.77
Gambella x B35	0.94	0.94	0.88	0.32	0.36	0.39	3.92	3.82	5.08	4.38	3.93	3.45

Appendix 7. Mean value of physiological traits

Water use efficiency							Relative water content					
Well watered				Drought stress			Well watered			Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	0.36	0.27	0.33	0.24	0.22	0.26	88.43	69.86	83.49	59.92	86.26	60.35
Meko x B35-120	0.49	0.64	0.6	0.29	0.28	0.24	84.21	79.42	81.17	70.2	81.25	76.06
Meko x B35-116	0.37	0.27	0.28	0.25	0.27	0.18	75.23	77.5	81.23	73.24	61.77	61.03
Meko x B35-117	0.19	0.26	0.22	0.13	0.12	0.15	71.57	67.32	72.87	57.56	52.79	49.37
B35	0.8	0.72	0.52	0.41	0.31	0.36	88.24	87.82	87.91	80.41	88.66	73.17
Teshale	0.3	0.26	0.3	0.18	0.17	0.12	87.27	72.62	68.71	62.65	64.67	56.48
Teshale x E36-1	0.47	0.36	0.26	0.23	0.27	0.25	80.05	69.63	87.67	72.69	65.56	73.14
Teshale x B35-2012	0.33	0.22	0.21	0.18	0.08	0.17	75.42	75.64	73.26	67.11	69.45	56.09
Teshale x B35-2011	0.4	0.44	0.28	0.25	0.29	0.26	78.34	76.52	80.97	80.16	76.36	65.25
E36-1	0.65	0.71	0.57	0.28	0.33	0.3	87.82	83.26	83.98	74.91	78.06	79.05
Gambella	0.29	0.25	0.24	0.17	0.13	0.14	80.67	65.61	82.27	57.94	53.63	52.02
Gambella x B35	0.24	0.24	0.16	0.08	0.14	0.14	66.25	67.55	72.45	53.11	54.31	43.6

Appendix 8. Mean value of root traits

Root length							Root dry weight					
Well watered				Drought stress			Well watered			Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	35	34	31.66	35.66	21.33	24.33	30.13	29.11	29.61	23.93	24.32	32.12
Meko x B35-120	57.66	45	53.33	45.66	39	37.66	35.92	39.35	37.63	33.02	30.43	31.72
Meko x B35-116	43.33	43.66	37.33	32	29	35	28.23	27.62	27.97	20.6	22.4	24.98
Meko x B35-117	31.33	32.33	38.66	26.33	26	24.33	23.27	27.22	26.28	17.65	17.65	17.71
B35	40.66	34.33	37.66	24.33	24.33	22.33	41.44	41.44	41.33	46.87	48.64	47.78
Teshale	37.33	43.66	38	23.33	25	26	24.5	29.11	26.44	19.14	20.59	19.86
Teshale x E36-1	42.33	39.33	44.33	39.33	27.33	36.66	31.69	31.71	31.86	23.2	24.02	25.74
Teshale x B35-2012	23.66	35.33	39.66	22.66	23.33	21.66	32	32.18	32.09	25.47	27.46	26.47
Teshale x B35-2011	39.33	46.33	45.33	34.66	37.33	39.33	33.37	34.97	35.27	31.48	29.29	25.86
E36-1	36.33	37	36.33	31.33	31	28.33	38.99	39.37	39.01	40.15	38.57	39.11
Gambella	34	29	34	27	29.33	22.33	25.75	28.31	27.12	18.52	22.77	19.69
Gambella x B35	34.66	33	31	28.66	25.33	25.33	25.12	30.62	27.86	20.6	22.18	24.72

Appendix 9. Mean value of yield components

Panicle weight						
Well watered				Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	143.03	163.83	157.97	119.82	140.83	158.22
Meko x B35-120	144.95	202.65	192.4	131.98	163.31	165.16
Meko x B35-116	150.61	124.2	137.78	125.52	124.59	105.22
Meko x B35-117	106.22	131.95	115.5	84.95	104.11	113.87
B35	92.12	85.68	117.47	92.29	92.91	97.71
Teshale	79.17	81.54	84.84	121.48	113.25	118.73
Teshale x E36-1	156.2	172.8	170.66	146.62	137.41	158.24
Teshale x B35-2012	124.5	117.21	120.68	102.77	105.11	114.63
Teshale x B35-2011	161.73	168.34	187.17	139.85	138.51	167.47
E36-1	138.48	143.59	160.33	137.14	121.07	111.82
Gambella	120.43	124.86	127.49	62.82	29.57	60.54
Gambella x B35	92.46	79.38	85.46	73.37	83.99	76.9

Appendex 10 Mean value of yield components

Hundred seed weight						
Well watered				Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	3.37	3.5	3.08	2.86	2.59	3.32
Meko x B35-120	4.78	3.62	4.2	2.99	3.62	4.29
Meko x B35-116	3.54	3.35	3.2	3.2	2.49	2.78
Meko x B35-117	4.13	3.18	3.77	2.29	2.21	1.87
B35	2.87	3.3	2.57	1.75	2.29	1.99
Teshale	3.3	4.15	3.14	2.69	2.34	2.36
Teshale x E36-1	3.16	4.17	3.45	3.3	2.94	2.83
Teshale x B35-2012	2.99	3.26	2.36	2.35	2.39	2.44
Teshale x B35-2011	3.56	3.66	3.11	3.24	3.67	2.5
E36-1	3.43	3.64	3.53	2.86	2.6	3.15
Gambella	3.29	3.25	3.39	2.61	2.15	2.26
Gambella x B35	3.19	2.89	2.44	1.35	2.11	1.87

Grain yield						
Well watered				Drought stress		
Genotypes	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meko	4190.37	4073.16	4663.2	3565.48	3993.73	4157.68
Meko x B35-120	4565.24	4995.42	4675.26	3298.37	4970.66	4280.23
Meko x B35-116	4821.4	4320.32	3952.22	4182.26	4161.18	3938.86
Meko x B35-117	3148.04	3686.8	3492.21	2457.98	2856.55	2767.55
B35	2496.5	2571.58	2848.9	2163.08	2297.65	2737.64
Teshale	4527.02	4547.77	3968.75	3229.24	3131.02	3432.91
Teshale x E36-1	4426.36	4406.03	4919.55	3758.12	4356.72	4285.28
Teshale x B35-2012	4238.43	3957.18	3043.44	2717.9	2792.4	2778.01
Teshale x B35-2011	4592.07	4831.84	4276.17	3262.09	4721.89	4273.85
E36-1	4029.42	3865.1	4392.9	3617.34	3450.56	3739.86
Gambella	4675.58	4209.84	3624.06	2939.45	3084.4	3171.92
Gambella x B35	3049.05	2237	2931.52	2962.05	2056.93	2821.42

Appendix 12. Mean value of grain yield

DECLARATION

I, the undersigned, declare that this is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been fully acknowledged. I cede copyright of the thesis in favor of Addis Ababa University.

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