Physiological and Agronomic Performance Evaluation of Stay Green (SG) Sorghum (*Sorghum bicolor* (L.) Moench) Varieties at Shewa Robit, Amhara Regional State, Ethiopia

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This is to certify that the Thesis prepared by Zelalem Getnet Tarekegne entitled: *Physiological and Agronomic Performance Evaluation of Stay Green (SG) Sorghum (Sorghum bicolor (L.) Moench) Varieties at Shewa Robit, Amhara Regional State, Ethiopia* and Submitted in partial fulfilment of the requirements for the Degree of Masters of Science (Biology: Botanical Science) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Physiological and Agronomic Performance Evaluation of Stay Green (SG) Sorghum (*Sorghum bicolor* (L.) Moench) Varieties at Shewa Robit, Amhara Regional State, Ethiopia

Zelalem Getnet Tarekegne, MSc. Thesis
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Drought is one of the major constraints limiting crop production worldwide. Crop growth models predict that this issue will be more severe in the future. Drought impairs normal growth, disturbs water relations, reduces water use efficiency and affects yield. Plants, however, have a variety of morphological, physiological and biochemical responses at cellular and whole organism level, making it a more complex phenomenon. A field experiment was conducted at Shewa robit to evaluate the physiological and agronomic performance of different SG sorghum accessions. To achieve this, three sorghum genotypes (Sorcoll 141/07, Sorcoll 146/07 and, Sorcoll 163/07) and a check (Afeso) accessions were used in the experiment. The experiment was laid down in a randomized complete block design (RCBD). The data on morphological, physiological and biochemical traits such as girth diameter, number of tillers, total green leaf area, chlorophyll content, rate of leaf senescence, relative water content, assimilation rate, WUE, SDW, RDW, RSR, leaf proline, nitrogen, protein, soluble sugar contents, and yield revealed significant difference among accessions at $P < 0.05$*. Afeso and Sorcoll 163/07 showed better stress tolerance and SG property. These accessions were also selected for their maximum grain yield per hectare, while accession Sorcoll 146/07 was selected for its early maturity. Correlation analysis revealed that GD, number of tillers, chlorophyll content, GLA, RWC, WUE, lower rate of leaf senescence, SDW, RDW and RSR had significantly positive relation with grain yield per hectare and have been found to be effective screening tools for stress tolerance and SG property. So, they can be used in sorghum breeding program.*

Key words/ phrases: Agronomic performance; Biochemical traits; Sorghum accessions; Stay-green; Stress tolerance.

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LIST OF ACRONOMYS

ATP:	Adenosine tri phosphate
CCM:	Chlorophyll content meter
CSA:	Central statistical agency
DAF:	Days after flowering
DAP:	Diammonium phosphate
GD:	Girth diameter
GLA:	Green leaf area
ICRISAT:	International crops research institute for the semi-arid tropics
LSD:	List significant difference
NG:	Number of green leaves
NSeL:	Number of senesced leaves
OA:	Osmotic adjustment
RSR:	Root to shoot ratio
Rubisco:	Ribulose - 1, 5 - bisphosphate carboxylase/oxygenase
RuBp:	Ribulose bisphosphate
RWC:	Relative water content
SG:	Stay green
SLA:	Specific leaf nitrogen
Sorcoll:	Sorghum collection
WUE:	Water use efficiency
YPH:	Yield per hectare
YPP:	Yield per panicle

CHAPTER ONE

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), is among the most important grain crops in the world including Ethiopia. Because of its multiple purposes and its ability to cope up with unfavourable growing conditions, sorghum will continue to feed the world's expanding populations. Moreover, it will be the crop of the future due to the changing global climatic trends and increase in use of marginal lands for agriculture (Paterson *et al*., 2008; CSA, 2011). Sorghum is widely grown in the high lands, low lands and semi-arid regions of Ethiopia; especially in moisture stressed parts where other crops can least survive (Tesfaye Tesso, *et al.*, 2008). However in Ethiopia, sorghum production has declined due to population growth; land degradation; use of traditional farm implements and global climate change (Asfaw Adugna, 2007). Moreover, drought is the major cause for underproduction of the crop (Yilma Kebede, 1991; Asfaw Adugna, 2007).

Drought is one of the most severe stresses which reduce crop production by affecting its growth, development and metabolic activity of plants (Bota *et al*., 2004). It is a serious problem that continues to challenge to agricultural scientists, plant physiologists and plant breeders (Bakheit, 1989; Atteya, 2003). The impact of drought depends on the intensity of the stress, duration of stress, developmental stage and genotypic difference of the crop (Prasad *et al.,* 2008). Plants including sorghum resist drought stress by either of drought escape, drought avoidance or drought tolerance mechanism. However, a plant may exhibit more than one of these strategies to cope with drought stress.

Drought response in sorghum has been classified into two distinct stages, pre-flowering and post-flowering (Tuinstra *et al*., 1996). Severe drought stress during post-flowering stages led to loss of chlorophyll and increased senescence of leaves (Bray, 1993). However, post-flowering drought resistance referred to as the stay-green trait (SG) has been reported in sorghum (Xu *et al*., 2000; Borrell *et al*., 2000a) and in maize (Monneveux *et al.*, 2006). Genotypes that possess SG trait are reported to have reduced lodging and resistance to stalk rots, higher levels of stem carbohydrates, and improved grain filling and grain yield under stress (Borrell *et al.*, 2000a). However, researches have indicated that sources of resistance to post-flowering water stress are less common than those found for pre-flowering.

In Ethiopia, many efforts have been made to address the drought problem in sorghum production. Breeding programme in Ethiopia has released a number of varieties from lowland areas which give reasonable yield in drought prone areas. Currently, sorghum breeding in Ethiopia is fully engaged in different research activities in sorghum drought tolerance. So far, two sources of SG, B-35 and E-36-1 were identified from the Ethiopian gene pools by ICRISAT and now in use in different part of the world to generate drought resistance sorghum varieties (Yilma Kebede, 1991; Asfaw Adugna, 2012). The Ethiopian sorghum germplasm is also noted worldwide as a source for useful genes such as cold tolerance, good grain quality, and disease and insect resistance (Yilma Kebede, 1991). Amsalu Ayana *et al.* (2001) also evaluated 415 accessions based on morphological criteria and showed presence of significant variation among Ethiopian sorghum accessions. Moreover, the cultural and ethnobotanical aspect of sorghum in Ethiopia has been well addressed by Firew Mekbib (2009).

However, only few works are done to screen post flowering drought tolerant SG sorghum varieties in the country. The BIO-EARN project has attempted to screen accessions for post flowering drought tolerance. Under this project Dagnachew Bekelle (2008), Zelalem Mengiste (2008) and Addisie Yalew (2010) evaluated sorghum accessions genetic diversity and post-flowering drought tolerance using few morphological and agronomic criteria. Dagnachew Bekelle (2008) has noted the presence of very high genetic diversity among Ethiopian sorghum germplasm accessions collected from the drought prone areas. On the other hand, Zelalem Mengiste (2008) has revealed the presence of variation in SG property among 165 Ethiopian sorghum accessions collected from the drought prone areas. Moreover Addisie Yalew (2010) has showed the presence of variation among seven SG sorghum varieties, under semi controlled glasshouse conditions using morphological and physiological criteria and identified three better SG accessions. However, the author has suggested the need to extrapolating the results in the field experiment and needs to include more parameters. Furthermore, the biochemical tests are not yet done. However, Pawar (2007) noted drought stress triggers many biochemical changes including accumulation of various stress metabolites in plants.

In the present field experiment, the responses of the recommended sorghum accessions to post flowering drought were evaluated in terms of morphological, physiological, biochemical and yield parameters. Also, the well known local sorghum accession was used as a check. In addition, research conducted to evaluate and select sorghum accessions for SG trait in Ethiopia were few. Therefore, it is crucial to screen sorghum local accessions for SG trait under field condition.

1.1. OBJECTIVES

1.1.1. General objective

- ✓ To evaluate the agronomic, biochemical and physiological responses of stay green (SG) sorghum varieties at Shewa robit.

1.1.2. Specific objectives

- To measure the morphological performance of the SG sorghum accessions.
- To evaluate the physiological traits of SG sorghum accessions.
- To study the biochemical responses of SG sorghum accessions.
- To find out the variation in yield and yield components of SG sorghum accession(s) associated with drought tolerance.
- To identify specific accession(s) with valuable SG traits that can be used in future sorghum breeding programmes.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Origin and distribution of *S. bicolor*

The origin of sorghum is not clearly known and there is no agreement among the different authors with regard to its origin. There is an assumption that the domestication has occurred 3000-5000 years ago (Laidlaw and Godwin, 2008). According to Smith and Frederikson (2000) there is also anthropological evidence that hunter-gatherers consumed the crop during the early 8000 BC. The primary centers of origin and diversity for sorghum are believed to be in sub-Sahara Africa extending from the extreme East to West Africa (Laidlaw and Godwin, 2008). However, at present day, the most accepted probable place seems to be the area extending from Ethiopia to Lake Chad (Aldrich *et al*., 1992). Members of the wild *S. bicolor* ssp. *verticilliflorum* are reported to be the immediate progenitors of the domesticated sorghum and are dry Savanna plants that were most probably domesticated west of the Ethiopian highlands (De Wet, 1978). Likewise, survey results of Amsalu Ayana *et al*. (2001) and Tesfaye Tesso *et al*. (2008) indicated that wild *S. bicolor* ssp. *verticilliflorum* found in five districts of western, eastern, northern, north western and south western Ethiopia.

2.2. Taxonomy in the genus Sorghum

2.2.1. Formal taxonomy

The taxonomy of sorghum is among the most unsettled despite the publication of a number of papers by different authors with continued modification (e.g., Garber, 1950; De Wet, 1978). Sorghum belongs to the family *Poaceae,* tribe *Andropogoneae,* subtribe *Sorghinae,* and genus *Sorghum* Moench (Clayton and Renvoize, 1986).

Moench coined the name sorghum by changing the original name "*Holcus*" that was given by Linnaeus in 1753 (House, 1985). According to House (1985) authors who contributed to the classification of sorghum are Piper (1916), Stapf (1917), Kuwada (1915, 1919), Marchal (1920) and Vinall (1926).

The most recent classification was done by De Wet (1978) based on experimental data, accumulated over two decades of biosystematic studies of herbarium specimens and seed collected across the range of section sorghum in Europe, Africa and southern Asia. The author divided it into five races (bicolor, caudatum, guinea, durra, and kafir). The race bicolor is found nearly everywhere sorghum is grown and is characterized by very loose, open panicles similar to wild sorghum. Caudatum race originated mostly from the region around Lake Chad to the Ethiopian border. The guinea race has its origins in West Africa and is grown in areas with higher rainfall. The kafir race is from southern Africa. Durra race has its origins around the edges of the Sahara.

2.2.2. Ethno taxonomy

Farmers in different parts of Ethiopia recognize each sorghum type commonly grown in their area by various vernacular/ folk names (Awegchew Teshome *et al*., 1997; Beyene Seboka and Van Hintum, 2006; Firew Mekbib, 2009). These names may be reflections of their morphological appearance e.g., Mishinga Worabisa (Oromo language, meaning hyena sorghum, due to the awn covering of the panicle), Cherekit (Amharic, moon like, due to its very white, shiny seeds that light as a moon) or the special purposes they offer, e.g., Marye (Amharic, my honey, due to its sweet taste), Gan seber (Amharic, pot breaker, due to its potency in making local beer). Moreover, names are given due to their ability in surviving biotic and abiotic stresses (e.g., Kitign ifere, unafraid of syphilis (Kitign/striga, mean to say tolerant to striga). They also recognize wild sorghums by different vernacular names. However, unlike the cultivated sorghum, most of the naming is related to their noxious nature. For instance, in Wello and Pawe areas farmers call them "Killo" (the fool, as it fools farmers through mimicry), "Yeseyitan ageda" (Satan's cane), "yeseyitan mashila" (Satan's sorghum) in Amharic to express their abhorrence to this weed. In Oromifa, it is called Seepo. In Tigray it is called "Kancha Seyitan" (Alamata area), "Kuchuye" (Adenkel area), "Seyitanzer" (in Subday), and "Aadar" (in Hagereselam and Humera areas). "Aadar" is similar to the naming in the adjacent region of Sudan.

2.3. Significance of *S. bicolor*

Sorghum is the fifth most important food crop in the world and a staple food crop in many regions of Africa, Asia and Latin America (ICRISAT, 2006). It is the second crop next to maize (*Zea mays*) grown across all agro-ecologies in Africa (Gebisa Ejeta and Grenier, 2005). In Ethiopia, sorghum stands fifth major cereal crop in terms of area and production next to teff (*Eragrostis tef*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*) and maize (*Zea mays*) (Paterson *et al*., 2008). It covers 16% of the total area allocated to grains (cereals, pulses, and oil crops) and 19.5% of the area covered by cereals of the country (CSA, 2011). It is also the second most important crop for Injera making next to teff (*Eragrostis tef*).

Rooney and Waniska (2000) provide a tremendous overview of the uses of sorghum in food and industry. Worldwide, Sorghum has been used for human food, animal feed, building material and fencing. It can be used to produce foods that are gluten free and in this respect the potential for new food items exists for both the US and Europe. The crop is also being exploited as a major distilled and non distilled alcohol producer, for example Africa Harvest is partnering with East Africa Breweries Ltd., (Rooney and Waniska, 2000). Sorghum is also thoroughly investigated as an energy crop for bioethanol and methane production (House, 1985). Furthermore, the straw of traditional tall sorghums is used to make palisades in villages or around homesteads. The plant bases are an important source of fuel for cooking and the stems of wild varieties are used to make baskets or fish traps. Dye extracted from sorghum is used in West Africa to colour leather red. In western Africa, Nigeria has emerged as a pioneer in the industrial utilization of sorghum (Wambui, 2012).

2.4. Drought stress

Drought stress is a major environmental factor that affects the growth and development of plants. Possibly the most commonly accepted definition of drought is offered by Beran and Rodier (1985): ‘drought is reduction in water availability in a specific time and on a specific region’. Thus, drought happens when an essential water shortage expands both in time and area. Due to global warming and climate change, drought is increasing throughout the world with time. Several studies showed that, due to climate change drought stress accounting for more than a 50% reduction in yields worldwide (Tsakiris and Vangelis, 2004; CSA, 2011)**.** Therefore water will become the most important vital substance in the near future. In recent years, understanding the effects of drought on plants has become a very important activity to design and implement better plant breeding and management techniques in agriculture and in determination of the fate of natural vegetation in the environment (Chaves *et al.,* 2003; Pirdashti *et al*., 2009; Wambui, 2012).

2.5. Effect of drought on plant growth and development

Cell division and cell growth are the two primary processes involved in plant growth and can be influenced by relatively mild drought stress. Cell division is considered to be less sensitive to drought than cell enlargement or growth. In this regard, leaf expansion is among the most sensitive growth processes to drought (Alves and Setter, 2004). The authors also showed that both cell expansion and production of cells contributed to a loss in leaf area depending on the developmental stage at which the leaf was stressed. However, both cell division and cell expansion were able to recover fully when stress occurred at early phases of leaf development, but at the final phase, these processes did not resume long enough to generate full size leaves (Alves and Setter, 2004). The general effects of mild drought are a reduction in leaf numbers, rate of expansion, and final leaf size. Under severe stress, the rate of leaf elongation decreases and leaf growth can cease. Drought stress can also influence total leaf area by affecting the initiation of new leaves. Continued drought stress can accelerate leaf senescence (De Souza *et al.*, 1997) and lead to death of leaf tissue, resulting in leaf drop. Decreased leaf senescence under drought stress is often termed as a tolerance mechanism, particularly under post flowering drought stress. In contrast, loss of leaf area can serve as a drought-avoidance mechanism, helps to prevent further water loss. On the other hand, retaining GLA (e.g. Sorghum) during grain filling has proved to be an excellent indicator of SG and has successfully been used to select drought resistant sorghums (Rosenow *et al.*, 1983).

Drought stress often decrease stem growth and plant height. When plants experience drought stress, stem diameter shrinks in response to changes in internal water status

(Katerji, *et al.,* 1994). Changes in stem diameter were well correlated with predawn leaf water potential under prolonged drought (Katerji *et al.,* 1994). Yatapanage and So (2001) used stem diameter data to predict leaf water potential in sorghum and showed promising results. Root growth is also very sensitive to water stresses. Xiong *et al.* (2006) noted that the response of root growth is variable. Under moderate moisture stress its growth can be greater because of increased partitioning of carbohydrates to roots, whereas, severe drought often limits root growth, more so when it is associated with increased soil temperatures.

In addition to the growth effect, drought influences the duration of plants developmental stage. In most cases, the length of time from floral initiation (panicle initiation) to anthesis (panicle exsertion) is decreased by moderate drought but is increased by severe stress. Drought stress during panicle development inhibits the conversion of vegetative to reproductive phase and plants remain vegetative until the stress is relieved (Craufurd *et al.,* 1993). Moreover, it delays the panicle initiation but also can cause the cessation of panicle development at any stage between panicle initiation and flowering. Severe drought inhibits panicle exsertion and also delays flowering (Prasad *et al.,* 2008).

2.6. Effect of drought on plants metabolism

2.6.1. Plant physiology

Relative water content (RWC) and leaf water potential are the primary plants physiological traits identified for drought tolerance and which are dependent on the intensity and duration of water stress (Abdalla and El-Khoshiban, 2007). The reduction in leaf RWC has been induced by the water deficiency in soil as a consequence of water loss via the stomata (Pirdashti *et al*., 2009). Various studies have showed that the decline in RWC lead to a reduction in leaf photosynthetic activity under water stress (Siddique *et al*., 2000; Ahmadi and Siosemarideh, 2005). It has been hypothesized that genotypes which keep their stomata open under water stress condition while maintaining adequate leaf RWC can be considered as suitable for dry regions (Ahmadi and Siosemarideh, 2005). In this regard, during field experiment, genotypes maintaining higher RWC at flowering and dough stage were identified as drought tolerance (Shivalli, 2000). Therefore, RWC could be an effective parameter used to evaluate drought tolerance and identification of high yielding genotypes (Xu *et al.*, 2000).

Previous studies have shown that drought significantly affects gas exchange parameters such as, leaf net CO_2 assimilation rate, stomatal conductance, and transpiration rate (Lawlor and Cornic, 2002). When the plants photosynthetic machinery becomes susceptible to photoinhibition, CO_2 assimilation is reduced leading to a decline in photosynthetic efficiency, thereby reducing crop productivity and yield (Chaves *et al*., 2003; Xu *et al*., 2008). During the initial onset of drought stress, the decline in assimilation rate is attributable to stomatal limitation (stomatal closure), because stomata are highly sensitive to changes in soil water deficit (Atteya, 2003). Moreover, stomatal response to leaf water potential and environment are important for regulation of transpiration and assimilation, and essential for CO_2 acquisition (Pirdashti *et al*., 2009). At later stages with increasing severity, drought stress causes non-stomatal limitations (tissue dehydration), which impairs metabolic activities (Atteya, 2003; Chaves *et al*., 2003; Bota *et al.*, 2004). Major metabolic changes caused by non-stomatal limitations are decline in regeneration of ribulose bisphosphate (RuBP) and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein content. This decreases Rubisco activity impairs ATP synthesis and photophosphorylation or decreased inorganic phosphorus. Sinclair and Jamiesson (2008) also have reported the assimilation rate of drought tolerant wheat cultivars was higher than those of susceptible ones during post-flowering stage. Thus, capability to maintain key physiological processes such as photosynthesis during post-flowering drought stress is found to be good indicator and screening tool for drought tolerance.

Both stomatal and non-stomatal limitations cause reductions in photosynthesis during drought stress (Ahmadi and Siosemardeh, 2005). However, these limitations also have a negative impact on plant biochemistry and primary photochemistry associated with photo system II (PSII) (Ashraf *et al*., 2007). According to Pirdashti *et al*. (2009) under well-watered conditions, tolerant as well as susceptible genotypes maintained high Fv/Fm (~0.82-0.83). However, drought stress has resulted in a decrease in Fv/Fm, which was evident only in drought susceptible genotypes due to photoinhibition associated with an over-reduction of PSII (Massacci and Jones, 1990). The ability to maintain high Fv/Fm under drought stress indicates a high efficiency of radiation use possibly for photochemistry and carbon assimilation. In turn, this would have implication to grain yield, hence a positive correlation was reported between Fv/Fm.

Effect of water stress on water use efficiency (WUE) depends on plant species and phenological stage of water stress imposition and severity. Crops productivity and WUE are associated with the temporal and spatial distribution of precipitation and available soil water status (Atteya, 2003; Chaves *et al*., 2003). Regulation of diffusive conductance is known to affect WUE by modulating both transpiration rate and assimilation rate, since regulation of assimilation rate also strongly affects WUE (Seghatoleslami *et al.*, 2008). Generally, WUE in plants tends to be high as an adaptation under stress conditions. Therefore, substitution of drought adopted crops with those with high water use efficiency ones is an efficient strategy in water shortage conditions.

2.6.2. Plant biochemical accumulation

Drought triggers many biochemical changes. The common response is the production and accumulation of compatible osmolytes, which are osmotically active (proline) and neutral organic compounds such as sugars, proteins, selected amino acids and quaternary ammonium compounds (Yancey *et al.*, 1982; Bohnert *et al.*, 1995). The accumulation of compatible osmolytes reduces the water potentials; thereby additional water is taken up from the environment.

Presence of chlorophyll is unique in plants as it traps light which is essential for the processes of photosynthesis. According to Rosa and Maiti (1990) chlorophyll has not always been shown to have a strong relationship with photosynthesis rate under normal conditions, but at lower light intensity, it becomes the deciding factor. Ashraf, *et al.* (2007) studied that majority of the chlorophyll loss in response to water stress occurred in mesophyll cells and this loss is mainly due to the reduction in the lamellar contents of the light harvesting chlorophyll a/b protein. The author also pointed that, leaf chlorophyll content was 14% higher in the non senescent genotype than in the senescent. According to Younis *et al*. (2000) drought tolerance was associated with increased chlorophyll content. The post rainy sorghum genotypes maintained higher chlorophyll a and total chlorophyll at 50% flowering and drought stages, however long term stress at vegetative and reproductive phases reduced the chlorophyll content (Shivalli, 2000; Younis *et al*., 2000). It leads a conclusion that, drought tolerance was associated with increased chlorophyll content. So, the analysis of chlorophyll content may act as a basic parameter to screen drought tolerance sorghum genotypes.

Accumulation of proline is a widespread plant response to environmental stresses. It has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm (Trovato *et al*., 2008). In addition to being an osmolyte, proline is a powerful antioxidant and inhibits programmed cell death (PCD). As a result of this, proline can decrease the negative effect of reactive oxygen species (ROS) in microbes, animals and plants (Chen and Dickman, 2005). Free proline functions as an osmoprotectant, a secondary metabolite, a protein stabilizer, a metal chelator, an inhibitor of lipid peroxidation (LPO), and a scavenger of OH and O_2 (Ashraf and Foolad, 2007; Trovato *et al*., 2008). The authors also showed that, significant accumulation of proline was observed during the heading and hard dough stages, which indicated that drought stress greatly, stimulated the increase in proline content. Therefore, (Prasad *et al.*, 2008) found that, the resistant sorghum lines accumulated high levels of proline, while the susceptible ones showing no significant accumulation.

Sugars are the main solutes that accumulate in different parts of sorghum and other plants in response to various environmental stresses Prado, *et al.* (2000). The development of leaf water deficit in fully expanded sorghum leaves resulted in increased amount of glucose, sucrose and fructose (Al Hakimi, *et al.*, 1995). The author also pointed out that, the accumulated total sugars have higher grain yields than others. However, as the water stress increased there was a proportionate decrease in total sugars. Accumulation of total soluble sugars and free amino acids under stress at all the growth stages of sorghum indicated the possibility of their involvement in osmotic adjustment (Pawar, 2007). So, soluble sugar content proved to be a better marker for selecting improvement of drought tolerance in different plants.

Nitrogen is a critical nutrient for the growth of healthy vegetative part. Part of the absorbed nitrogen is stored in plant leaves in the form of vegetative part. Hence, determination of plant nitrogen is crucial to optimize crop yield (Muchow and Sinclair, 1994). The accumulation of specific leaf nitrogen varies in sorghum plants at different growth stages. At early growth stage, the stay green hybrids partitioned more carbon and nitrogen to leaves compared with their senescent counterparts, resulting in higher specific leaf nitrogen (Khannan *et al.,* 1994; Borrell and Hammer, 2000). The authors also wrote that, after anthesis, higher specific leaf nitrogen delays the onset and reduced the rate of leaf senescence and this is associated with stay green crops taking up more nitrogen from the soil compared with senescent crops. The specific leaf nitrogen in stay green sorghum hybrids remained above the threshold senescence level for longer than in senescent hybrids for at least three reason: i) The leaf nitrogen benchmark at anthesis was higher in stay green than in senescent hybrids, ii) Nitrogen uptake during grain filling was higher in stay green than in senescent hybrids, iii) The remobilization of nitrogen from leaves of stay green hybrids during grain filling is low compared to senescent hybrids (Muchow and Sinclair, 1994; Borrell and Hammer, 2000). So, as a screening tool, sorghum accession with higher nitrogen accumulation under post flowering drought stress may give better grain yield than the accessions with low nitrogen content.

2.7. Effect of drought on biomass, yield and yield components

Under mild drought stress, pattern of resource allocation generally favours root growth rather than shoot growth. Severe stress conditions often decrease root growth. Timing of drought stress also has great influence on partitioning of carbohydrates and nitrogen. If drought stress occurs during early vegetative growth stages, there is a shift of partitioning toward roots rather than shoots, increasing the root-to-shoot ratio. This increase is due mainly to decreased shoot growth rather than increased root weight. Root mass rarely increases under stress, whereas root length and root volume often increase in response to mild stress. If drought stress occurs during the reproductive phase, there is no influence on the root-to-shoot ratio, but flowering and seed-set are slowed down of drought stress occurs after flowering, there is generally increased partitioning of resources toward seed filling (Wardlaw and Willenbrink, 2000).

Yield is mainly a function of various components which can broadly be divided into the number of plants (germination), dry matter production (growth, tillers, potential reproductive sites), seed numbers (reproductive processes and seed-set), and seed size (product of seed-filling rate and seed-filling duration). Drought mainly influences yield by limiting seed numbers by either influencing the amount of dry matter produced by the time of flowering (this is particularly true for determinate plant types) or by directly influencing pollen or ovule function, which leads to decreased seed-set. Secondly, drought influences seed filling mainly by limiting the assimilate supply, leading to smaller seed size and lower yields (Frederick *et al.,* 1991; Wardlaw and Willenbrink, 2000).

2.8. Mechanisms of drought resistance

Drought resistance can be defined as the ability of the plant to withstand water deficit while maintaining appropriate physiological activity. Generally, all plants including sorghum that are growing in water-limiting environments utilize various adaptive mechanisms (Yilma Kebede, 1991). However, it is a very complex mechanism; it not only depends on features of defence mechanisms but also depends on the response to several environmental signals (Xiong and Ishitani, 2006). The literature suggests classification of drought resistance into three separate categories: drought avoidance, drought tolerance, and drought escape. These drought-resistance strategies are not mutually exclusive. A plant may exhibit more than one of these strategies to cope with drought stress.

2.8.1. Drought escape

Drought escape is the ability of a plant to complete its life cycle prior to drought exposure or becomes dormant during drought stress (Turner, 1980). It involves rapid phenological development such as, early flowering and early maturity, developmental plasticity (variation in duration of growth period) and remobilization of pre-anthesis assimilates to grain (Turner, 1980). It is mainly demonstrated by desert ephemerals and some short duration dry land crops which have a condensed growth cycle and reach maturity before drought occurs. Crop species displaying this type of adaptation are often photoperiod-sensitive so that flowering coincides with the average date of the end of the rainy season (Prasad *et al.*, 2008). However, cultivars that mature extremely early tend to be lower in yield because the plants have a shorter growth period to flower and store nutrients in the grain. Furthermore, there is a positive relationship between long growth period and yield potential (Turner, 1980).

2.8.2. Drought avoidance

Drought avoidance is the ability of a plant to maintain normal physiological function by postponing tissue dehydration. This mechanism may be achieved by increasing water uptake of the root system and/or reducing water loss from transpiring leaves. Plants have developed multiple ways of acquiring water, including developing extensive, deep root systems, increasing root branching and surface areas to exploit large soil volumes for water absorption. Research in different plants has shown that a shallow and less branch rooted species had lower drought tolerance than species with well developed and deeper root systems (Marcum *et al.* 1998; Huang and Fu, 2001).

Another important avoidance mechanism is the ability of plants to reduce water loss through transpiration. Most transpirational water loss is through stomata in leaf epidermal surfaces. Stomatal closure is one of the most sensitive responses to drought stress. Stomatal closure can be affected by many factors. It has been found to be induced by increasing leaf concentration of abscisic acid (ABA) (Davies *et al*., 1994; Davies *et al.*, 2002). Roots are important sites for the synthesis of ABA, which is transported to shoots and initiates a signal cascade in guard cells that alters the membrane transport of several ions, and as a result, guard cells lose their turgor and stomata close. This results in changes of stomatal conductance, transpiration rate, and photosynthesis (Bray, 1993; Bohnert *et al.*, 1995). In addition to stomatal regulation of water loss, modification of shoot characteristics such as leaf shedding or folding, or the development of a thick cuticle reduces leaf transpiration (Johnston *et al.*, 2002). The authors also showed that, the modification of shoot is positively related to leaf thickness, epicuticular wax content, and tissue density but negatively related to stomatal density and leaf width.

2.8.3. Drought tolerance

Drought tolerance can be defined as a plant's ability to maintain physiological functions when little or no water is available to the plant. One of the important factors controlling cell tolerance to desiccation or dehydration is the cell's capability to maintain adequate turgor pressure during drought stress. This is done by increasing the concentration of compatible solutes within the cell. By increasing compatible solutes, a plant cell can lower its osmotic potential and thus, water potential, which prevents water loss to intercellular spaces or can increase water movement into cells with low water potential due to osmotic adjustment (Nilsen *et al.,* 1996). In addition to osmotic adjustment, other mechanisms such as cell wall elasticity also play important roles in maintenance of cell turgor pressure and desiccation tolerance. As a result of increased cell wall elasticity, plants can maintain turgor for a longer period of time under drought stress (Auge *et al.,* 1987; Samntaaria and Fukai, 1990).

Drought tolerance mechanisms also involve changes in various metabolic processes. A relatively drought sensitive metabolic function is photosynthesis; drought stress inhibits carbon fixation. While light absorption continues under drought stress, reduced electron transport to carbon fixation leads to an accumulation of excessive energy that can be dissipated by reducing molecular oxygen, thus generating active oxygen species, singlet oxygen ($1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH). These species can cause cellular damage by interacting with lipids, nucleic acids, and proteins. It has also been suggested that oxidative stress resulting from drought can negatively impact on quality and physiological functions of plants (Huang and Fu, 2001; Zhang *et al.,* 2004). However, many plant species can defence the oxidative stress by scavenging those active species, through increasing the activity of superoxide dismutase (SOD), catalase (CAT), hydrogen peroxidase (POD), and ascorbate peroxidase (APX) (Zhang *et al.*, 2004).

In response to drought stress, plants exhibit significant changes in protein composition, protein synthesis, and expression, including synthesis of stress-inducible proteins (Close, 1996). Synthesis of stress-induced proteins such as late embryogenesis abundant (LEA) proteins in response to drought stress has been associated with increased adaptive ability and tolerance to drought stress in various plant species (Dure, 1993; Han and Kermode, 1996). The function of these proteins involved in drought tolerance remains unclear. However, some studies suggest that these proteins may act in the stabilization of membranes and as molecular chaperones, which prevent denaturation of other proteins (Dure, 1993; Close, 1996).

2.8.4. Stay-green or delayed senescence

Plant leaves senesced early in response to drought stress particularly when the stress occurs during the post flowering stages. Some genotypes tolerate drought during grain filling by keeping their leaves green; these cultivars are termed as stay-green types. Stay-green genotypes retain chlorophyll in their leaves and maintain the ability to carry out photosynthesis longer than the senescent types, and are often shown to have a yield benefit (Borrell *et al.*, 2001). These stay-green genotypes are different from some cultivars which have cosmetic stay-green, i.e., the cultivars which have green leaves but do not sustain photosynthesis for longer periods under drought conditions. It is also possible that stay-green genotypes can store large amounts of carbohydrates in stems, as these genotypes also often exhibit decreased lodging and have resistance to diseases such as charcoal rot [caused by *Macrophomina phaseolina* (Tassi) Goid].

The physiological basis of stay-green trait is not well understood and needs further investigation. However, Stay-green genotypes are known to have more sugars in stems and contain higher cytokinin content (McBee and Miller, 1982). Borrell *et al.* (2001) showed that stay-green types assimilate more nitrogen and have greater specific leaf nitrogen content. However, it is unclear if the above mentioned traits are a consequence of stay-green or a secondary trait that is associated with general adoption of stay-green (Xu *et al.*, 2000). The stay-green trait has been exploited in several crops. However, the progress of this trait in breeding is more obvious in corn and sorghum. In sorghum, several genotypes have been identified to possess stay-green trait (e.g., B-35). These genotypes have been used as a source of stay-green in several sorghum cultivars (Xu *et al.*, 2000).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the study area

The present field experiment was conducted during 2013/2014 cropping season at Shewa robit. Shewa robit is a town in North-central Ethiopia, located in the northern Shewa Zone of the Amhara Region. It is one of the zones in the Amhara Regional State, and located 200 km North of Addis Ababa. The farm is situated at 10°00′N latitude and 39°53′E, longitude with an altitude of 1280 meters above sea level.

Fig. 1. Location of study area; from World Google Maps (June 2014)

According to the data from the Central Statistical Agency in 2005, Shewa robit has an estimated total population of 24,886 of whom 13,021 were men and 11,865 were women. The inhabitants include members of the Argobba people. It is the largest settlement in Kewet Woreda (http://en.wikipedia. org/wiki/Shewa_Robit). Shewa robit was selected as research site for this study because this area is one of the most drought prone areas and also represents the major sorghum growing areas in the region.

3.2. Weather during the crop growth period

The data condition during the crop growth period from July to December 2013 was collected from Ethiopian meteorological agency and presented in Table 1. The rainfall received during cropping period ranged from 150.50 mm (July) to 0.00 mm (January), while the maximum and minimum temperature were 34.204 ^{o}C (May) and 9.62 ^{o}C (December) respectively.

Table 1. Monthly meteorological data during crop growth period (2013) at Shewa robit, Amhara Regional State

No	**Months**	**Traits**		
		Rain fall (mm)	Min. temperature (^{o}C)	Max. temperature (^{o}C)
1	January	0.00	13.50	30.35
2	February	69.00	15.90	28.90
3	March	74.00	16.10	30.20
4	April	48.00	18.50	32.72
5	May	35.00	19.30	34.20
6	June	85.00	18.20	37.70
7	July	150.00	17.25	33.64
8	August	100.53	17.54	33.15
9	September	60.02	25.84	31.14
10	October	10.53	12.39	32.66
11	November	21.54	11.14	30.68
12	December	0.50	9.62	29.25

3.3. Planting material

In this study, three SG sorghum accessions were selected to evaluate the physiological and agronomical performance under field condition. These accessions were Sorcoll-141/07, Sorcoll-146/07 and Sorcoll-163/07. A local accession (Afeso) was used as a check, since it is a known source of sorghum genotype trait being used in local farmers and its seed was obtained from farmers. Likewise, evaluating local accessions for sorghum genotype trait would play a role in crop improvement by serving as source material. Because local accessions are still the backbone of agricultural production in the developing world, which are adapted to various environments and preferred by farmers for various traits under difficult conditions (Brush, 2000). The other three accessions were selected based on different morphological and physiological characters. They were selected as a result of a series of previous screening experiments by Dagnachew Bekelle (2008), Zelalem Mengiste (2008) and Addisie Yalew (2010) under the BIO-EARN project. It was from these researches that the present study obtained the accessions considered and these accessions were proposed for further approval of sorghum genotype property.

3.4. Soil and fertilizer material

Crops previously grown on the experimental area were maize (*Zea may*), sorghum, Timbaho (Tobacco), teff and Masho *(Vigra radiata*). Some physical and chemical properties of the soil were analysed by collecting composite samples from each of the three plots at three depths i.e. 0-15, 15-30 and 30-45 cm. The soil pH was determined according to Juo (1978) and its electric conductivity was measured by using calibrated conductivity meter, while the texture class of the soil was determined by soil textural triangle, following the method of Juo (1978). Based on the analysis result, the pH and electric conductivity of the composite soil sample was 7.5 and 979 µScm^{-1} respectively, while the texture class of the mixed soil was silt clay loam. Urea [CO $(NH_2)_2$], and diammonium phosphate (DAP) were added to supplement the nutrient requirement of the plant.

3.5. Experimental design and layout

The field experiment was laid out in randomized complete block Design (RCBD) with three replications (three farm lands). The first and second farm lands covered an area of 484 m^2 (22 m * 22 m) while the third farm land was 419 m^2 (22 m * 19 m). Each farm land was divided in to four equal parts to sow four different sorghum varieties. There were 2 m gap in between the accessions. It helps to manage and identify varieties easily. Inter-row distance was 0.75 m and helps to create conducive environment for the proper management of the plots, while intra-row distance was 0.20 m and it helps to avoid competition.

3.6. Experimental management

Land was prepared according to the local practice. It was ploughed three times using oxen before planting and the last ploughing was used for sowing. Sowing of *Sorghum bicolour* was done on July 8/2013 and recommended rate of fertilizer was applied on the same day with the rate of 100/50 kg per hectare DAP and urea. Tinning was done 15 days after sowing. Weeding and all other recommended crop protection activities were done manually, similar to the farmers’ practice recommended for sorghum cultivation.

3.7. Data collection

3.7.1. Phenological traits

Plant height: The plant height was determined from five randomly selected plants at 14, 30 and 42 days after flowering (DAF) by measuring the height from ground level to the base of youngest fully opened leaf. After panicle emergence height was recorded from the base of the plant to the tip of the panicle at physiological maturity and expressed in centimeter.

Girth diameter and number of tillers: Girth diameter was measured using calliper at the widest point (10 cm) above the ground along the lower length of the stem, and number of basal tillers per plant also counted at 14, 30 and 42 DAF.

Number of green leaves (NG) and number of senesced leaves (NSeL): They were determined on five randomly selected plants at 14, 30 and 42 DAF. Number of green leaves (More than 50% green portion) was counted. NSeL was also counted when more than 50% of its area had senesced at the same date as NG measurement was taken.

Green Leaf Area (GLA): Leaf area of five tagged plants was measured by adopting Stickler's linear measurement method (Stickler *et al.*, 1961). The length and breadth of fully opened leaf lamina was measured from the leaf base to the tip and maximum width, respectively at 14, 30 and 42 DAF. The product of leaf length, breadth and the factor 0.747 was the leaf area and expressed as cm^2 per plant as given below:

- Leaf area (cm^2) = l * b * 0.747.. (1)

Determination of greenness (chlorophyll content): at physiological maturity leaf chlorophyll contents of all studied varieties were measured with Chlorophyll Content Meter (CCM-200 pulse) at 30 DAF. The readings were taken between 10:00 and 12:00 hours of the day. A mean of 30 reading per plot was taken from five tagged plants. The readings were taken at the base of the leaf lamina, middle and towards the 1/3 of the tip of both the 2nd and flag leaves. The values were calculated according to the amount of light transmitted by the leaf area (2 mm * 3 mm) in two wave length regions in which the absorption of chlorophyll is different. Higher CCM-200 reading values represent higher total chlorophyll contents**.** Individual plant chlorophyll content was represented by the average of the six measurements.

Days to 50 percent flowering and physiological maturity (days): The number of days required to 50 percent anthesis (flowering) were noted when 50 percent of plants in each plot flowered and expressed in days. The number of days after sowing to the date on which the seeds of most of the plants in a plot showed the appearance of black spot on the hilum of seed was taken as the indication of physiological maturity.

3.7.2. Physiological traits

Relative water content (RWC): RWC was estimated following the procedure of Barrs and Weatherly (1962) at 30 DAF. Second or third fully expanded leaf from the top was brought in polyethylene bags and kept in an ice box from the field. Immediately, twenty leaf discs were weighted on an electronic balance, and fresh weight was determined. The weighted leaf discs were floated over night in a Petri-dish containing distilled water and subsequently blotted gently and weighted again (turgid weight). After taking turgid weight, the leaves were oven dried at 80°C for 48 hours and dry weight was recorded separately. The RWC was calculated using the following formula and expressed in percent.

- Relative water content (%) = (FW-DW) / (TW-DW) * 100......................... (2)

Leaf gas exchange: Leaf gas exchange parameters such as, net photosynthesis (A), transpiration rate (E) and stomatal conductance (Gs) were measured simultaneously using Lc pro+ photometer. The readings were taken between 10:00 and 12:00 hours of the day. A mean of 30 readings per plot were taken from five tagged plants at the base of the leaf lamina, middle and towards the 1/3 of the tip of both the second and flag leaves. Gas exchange measurements were performed on fully expanded and sun-exposed leaves throughout the natural photoperiod.

Fig.2. Measurements of leaf gas exchange using Lc pro+ photometer on the field at 30 DAF

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

Chlorophyll fluorescence: The potential quantum yield of all accessions was measured by a Plant Efficiency Analyzer (PEA) (Hansatech Instruments Ltd., England). Chlorophyll fluorescence was determined from the youngest and fully expanded leaves five randomly selected plants at 30 DAF. Leaves were covered with clips and kept in dark for 30 minutes before measurements. The transients were induced by red light of 3000 μmol m^{-2} S^{-1} provided by an array of six light emitting diodes, which focused on the sample surface to give homogenous illumination over exposed area of sample surface and maximal quantum yield. After 30 minutes quantum yield of PS II (Fv/Fm) was measured.

3.7.3. Biochemical traits

Estimation of chlorophyll content: Total chlorophyll, chlorophyll 'a' and chlorophyll 'b' content of sorghum leaf were estimated according to Witham *et al.* (1971) at 30 DAF. The leaf sample was taken from third or fully expanded leaf from the top of five tagged plants and transported in to ecophysiology laboratory preserved in polyethylene bag and ice box. One gram of leaf was homogenised in cold mortal with 5 ml of pre-chilled 80% acetone. The mixture was centrifuged at 4000 rotation per minute (RPM) for five minutes. The supernatant was transferred to a test tube (volumetric flask) and made up to 20 ml with 80% acetone. The absorbance of the extract was read in spectrophotometer at 645 and 663 nm against 80% acetone blank. The values were expressed as ml chlorophyll per gram fresh weight of sample. The total chlorophyll, chlorophyll a and chlorophyll b contents were calculated by using the following formula:

- Total chlorophyll $= [20.2 (A645) + 8.02 (A663) * V]/ (1000 * W * a)$.....(3)
- Chlorophyll - a $= [12.7 (a663) - 2.69 (A645) * V]/ (1000 * W * a)$..........(4)
- Chlorophyll - b $= [22.9 (A645) - 4.68 (a663) * V]/ (1000 * W * a)$..........(5)

Where:

- A645 = Absorbance of the extract at 645 nm
- A663 = Absorbance of the extract at 663 nm
- W = Fresh weight of the sample (g)
- A = Path length of cuvette (cm^2)
- V = Final volume of the chlorophyll extract (ml).

Estimation of free proline content: Proline content was estimated by the method of Bates *et al.* (1973) at 30 DAF. A known quantity of fresh leaf sample (0.5 g) was taken from fully expanded third leaf from the top and homogenized with 10 ml 3 per cent sulfosalicylic acid. The extract was filtered through whatman No. 1 filter paper and the filtrate was used for proline estimation. An aliquiout of 2 ml from each sample was taken in separate test tube and in each test tube 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added and then boiled in a hot water bath for one hour. Acid nintydrin was prepared by dissolving 1.25 g of ninhydrin with a mixture of 30 ml glacial acetic acid and 20 ml 6M phosphoric acid. Then, the test tubes were transferred to ice water bath for one hour for cooling. 4 ml of toluene was added, shaken thoroughly and allowed to form two separate layers. The upper toluene layer containing the colour complex due to proline ninhydrin reaction was taken in to a separate test tube and absorbance was read in spectrophotometer at 520 nm. The proline concentration was determined from the standard curve and expressed in moles of proline per gram fresh weight of the leaf.

- **µmole proline g^{-1}sample** = [(µg proline / ml * ml toluene) / 115.5µg / µmole] / [(g sample / 5)]........................ (6)

Fig.3. Determination of proline content, (A) Sample extraction, (B) Standard solution (C) Formation of two layers after toluene was added and (D) Reading the absorbance by spectrophotometer

Estimation of total soluble sugar: The amount of total soluble sugars present in dried leaf sample was estimated by phenol sulphuric acid method (Dubois *et al.*,

1956) at 30 DAF. 0.5 mg sample was weighed and added into 100 x 12 mm glass test tube containing 1 ml of water. Alternatively; 1 ml of aqueous extract was used. 1 ml of 5 % phenol solution was added, mixed thoroughly and 5 ml of concentrated sulphuric acid was added immediately. The content of the test tube was vortexed for 10 second and allowed to stand at room temperature for an hour. The blank was run with water instead of phenol solution. The mixture was centrifuged for 10 minutes at 4600 RMP. Absorbance of the sample was measured at 490 nm. The sugar content was determined based on the standard curve.

Determination of total nitrogen content: Nitrogen content was estimated by modified micro Kjeldhal method (Jackson, 1967) at 30 DAF with three steps (digestion, distillation and titration).

i. Digestion; 0.3 g of dried and ground sample was placed in Kjeldhal tubes, to which 3 ml concentrated H_2SO_4 and a pinch of catalyst mixture were added. The contents were digested on a Kjeldhal digestion apparatus until a clear solution was obtained. The contents were allowed to cool and further used for distillation.

ii. Distillation; The digested solution was added to the micro Kjeldhal distillation apparatus. About 10ml of 40 per cent NaOH was added and allowed for distillation. Simultaneously, a beaker containing 20 ml of 2 per cent boric acid with 3 drops of mixed indicator was placed at the outlet of the condenser. Steam from the boiler was allowed to pass through the sample and ammonia released was captured in boric acid mixed indicator. Distillation process was continued for six minutes and then the contents were titrated.

Titration; After the completion of distillation process, the samples were titrated against the standard sulphuric acid (0.1 N) carefully until orange red colour was developed. The sample nitrogen content was calculated using the following formula;

- $$\text{Nitrogen \%} = \frac{[(\text{Sample titre valve} - \text{Blank titre valve}) * \text{Normality of } H_2SO_4\ (0.1) * \text{Molar equivalency of N } (0.014)]}{\text{Dried sample weight (g)}} * 100 \quad \text{.... (7)}$$

From nitrogen content, protein content was calculated as:

- **Protein (%)** = % Nitrogen * Conversion factor (6.25)..................................(8)

Fig.4. Determination of leaf nitrogen, (A) Digestion of a sample, (B) Distillation, (C) Titration and (D) colour difference after and before titration

3.7.4. Biomass, yield and yield components

Biomass: 153 days after sowed, plants were harvested and separated into shoot and root parts. These parts were dried at 80^0C in an oven until constant dry weight was obtained. Consequently the dry weights were determined using a triple balance (plate 3a). Root to shoot ratio was determined from dry shoot and root biomass.

Grain weight and grain number per panicle: Dried panicles with mature seeds were taken from five previously tagged plants. Subsequently the panicles were allowed to air dry at room temperature (25°C) to remove excess moisture. Once the panicles were dried the seeds were threshed mechanically (using hand). Grain weight per plant was determined weighing all the seeds per panicle using sensitive balance.

To determine the grain number per panicle, 1000 grains were counted from each of previously tagged five plants. The weight of 1000 grain was recorded separately in grams and the grain number per panicle was calculated from the grain weight per panicle and the corresponding 1000 grain weight as follow;

- Grain number per panicle = [Grain wt plant^{-1} (g)/ 1000 grain wt (g)] * 1000...(9)

Grain yield (kg ha^{-1}): All the panicles of each accession in every plot were collected, sun dried, threshed, cleaned and weight of the grains was recorded and expressed as kg per plot and converted to kg per hectare.

3.8. Statistical analysis

All the collected data were subjected to analysis of variance using SPSS Software (Version 20, SPSS Inc., Chicago USA) to see variations between sorghum accessions. The accession means were separated using the least significant differences (LSD) test at 5% level of significance. The correlations between the studied traits were analysed using Pearson test, whereas all the graphs were generated with the Sigma Plot 8.0 (Systat Software, Inc.).

CHAPTER FOUR

4. RESULTS

4.1. Morphological traits

4.1.1. Plant height and girth diameters

Based on analysis of variance, plant height and girth diameter of the studied accession showed significant differences at $p < 0.05$ (Table 2 and Fig.5). Among the accession highest plant height was recorded in check accession (Afeso), while Sorcoll 146/07 had significantly lower height. On the other hand, all accessions showed reduction in girth diameter through time at 14, 30 and 42 DAF measurements. Its decreasing rate varied among accessions. Sorcoll 163/07 had significantly highest girth diameter than the other accessions except insignificant difference with Afeso, while Sorcoll 146/06 had significantly lowest girth diameter at 42 DAF.

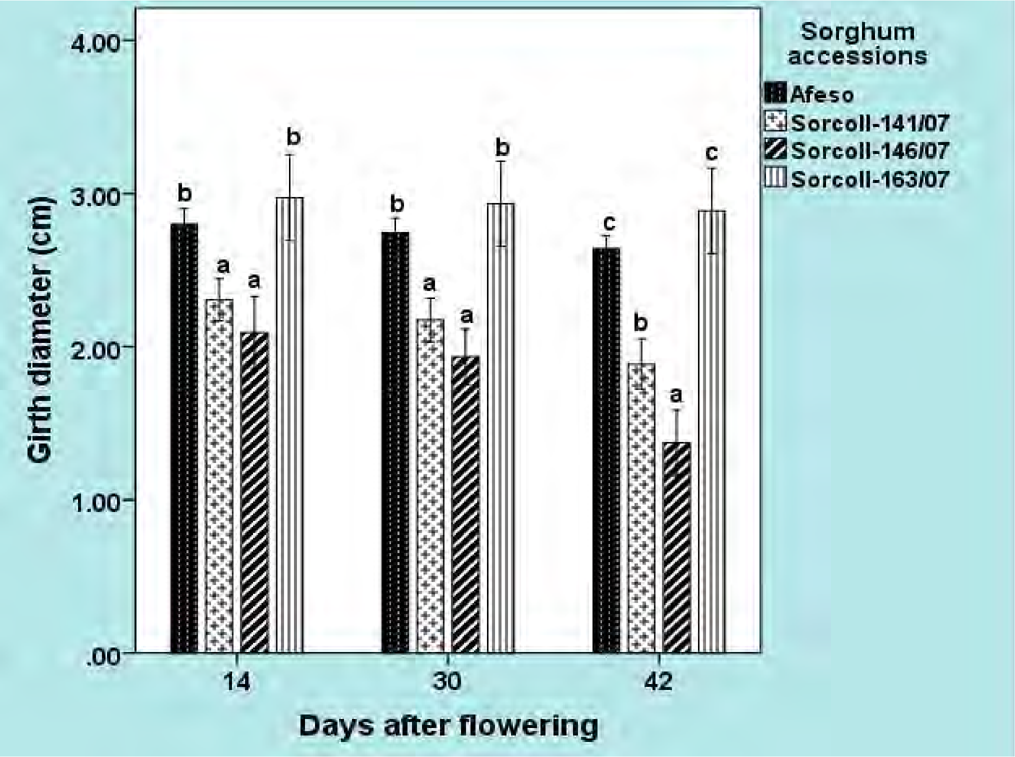

Fig.5. Pattern of girth diameter of *S. bicolor* accessions at 14, 30 and 42 DAF. Bars represented by the same letter are not significantly different between accessions at $p < 0.05$. Data represent the mean + SE of the three replicates.

4.1.2. Number of tillers

Analysis of variance revealed that the studied sorghum accessions were significantly different in number of tillers at $P < 0.05$. Among the accessions, Sorcoll 163/07 and the check (Afeso) had significantly higher tillers number than all the accessions. On the other hand, lowest number of tiller was recorded in Sorcoll 141/07 and followed by Sorcoll146/07.

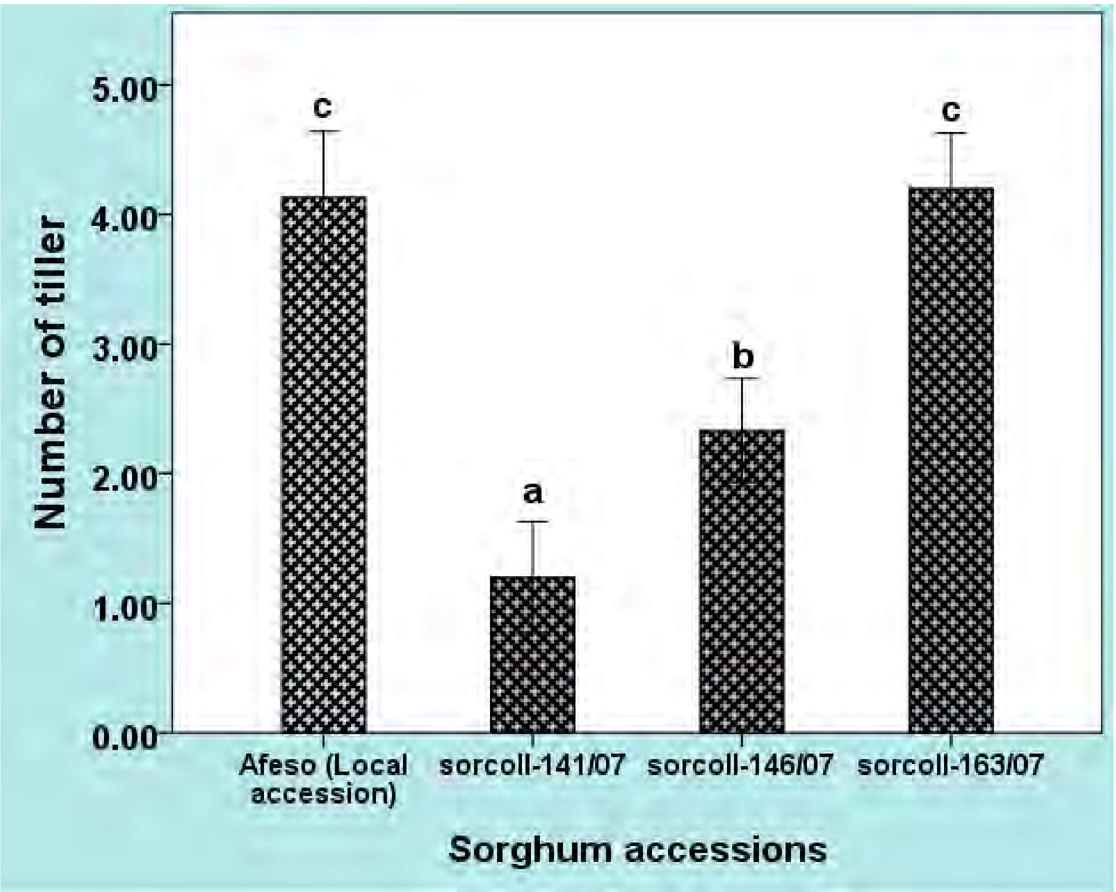

Fig.6. **N**umber of tillers of four *S. bicolor* accessions. Bars represented by the same letter are not significantly different at $p < 0.05$. Data represent the mean + SE of the three replicates

4.1.3. Number of green leaves

There was also a significant difference in number of green leaves (NG) on the studied accessions at $p < 0.05$ (Fig.7). With increasing sampling dates, the accessions showed a reduction in NG across all accessions. Especially, Sorcoll 146/07 and Sorcoll 141/07 showed a high rate of reduction on the number of green leaves (Fig.7). At 14 DAF, accession Afeso had significantly higher number of green leaves from other accessions. The difference among other accessions was not significant. At sampling date of 30 and 42 DAF, Sorcoll 163/07 showed the highest NG next to the check (Afeso), while Sorcoll 146/07 showed the least NG followed by Sorcoll 141/07. At 42 DAF, NG counts showed significant differences between accessions.

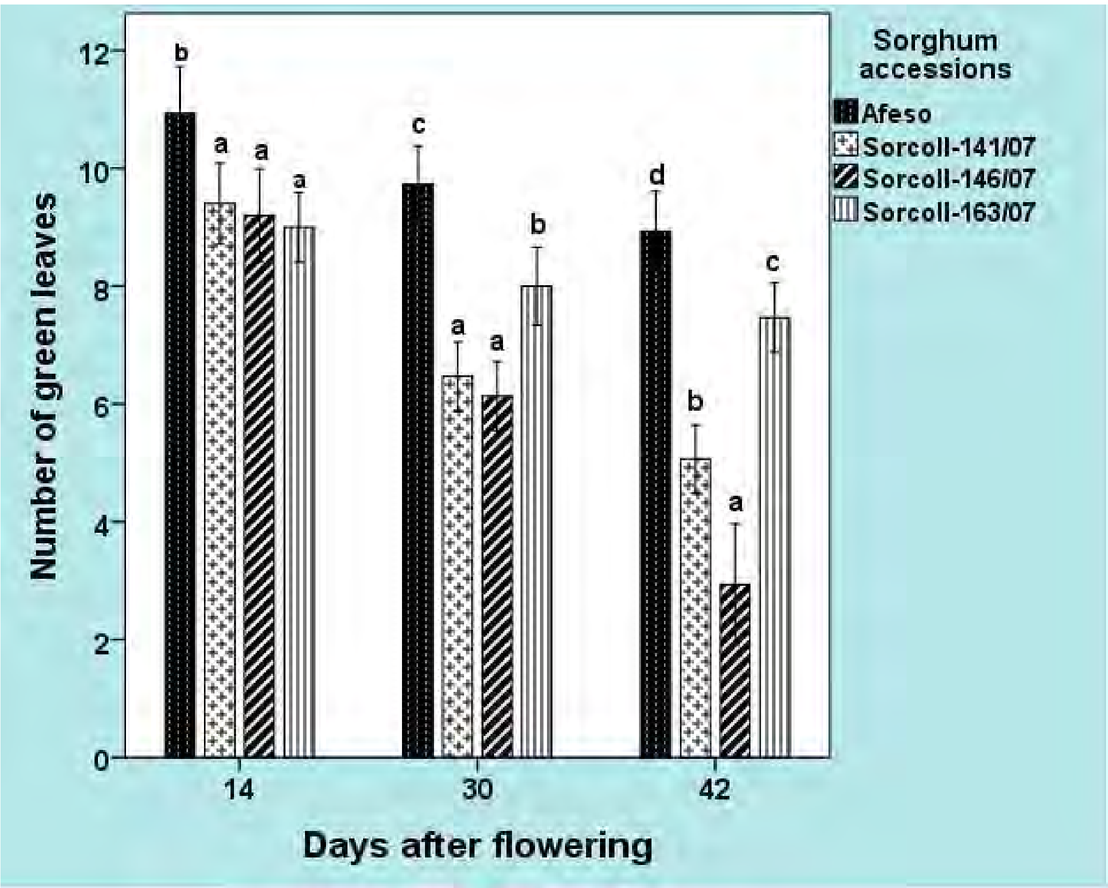

Fig.7. Pattern on the NG of sorghum accessions at 14, 30 and 42 DAF. Bars designated with the same letter are not significantly different between accessions at $p < 0.05$. Bars represent mean+ SE of the three replicates

4.1.4. Green leaf area (cm^2)

According to the analysis of variance, the accessions showed significant difference in green leaf area (GLA) at $p < 0.05$ (Fig.8). With increasing sampling dates the accessions showed a reduction in GLA. Among the groups, Sorcoll 141/07 and Sorcoll 146/07 showed a high rate of reduction on GLA, while Sorcoll 163/07 and the check (Afeso) maintained more or less similar GLA throughout the measurement period. At 42 DAF measurements, Sorcoll 141/07 and Sorcoll 146/07 showed a sharp decline in GLA. The lowest leaf area was recorded in Sorcoll 146/07 followed by Sorcoll 141/07, while the highest was observed in Afeso and Sorcoll 163/07.

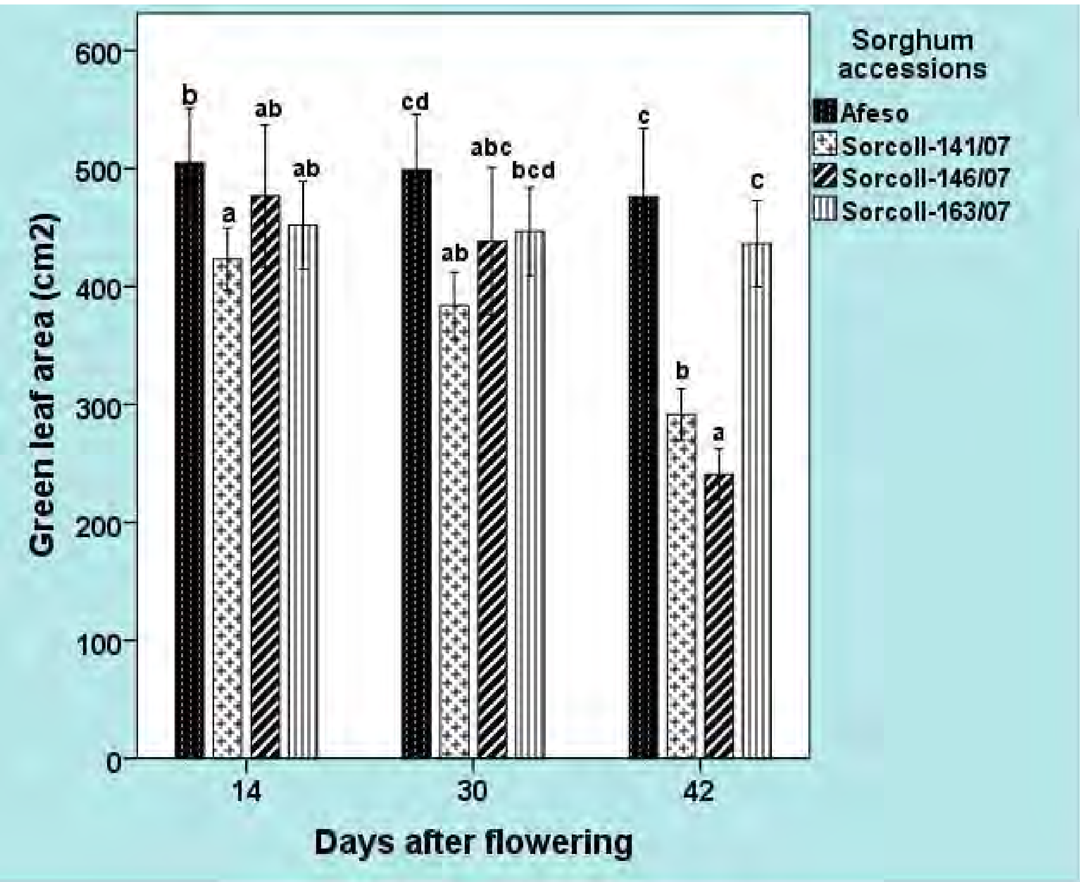

Fig.8. Pattern of green leaf area of sorghum accessions at 14, 30 and 42 DAF. Bars designated with the same letter are not significantly different between accessions at $p < 0.05$. Bars represent mean+ SE of the three replicates

4.1.5. Rate of leaf senescence

The studied accessions showed significant difference in the rate of leaf senescence at $p < 0.05$ (Fig.9). The NSeL showed an increasing trend with time. However, its increasing rate was the lowest in the check accession (Afeso) and the highest in Sorcoll 146/07 at 14 and 30 DAF. A significant increase in number of senesced leaves was also observed in Sorcoll 141/07. In addition, Sorcoll 141/07 and Sorcoll 146/07 commenced senescing at 42 DAF, whereas the remaining genotypes did not.

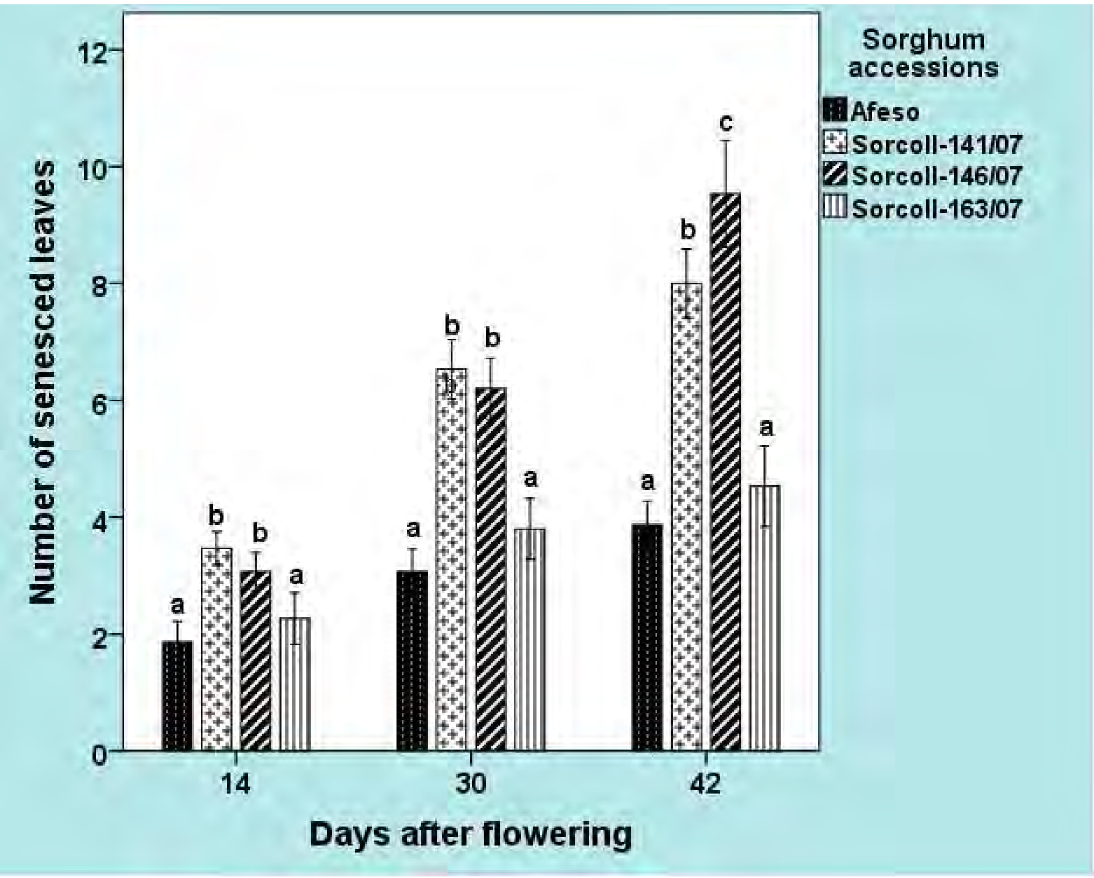

Fig.9. Pattern of leaf senescence of sorghum accessions at 14, 30 and 42 DAF. Bars designated with the same letter are not significantly different between accessions at $p < 0.05$. Bars represent mean+ SE of the three replicates

4.1.6. CCM-200 plus readings

The analysis of variance indicated that there was a significant variation in CCM-200 reading at $p < 0.05$ (Fig.9). Compared to the other accessions, Sorcoll 163/07 exhibited the highest CCM-200 reading value followed by the check (Afeso), however, the difference between accessions was not significant at $p < 0.05$. On the other hand Sorcoll 146/07 showed the lowest CCM-200 value followed by Sorcoll 141/07 and the two accessions were significantly lower than the rest accessions.

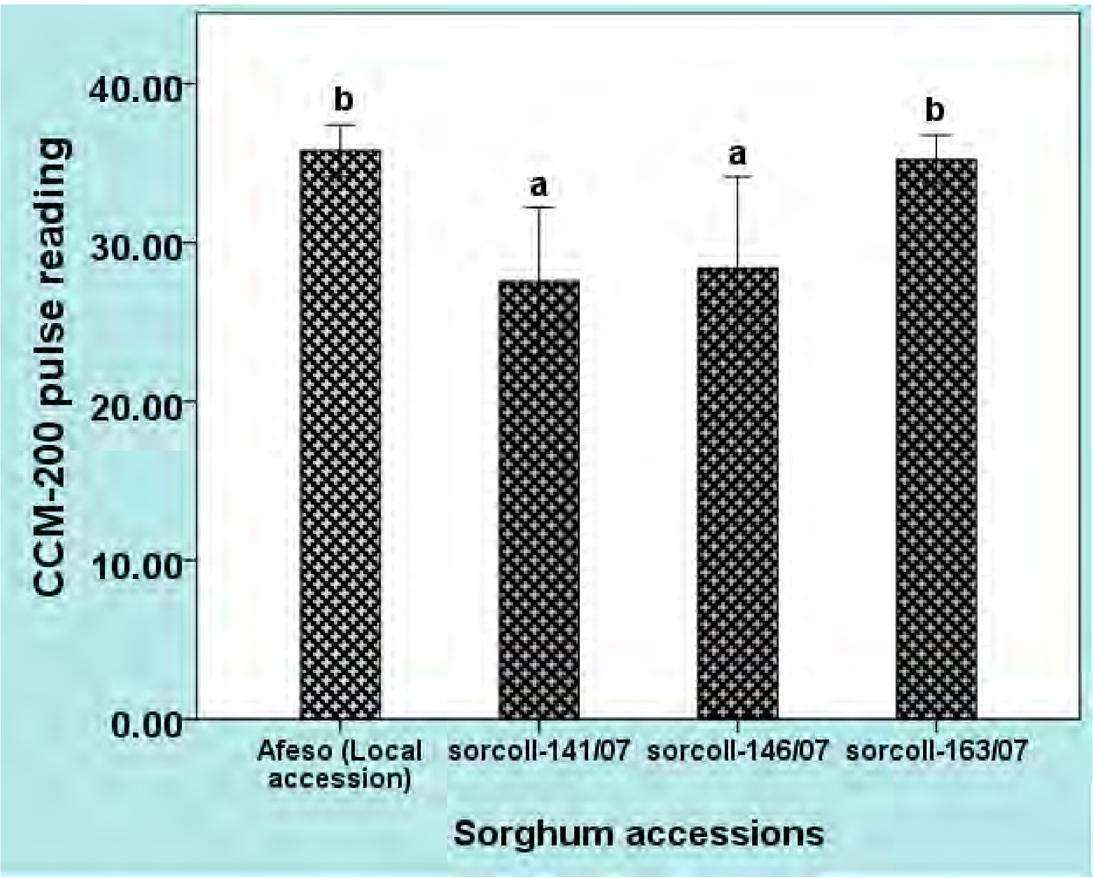

Fig.10. CCM-200 reading of four *S. bicolor* accessions. Bars designated by the same letter are not significantly different at $p < 0.05$. Bars represent mean + SE of the three replicates

4.1.7. Days to 50% flowering and maturity

The studied accessions showed significant difference for days to 50% flowering and physiological maturity at $p < 0.05$ (Table 2). The LSD test showed that, all accessions were significantly different with each other. Next to the check, Sorcoll 163/07 had higher number of days to 50% flowering and physiological maturity with the mean value of 66 and 110 days, respectively, while Sorcoll 141/07 recorded significantly lower days than the rest with the mean value of 64 and 87 days for 50% flowering and maturity respectively.

Table 2. Mean value of morphological and agronomic traits on the studied sorghum accessions.

	Accessions			
Trait	**Afeso**	**Sorcoll 141/07**	**Sorcoll 146/07**	**Sorcoll 163/07**
H (cm)	349.04d	331.60c	279.46a	267.50a
GD (cm)	2.73c	2.12b	1.80c	2.93c
Tiller	4.13c	1.20a	2.33b	4.20c
NSeL	6.22a	12.67c	12.44c	7.58b
NG	9.87b	6.98a	6.09a	8.162b
GLA (cm)	493.33c	366.14a	385.22a	444.91b
CCM-200	35.79b	27.55a	28.39a	35.24b
50% flowering	72.33d	68.67c	64.00a	66.33b
Maturity (days)	117.67c	101.00b	87.33a	113.33c

Key: Means followed by the same letter in a row are not significantly different at $p < 0.05$. Where, H = Plant height, GD = Girth diameter, GL = Number of green leaves, NSeL = Number of senesced leaves, GLA = Green leaf area

4.2. Physiological traits

4.2.1. Relative water content

Based on the analysis of variance, the accessions showed significant difference in mid day RWC (Fig.11). Among the accessions the highest value was recorded in Afeso (82.05%) followed by Sorcoll 163/07 with a mean value of 78.37%, while the lowest RWC (60.53%) was recorded in Sorcoll 141/07 followed by Sorcoll 146/07 (68.36%). The LSD test showed that all accessions differed significantly with one another except Sorcoll 163/07 and the check (Afeso).

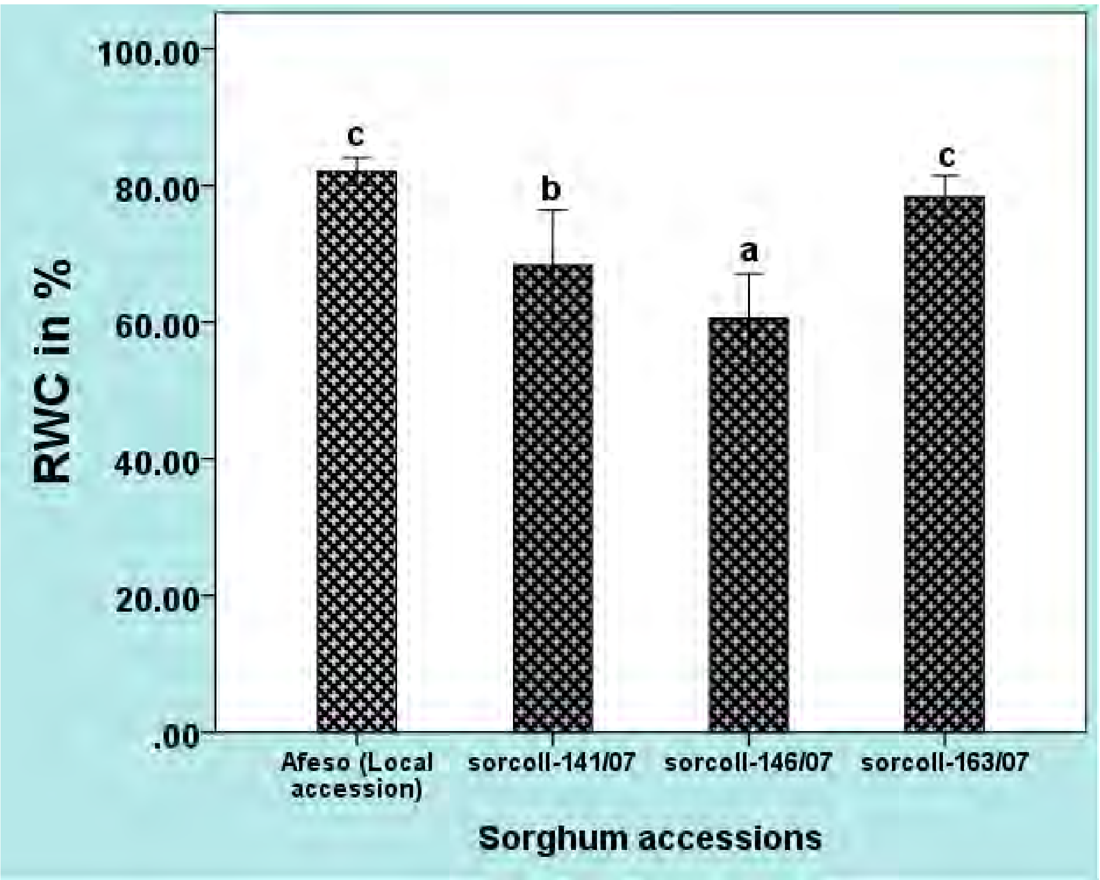

Fig.11. RWC of four *S. bicolor* accessions grown under field conditions during the main rainy season. Bars designated by the same letter are not significantly different at $p < 0.05$. Bars represent mean + SE of the three replicates

4.2.2. Assimilation rate

The analysis of variance on photosynthetic assimilation rate of the tested accessions showed significant variation at $p < 0.05$ (Fig.12A). Sorcoll 163/07 had the highest assimilation rate of 1.86 µmole^{-2} S^{-1} followed by check 1.71 µmole^{-2} S^{-1}, and the lowest was recorded in Sorcoll 141/07 with the mean value of 0.87 µmole^{-2} S^{-1} followed by Sorcoll 146/07 (1.00 µmole^{-2} S^{-1}). Between groups, Sorcoll 163/07 had significantly higher assimilation rate than other accession except the check, while Sorcoll 141/07 was significantly lower than all accessions except Sorcoll 146/07.

4.2.3. Transpiration rate

The accessions exhibited significant difference in transpiration rate at $p < 0.05$ (Fig.12B). The check accession (Afeso) had significantly higher transpiration rate than the other accessions. Relatively, Sorcoll 163/07 revealed the lowest transpiration rate (3.23 mmol m^{-2} S^{-1}) followed by Sorcoll 141/07 (3.83 mmol m^{-2} S^{-1}). The highest transpiration rate was observed in the check with a mean value of 4.33 mmol m^{-2} S^{-1} followed by Sorcoll 146/07 (4.16 mmol m^{-2} S^{-1}).

4.2.4. Water use efficiency

The studied accessions showed significant difference in WUE at $p < 0.05$ (Fig.12C). Among the accessions, Sorcoll 163/07 had higher WUE with the mean value of 0.60 and it differed significantly from all other accessions. Sorcoll 141/07 and Sorcoll 146/07 had significantly lower WUE than the rest with the mean values of 0.22 and 0.21, respectively.

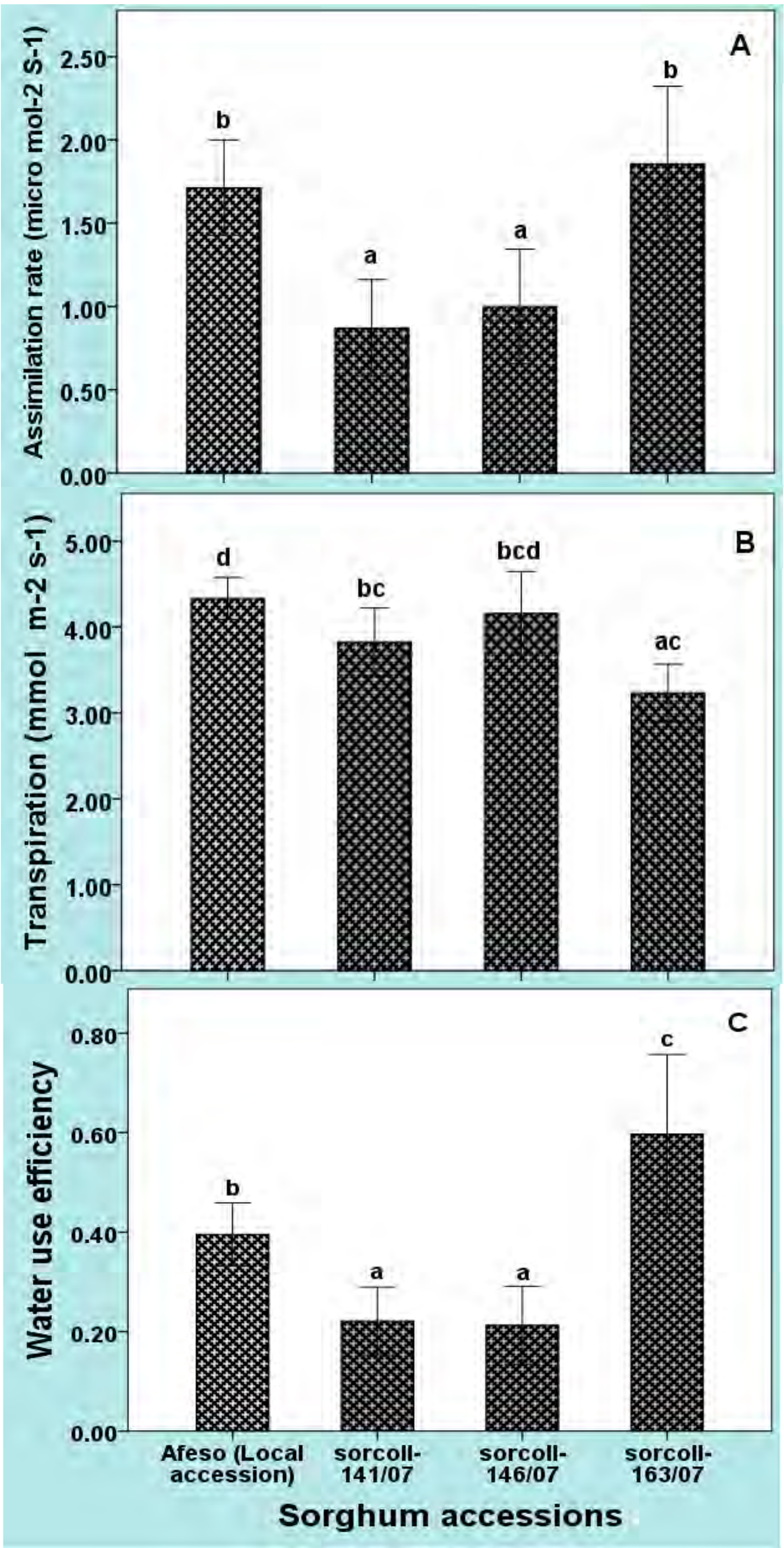

Fig.12. Assimilation rates (A), transpiration rate (B) and water use efficiency (C) of four *S. bicolor* accessions at 30 DAF. Bars represented by the same letter are not significantly different at $p < 0.05$. Data represent the mean + SE of the three replicates

4.2.5. Chlorophyll fluorescence

The potential quantum yield (Fv/Fm) of the accessions was significantly different at $p < 0.05$ (Fig.13). Based on the LSD test, Sorcoll 141/07 and Sorcoll 146/07 had no significant variation and showed significantly lower potential quantum yield than the rest, while the check accession had the maximum yield followed by Sorcoll 163/07.

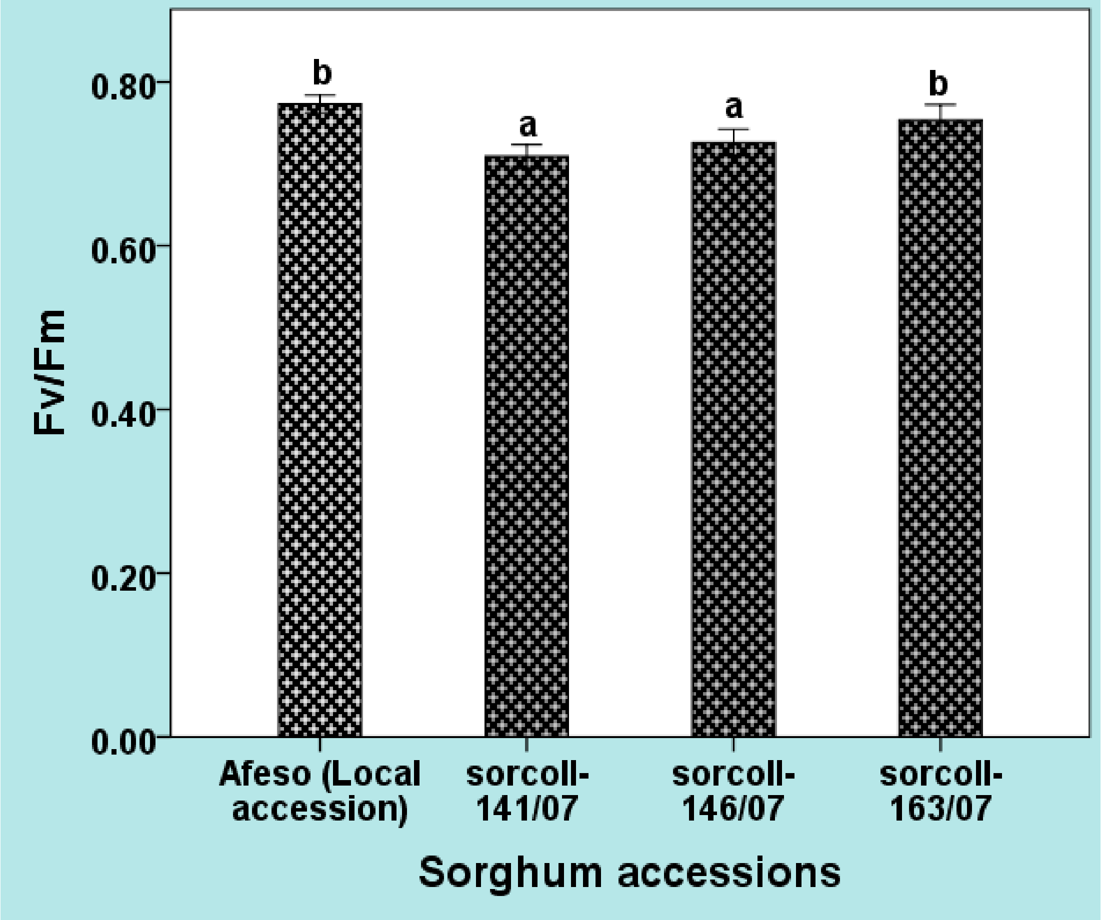

Fig.13. Potential quantum yield of photosystem II of four *S. bicolor* accessions at 30 DAF. Bars represented by the same letter are not significantly different at $p < 0.05$. Data represent the mean + SE of the three replicates

4.3. Biochemical traits

4.3.1 Chlorophyll content

Chlorophyll content (Total chlorophyll, chlorophyll ‘a’ and chlorophyll ’b’) contents were significantly different between the studied accessions at $p < 0.05$ (Table 3). Sorcoll 163/07 exhibited the highest chlorophyll content followed by the check (Afeso) as compared to other accessions. It was significantly differed with all accession except the check. However, the minimum chlorophyll content was measured in Sorcoll 146/07 and it differed significantly with all of the accessions.

4.3.2. Free proline content

A statistically significant variation in proline content was recorded among the studied accessions at $p < 0.05$. (Table 3). Sorcoll 163/07 showed the maximum proline content and the lowest was recorded in Sorcoll 146/07. All accessions were significantly different one another in proline content. Sorcoll 163/07 had the highest proline content value (3.96 µmol g^{-1}), followed by the check accession, Afeso (3.88 µmol g^{-1}), while Sorcoll 141/07 ranked third with a value of 2.84 µmol g^{-1}, and the last was Sorcoll 146/07 with proline content of 2.57 µmol per gram of fresh sample.

4.3.6. Leaf soluble sugar

Significant difference was recorded in leaf soluble sugar content between the studied genotypes at $p < 0.05$ (Table 3). Next to the check accession, Sorcoll 163/07 registered the maximum soluble sugar content (9.19 µg ml^{-1}). The lowest sugar content was recorded in accession Sorcoll 141/07 (6.75 µg ml^{-1}) followed by Sorcoll

146/07 (8.10 µg ml^{-1}). LSD test showed that, all accessions showed significant difference one another except the check (Afeso) and Sorcoll 163/07 at $P < 0.05$.

4.3.4. Leaf nitrogen and protein content (%)

Leaf nitrogen and protein contents showed significant difference in the studied sorghum accessions at $p < 0.05$ (Table 3). Among the accessions, Sorcoll 163/07 had significantly higher contents over all the genotypes, with the mean value of 6.45% and 40.28% of nitrogen and protein contents, respectively. On the other hand, the lowest contents were recorded in Sorcoll 146/07 with the mean value of 2.29% 18.28%, respectively. However, the difference between Sorcoll 141/07 and Sorcoll 146/07 was not significant at $p < 0.05$.

Table 3. Mean value of biochemical trait on the tested *S. bicolor* accessions

	Traits measured				
Accession	Total chl (mlg^{-1}).	Proline µmol g^{-1}	Soluble sugar	Nitrogen (%)	Protein (%)
Afeso	1.42c	3.89c	9.51c	5.59b	34.94b
Sorcoll 141/07	1.30b	2.84b	6.75a	3.13a	19.54a
Sorcoll 146/07	0.78a	2.57a	8.10b	2.92a	18.28a
Sorcoll 163/07	1.43c	3.96d	9.20c	6.45c	40.28c

Key: Means followed by the same letter in a column of the same accession has no significant difference at $p < 0.05$.

4.4. Biomass

SDW, RDW and RSR were significantly different between the studied accessions at $p < 0.05$ (Fig.14A, B and C). Sorcoll 146/07 produced the least SDW, while the check accession (Afeso) had significantly higher SDW than the other accessions. The check accession (Afeso) also demonstrated the highest RDW followed by Sorcoll 163/07, while Sorcoll 146/07 had the least RDW. On the other hand, Sorcoll 163/07 exhibited the highest RSR followed by the check accession whereas; Sorcoll 141/07 and Sorcoll 146/07 produced the least RSR.

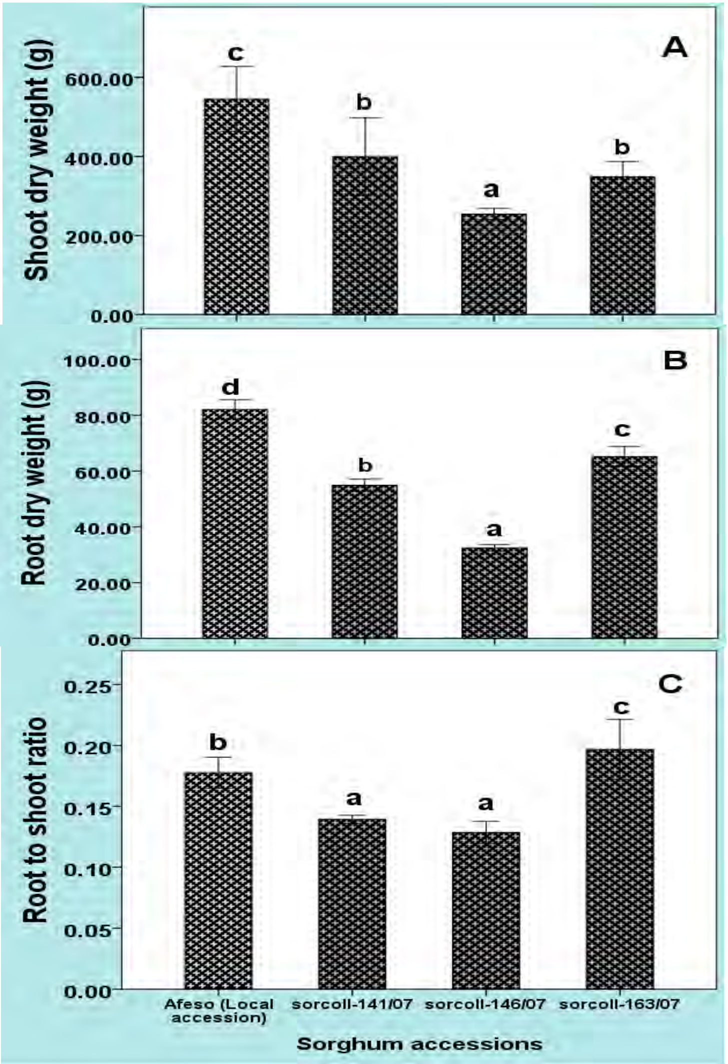

Fig.14. SDW (A), RDW (B) and RSR (C) of *S. bicolor* accessions. Bars represented with the same letters are not significantly different at $P < 0.05$. Bars represent mean + SE of the three replicates

4.5. Yield and yield components

Based on analysis of variance, a significant difference was observed in mean grain yield per panicle of the studied accessions at $p < 0.05$ (Fig.15A and Fig.16). All accessions had lower yield than the check accessions (Afeso). Moreover, the check accession recorded the maximum grain yield per panicle (161.07 g) followed by Sorcoll 141/07 (128.61 g). The lowest grain yield was recorded in Sorcoll 146/07 (85.73 g) followed by Sorcoll 163/07 (114.62 g).

Number of grain per head also showed significant difference among the tested genotypes ($p < 0.05$) (Fig.15B). Compared to the other accessions, the check accession exhibited the highest number of grains per panicle followed by Sorcoll 163/07, while the minimum was recorded in Sorcoll 141/07.

Significant difference in yield per hectare was also showed by the tested genotypes at $p < 0.05$ (Fig.15C). Sorcoll 163/07 registered the maximum grain yield (3978.33 kg ha^{-1}) followed by Afeso (3867.34 kg ha^{-1}), while the lower grain yield was recorded in genotype Sorcoll 141/07 (2953 kg ha^{-1}) followed by Sorcoll 146/07 (1967 kg ha^{-1}). All accessions showed significant differences in grain yield one another at $P < 0.05$.

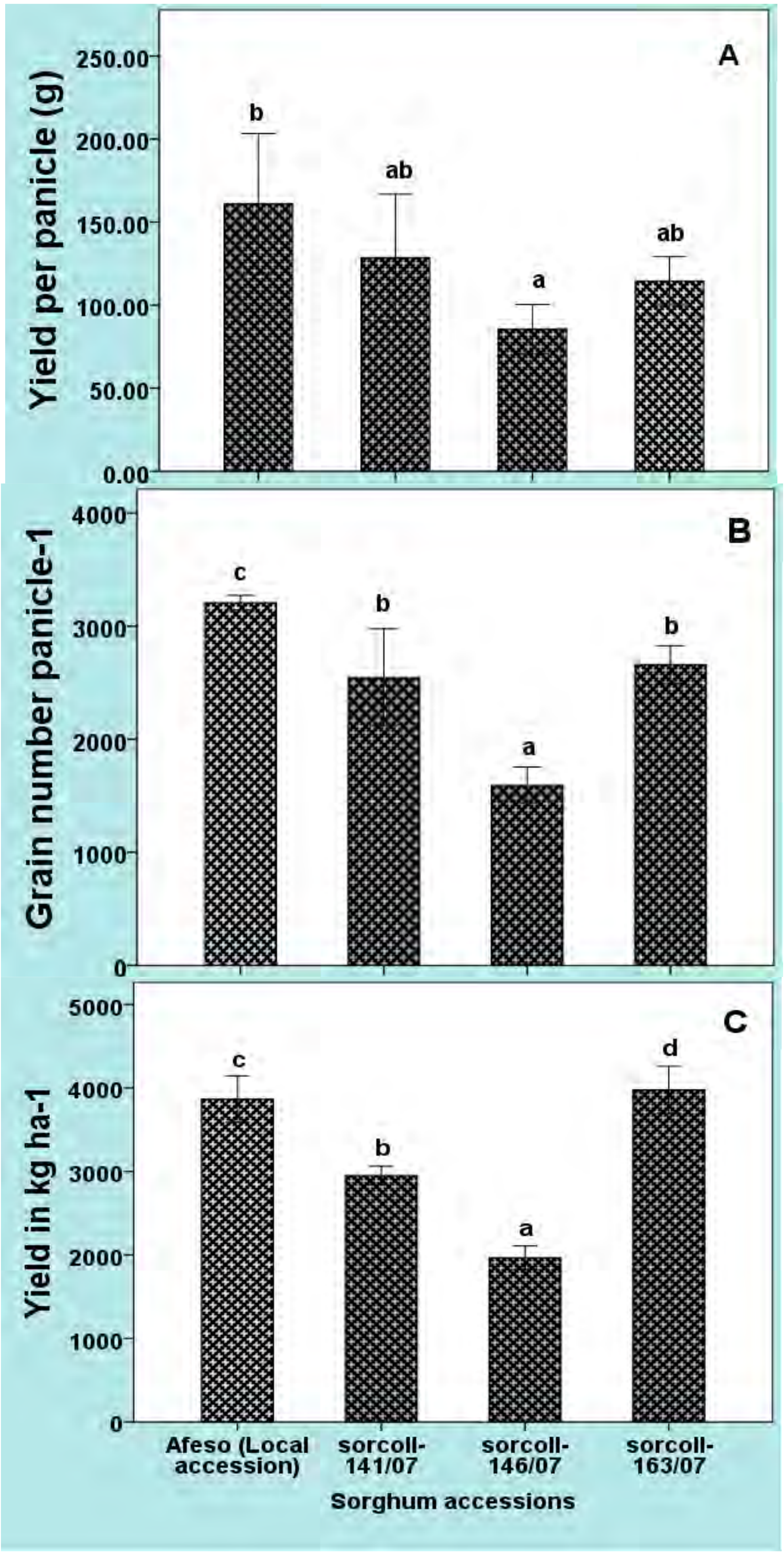

Fig.15. Grain weight (A), grain number per panicle (B) and yield per hectare (C) of four sorghum accessions. Means followed by the same letters are not significantly different at $P < 0.05$. Bars represent mean + SE of the three replicates

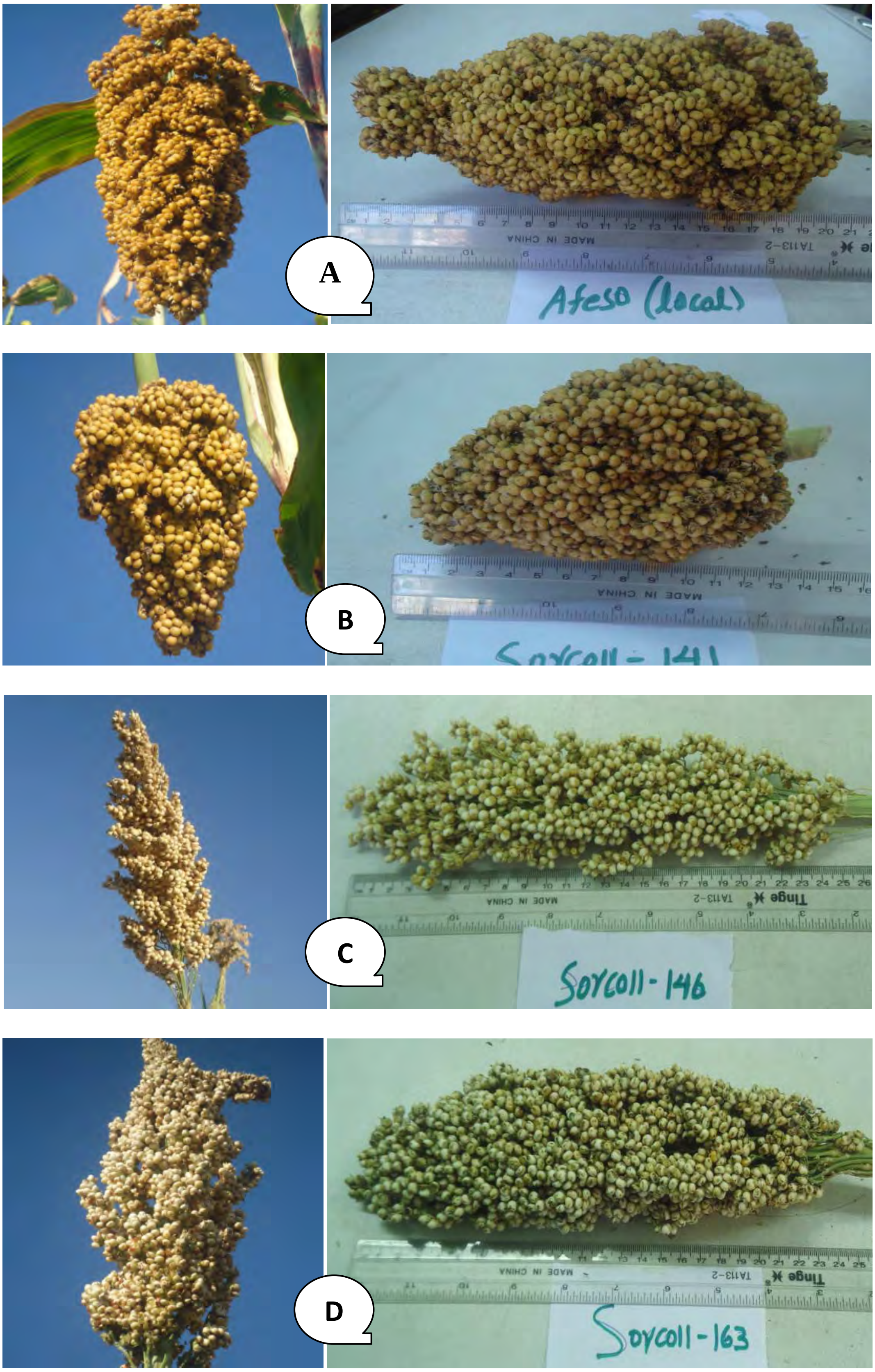

Fig.16. Picture of panicles Afeso (A), Sorcoll 141(B), Sorcoll 146 (C) and Sorcoll 163 (D) captured on the study area (Shewa robit) at harvesting date.

Table 4. Correlation among different morphological, physiological and biochemical traits of four *S. bicolor* accessions under seasonal post flowering drought stress condition at Shewa robit.

Traits	**H**	**GD**	**Tiller**	**NSeL**	**GLA**	**CCM**	**flower**	**Maturity**	**RWC**	**A**	**E**	**WUE**	**Flour**	**Chl**	**Proline**	**N**	**Sugar**	**SDW**	**RDW**	**RSR**	**YPH**
H	1	.095	-.110	-.118	.183	.047	-.250	-.255	.212	-.003	.482**	-.194	.101	.368**	.095	-.164	-.078	.672**	.457**	.575**	-.296
GD		1	.563**	-.589**	.528**	.413*	.304	.624*	.555**	.577**	-.156	.644**	.380**	.637**	.798**	.680**	.322*	.535**	.657**	.511**	.732**
Tiller			1	-.729**	.508**	.624**	.144	.636*	.422**	.484**	-.071	.493**	.559**	.393**	.744**	.665**	.573**	.281	.547**	.284	.615*
NSeL				1	-.391**	-.640**	-.666*	-.843**	-.575**	-.516**	-.033	-.464**	-.593**	-.606**	-.838**	-.719**	-.550**	-.500**	-.732**	-.516**	-.750**
GLA					1	.196	.048	.334	.229	.179	.033	.178	.327*	.182	.516**	.528**	.418**	.316*	.518**	.215	.386
CCM						1	.166	.687*	.630**	.468**	.116	.425**	.279	.628**	.590**	.501**	.257	.280	.498**	.339*	.740**
Flower							1	.710**	.831**	.155	-.643*	.353	.283	.453	.541	.510	.101	.549	.569	.444	.589*
Maturity								1	.824**	.466	-.654*	.618*	.316	.820**	.897**	.852**	.386	.805**	.938**	.608*	.942**
RWC									1	.387**	-.075	.368**	.338**	.616**	.620**	.388*	.132	.385**	.609**	.489**	.755**
A										1	.069	.926**	.488**	.489**	.563**	.469**	.233	.386**	.428**	.501**	.463
E											1	-.259*	.000	-.048	-.165	-.053	-.029	.218	.003	.337*	-.749**
WUE												1	.471**	.486**	.595**	.504**	.242	.270	.383**	.306	.663*
Flour													1	.381**	.593**	.394*	.453**	.293*	.498**	.316	.208
Chl														1	.752**	.603**	.162	.690**	.795**	.592**	.860**
Proline															1	.873**	.535**	.529**	.847**	.483**	.964**
N																1	.533**	.389*	.717**	.405*	.883**
Sugar																	1	.210	.371**	.065	.373
SDW																		1	.788**	.864**	.753**
RDW																			1	.706**	.915**
RSR																				1	.516
YPH																				.	1

Key: Where, ** and *= significant at p= 0.01 and p=0.05 levels (N=12 - 60) respectively, H=Plant height, GD=Girth diameter, NG= Number of green leaves, NSeL= Number of leaf senesced, GLA= Green leaf area, CCM=Chlorophyll content meter, RWC= Relative water content, A=Rate of photosynthesis, E=Rate of transpiration, WUE=Water use efficiency, Flour=Potential quantum yield, Chl=Total leaf chlorophyll content, N=Leaf nitrogen content, SDW= Shoot dry weight, RDW= Root dry weight, RSR= Root to shoot ratio and YPH= Yield per hectare

CHAPTER FIVE

5. DISCUSSION, CONCLUSION AND RECOMENDATIONS

5.1. Discussion

5.1.1. Morphological traits

5.1.1.1. Plant height, girth diameter and number of tiller

In the present study, environmental factors had no significant effect on the height of the accessions at 14 30 and 42 DAF (Table 2). However, significant difference in plant height was observed among accessions due to genetic variation, because plant height is basically a genetically controlled character. The result was in agreement with Pawar, (2007) and Addisie Yalew (2010). In contrast to the present study, several reports indicated that plant height is reduced due to environmental stress factors. It may be influenced by environmental conditions and management practices (Sutro and Tirtoutom, 1989).

The correlation analysis indicated that plant height was positively correlated with NG, chlorophyll content, SDW, RDW and RSR under drought condition. However, it had no definite relationship with grain yield. In agreement with the present finding Hiremath and Parvatikar (1985) and Pawar (2007) also could not find any relationship between plant height and grain yield of sorghum accessions. In contrast, Bakheit (1989); Blum *et al*. (1989) were found a positive relation between plant height, dry matter production and grain yield. This may be because of increased translocation of stored photosynthates from the stem reserves when the current photosynthesis ceases due to environmental stress factors, particularly during grain filling and grain development period (Erick and Musick, 1979). Negative correlation was also observed between these parameters (Pawar and Jadhav, 1996).

In the present study, environmental factors showed significant effect on girth diameter of the tested accessions (Fig.5). This may be due to the difference in accessions' strategies and responses to growing conditions. Sorcoll 163/07 had higher girth diameter and less reduction under field growing conditions while, Sorcoll 146/07 had lower girth diameter and relatively higher rate of reduction on the girth diameter when the environmental stress is severe through time. The increase/ decrease in girth diameter of the accessions might further explain difference in osmotic regulation, which enable them to maintain cell turgor to assist growth under severe conditions (Shao *et al*., 2008). In this regard, Sorcoll 163/07 was a better SG accession.

Tillering is an important agronomical trait as it has a major impact on leaf area development of *S. bicolor* (Lafarge *et al.,* 2002; Van Oosterom *et al.,* 2008). Similar to Lafarge *et al.* (2002) report the number of fertile tillers per plant can vary from zero to around four depending on growing conditions and variety. The present study show significant difference in the number of tillers among accessions (Fig.6). It can be suggested that the higher potential of Sorcoll 163/07 and the check (Afeso) for tiller formation might be due to the genetical background of the accessions. The results are in conformity with the study of Dolciotti *et al.* (1998), Abdalla and El-Khoshiban (2007) and Addisie Yalew (2010),

5.1.1.2. Leaf and leaf related morphological traits

Green leaf area (GLA), number of green leaf and number of senesced leaves are interrelated plant characteristics. High GLA was attributed to presence of stay green trait, which reduced the senesced leaves and increase number of green leaves by maintaining higher green leaf area. In the present study, the accessions significantly differ with respect to GLA (Table 2). In this regard, Sorcoll 163/07 exhibited the maximum GLA next to the local check (Afeso). The present study also showed that the environment had significant effect on GLA of the tested accessions (Fig.8). Afeso and Sorcoll 163/07 had lower rate of reduction in GLA and were being better SG accessions, maintaining higher GLA at both early and late stages of measurement. However, on the other accessions (Sorcoll 146/07) most of the GLA were lost when the environmental stress is severe especially at 42 DAF. Similar observations are also made by several workers (Duncan *et al.,* 1981; Pawar, 2007).

Continuous severe environmental stress accelerates leaf senescence of the accessions (Fig.9 and Table 2). This fact was evidenced by rapid decline in chlorophyll content and GLA (Fig.8 and Fig.13). Significant increment in the rate of senescence under harsh environmental condition has been also reported by De Souza *et al.* (1997). Rate of leaf senescence of the accessions was negatively correlated with the loss of chlorophyll (Table 4), since, the coexisting yellowing of leaves are convenient and distinctive indicators of leaf senescence (Wiedemuth *et al*., 2005). Afeso and Sorcoll 163/07 showed lower rate of leaf senescence at 42 DAF, in reference to the check. Sorcoll 163/07 showed SG phenotype, characterized by an extended green leaf period. This can arise from delays in the beginning of senescence and/or in senescence progress, as previously reported in rice varieties (Rampino *et al*., 2006). Afeso, and Sorcoll 163/07 not only had higher chlorophyll content (CCM reading value) but also sustained green leaves longer throughout post-flowering condition than the remaining accessions. Sorghum plants with the SG trait resist premature senescence when subjected to stress factors during grain-filling stage (Borrell *et al*., 2000a). In contrast, Sorcoll 146/07 is considered as senescent accessions than the remaining three accessions because this accession revealed the highest rate of senescence even at 30 DAF. The observed remarkable variation might be due to genetic variation for leaf senescence, and differences in series of biochemical and physiological changes occurring during senescence (Lim *et al.*, 2007). Therefore, leaf senescence could provide complementary information about stress tolerance and for screening genotypes with SG for breeding purpose.

The correlation analysis showed that GLA showed a positive relation with GD, quantum yield (Fv/Fm), SDW, RDW and with all studied biochemical traits (nitrogen, protein, proline and soluble sugar contents. Similar result was observed by Rosenow *et al.* (1983), Borrel *et al.* (2000) and Pawar (2007).

In the present study, CCM-200 pulse values differed significantly among accessions (Fig.10 and Table 2) and the results are in conformity with various studies on different plant species (Massacci *et al*., 1990; Xu *et al*., 2000; Rao *et al*., 2001; Silva *et al*., 2007 and Sibel and Birol, 2007). Reduction in the content of photosynthetic pigment during stress might be attributed to the ultra-structural deformation of plastids (Abdalla and El-Khoshiban, 2007), or due to loss of chloroplast membrane integrity (De Silva *et al*., 1979). However under environmental stress factors the check and Sorcoll 163/07 recorded the maximum CCM-200 values. Such phenomenon of retaining higher chlorophyll contents has been suggested as a stress tolerance mechanism in sorghum genotypes with SG property (Thomas and Howarth, 2000). While Sorcoll 141/07 and Sorcoll 146/07 had relatively lower CCM-200 value than other accessions, which is relatively undesirable trait and is a negative consequence of environmental stress factors. However, it has also been considered as an adaptive feature in plants grown under adverse climatic conditions, usually exposed to an excess of excitation energy (Pic, *et al.*, 2002). Therefore, the variation in chlorophyll content might reflect genetic difference among sorghum accessions which is ascribed to the presence of SG genes (Wiedemuth *et al*., 2005). The chlorophyll stability index is associated with desiccation tolerance under seasonal drought condition and can be used as one of the reliable selection criterion in rapid screening for SG sorghum genotypes adapted to drought tolerance.

The correlation of the present study indicate that, CCM-200 pulse reading had significantly positive relation with GD, GLA, A, WUE, RWC, protein nitrogen, SDW, RSR and YPP. But no significant relation with grain yield and it also had negative relation with NSeL. These results are in conformity with those obtained by Kadam *et al*. (2002), Awari *et al*. (2003) in sorghum and Sairam, (1994) in wheat.

5.1.1.3. Days to 50% flowering and maturity

Another important character of crop plants, which has relevance to the performance of the genotype in terms of productivity, is the number of days taken for different phenological stages. Significant differences were observed in days to 50 % flowering and physiological maturity in the present study (Table 2). Next to the check accession, Sorcoll 163/07 had taken more number of days to 50% flowering and maturity. The number of days taken for grain filling was also higher. In agreement with the present study Rosenow *et al.* (1983), Craufurd *et al.* (1993) and Prasad *et al.* (2008) showed that sorghum accessions which have delayed maturity and higher number of days to grain filling under drought stress are better SG genotypes. So in this regard Sorcoll 163/07 and the check are better SG accessions. However, the early matured accession Sorcoll 146/07 had significantly lower number of days to 50% flowering, maturity and the number of days for grain filling under drought stress environment. Thus, such traits can be considered as ways of drought escape mechanism on several crop plants (Turner, 1980).

The correlation of the present study revealed that, day to 50% flowering had significantly positive relation with physiological maturity, RWC and YPH. On the other hand physiological maturity also had significantly positive correlation with GD, CCM, RWC, WUE, chlorophyll, protein, proline and nitrogen contents, SDW, RDW RSR. However both 50% flowering and maturity had negative relation with plant height, NSeL and transpiration rate (Table 4). These results are in conformity with those obtained by McBee and Miller (1982) and Prasad *et al.* (2008).

5.1.2. Physiological traits

5.1.2.1. Relative water content

In the present study, RWC differed significantly among the tested accessions (Fig.11). This is in agreement with reports on various plants (Siddique *et al*., 2000; Ahmadi and Siosemarideh, 2005; Pirdashti *et al*., 2009). At 30 DAF measurements, the check (Afeso) maintained the greatest mean RWC (82.05%) than other accessions. This is because of the fact that better SG accessions keep the stalk transpiration system functioning under severe drought conditions (Xu *et al*., 2008). Next to the check higher RWC was recorded in Sorcoll 163/07 than the other accessions. This may be due to the maintenance of better water status. Sorcoll 141/07 had lower RWC (60.53%) than other accessions. The difference between accessions in RWC in reaction to terminal drought could be the difference in adaptation of the accessions, which is mainly a function of intensity of stress and genotypes as reported by Abdalla and El-Khoshiban (2007). In this regard, Sorcoll 163/07 revealed a better SG property owing to the possession of higher RWC during post-flowering terminal drought (Xu *et al*., 2008). RWC has been used for evaluation of SG expression for years by sorghum breeders to measure post-flowering drought tolerance (Rosenow and Clark, 1981). Our result has also confirmed that measuring RWC is a potential tool for screening genotypes under post flowering drought stress.

The correlation study showed that, there is positive relation between RWC and GD, GLA, 50 percent flowering, physiological maturity, WUE, grain yield, chlorophyll, proline, nitrogen and protein contents. But it negatively related with E and NSeL (Table 4). Similar result has been reported by Rao *et al*. (2001) and (Salunke *et al*., 2003).

5.1.2.2. Gas exchange parameters and water use efficiency

Accessions showed a significant difference in assimilation rate under field condition (Fig.12A). In support of this, significant differences in assimilation rate has been reported in genotypes of diverse crops (Lawlor and Cornic, 2002; Ashraf *et al*., 2007; Prasad *et al*., 2008). Sorcoll 163/07 had significantly higher assimilation rate (1.71 μmol^{-2} S^{-1}), while Sorcoll 141/07 had significantly lower assimilation rate than the other accessions. Lower assimilation is possibly owing to the loss of function and integration of photosynthetic apparatus as a result of stress factors (Kasier, 1987). The observed lower assimilation might be due to a decrease in RWC, which leads to turgor loss of guard cells, causing passive stomatal closure, which in turn reduces supply of CO_2 to the fixation site (Ahmadi and Siosemarideh, 2005; Miyashita *et al*., 2005).

Moreover, a decrease in assimilation rate due to stress factors implies the importance of stomatal limitation to assimilation rate in the examined accessions of sorghum. In our investigation the reduction might not only be attributed to stomatal factors but might also be due to non-stomatal factors. Since under stress, decline in CO_2 uptake is mainly considered to be due to non-stomatal factors (Atteya, 2003; Chaves *et al*., 2003). The CO_2 uptake of Sorcoll 141/07 is almost below one, which may suggest that factors other than stomatal limitation might have been involved. However, Sorcoll 163/07 and the check, Afeso showed less sensitivity to stress in comparison to other accessions. Genotypes possessing SG trait maintain more photosynthetically active leaves than accessions not containing this trait under drought condition (Borrell *et al*., 2000b). Thus, assimilation rate is useful criterion in identifying stress tolerant accessions from sensitive SG types.

The correlation study showed that assimilation rate had significantly positively correlated with many traits like GD, NG, RWC, WUE, chlorophyll florescence, chlorophyll content and all tested biochemical traits, while assimilation rate was negatively related with NSeL (Table 4).

In the present study significant difference on transpiration rate among accessions was observed (Fig.12B). Similar results are reported by Lawlor and Cornic (2002) and Tsuji *et al*. (2003). Better yield bearing accession Sorcoll 163/07 had the least transpiration rate than all other accessions. Stomatal closure is a probable factor responsible for the reduction in transpiration under drought condition because; stomatal closure is a well-known and most efficient way of reducing transpirational water loss (Cornic, 2000). Moreover, the check (Afeso) had higher transpiration rate. It has been reported that crops with SG characteristics have enhanced transpiration efficiency, which enables the plant to set a higher yield potential during anthesis that ultimately leads to higher grain yield (Borrell *et al.*, 2001).

Sorcoll 163/07 and the check (local accession) had higher WUE (Fig.12C). These two accessions showed comparatively higher WUE, suggesting improved drought tolerance achieved by optimizing CO_2 uptake per unit water lost through transpiration. Possibly, this is done through maintaining better RWC and opening of stomata partially, this feature allowing a relatively higher assimilation and transpiration (Cornic, 2000). Since, stomatal regulation controls the exchange of water and carbon between the leaf and the atmosphere it in turn would affect WUE (Condon *et al*., 2002). Furthermore, the lower WUE of Sorcoll 141/07 and Sorcoll 146/07 were perhaps due to extensive stress-induced leaf senescence (Fig.8). Therefore, WUE indicates the tissue water relation of sorghum accessions and suggests difference in adaptation strategies among the accessions to stress.

5.1.2.3. Chlorophyll fluorescence

Massaci and Jones (1990) reported that distinguishing the decrease in photosynthesis is either due to stomatal or non stomatal factors, the ratio of Fv/Fm gives a direct estimate of PSII chemistry. The authors also showed that, a sustained decrease in Fv/Fm is believed to indicate the occurrence of photoinhibitory damage in response to many environmental stresses including water deficit stress. In the present study significant difference on potential quantum yield of photosystem II (Fv/Fm) was recorded among accessions (Fig.13). As a result, it supports the suggestion that, stress during post-flowering stage in crop plants had a direct effect on PSII photochemistry. The result is in agreement with Ashraf *et al*. (2007) report, while in contradictory with findings of Addisie Yalew (2010). The accessions such as Sorcoll 163/07 and the check maintained higher Fv/Fm relative to the other accessions at 30 DAF measurements. The ability of these genotypes in maintaining high Fv/Fm under terminal drought condition might indicate high efficiency of radiation use for photochemistry and carbon assimilation (Massaci and Jones, 1990). On the other hand, Sorcoll 146/07 and Sorcoll 141/07 scored significantly lower Fv/Fm under post flowering drought condition. Thus, stress induced reduction in Fv/Fm is indicative of photoinhibition associated with PSII, since the value recorded is below the response of plants under normal condition (i.e. + 0.83) (Piradshti *et al*., 2009; Praba *et al*., 2009).

5.1.3. Biochemical traits

5.1.3.1. Chlorophyll contents

Higher photosynthetic rate under limited water supply conditions is one of the factors for realizing higher grain yield because, it is expected to provide the raw material and the energy required for growth and development. Results of the present study indicated that the accessions showed significant difference in chlorophyll contents (Table 3). The data revealed that the genotypes Sorcoll 163/07 and the check had the maximum chlorophyll ‘a’ and chlorophyll ‘b’ contents. Since, chlorophyll ‘a’ is essential for the conversion of light energy into chemical energy (carbohydrates) and consists of the major portion of both pigment system I and pigment system II, it would be advantageous for efficient exploitation of available radiant energy. Rosa and Maiti (1990) and Pawar (2007) also reported that there was a considerable difference in total chlorophyll content under stress but it was not apparent under irrigated conditions.

Chlorophyll contents had meaningful positive correlation with CCM reading, WUE, RWC, leaf nitrogen and proline contents. In agreement with the present study, Murthy *et al*. (1986) reported that variations in photosynthesis are associated with leaf protein content, nitrogen and chlorophyll content per unit area.

5.1.3.2. Free proline and soluble sugar accumulation

The tolerance mechanism in stress reaction may be associated with accumulation of osmoprotectants such as proline and soluble sugars. Free proline content has been shown to accumulate upon desiccation in leaves of many plant species. In the present study, leaf proline content of the tested accessions showed significant variation (Table 3). The maximum proline content was recorded on better yield bearing accessions, Sorcoll 163/07 and the check (Afeso), while the minimum was recorded on Sorcoll 146/07. In agreement with the present study Jenks *et al*. (1994) and Pawar (2007) reported that high yielding sorghum hybrids accumulated more proline as compared to other varieties under stress. Blum and Ebercon (1976) also showed the proline content of stay green accessions were higher than the drought susceptible accessions with a decrease in the soil moisture content and RWC in all the genotypes. It has been suggested by Jones *et al*. (1980) that the accumulation could make useful contribution to the osmotic adjustment, if it is confined to cytoplasm, but the direct evidences for this are lacking.

The correlation studies indicated that the proline content had significant positive correlation with GLA, quantum yield, chlorophyll content and grain yield (Table 4). This clearly indicates that the accession with a higher proline accumulation under a water limited environment had a better stay green trait by preventing the water in the plant from loss. These results are in conformity with the findings of Bhaskaran *et al*. (1985), who stated that proline accumulation increased in response to water stress. Similarly, Pawar (2007) also reported correlation of proline accumulation with grain yield in water limited environment.

In the present study, accumulation of soluble sugar was found on the tested sorghum accessions. Similar results are reported by Yancey *et al. (*1982) and Al Hakimi *et al*. (1995). The accumulation in leaf sugar concentration under post flowering drought stress may be as a result from the degradation of starch (Fischer and Höll, 1991). Starch may play an important role in accumulation of soluble sugars in cells. On the other hand leaf soluble sugars showed a significant difference among the accessions. Next to the check (Afeso), Sorcoll 163/07 had the maximum total soluble sugars (Table 3). The genotypes which had higher content of total sugars also performed better with respect to yield and yield components and other characters which could probably be due to the maintenance of leaf water status through osmoregualtion (Mohammadkhani and Heidari, 2008).

Correlation study also indicated a positive association of total sugars with total chlorophyll content of the tested accessions (Table 4). This could be another reason for higher yield in these genotypes. Premchandra *et al*. (1995) also reported that total soluble sugars are the major solutes contributing to osmotic potential in sorghum.

5.1.3.3. Leaf nitrogen contents

The data on nitrogen and protein content showed significant difference among accessions, the maximum was found in Sorcoll 163/07 and the check accession, Afeso (Table 3). These two genotypes also had better grain yield and total dry weight

(Fig.14 and Fig.15). However, Sorcoll 141/07 and Sorcoll 146/07 had least amount of leaf nitrogen and protein contents. These results are in conformity with the results of Roy and Wright (1974) and Pawar (2007). The authors found maximum accumulation of nitrogen under post flowering drought condition on better SG genotypes. Moreover, Muchow and Sincliar (1994) reported that nitrogen supply can affect plant growth and productivity by altering both leaf area and photosynthetic capacity. The authors also found increased radiation use efficiency (RUE) with an increase in leaf nitrogen content.

Further, Correlation of the present study also indicated the significant positive correlation of leaf nitrogen with GLA, RWC, RSR and grain yield indicating the importance of leaf nitrogen for higher yield. It also had positive correlation with chlorophyll ‘a’ and total chlorophyll contents (table 4). This is expected because; nitrogen is one of the components of chlorophyll molecule. The higher nitrogen content in the leaf naturally increases chlorophyll content which in turn increases the photosynthetic rate (Borrell and Hammer, 2000).

5.1.4. Biomass and root related traits

The major effect of terminal drought is mainly observed in the form of decrease in SDW and increase in RDW and RSR (Seghatoleslami *et al*., 2008). In the present study, a significant difference was observed among accessions in SDW and RSR under drought stressed environment (Fig.14A) and the outcome is also in agreement with Abdallah (2009). All accessions showed higher SDW except Sorcoll 146/07. The variation in SDW under drought stress conditions would help to screen tolerant accessions from sensitive, because most of the time tolerant genotypes had higher SDW than susceptible ones under terminal drought conditions (Salem, 2003).

In the present study, better drought tolerance accessions (Sorcoll 163/07 and Afeso) had higher RDW, while the drought susceptible accessions (Sorcoll 146/07) was recorded lower RDW (Fig.14B). According to Reddy *et al*. (2003), most crop plants subjected to drought stress usually show an enhanced allocation to roots than to shoots, thus leading to higher RDW. Because root development has long been recognized as an important factor determining the adaptability of a given plant species to post flowering drought stress (Reddy *et al*., 2003). Kramer and Boyer (1995) reported that the roots were usually the site of the highest resistance in the pathway for liquid phase movement of water through the soil-plant atmosphere continuum. So, the efficiency of soil water uptake by the root system is, therefore a key factor in determining the rate of transpiration and tolerance to drought. In this respect, the result of this study is similar to the result reported by Addisie Yalew (2010).

RSR also differed significantly among genotypes under terminal drought (Fig.14C). Sorcoll 163/07 showed the maximum RSR possibly as a result of higher RDW. The difference in RSR among accessions might be a consequence of differences in adaptation of the accessions for survival under drought conditions. The higher RSR value of Sorcoll 146/07 and Afeso under severe terminal condition could be due to the shifts in photo assimilates partitioning which favour root growth than the shoots (Salem, 2003; Bota *et al*., 2004). In addition, it might be due to environmental factors including water deficit which promote lesser biomass allocation to the shoot than to roots (Salem, 2003), as a consequence root to shoot ratios of the accessions have increased (Anwar *et al*., 2003). A reduction in shoot growth coupled with continued root growth would result in an improved plant water status under extreme drought conditions (Passioura, 2002). In general, terminal drought occurring during post-flowering (i.e. early grain development) reduces final dry matter production (Khanna *et al*., 1994). So, the production of maximum biomass under stress condition indicates, dry matter production can be used as selection criterion for drought tolerance in sorghum.

5.1.5. Yield and yield components

Yield is a complex polygenic trait which is highly influenced by the environment and season. In the present study, the accessions varied significantly on yield per panicle (Fig.15B and Fig.16). Higher grain yield per panicle in SG types have been directly attributed to maintaining photosynthetic capability during the grain filling period (Borrell *et al*., 2000b). Sorcoll 163/07 and the check (Afeso), which has resulted in high WUE could have favoured high assimilation and finally high grain yield under terminal drought stress (Fig.12A and 12C). Similarly, the minimum grain yield per panicle of Sorcoll 146/07 might be attributed to the lowest assimilation rate (Sinclair and Jamieson, 2008). Similar results are reported by Borrell *et al*. (2000b) and Addisie Yalew (2010).

Moreover, the reduction of grain yield per panicle might be as a result of fewer seeds per unit area and smaller size of the panicle as it has been reported in soybean (Praba *et al*., 2009). Perhaps, drought induced leaf senescence might affect CO_2 uptake, and that in turn could reduce grain yield. Similarly, studies on sorghum by Addisie Yalew (2010) and on maize varieties by Monneveux *et al*. (2006) have indicated that drought greatly reduces grain yield, which was dependent on the level of senescence due to the stress during grain filling. Thus, the result helps to infer the existence of genotypic difference in grain yield per panicle. Since, the variation in grain yield per panicle has found to be a function of terminal drought (Khannan *et al*., 1994).

A large variation in grain yield per hectare was observed, ranging from 1910-4091 kgha^{-1}. Accession Sorcoll 163/07 recorded higher yield followed by the check (Afeso) and the lowest was recorded by Sorcoll 146/07 (Fig.15C). It is well documented that terminal drought during panicle development consistently reduces the final grain yield of drought susceptible accessions (Manjarrez, 1989). The reduction in grain yield may be attributed to the components of yield that are influenced by moisture as well as drought stress during the later stages of crop growth.

Praba *et al*. (2009) also reported that the number and weight of grains per panicle greatly contributed to the total grain yield. However, in the present study, yield per panicle of drought accessions Sorcoll 146/07, were higher than the better SG genotype Sorcoll 163/07 (Fig.15A, Fig.15B and Fig.16). However in case of yield per hectare of cultivated land the reverse is true, this is because Sorcoll 163/07 had higher number of fertile basal tiller than all tested accessions, while Sorcoll 146/07 had the least number of tiller among accessions (Fig.6). So, the higher tillers lead the accession to be superior in terms of productivity. These findings are in agreement with the results of Rao *et al*. (2001).

The correlation study showed that grain yield had significantly positive relation with GD, NG, GLA, CCM, chlorophyll content, proline accumulation, SDW, RDW and RSR. Possession of high GLA might be one of the reasons contributing to the higher yield per panicle as observed in Sorcoll 163/07 (Table 4). Moreover, the observed high grain yield of Sorcoll 163/07 was consistent with high assimilation rate. However grain yield per panicle had negative relation with NSeL.

5.2. Conclusion

The results of the present study showed that the studied sorghum accessions demonstrated variation in response to most of the measured parameters under post-flowering terminal drought. Thus, the variations might elucidate the existence of genotypic difference among sorghum accessions in response to post-flowering drought stress.

Sorghum accessions such as Sorcoll 163/07 and Afeso appeared to be more resistant to severe post flowering drought stress than Sorcoll 141/07 and Sorcoll 146/07; this can be ascribed to maintaining high value of GLA, RWC, CCM value, assimilation rate, WUE, delayed leaf senescence, RDW and RSR. They also had higher accumulation of biochemical metabolites like (chlorophyll, soluble sugar, free proline, leaf nitrogen and leaf protein contents) and higher grain yield.

The results also showed that sorghum accessions had a remarkable array of contrasting behaviours in response to post-flowering terminal drought conditions. In addition, because of their relationship with other traits and their measurement simplicity, relative greenness (CCM value), day to physiological maturity, total green leaf areas, rate of leaf senescence, relative water content, proline content, nitrogen contents and grain yield are found to be traits that have strong correlation with drought tolerance and stay-green in sorghum.

On the basis of the various morphological, physiological and biochemical traits measured, Sorcoll 163/07 and Afeso was found to show better stay-green property and higher grain yield under post flowering drought stress, next to Afeso, while Sorcoll 146/07 was an early matured genotype.

5.3. Recommendations

It is recommended that further work on identification of desirable traits of the selected accessions through marker assisted breeding is compulsory, so that utilization of morphological, physiological and biochemical traits for selection of drought resistance could be further integrated with the inheritance/genetics and molecular background of the accessions selected. From the studied agro-physiological traits, relative greenness (CCM value), day to physiological maturity, GLA, NSeL, RWC, proline content, nitrogen contents and grain yield parameters are recommended as potential screening tools for SG property due to their measurement simplicity and strong correlation with other traits.

The study was carried out on farmers' field without adaptation of the tested accessions to the environment. As a consequence, the tested accessions showed lower performance almost in all tested parameters than the check except Sorcoll 163/07. In addition to the genetic variation, this may happen due to environmental adaptation problem. So, extrapolating the results would require careful consideration of environmental adaptations on the experimental field. Therefore, testing the accessions selected as SG materials after adaptation to the field should follow.

Moreover, the better performances of the local accession indicate that still additional better SG accessions are available on different part of the country including the study area. Therefore, further screening of SG and better yield bearing sorghum accession must follow.

Finally, as a genetic aspect for breeding purpose, the desired SG and better yield traits of Sorcoll 163/07 and Afeso need to identified and used for further work.

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APPENDICES

Appendix Table 1. Row data on morphological trait at 14, 30 42 DAF measurements

Sorghum Accessions	Plots	Phenological traits											
		Plant height (cm)			Girth diameter (cm)			NSeL			Green leaf area (cm^2)		
		14 DAF	30 DAF	42 DAF	14 DAF	30 DAF	42 DAF	14 DAF	30 DAF	42 DAF	14 DAF	30 DAF	42 DAF
Check accession (Afeso)	**Plot one**	350	339	338	3	3	3	2	3	4	645	643	640
		370	363	362	3	3	3	2	4	4	560	558	550
		350	360	358	3	3	3	2	3	4	377	367	356
		345	354	354	3	3	3	3	4	5	490	487	476
		350	355	354	3	3	3	3	4	5	450	462	460
	Plot two	320	310	310	3	3	3	2	3	4	390	385	380
		365	366	360	3	3	2	1	2	3	400	381	370
		365	374	375	3	3	3	2	3	4	590	588	578
		342	347	340	3	3	3	2	2	4	470	462	452
		342	345	347	3	3	3	1	3	4	530	521	511
	Plot three	321	325	330	3	3	3	2	3	3	650	643	640
		360	364	360	3	3	2	2	3	4	530	514	500
		365	367	369	3	3	3	1	4	4	500	498	487
		330	345	340	3	3	3	2	3	4	501	493	482
		330	346	345	3	3	3	1	2	2	490	483	260
Sorcoll - 141/07	**Plot one**	315	316	316	2	2	2	4	5	6	360	334	250
		310	310	310	2	2	2	4	8	9	500	466	350
		311	312	310	2	2	2	4	7	9	450	419	320
		312	313	310	2	2	2	4	8	9	459	415	320
		312	314	310	2	2	2	4	7	9	489	442	340
	Plot two	373	373	374	3	3	3	3	6	10	380	333	250
		323	323	325	2	2	2	3	6	8	450	407	320
		361	362	360	2	2	2	4	7	8	350	293	260
		353	353	350	2	2	2	3	5	7	430	398	300
		352	352	350	2	2	2	3	6	8	450	409	300
	Plot three	344	345	345	3	3	1	3	6	7	450	430	320
		325	316	315	2	2	2	3	7	8	420	387	300
		336	337	336	2	2	2	4	7	8	420	376	280
		327	327	325	2	2	2	3	6	7	360	332	220
		325	326	328	2	2	2	3	7	7	380	318	240

Sorghum Accessions	Plots	Phenological traits											
		Plant height			Girth diameter			NSeL			Green leaf area		
		14 DAF	30 DAF	42 DAF	14 DAF	30 DAF	42 DAF	14 DAF	30 DAF	42 DAF	14 DAF	30 DAF	42 DAF
Sorcoll 146/07	**Plot one**	325	325	325	3	3	2	4	7	12	702	665	300
		309	309	309	2	2	1	4	7	11	462	411	200
		210	210	210	2	2	1	3	5	13	412	374	200
		254	255	254	3	2	1	3	5	9	612	585	320
		253	254	250	2	2	1	3	6	8	555	512	230
	Plot two	301	301	300	1	1	1	2	6	9	420	387	200
		302	302	300	1	2	1	3	6	9	360	330	200
		255	255	250	2	2	1	3	5	10	352	301	210
		284	285	280	2	2	1	2	6	8	410	379	250
		290	290	290	2	2	2	3	7	10	400	321	220
	Plot three	312	313	312	2	2	2	3	5	9	646	616	240
		304	306	300	2	2	2	3	6	9	542	512	300
		232	233	230	2	2	2	3	7	8	411	379	250
		283	283	280	2	2	2	3	7	7	410	376	240
		283	284	285	2	2	2	4	8	11	456	427	250
Sorcoll 163/07	**Plot one**	251	260	260	3	3	3	3	4	5	540	538	527
		280	286	286	2	2	2	3	3	3	421	411	400
		260	255	255	3	3	3	2	3	3	375	374	360
		255	265	265	3	3	3	2	4	5	435	431	420
		270	263	263	3	3	3	3	4	5	420	415	400
	Plot two	286	297	295	3	3	3	2	4	5	530	527	520
		254	268	268	4	4	3	3	5	7	471	461	450
		245	250	250	2	2	2	1	3	4	310	303	300
		260	270	271	4	4	4	9	8	7	515	506	490
		280	273	272	4	4	4	10	9	8	480	474	464
	Plot three	270	279	278	3	3	3	10	8	7	515	506	490
		265	277	277	3	3	3	7	6	6	512	512	500
		252	253	251	3	3	2	9	8	7	455	453	443
		280	270	270	3	3	3	8	7	7	360	356	350
		269	269	265	3	3	3	9	7	7	440	431	430

Appendix Table 2. Mean value of phenological traits measured at 14, 30 and 42 DAF

Accessions	Plots	Phenological traits						
		Plant height (cm)	Girth diameter (cm)	Number of tillers	NG	NSeL	GLA (cm^2)	SPAD reading
Check accession (Afeso)	**Plot One**	342.33	2.70	5	8	6	642.58	38.17
		365.00	2.53	4	10	7	556.12	39.73
		356.00	2.87	5	9	6	366.52	35.93
		351.00	2.70	2	8	9	484.21	37.50
		353.00	2.57	4	9	9	457.38	38.00
	Plot Two	313.33	3.03	3	11	6	385.04	33.00
		363.67	2.50	4	11	4	383.57	32.87
		371.33	2.97	5	11	6	585.46	34.00
		343.00	2.77	3	9	5	461.38	33.29
		344.67	2.76	4	12	5	520.80	33.29
	Plot Three	325.33	2.77	5	10	6	644.35	34.50
		361.33	2.47	5	9	6	514.50	36.00
		367.00	2.87	4	11	6	494.90	36.20
		338.33	2.67	5	10	6	491.99	35.50
		340.33	2.77	4	9	4	411.16	36.00
Sorcoll-141/07	**Plot One**	315.67	2.03	0	5	11	314.59	20.73
		310.00	2.07	2	6	15	438.81	20.90
		311.00	2.07	1	6	14	396.34	20.37
		311.67	2.02	0	7	15	397.92	20.67
		312.00	2.06	1	5	14	423.80	20.69
	Plot Two	373.33	2.97	2	8	12	321.07	34.67
		323.67	2.07	2	8	12	392.23	33.43
		361.00	2.03	2	8	14	300.90	34.60
		352.00	2.00	0	8	10	376.05	34.00
		351.33	1.99	1	7	12	386.41	34.00
	Plot Three	344.67	2.13	2	7	11	399.92	27.25
		318.67	2.03	1	8	13	369.03	25.00
		336.33	2.08	1	8	14	358.68	31.00
		326.33	2.17	1	7	11	303.93	28.00
		326.33	2.13	2	7	12	312.53	27.00

Accessions	Plots	Phenological traits						
		Plant height (cm)	Girth diameter (cm)	Number of tillers	NG	NSeL	GLA (cm^2)	SPAD reading
Sorcoll 146/07	Plot One	325.00	2.60	1	5	15	555.83	21.03
		309.00	1.73	2	5	15	357.60	19.17
		210.00	1.37	2	4	12	328.82	23.77
		254.33	1.93	3	4	11	505.53	21.30
		252.33	1.77	2	6	12	432.31	21.40
	Plot Two	300.67	1.17	3	7	11	335.59	30.80
		301.33	1.53	1	8	12	296.74	32.10
		253.33	1.80	3	6	11	287.61	43.43
		283.00	1.60	2	8	11	346.40	34.00
		290.00	1.87	3	8	13	313.58	35.00
	Plot Three	312.17	1.85	3	5	11	500.73	26.00
		303.17	2.15	3	6	12	451.31	25.60
		231.50	1.77	3	7	13	346.73	33.58
		282.00	2.00	2	6	12	342.01	28.00
		284.00	1.87	2	6	16	377.53	28.50
Sorcoll 163/07	Plot One	257.00	3.03	5	11	9	534.89	37.00
		284.00	2.07	4	8	7	410.60	38.00
		256.67	2.83	3	8	6	369.82	37.00
		261.67	2.90	5	9	8	428.78	37.00
		265.33	2.97	4	9	9	411.58	38.00
	Plot Two	292.67	3.00	3	8	8	525.78	35.17
		263.33	3.47	5	7	10	460.79	32.00
		248.33	2.23	4	7	5	304.32	33.00
		267.00	3.80	4	8	10	503.53	33.40
		275.00	3.92	3	9	5	472.67	33.40
	Plot Three	275.67	2.97	4	8	11	503.53	35.00
		273.00	2.77	5	6	6	507.97	36.00
		251.83	2.48	4	8	9	450.49	34.00
		273.33	2.82	5	7	6	355.17	35.00
		267.67	2.71	5	8	5	433.78	35.00

Appendix Table 3. Row data on physiological traits at 30 DAF measurements

Sorghum accessions	Plots	Physiological traits				
		RWC	A	E	WUE	Fv/Fm
Check accession (Afeso)	Plot One	77.17	1.50	3.58	0.42	0.74
		82.26	1.50	4.53	0.33	0.75
		82.62	1.30	3.59	0.36	0.78
		77.17	1.00	3.80	0.26	0.75
		82.26	2.00	3.90	0.51	0.75
	Plot Two	89.79	2.79	4.33	0.64	0.80
		82.01	2.00	5.03	0.40	0.79
		79.93	2.00	4.44	0.45	0.77
		89.79	2.30	4.50	0.51	0.79
		82.01	1.50	4.50	0.33	0.80
	Plot Three	81.41	2.00	4.33	0.46	0.77
		80.85	2.00	5.03	0.40	0.78
		81.26	1.00	4.44	0.23	0.78
		81.41	1.00	4.50	0.22	0.77
		80.85	1.80	4.50	0.40	0.78
Sorcoll 141/07	Plot One	80.54	0.74	3.18	0.23	0.69
		40.67	0.17	3.81	0.04	0.73
		73.98	0.08	2.68	0.08	0.71
		80.54	0.50	3.50	0.14	0.71
		40.67	0.60	3.60	0.17	0.72
	Plot Two	81.88	1.72	5.57	0.31	0.66
		77.50	1.02	4.29	0.24	0.74
		72.96	1.22	3.74	0.32	0.75
		81.88	0.50	4.50	0.11	0.70
		77.50	0.70	4.00	0.18	0.71
	Plot Three	58.33	1.70	4.36	0.39	0.67
		59.40	1.50	4.03	0.37	0.74
		81.77	1.30	3.15	0.41	0.73
		58.33	0.80	4.00	0.20	0.70
		59.40	0.60	3.00	0.20	0.69

Sorghum accessions	Plots	Physiological traits				
		RWC	A	E	WUE	Fv/Fm
Sorcoll 146/07	**Plot One**	44.72	1.28	4.95	0.26	0.72
		60.12	0.34	3.05	0.11	0.69
		65.92	0.16	2.62	0.06	0.75
		50.98	0.50	4.00	0.13	0.68
		56.50	0.60	3.00	0.20	0.69
	Plot Two	45.34	1.50	5.56	0.27	0.78
		64.32	1.50	4.90	0.31	0.76
		85.03	2.50	4.22	0.59	0.69
		45.34	1.50	4.00	0.38	0.72
		68.99	1.50	4.50	0.33	0.73
	Plot Three	72.07	0.80	5.23	0.15	0.75
		68.93	0.44	4.00	0.11	0.75
		69.86	0.80	3.40	0.24	0.72
		49.69	0.90	4.00	0.23	0.73
		60.15	0.70	5.00	0.14	0.73
Sorcoll 163/07	**Plot One**	78.90	0.98	3.48	0.28	0.72
		81.93	0.39	2.39	0.16	0.77
		78.81	0.32	4.67	0.07	0.69
		78.90	1.50	3.50	0.43	0.70
		81.93	2.60	3.00	0.87	0.72
	Plot Two	81.73	2.64	3.36	0.78	0.77
		76.60	2.50	2.57	0.97	0.82
		60.38	2.94	3.30	0.89	0.78
		81.73	2.90	3.00	0.97	0.76
		76.60	2.50	3.00	0.83	0.77
	Plot Three	82.35	1.83	3.42	0.54	0.75
		79.31	1.42	2.30	0.62	0.79
		74.64	1.63	4.00	0.41	0.74
		82.35	1.80	3.00	0.60	0.76
		79.31	1.90	3.50	0.54	0.77

Appendix Table 4. Biochemical traits at 30 DAF measurements

Sorghum accessions	Plots	Biochemical traits						
		Total chl (ml/g)	Chl ‘a’ (ml/g)	Chl ’b’ (ml/g)	Leaf proline (mol/g)	Soluble sugar (µg/ml)	Nitrogen content (%)	Protein content (%)
Check accession (Afeso)	Plot One	1.31	0.78	0.74	3.82	9.55	6.16	38.50
		1.38	0.83	0.78	3.95	9.57	6.16	38.50
		1.44	0.86	0.81	3.83	9.59	3.13	19.54
		1.44	0.86	0.81	3.87	9.39	5.15	32.20
		1.54	0.90	0.85	4.03	9.39	5.15	32.16
	Plot two	1.41	0.85	0.79	3.75	7.55	6.95	43.46
		1.40	0.84	0.79	4.07	7.57	5.23	32.67
		1.43	0.82	0.84	3.87	7.59	5.93	37.04
		1.40	0.84	0.79	3.79	7.39	6.00	37.70
		1.45	0.89	0.85	3.95	7.55	6.10	37.74
	Plot Three	1.36	0.81	0.76	3.87	11.54	6.53	40.83
		1.39	0.84	0.78	3.88	11.56	5.69	35.58
		1.44	0.84	0.83	3.73	11.58	4.53	28.29
		1.42	0.85	0.80	4.03	11.38	5.60	34.90
		1.45	0.90	0.83	3.87	11.38	5.60	34.90
Sorcoll- 141/07	Plot one	1.12	0.67	0.64	3.01	6.87	3.13	19.54
		1.15	0.67	0.66	2.90	6.85	3.83	23.92
		1.15	0.67	0.66	2.80	6.83	2.89	18.08
		1.15	0.67	0.67	2.67	6.87	3.30	20.51
		1.20	0.70	0.68	2.82	6.87	3.25	20.51
	Plot Two	1.44	0.87	0.80	2.86	4.88	2.89	18.08
		1.44	0.87	0.81	2.74	4.86	3.13	19.54
		1.43	0.85	0.81	2.98	4.84	2.89	18.08
		1.45	0.87	0.81	2.82	6.87	2.97	18.60
		1.45	0.87	0.81	2.82	6.87	2.97	18.56
	Plot Three	1.27	0.76	0.72	2.98	8.87	3.01	18.81
		1.30	0.78	0.74	2.86	9.07	3.48	21.73
		1.30	0.76	0.75	2.94	6.99	2.89	18.08
		1.30	0.78	0.75	2.74	6.87	3.10	19.50
		1.30	0.76	0.75	2.66	6.87	6.16	19.60

Sorghum accessions	plots	Biochemical traits						
		Total chl (ml/g)	Chl ‘a’ (ml/g)	Chl ’b’ (ml/g)	Leaf proline (mol/g)	Soluble sugar (µg/ml)	Nitrogen content (%)	Protein content (%)
Sorcoll-146/07	Plot One	0.56	0.34	0.32	2.70	8.35	3.13	19.54
		0.54	0.31	0.32	2.44	8.35	2.66	16.63
		0.55	0.31	0.33	2.49	8.15	2.89	18.08
		0.57	0.33	0.33	2.42	7.95	2.90	18.00
		0.56	0.34	0.32	2.74	8.15	2.88	18.10
	Plot Two	1.02	0.60	0.59	2.58	8.35	3.13	19.54
		1.03	0.60	0.59	2.62	8.35	2.85	17.79
		1.01	0.61	0.57	2.54	6.16	2.89	18.08
		1.02	0.61	0.58	2.58	7.95	2.98	18.50
		1.04	0.60	0.59	2.58	8.15	2.90	18.46
	Plot Three	0.77	0.44	0.45	2.60	8.35	3.13	19.54
		0.76	0.43	0.45	2.58	6.35	2.75	17.21
		0.75	0.44	0.44	2.50	8.15	2.89	18.08
		0.77	0.43	0.46	2.66	10.54	2.95	18.30
		0.75	0.44	0.44	2.58	8.15	2.89	18.25
Sorcoll 163/07	Plot One	1.42	0.87	0.79	4.04	9.15	7.33	45.79
		1.40	0.85	0.78	4.03	9.35	4.53	28.29
		1.41	0.85	0.79	3.87	9.25	7.33	45.79
		1.40	0.84	0.78	3.79	7.15	6.40	40.00
		1.42	0.87	0.79	3.95	6.97	6.38	39.80
	Plot Two	1.46	0.88	0.82	3.95	9.25	6.63	41.42
		1.45	0.88	0.81	3.94	9.15	5.93	37.04
		1.45	0.87	0.81	4.02	9.35	7.00	43.75
		1.46	0.88	0.82	3.95	9.25	6.50	40.70
		1.48	0.89	0.83	4.11	9.15	6.48	40.76
	Plot Three	1.44	0.88	0.80	4.03	8.97	6.86	42.88
		1.42	0.86	0.80	3.98	9.15	5.23	32.67
		1.42	0.85	0.80	3.95	9.35	7.19	44.92
		1.43	0.86	0.80	3.91	11.24	6.40	40.15
		1.45	0.89	0.81	3.87	11.14	6.44	40.15

Appendix Table 5. Row data on biomass and yield components at 30 DAF measurements

Sorghum accessions	Biomass, yield and yield components						
	Plots	SDW (g)	RDW (g)	RSR	Grain number per panicle	YPP (kg)	YPH (kg)
Check accession (Afeso)	Plot One	429.00	84.00	0.20	3243	132.65	3979
		422.00	85.79	0.20	3444	297.00	
		469.50	61.00	0.13	3428	182.48	
		435.00	82.00	0.19	3241	202.00	
		421.00	84.00	0.20	3250	206.00	
	Plot Two	511.00	84.00	0.16	3149	126.19	3755
		560.00	88.00	0.16	3140	142.54	
		597.00	85.00	0.14	3145	119.54	
		400.00	79.00	0.20	3160	130.00	
		412.00	82.00	0.20	3156	128.00	
	Plot Three	486.00	83.00	0.17	3202	129.23	3868
		511.00	86.00	0.17	3010	170.00	
		529.50	85.00	0.16	3060	150.00	
		421.00	82.00	0.19	3240	150.00	
		422.00	81.00	0.19	3241	148.00	
Sorcoll-141/07	Plot One	382.50	55.00	0.14	2008	94.11	2998
		390.00	53.00	0.14	2000	110.91	
		350.00	52.00	0.15	2001	67.11	
		370.00	53.00	0.14	2023	91.00	
		380.00	55.00	0.14	2019	90.00	
	Plot Two	400.00	53.00	0.13	3604	185.83	2908
		405.00	56.00	0.14	3654	167.30	
		400.00	53.00	0.13	3645	173.00	
		400.00	52.00	0.13	3600	179.00	
		389.00	55.00	0.14	3550	173.00	
	Plot Three	452.00	66.00	0.15	2013	136.00	2953
		407.00	58.00	0.14	2012	139.00	
		421.00	60.50	0.14	2015	140.00	
		380.00	52.00	0.14	2014	134.00	
		390.00	51.00	0.13	2024	135.00	

Sorghum accessions	Biomass, yield and yield components						
	Plots	SDW (g)	RDW (g)	RSR	Grain number per panicle	YPP (kg)	YPH (kg)
Sorcoll- 146/07	**Plot One**	224.00	31.00	0.14	1275	57.29	1910
		267.50	33.00	0.12	1280	90.80	
		239.80	32.00	0.13	1260	75.00	
		244.00	34.00	0.13	1270	74.00	
		243.00	31.00	0.13	1265	74.00	
	Plot Two	278.50	37.93	0.14	1955	128.58	2024
		274.50	28.60	0.10	1954	84.11	
		243.50	33.00	0.14	1952	81.90	
		266.00	32.00	0.13	1959	96.00	
		264.00	31.00	0.12	1960	100.00	
	Plot Three	250.25	34.45	0.14	1556	90.50	1968
		269.50	31.00	0.12	1555	87.50	
		241.00	32.15	0.13	1546	77.50	
		254.00	34.00	0.13	1564	86.00	
		253.00	32.00	0.13	1534	84.00	
Sorcoll- 163/07	**Plot One**	336.00	75.00	0.22	2386	104.88	4091
		273.60	64.00	0.23	2400	83.88	
		324.00	63.00	0.19	2345	107.12	
		313.00	64.00	0.22	2367	99.00	
		309.00	72.00	0.21	2390	97.00	
	Plot Two	310.00	65.00	0.15	3051	141.16	3864
		355.50	75.00	0.21	3040	144.20	
		359.10	63.00	0.18	3060	106.54	
		342.00	54.00	0.18	3070	128.00	
		340.00	64.00	0.18	3059	132.00	
	Plot Three	391.00	64.00	0.16	2533	123.00	3980
		312.50	74.00	0.24	2560	114.00	
		341.00	64.00	0.19	2554	106.80	
		350.00	64.00	0.19	2555	116.00	
		346.00	54.00	0.20	2547	112.00	

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 the materials used in this thesis have been duly acknowledged.

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Name Signature Date

This work is done under the supervision of:

Name	Signature	Date
Masresha Fetene (Prof.)	__________________	________________