Physiological Indices for Drought Tolerance in Stay-green Sorghum (*Sorghum bicolor* L. Moench) Accessions

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GRADUATE PROGRAMS

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ABSTRACT

Physiological Indices for Drought Tolerance in Stay-green Sorghum (*Sorghum bicolor* L. Moench) Accessions

Eyerusalem Arusi Morka, Msc. Thesis

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Drought is one of major stress factor, which results in a profound effect on plant growth and yield. The effect becomes severe mainly where rainfed agriculture practiced. Having this thought, assessing of genotypes that can better tolerate such condition and methods that can help for screening are important in reducing associated yield loss. The study was carried out at Addis Ababa University, College of Natural Science, Addis Ababa in glasshouse using randomized complete block design (RCBD) with 3 replications. Five Sorghum accessions Sorcoll 141/07, Sorcoll 146/07, Sorcoll 163/07, Sorcoll 060/07 and E36-1 (check) were used and grown under well watered and post- flowering water stress condition. Data were collected for growth parameters of specific leaf area (SLA) and specific leaf weight (SLW), Biochemical parameters such as leaf proline, total soluble sugar (TSS), leaf nitrogen (N%) and protein content yield per panicle and drought indices such as tolerance (TOL), mean productivity (MP), yield stability index (YSI), yield index (YI), stress tolerance index (STI), stress susceptibility index(SSI), geometric mean (GM) and harmonic mean (HM). Based on SLA and SLW value, Sorcoll 141/07 and Sorcoll 060/07 were observed to be drought tolerant. Proline, TSS, N, Protein, yield per panicle, TOL, SSI, MP, STI, GM, HM value had revealed Sorcoll 060/07 and E36-1 tolerant accessions whereas Sorcoll 146/07 was sensitive. Correlation result showed that there existed a positive and negative association of proline and SLW with yield at $P < 0.05$*. Future studies will be required to assess the association of stress induced osmolytes with SG trait and QTL identification for better utilization of these traits in breeding program.*

Key words/phrases: Sorghum; drought tolerance; growth and biochemical parameters; drought indices.

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LIST OF ACRONYMS

CSA:	Central Statistical Agency
FC:	Field Capacity
GM:	Geometric Mean
HM:	Harmonic Mean
LSD:	Least Significance Difference
MP:	Mean Productivity
N:	Nitrogen
PC:	Principal Component
PCA:	Principal Component Analysis
RCBD:	Randomized Complete Block Design
SG:	Stay-green
SLA:	Specific Leaf Area
SLW:	Specific Leaf Weight
SSI:	Stress Susceptibility Index
STI:	Stress Tolerance Index
TOL:	Tolerance
WS:	Water Stressed
WW:	Well Watered
YI:	Yield Index
YSI:	Yield Susceptibility Index

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Water is one of the crucial requirements for plants to survive, grow and develop and drought stress due to water deficit is one of the most important abiotic stresses that affect plant growth and ultimately limits crop production. The shortage of water in the soil reserve results in limitation of available water in the plant cell and leads to loss of cell turgidity and closure of stomata. This condition limits CO_2 assimilation and nutrient uptake which is driven by transpiration and causes lower chemical energy production which required for different processes to take place in the plant. Drought is the most limiting factor for crops worldwide and causes a serious loss of yield and productivity in arid and semi arid regions (Waseem *et al*., 2011).

Drought is the most important problem of dry land regions and leads to poverty, food insecurity and malnutrition in sub Saharan African countries like Ethiopia. The late onset, below normal amount and erratic nature of the rain highly affects the rainfed agriculture and the livelihood of peoples in the region (Dereje Assefa *et al*., 2007).

Sorghum is one of an important cereal crops cultivated in dryland regions and serve as a stable food and animal feed for more than 500 million people in 30 countries (Kumar *et al.*, 2011). The unique ability of sorghum to grow in resource poor areas under low rainfall condition makes the crop chosen by peoples living in these areas (Leder, 2004). Due to this, 90 % of sorghum growing areas are found in developing countries especially in Africa and Asia.

Sorghum is a cereal crop which belongs to Poaceae (Gramineae) family and fifth most important cereal crop which grows on 40 million ha in 105 countries of Africa, Asia, Oceania and the Americans. In Ethiopia, it is one of the warm weather adapted cereal crops and grows in northwest and eastern parts of the country over wide range of agro-ecological conditions (Abu Tefera, 2013). It is an important stable food crop and source of proteins, calories and minerals for millions of people in arid and semi arid tropics of Africa and Asia (Nagarjuna, 2007). In terms of production, sorghum is the 5th important crop in the world, the 2nd cereal in Africa and the 4th major cereal produced in Ethiopia following Teff, Wheat, and Maize (Alemayehu Seyoum *et al*., 2012).

Asfaw Adugna (2007) explained that sorghum is the most widely growing cereal and stable food for millions of poor Ethiopians. Food insecurity is a major event of moisture scarce area but, sorghum has wider adaptation and can grow especially in such areas were other crops cannot survive and give various uses such as food, feed, fuel and materials for construction of traditional houses. It is believed that sorghum was domesticated in Ethiopia and surrounding countries before 4000 -3000 BC. Genus Sorghum is a widely diversified and all cultivated sorghum belongs to *Sorghum bicolor* subsp. *bicolor* (Mutava, 2009). Sorghum is widely diversified crop (more than 30,000 varieties) found across the world (Leder, 2004). Sorghum richness and visible genetic diversity for drought tolerance makes it an excellent crop model for evaluation of genetic and physiological mechanisms of drought tolerance (Mutava, 2009).

Plants differ in their tolerance level, which differ with their ability to use all drought resistance and protective mechanisms (Zivcak *et al*., 2009). Therefore, there is difference in their response for period of moisture shortage. These are: (1) drought escape; through early maturing and developmental elasticity to complete the life cycle before the onset of water stress, (2) drought avoidance; by reducing leaf area, stomatal closure to reduce water loss and maintain high tissue water potential under soil moisture stress, (3) drought tolerance in which plants survive desiccation without injury on protoplasm and keep the capacity of protoplasm for normal growth when the cell is rehydrated and this is achieved by osmotic adjustment (Taiz and Zeiger, 2006). Even if these drought tolerance mechanisms are divided into three, plants can utilize and respond to drought by combining these mechanisms (Mutava, 2009).

Sorghum is tolerant to drought better than most of other grains and characterized by having well developed and finely branched root system that are efficient in water absorption. It also characterized by the development of small leaf area and folding during warm condition and waxy cover to prevent desiccation and stomatal closure to limit water loss (Plessis, 2008).

There are four major growth stages in sorghum that are susceptible to drought. These are: germination, post-emergence, pre-flowering and post-flowering. Of these, post-flowering drought is the most limiting factor for sorghum production across the world. Climatic variations like availability of moisture along with genetic ability determine the drought tolerance characteristics of the crop (Kumar *et al*., 2011). Sorghum genotypes respond differently to severe drought stress at post-flowering stage. Genotypes that are sensitive to terminal drought characterized by pre-mature leaf and plant senescence; stalk collapse and lodging; charcoal rot and reduced grain number and size. Other genotypes are tolerant to terminal drought and are called non senescent or stay-green. Sorghum genotypes with stay-green traits retain photosynthetically active leaves under water deficit condition during grain filling period (Nagarjuna, 2007).

Osmotic adjustment is an important physiological adaptation in response to drought tolerance and it involves accumulation of solutes such as sugars, amino acids (proline) within a plant tissue in response to developing drought stress. These help to maintain water potential of the plant and prevent desiccation. Osmotic adjustment maintain the relative water content (Blum, 2005), opening of stomata and prolong photosynthesis at lower water potential (Anant *et al*., 2011), prevent chlorophyll degradation or leaf senescence and plant death (Blum, 2005; Anant *et al*., 2011), N loss and sustain yield under drought condition (Blum, 2005). In order to assess the drought tolerance of genotypes, studying the physiological indices of the crop and the contribution of osmotic adjustment for drought tolerance and yield is very important for selection of tolerant genotypes.

The relative yield performance of genotypes under drought stressed and non-stressed environments can be used as an indicator to identify drought resistant varieties in breeding program for drought susceptible areas (Raman *et al*., 2012). Ability of the cultivars to grow and yield fairly under drought stressed condition determines the stability of yield production. Several drought indices that are based on drought resistance or susceptibility of genotypes have been suggested and calculated from mathematical relationship between yield under drought and normal condition. These includes: stress tolerance index (STI), stress susceptibility index (SSI), tolerance (TOL), mean productivity (MP), yield index (YI), yield stability index (YSI), geometric mean (GM) and harmonic mean (HM) (Raman *et al*., 2012), yield index (YI) Lin *et al*., 1986 cited in Aliakbari (2013). Banayjedi *et al*. (2012) indicated

STI, MP and GM indices are essential in assessing performance of genotypes under stress condition. Depending on the correlation and biplot analysis, Aliakbari *et al*. (2013) found TOL and MP have potential to select drought tolerant genotypes. Also YSI and YI are indices suggested by breeders for screening drought tolerant genotypes (Aliakbari *et al*., 2013). Karimizadeh *et al*. (2011) suggested SSI and STI indices as best tools in screening drought resistant genotypes.

Therefore, studying the responses of accessions for drought tolerance and evaluating the effectiveness and reliability of physiological indices can help to select suitable genotype for drought prone areas and increase the production status of the area.

1.2. Objectives of the study

The study was carried out with the following objectives:

1.2.1. General objective

- To evaluate drought tolerance in sorghum accessions with stay-green traits by using drought indices.

1.2.2. Specific objectives

- To estimate the amount of free proline of each accessions under each treatment.
- To estimate the amount of total soluble sugars of each accessions under each treatment.
- To examine the leaf nitrogen and protein content in response to drought stress.
- To assess significance of stay-green trait for yield effect under terminal drought condition.
- To identify the best drought indices which help to select accession that can grow under drought prone areas.

CHAPTER TWO

2. LITERATURE REVIEW

2.1.Effect of drought on plant growth

The effect of water stress starts from plant cells. Every process within a plant cell affected by water stress. Water availability is the major factor that affects plant growth and development (Waseem *et al*., 2011). Water deficit is a condition at which plants suffer from lack of enough water in the soil which reduced as a result of limited precipitation. The phenomena result growth inhibition and also other metabolic and physiological changes (Vajrabhaya *et al*., 2001).

All stages of plant growth from seed germination to plant maturation are dependent on water (Samar Raza *et al*., 2012). The establishment in the field also affected by water stress (Jiregna Gindaba *et al*., 2004; Ulemale *et al*., 2013). The decline in plant growth is the most outcome of water unavailability in the plant and the drop of water potential results for retardation of leaf and stem elongation (Younis *et al*., 2000). The inhibition of cell expansion due to turgor loss of cells and reduction of carbon assimilation are the major reasons for reduction in growth as a consequence of drought. Turgor loss due to water stress inhibits turgor dependent activities such as leaf expansion and root elongation (Taiz and Zeiger, 2006).

2.1.1. Specific leaf area (SLA)

Leaf area is one of important factor that can determine CO_2 assimilation. Concentration of more nutrients especially nitrogen is important to endure drought stress. SLA shows the thickness of the leaf and the extent of photosynthetic machine potential for greater assimilation (Painawadee *et al*., 2009).

Plants with higher SLA have higher metabolic activity than those with lower SLA. Study showed that flag leaf area has a strong positive correlation with grain yield but, negative relation with specific leaf weight (Ali *et al*., 2009). Pawar (2007) showed that SLA decreases continuously from 30-90 days after sowing. However, more leaf area might cause more water losses due to more evapotranspiration from the leaf surface. Ali *et al*. (2009) suggested that flag leaf area could contribute towards water stress tolerance. Painawadee *et al*. (2009) also found drought stress significantly decreased the SLA of peanut and stated that lower SLA value indicates the higher drought resistant genotype.

2.1.2. Specific leaf weight (SLW)

Specific leaf weight has a great association with photosynthesis products. Borrell *et al*. (2001) found that SLW associates with specific leaf nitrogen (SLN) and leaf nitrogen concentration (LNC) and three variables are interdependent to each other. B-35 stay-green variety has thicker leaf as compared to senescent hybrid. A small remobilization of assimilates out of leaf in stay-green promotes relatively thicker leaf than senescent hybrids. The negative correlation of Specific leaf weight with grain yield suggests that high leaf area and lower specific leaf weight help for the selection of the genotypes with higher grain yields under water stressed condition (Ali *et al*., 2009).

2.2. Effect of drought on plant physiology

Drought causes harmful effects on plant life. Reduction of growth occurs as a result of various physiological responses including change in ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon assimilation and utilization (Kizilimak, 2012). Growth and physiological activity (photosynthesis) are the two processes most critical for crop yield are among the first plant functions to be inhibited by water deficit. A prolonged decline in photosynthetic activity will lead to nutrient shortage and growth limitation (Opik and Rolfe, 2005). However, when water deficit develop slowly, it allow changes in developmental process (Taiz and Zeiger, 2006).

Photosynthesis has a great role in determining crop productivity in all species. The most important effect of drought on photosynthesis expressed by reduction in growth and productivity (Zlatev and Lidon, 2012). Water stress leads for closure of stomatal and reduced transpiration rates, a decrease in the water potential of plant tissues, decrease in photosynthesis and growth inhibition (Yordanov *et al*., 2003). The severity of drought stress determines the level of physiological effects on the plant. Photosynthesis is less sensitive to mild water stress than leaf expansion. Water deficit leads to turgor loss of guard cells and closure of stomata and it consequences for limitation of CO_2 intake and reduction of internal CO_2 concentration, finally reduce photosynthesis product. It also result damage on chloroplast as a result of excess excitation energy which is not quenched by chloroplast and results for production of reactive oxygen species (ROS) (Waseem *et al*., 2011). Ultrastructural changes in chloroplast can also affect photosynthesis electron transport and CO_2 assimilation and hence impairment of adenosine triphosphate (ATP) synthesis and Ribulose-1,5-bisphosphate (RuBP) generation (Mutava, 2009).

Delay of leaf senescence increase the total photosynthesis within a life of annual plant by extending active photosynthesis, as a result it can increase carbon assimilation to grain at grain feeling period and maximize mass per grain (Spano *et al*., 2003). Also Yoshida (1972) cited in Pawar (2007) discussed photosynthetic rate of single leave affected by different leaf characters such as leaf thickness or specific leaf weight, leaf nitrogen content, stomatal density, carboxylating enzymes, inter veinal distance and leaf vein frequency. Therefore Plant growth and the existing physiology can be assessed by studying traits such as SLA and SLW.

2.2.1. Biochemical response of plants for drought

Osmotic adjustment is an important physiological adaptation for drought tolerance and it is an accumulation of solutes in the cell in order to decrease water potential and maintain cell turgor and volume (Taiz and Zeiger, 2006; Bahadur *et al*., 2011; Oliveira *et al*., 2012). Accumulation of solutes occur when a decline in water potential occur in the root (Oliveira *et al*., 2012). Adjustment accomplished by accumulation of various solutes such as sugar, organic acids, amino acids (proline) and also inorganic ions (Taiz and Zeiger, 2006; Oliveira *et al.*, 2012).

Bahadur *et al.* (2012) stated that osmotic adjustment play a significant role in maintaining turgor potential and other associated processes such as stomatal opening, photosynthesis, shoot growth and growth of roots to deeper soil layers which assist supply of water. Osmotic adjustment had a significant role in maintaining stomatal conductance and accumulation of solutes that extend time for CO_2 assimilation and increase the net assimilation rate under drought condition. Results of study showed that grain yield from genotypes that have higher osmotic adjustment ability were less affected than those with lower ability of osmotic adjustment. Also yield of wheat genotypes with higher osmotic adjustment showed lower yield reduction as a result of drought induced than those with lower level of osmotic accumulation (Zivcak *et al.*, 2009).

2.2.1.1. Proline

Proline production triggered by various types of stresses and it play a great role in drought tolerance but, the accumulation depend on the type and intensity of the stress. Proline is one of compatible solutes which accumulate in the vacuole to avoid effect on other cells, protect photosynthetic apparatus and enzymes that involve in detoxification of reactive oxygen species (ROS), stabilize proteins by serving as molecular chaperons to prevent denaturation effect of stress on protein (Szabado and Savoure, 2009).

Oraki *et al*. (2012) reported that proline content increase with drought stress especially with severe water stressed condition. Pawar (2007) also showed that the progress of proline increment with crop growth and 13.92 and 19.59 µ mol g fr. wt^{-1} of proline were obtained on 30 and 90 days after sowing. Ulemale *et al*. (2013) showed the proline content increase with stress tolerant genotypes of chickpea. Drought tolerant lines of rice showed an increase in 15 fold in proline accumulation after 5 weeks drought stress than the control which showed only an increase in 5 fold (Vajrabhaya *et al*., 2001).

Results from Moayedi *et al*. (2011) showed that accumulation of proline depends on the developmental stage of the plant. The amount of proline produced increased by 22 %, 47 % and 114 % by water stress which occurs from one leaf stage to floral initiation; from floral initiation to anthesis and after anthesis respectively.

Also Geravandi *et al*. (2011) found that the proline concentration produced by genotypes under drought stress scored an average of 23.2 % higher amount than irrigated genotypes. Also Geravandi *et al.* (2011) and Pawar (2007) reported that there is a positive significant correlation between proline concentration and grain yield and suggested proline concentration as selection criteria for grain yield under drought stressed condition. A finding from Pawar (2007) had showed that the level of increase in proline was lower in low yielder genotypes.

2.2.1.2. Total soluble sugar

Accumulations of soluble sugars in plants give drought tolerance ability and protect cells from damage in two ways. Sugars create hydrophilic interaction with membrane protein because of their OH group, these prevent protein denaturation during desiccation. Also sugars form biological glass in the cytoplasm of dehydrated cell and this can prevent the movement of reactive compounds in the cell and extend the life of dehydrated tissue (Bahadur *et al*., 2011). Under severe drought stress condition, total soluble sugars play a great role in osmotic adjustment (Oliveira *et al*., 2012).

Oraki *et al*. (2012) reported that soluble sugars content also increased with increased level of drought stress. Mohammadkhani and Heidari (2008) reported that the seedling of maize variety showed an increase in total soluble sugar content as the water potential becomes lower. Keyvan (2010) reported that soluble carbohydrate of flag leaf obtained from drought stress at grain filling stage is higher in cultivars of wheat than the amount on other stages.

Drought tolerant lines of rice also accumulate total soluble sugar and the accumulation were higher in drought tolerant lines by 4 fold but, the amount in control was higher by 2.5 fold (Vajrabhaya *et al.*, 2001).

Although there exist a compatibility question with osmolytes produced in response to drought, difference in level and type of osmolytes produced. Even though the osmolytes are from different chemical groups, they can use interchangeably (Yancey, 2005).

2.2.3. Stay-green and its role for drought tolerance

Senescence is the process that shows the end of developmental stages in annual crops (monocarpic plants) and it is degradation and loss of leaf chlorophyll which result decrease in photosynthesis assimilation. Senescence is genetically controlled process but, it induced by various abiotic and biotic stress factors (Jiang *et al*., 2004). Drought tolerance of crops is an important character to reduce yield loss by water loss and genotypes differ in their level of tolerance. Those genotypes susceptible for post-flowering drought affected by premature leaf senescence and results loss of crops grain yield and quality; lodging and charcoal rot disease. (Bineyam Kassahun *et al*., 2010). In contrast with this, there are genotypes with stay-green trait which is most desirable trait for post-flowering drought tolerance. Stay-green trait can increase grain yield and fodder quality by delaying onset and rate of leaf senescence (Reddy *et al*., 2007), maintain photosynthetically active leaf under drought stress (Jiang *et al*., 2004; Rosyara *et al*., 2007; Bineyam Kassahun *et al*., 2010) and able to translocate carbohydrate under terminal drought (Mutava, 2009).

Result from study of Spano *et al*. (2003) showed that durum wheat mutant with stay-green trait increased the seed weight by 10-12 % as a result of extended period for photosynthesis which increase the translocation of assimilate during grain feeling stage which is a limitation in senescent types.

Drought stress result a decrease in seed yield and the same is true for sorghum also and Mutava (2009) stated that drought occurring during post-flowering cause a significant yield reduction than other developmental stages (Groene, 2008).

2.2.3.1. Leaf Nitrogen content

Drought stress can induce as well as accelerate leaf senescence (Chen *et al*., 2013). At grain feeling stage, if the demand for grain doesn’t covered by current assimilation, remobilization of assimilates (N) from other organ will proceed (Thomas *et al*., 2002). Stay-green is a trait which expressed by the balance between N demand of grain and N supply at grain feeling stage (Borrell *et al*., 2001). Amount of leaf Nitrogen and chlorophyll stability at grain filling determined the non senescence trait of stay-green genotypes.

Borrell *et al*. (2001) stated that sorghum genotypes with stay-green trait (B35 and KS19 which are the source of stay-green trait) have higher N in their leaf after anthesis, mid grain filling and maturity than senescent hybrids. Stay-green genotypes have higher specific leaf nitrogen (SLN) as a consequence of higher leaf nitrogen benchmark, nitrogen uptake at grain feeling stage and less N remobilization from their leaf than senescent genotypes. In the absence of available water in the soil, plants uptake of soil nitrogen become limited and it results a breakdown of leaf chlorophyll and translocation of leaf nitrogen to developing leaf (Bineyam Kassahun *et al*., 2010).

2.3. Effect of drought on crop yield

Drought is the most important limiting factor in determining the yield stability of crops which severely affect grain yield and quality (Groene, 2008). However, the extent of grain yield losses due to drought stress depends on the stage of the crop and the timing, duration and severity of drought stress (Kumar *et al*., 2011). Yield of the plant depend on the amount of photosynthetic carbon assimilation. The effect of drought on photosynthetic activity ultimately expressed by crop yield and productivity. Younis *et al*. (2009) illustrated that seed yield and yield component of sorghum reduced because of drought which occurs at vegetative as well as reproductive growth stages. Crop yield express the performance and genetic potential of the crop. Thus, yield under stress serve as important criteria for the selection of drought tolerant genotypes (Blum, 2011).

2.3.1. Drought indices

Drought indices are best ways for identification of stress tolerant and susceptible genotypes under water stressed conditions. Different studies have been done on these methods especially on wheat, rice, maize, etc., but a little have been done on sorghum.

Banayjedi *et al*. (2012) result showed that STI is effective for identification of genetically potential varieties for drought stress. In addition, MP was better in selection of stress tolerant genotypes than SSI and TOL but, it favors high yielding genotypes when the yield between stressed and unstressed has a wide difference (Shirinzadeh *et al*., 2010). Shirinzadeh *et al*. (2010) showed the existence of positive correlation between STI value and yield and suggested STI for selection of high yielding genotype under stressed and normal condition and the higher STI value tells the tolerance of the genotype. SSI evaluates the yield loss as a result of stress in comparison with favorable growth condition (Fischer and Maurer, 1978 cited in Raman *et al*., 2012). GMP helps to test drought response of genotypes by considering drought variability over environment and time (Ramirez and Vallejo, 1998 cited in Raman *et al*., 2012).

The positive correlation relation result between STI and grain yield indicates that STI have the ability to identify high yielder or drought tolerant genotypes under stressed and normal condition than sensitive genotypes (Geravandi, 2011). Ali *et al*. (2013) found High values of MP, GMP, HAR and STI indices can identify tolerant genotypes.

2.4. Mechanism of drought tolerance

Under drought stress condition, plants develop different physiological and morphological mechanisms to withstand the impacts but, the mechanisms are complex (Waseem *et al*., 2011), altered by environmental factors and the developmental stage of the plant at a time drought occurs. These are: drought escape, avoidance and tolerance.

2.4.1. Drought escape

Drought escape can be expressed by consistence of crop cycle along with water availability due to genotypic variation in phenology. Drought escaper's plants have the ability to complete their life cycle before the onset of drought condition. This mechanism achieved by early maturity and plants have high degree of developmental elasticity. Drought escape mechanism depends on the success of reproduction before the occurrence of drought stress through adjusted growth and development. This plants start growth early by rapid germination, flower early to complete maturity before the onset of late season drought stress (Verslues and Juenger, 2011).

Franks (2011) showed that *Brassica rapa* grown under low water condition flower earlier than high water condition and to achieve this, the process assisted by high stomatal conductance to obtain high carbon assimilate.

2.4.2. Drought avoidance

By definition drought avoidance is the ability of the plant to maintain relatively high water content under soil or atmospheric drought stress. Avoidance achieved either by reducing amount of water loss or by increasing the capacity of water uptake of roots. So this strategy helps to reduce water loss to lower level and promote relatively high carbon assimilation. Therefore, plants are able to avoid tissue dehydration and can accumulate high carbon in their tissue (McCann *et al*., 2008; Blum, 2011). Mechanism of avoidance involves: Stomatal closure, increase in root to shoot ratio, solute accumulation.

Plants close their stomata hydropassively by the direct loss of water from the guard cells and by ABA hormone which produced when roots perceive drought. Closure of stomata aids the plants to avoid severe effect of drought, which result wilting and death (Arve *et al*., 2011).

An increase in root elongation enables plants to extract water from the deepest part of the soil. In plants defense mechanism, promotion of root growth to deeper soil considered as the second line of defense to avoid drought (Taiz and Zeiger, 2006). Accumulation of solutes (means for osmotic adjustment) helps to maintain higher relative water content at lower water potential and advance growth (Blum, 2005).

2.4.3. Drought tolerance

Drought tolerance is the ability of plant to maintain tissue turgidity and extending plant growth under severe drought condition (Oliveira *et al*., 2012). Tolerance achieved through osmotic adjustment (accumulation of solutes, free amino acids in the roots and shoots of the plant to maintain cellular turgidity (Mohammadkhani and Heidari, 2008). Drought tolerant plants produce solutes, protective proteins and active defense mechanisms for oxidative damage as a result of drought (Verslues and Juenger, 2011). These all enables to maintain low water potential in the leaf and it enhance turgidity and water potential gradient that enable the plant to extract more water from the soil. These all result a progress in stomatal conductance, photosynthesis, cell elongation and finally growth at low water available condition (Taiz and Zeiger, 2006).

2.5. Origin and geographic distribution of sorghum

According to Doggett (1965) cited in Etuk *et al*. (2012), sorghum was domesticated in Ethiopia before 5000 and more years ago from wild sorghum. Also Vavilov found Ethiopia as a center of origin for sorghum. *Sorghum bicolor* (L.) Moench known by its different name in different country: kaffir corn in South Africa, durra in Sudan, great millet and guinea corn in West Africa, Mtama in East Africa, joha ala cholam in India and kaoliang in China. According to Norman (1995) cited in Etuk *et al*. (2012) grouping cultivable sorghum divided into five groups called bicolor, guinea, caudatum, kafir, and dura and their area of dominance listed in Table 1.

Genus sorghum is a widely diversified all cultivated sorghum belongs to *Sorghum bicolor* subspp *bicolor* (Mutava, 2009). Sorghum is a tropical grass which widely distributed and grows in semi arid regions across the world. The major growing areas in Africa extend from west to eastern region (Ethiopia, Sudan and Somalia). It is an important crop in Kenya, Burundi, Rwanda, Uganda, Tanzania, Mozambique, Malawi, Botswana, Lesotho, and South Africa. Also it grow in India, Pakistan, china, Australia, Mexico, United States, Nebraska, Colombia, Argentina, Sao Paulo, brazil, Venezuela, France and Italy (Leder, 2004).

2.6. Sorghum production

In Ethiopia, Sorghum covers 19 % of total cereals produced and the production is also increasing through time by land expansion and yield improvement (Demeke and Di Marcantonio, 2013). Sorghum is the 4th largest cereal produced at different agro ecological conditions found in Ethiopia. Sorghum produced in different farming system Such as a sole, mixed farming with maize and intercropping. But the production was hindered by different factors such as low inputs, weeds (especially striga), and rainfall timing, amount and distribution are major problems for low production of sorghum in Ethiopia (Schneider and Anderson, 2010). However, according to 2012/13 report by CSA (2013) shows, sorghum cultivation covers 1,711,485 hectare of land and the production has reached 36,042,619 quintals (Table 2.).

Table 1. Different Sorghum races and area of dominance

Races	**Area of dominance**
Bicolor	African savannah, South East Asia
Guinea	West Africa Savannah, India, South East Asia
Caudatum	Tropical Africa
Kaffir	Africa, South of Equator
Durra	Near East and India

Source: Norman *et al*. (1995) cited in Etuk *et al*. (2012)

Table 2. Area, production and yield of crops for private peasant holdings for Meher season 2012/13 (2005 E.C.).

Crops	**Area of Production (hectares)**	**Production (quintals)**
Maize	2,013,044.93	61,583,175.95
Teff	2,730,272.95	37,652,411.66
Sorghum	1,711,485.04	36,042,619.65
Wheat	1,627,647.16	34,347,061.22
Barley	1,018,752.94	17,816,522.00
Total	**9,101,203.02**	**187,441,790.48**

Source: CSA, (2013).

2.7. Nutritional value and consumption of sorghum

Sorghum is one of important cereal crops, which serve as a source of proteins, carbohydrates, minerals, for millions of resource poor farmers found in Africa and Asia. Fifty percent of grain sorghum produced used for food in the form of flat bread (Injera) and porridge by peoples in Africa and Asia but, in America 33 % of sorghum grain used as feed for animals (Kumar *et al*., 2011). This crop is highly rich in mineral but, the bioavailability of these minerals vary about 1 % for some form of iron up to maximum of 90 % for sodium and potassium (Mohammed *et al*., 2011). Ethiopia is one of major sorghum producing countries and it is an important crop, which used for making of Injera with Teff, it also consumed in bread and local beverage drinks. In Ethiopia, sorghum serves as a major food crop in resource poor and semi drier part of the country. Households from Eastern and northwest regions of the country get their 10 % daily caloric intake from the sorghum. Fodder is also used for animal feed. Major nutritional values of sorghum are listed in Table 3, in comparison with other drought tolerant crops maize and finger millet (Leder, 2004).

Table 3. Nutritional composition of Maize, Sorghum and Finger Millet.

	Maize	**Sorghum**	**Finger Millet**
Protein (g)	9.20	10.90	6.00
Fat (g)	4.60	3.20	1.50
Carbohydrate (g)	73.00	73.00	75.00
Crude fiber (g)	2.80	2.30	3.60
Ash (g)	1.20	1.60	2.60
E(kcal)	358.00	329.00	336.00
Ca (mg)	26.00	27.00	336.00
Fe (mg)	2.70	4.30	5.00
Thiamin (mg)	0.38	0.30	0.30
Niacin (mg)	2.83	2.00	1.40
Riboflavin (mg)	0.20	0.14	0.10

Source: Leder, (2004).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of growth conditions

The experiment was conducted in glasshouse found at Addis Ababa University, College of Natural Science from December 2013 to May 2014 (Fig. 1). During the experiment, temperature and relative humidity were measured by hanging thermo-hydrometers in the glasshouse. The average minimum and maximum temperatures recorded were 13.4 ^{0}C and 36.7 ^{0}C, respectively. The maximum and minimum relative humidities during the experiment were 11.8 % and 73 % respectively (Fig. 2).

3.2. Experimental design and treatments

The experiment was carried out using randomized complete block design (RCBD) with three replications (Table 5). Five sorghum accessions used in the study were Sorcoll 163/07, Sorcoll 146/07, Sorcoll 141/07, Sorcoll 060/07 and E-36-1 was the check and it's known by its stay-green trait. Each accession was grown under different water levels (Table 4). Treatments were assigned to each plots randomly using lottery method. The four accessions were selected from previous study by Addisie Yalew (2010) held under BIO-EARN project and suggested for their stay-green trait and better grain yield. Sowing was done on December 5, 2013 and 10 seeds from each accession were sown in each bucket. Tinning was done after seedlings were well established and only one healthy seedling per bucket was allowed to grow, so as to avoid competition. Dates of germination and flowering for each accession are listed on Appendix 1.

3.3. Water level determination

Soil moisture was determined by flooding the buckets with water to create saturated condition. Then, the buckets were left over night to drain excess water as a result of gravitational force and weight was taken. At this condition it’s believed that the soil water was near to field capacity (FC). Soil was weighed after 48 hours in order to replenish water lost via evaporation from the reference field capacity. Water level at field capacity called well watered and water stress level was determined by replenishing evapotranspirationally lost water to well water level. Field capacity of the soil was maintained until the plants were exposed to water stressed condition at post-flowering stage.

The amount of water applied was calculated as follows:

1. Dry weight of the soil was taken (Md).
2. The bucket was weighed alone (Mb).
3. Then, the soil was saturated with water.
4. The soil with bucket was weighed at field capacity; this gives wet mass of the soil with bucket (Mwb).
5. Then, weight of bucket was subtracted from wet mass of the soil with bucket, which gives wet mass of the soil (Mw), Mwb-Mb = Mw.
6. Mm-Md= amount of water at field capacity (FC).
    - Well watered (WW) =100% = FC.
    - Water stressed (WS) = 50% * FC.

Fig. 1. Partial view of the glasshouse experiment at post-flowering stage.

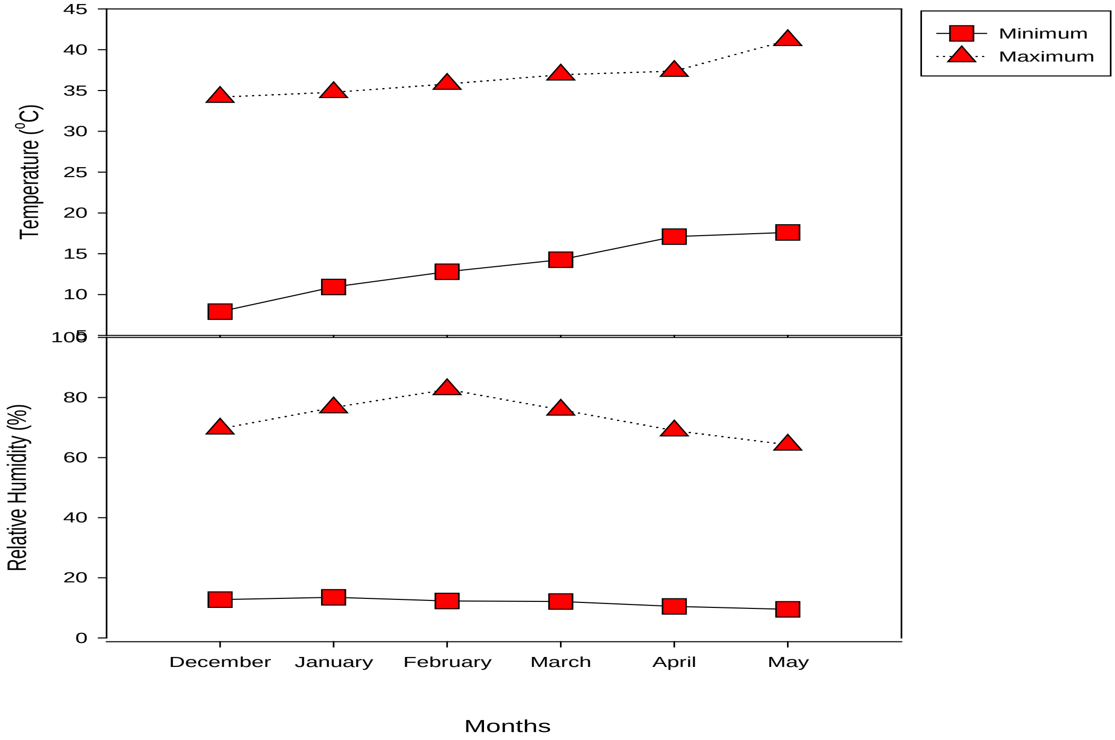

Fig. 2. Average minimum and maximum temperatures and RH of experimental time.

Table 4. Treatments combinations

Accessions	**Water level**	**Treatments (T)**
Sorcoll 163/07	Well watered (WW)	T_1= Sorcoll 163/07 * WW
Sorcoll 146/07	Water stressed (WS)	T_2= Sorcoll 163/07 * WS
Sorcoll 141/07		T_3= Sorcoll 146/07 * WW
Sorcoll 060/07		T_4= Sorcoll 146/07 * WS
E-36-1		T_5= Sorcoll 141/07 * WW
		T_6= Sorcoll 141/07 * WS
		T_7= Sorcoll 060/07 * WW
		T_8= Sorcoll 060/07 * WS
		T_9= E-36-1 * WW
		T_{10}= E-36-1 * WS

Table 5. Treatment allocation within a block or replication (R) and experimental layout.

R_1	R_2	R_3
T_6	T_{10}	T_8
T_2	T_5	T_3
T_8	T_9	T_4
T_7	T_1	T_6
T_4	T_3	T_{10}
T_{10}	T_7	T_2
T_3	T_8	T_5
T_9	T_6	T_1
T_1	T_4	T_7
T_5	T_2	T_9

Key: R = replication, T = treatment

3.4. Growth media

Growing medium was prepared from a mixture of soil, sand and compost with 2:1:1 ratio. Ninety buckets with a diameter of 30 cm and depth of 30 cm were used for the study. Eight perforates were made to all buckets, so as to maintain uniform percolation of excess water. Each bucket was filled with 18 kg or 21,195 cm^3 of soil, sand and compost mixtures.

Agronomic activities such as weeding, fertilizing and tillage were done so as to break hard pans and maintain aeration. Aphid infestation was occurred at grain feeling stage. Managements like water splash and pesticide called Karate were done by consulting Entomologist from the department of zoological sciences.

3.5. Data collection

3.5.1. Growth parameters

3.5.1.1. Specific leaf area (SLA)

Data about specific leaf area were taken at post-flowering stage when maximum growth was attained. Two fully expanded leaves were selected randomly from the middle part of a single plot and a total of 6 leaves were taken from the 3 replica. Samples were immediately brought to ecophysiology laboratory. Leaf area was determined by following methods of Sticklers linear measurement method Stickler *et al*. (1961) as used by Pawar (2007). Leaf area was determined by measuring the length and width of the leaf by scale. Width was measured from the fairly wider part. Then, samples were oven dried for 24 hour at 70 ^{0}C for determination of dry weight. Weight was taken by sensitive balance. Finally, SLA was calculated by the formula below. Data regarding to growth parameters are listed on Appendix 2.

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$$\mathrm{SLA} = \frac{\text{Leaf area (dm2)}}{\text{Leaf dry weight (mg)}}$$

3.5.1.2. Specific leaf weight (SLW)

The same procedures were used for determination of SLW, since it’s the reciprocal of SLA

$$\mathrm{SLW} = \frac{\text{Leaf dry weight (mg)}}{\text{Leaf area (dm2)}}$$

$$\text{Leaf area (dm2)} = \mathrm{L} * \mathrm{W} * \mathrm{cf}$$

L= Length of the leaf and W= width of the leaf

cf = correction factor (0.747)

3.5.2. Biochemical parameters

3.5.2.1. Proline determination

Analysis of proline content in the leaves was measured following method of Bates *et al*. (1973). Reagents used in this analysis were; aqueous sulfo salicylic acid, toluene, glacial acetic acid, acid ninhydrin (prepared by warming 1.25 g ninhydrin , 30 ml glacial acetic acid and 20 ml of 6M phosphoric acid). Agitated until dissolved and kept cool (the reagents remained stable for 24 hours).

As proline production occurs in response to stress, proline analysis of leaf samples was done at post-flowering stage after drought stress was induced. Fresh plant sample (0.5 g) was homogenized with 10 ml of 3 % aqueous sulfo salicylic acid and the homogenate was filtered using whatman No.2 filter paper. The filtrate was used for proline concentration determination. Two ml of the filtrate was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube and allowed to react for 1 hour in a waterbath at 100 ^{o}C. It was then cooled, producing radish color. The reaction mixture was extracted with 4 ml of toluene, mixed vigorously in a test tube and stirred for 15-20 second until separate layers were formed (Fig. 3). The content from each tube was transferred to funnel. Chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction was separated from aqueous phase to other test tube and warmed at room temperature and absorbance was read at 520 nm by spectrophotometer. The proline concentration was determined from standard curve constructed from known concentration of proline and expressed in moles of proline per g fresh weight of the leaf.

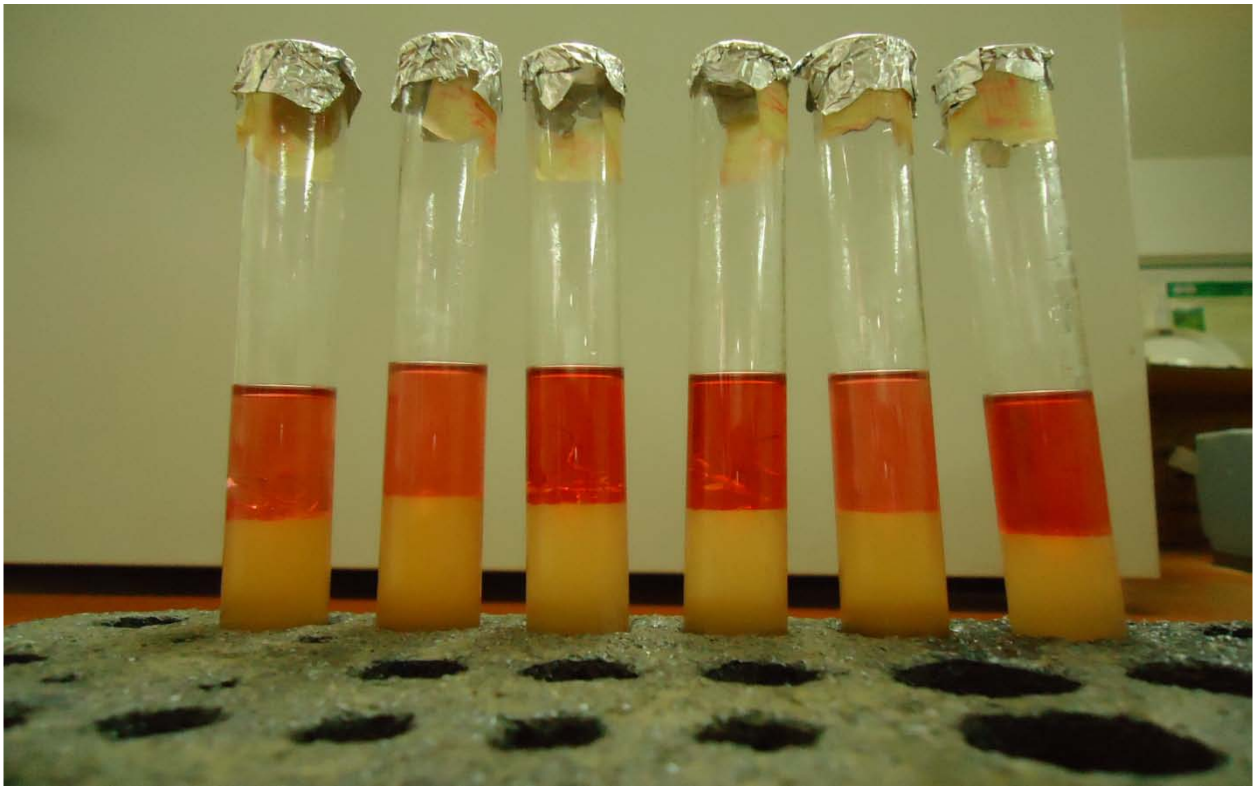

Fig. 3. Leaf proline analysis, forming Chromophore after extraction with toluene

3.5.2.2. Total soluble sugar (TSS)

Total soluble sugar present in the leaf was determined following the methods used by Pawar (2007). Ethanol (80 %) and anthrone reagent (2 %) which prepared by dissolving 0.2 g of anthrone in 10 ml of concentrated sulphuric acid were used.

Dry leaf sample (0.1 g) was mixed with 10 ml of 80 % ethanol in the test tube by stirring the test tubes for 3-5 minutes. The test tubes were kept on hot waterbath and boiled for 10 minutes, then cooled and filtered by Whatman No.1 filter paper. The extract was used for TSS estimation by using anthrone reagents. The alcohol was evaporated in hot waterbath until the content of the test tube was reduced to 1-2 ml. Then, the volume was increased to 25 ml by adding distilled water. From this extract, 1 ml was pipetted to other test tubes. The test tubes were kept in cold places for 15-20 minutes. Then 4 ml of anthrone reagent was added to all test tubes containing the samples, boiled on hot waterbath for exactly 7.5 minutes, removed and cooled in ice for 10 minutes. Finally the absorbance was measured at 630 nm by spectrophotometer. Total soluble sugar content was calculated by using glucose standard curve constructed with known concentration and expressed in mg per g dry weight of leaf sample. TSS content was also measured at post-flowering Stage.

3.5.2.3. Leaf Nitrogen and protein

Leaf nitrogen content was determined using modified micro Kjeldhal procedure used by Pawar (2007) adopted from Jackson (1967). Reagents required for N analysis were; standard H_2SO_4 (0.1N %), NaOH (40 %), Boric acid (2 %), salt mixture (prepared from 250 g of K_2SO_4 mixed with 50 g of $CuSO_4$ and 50 g of metallic selenium) and mixed indicator (prepared by dissolving 0.3 g of bromocresol green and 0.2 g of methyl red in 400 ml of 90 % ethanol. The procedure involves the following processes:

1. Digestion

Dried leaf sample (0.3 g) was mixed in kjeldhal tubes with 7 ml of concentrated H_2SO_4, 3 ml of 30 % H_2O_2 and 2 gm of salt mixture. The content was digested for 1 hour and 50 minutes until a clear solution was obtained. The solution was cooled and taken to the distillation process.

2. Distillation

The digested solution was taken to the distillation apparatus. Ten ml of 40 % NaOH was added and made to distill. Along with this, 20 ml of 2 % boric acid mixed with 1.28 ml of indicator was placed at the outlet of condenser. Steam from the boiler was passed through sample. The ammonia released from the solution was captured by boric acid plus indicator. The process was continued for 5 minutes and followed by Titration.

3. Titration

The content was titrated against the standard H_2SO_4 (0.1N) until bluish green color was turned to pink (Fig. 4). The N content in the sample was calculated by using the following formula:

$$\text{N (\%)} = \frac{\text{(sample titre value - black titre value)} * \text{N of H2SO4} * 14 * 100}{\text{Sample weight (g)} * 1000}$$

$$\text{Protein (\%)} = \text{\% Nitrogen x conversion factor (6.25)}$$

Fig. 4. Leaf N analysis, showing a pink color to the left after titrated with 0.1N H_2SO_4.

3.5.3. Drought indices

Physiological indices were estimated using the following formulas. [Fernandez (1992) and Bouslama and Schapaugh (1984) as cited in Aliakbari *et al*. (2013); Rosielle and Hamblin (1981) as cited in Shirinzadeh *et al*. (2010); Fischer and Maurer (1978) as cited in Raman *et al*. (2012)]. All computation for the drought indices were done after harvesting. Data are listed on Appendix 3.

1. Stress tolerance index (STI) $= \frac{\text{Ys} * \text{Yp}}{(\mu\text{p}2)}$ ……………. Fernandez, (1992)
2. Stress susceptibility index (SSI) $= \frac{1 - (\text{Ys}/\text{Yp})}{\text{SI}}$ ……...Fischer and Maurer, (1978)

   Stress index (SI) $= 1 - \mu\text{s}/\mu\text{p}$
3. Stress tolerance (TOL) = $\text{Yp} - \text{Ys}$ ………..………. Rosielle and Hamblin, (1981)
4. Mean productivity (MP) $= (\text{Ys} + \text{Yp})/2$ ....………Rosielle and Hamblin, (1981)
5. Yield stability index (YSI) = Ys/Yp ....Bouslama and Schapaugh, (1984)
6. Yield index (YI) = $\text{Ys}/\mu\text{s}$ ....Gavuzzi *et al*., (1992)
7. Geometric mean (GM) $= \sqrt{\text{Yp} * \text{Ys}}$ ………………Fernandez, 1992
8. Harmonic mean (HM) $= \frac{[2 * (\text{Yp} * \text{Ys})]}{\text{Yp} + \text{Ys}}$ …………Fernandez, 1992

Where Ys, Yp, represent yield under stressed and normal condition, µs and µp represent the mean yield under stressed and normal conditions, respectively.

3.6. Data analysis

All data were subjected to analysis of variance (ANOVA) using SAS 9.1.3. Mean comparisons were performed using LSD test at $P \leq 0.05$. All graphs were generated using sigma plot version 11.0.

CHAPTER FOUR

4. RESULTS

4.1. Growth parameters

4.1.1. Specific leaf area (SLA)

Analysis of variance revealed that SLA was significantly affected by water stress, sorghum accessions and interaction between them at $P < 0.01$. SLA obtained from Sorcoll 146/07 and Sorcoll 060/07 was significant under both water levels. However, Sorcoll 163/07, Sorcoll 141/07 and E36-1 showed non significant difference in their SLA under both conditions. The performance of accessions showed the same trend for both conditions. Under well watered condition, Sorcoll 141/07 scored the highest SLA value and followed by Sorcoll 060/07, E36-1, Sorcoll 146/07 and Sorcoll 163/07. Under water stressed condition, Sorcoll 141/07 was also the highest SLA followed by Sorcoll 060/07 and E36-1. SLA of Sorcoll 141/07 was significantly higher than the check under both conditions (Table 6).

4.1.2. Specific leaf weight (SLW)

SLW was significantly affected by water stress and accessions at $P < 0.01$. SLW obtained from the two water regimes was nonsignificantly different for Sorcoll 163/07, Sorcoll 141/07 and E36-1. Since SLW is the resprocal of SLA, the rank of SLW was in different direction as compared to SLA. Under well watered condition, the highest SLW was recorded by Sorcoll 163/07. However, under water stressed, Sorcoll 146/07 was the highest in its value and Sorcoll 141/07 showed the lowest SLW value.

Table 6. Mean sum square for growth, biochemical and yield parameters

Source of variation	**Mean sum square**						
	SLA	**SLW**	**Proline**	**TSS**	**N**	**Protein**	**Yield/ panicle**
Accessions	0.0073**	28.57**	1071.79**	6.20**	19329.12**	755043.84**	118.75
Water level	0.0014**	5.94**	5083.22**	2.30**	5.11	199.49	714.83**
Interaction	0.0010*	7.37**	923.49**	0.68**	295.36	11537.46	75.86
Error	0.0012	4.72	298.73	0.31	1299.79	50773.26	238.67
CV (%)	5.8300	6.37	18.47	5.37	15.62	15.62	14.00

Key: ** significant at $P < 0.01$, * significant at $P \leq 0.05$. Where, SS= sum square, SLA= specific leaf area, SLW= specific leaf weight, TSS, total soluble sugar, N= nitrogen, CV= coefficient of variance.

Table 7. SLA and SLW of sorghum accessions under well watered and water stressed condition.

Accessions	**Water level**	**Parameters**	
		SLA (dm^{2} g.$^{-1}$)	**SLW (g. dm^{-2})**
Sorcoll 163/07	WW	0.123667^{d}	8.1003^{a}
Sorcoll 146/07	WW	0.128667^{cd}	7.8163^{ab}
E36-1	WW	0.140667^{bc}	7.1203^{bc}
Sorcoll 060/07	WW	0.151333^{ab}	6.6237^{c}
Sorcoll 141/07	WW	0.159333^{a}	6.2763^{c}
LSD (5%)		0.014700	0.8582
Sorcoll 141/07	WS	0.152667 ^{a}	6.5613 ^{c}
Sorcoll 060/07	WS	0.134667 ^{b}	7.4333 ^{bc}
E36-1	WS	0.133667 ^{b}	7.5207 ^{b}
Sorcoll 163/07	WS	0.121667 ^{b}	8.2330 ^{b}
Sorcoll 146/07	WS	0.094000 ^{c}	10.6363 ^{a}
LSD (5%)		0.013600	0.9091

Key: Means with the same letters have non significant difference at $P \leq 0.05$. Where WW= well watered, WS= water stressed, SLA= specific leaf area, SLW= specific leaf weight.

4.2. Biochemical parameters

4.2.1. Proline

Proline production was significantly affected by water stress, accessions and interaction between the two at $P < 0.01$. The difference in proline concentration between accessions was not significant under well watered condition. However, under water stressed condition, all accessions produced significantly different amount of proline. Even if there existed no statistical difference under well watered condition, Sorcoll 060/07 yielded the highest proline concentration under well watered (Fig. 5). Though, water stress condition, E36-1 produced the highest proline concentration and followed by Sorcoll 060/07. The difference between Sorcoll 163/07, Sorcoll 146/07 and Sorcoll 141/07 was non significant at $P < 0.05$. Under water stressed, all accessions yielded lower proline concentration than the check and Sorcoll 141/07 was the lowest in its amount of proline.

4.2.2. Total soluble sugar (TSS)

Total soluble sugar was significantly affected by water stress and sorghum accessions at $P < 0.01$ (Table 6). All accessions produced significantly higher amount of TSS under water stress than well watered condition except Sorcoll 141/07. Under well watered condition, Sorcoll 060/07 produced the highest TSS. Under water stressed condition, there was a significant difference among all accessions. Sorcoll 060/07 yielded the highest TSS and followed by E36-1, Sorcoll 146/07, Sorcoll 163/07 and Sorcoll 141/07, respectively and they were significantly lower than the check. Sorcoll 060/07 was the only accession which produced the higher TSS than the check. Sorcoll 141/07 revealed the lowest TSS.

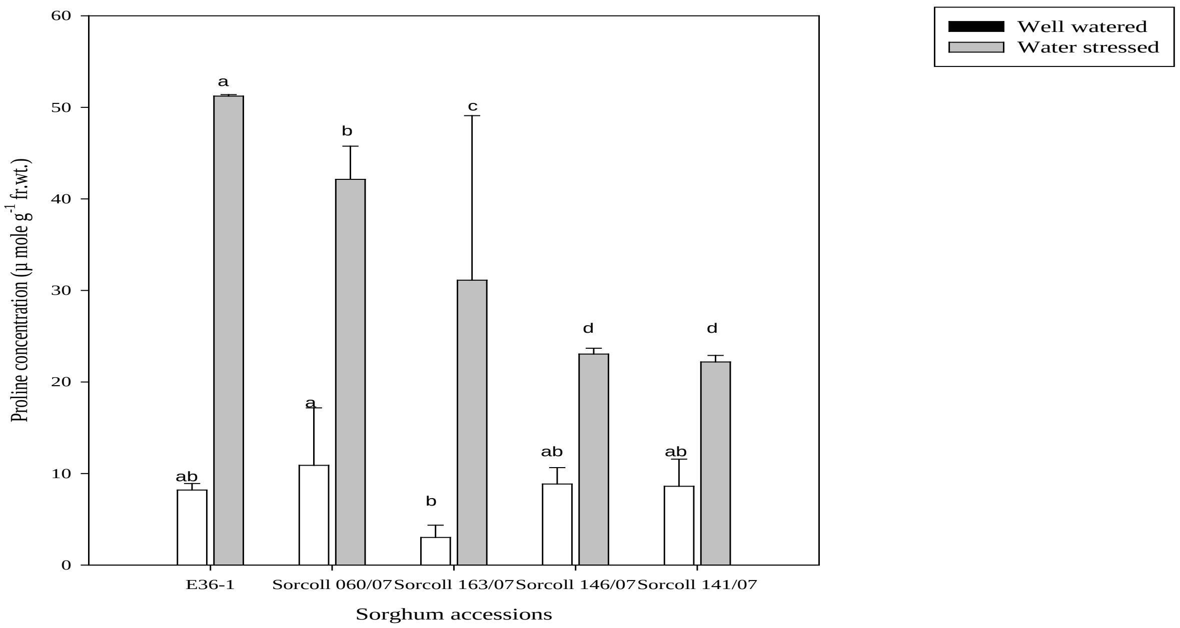

Fig. 5. Proline concentration of leaf under well watered and water stressed condition.

Means assigned with the same later have no significant difference at P ≤ 0.05. Bars represent mean±SE.

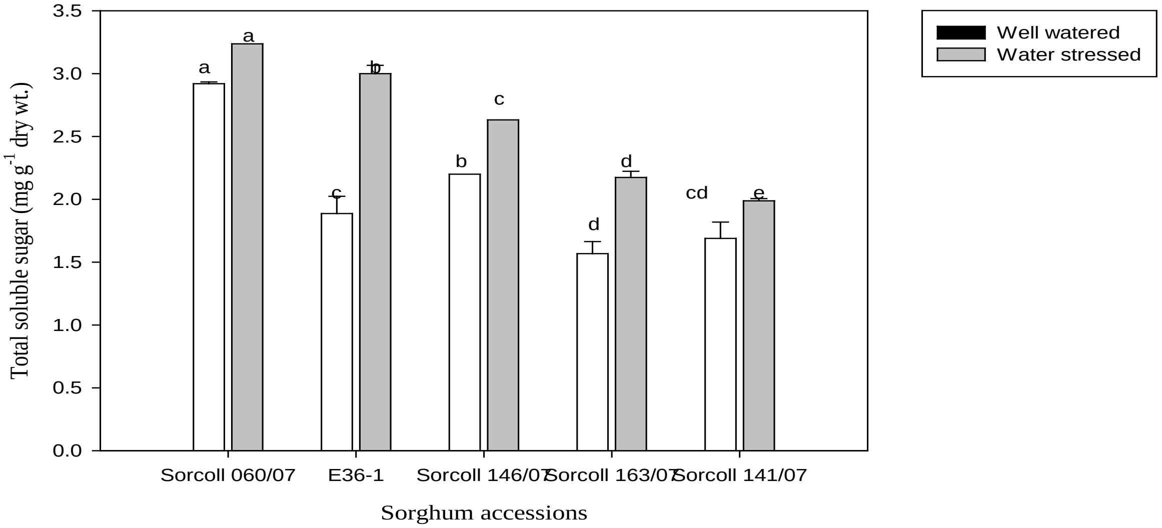

Fig. 6. Total soluble sugar content of sorghum accessions under well watered and water stressed condition. Means assigned with the same later have no significant difference at P ≤ 0.05. Bars represent mean ± SE. Where, TSS= total soluble sugar.

4.2.3. Leaf nitrogen and protein content

Analysis of variance showed that accessions had significant effect on the leaf nitrogen and protein content at $P < 0.01$ but, water stress and the interaction were insignificant. Under well watered condition, Sorcoll 060/07 produced the highest leaf N and Sorcoll 146/07 yield the lowest. Under water stressed condition, Sorcoll 060/07 also yield the highest N followed by Sorcoll 163/07, Sorcoll 141/07, E36-1 and Sorcoll 146/07 sequentially but difference among them was non significant. In contrast with others, water stress had less effect on Sorcoll 163/07 and Sorcoll146/07 which showed higher N content under water stress than well watered condition. Since leaf protein analysis was determined from the result obtained from leaf N, the performance of accessions were similar to that of leaf N as indicated on Table 8.

Table 8. Leaf N and protein content of five sorghum accessions under well watered and water stressed condition.

Accessions	**Water level**	**Parameters**	
		N (%)	**Protein (%)**
Sorcoll 060/07	WW	102.250 ^{a}	639.060 ^{a}
Sorcoll 141/07	WW	50.355 ^{b}	314.720 ^{b}
Sorcoll 163/07	WW	46.690 ^{b}	291.810 ^{b}
E36-1	WW	29.882 ^{c}	186.760 ^{c}
Sorcoll 146/07	WW	26.767 ^{c}	167.300 ^{c}
LSD (5%)		4.303	26.893
Sorcoll 060/07	WS	94.314 ^{a}	589.46 ^{a}
Sorcoll 163/07	WS	58.052 ^{b}	362.82 ^{b}
Sorcoll 141/07	WS	49.025 ^{bc}	306.41 ^{bc}
E36-1	WS	29.412 ^{c}	183.82 ^{c}
Sorcoll 146/07	WS	29.268 ^{c}	182.93 ^{c}
LSD (5%)		20.290	126.81

Key: Means with the same letters have insignificant difference at $P \leq 0.05$. WW= well watered, WS= water stressed, N= nitrogen.

4.3. Yield and drought indices

4.3.1. Yield per panicle

Analysis of variance showed that there existed a significant effect of water on yield at $P < 0.01$. However, the effect of accessions and the interactions was insignificant. Under well watered condition, E36-1 achieved the highest yield. Sorcoll 146/07, Sorcoll 060/07, Sorcoll 141/07 and Sorcoll 163/07 produced grain yield in decreasing order following E36-1. The difference in yield among all accessions was not significant (Table 9). Under water stressed condition, a higher yield was recorded by Sorcoll 060/07 followed by E36-1.

Table 9. Yield of the five accessions under well watered and water stressed condition

Accessions	**Water level**	**Yield/panicle (g)**
E36-1	WW	32.272 ^{ns}
Sorcoll 146/07	WW	30.667 ^{ns}
Sorcoll 060/07	WW	29.540 ^{ns}
Sorcoll 141/07	WW	29.200 ^{ns}
Sorcoll 163/07	WW	26.083 ^{ns}
LSD (5%)		7.570
Sorcoll 060/07	WS	23.960 ^{a}
E36-1	WS	21.750 ^{ab}
Sorcoll 141/07	WS	19.972 ^{abc}
Sorcoll 163/07	WS	17.833 ^{bc}
Sorcoll 146/07	WS	15.434 ^{c}
LSD (5%)		4.657

Key: Means with the same letters have no significant difference at $P \leq 0.05$. Where, WW= well watered, WS= water stressed.

4.3.2. Drought indices

The assessment of appropriate drought indices for selection of tolerant accessions were calculated based on yield under stressed and unstressed condition (Table 10). According to

TOL value, Sorcoll 060/07 and Sorcoll 141/07 showed the highest tolerance, Sorcoll 146/07 and E36-1 were the least tolerant. Based on STI value, Sorcoll 060/07 and Sorcoll 141/07 scored the highest STI and drought tolerance under well watered condition but, Sorcoll 163/07 and Sorcoll 146/07 were the least in STI value and tolerance level under well watered condition, respectively. E36-1 was observed average. The same ranks were observed for YSI too. MP, GM and HM values showed positive correlation among them and analyze the yield gap between stressed and unstressed. Based on this, Sorcoll 060/07 and E36-1 were the tolerant accessions. Sorcoll 141/07, Sorcoll 163/07 and Sorcoll 146/07 were less tolerant and sensitive, respectively. YI observed about similar to all accessions.

Principal component analysis (PCA) was run for all indices to analyze the existing association between any two indices. Based on Eigenvalue (>1) (Fig. 7) result, the PCA assessment showed that two components had higher variation. The first component showed 56.17 % of the total variation and this component was named as drought tolerant (Table 11). The second component explained 32.04 % of the total variation and this component was also called stress susceptible dimension. Based on this, selection of accessions with high PC1 and low PC2 value are suitable for both stressed and unstressed conditions. As it was indicated on Table 11, the positive loading of each indices revealed that indices GM, HM, MP, STI, Ys, Yp and YSI had higher correlation with PC1. TOL, SSI and Yp showed a positive correlation with PC2. According to biplot analysis (Fig. 8), TOL and SSI explained the highest PC2. Accessions with high value of these indices could be susceptible for drought stress.

Table 10. Drought indices of five SG sorghum accessions grown under well watered and stressed condition

Accessions	Ys	Yp	TOL	MP	YSI	YI	STI	SSI	GM	HM
Sorcoll 060/07	23.960(1)	29.540(3)	5.580(5)	26.750(2)	0.810(1)	1	0.816(1)	1.005(2)	26.603(1)	26.458(1)
Sorcoll 141/07	19.806(3)	27.750(4)	7.944(4)	23.778(3)	0.708(2)	1	0.724(2)	1.021(1)	23.403(3)	23.036(3)
E36-1	21.750(2)	32.272(1)	10.522(2)	27.011(1)	0.705(3)	1	0.678(3)	0.904(5)	26.364(2)	25.739(2)
Sorcoll 146/07	15.434(5)	30.667(2)	15.234(1)	23.050(4)	0.506(5)	1	0.501(5)	0.994(3)	21.703(5)	20.445(5)
Sorcoll 163/07	18.000(4)	27.533(5)	9.533(3)	22.767(5)	0.658(4)	1	0.652(4)	0.994(3)	22.232(4)	21.712(4)

Key: Ys=yield under water stressed, Yp= yield under well watered, TOL= tolerance, MP=mean productivity, YSI= yield stability index, YI= yield index, STI= stress tolerance index, SSI= stress susceptibility index, GM= geometric mean and HM= harmonic mean

Table 11. Variables load to each components

Components	Total variance %	Ys	Yp	TOL	MP	YSI	YI	STI	SSI	GM	HM
1	56.167	.867	.670	-.105	.972	.342	.644	.926	-	.992	.985
2	32.043	-.466	.717	.963	.193	-.910	-.306	-.110	.824	-	-.107

Key: Ys=yield under water stressed, Yp= yield under well watered, TOL= tolerance, MP=mean productivity, YSI= yield stability index, YI= yield index, STI= stress tolerance index, SSI= stress susceptibility index, GM= geometric mean and HM= harmonic mean.

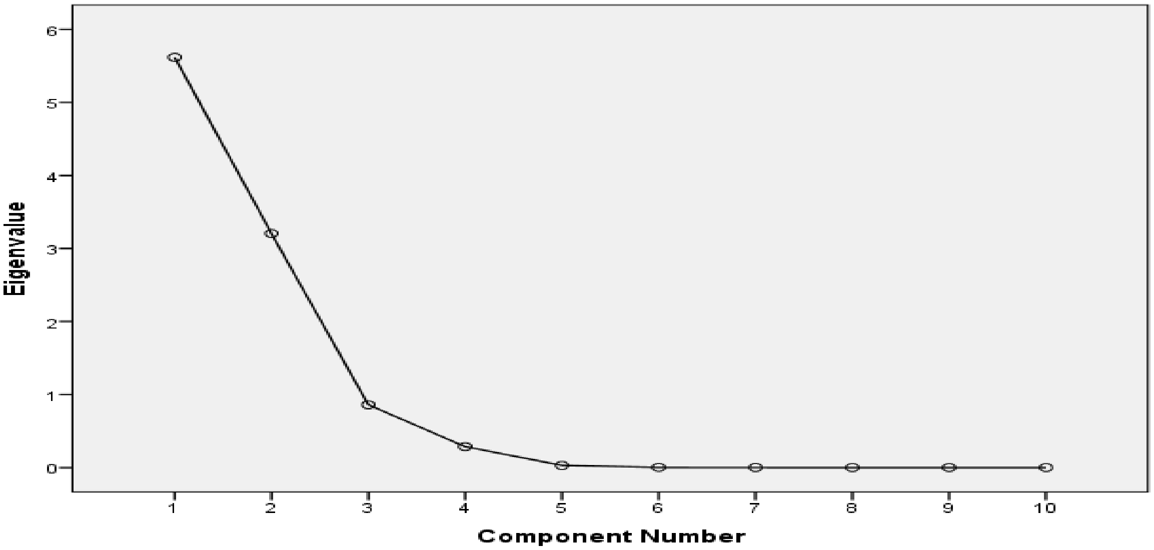

Fig. 7. Scree plot of Eigenvalue with corresponding components

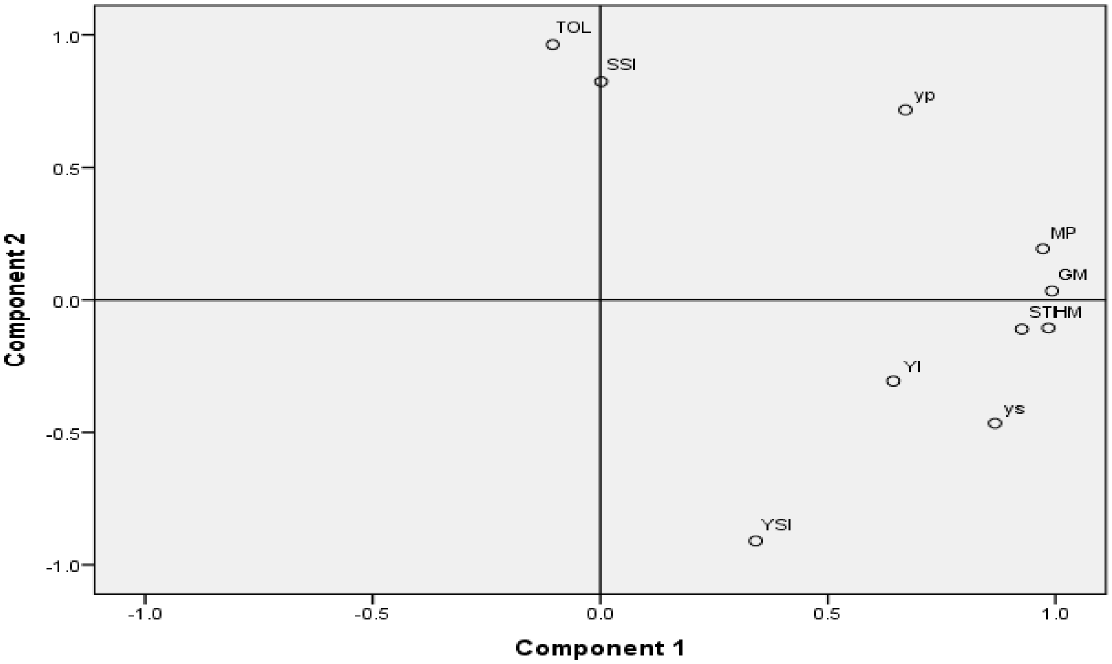

Fig. 8. Biplot of the first two principal components, summarizing the relation between drought tolerant and susceptible indices. Where Ys=yield under water stressed, Yp= yield under well watered, TOL= tolerance, MP=mean productivity, YSI= yield stability index, YI= yield index, STI= stress tolerance index, SSI= stress susceptibility index, GM= geometric mean and HM= harmonic mean.

4.4. Correlation analysis

4.4.1. Relationship among parameters

In order to analyze how each parameter interrelated to a given condition, correlation analyses were performed for each water level. Under well watered condition, all parameters showed weak correlation with yield. SLA showed a strong negative correlation with SLW (Table 12). TSS was positively correlated with N and protein. N and protein showed a strong positive correlation. However, under water stressed condition, biochemical parameters were positively associated with each other. Among these, proline showed a positive correlation with a yield (Table 13). SLW also showed a negative correlation with a yield and SLA.

4.4.2. Correlation among drought indices

The correlation analysis of drought indices showed a high positive correlation of Ys with MP, YSI, STI, GM and HM and low positive with YI (Table 14). Apart from this, TOL was negatively correlated with Ys. Yp showed a strong positive association with TOL, MP, GM, and weak positive correlation with SSI and HM but, it showed negative correlation with YSI. Indices MP, YI, STI, GM and HM showed the existence of positive correlation among themselves. TOL had positive association only with SSI and both had negative correlation with YSI and the same was true between SSI and YI.

Table 12. Simple correlation of parameters under well watered condition

	SLA	SLW	Proline	TSS	%N	Protein	Yield
SLA	1.0000	-0.9944**	0.47927	0.23831	0.43961	0.43961	0.29554
		<.0001	0.07070	0.39240	0.10110	0.10110	0.28490
SLW		1.0000	-0.48210	-0.24465	-0.42469	-0.42469	-0.30119
			0.06880	0.37950	0.11460	0.11460	0.27530
Proline			1.00000	0.37249	0.21985	0.21985	0.42657
				0.17150	0.43110	0.43110	0.11280
TSS				1.00000	0.68560*	0.68560*	0.06930
					0.00480	0.00480	0.80610
%N					1.00000	1.00000**	-0.11729
						<.00010	0.67720
Protein						1.00000	-0.11729
							0.67720
yield							1.00000

Key: **and*significant at P=0.01 and P=0.05. Where, SLA= specific leaf area, SLW= specific leaf weight, TSS= total soluble sugar, N= nitrogen.

Table 13. Simple correlation of physiological parameters under water stressed condition

	SLA	SLW	Proline	TSS	%N	Protein	Yield
SLA	1.00000	-0.9857**	0.20344	-0.16044	0.31086	0.31086	0.51013
		<.0001	0.46710	0.56790	0.25940	0.25940	0.05200
SLW		1.0000	-0.29625	0.10200	0.36098	-0.36098	-0.57709*
			0.28370	0.71760	0.18620	0.18620	0.02430
Proline			1.00000	0.73607*	0.14865	0.14865	0.55699*
				0.00180	0.59700	0.59700	0.03100
TSS				1.00000	0.24869	0.24869	0.46814
					0.37140	0.37140	0.07840
%N					1.00000	1.00000**	0.50697
						<.00010	0.05380
Protein						1.00000	0.50697
							0.05380
yield							1.00000

Key: **and*significant at P=0.01 and P=0.05. Where, SLA= specific leaf area, SLW= specific leaf weight, TSS= total soluble sugar, N= nitrogen

Table 14. Simple correlation among drought indices

	Ys	**Yp**	**TOL**	**MP**	**YSI**	**YI**	**STI**	**SSI**	**GM**	**HM**
Ys	1	0.235	-0.574*	0.764**	0.759**	0.580*	0.848**	-0.315	0.861**	0.925**
Yp		1	0.661**	0.807**	-0.443*	0.264	0.487	0.490*	0.694**	0.578
TOL			1	0.090	-0.959**	-0.225	-0.245	0.656**	-0.080	-0.227
MP				1	0.168	0.528*	0.839**	0.134	0.985**	0.946**
YSI					1	0.337	0.418	-0.652**	0.328	0.461*
YI						1	0.635*	-0.519*	0.566*	0.584*
STI							1	0.032	0.886**	0.905**
SSI								1	0.039	-0.046
GM									1	0.988**
HM										1

Key: ** and * significant at P=0.01 and P=0.05. Where Ys=yield under water stressed, Yp= yield under well watered, TOL= tolerance, MP=mean productivity, YSI= yield stability index, YI= yield index, STI= stress tolerance index, SSI= stress susceptibility index, GM= geometric mean and HM= harmonic mean

CHAPTER FIVE

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

5.1.1. Growth parameters

It is a well documented fact that SLA is an important growth parameter in response to favorable and unfavorable condition (Schulze *et al*., 2005), associated with physiological activity (photoassimilation) (Pawar 2007). Since SLA tells the amount of leaf area invested for light capture, the maximum area is attained after physiological maturity (Borrell *et al*., 2000).

SLA indirectly tells the leaf thickness. Pawar (2007) discussed that an increase in SLW shows a decrease in leaf area. The lower SLA value obtained in the present study can be suggested as a reduction of growth in response to water stress and it is one of tolerance mechanisms under water stressed condition (Plessis, 2008; Muhammad *et al*., 2011). Sorcoll 141/07 has higher SLA than the other accessions. However, this accession observed with low yield. This might associate with high transpirational water loss as a result of invested higher leaf area than expected high light capture. SLW values also observed higher for accessions Sorcoll 163/07 and Sorcoll 146/07 and negative relation with yield.

In contrast with this, Sorcoll 141/07 and Sorcoll 060/07 have better SLA and thus yield per panicle (Sorcoll 060/07). Also stay-green accessions are known by having thicker leaf (Borrell *et al*., 2000). Sorcoll 146/07 and Sorcoll 163/07 had showed higher SLW, this might be due to existence of less remobilization of assimilates (Borrell *et al*., 2000; Nagarjuna, 2007). In conformity with the present finding, Ali *et al*. (2009) suggested that low SLW and higher SLA genotypes might help to select genotypes that can give high grain yield under significant drought stress.

5.1.2. Biochemical parameters

Proline and soluble sugars play a great role in osmotic adjustment for drought tolerance. According to Zhang *et al*. (1999) solutes are accumulated during stress serve as an aid to cope with the existing condition. The result of this study shows that the mean value of proline produced for all accessions is higher under water stressed condition. This shows that accumulation of proline has a great importance in withstanding the effect of desiccation (Ulemale *et al*., 2013). In agreement with this research, an increase in proline production have been observed in different crops (Mohammadkhani and Heidari, 2008; Zivcak *et al*., 2009; Keyvan, 2010; Oraki *et al*. 2012). Proline is one of osmoprotectant that accumulates in a lot of crops in response to different kinds of stresses including drought (Trovato *et al*., 2008; Mafakher *et al*., 2010). According to Taize and Zeiger (2006), proline is a compatible solute which plays a great role in osmotic adjustment through osmotic balance in cytoplasm and vacuole and protection of membrane and proteins from oxidative damage during water stress (Schulze *et al*., 2005). Moreover, genotypes differ in their level of proline production. In argument with this, Zhang *et al*. (1999) reviewed that there existed genotypic difference between cultivars and species. The present study illustrates that E36-1 has the highest proline concentration and Sorcoll 141/07 has the lowest. This finding agrees with Oraki *et al*. (2012). The observed genetic variation can be the existing capability of accessions in maintaining their level of proline biosynthesis pathway than the degradation.

The result which has been reported by kanani *et al*. (2013) showed that tolerant genotypes produced higher yield and proline than drought susceptible genotypes. This showed that proline accumulation in response to induced stress has a great importance in improving the yield under stress condition.

Total soluble sugars are also one of the compatible solutes used by plants for osmotic adjustment under water deficit condition. An accumulation of sugars as tolerance mechanism under water stressed condition have been reported by researchers in several crops (Mohammadkhani and Heidari, 2008; Farooq *et al*., 2009). Similarly, in present study amount of total soluble sugar produced by sorghum accessions is higher under water stressed condition.

Accessions also differ in their level of sugar production. In this study, Sorcoll 060/07 has the highest TSS and Sorcoll 141/07 has the lowest. Even if the existing correlation between TSS and yield is significant at $P < 0.1$, presence of higher soluble sugar helped to improve the yield trait. Thus, Sorcoll 060/07 was high yielder. It was suggested that an increase in sugar level could be the result of starch degradation under water deficit condition (Fox and Geiger, 1986 as cited in Yordanov *et al*., 2000).

Naser *et al*. (2009) discussed that an increase in soluble sugars may contribute to enhance drought stress tolerance. Also literatures argued that sugars are vital in membrane stabilization (Xoconostle-Cazares *et al*., 2011), maintaining turgidity and osmoprotection (Mohammadkhani and Heidari, 2008). Under water stressed condition, sugars play a great role in protecting a cell through replacing the hydroxyl group of protein and prevent protein denaturation. Under sever condition, sugars form glassy matrix in the cytoplasm through which proteins are protected (Opik and Rolfe, 2005; Mohammadkhani and Heidari, 2008).

Nitrogen is one of essential nutrients that constituents organic molecules such as proteins, nucleic acids, pyramidines, etc, which are basic for plant growth and development (Barker and Pilbeam, 2007). The major symptom during water stress is chlorosis (degradation of chlorophyll) which in turn leads to early senescence as a result of N loss from the leaf. Therefore, strategies that can extend the onset of senescence are beneficial (Xu *et al*., 2000). In the present study, the effect of water deficit stress on leaf N is insignificant. This is because, the accessions are known by their stay-green trait. This is in agreement with other studies reported on stay-green trait as drought tolerance mechanism (Harris *et al*., 2007; Borrell *et al*., 2014).

Stay-green trait is associated mainly with a delay of leaf senescence which has a direct relation with leaf nitrogen, especially N containing molecules. It is revealed that this trait is very important for post-flowering drought tolerance. Similarly, Pawar (2007) reported that stay-green genotypes maintain green leaf area for longer time. The mechanism of staying green associates with N. The report of Borrell and Hammer (2000) as cited in Nagarjuna (2007) explains stay-green is a balance in N demand from green leaves and N supply during grain feeling.

In this study, Sorcoll163/07 and Sorcoll146/07 showed higher percent of N under water stress than well watered condition. This might be either because of blocking of remobilization of N from leaf to other organ or an increase in the uptake of N from the soil (Distefeld *et al*., 2014). Hence, having higher leaf N is helpful in copping chlorophyll degradation during drought and maintenance of photosynthetically active leaf for extended period of time. Even though majority of leaf N found in enzyme Rubisco, having higher leaf N can only increase the photosynthesis, if the carbon assimilation is occurring at maximum rate (Meziane and Shipley, 2001).

5.1.3. Yield and drought indices

It has been reported that post-flowering stage is the most sensitive that results in a significant yield loss. The reduction in yield as a result of drought depends on the time of drought occurrence (Farooq *et al*., 2009). Scholars have reported that stay-green genotypes are tolerant to post-flowering drought (Borrell *et al*., 2003; Bineyam Kassahun *et al*., 2010). In argument with this, this study showed that stay-green sorghum accessions are insignificantly affected by induced stress. Haussmann *et al*. (2002) as cited in Ngugi *et al*. (2013) pointed out high yielding E36-1. Sorcoll 060/07 produce higher yield with low yield loss due to the stress than the others. The ultimate effect of drought is a reduction in yield, so that selection of accessions with a minimum yield loss can be achieved through selection of drought indices suitable for stressed conditions. The suitability of the indices depends up on their correlation with the yield under well watered and stressed conditions. Based on TOL and SSI values, genotypes with high value of these indices showed susceptibility (Shirinzadeh *et al*., 2010).

In present study, Sorcoll 146/07 and Sorcoll 141 scored the highest TOL and SSI values, respectively. From the correlation point of view, due to the existence of negative correlation of yield with TOL and SSI, these accessions can only give better yield under well watered condition. Similar findings are reported by (karimizadeh *et al*., 2012; Aliakbari *et al*., 2013). In argument with the present study, the positive relation of MP, GM, HM under both experimental conditions has been reported by scholars (Moosavi *et al*., 2008; karimizadeh *et* *al*., 2012; Aliakbari *et al*., 2013). Accordingly, Sorcoll 060 /07 and E36-1 are found to be tolerant accessions. The same is true for STI. This finding showed that MP, GM, HM and STI have high value of component one. Shirinzadeh *et al*. (2010) recommended these indices as suitable for selecting drought tolerant genotypes. karimizadeh *et al*. (2012) suggest that selection based on high PC1 and low PC2 is suitable for stressed and unstressed environment. But if the existing stress is sever; TOL, SSI, and STI are more suitable in selecting high yielder genotypes.

5.1.4. Relationship among parameters

Under well watered condition, measured parameters showed less correlation. N, protein and TSS content of leaf have showed positive association. This might be occurred because the accessions are capable in minimizing dilution effect as a result of dry weight produced. Similar correlation between parameters has been reported by Nagarjuna (2007). In contrary, the correlation analysis under water stressed condition revealed that there existed positive correlation between proline with yield as well as TSS. This result is in conformity with keyvan (2010) and Pawar (2007). As Naser *et al*. (2009) has indicated, the positive association is because of the level of proline produced depends on the amount of carbohydrate available. Moreover, the correlation of TSS with yield is positive at $P < 0.1$. This clearly shows that the accumulation of proline and TSS is due to result of drought stress (Nagarjuna, 2007). Due to the inverse relation of SLW and SLA, there existed a strong negative association. Yield also had a negative correlation with SLW, this might be due to existence of week or less remobilization of assimilate from leaf to grain. This finding argued with review of Borrell *et al*. (2000) and Pawar (2007).

5.2. Conclusion and Recommendations

The finding of this study showed that the accessions responded differently due to induced post-flowering drought stress. The amount of leaf area invested for light capture and level of assimilates remobilization are the two important factors that associate with SLA and SLW. There existed difference among accessions; however the trait is also an adaptive mechanism to existing condition. Therefore, higher SLA and lower SLW can help in selection of drought tolerant accessions.

The induced water stress had resulted in an increase in accumulation of proline and TSS. There was a significant difference among all accessions. E36-1 and Sorcoll 060/07 were higher in their leaf proline accumulation. There existed a positive association of leaf proline content with yield under stress condition. Result of leaf TSS was higher for Sorcoll 060/07. Sorcoll 141/07 was observed yielding lower proline and TSS. Leaf N and protein had showed insignificant effect of water stress, but genotypic variation revealed significant difference among accessions. This indicated that stay-green genotypes have higher leaf nitrogen and can maintain green leaf area under stressed condition. Based on this, Sorcoll 060/07 was observed with highest leaf N and protein than the check. Yield per panicle was also observed that it was significantly affected by genotypic potential than water stress and Sorcoll 060/07 was high yielder.

The investigation of drought indices had revealed the suitability of TOL, SSI and MP, STI, GM, HM for selection of accessions for favorable and stressed environment. Based on lower TOL and higher MP, GM, HM value, Sorcoll 060/07 and E36-1 were found tolerant for water stress environment. The higher TOL value indicated that high yielding potential of Sorcoll 146/07 and Sorcoll 141/07 can be achieved only under favorable environment. Therefore it is possible to conclude that selection based on these indices will help to achieve the maximum yielding potential of accessions and it is possible to recommend these indices for screening programs of genotypes for various kinds of stresses.

Since the current research was done under semi controlled condition, the consistency of observed traits should be studied under field condition. The results obtained had showed an importance of biochemical traits for drought tolerance and also for yield. Therefore, future studies will be required to assess the role of stress induced accumulation of osmolytes association to the stay-green trait, QTL study for these traits and utilization in breeding program for development of tolerant genotypes.

REFERNCES

Abu Tefera (2013). Grain and feed annual, Ethiopia. Gain report: Annual report by United State Department of Agriculture (USDA).

Addisie Yalew (2010). Evaluation of Sorghum (*Sorghum bicolor*) genotypes for post- flowering drought resistance (Stay-green Trait). M. Sc thesis, Addis Ababa University, Addis Ababa.

Alemayehu Seyoum T., Dorosh, P. and Sinafikeh Asrat S. (2012). Crop production in Ethiopia: Regional patterns and trends. IFPRI (International Food Policy Research Institute), pp. 1-2.

Ali, M.A., Abbas, A., Niaz, S. Zulkiffal, M. and Ali, S. (2009). Morpho-physiological criteria for drought tolerance in sorghum (*Sorghum bicolor*) at seedling and post-anthesis stages. *Int. J. Agric. Biol*. **11**:674-680.

Aliakbari, M., Saed-Moucheshi, A., Hasheminasab, H. and Pirasteh-Anosheh, H. (2013). Suitable stress indices for screening resistant wheat genotypes under water deficit conditions. *Int. J. Agron. Plant Prod*. **4**:2665-2672.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Physiology* **24**:1-15.

Arve, L.E., Torre, S., Olsen, J.E. and Tanino, K.K. (2011). Stomatal responses to drought stress and air humidity. **In:** *Abiotic Stress in Plants-Mechanisms and Adaptations*, pp. 267-280, (Shanker, A. ed.), InTech.

Asfaw Adugna (2007). The role of introduced sorghum and millets in Ethiopian agriculture. *SAT eJournal | ejournal. icrisat.org*. **3**:1-4.

Bahadur, A., Chatterjee, A., Kumar, R., Singh, M. and Naik, P.S. (2011). Physiological and biochemical basis of drought tolerance in vegetables. *Veg. Sci*. **38**:1-16.

Banayjedi, M.E., Ehteshami, S.M., Abdi, B.D.H. and Rohami, M. (2012). Evaluation of drought tolerance indices and their relationship with grain yield of wheat genotypes. *J. Basic. Appl. Sci. Res.* **2**:1230-1232.

Barker, A.V. and Bryson, G.M. (2007). Nitrogen. **In**: *Handbook of Plant Nutrition*, 23 pp. (Barker, A.V. and Pilbeam, D.J. eds), CRC press, Boca Raton.

Bates, L.S., Waldren, R.P. and. Teare, L.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil* **39**:205-207.

Bineyam Kassahun, Bidinger, F.R., Hash, C.T. and Kuruvinashetti, M.S. (2010). Stay-green expression in early generation sorghum [*Sorghum Bicolor* (L.) Moench] QTL introgression lines. *Euphytica* **172**:351–362.

Blum, A. (2005). Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive. *Aust. J. Agric. Res.* **56**:1159–1168.

Blum, A. (2011). *Plant Breeding for Water-Limited Environments*. Springer, New York, USA.

Borrell, A., Hammer, G. and Oosterom, E.V. (2001). Stay-green: A consequence of the balance between supply and demand for nitrogen during grain filling? *Ann. Appl. Biol.,* **138**:91-95.

Borrell, A., Oosterom, E.V., Hammer, G., Jordan, D. and Douglas, A. (2003). The physiology of “stay-green” in sorghum. *Proceedings of the 11th Australian Agronomy Conference*, 2-6 February, Geelong, Victoria.

Borrell, A.K., Mullet, J.E., George-Jaeggli, B., van Oosterom, E.J., Hammer, G.L., Klein, P.E. and Jordan, D.R. (2014). Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. Oxford University Press, pp. 1-13.

Central Statistical Agency (CSA) (2013). Report on area and production of major crops, private peasant holdings, Meher season. Addis Ababa, March, Ethiopia.

Chen, W., Liu, H., Zhang, J., Fu, Z., Liu, Y., Liu, Y., Yang, H. and Huang, Y. (2013). Genetic analysis and assessment of stay-green traits in hybrids of temperate and tropical maize germplasm. *Medica* **58**:260-265.

Demeke, M. and Di Marcantonio, F. (2013). Analysis of incentives and disincentives for sorghum in Ethiopia. Technical notes series, MAFAP, FAO, Rome, 6 pp.

Dereje Assefa, Maru Belay, Diress Tsegay and Mitiku Haile (2007). Transplanting sorghum as a means of ensuring food security in low rainfall sorghum growing areas of northern Ethiopia. DCG Report No. 48.

Distelfeld, A., Avni, R. and Fischer, A.M. (2014). Senescence, nutrient remobilization, and yield in wheat and barley. *J. Exp. Bot.*, pp. 1-16.

Etuk, E.B., Ifeduba, A.V., Okata, U.E., Chiaka, I., Okoli, Ifeanyi C., Okeudo N.J., Esonu B.O., Udedibie, A.B.I. and Moreki, J.C. (2012). Nutrient composition and feeding value of sorghum for livestock and poultry. *J. Anim. Sci. Adv.* **2**:510-524.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. **In**: *Sustainable Agriculture*, 155, 169 pp., (Lichtfouse E., Navarrete M., Debaeke P., Souchere V. and Alberola C. eds), Springer.

Franks, S.J. (2011). Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytologist* **190**:249–257.

Gavuzzi, P., Rizza, F., Palumbo, M., Campanile, R.G., Ricciardi, G.L. and Borghi, B. (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Can. J. Plant Sci*. 523-531.

Geravandi, M., Farshadfar, E. and Kahrizi, D. (2011). Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. *Russian J. Plant Physiol*ogy **58**:69-75.

Groene, G.A. (2008). Evaluating sorghum and maize germplasm for post-anthesis drought tolerance. M. Sc. Thesis, Kansas State University, Manhattan, Kansas.

Harris, K., Subudhi, P.K., Borrell, A., Jordan, D., Rosenow, D., Nguyen, H., Klein, P., Klein, R. and Mullet, J. (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *J. Exp. Bot*. **58**:327–338.

Jiang, G.H., He, Y.Q., Xu, C.G., Li, X.H. and Zhang, Q. (2004). The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by japonica cross. *Theor. Appl. Genet*. **108**:688–698.

Jiregna Gindaba, Rozanov A., Legesse Negash. Response of seedlings of two Eucalyptus and three deciduous tree species from Ethiopia to severe water stress. *Forest Ecology and Management*. **201:**119–129.

Kanani, S.M., Kasraie P. and Abdi H. (2013). Effects of late season drought stress on grain yield, protein, proline and ABA of bread wheat varieties. *Intl. J. Agron. Plant Prod*. **5**:2943-2952.

Karimizadeh, R., Mohammadi, M., Ghaffaripour, S., Karimpour, F., and Shefazadeh M.K. (2011). Evaluation of physiological screening techniques for drought-resistant breeding of durum wheat genotypes in Iran. *Afric. J. Biotech*. **10**:12107-12117.

Keyvan, S. (2010). The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *J. Anim. Plant. Sci*. **8**:1051-1060.

Kumar, A.A., Reddy, B.V.S., Sharma, H.C., Hash, C.T., Rao, P.S., Ramaiah, B. and Reddy, S. (2011). Recent advances in sorghum genetic enhancement research at ICRISAT. *American. J. Plant. Sci*. **2**:589-600.

Leder, I. (2004), Sorghum and Millets, in cultivated plants, primarily as food sources. **In**: *Encyclopedia of Life Support Systems (EOLSS)*, pp. 2-10, (Fuleky G. ed), UNESCO, Eolss Publishers, Oxford, UK.

Li, G., Zhang, Z.S., Gao, H.Y., Liu, P., Dong, S.T., Zhang, J.W. and Zhao, B. (2012). Effects of nitrogen on photosynthetic characteristics of leaves from two different stay-green corn (*Zea mays* L.) varieties at the grain-filling stage. *Can. J. Plant Sci*. **92**:671-680.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C. and Sohrabi, Y. (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian J. crop Sci*. **4**:580-585.

Mansouri-far, C. and Sanavy, S.A.M.M. (2004). Effect of drought and nitrogen stress on chlorophyll characteristics of maize (*Zea mays* L.) leaf. *Proceedings of the 4th International Iran & Russia Conferenc*, pp. 206-212.

McCann, S.E. and Huang, B. (2008). Evaluation of drought tolerance and avoidance traits for six creeping bentgrass cultivars. *Hortscience* **43**:519–524.

Meziane, D. and Shipley B. (2001). Direct and indirect relationships between specific leaf area, leaf nitrogen and leaf gas exchange. Effects of irradiance and nutrient supply. *Ann. Bot.* **88**:915–927.

Moayedi, A.A., Nasrulhaq-Boyce, A. and Tavakoli H. (2011). Application of physiological and biochemical indices for screening and assessment of drought tolerance in durum wheat genotypes. *Aust. J. Bot*. **5**:1014-1018.

Mohammadkhani, N. and Heidari, R. (2008). Drought-induced accumulation of soluble sugars and proline in two maize varieties. *World Appl. Sci. J.* **3**:448-453.

Mohammed, N.A., Ahmed, I.A. M. and Babiker, E.E. (2011). Nutritional evaluation of sorghum flour (*Sorghum bicolor* L. Moench*)* during processing of Injera. *Int. J. Biol. Life Sci.* **7:**1.

Moosavi, S.S., Yazdi Samadi, B., Naghavi, M.R., Zali, A.A., Dashti, H., Pourshahbazi, A. (2008). Introduction of new indices to identify relative drought tolerance and resistance in Wheat Genotypes. *Desert* **12**:165-178.

Nagarjuna, K.N. (2007). Physiological investigation on stay green trait in sorghum with varying levels of Nitrogen. M. sc. Thesis, Dharwad University of Agricultural Sciences, Dharwad, pp. 1-2, 19-21, 43.

Naser, L. Kourosh, V., Bahman, K. and Reza A. (2009). Soluble sugars and proline accumulation play a role as effective indices for drought tolerance screening in Persian walnut (*Juglans regia* L.) during germination. *Fruits* **65**:97-112.

Ngugi, K., Kimani, W., Kiambi, D. and Mutitu, E.W. (2013). Improving drought tolerance in *Sorghum bicolor* L. Moench: Marker-assisted transfer of the stay-green quantitative trait loci (QTL) from a characterized donor source into a local farmer variety. *Int. J. Scientific Research in Knowledge* **1**:154-162.

Okiyo, T., Gudu, S., Kiplagat, O. and Owuoc, J. (2010). Effect of post anthesis moisture stress on performance of stay green sorghum hybrid in eastern province of Kenya. *Twelveth KARI biennial conference*, 8- 12 November, Nairobi, Kenya, pp. 1-6.

Oliveira, A.B., Alencar, N.L.M. and Gomes-Filho, E. (2012). Physiological and biochemical responses of semiarid plants subjected to water stress. **In**: *Water Stress*, pp. 1-16, (Rahman, I.M.M. ed.), InTech.

Opik, H. and Stephen, R. (2005). *Physiology of Flowering Plants*. 4th Edn. Cambridge University Press, New York, 349 pp.

Oraki, H., Khajani, F.P. and Aghaalikhana, M. (2012). Effect of water deficit stress on proline contents, soluble sugars, chlorophyll and grain yield of sunflower (*Helianthus annuus* L.) hybrids. *Afric. J. Biotech*. **11**:164-168.

Painawadee, M., Jogloy, S., Kesmala, T., Akkasaeng, C. and Patanothai, A. (2009). Identification of traits related to drought resistance in Peanut (*Arachis hypogaea* L.). *Asian J. Plant Sci*. **8**:120-128.

Pawar, K.N. (2007). *Physiological indices for drought Tolerance in rabi sorghum*. Ph.D. Dissertation. Dharwad University of Agricultural Sciences, Dharwad, pp. 1-11, 26-38, 74-107.

Plessis, J.D. (2008). Sorghum production. Republic of South Africa Agriculture Department, Pretoria. www.nda.agric.za/publications.

Raman, A., Verulkar, S.B., Mandal, N.P., Variar, M., Shukla, V.D., Dwivedi, J.L., Singh, B.N., Singh, O.N., Swain, P., Mall, A.K., Robin, S., Chandrababu, R., Jain, A., Ram, T., Hittalmani, S., Haefele, S., Piepho, H. and Kumar, A. (2012). Drought yield index to select high yielding rice lines under different drought stress severities. *Rice* **5**:1-12.

Reddy, B.V.S., Ramaiah, B., Kumar, A.A. and Reddy, P.S. (2007). Evaluation of sorghum genotypes for the stay-green trait and grain yield. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Andhra Pradesh, India, pp. 1-4.

Rosyara, U.R., Khadka, K., Subedi, S., Sharma, R.C. and Duveiller, E. (2007). Heritability of stay-green traits and association with spot blotch resistance in three spring wheat populations. *J. Genet. & Breed*. **61**:00-00.

Sammar Raza, M.A., Saleem, M.F., Khan, I.H., Jamil, M., Ijaz, M., and Khan, M.A. (2012). Evaluating the drought stress tolerance efficiency of wheat (*Triticum aestivum* L.) Cultivars. Russian *J. Agric. and Socio-Economic Sci*. **12**:41-46.

Schneider, K. and Anderson, L. (2010). Yield gap and productivity potential in Ethiopian agriculture: staple grains & Pulses. Evans School Policy Analysis and research (EPAR), University of Washington.

Shirinzadeh, A., Zarghami, R., Azghandi, A.V., Shiri, M.R. and Mirabdulbaghi, M. (2010). Evaluation of drought tolerance in mid and late mature corn hybrids using stress tolerance indices. *Asia. J. Plant. Sci*. **9**:67-73.

Shulze, E.D., Beck, E. and Muller-Hohenstain, K. (2005). *Plant Ecology*. Springer, Berlin, Heidelberg, p. 132, 134.

Spano, G., Fonzo, N.D., Perrotta, C., Platani, C., Ronga, G., Lawlor, D.W., Napier, J.A. and Shewry, P.R. (2003). Physiological characterization of `stay-green' mutants in durum wheat. *J. Exp. Bot*. **54**:1415-1420.

Statistical Analysis System (SAS) (2003). Institute Inc., Cary, NC, US.

Szabados, L. and Savoure, A. (2009). Proline: a multifunctional amino acid. *Trends in Plant Sci.* **15**:89-97.

Taiz, L. and Zeiger, A. (2006). *Plant Physiology*. 4th Edn. Sinauer Associates, Inc., Sunderland, Massachusetts, pp. 592-593.

Thomas, H., Ougham, H., Canter, and Donnison, I. (2002). What stay-green trait mutants tell us about nitrogen remobilization in leaf senescence. *J. Exp. Bot*. **53**:801-808.

Trovato, M., Mattioli, R. and Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei* **19**:325 – 346.

Ulemale, C.S., Mate, S.N. and Deshmukh, D.V. (2013). Physiological indices for drought tolerance in Chickpea (*Cicer arietinum* L.). *World. J. Agric. Sci*. **9**:123-131.

Vajrabhaya, M., Kumpun, W. and Chadchawan, S. (2001). The Solute Accumulation: The Mechanism for Drought Tolerance in RD23 Rice (*Oryza sativa* L.) Lines. *Sci. Asia* **27**:93-97.

Verslues, P.E. and Juenger, T.E. (2011). Drought, metabolites, and Arabidopsis natural variation: a promising combination for understanding adaptation to water-limited environments. *Current Opinion in Plant Biol*. **14**:240–245.

Waseem, M., Ali, A., Tahir, M., Nadeem, M.A., Ayub, M., Tanveer, A., Ahmad, R. and Hussain, M. (2011). Mechanism of drought tolerance in plant and its management through different methods. *Cont. J. Agric. Sci*. **5**:10-25.

Xoconostle-Cazares, B., Ramirez-Ortega, F.A., Flores-Elenes, L. and Ruiz-Medrano, R. (2011). Drought tolerance in crop plants. *Am. J. Plant Physiol.* pp. 1- 16.

Xu, W., Rosenow, D.T. and Nguyen, H.T. (1999). Stay-green trait in grain sorghum, relationship between visual rating and leaf chlorophyll concentration. *Plant Breeding* **8**: 254-256.

Yancey, P.H. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol*. **208**:2819-2830.

Yordanov, I., Velikova, V. and Tsonev, T. (2003). Plant responses to drought and stress tolerance. *Bulg. J. Plant physiol.* 187–206.

Younis, M.E., El-shahaby, O.A., Abo-Hamed, S.A. and Ibrahim, A.H. (2000). Effects of water stress on growth, pigments and $^{14}CO_2$ assimilation in three sorghum cultivars. *J. Agr. Crop Sci*. **185**:73-82.

Zhang, J., Nguyen, H.T. and Blum, A. (1999). Genetic analysis of osmotic adjustment in crop plants. *J. Exp. Bot*. **50**:327-338.

Zivcak, M., Repkova, J., Olsovska, K. and Brestic, M. (2009). Osmotic adjustment in winter wheat varieties and its importance as a mechanism of drought tolerance. *Cereal research communications*. **37**:569-572.

Zlatev, Z. and Lidon, F.C. (2012). An overview on drought induced changes in plant growth, water relations and photosynthesis. *J. Food Agric.* **24**:57-72.

APPENDICES

Appendix 1. Date of sowing, germination and flowering of five sorghum accessions

Accessions	Date of Sowing	Date of Germination	Flowering Date
Sorcoll 060/07	05/12/2013	15/12/2013	23/04/2014
Sorcoll 141/07	05/12/2013	15/12/2013	18/04/2014
E36-1	05/12/2013	13/12/2013	14/03/2014
Sorcoll 146/07	05/12/2013	15/12/2013	16/03/2014
Sorcoll 163/07	05/12/2013	13/12/2013	14/03/2014

Appendix 2. Raw data for growth, biochemical and yield parameters

Accessions	Water level	Rep	SLA	SLW	Proline	TSS	N (%)	Protein	Yield/panicle
Sorcoll 060/07	WW	1	0.155	6.462	18.220	2.896	101.000	631.250	32.240
Sorcoll 060/07	WS	1	0.133	7.507	37.932	3.243	93.000	581.250	26.660
Sorcoll 141/07	WW	1	0.159	6.272	14.458	1.464	50.355	314.719	30.500
Sorcoll 141/07	WS	1	0.148	6.779	21.407	2.018	48.000	300.000	25.167
E36-1	WW	1	0.138	7.258	8.525	1.648	28.000	175.000	32.750
E36-1	WS	1	0.131	7.606	51.153	3.114	28.000	175.000	20.750
Sorcoll 146/07	WW	1	0.141	7.068	6.729	2.196	29.415	183.844	32.367
Sorcoll 146/07	WS	1	0.089	11.234	21.847	2.627	28.000	175.000	13.667
Sorcoll 163/07	WW	1	0.118	8.496	1.966	1.734	46.690	291.813	25.500
Sorcoll 163/07	WS	1	0.126	7.941	26.542	2.088	72.370	452.313	18.000
Sorcoll 060/07	WW	2	0.156	6.406	9.508	2.944	102.250	639.063	27.240
Sorcoll 060/07	WS	2	0.137	7.313	39.136	3.231	94.314	589.463	21.660
Sorcoll 141/07	WW	2	0.164	6.105	6.508	1.913	49.000	306.250	30.100
Sorcoll 141/07	WS	2	0.155	6.466	21.525	1.958	49.025	306.406	17.000
E36-1	WW	2	0.136	7.339	6.831	2.124	29.882	186.763	23.500
E36-1	WS	2	0.149	6.705	51.000	2.885	29.415	183.844	21.500
Sorcoll 146/07	WW	2	0.130	7.682	12.407	2.203	21.472	134.200	28.967
Sorcoll 146/07	WS	2	0.101	9.855	23.966	2.635	28.948	180.925	17.200

Continued

Sorcoll 163/07	WW	2	0.122	8.187	1.390	1.401	46.690	291.813	25.750
Sorcoll 163/07	WS	2	0.122	8.194	31.661	2.258	29.415	183.844	16.500
Sorcoll 060/07	WW	3	0.143	7.003	4.932	2.920	103.500	646.875	29.140
Sorcoll 060/07	WS	3	0.134	7.480	49.339	3.237	95.628	597.675	23.560
Sorcoll 141/07	WW	3	0.155	6.452	4.831	1.688	51.710	323.188	27.000
Sorcoll 141/07	WS	3	0.155	6.439	23.610	1.988	50.050	312.813	17.750
E36-1	WW	3	0.148	6.764	9.220	1.886	31.764	198.525	40.567
E36-1	WS	3	0.121	8.251	51.525	2.999	30.820	192.625	23.000
Sorcoll 146/07	WW	3	0.115	8.699	7.424	2.199	29.415	183.844	30.667
Sorcoll 146/07	WS	3	0.092	10.820	23.339	2.631	30.856	192.850	15.434
Sorcoll 163/07	WW	3	0.131	7.618	5.678	1.568	46.690	291.813	27.000
Sorcoll 163/07	WS	3	0.117	8.564	35.153	2.173	72.370	452.313	19.000

Appendix 3. Raw data for the drought indices

Accessions	**Rep**	**Ys**	**Yp**	**TOL**	**MP**	**YSI**	**YI**	**STI**	**SSI**	**GM**	**HM**
Sorcoll 060/07	1	26.660	32.240	5.580	29.450	0.827	1.113	0.984995	0.916	29.318	29.186
Sorcoll 141/07	1	25.167	30.500	5.333	27.834	0.825	1.271	0.996794	0.611	27.705	27.578
E36-1	1	20.750	32.750	12.000	26.750	0.634	0.954	0.652482	1.124	26.068	25.404
Sorcoll 146/07	1	13.667	32.367	18.700	23.017	0.422	0.886	0.470363	1.163	21.032	19.219
Sorcoll 163/07	1	18.000	25.500	7.500	21.750	0.706	1.000	0.605473	0.849	21.424	21.103
Sorcoll 060/07	2	21.660	27.240	5.580	24.450	0.795	0.904	0.676152	1.084	24.290	24.132
Sorcoll 141/07	2	16.500	25.750	9.250	21.125	0.641	0.833	0.551741	1.255	20.612	20.112
E36-1	2	21.500	23.500	2.000	22.500	0.915	0.989	0.485116	0.261	22.478	22.456
Sorcoll 146/07	2	17.200	28.967	11.767	23.084	0.594	1.114	0.529772	0.818	22.321	21.584
Sorcoll 163/07	2	17.000	30.100	13.100	23.550	0.565	0.944	0.674991	1.257	22.621	21.728
Sorcoll 060/07	3	23.560	29.140	5.580	26.350	0.809	0.983	0.786763	1.014	26.202	26.055
Sorcoll 141/07	3	17.750	27.000	9.250	22.375	0.657	0.896	0.622352	1.197	21.892	21.419
E36-1	3	23.000	40.567	17.567	31.784	0.567	1.057	0.89586	1.328	30.546	29.356
Sorcoll 146/07	3	15.434	30.667	15.234	23.050	0.503	1.000	0.503261	1.000	21.755	20.533
Sorcoll 163/07	3	19.000	27.000	8.000	23.000	0.704	1.056	0.676706	0.856	22.650	22.304

DECLARATION

I, the undersigned, declare that this thesis is my original work and it has not been presented in other universities, colleges or institutions for similar degree or other purpose. All sources of materials used for this study have been appropriately acknowledged.

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