MORPHO-PHYSIOLOGICAL AND SOME BIOCHEMICAL RESPONSES OF SORGHUM [*Sorghum bicolor* (L.) Moench] LANDRACES GROWN UNDER DIFFERENT IRRIGATION LEVELS WITH SPECIAL EMPHASIS ON POST-FLOWERING DROUGHT STRESS

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Declaration

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by Yemane G.Egziabher W.Gerima entitiled: *Morpho-Physiological and Some Biochemical Responses of Sorghum [Sorghum bicolor (L.) Moench] Landraces Grown under Different Irrigation Levels with Special Emphasis on Post-flowering Drought stress* and submitted in fulfillment of the requirements for the degree of Doctor of philosophy (Biology: Botanical Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Morpho-physiological and some biochemical responses of sorghum (*Sorghum bicolor* (L.) Moench) landraces grown under different irrigation levels with special emphasis on post-flowering drought stress

Yemane G.Egziabher W. Gerima, PhD Dissertation

Addis Ababa University, 2014

Morpho-physiological and biochemical investigations were conducted on five selected sorghum (Sorghum bicolor (L.) Moench) landraces (SorColl 60, SorColl 146, SorColl 163, SorColl 178 and SorColl 179) and three cultivars (Abshir, E 36-1 and B 35) to evaluate and screen for drought resistant and stay green potential landraces. The main objective of the study was to enrich parental lines with stay green trait. The study was undertaken both at field (Melkassa Research Station, Melkassa) and greenhouse (College of Natural Science, Addis Ababa University) conditions in 2011 and 2012. A split plot and randomized complete block designs with three replications were used for the field and greenhouse experiments, respectively. Irrigation levels 0%, 25%, 55%, 65% and 80% of total available water (TAW)at field and FC at green house during pre-flowering and a post-flowering progressive drought stress during the field study were applied.

Results demonstrated that all sorghum varieties exhibited different values of the phenotypic traits considered at non stress level except proline content analysis, indicating genetic variation among landraces. Moreover, all traits examined were with different varieties scoring highest value, and high performance at full irrigation had no synchronization with stay green trait. Imposing different irrigation level at pre-flowering resulted in delayed reproductive stage occurrence in the field study. Culm height was more affected by pre-flowering drought stress than by post-flowering. Culm height was relatively more resistant to pre-flowering drought effect in varieties E 36-1, SorColl 163 and Abshir. SorColl 60, E 36-1 and SorColl 163 demonstrated an improvement in culm height over other varieties.Third leaf area, biomass, senescence rate, assimilation rate responses were more sensitive to drought, but SPADR was not. SorColl 60, Abshir followed by SorColl 163 third leaf area decreased least relative to other varieties. Senescence rate evaluation after grain filling revealed an increasing rate with increasing *drought stress. SorColl 163, SorColl 146 and E 36-1were with least senescence rate in post grain filling stage. At 80% water deficit level, the maximum per cent shoot weight loss from the controls was recorded for SorColl 179 (60%) followed by Abshir and E 36-1. SorColl 146 had the lowest percent shoot weight loss (36%) as compared to other varieties. Root biomass of E 36-1 and SorColl 163 increased at severe and mild drought stress levels respectively, while root biomass of SorColl 60 and SorColl 179 went diminishing with increasing water deficit levels. E 36-1, SorColl 178 and SorColl 163 demonstrated a sharp increase in root-shoot biomass ratio indicating relatively a higher dry mater partitioning to roots, while SorColl 179 had the least. In all sorghum varieties under non stress condition /full irrigation/, root length density (RLD) distribution was skewed (greater than 50%) to the first two upper soil depths. The skewed RLD distribution was improved with increasing drought stress from 70-60% in non stress condition to 55-47.5% at 80% on the upper depth. High drought tolerance index (DTI) value was recorded in Abshire, SorColl 178 and SorColl 60. Abshir had high DTI that increased with increasing drought effect; while SorColl 179 demonstrated least index value that further declined with increasing drought stress. Assimilation rate decreased with increasing drought stress and at 55% water deficit level, SorColl 146 (48.4 μmole CO_2/m^2s), E 36-1(43.4 μmole CO_2/m^2s) and SorColl 163 (40 μmole CO_2/m^2s) maintained rate of assimilation better than others and at both 65% and 80 % levels A_N was insignificantly affected by the drought stress.Yield in terms of seed weight per panicle was observed highest in the check E 36-1 followed by SorColl 163 and SorColl 60 whereas SorColl 178 performed least. The study on interrupted drought in the phases of the reproductive stage revealed that yield loss was due to different factors in the reproductive phases considered. Higher yield loss was found due to drought imposed at panicle initiation and seed filling. Loss in seed count had least contribution in E 36-1, SorColl 146 and SorColl 163.Seedlings of Sorghum varieties were found decreasing in height, biomass, assimilation rate but increasing in Chlorophyll a and b and proline content with increasing drought stress. Sorghum landraces SorColl 146, SorColl163, SorColl 60 relatively performed better, whereas SorColl 179, E 36-1, SorColl 178, Abshir, and B 35 were negatively affected by the drought stress. Green house root-shoot* *ratio of SorColl 146, SorColl 178, SorColl 179 and SorColl 163 were higher than others at the most severe drought stress (65%). Third Leaf area of SorColl 179, SorColl 163, and E 36-1 SorColl 178 was less affected over other genotypes. Unlike the results from the field condition, assimilation rate of SorColl 163 and SorColl 146 including the checks were affected highest at seedling and greenhouse condition. A gentle increase in Chl a and Chl b content was recorded with increasing drought but concentrations at 65% were relatively lower than 25% and 55% irrigation levels. The highest reading of Chl a content was recorded in B 35, SorColl 163 and SorColl 60 but SorColl 146 and SorColl 179 had least content and with a sharp decrease in Chl a. On the other hand, Chl b content in SorColl 179, SorColl 146 and B 35 was highest but SorColl 146, SorColl 163, E 36-1 had least and values decreased up to 55% field capacity. Proline content also increased with increasing drought stress in the extracts of all the three organs from control plants. In all organs, there was small amount (about 2-5 μgm/gm) and insignificant variation among the varieties (except SorColl 60 (14.17μgm/gm) and SorColl 146 (8.95μgm/gm) in root) at non stress condition and slow induction in proline was recorded in the mild drought stress. With increasing drought stress, proline content increased sharply in the stem followed by root extract. E 36-1, SorColl 163, B 35 and Abshir demonstrated the highest content in all the organs at the severe drought stress levels, indicating that all the check varieties had active response. It is concluded that of the grain sorghum landraces studied, SorColl 163 had a stay green trait that is found to give high yield under both deficit irrigation before flowering and progressive drought stress in post-flowering period. SorColl 146 performed in many traits next to SorColl 163 but with reduced yield. Thus, SorColl 146 may be described to have cosmetic stay green trait under drought stress. SorColl 60 had inconsistent responses but had better yield than SorColl 146 under severe drought stress. The study recomends that SorColl 163 be used to transfer drought resistant traits to other sorghum lines along with the currently used lines, B 35 and E 36-1.*

Key words*: grain sorghum, stay green, pre-flowering drought stress, post-flowering drought stress, biomass, leaf area, senescence, photosynthesis, SPAD, RLD, yield, proline, chlorophyll content, drought tolerance.*

Dedication

In the memory of my beloved parents, Ato G.Egziabher W.Gerima and W/ro Mamit G.Medhin who had been craving, but regretfully did not live, to see this educational achievement, which is resulted from their gift of many years of love to me.

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List of Symbols and abbreviations

Symbols	**Meaning**
ABA	abcisic acid
Amh.	*Amharic*
A_N	CO_2 assimilation rate
asl.	above sea level
ATP	Adenosine triphosphate
Ca	atmospheric CO_2 concentration
C_i	sub-stomata CO_2 concentration
CAT	catalase
DAF	days after flowering
DAP	Diammonium phosphate
DBF	days before flowering
DTI	drought tolerance index
dsl	drought stress level
E	transpiration rate
EIAR	Ethiopian Institute of Agricultural Research
FC	field capacity
g_m	mesophyll conductance
g_s	stomata conductance to H_2O
ICRISAT	International Crops Research Institute for Semi Arid Tropics
LA	leaf area
GH	greenhouse
GLA	green leaf area
GLAM	green leaf area at maturity
HI	harvest index
Oro.	*Oromifa*
P5CS/P5CR	Δ-pyrroline-5-carboxylate synthase/reductase
Pds	progressive drought stress
PEPCase	phosphoenolpyruvate carboxylase

POX	proline oxydase
RLD	root length density
RuBP	Rubilous bisphosphate
SPAD	soil plant analytical development
SPADR	soil plant analytical development reading
SPADCMR/SCMR	soil plant analytical development chlorophyll meter reading
TAW	total available water
Tig.	*Tigrigna*
Wdl	water deficit level
WUE	Water use efficiency

1 INTRODUCTION

1.1 Background and Justification

Grain sorghum belongs to the tribe Andropogonae Dumort., subtribe Sorghinae, the Poaceae (grass) family, genus *Sorghum* Moench, and with the scientific name *Sorghum bicolor* ssp *bicolor L. (Moench) (*Phillips, 1995*)*. The genus Sorghum is very diverse and all cultivated sorghums belong to *Sorghum bicolor* ssp. *bicolor L. (Moench)* which is divided, based on morphology, into five races (bicolor, caudatum, guinea, durra, and kafir) (Harlan and de Wet, 1972; Phillips, 1995), along with them there are intermediate races resulting from all possible inter-racial crosses. The race bicolor is relatively with small grain, found nearly everywhere but in Ethiopia infrequently grown in eastern and northern regions. Caudatum race is common in the region around Lake Chad extending to the south and west Ethiopia including the hot valleys. The guinea race is mostly found in West Africa and India growing in areas with higher rainfall; Gamogofa is the region where it grows in Ethiopia. Durra race extends to the edges of the Sahara and India and it is economically the most important race in Ethiopia, which is cultivated in the eastern and northern highlands (Phillips, 1995). The Kafir race is primarily from southern Africa and not yet cultivated in Ethiopia. Many varieties of sorghum were created through the practice of disruptive selection, whereby selection for more than one level of a particular character within a population occurs (Doggett, 1970). Disruptive selection must have resulted in sorghum types with vastly different characteristics in height, inflorescence type, including end use (food, fodder, fiber, building materials, etc).

Many agree that sorghum is originated and domesticated in the North-East quadrant of Africa, most likely in the Ethiopian-Sudan border (Doggett, 1988). Archeological evidence suggests that Sorghum consumption began by hunter-gatherers as early as 8000 BC (Smith and Frederickson, 2000), and domestication was practiced around 4000- 3000 BC in Ethiopia and surrounding countries (Dillons *et al*., 2007). The presence of wild sorghums and their cultivated forms and their ecotype differentiation of sorghum into different races and their presence in different parts of the country supports that Ethiopia is one of the centre of origin and diversity for sorghum (Doggett, 1988; Firew Mekbib *et al*., 2009). Sorghum importance was recognized and the improved varieties were distributed along trade and shipping routes to other parts of Africa, and through the middle East to India (approx. 1500 – 1000 BC), and along the Silk Route from Asia Minor to China (Dicko *et al*., 2005) and late 1800s to early 1900s is the time transported to America (Doggett, 1965).

Sorghum (*Sorghum bicolor* L. (Moench)), a cereal grain, is the fifth most important cereal crop in production after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.) in the world (Doggett, 1970; FAO, 1985). Worldwide it is cultivated on 43,727,353 ha with a total production of 58,884,425 Mt (FAO, 2005). Globally, more than 50% sorghum is grown directly for human consumption and the rest is used primarily for animal feed, alcohol production and industrial products (Awika and Rooney, 2004; FAO, 2007). In USA, it is the main source of ethanol used as bio-fuel, constituting 40% of American ethanol from source grain. Sorghum is grown predominantly in low-rainfall (600-800mm per annum), arid to semi-arid environments of sub-Saharan Africa, Asia, America and Australia, supporting as dietary staple of more than 500 million people in more than 30 countries (Reddy *et al*., 2010).

Sorghum is a staple food grain in many semi-arid and tropic areas of the world, notably in Sub-Saharan Africa because of its good adaptation to hard environments and its good yield of production (Dicko *et al*., 2006; FAO. 2007). It is the second major crop (after maize) across all agroecologies in Africa. Nowadays, sorghum is attracting industries beyond animal feed elsewhere and human consumption in Africa. It is gaining commercial value in malting and brewing industries like in Nigeria, Ghana, Uganda and Kenya. These brewing industries are buying sorghum on agreed prices and Nigeria is consuming about 152, 000MT per annum (Taylor, 2005). It is believed that such higher prices will sensitize increase in sorghum production. Globally, International Crops Research Institute for Semi Arid Tropics (ICRISAT) is involved in diversification of sorghum breeding populations, and ultimately provision of cultivars with better performance to farmers, through the incorporation of traits and genetic having germplasm from the world.

Sorghum in Ethiopia has an indigenous knowledge-based classification and naming, since its existence from antiquity (Firew Mekbib, 2007). Among the many common names in Ethiopia are *Mashila* (Amh. and Tig.), *Mishinga* (Oro.) and *Mishela* (Tig). There are also many farmer' varieties or folk species named after many issues in their localities. Firew Mekbib (2007) identified many folk species for *mishinga* with different origins for naming, for example 16 were named after the introducer and/or geographical origin, 37 according to botanical traits, and 8 to use related traits. Yemane Tsehaye *et al.* (2009) also identified 31 locally named sorghum varieties in south and central Tigray. According to Firew Mekbib (2007), the crop is intimately associated with the life of the farmers and the associations are reflected in farmers' activities such as seed selection and utilization using folksongs. Amsalu Ayana and Endashaw bekele (2000) found sorghum accessions variation highly pronounced for agronomically important characters, which were also influenced by environmental factors. Sorghum ranks fourth in area coverage after tef (*Eragrostis tef* (Zucc.) Trotter), maize (*Zea mays* L.), and wheat (*Triticum aestivum* L.) (CSA, 2005). The total production of grain sorghum year to year is also increasing in Ethiopia (CSA, 2005; Waddington *et al*., 2009) and sorghum production per hectare exceeded world averages in years between 2005 and 2008 (Waddington *et al*., 2009). In 2012/2013 sorghum in Ethiopia is produced on an area of 1711485.04 ha with a total production of 1764286.2 Mt with yield of 21.06 quintal per ha (CSA, 2013). It is grown mostly in the northern and eastern regions on 1,253,620 ha with a total production of 1,715,954 tons (CSA, 2005) and it accounts 14.2% and 13.6% of the crop area and production respectively. There are over 3,674,865 farmer households dependent on sorghum production in the last two regions. Grain sorghum is a major cereal crop with multi-purposes in lower and mid altitude regions of Ethiopia. It is a staple food crop in the rural areas where it grows. As barley is to the peoples in the highlands above 3600m asl. (Zemede Asfaw, 1990; Masresha Fetene *et al*., 2012), grain sorghum is the main staple food for the people in the lower altitudes. Grain sorghum in Ethiopia is used primarily to prepare local foods such as *'injera'*, bread, thick porridge, soup, boiled grains and pop, medicinal values for some landraces is also common (Rooney and Murty, 1982). *Injera* a thin unleavened, round and flat pancake-like national dish to Ethiopians is a daily habit and an exercise in every door, which is prepared from sorghum or mixed with other cereals next to *Eragrostis teff* (tef). Local beer called *tela* is also prepared from sorghum, the preferred landrace *'gan-seber'*, to mean pot breaker, is known for its high alcohol production. Its straw is also used for animal feed, house construction and bio-fuel source.

Sorghum research in Ethiopia began in the mid 1950s at the Alemaya College of Agriculture (ESIP, 1978). The Ethiopian Sorghum Improvement Program (ESIP) was established in 1973. Despite many problems encountered, ESIP released improved varieties in 1977 and started working directly with farmers (ESIP, 1978). Studies undertaken include survey research to quantify the trend in productivity, the level of and reasons for adoption of improved varieties, yield performance and preference evaluation of landraces and improved varieties. Despite the long time journey on sorghum research in Ethiopia, farmers have been benefited less from research out comes.

To date, Ethiopia has research centers for sorghum development at Melkassa and other additional sites and enormous diversity or various germplasm collections have been made by the Institute of Biodiversity Conservation and Research (IBCR). Ethiopia is 3rd top cereal producer in Africa next to south Africa and Egypt (FAO, 2013). Whereas, like in other regions drought leading to water stress in plants has been a major problem in reducing crop productivity including sorghum in Ethiopia (Rosenow *et al*., 1996). Whereas, crops such as sorghum adapted to semi-arid regions, and contain various traits that enable to escape and resist drought are potentially important to alleviate the drought effect. Genetic improvement for drought tolerance is a long term strategy and hence the selection for drought resistance has to be either drought escape or drought avoidance or drought tolerance assumes greater importance (Neumann, 2008; Prasad and Staggenborg, 2008). The primary involvement of the physiological evaluation for screening elite parental lines to be used in genetic improvement has no another alternative (Reynolds and Trethowan, 2007). A number of physiological traits have been associated with enhancement of drought tolerance (Ludlow and Muchow, 1990). Field screening using morphological and agronomic traits for drought resistance has led to the identification of two distinct drought stress responses in sorghum, namely pre-flowering and post-flowering (Rosenow and Clark, 1981). Recently, in sorghum cultivation, the stay green property is found to be the most important in the sub-Saharan environment, improving production under post-flowering and terminal drought. The adaptation of grain sorghum to a wide range of environmental conditions has led to the evolution and existence of extensive genetic variation for drought tolerance (Dogget, 1988; Blum *et al*., 1997). The genetic base that has been used in sorghum breeding, however, is very narrow and can be traced to few parents. There is a need to expand this by looking at other existing lines and evaluating them for traits that can be used in sorghum improvement. The indigenous crop plant species in Ethiopia, their wild relatives, the wild and weedy species which form the basis of Ethiopia's plant genetic resources are highly prized for their potential value as sources of important variations for crop improvement programmes (Melaku Worede, 1991). So far, Ethiopian sorghum varieties have been used by different researchers elsewhere for their best source of stay green trait and are hopping these and other varieties (not checked) to offer high contribution in world food security in the drier regions. Understanding the genetic, physiological, molecular and biochemical basis of such drought-resistance mechanisms is fundamental to the development of new strains that are better adapted to dry conditions. Keeping leaves alive longer is a fundamental strategy for increasing crop production, particularly under water-limited conditions. Our study, therefore, is designed to investigate morphological, physiological and biochemical adaptations with stay-green drought resistance trait in indigenous sorghum landraces under deficit irrigation.

1.2 Objectives

1.2.1 General Objectives

The research had the following major objectives:

- ❖ To investigate physiological and morphological responses of elite sorghum landraces with potential stay green property grown both under pre-flowering different soil water potential and post-flowering progressive water deficit conditions.
- ❖ To identify the most sensitive phases in reproduction stage to short period drought stress exposure of the sorghum genotypes.
- ❖ To assess the biochemical responses in different levels of drought at seedling stage

1.2.2 Specific Objectives

- ➢ To find out the variation in various morphological and physiological traits including attributes to yield among grain sorghum varieties grown both under pre-flowering different deficit irrigation and post-flowering progressive drought stress at field condition.

- To identify and recognize the lower limit of soil water potential below which the sorghum landraces with stay green trait cannot give crop yield.
- To investigate root biomass, length and distribution in responses to drought stress and evaluate the associations with stay green trait.
- To determine the roles of stay green in sorghum varieties exposed to drought stress at seedling, a pre-flowering and post-flowering stages
- To identify the most sensitive phase in sorghum varieties to short period drought stress during reproductive development
- To quantify the impact of drought on the morphological and yield traits (percent seed-set, seed numbers and seed weight) of grain sorghum imposed at different phases of reproductive stage.

1.3 Rationale and justification of the study

The exercise of traditional farmers in selecting crop varieties for their better performance in yield under optimal or stress conditions since the antiquity is the basis for the modern parental lines development in crop improvement programmes. However, the struggle of conventional breeders to come up with high yielding varieties faced difficulties to go beyond improving yield of crops. Farmers, especially from the developing world, have been benefited less from research outcomes due to complex nature of the concept of crop yield. This is because breeders have been emphasizing particular innate phenology of crops and harvesting time (Araus *et al*., 2008), for it depends on plant growth, development and differentiation. Rate of plant growth, development and differentiation are dependent on both genetic and environmental factors, which demand multidisciplinary approach to evaluate. Therefore, the integration of different disciplines such as molecular, genetics, physiology, biotechnology and breeding in developing vigor parental lines using many characters as selection indices for obtaining high yields consistently is becoming promising and a common interest. Plant improvement for drought environments requires more complete knowledge of the genetic basis of both the morphological traits and the physiological parameters involved in the plant responses under water-limited conditions. Sorghum is among the potentially important crops that may help in feeding many populations in the drier regions of world, with the context of climate change, because it is recognized as relatively hardy and photosynthetically efficient plant in marginal environment. Despite of the C_4 mechanism of its CO_2 fixation, the yield levels are still very low due to abiotic factors including drought (Ghannoum (2009). Hence, in spite of its multipurpose help for the majority poor farmers in drier regions of the world, genetic efforts made for increasing yield levels are inefficient due to inadequate involvement of physiological indices and lack of sufficient vigor parental lines.

2 LITERATURE REVIEW

Toward the year 2050, the world population is projected to reach around 9.2 billion (Pretty, 2008; Singh, 2012), which demands doubling global agricultural production, and farm productivity would need to increase by 1.8% each year. On the other hand, the agricultural production base especially land, water, and biodiversity are fast shrinking and degrading (Pretty, 2008; Singh, 2012). For instance, by 2025, 30% of crop production will be at risk due to the declining water availability (Singh, 2012). Thus, in order to meet the ever-intensifying demand for food and primary production, more and more is to be produced from less and less of the finite natural and nonrenewable resources.

Drought is the most economically important abiotic stress attracting the attention of agronomists, physiologists and genetists better than any time because of the increasing demand of crop production due to world population pressure and unreliable rainfall due to the global climatic change (Pretty, 2008). Many researches have been undertaken for developing drought tolerant varieties using a number of morpho-phenological, physiological, and biochemical parameters as selection criteria for higher yields under drought situation (Mojaddam *et al*., 2012). Therefore, under this section available literature on morphological, physiological and biochemical parameters with great emphasis to stay green trait, which contribute for tolerance to post-flowering drought stress are reviewed.

Plant physiological processes are directly affected by the amount of water that plants can extract from the soil. Water stress limits plant growth and crop production more than any other single environmental factor (Boyer, 1982). Stomata closure, together with leaf growth inhibition, are among the first processes affected in response to drought, to protect plants from extensive water losses and reducing the source of assimilates (photosynthetic rate) and, consequently, the resulting biomass. Therefore, many studies have focused their attention on the importance of drought as one of the most important constrains limiting growth in plants and crops and the ecosystem productivity worldwide (Passioura, 1996).

2.1 Definition of drought stress

Any situation where the external constraints limit the rate of dry-matter production of all or part of the vegetation below its 'genetic potential' is stress (Grime, 1979; Larscher, 1995). The stress effects depend on the degree and duration of the stress, developmental stage of the plant, genotypic capacity of species and environmental interactions (Levitt, 1980). The difference in growth rate even deviation to some extent from the normal situation, not every deviation of a factor from its optimum because of the flexibility of normal metabolism, can cause a dramatic effect on the growth and performance of plants (Jaleel *et al*., 2007). There are difficulties in quantifying the 'genetic potential' of plants and therefore measuring the impact of stress. So, 'genetic potential' is always considered the performance of plants under prevailing interacting environmental conditions, since stress factors do not usually operate alone so that interactions between and variation of stresses are the norm in the natural environment. Stress may also have a greater effect during certain phases of the plant's life cycle than others. For instance, seedling establishment and floral development are often particularly sensitive stages in most crops.

The moisture level and temperature acting together are the two important environmental factors that determine largely the climate of a region and the global distribution of plants and animal life (Taiz and Zeiger, 2010).

On a global basis, water deficit is a major cause limiting productivity of agricultural systems and food production (Boyer, 1982). In cereal crops which provide the major carbohydrate staples for humans, even intermittent water stress at critical stages may result in considerable yield reduction (Ludlow and Muchow, 1990). The insufficiency of moisture for human and ecological sustainability has been described on several levels. Three types of drought are recognized in plant science, each involving a different scientific domain.

Meteorological drought can be brought about when there is a prolonged period with less than average precipitation, which usually precedes the other kinds of drought. Secondly, Hydrological drought is named after water reserves available in sources such as aquifers, lakes, and reservoirs fall below the statistical average, usually arises due to over usage of water reserves even in times of average (or above average) precipitation. This is believed to be a serious threat to the future farming in drier regions. The third is most important to our interest and it is called Agricultural drought which explains the insufficient moisture for maximum or potential growth of crops, range or plantations. This condition can arise, even in times of average precipitation, owing to specific soil conditions, topography or biotic factors. Agricultural drought can be expressed on a very wide range of plant growth reductions up to complete crop failure (Quiring and Papakryiakou, 2003). It does not necessarily imply that plants must wilt or die or fail in any spectacular manner (Quiring and Papakryiakou, 2003), it can cause small reductions in yield when it is mild. The effect of agricultural drought is due to the occurrence of cellular water deficit in the plant, when the rate of transpiration exceeds water uptake by plants (Yoshida, 1981), this occurs when water potentials in the rhizosphere are sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development. The cellular water deficits result in an increased concentration of solutes, loss of turgor, change in cell volume, disruption of water potential gradients, change in membrane integrity, denaturation of proteins and several physiological and molecular components (Grifth and Parry, 2002; Lawlor, 2002).

According to Maunder (2005), sorghum is a potential crop that will satisfy the doubling demand of developing world population by 2020, predicted to have 25% of world population experience severe water scarcity by then. This is because sorghum is considered relatively the potential drought-resistant cereal and a model crop for evaluation of drought resistance mechanisms, but the genetic and physiological mechanisms involved in its expression are poorly understood (Tuinstra *et al*., 1998; Ejeta and Knoll, 2007) and they recommended research efforts to focus on specific genomic regions associated with drought tolerance for the development of clear understanding of the physiology and biochemistry associated with this complex trait.

2.2 Plant drought adaptation mechanisms

Plant stress responses are very complex (Barnabas *et al*., 2008). Plants have evolved three strategies of coping with water deficit (Turner, 1997) that are involved together or separately, which makes studying uneasy. Plants adjust evolutionarily to environmental stress by rapid plant development or developmental plasticity to overcome drought stress, which is drought escape. Plants are also capable of tolerating drought by reducing water loss and maintaining high tissue water potential by increasing absorption, where drought is tolerated at high tissue water potential and is called dehydration avoidance. The third is a drought tolerance at low tissue water potential (dehydration tolerance). A tolerance to drought is demonstrated by plants maintaining turgor pressure under conditions of low soil moisture. The term drought resistance is also used to represent the plant strategy of drought tolerance, avoidance, escape and its capacity to recover from drought stress effects (Ashley, 1993). A large number of physiological, molecular and biochemical processes at the cellular or whole plant level are altered in response to drought and play an important role in mitigating stress (Bhargava and Sawant, 2013). Drought stress resistance is, therefore, a complex interaction of physiological, morphological, phenological, and environmental factors (Tuinstra *et al.*, 1998; Jaleel *et al.,* 2007). Several physiological, phenological, morphological, cellular, and biochemical approaches in selecting for drought resistance have been attempted (Acevedo *et al.*, 1979).

2.3 Evaluation of plant traits for drought resistance

Plant traits conferring resistance to drought stress differ according to the plant species and developmental stage as well as the degree and periods of drought stress imposed (Levitt, 1980; Passioura, 1996). The plant traits that confer drought resistance depend on the type of drought. The investigation of biochemical, physiological and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions (Anjum *et al.*, 2011). The following describes the physiological and morphological traits likely to be useful against the three common types of drought. Drought may occur naturally at any stage of plants and based on time of occurrence in annual plants, two types of drought, intermittent and terminal stress, are known, where the former occurs for short period at any stage whereas the later occurs towards the end of the growing season, which includes pre-and post-flowering stages (Neumann, 2008). Severe drought cycles has been occurring at seedling, pre-flowering and post-flowering stages affecting sorghum growth and yield in arid regions (Borrell *et al*., 2000b). In rice three drought stages have been recognized including vegetative stage in addition to the above (Kamoshita *et al*., 2008). Plant traits that have been considered to confer resistance for intermittent drought stress effect in grain sorghum include early matching maturity to water supply, leaf maintenance, rooting depth and density, osmotic adjustment of roots and shoots, early vigor, increased leaf reflectance and dehydration resistance (Ludlow and Muchow, 1990) and besides the above, resistant traits for terminal stress also include mobilization of pre-anthesis photosynthate to the kernels.

Drought often delays developmental events because of the inhibition of growth by water deficit (Blum, 1996), drought affected stand losses may occur soon after full emergence before seedling establishment. Nine stages that can be used in understanding the growth and development of grain sorghum are recognized (Vanderlip, 1993), which include emergence, three-leaf stage, five-leaf stage, growing point differentiation, flag leaf visible, boot stage, half-bloom stage soft-dough, hard-dough and physiological maturity. According to Vanderlip (1993), these stages are labeled from stage 0 (emergence) to stage 9 (physiological maturity) with each stage having some distinctive characteristics. Time required to reach each stage depends both on the hybrid and the environment in which it is growing. Seedling establishment is a critical stage, when stable rooting and initial green leaf area attained, although in case of sorghum seeding establishment is different in that it depends mainly on the development of crown roots (adventitious roots) to support the plant before the seminal root looses its functionality (Blum *et al*., 1977a and b), while in wheat seminal roots are functional for most of plant life.

The greater availability of meristems (actively growing parts) in late flowering correlates with higher impact of drought (Blum, 1996) and the greater viability of these parts is expressed better tolerance to desiccation. The leaf area is also determined by phenology, stem morphology, rates of leaf immergence and potential leaf size. Therefore, any drought effect on these would modify leaf area. Some studies indicated that the slow growth of leaves help sorghum plants reduce rate of transpiration under drought condition.

Sorghum is mostly vulnerable to drought stress at post-flowering stage where reduction of grain yields by more than 50% is likely to be realized (FAO,1999). Post-flowering drought adaptation in sorghum is found associated with the stay-green phenotype (Borrell *et al*., 2000b; Harris *et al*., 2007); and is an essential trait for increasing sorghum production. It is also important trait in other cereals in the semi arid regions (Thomas and Howarth, 2000). Stay-green is becoming a trait of interest, because an active green canopy is essential to continued grain filling under progressive post-flowering drought (Tenkouano *et al*., 1993; Walulu *et al*., 1994; van Oosterom *et al*., 1996; Borrell *et al*., 2000a and b; Borrell and Hammer, 2000; Thomas and Howarth, 2000; Xu *et al*., 2000), which is the most important drought stress in arid and semi arid regions. Stay green is a character of greater green leaf area duration during grain filling that appears to be cumulative expression of three distinct factors; green leaf area at flowering time, onset of senescence and subsequent rate of senescence (van Oosterom *et al.*, 1996). However, ‘rate’ rather than ‘onset’ of leaf senescence was found the most important component of stay-green (Harris *et al.*, 2007). The stay green trait is important as it improves genotype adaptation to post-flowering drought stress, particularly in environments where the crop depends on stored soil moisture to fill the grains (Rosenow *et al.,* 1977), for severe stress that occurs during grain filling may result in drastic reduction of photosynthetic rate followed by leaves desfunctioning, hence non-senescent forms with high stem and leave food reserves play great advantage over other during this time (Borrell and Hammer, 2000; Yang *et al*, 2001). It is also found associated with resistance to charcoal stalk rot [*Macrophomina phaseolina* (Tassi) Goid] (Rosenow, 1984), lodging (Resenow *et al.*, 1995) and superior ruminant nutritional quality of Stover due to a higher content of basal stem sugars (Duncan, 1984; van Oosterom *et al.*, 1996).

According to Borrell *et al.* (2001) staying green longer during grain filling is as a result of the balance between nitrogen demand by the grain and nitrogen supply, but stay green genotypes also found commonly containing increased sugar content cytokinin (Thomas and Howarth, 2000).. This confirms nitrogen dynamics may not be the sole reason for increased leaf longevity.

The benefits of stay green phenotypes have been described in sorghum (Borrell *et al.*, 2000a and b), where the rate of leaf senescence was shown to be negatively correlated with yield under conditions of terminal water deficits (Borrell *et al.*, 2000a and b). According to Borrell and Hammer (2000) if ever rapid leaf senescence occurs in these genotypes, it is believed to be indicative of reserve mobilization to the grain. As greenness per se does not guarantee continued C assimilation (Thomas and Howarth,

2000) and may be detrimental if it indicates a weak grain sink and/or an inability to remobilize stem reserves (Blum *et al.*, 1997). Lopes *et al.* (2011) reviewed problems of stay green when it interacts with other variables. According to them, for example, stay green can result from late flowering or low reproductive sink strength, and stay green phenotypes are highly prone to variation in response to environmental factors (Rosenow and Clark, 1995). Stay-green genotypes also appear to have higher leaf-nitrogen concentration (specific leaf nitrogen) at flowering and maintain it during grain filling (Borrell and Hammer, 2000), which is possibly associated with a higher transpiration efficiency in the best stay-green hybrids (Borrell *et al.*, 2000b). Other genotypes, however, may demonstrate seemingly stay green trait with non functional green leaves (Thomas and Howarth, 2000), who identified five stay green types with three cosmetic forms. Conventional breeding for best stay-green trait in sorghum has been developed so far primarily based on two genetic stocks; B 35 and E 36-1, sources were from Ethiopia (Borrell *et al.,* 2000a). Xu *et al*. (2000) after scoring the stay green trait of stressed plants, a strong correlation was found among total leaf chlorophyll contents from SPAD values and a Spectrophotometer method as well as visual stay green ratings as indicator of leaf senescence, where visual stay green ratings were a reliable indicator of leaf senescence and useful in evaluating progeny when breeding for drought tolerance.

The chlorophyll meter SPAD-502 has been used as simple, rapid, and non destructive estimation of chlorophyll contents (Fontes and de Araujo, 2006). Several authors have shown a relationship between chlorophyll and N contents in plant leaves (Wang *et. al*, 2004), and chlorophyll contents from SPADR have been used as an alternative measure of plant N status (Fontes and Araujo, 2006). For example, A SPAD meter was used in quantitative measure of the N requirement of the tomato plants after appropriate SPAD critical values were established (Cezar *et al.*, 2006).

Green down measurement by visual rating has been used to evaluate senescence rate in sorghum plants under drought stress treatment (Borrell *et al.*, 2000a and b). The leaf tip of cereals consists of the oldest cells while the youngest cells are found at the leaf base (Mullet, 1988), hence progression of senescence begins from tip and they are ideal for studies. Severe drought enhances senescence, which in turn reduces TLA and production (Borrell, 2000a and b). Rather than cell stress, senescence can be considered as a whole plant mechanism which reduces leaf area in the presence of drought stress in order to reduce transpiration and remobilize assimilates to seeds or growing regions (Munne-Bosch and Alegre, 2004).

Most common observation concerning roots under root stress is the increase in root/shoot dry matter weight ratio, which results from the relatively greater decrease in shoot growth than in roots and sometimes due to root weight increase (Blum, 1996). The ABA accumulation in roots as a result of drought was considered to decrease shoot growth while sustaining root growth by Sharp and LeNoble (2002), which is a controversial as there are evidences supporting the transport of ABA from the root to the leaves to induce stomata closure during water deficit development in the soil (Hartung *et al.*, 2002; Taiz and Zeiger, 2010). Increase in wheat root-shoot biomass ratio due to drought stress was explained by Zhang (2009) as a result of the inhibition of some materials with less drought resistance growth in aerial part, while root was still growing. This is because the root is less stressed than shoot as drought stress develops slowly.

Root system architecture and root distribution are also key determinants of the ability of a plant to access water and nutrients in water limiting conditions (Lynch, 2007; Songsri *et al.*, 2008). Enhanced water uptake through deeper and increased root length density (RLD) in lower soil depths have also been demonstrated to offer dehydration avoidance in grain sorghum (Songsri *et al.*, 2008). However, the magnitude and consequences of their differences have not always been associated in different plant species (Smucker, 1991). The physiological basis of the plasticity of root growth in response to environmental variables is very poorly understood and demands integrated study (Lynch and Brown, 2012)

Photosynthesis is often used as a criterion of crop dehydration tolerance or in general drought resistance. A decline in the photosynthetic rate under drought stress conditions could be attributed either to a decrease in stomata conductance and/or to non-stomata limitations (Cornic and Massacci, 1996). Both Stomata and non stomata limitations to photosynthesis have been observed in lilies (Zhang *et al.*, 2012), and chickpea cultivars (Mafakheri *et al.*, 2010) due to drought stress. Most researchers agree that the stomata closure and the resulting CO_2 deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano, 2002). However, some authors assert that impaired ATP is a likely explanation for decreased photosynthesis under water stress (Lawlor, 2002; Lawler and Conic, 2002). It has been found out that contents of RuBP and ATP decrease early in drought development, at relatively high gs (higher than 150 mmol H_2O $m^{-2}s^{-1}$) (Flexas and Medrano, 2002), indicating that RuBP regeneration and ATP synthesis are impaired. The photosynthetic systems of C4 plants like sorghum are as sensitive to drought induced inhibition as they are in C3 plants (Ripley *et al*., 2010) even more susceptible (Ibrahim *et al*., 2008; Ghannoum, 2009). This is because C4 plants have strong variation in the C4 pathway due to phylogenetic diversity, from which taxa they inherited their C4 (Christin *et al*., 2009; Taylor *et al*., 2010 and Sage and Zhu, 2011). Compared to the other varieties from ICRISAT, Jagtap *et al*. (1998) identified E 36-1 with high yield sustaining a higher maximal rate of photosynthetic oxygen evolution under water deficit, heat and light stresses. It was suggested that the high stability to stress-induced damage, or acclimation of photosynthesis to the individual components drought stress or the markedly higher levels of the chloroplastic chaperonin 60 (cpn 60) found, are the causes for the high yield of E 36-1. Positive correlation between biomass production and net CO_2 assimilation rate (so photosynthetic capacity) has been found in maize (Ashrafi *et al*., 2007) which entails a potential selection criterion for drought tolerance in maize.

Rate of photosynthesis is influenced by the relationships between stomata resistance, leaf water potential, leaf temperature, and environmental factors such as temperature and humidity in plants growing in arid or semiarid conditions. A decrease in A_N under mild drought stress was found associated with reduced stomata conductance in durum wheat (El Hafid *et al*., 1998) and soya been seedling (Berhanu Amsalu *et al*., 2012), but in the former possible factors determining the drought-resistance of a cultivar were assumed to be with lower sensitivity of CO_2 exchange rate. The down regulation of the photochemistry due to drought at early vegetative growth stages has been regarded as the main photoprotective/ regulative mechanisms in sweet sorghum (Zegada-Lizarazu and Monti (2013).

Analysis of biochemical responses has been used as one way of evaluating drought tolerance in crops. The amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments, and therefore low concentrations of chlorophyll can directly limit photosynthetic potential and hence primary production (Filella *et al.,* 1995). In this case, pigmentation can be directly related to stress physiology, as chlorophyll a and b concentrations change with the effect of abiotic stresses and during senescence (Larscher, 1995; Penuelas and Filella, 1998). It is both increase in chlorophyll a and chlorophyll b contents (Valentovic *et al*., 2006 and Jabeen *et al*., 2008) as well as reduced contents (Nyachiro *et al*., 2001; Mafakheri *et al*., 2010), recorded as drought stress effect and both conditions ultimately reduce the amount of grain yield due to the negative effect of the drought stress.

The synthesis and/or accumulation of compatible osmolytes, which are osmotically active (proline) and neutral organic compounds such as sugars, selected amino acids, and quaternary ammonium compounds, have been identified in response to water deficit stress. Many plant species naturally accumulate proline and protein as major organic osmolytes when subjected to different abiotic stresses such as sorghum (Waldren *et al*., 1974, Blum and Ebercon, 1976*;* Bhaskaran *et al*., 1985; Yadav *et al*. 2005), wheat (Hamada 2000, Bowne, 2012) and in salt-stressed *Catharanthus roseus* (Jaleel *et al*. 2007), maize (Jabeen *et al*., 2008) and rice (Mostajeran and Rahimi-Eichi, 2009). Rahdari *et al.* (2012) found proline concentration increasing with increasing drought stress, but free proline did not accumulate significantly in leaves of sorghum [*Sorghum bicolor* (L.) Moench until plants were severely stressed (Jabeen *et al*., 2008). Moreover, proline levels increase as leaf water potential and relative water content fell was not apparently different among different sorghum lines with change in plant water status (Sivaramakrishan *et al*., 1988). On the otherhand, a drought study on bean (*Vicia faba* L.) by El-Tayeb, (2006) revealed an increase in proline and soluble sugars as well as catalase (CAT) and peroxidase (POX) activity with much higher accumulation in the resistant form and had better protection against drought-induced oxidative stress. Proline and sugars produced during drought condition improve stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions (Rudolph *et al*. 1986; Thomas and James, 1999). Proline accumulation with increasing drought has been explained as a result of both induction of proline biosynthesis and/or inhibition of its oxidation (Hong *et al*., 2000,). Other studies also found out that abiotic stresses other than water deficit stress such as ultra violet light in wheat (Demer, 2000), salt stress in rice (Lin *et al*. 2004) caused an increase in proline content.

Grain yield or attributes are among the most important trait in screening drought tolerant cultivars. In fact, selecting for other drought traits without considering yield will not be useful (Schaffert *et al*., 2010) in the production enhancement program. Grain number and weight per panicle are the main components of yield. Yield stability in terms of seeds per panicle in the most severe water deficit stress levels under post-flowering water stress is manifested by a stay green phenotype and normal grain filling in sorghum (Xu *et al*, 2000). Saeedipour and Moradi (2011) recorded severe water stress deleterious effect on grain yield of both susceptible and tolerant wheat cultivars, led to a smaller kernels and lesser aerial biomass at maturity but more severe in susceptible.

3 MATERIALS AND METHODS

The materials and techniques used during the course of investigation under deficit irrigation to screen sorghum parental lines with stay green trait and associated morphological, physiological and biochemical performance that help in enhancing the stay green trait and yields in drought prone areas conducted at field and greenhouse conditions are described in this section.

3.1 Field study

The field study was conducted having two major experiments. One to evaluate performance of potential stay green sorghum landraces in pre and post-flowering drought stress and the second was to identify the most sensitive reproductive phase to interrupted drought stress at reproductive stage.

3.1.1 Study Area description

3.1.1.1 Location of experimental site

The field experiment was conducted at Melkassa Research Station (MRS) in 2011 during off season. MRS is one of the research centers of the Ethiopian Institute of Agricultural Research /EIAR/ in Ethiopia. It is the vanguard sorghum development center of EIAR situated in the central part of the rift valley, about 110km south east of Addis Ababa, 15 km south-east of Nazareth /Adama/ City (Figure 1). It is located at longitude 39.31^{o}E and latitude 8.43^{o}N. It has an elevation of 1550 m above sea level.

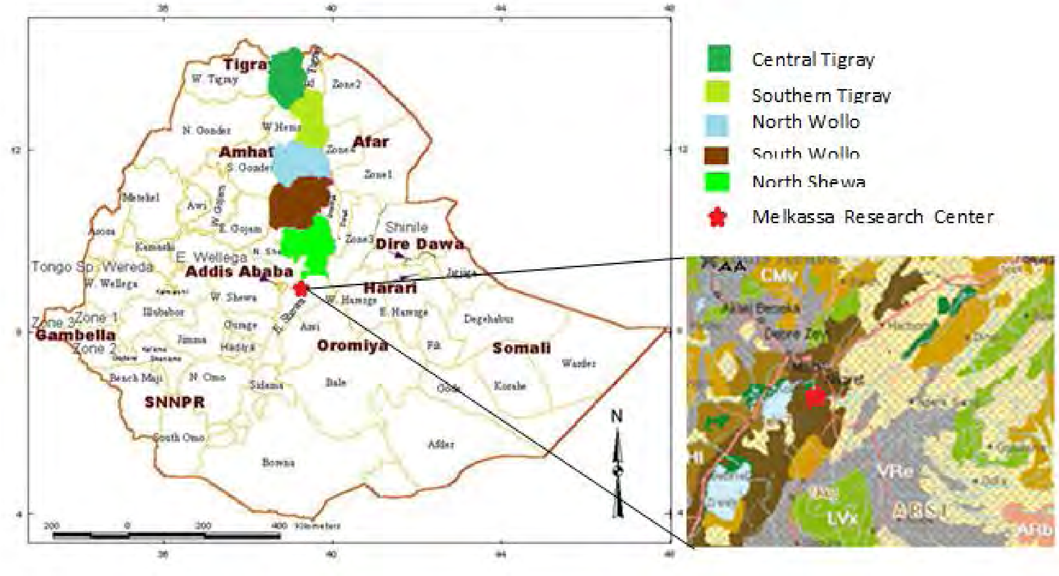

Figure 1 **Site of collection of sorghum landraces and the study site**

3.1.1.2 Weather condition of the experimental site

Weather data were obtained from MRS, Soil and Water Management Division collected from meteorology station located within the study site. The meteorological data recorded for 10 years including the crop growth period (2011) are presented in Figure 2. The main rainy season of the study area falls to the months of June to September. The major annual rainfall was around 800 mm (Figure 2) and with average minimum and maximum temperature of 31.9°C and 10.6°C respectively. The highest evaporative demand occurs during the months of March, April and May. During these months, the mean maximum temperature was around 30°C while the mean relative humidity drops below 50%. The study site is believed to be ideal for evaluating the expression of adaptive traits for terminal moisture-deficit conditions, as the humid period of the year is narrow.

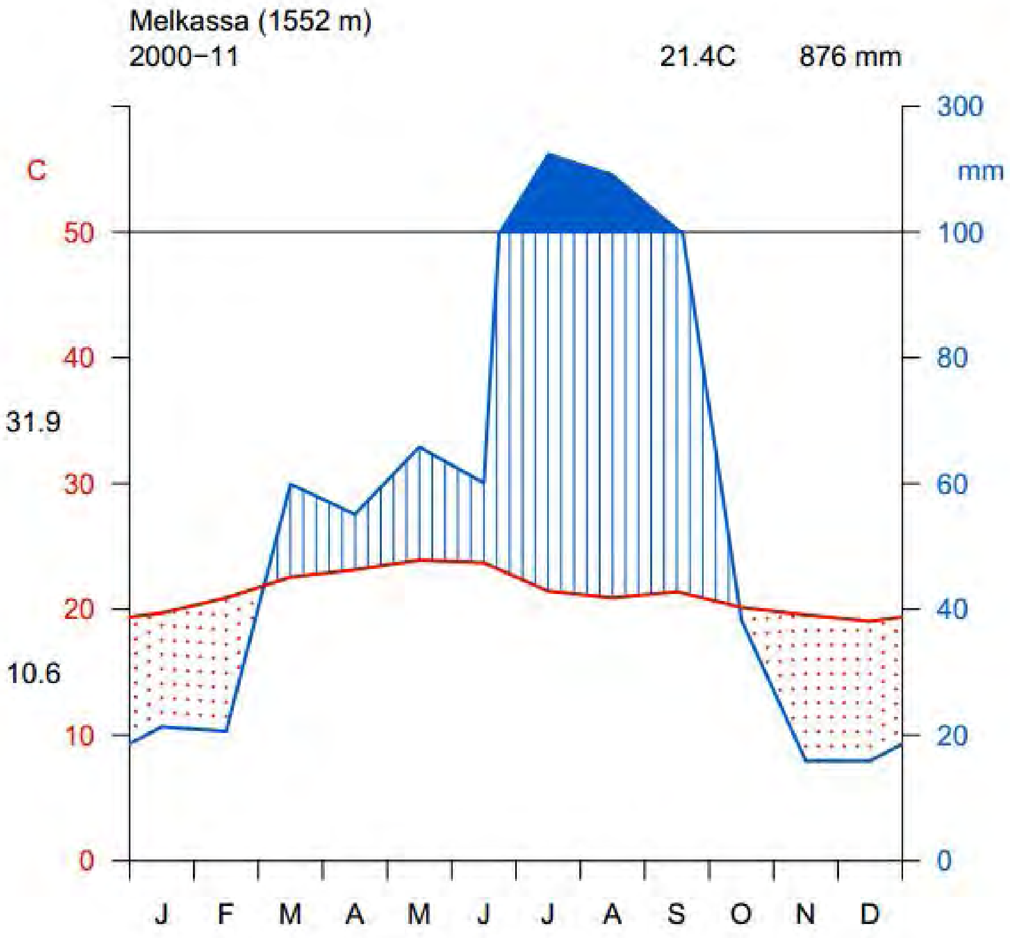

Figure 2 Melkassa Research Station monthly average climate for the years 2000 – 2011

3.1.1.3 Soil property of the experimental site

MRS is characterized by a gently sloping plain with a slope ranging from 0% to 5% comprising a foot slope of the rift valley near Awash River. The dominant soil texture in this area is Andosol, with clay loam texture (Figure 1). To determine the soil fertility and physical properties, soil samples were collected using augur from five sites at two depths i.e. 0–20 and 20–40 cm from the experimental plots before sowing (Piper, 1966; Jackson, 1967). The soil samples from upper surface and lower depth were separately submitted to the laboratory of JIJE Analytical Testing Service Laboratory, Addis Ababa. The soil samples from each depth were made a composite of two (composites from 0–20 and 20–40 cm) and they were analyzed in replica of three for each parameter for both depths. The averages of the three replicas for the physical and chemical characteristics of the soil are presented in Table 1. Melkassa soils are found to be with favorable clay loam texture on upper surface and a loam soil at the relatively lower profile, both with high silt content. The profile has got a hard plate at depth of 1.20m, created a challenge digging down through during access tube insertion and pond formation. The bulk density was determined from three samples at Melkassa soil laboratory to be 1.1g/cm^3.

Table 1 Chemical and Physical properties of soil from the experimental site at Melkassa

S.No.	Properties	Values obtained at depth		Method employed
		0-20 cm	20-40cm	
Chemical properties				
1	Total N (%)	0.15	0.13	Kjeldahi
2	Available P (mg/kg soil)	11.19	5.69	Olsen *et al*., (1954)
3	Available K (Cmol(+)/Kg Soil)	3.02	2.89	Ammonium acetate extraction, flame photometry for determination
4	Organic carbon (%)	1.65	0.87	Walkley-Black
5	pH (1:2.5 suspension)	7.41	7.51	Potentiometric-Water Extract
	Electrical conductivity (1:1 extract)/(dsm)	0.11	0.14	Conductivity Water Extract
Physical properties				
1	Clay proportion	32	27.67	Hydrometer
2	Silt proportion	40.33	38.67	Hydrometer
3	Sand proportion	27.68	33.67	Hydrometer
4	Texture	Clay loam	loam	Hydrometer

3.1.2 Crop management

3.1.2.1 Source of plant materials

The study included landraces SorColl 60/97, SorColl 146/97 SorColl 163/97 SorColl 178/97 SorColl 179/97 selected from collections of drought prone zones of northern Ethiopia, Tigray and Amhara regional states, obtained from Eco-physiology stream, Addis Ababa University. The checks E 36-1 with stay green trait and Abshir (P9403) an early maturing drought resistant were obtained from MRS. Selection for the landraces have been undertaken based on their superiority in the combined traits of Nitrogen content, SPAD reading, senescence rate from the findings of Zelalem Mengiste (2008).

3.1.2.2 Experimental layout

The two experiments were carried out in a split plot design consisting of factorial combinations of seven sorghum genotypes, sown on the plots according to a randomized complete block design. For Experiment I, six water regime treatments or irrigation levels (control, zero, 25%, 55%, 65% and 80% of total available water (TAW)) were imposed before flowering followed by a progressive drought stress during post-flowering except the controls. The six irrigation treatments were applied to main plots and subplots having five different landraces including two cultivars with drought resistant trait before progressive stress. Each main plot had five sub-plots, where the sorghum varieties were allocated along lines or small ridges (Figure 3). The three subplots representing controls were allocated in the second block used in common with the second experiment. Different irrigation frequencies were assigned to five sub-plots in each plot and the former were put in a randomized complete block design. Each main plot had area of 4.5m by 18 m, with a 2.8 m buffer zone between them. The subplots had 2m width by 4.5 m (7 rows) length with 0.75m distance among the rows. Subplots were separated by a 2.8 m roadway adjoining a 4-m cropped buffer zone as described by Borrell *et al.*, (2000). The plots in the experimental blocks were shielded by buffer zone of lately sown sorghum plants to avoid variation among experimental plants as a result of the influence of external condition. Furrow irrigation was used to water these sorghum plants acting as buffer zone, which is the most common irrigation practice in the research center.

Figure 3 **Field experiment layout**

3.1.2.3 Crop husbandry/ Plant growth and treatments/

The experiment was conducted during off season in 2011. Land for the experiment was ploughed by a tractor and the experimental field was brought to a fine uniform soil structure by harrowing and leveled with wooden flat timber before seed planting.

Thereafter, the drip irrigation system was installed based on the lay out with the help of an expertise from *Biruh-Tesfa* irrigation system manufacturing Plc. The seeds were sown on 17th February 2011, forming thin shallow furrows, roughly depth of 5cm, with a finger along the dripper tube dibbling at a distance of 75 cm between the rows as recommended by (Asfaw Adugna, *et al*., 2005; Wilson and Myers, 1954). At the time of dibbling care was taken to place the seeds at uniform depth, roughly 5cm as recommended by Asfaw Adugna *et al*. (2005). A recommended dose of fertilizer at 100 kg /ha^{-1} of N and P_2O_5 was given only at the time of sowing in the form of DAP and supplemented with Urea during harrowing to ensure establishment. All the recommended package of practice as per the research center was applied to protect the crop from pests, other pathogens and weeds. Pests were controlled by spraying pesticides called Endosolphan and Pylotrin with the recommended dose and date interval since early establishment consulting expertise from Crop Protection Division. During hoeing and weeding, covering roots with soil at the base of sorghum plants to avoid uprooting were additional activities. Birds of prey and night gazelles were kept away having guards during both day and night times. In addition, starting from the time of grain filling, panicle of tagged sorghum plants were covered with cotton bags (20cm X 16cm) to secure protection. Rain shower was prevented using plastic rainout shelter that was being regulated manually.

Drip irrigation was applied immediately after sowing i.e. having seeds covered with thin soil layer, which continued every other day. Thinning was done in the first weeding time, 15 days after sowing to retain one seedling per spot with the distance of 20cm i.e. ten sorghum plants in each line, a recommended interval (Asfaw Adugna, *et al*. 2005; Wilson and Myers, 1955). Four weeks after sowing (AS), the sorghum seedling were subjected to deficit irrigation following the irrigation schedule developed to meet the requirement of predetermined moisture levels, the treatment developed (Table 3). Drip irrigation technique was preferred to others because it helps to control the amount of water to apply easier and irrigation efficiency with drip systems ranges from 75-95% compared to 25-50% for surface (furrow); 70-80% solid set sprinklers and 65-75% for portable sprinkler systems (Smajstrla *et al.*, 2002).

The method runs water through plastic tubes that release the flow through small holes directly to crop roots or stems any time from source. The irrigation system had three barrels as source of water installed on wooden stand at 1.5m high from the ground. Appropriate water lifting device, hand pump (Kick Start irrigation pump, Kenya) was used to fill the barrels from a temporary made pond. At the base of the barrels, filters with control valves were attached, which was further extended to the main blind integral pipes with a diameter of 16cm. The blind pipes run away from the barrels along the walk roads between the subplots and were further branched in each subplot to lateral lines finally divided into seven to internal drippers with inbuilt emitters. The internal drippers had double coated emitters 20 cm far apart that discharge 2 gallons (7.57L) per hour each. The extended or branched blind tubes supplying water to the seven 2m long drippers in each subplot had one small control valve with which water flow to each subplot was controlled. When the calculated water was irrigated to a given subplot with an area of 4.5 X 2m, the water flow was blocked using the control valve. Subplots with replica of the same water regime were irrigated and stopped at the same time when the calculated volume of water from the barrels was obtained.

During post-flowering progressive drought stress imposition, the 2m long internal drippers were detached from the lateral blind tube and to permanently withheld water the site of attachment was locked by a cap. Progressive drought stress imposition was done when 50% (5 sorghum plants) of the sorghum plants of a landrace reached 50% flowering stage. Time of imposition of progressive drought stress to sample sorghum plants was different because they had different dates to 50% flowering. The barrels were always filled with water from temporary pond when water is in demand for irrigation. Filters were always cleaned before irrigation or even during irrigation when found necessary and the system were checked for water leakage and blockage of emitters. Flushing method was applied frequently to clean the system.

3.1.3 Management of soil moisture content

In experiment I, sorghum varieties were grown under different water regimes from full to different levels of deficit irrigation below the water requirement of the crop. Both gravimetric and volumetric using neutron probe 503DR- 1.5” Hydroprobe (ICT International Pty Ltd, Australia) were used to measure water content of the soil during the experimentation. The sphere of influence of the neutron-probe instrument would extend into the air above the soil surface (Zermeno-Gonzalez *et al.*, 2012) that may increase errors or/and health hazard to data collector. To minimize this, measurements in the top 15 cm of the soil were determined gravimetrically and converted to volumetric values having the bulk density measured.

Analysis of initial soil water content was done and checked for the uniformity among sub plots. Moreover, the neutron probe reading at the experimental block was checked at dry soil and fully hydrated or saturated soil and the results were in line with that of research station calibrated. The site is coded by the research center as Block (Field) 2. The neutron probe calibration had a regression line equation y= 2.277x+1.201 (R=0.901). Moreover, the research center has known total available water (TAW) with increasing depth determined from many recordings (see Table 3), the last four columns are calculated from TAW value in column three.

Table 2 Research site field capacity and total available water (after MRS, Department of Soil and water) and corresponding deficit levels from TAW.

Depth, mm	FC, mm	PWP, mm	TAW, mm	0% deficit	25% deficit	55% deficit	65% deficit	80% deficit
150			25.29	25.29	18.97	11.38	8.85	5.06
300			22.81	22.81	17.11	10.26	7.98	4.56
450	123.60	72.60	51.00	51.00	38.25	22.95	17.85	10.20
600	185.60	109.10	76.50	76.50	57.38	34.43	26.78	15.30
750	247.20	145.20	102.00	102.00	76.50	45.90	35.70	20.40
900	309.00	181.50	127.50	127.50	*95.63*	57.38	44.63	25.50
1050	370.80	217.80	153.00	153.00	114.75	68.85	53.55	30.60

Irrigation scheduling was produced ahead of time based on levels of water deficit set to be regulated from total available water of the soil (Table 2). Five water deficit levels control or 0%, 25%, 55%, 65% and 80% were developed for experimentation. Control and 0% TAW coded plants received the same until anthesis but after anthesis 0% TAW grown plants were imposed to post-flowering progressive drought stress. The different water regimes were induced by minimizing the time/ amount of irrigation based on the moisture levels set relative to total available water at different depths of the soil. Therefore, the amount of water to be irrigated was increasing in each water regime based on the speculated root depth increments with time because the volume of soil to be irrigated increases correspondingly.

Maintaining preset level of soil moisture for the deficit and to meet the sorghum water requirement growing at full irrigation was achieved by irrigation every other day. The duration of irrigation water application for a given drip system was computed as follows and converted to liters and then to number of barrels to make the management easy:

$$\text{Duration (hr)} = \frac{\text{Water requirement (mm)}}{\text{Application rate (mm hr}^{-1}\text{)}} \qquad [1]$$

3.1.3.1 Gravimetric Method

The soil water content near to the surface with the depth of 15cm was measured by gravimetric method. Soil samples near to the access tubes within the radius of 20cm were repeatedly taken using cans. The cans were immediately covered with plastic bags and wet weight was measured in the laboratory of MRS soil laboratory. After 24 hours exposure to a temperature of 105 ^{0}C, cans were withdrawn from the oven and dry weight was measured, it was always followed by can weight measurement. The soils sampled for measurement were always replaced in the same position in the soil at original site. Dry gravimetric samples collected were weighing more than 65 grams with the sampler (cans with average volume of 30cm^3) and the three replicas recorded were averaged and the percent of water contained in the soil was determined by:

$$H_2O \text{ in } \%_{\,(0\text{-}150\text{mm})} = \frac{\text{(Wet weight - Dry weight of soil)}}{\text{volume of soil}} \quad [2]$$

The bulk density was determined to be 1.1 using the formula:

$$\text{Bulk density} = \frac{\text{Soil mass}}{\text{Volume of soil}} \quad [3]$$

Multiplying Equation [2] by Equation [3] enabled to determine the volumetric content of total available water in the first 15 cm depth.

3.1.3.2 Volumetric Method

For the volumetric measurement of soil moisture below 15cm, a vertical hole with depth of 150cm that closely fits the diameter of the access tubing was drilled in representative subplots using soil auger. The thin-walled 150cm long aluminum access tubes were inserted to the holes from which soil moisture data was collected. The access tubes were inserted in subplots with an alternate fashion and each water regime was represented by two access tubes. They were placed in the plant line one tube under the dripper and another is placed half way between the drippers. The access tubing was left so as to protrude about 10 cm above the soil surface and was covered with a stopper (plastic cup) between readings to keep water and debris out. Measurement was made by lowering the probe with a cable down in the access tube to reach the target depth of the soil profile. Clips with 15 cm interval on the cable allow the cable to be set at pre-selected depths into the soil profile. During measurement, the data collector was shielded from radiation hazard emitted, for about 15 seconds at intervals, by moving at least 100 cm away from the site of access tube inserted. This is because the zone of influence for 5-MeV neutrons is roughly spherical with a radius of about 15 cm in wet soil and 40 cm in dry soil (Zermeno-Gonzalez *et al*., 2012). About three neutron counts were made at each selected depth and results were read immediately from the LCD and recorded manually. Immediately after taking the neutron counts, soil samples were collected to obtain the soil moisture contents. Neutron probe measurements are not directly comparable with the gravimetric water content measured (weight of water in the sample/weight of dried sample), and calibration curve equation developed for the experimental block (Block 2) were used to convert the count ratios (count in soil/count into volumetric water content). The study site was coded by the research center as Block (Field) 2 and had a neutron probe calibration/ a regression line equation:

$$y= 2.277x+1.201 \quad (R=0.901) \qquad [4]$$

Coverage for vertical measurements ranged from 0 cm to 105 cm with measurements made at 7 discrete depths (105, 90, 75, 60, 45, 30 and 15 cm) and calculations for the volume of water to be irrigated was made based on the expected depth of roots at different stages of the sorghum plants. Volumetric soil moisture measurements were made at the following depths and it was undertaken bottom-upside, the starting depth being different at different ages of the plant, based on a new column colonized by roots (new root zone):

Column: Depth (cm):

1 . . . 90-105 (Neutron probe measurement)
2 . . . 75-90 (Neutron probe measurement)
3 . . . 60-75 (Neutron probe measurement)

4 . . . 45-60 (Neutron probe measurement)

5 . . . 30-45 (Neutron probe measurement)

6 . . . 15-30 (Neutron probe measurement)

7 . . . 0-15 (Gravimetric sample converted to volumetric data)

The surface gravimetric samples were collected on the same day as the neutron readings were recorded at early morning before irrigation and the water requirement was determined for irrigation to be applied a day after, for watering was applied every other day and cans with moist soils should also be in the oven for 24 hrs.

Measuring the soil moisture using Neutron probe for depths below 15cm and gravimetric method for the 15 cm depth from the soil surface helped to determine the magnitude of water entry during irrigation. Finally, starting from 50% flowering date for each genotype progressive water stress was imposed by complete irrigation withholding to plants under investigation except for the control, which obtained full irrigation until maturity.

The rain shower of the “*belg*” (short) and “*meher*” (main) rainy seasons have been avoided using rain out shelter made of plastic as they were also negligible (Figure 2). The plastic was manipulated manually using robes to cover and uncover the sorghum plants.

3.1.4 Data collection

3.1.4.1 Morphological evaluation

i. Plant Height

Plant height measurement was taken at pre-flowering stage and the second is at maturity. The plant height was recorded on three tagged plants from each replica, total of nine plants for each landrace and treatment. Measurement was taken from the base of each plant using measuring tap fixed with long stick to keep firmness. For the first experiment, three measurement records were possible for those under booting and flowering, up to the base of the sheath of flag leaf (Culm height), up to the base of the panicle (peduncle height) and up to the tip of the panicle (panicle height).

ii. 3rd leaf area

In our study, third leaf area (3rd LA) has been considered in experiment I to evaluate the response variation to drought stresses among sorghum varieties. To avoid the variability in area from leaf to leaf for all experimental plants 3rd leaf was considered. Area of 3rd leaves from three tagged plants was measured by adopting Stickler’s linear measurement method (Stickler *et al.*, 1961) and expressed in cm^2. Leaf area calculations of many leaves by multiplying length and breadth over estimated by approximately 0.254, hence a correction factor of 0.75 is necessitated to get the average 3rd LA.

3rd LA (cm^2) = Length of the 3rd leaf x Breadth of the 3rd leaf x 0.75………….. [5]

A total of nine third leaves from nine plants, three from each sub plot were measured to get an average 3rd LA for each genotype for each stress level.

3.1.4.2 Biophysical properties

i. Soil Plant Analytical Development (SPAD)

SPAD Reading (SPADR) was taken using SPAD Chlorophyll meter (SPAD 502c; Minolta company ltd., UK) before and after plants were imposed to progressive drought stress. Measurement was taken on three sampling positions on upper side of the 3rd leaf blade, at leaf base, mid way and near the leaf tip (apex). The reading of greenness or relative chlorophyll content of the leaves was taken between 10.00 and 12.00 hours of the day. A mean of 27 reading per treatment was taken from nine tagged plants (three readings from each plant) from three plots (total nine plants) at two weeks intervals for each treatment and sample in the 2011 season. SPAD reading have been recorded at four different times and values from the first and last measurements are presented for experiment I and experiment II had only once.

ii. Leaf senescence rate

The rate of green down as a result of drought was determined by means of visual rating only in Experiment I. The distance within individual plants and between lines was carefully maintained equally in order to maintain the soil moisture at the desired level and secure equal distribution in the soil. Early in crop growth, three from the 10 representative plants were tagged from the center of rows in each subplot. During the study tagged plants were strictly observed every two weeks interval and sorghum plants had no clear difference in senescence rate exposed to different water regimes during pre-flowering and more emphasis was given to the imposition of post-flowering progressive stress. The senesced portion in the leaves was recorded twice in two weeks interval before and soon after grain filling stage because the greenness is vital before grain filling and leaves with greater than 50% yellowed portion from the tip were considered as senesced leaves. Leaf yellowing which seem to be as a result of biotic effect was excluded from the rating. It is also observed that severe drought and age made leaves at the base of the stem to senesce and defoliated, therefore missing leaves from the node at lower regions of the stem were considered together with senesced leaves. Since they had different days of maturity, senescence rate was determined at different times where the early maturing (Abshir and E 36-1) first, the intermediate forms next and late maturing forms last. Finally, senescence rate was determined by proportion of number of leaves senesced in each tagged plant following Borrell *et al.* (2000a).

3.1.4.3 Dry Matter accumulation and partitioning

Three randomly selected plants for each genotype were used to record both fresh and dry matter production in sorghum plants grown under different water deficit levels and progressive drought stress. The biomass was measured by destructive harvest at final maturity stage. The shoot and root parts of the sample plants randomly identified was collected having all components attached from above ground and up rooted by digging down from below ground respectively. Finally, they were separately put in sacks and labeled. The fresh shoot and root components were immediately weighed using a sensitive balance (Sartorius-AG-GOTTINGEN, Germany) in the soil laboratory of MRS and fresh biomass of both parts was recorded. Thereafter, root and shoot samples were dried in an oven at 72°C for 48 hours until constant weight was attained in the soil laboratory at MRS to compare among the landraces. Finally, total biomass produced per plant and root-shoot ratio performance was determined.

3.1.4.4 Root Length Density

Although root observations are generally work-intensive and expensive; they are the major heritable components involved in dehydration avoidance. Soil samples for root measurements were taken from each plot after plants were exposed to progressive stress during harvest. Root was sampled using the coring tube. The core sampler used for sampling had a tube with 2.5 cm diameter and 1.15 m long. The core was graduated into 0.2 m lengths and was hammered manually down with plastic hammer to the target depth during sampling. The sampler had a sharp cutting edge in the inner tube designed in a way to reduce compaction. Any loose plant residue or debris on the soil surface was brushed away from the sampling site before samples were taken. The sampling tube was centered over the base of the shoot and sample was taken at four depths, i.e. at every 0.20m depth up to 0.80m. The samples were carefully removed from the soil cores and placed in labeled plastic bags. The collected samples were soaked and separately washed using a 5% sodium hexametaphosphate $(NaPO_3)_6$ and running tape water was used to wash soil particles away and rinse roots in presence of three different sieves (2mm, 1mm and 0.5mm diameters) below overlapping with wider mesh on top to trap or filter roots out from flowing water. The cleaned root samples were collected in a labeled petri-dish having small amount of distilled water to keep the roots moist and make separation of each root easy during scanning. Root length was measured using a WINRHIZO scanner and the WINRHIZO Pro 2008a software (REGENT Instruments Inc., QC, Canada).

Data collection was preceded by scanning the roots. The cleaned roots were spread out over a transparent water filled tray (2-4mm water depth). The tray with the roots was then placed on a scanner bed and shortly roots were scanned. Scanning was performed with a resolution of 400dpi as recommended in the manual. The digital output images were stored in computer as a TFF file. Images were then analyzed using Whinrhizo software. The values were converted to excel for statistical manipulation to find out the RLD of the replicated samples. Root samples were not divided in to subsamples to minimize overlap because our samples had densities less than one cmcm^{-3}, scanning density range in root research does not exceed 5% discrepancies (Himmelbauer *et al.*, 2004). RLD was calculated as the ratio between root length (cm) and soil volume (cm3). RLD from the first (0–20 cm) and second (20–40 cm) layers were added together and defined as a single 0 to 40 cm layer (RLD$_{0\text{ to }40\text{ cm}}$), while RLD at the deeper layers (third to fifth) were combined to form a single 40 to 80 cm layer (RLD$_{40\text{ to }80\text{ cm}}$). The percentage of RLD in the 40 to 80 cm layer (%RLD$_{40\text{ to }80\text{ cm}}$) was calculated as:

$$\%\,\text{RLD}_{40\text{ to }80\text{ cm}} = \frac{\%\text{RLD}_{40\text{ to }80\text{ cm}}}{\text{RLD}_{40\text{ to }80\text{ cm}} + \text{RLD}_{0\text{ to }40\text{ cm}}}\text{ X }100 \qquad [6]$$

Drought tolerance indices (DTI) were calculated for the sorghum varieties from %RLD$_{40\text{ to }100\text{ cm}}$ values under each stress conditions to that of under well-watered (FC) condition as suggested by (Nautiyal *et al*. 2002), using the relationship as follows:

$$\%\,\text{RLD}_{40\text{ to }80\text{ cm}} = \frac{\text{stress treatment}}{\text{non-stress treatment}}\text{ X }100 \qquad [7]$$

3.1.4.5 Physiological parameters

The field and green house CO_2 assimilation rate (A_N), transpiration rate (E) and carbondioxide concentration within the intercellular space (Ci) was determined using portable infrared gas analyzer (IRGA), Li-cor 6400 (Lincoln, Nabraska, USA). Measurement was taken in the time interval from 8:00 to 12:00AM in three tagged plants in the field and plant pots in the green house for each variety and deficit treatment. During measurement a light intensity of 2000 mol $m^{-2}s^{-1}$ from Li-Cor 6400 light source and 350 ppm CO_2 and a temperature of 25 ^{O}C were set. The Li-cor 6400 chamber had area of 6 cm^2. When samples were clumped by the cuvvet to start measurement, gaskets surrounding the leaf area were always checked for leakage and corrections were made by replacing damaged parts. Humidity in Li-6400 is controlled by two mechanisms, manually by adjusting the incoming air in the leaf chamber, routed through the drier or humidifier, and controlling the flow rate of air through the chamber. CO_2 mixer allows control of the CO_2 concentration, which is the basis of the photosynthetic CO_2 response curves (Flexas *et al.,* 2007).

3.1.4.6 Yield and its attributes

For both experiments, harvesting was done examining the physiological maturity of the seeds of different genotypes. The three representative plants for each genotype and water regime in the first experiment and three representative plants for each short period drought stress were randomly selected and tagged for different yield component evaluation. Panicles from plants grown in each subplot treated the same water regime were separately put in a cotton bag and labeled after measuring the width and length of each panicle. Thereafter, they were sun dried and panicles from the second experiment were oven dried at 40°C. Finally, they were threshed, cleaned. The parameters considered were panicle size (width and length), 1000 seeds weight and grain weight per panicle, seed count per panicle. 1000 seed and grain weight per panicle. These were weighed using a tri-beam balance (ACS System Electronic Scale, China). In Experiment II, unfilled sites per panicle were counted visually and great attention was given to the space and size of the glumes to know whether seed was aborted at early seed set and filling or withered later during maturity.

i. Panicle length

The mean length of panicles in cm was recorded for the length of the panicle from base to the tip for each genotype.

ii. Grain weight per plant

Panicles were threshed completely after sun dried and grains were separated. The mean value of the three panicles was expressed as grain weight per plant (g plant^{-1}).

iii. Thousand grain weight

Thousand grains counted from the seeds collected after threshing the three plants under each water regime was taken for 1000 grain weight. The weight of 1000 grains from each panicle was recorded separately and the average weight was expressed in grams as 1000 seeds weight for each genotype grown in each water regime.

iv. Grain number per panicle

As it was so laborious to count all seeds from panicles of 7 varieties the water regimes, grain number per panicle was calculated from the grain weight per panicle and the corresponding 1000 grain weight as follows:

$$\text{Grain number per panicle} = \frac{\text{Grain weight per plant (g)}}{\text{1000 grain weight (g)}} \quad \text{X 100} \qquad [8]$$

For small panicles from severe water regimes direct count was also done for counter check and it was equivalent to the value calculated.

3.2 Sorghum responses to interrupted drought stress at reproductive stage

3.2.1 Plant Growth and Treatments

Land preparation for experiment II was made in block 2 next to block 1. The lay out as well as crop management for this experiment was the same as field experiment I (Figure 2). All sorghum plants in experiment II at the vegetative stage received full irrigation before booting. The treatment began shortly after booting. It was consisted of factorial combination of seven sorghum varieties and four intermittent irrigation scheme, drought periods, applied at different phases of sorghum reproductive stage except the control plants that received full irrigation throughout. The control plants were used for both experiments I and II.

Sorghum plants were exposed to drought by stopping irrigation for about 10 - 30 days (Table 3) within the days of reproduction. The plants were completely irrigated following the imposed incident drought until maturity in the first three treatments. These were (a) Full irrigation (controls) throughout the plant life up to maturity, (b) imposed when the plants were at boot leaf until panicle emergence (10 days before flowering (DBF), (c) imposed when the plants were at blooming until seed set (Flowering time), (d) applied when the plants were at seed set to seed fill (15 DAF), and (e) applied when plants were within late seed fill to maturity or harvest (45 DAF).

Table 3 Period of drought stress treatment at reproductive stage

Sorghum varieties	Days after sowing and time of withholding water				
	days from planting and withholding of water (10 days)	flowering date	Flowering to seed set (15 days)	Seed set to seed fill (15 days)	Seed fill to maturity (45 days)
Abshir	74	91	100	139	169
E-36-1	60	85	95	135	165
SorColl 60	87	102	112	152	182
SorColl 146	85	101	110	130	160
SorColl 163	88	106	115	145	175
SorColl 178	75	90	100	140	170
SorColl 179	80	95	105	135	165

* Control plants were irrigated continuously.

3.2.2 Data collection

In the Experiment II, special emphasis was given to the response in production attributes. Sorghum varieties were clustered into three based on days to 50% flowering, for strict follow up. The time of booting was the main triggering stage for the start of the events of drought treatments. Three plants with uniform stages excluding those at the margins were tagged in each replication for each treatment and data was collected from these tagged plants. Thereafter, flowering stage, seed set and seed filling and physiological maturity were noted on tagged plants for the other successive drought installments.

3.2.2.1 Morphological evaluation

At maturity, plant height was measured from tagged plants using measuring tape fixed to long stick (see also in project I, methodology section). The tagged plants were harvested for the shoot and root biomass. The fresh weight soon after harvest and dry weight after oven dried at 72°C for 24 hours was collected like the previous experiment. The panicles were oven dried at 40°C then the unfilled reproductive sites were counted with especial emphasis to the space size of glumes. Finally, before the panicles were hand threshed, length and width were measured and the seeds obtained were counted and weighted.

3.2.2.2 Yield and related attributes response

The parameters considered were panicle size (width and length), unfilled sites per panicle, 1000 seeds weight and grain weight per panicle, seed count per panicle. 1000 seed and grain weight perpanicle were weighed using a tribeam balance (ACS System Electronic Scale, China). Panicle length, Grain weight per plant, Thousand grain weight, Grain number per panicle, Grain yield (kg ha^{-1}) were determined like in section I where three sorghum plants were considered for the average value of each drought treatment and genotype. Unfilled sites per panicle were counted visually and great attention was given to the space and size of the glumes to know whether seed was aborted at early seed set and filling or withered later during maturity. As birds of prey were a threat in the study area in reducing yield sorghum plants protected with cotton bags were solely used for yield evaluation.

3.3 Sorghum seedling response to water deficit regimes

3.3.1 Plant growth and treatment

The experiment was a randomized complete block design with triplicate replication having five irrigation levels and eight sorghum varieties. Seeds of the eight sorghum varieties (B 35 was included here) were sterilized once in 70% ethanol for 2-3 min and then rinsed 4-5 times with sterile distilled-water. Surface sterilized seeds were germinated in Petri dish in Ecophysiology laboratory, College of Natural Science, Addis Ababa University.

After a week, five healthy germinated seeds were transplanted into labeled plant pots (10L volume and 25 cm depth, diameter of 40 on top and 20cm at the bottom) filled with soil mixture of clay: sand: humus (2:1:1) in the green house, College of Natural Science, Addis Ababa University. Prior to transplanting five water regimes (100% (control), 25% and 55%, 65% and 80% field capacity) were established by determining the field capacity of the soil. Plant pots were weighed using a digital balance (ACS System Electronic Scale, China) every other day to maintain the water regimes by compensating the lost water. Maintaining the soil moisture level in each plant pot as determined ahead was continued during the experiment and measurement was undertaken between 8:00-10:00 in the morning. Soil moisture status in the plant pots had been checked three times with time intervals using gravimetric soil moisture analysis method both before and after watering. The value of the gravimetric measurements for the five water regimes was 60-50, 40-45, 30-35, 25-30, and 20-25 before watering and 55-65, 45-50, 35-40, 30-35 and 25-30 after watering for the control, 25%, 55% and 65% water deficit regimes, respectively (Figure 4). Transplanted seedlings were tinned to two plants per pot after two weeks, except at the most severe drought stress, 80% field capacity where establishment was impossible.

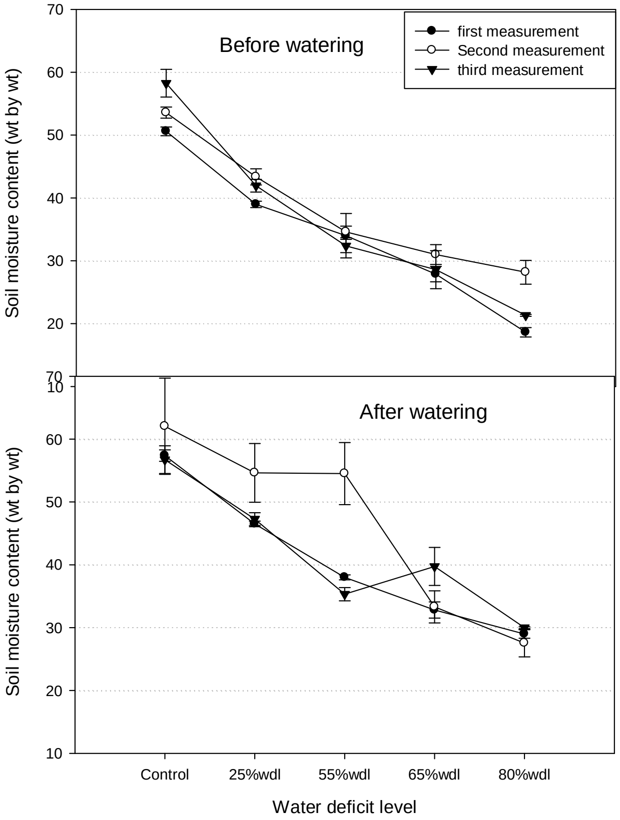

Figure 4 **Three checking soil moisture level measurements before (A) and after (B) irrigation for the corresponding percent field capacity**

Growth and physiological parameters was measured twice. The biochemical assay was done after a month when they could develop leaves enough for photosynthesis and transpiration measurement using the Li-cor 6400. Sorghum seedling leaves, stem and roots were harvested and stored at -25 °C prior to the analysis. The glasshouse had an average maximum and minimum temperature of 30/20 ^{0}C and a relative humidity with 10% to 80% on daily course basis.

3.3.2 Data collection

3.3.2.1 Morphological parameters

Plant height measurement was taken using measuring tape starting from the base of the stem to the tip of the longest leaf. 3rd leaf area was determined from the measure of the widest area and leaf length multiplied by a correction factor of 0.75. The wet and dry biomass of shoot and root of seedlings was determined using digital balance (ACS System Electronic Scale, China). Oven drying of the samples was carried out at 72 ^{0}C for 48 hours until constant weight was attained using an oven (Heraeus, RT 500) at Ecophysiology Laboratory, Addis Ababa University.

3.3.2.2 Physiological Parameters

Measurement of SPAD chlorophyll value was done in the green house using chlorophyll content meter (Apogee CCM-200 Plus, China) between 3:00-5:00 AM. Assimilation rate, transpiration and conductance were measured in the green house using Li-Cor 6400 portable IRGA (Lincoln, Nabraska, USA) similar to that of section I.

3.3.2.3 Biochemical analysis

The fully expanded third leaf, stem and root parts for proline biochemical assay and only leaf part for chlorophyll a and b content analysis have been taken from three sorghum seedling plants for each water deficit stress. A 100mg like in Abraham *et al.*, (2010) of the organs was weighed using sensitive electrical balance (Electrical balance, Adam PGW 253i) and the samples were immediately rolled in aluminum foil and put in ice box after labeling. Finally, the samples were brought and kept in a deep freezer at -25 ^{0}C at Ecophysiology laboratory, Addis Ababa University until experimentation was conducted.

i. Chlorophyll a and b Content Determination

The chlorophyll concentrations were determined following Arnon (1949). The 100 mg leaf were opened from the aluminum foil and ground using liquid nitrogen having samples put in polyethylene bags (to avoid contact) with the help of mortar and pistil. Finally, they were taken out from the polyethylene bags and further ground adding 2 ml ethanol (100%) in Plant Molecular and Biological Laboratory, Addis Ababa University. The solution was transferred into a 2-ml micro-centrifuge tube, from which 200 µl was taken and added into new micro-centrifuge tube with 1mL ethanol. After they were well mixed, the mixture was incubated at 4^{0}C for 1 h. Thereafter, they were centrifuged in micro-centrifuge (MIKRO 20, Germany) at 13,000×*g* for 5 minutes at 4^{0}C. 1 ml supernatant was taken and used to determine chlorophyll content using Ultraviolate-visible spectrophotometer (UV-7804C Model) at λ645 nm and λ663 nm at Bio-medical laboratory, Addis Ababa University. To determine the chlorophyll content, the following calculations has been employed after Arnon's equations:

$$A663 = A663\ \mathrm{ChlA} + A663\ \mathrm{ChlB} \quad [9]$$

$$A645 = A645\ \mathrm{ChlA} + A645\ \mathrm{ChlB} \quad [10]$$

where A is the absorbance; ChlA is chlorophyll A; and ChlB is chlorophyll B.

For each chlorophyll, the absorbance $A = \varepsilon lC$, where ε is the specific absorption coefficient of the chlorophyll considered, l is the light path, 1 cm, and C is the chlorophyll concentration. For chlorophyll A, ε values are 82.04 at 663 nm and 17.75 at

645 nm. For chlorophyll B, ε values are 9.27 at 663 nm and 45.6 at 645 nm (with ε expressed in L/g cm). The relation is simplified as:

$$C\mathrm{a} = 12.7A663 - 2.63A645 \quad [11]$$

$$C\mathrm{b} = 22.9A645 - 4.68A663 \quad [12]$$

where Ca is the chlorophyll A concentration and Cb is the chlorophyll B concentration, both are expressed in mg/l.

ii. Free proline content determination

Proline content was estimated by the method of Bates *et al.* (1973). Like the experiment for chlorophyll content determination, fresh leaf, stem and root was cut from a seedling uprooted from the plant pot were used. The 100mg samples of the leaf, stem and root were unrolled from the aluminum foil and they were snap freeze immersing directly in liquid nitrogen and 3% sulfosalicylic acid (5 µL/mg fresh weight) was added and ground using mortar in Plant Molecular and Biological laboratory, Addis Ababa University. The samples were kept on ice until grinding with all samples of each organ was finished. The samples were centrifuged with maximum speed of 13,000×*g* for 5 min at room temperature using Micro-centrifuge (MIKRO 20, Germany). A reaction mixture: 100 µL of 3% sulfosalicylic acid, 200 µL glacial acetic acid, 200 µL acidic ninhydrin was prepared in separate tubes and a 100 µL from the supernatant of the plant extract was added and incubated at 96 ^{0}C in water bath (Water bath thermostatic oscillator for, XMTD-204) for 60 minutes after mixing the reaction well and the reaction was stopped putting on ice. A 1 ml toluene was added to the reaction mixture and vortexed for 20 s using vortex (Vortex-QILINYONG-5, China). They were left on the bench for 5 minutes to allow the separation of the organic and water phases. The colored supernatant, the chromophore containing toluene, was removed into a fresh tube for absorbance measurement at 520 nm using Ultraviolate-visible spectrophotometer (UV-7804C Model), toluene was used as a reference/ blank. A serial dilution of 0.1, 0.2, 0.3, 0.4 and 0.5 µmol concentrations of pure L-proline bought from market were prepared using electrical balance (Electrical balance, Adam PGW 253i), treated equally like the samples and used for conversion of absorbance values into proline content for a standard curve generation (Figure 5). The proline concentration of the samples was determined using a standard concentration curve equation developed and calculated on fresh weight basis and expressed as microgram per gram FW.

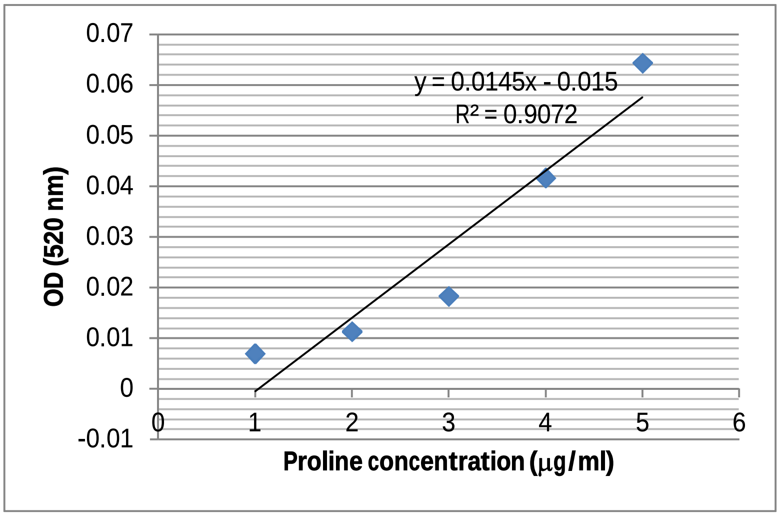

Figure 5 Standard concentration curve for proline

3.4 Data Analysis

The different number of measurements taken for each trait was averaged in to three replica (source three blocks) and was analyzed using descriptive statistics for the percent losses from controls as a result of drought effect for the height, third leaf area, senescence rate (only for experiment I), and presented in graphs using Sigma Plot 11.0, USA. Means were compared using multivariate analysis (MANOVA) using SAS 9.2 and MINITAB 16.2 and the level of significance of all results was tested at P = 0.05, i.e. mean separation was done using LSD tests at 5 per cent probability level in similar statistical package as suggested by Panse and Sukhatme (1967). Correlation analysis was also carried out using both softwares to determine the degree of relationship between morphological, physiological, growth parameters as well as biophysical, biochemical parameters and yield and yield components.

4 RESULTS

4.1 Physiological and morphological characterization of elite sorghum landraces under terminal drought stress

Analysis of variance showed that plant height, SPADR, senescence rate, dry shoot and root biomass, RLD at 61-80cm, photosynthesis, transpiration, total seed weight per panicle, panicle with seed weight seed count per panicle, 1000 seed weight and yield (Kg/ha) were significantly different (P<0.01) in both water deficit levels and among the varieties (see annex 2). RLD at depths 0-20cm, 21-40cm, 41-60cm and 61-80cm did not show significant difference among water deficit levels. Plant height in plants exposed to progressive drought stress, third leaf area in non stressed to progressive stress, senescence rate after grain filling, dry root biomass, assimilation rate, transpiration rate, total seed weight, panicle weight and 1000seed weight traits had significant varietal and water treatment interaction and mean separation values among varieties are presented in tables with the figures.

4.1.1 Plant growth response

4.1.1.1 Plant height

The plant height had been affected by the different moisture stress levels (Figures 6 and 7; Tables 4 and 6). The height of the sorghum plants under treatment decreased with increasing drought stress and they had significant (P<0.01) variation. At first height measurement SorColl 60 and SorColl 146 had higher plant height (above 200cm) over all other varieties in the mild water deficit stress levels followed by SorColl 178. The first two experimental plants were at vegetative stage indicating that they had potential for further growth in culm height and significantly increased at the second measurement (Figure 7). The lowest plant height (about 150 cm) was recorded in Abshir and E 36-1 and they were at reproductive stage in the same water deficit levels. The negative effect increased with increasing water deficit stress as a result of progressive stress and varieties had significant (P<0.01) variation (Table 5).

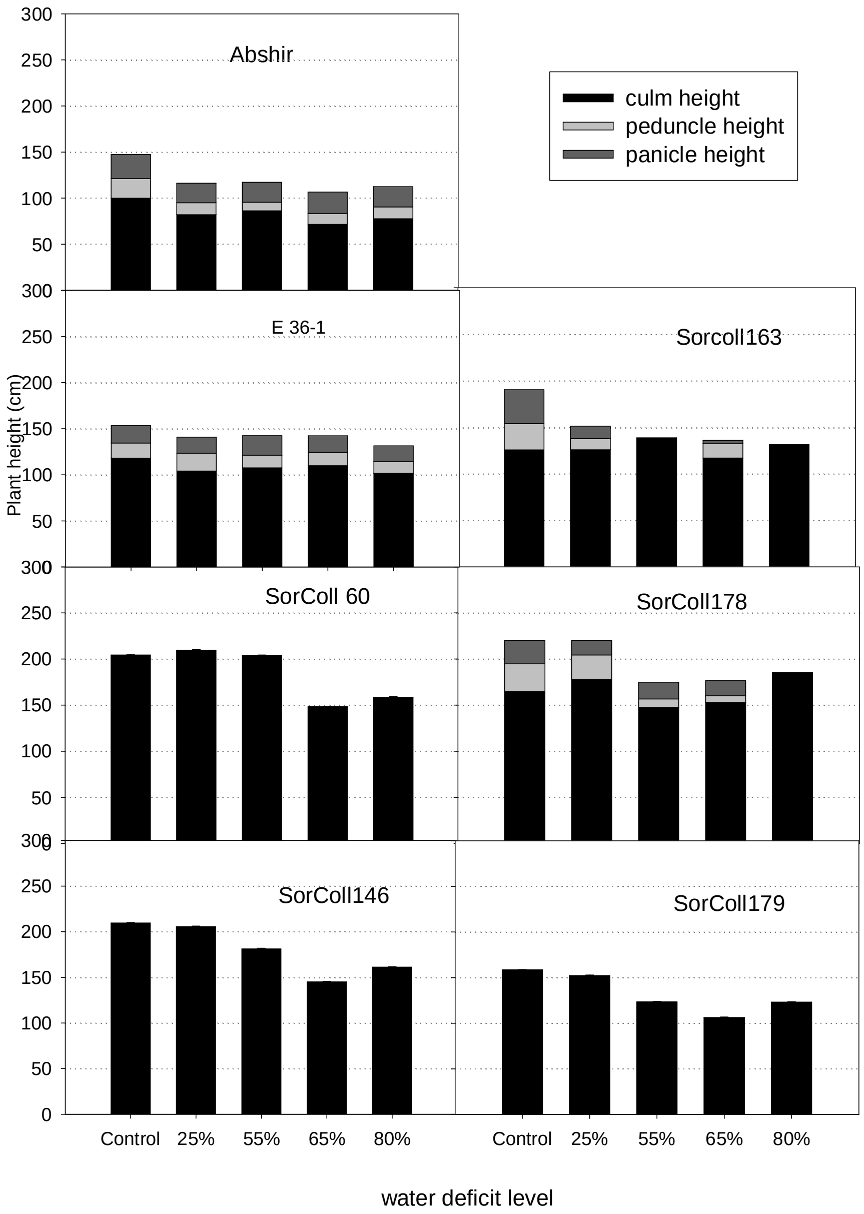

Figure 6 **Plant height measured before progressive drought stress was imposed.**

In most, percent loss in plant height increased with increasing severity of water deficit stress due to withholding water /up on application of progressive water stress/ (Figure 6), where treatment*variety was significant (P<0.01). Plant height was reduced least from controls in E 36-1 (8% at 25% water deficit level (wdl) to 14 % at 80% wdl) followed by SorColl 163 (4% at 0% to 14% at 80% wdl) before exposure to progressive stress. SorColl 179 and SorColl 146 followed by Abshir were relatively highly affected in height over the others in the 65% and 80% wdl. Although sorghum varieties-treatment interaction was insignificant (P=0.12), the last two water regimes reduced plant height significantly (before progressive water stress was applied relative to the control plants.

Table 4 Per cent loss in sorghum plants height at different levels of drought stress

WDL	Abshir	E 36-1	SorColl 60	SorColl 146	SorColl 163	SorColl 178	SorColl 179
0%	1.44	-0.94	0.49	-0.69	4.31	6.01	-2.24
25%	21.07	8.19	-2.55	1.86	12.56	0.26	3.99
55%	20.39	7.11	0.27	13.52	9.88	20.81	22.14
65%	27.63	7.23	27.45	30.75	11.14	20.10	32.94
80%	23.64	14.29	22.50	23.06	14.65	16.00	22.28

Water stress also delayed maturity in all varieties (Figure 6), the variation in different water deficit levels was seen in SorColl 163 and SorColl 178, for they demonstrated heterogeneous date of flowering date.

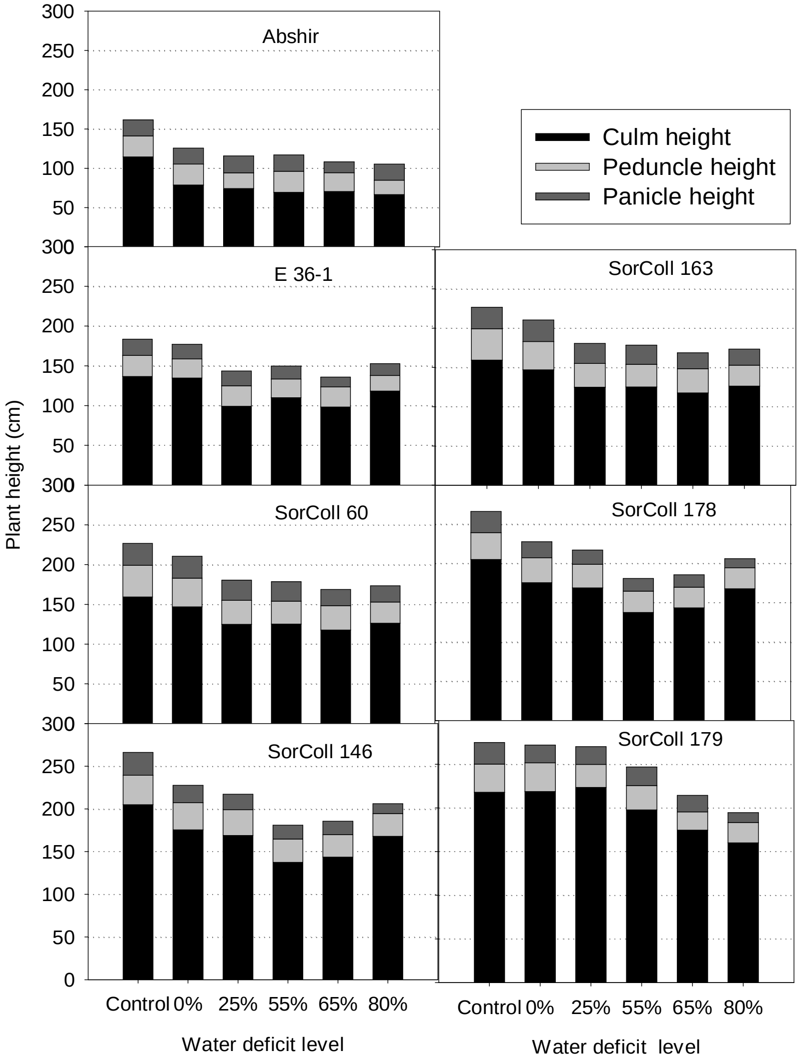

Figure 7 **Plant height measured at physiological maturity.**

In the second height measurement (Figure 7, Table 5 and Table 6) of the sorghum varieties, the effect of drought as a result of both treatments could lead to significant (P<0.01) plant height loss including plants grown at 55% wdl.

Table 5 Mean values of plant height for each variety measured after exposure to progressive drought stress

variety	control	Imposed to pds	25% wdsl	55%wdsl	65%wdsl	80%wdsl
Abshir	161.7fghij	125.7j	115.8j	117j	108.1j	111.7j
E-36	183.6cdefghij	149.7ghij	143.4hij	177.1defghij	135.9ij	139.3hij
SorColl60	270.4a	244.6abcde	251.7abcdefghij	245.1abcde	175.6defghij	186.9bcdefghij
SorColl 146	287.2a	277.6a	259.5abc	238abcdef	243.8abcde	181.7cdefghij
SorColl 163	226.7abcdefg	210.6abcdefghi	180.6cdefghij	158.4ghij	165.8efghij	178.3defghij
SorColl 178	266.2ab	249.6abcd	216abcdefgh	183.9cdefghij	181.6cdefghij	209abcdefghi
SorColl 179	274.9a	223.1abcdefg	185.8abcdefghij	168efghij	141hij	128.6j

Means that shared the same letter are not significantly ($p < 0.05$) different.

Table 6 **Per cent loss in sorghum plants height due to different water deficit regimes plus progressive drought stress**

WDL	Abshir	E 36-1	SorColl 60	SorColl 146	SorColl 163	SorColl 178	SorColl 179
0%	11.30	5.61	5.08	2.02	5.27	14.47	2.14
25%	18.28	3.22	6.55	10.41	10.00	18.43	2.80
55%	17.42-	-11.70	5.48 +	15.08+	10.99+	32.01+	11.20-
65%	23.69	14.30	24.55	17.89	10.84	30.29	22.87
80%	25.65	4.56	31.71	35.66	13.41	22.56	30.01

Plant height loss was not remarkably different from the measurement after application of progressive stress (highest percent loss recorded at 65% wdl is 30% and 35% at 80% wdl), even an improvement in height loss was also seen. E 36-1 and SorColl 163 were least affected after exposure to progressive stress (Table 6). SorColl 163 demonstrated insignificant levels of height reduction among the stressed sorghum landraces. Hence, based on the photosynthate allocation for both cells elongation and expansion, SorColl 163 is least affected landrace by the terminal drought. It is also necessary to emphasize from our result that post-flowering progressive drought stress had little influence on the height of plants relative to the different levels of drought imposed at pre-flowering.

Table 7 **Post-flowering progressive drought stress effect on sorghum varieties (negative values indicate % reduction in culm height relative to control)**

WDL	Abshir	E 36-1	SorColl 60	SorColl 146	SorColl 163	SorColl 178	SorColl 179
0%	-9.86	-6.55	-4.59	-2.71	-0.96	-8.46	-4.38
25%	2.79	4.97	-9.1	-8.55	2.56	-18.17	1.19
55%	2.97	18.81	-5.21	-1.56	-1.11	-11.2	10.94
65%	3.94	-7.07	2.9	12.86	0.3	-10.19	10.07
80%	-2.01	9.73	-9.21	-12.6	1.24	-6.56	-7.73

Progressive water stress imposed after flowering stage affected sorghum varieties differently. Abshir, SorColl 60, E 36-1, SorColl 163and SorColl 179 were relatively negatively affected less than SorColl 178, SorColl 60 and SorColl 146 (Table 6).

All sorghum varieties grown under full irrigation demonstrated remarkable height loss due to progressive water stress with different degrees, which demonstrates the acclimation process must have helped to those exposed to mild drought stress levels. Among the varieties SorColl 163 was less affected, whereas SorColl178, SorColl 60 and SorColl 146 were more affected by the progressive stress at most water deficit levels.

In addition to other traits, probably the drought escaping (Abshir), stay green (E 36-1 and SorColl 163) and large root biomass (SorColl 179) might have contributed in maintaining the height growth under post-flowering drought stress. Among others SorColl 163 avoided reduction in height due to post-flowering drought stress most over other genotypes, depicting a possible continuation of photosynthesis that could help in biomass production satisfying the sink grain filling, which is best indication for the existence of the stay green trait among the experimental sorghum varieties. Pre-flowering drought stress effect was found more pronounced on the vegetative part of the culm height than the other two components followed by the peduncle height.

Panicle height is relatively more resistant to pre-flowering water stress effect over the other parts of the culm (Figure 6). During the post-flowering progressive drought stress reproductive components of the culm (peduncle and panicle) didn’t stop growing.

4.1.1.2 3rd Leaf area

Sorghum varieties included in this study had third leaf area that can be categorized in to three groups, those with the widest SorColl 146 and SorColl 179, medium range Abshir, E 36-1 and SorColl 163 and the third includes E 36-1 and with relatively smallest area SorColl 178 , which are within the range of 500-700cm^2, 500-550 cm^2 and 350-500cm^2, respectively. In our study, it is observed that the varieties responded differently with respect to leaf area at all the measurements taken (Figure 8 and Table 8). Third leaf area tends to decrease insignificantly ($P>0.05$) with increasing water deficit stress in the different water regimes visa vise that of control plants in the first measurement. Third leaf area measured at maturity after progressive stress application demonstrated significant reduction ($P<0.05$), confirming that progressive drought negatively affected leaf area in all landraces (Figure 8 and Table 8).

With increasing drought stress, the third leaf area loss in the sorghum varieties in an order of increasing severity were: E 36-1, SorColl 60, Abshir, SorColl 163, SorColl 146, SorColl 178 and SorColl 179. The check, E 36-1, with stay green trait is found to decrease least relative to other varieties (Figure 8). Sorghum varieties SorColl 60, Abshir followed by SorColl 163 are found to decrease least relative to other varieties (Figure 8). Landraces SorColl 179 and SorColl 178 demonstrated significant reduction in third leaf area with increasing water deficit stress in the different water regimes. The leaf area measurement depicts that all the experimental plants before application of progressive stress had larger leaf area than at maturity stage, indicating progressive water deficit stress caused higher negative impact on leaf area. In general, withholding water later in post-flowering in all the treatments aggravated leaf area reduction except in those with stay green property. Therefore, the early exposure of plants to water deficit stress couldn’t help plants as means of acclimatization for leaf area maintenance but the stay green property might have contributed. However, in most varieties a tendency of increasing leaf area was seen at severe wdsl (Figure 8 and table 8).

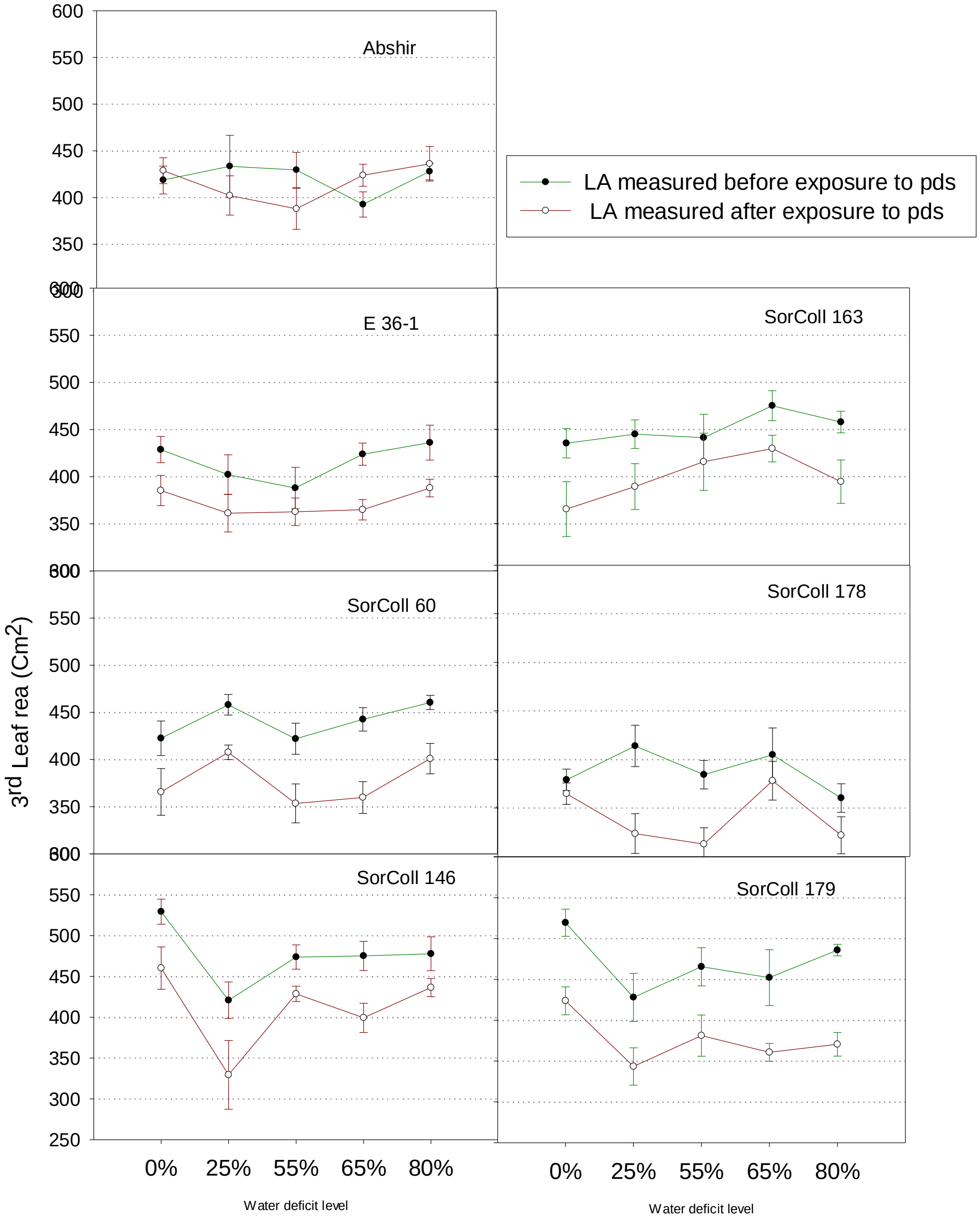

Figure 8 Third leaf area measured before and after (at maturity) progressive stress was applied.

Water deficit at 25% responded differently by plants with larger leaves and medium types in that the former demonstrated a sharp decrease at 25 % followed by gentle slope. On the other hand the medium sized kept a smooth parallel relationship.

Table 8 **Mean values for third leaf area measured after exposure to progressive drought stress**

variety	control	Imposed to pds	25% wdsl	55%wdsl	65%wdsl	80%wdsl
Abshir	525.1abcd	345.8def	362.8bcdef	400.9bcdef	389.3bcdef	321.8ef
E-36	460.5abcdef	415.4bcdef	365bcdef	460.2abcdef	415.5bcdef	473.8abcdef
SorColl60	538.5abc	427.6bcdef	387.9bcdef	329.4ef	429.5bcdef	378bcdef
SorColl 146	512.3abcde	364.5bcdef	365.7bcdef	428.7bcdef	394.4bcdef	393.4bcdef
SorColl 163	553.3ab	381.7bcdef	407.6bcdef	399.2bcdef	364.6bcdef	431.2bcdef
SorColl 178	536.6abcd	385.3bcdef	353.4cdef	436.3abcdef	323.6ef	410.7bcdef
SorColl 179	625.2a	361.4bcdef	359.7cdef	365.3bcdef	312.8f	420.5bcdef

Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.2 Biophysical response

4.1.2.1 Soil Plant Analytical Development Reading (SPADR)

The result from SPAD readings indicated maximum values (50-60) at early measurements in all the varieties both pre and post-flowering drought stresses (Figure 9). The varieties had significant ($P<0.05$) variation in SPAD value (58-55) even at early time of pre-flowering measurement with the relatively least SPAD reading values (54) of SorColl 179. However, the SPAD value lowered (56-54) slightly due to the effect of post-flowering recorded with least value (50) in SorColl 179 and the variety*treatment interaction was not significant ($P>0.05$). Therefore, increasing in drought stress both during pre-flowering and post-flowering decreased the SPAD reading value with gentle slope (Figure 9).

E 36-1, Abshir and SorColl 146 followed by SorColl 163 had higher SPAD reading in most of the measurement times. Especially SorColl 163 revealed minimal SPAD value difference among the value of different measurements at different water stress regimes. Moreover, SorColl 163 and Abshir were found to maintain their SPAD value better than others at the severe drought stresses. On the other hand, SorColl 146 demonstrated a relatively sharp decrease in SPAD value followed by SorColl 178 over the others with increasing drought stress after post-flowering progressive drought stress. Late SPAD measurement of all varieties together underestimated the chlorophyll content of early maturing varieties (Abshir and E 36-1), for they were totally matured earlier.

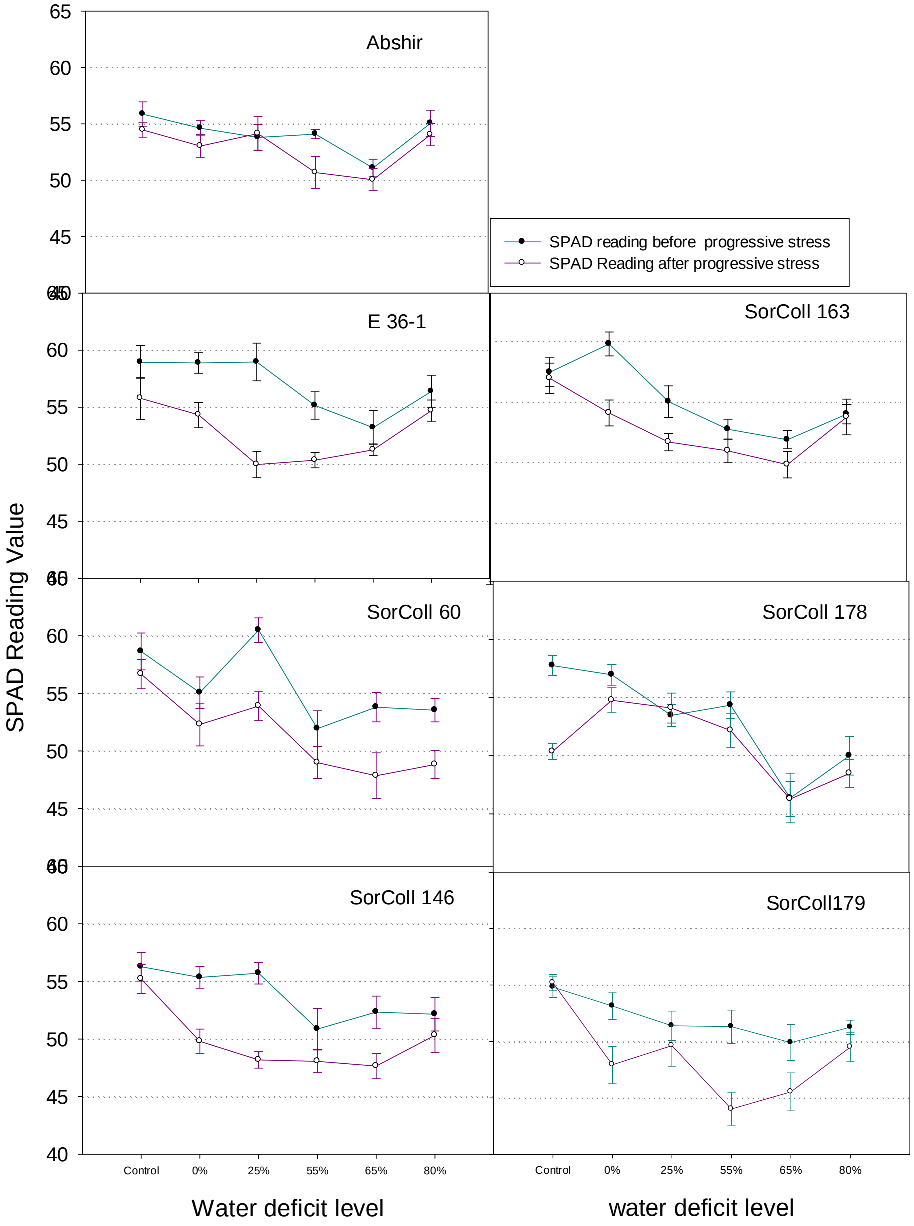

Figure 9 SPAD Chlorophyll reading.

4.1.2.2 Leaf senescence rate

In our study, based on visual rating, the senescence rate was found to increase with increasing both the level of water deficit regimes and time of exposure to the progressive water deficit stress at post-flowering stage (Figure 10). The effect of different (mild to severe) water regimes on varieties imposed before flowering stage resulted significant ($P<0.01$) variation and varieties had in the order of increasing rate of senescence: SorColl 179 (22.2-35.3%), E 36-1 (24.6-35.1%), SorColl 163 (25.2-39.4%) Abshir (26.2-40.1%), SorCol1146 (28.2-39.4%) SorColl 60 (27.7-41.7%) and SorColl 178 (38-46.9%) (Figure 10). However, the pre progressive drought stress effect had insignificant ($P>0.05$) treatment*variety intraction. Moreover, the effect was mild as the result includes the lower leaf defoliation as a result of age, which corresponds to an average of 15-20% of the total green leaf area.

With holding water during post-flowering remarkably aggravated the rate of the senescence in the genotypes and they had significant ($P<0.01$) difference. The measurement of senescence rate soon after grain filling revealed in an increasing order: SorColl 163 (35.6-48.5%), SorCol1146 (48.4-51.5%), E 36-1 (45.9-57.0%), SorColl 179 (47.05-50%), Abshir (50.3 -65.3%), SorColl 60 (53.8 65.1%) and SorColl 178 (51.7-74.8%) (Figure 10 and Table 9). Generally, having significant ($P<0.01$) treatment*variety interaction, the varieties were highly resistant (with senescence proportion less than 65% except in case of SorColl 178 with (74.8%) to senescence rate even to the progressive drought effect imposed at post-flowering stage. SorColl 163 maintained greenness even better than the check, E 36-1 followed by SorColl 179 and SorColl 146 under exposure to both pre and post-flowering drought stresses. Whereas, the varieties SorColl 60 and SorColl 178 demonstrated relatively highest rate of senescence both before and after flowering stage drought stresses.

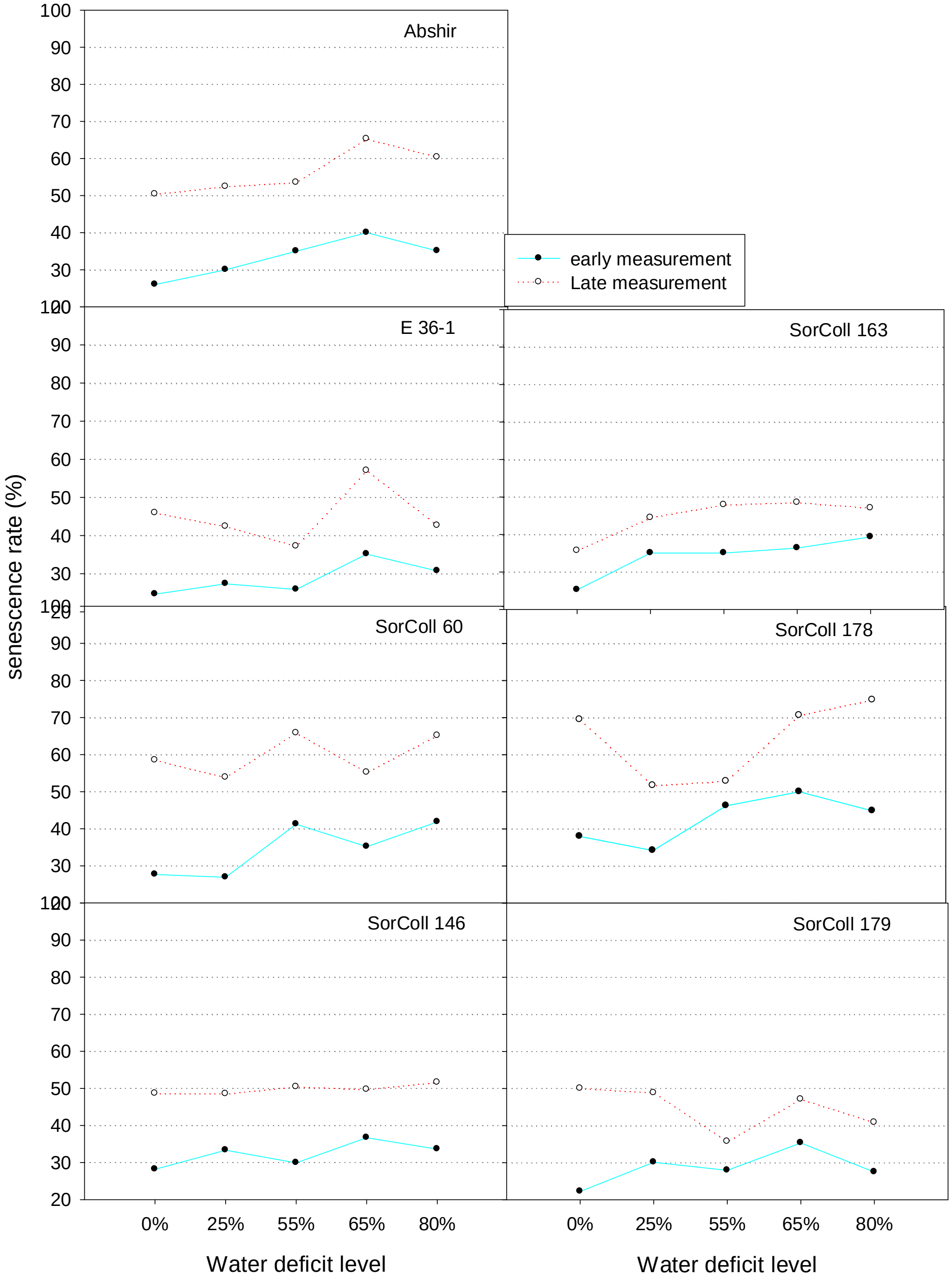

Figure 10 Senescence rate measured before and after progressive drought stress imposed.

Table 9 Senescence rate of sorghum varieties evaluated after grain filling

variety	control	Imposed to pds	25% wdsl	55%wdsl	65%wdsl	80%wdsl
Abshir	33.3efghij	50.5abcdef	52.4abcdef	53.5abcdef	65.3abcd	60.3abcde
E-36	28.9fghij	46bcdefgh	42.3cdefghij	37.2efghij	57abcde	42.4cdefghij
SorColl60	20.9ghij	58.5abcdeabcde	53.9abcdef	65.8abcd	55.2abcdef	65.1abcd
SorColl 146	19.2hij	48.5abcdef	48.5abcdef	50.4abcdef	49.6abcdef	51.6abcdef
SorColl 163	18.2ij	35.7efghij	44.3bcdefghi	47.9abcdefg	48.5abcdef	47.1bcdefg
SorColl 178	15.6j	69.4abc	51.7abcdef	52.8abcdef	70.6ab	74.8a
SorColl 179	15.3j	50abcdef	48.9abcdef	35.7efghij	47.1bcdefg	40.8defghij

Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.3 Dry Matter accumulation and partitioning

Three randomly selected plants for each genotype were used to record both fresh and dry matter production under different water deficit levels and subsequent progressive drought stress. The weight of the samples was recorded separately at harvest. The sample plants were separated into shoot and roots and dried in an oven at 72°C for 48 hours by checking until constant weight was attained. Total biomass produced per plant was determined summing up both shoot and root biomass.

4.1.3.1 Total biomass production per plant

At full irrigation, maximum total weight plant^{-1} (4720.23 gm wet and 1679.37 gm dry) was recorded in SorColl 179 followed by SorColl 60 (2603.07gam wet weight and 1327.80gm dry weight plant^{-1}) (Figure 11). The lowest total biomass was recorded in Abshir (779.30 gm wet and 470.31 gm dry plant^{-1}) at the same water regime. However, the varieties demonstrated a significant ($P<0.01$) biomass weight loss with increasing water deficit levels. At 80% water deficit level, the maximum per cent weight loss from the controls was demonstrated in SorColl 179 (60%) followed by early maturing (Abshir and E 36-1) and SorColl 146 had the lowest percent weight (36%) loss as compared to other varieties (Figure 11). At relatively mild water deficit stresses (25% and 55%), SorColl 163 and SorColl 146 recorded the least and stable percent weight loss.

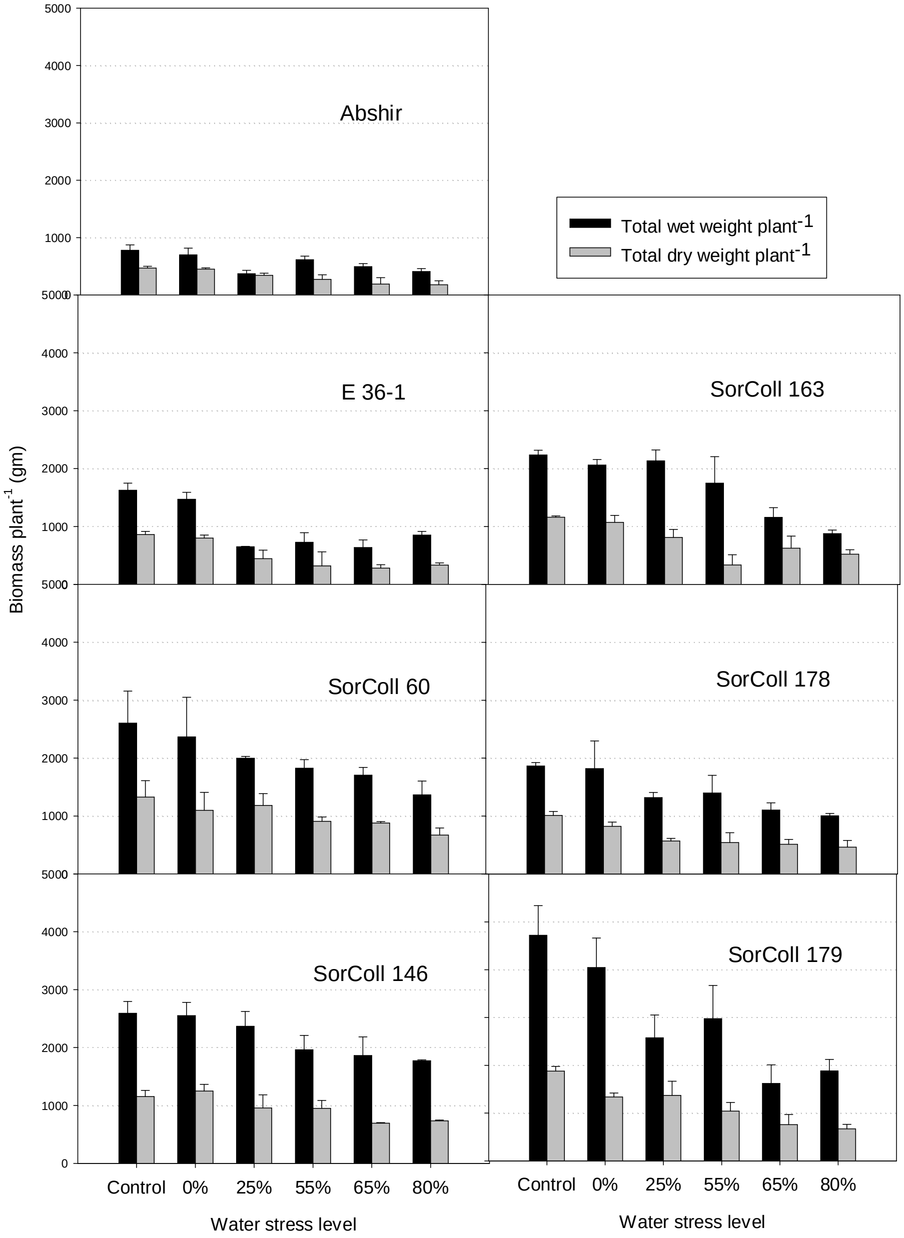

Figure 11 Total wet and dry shoot biomass production of sorghum plants grown at different water regimes.

4.1.1.1 Shoot biomass

At non stress condition, Abshir and E 36-1 had the least shoot biomass followed by SorColl 178, whereas SorColl 179, SorColl 146, SorColl 60 followed by SorColl 163 had highest shoot biomass (Figure 12). All the sorghum varieties demonstrated significant (P<0.01) shoot weight loss as a result of the commutative effect of both water deficit stress treatments, however the interaction with treatment was insignificant (P>0.05). The highest (greater than 50%) loss was recorded in E 36-1, SorColl 163 and SorColl 178 at the last three water deficit regimes (Figure 12). The sorghum varieties exposed only to progressive water stress were relatively with higher shoot biomass than others grown under deficit irrigation during pre-flowering stage.

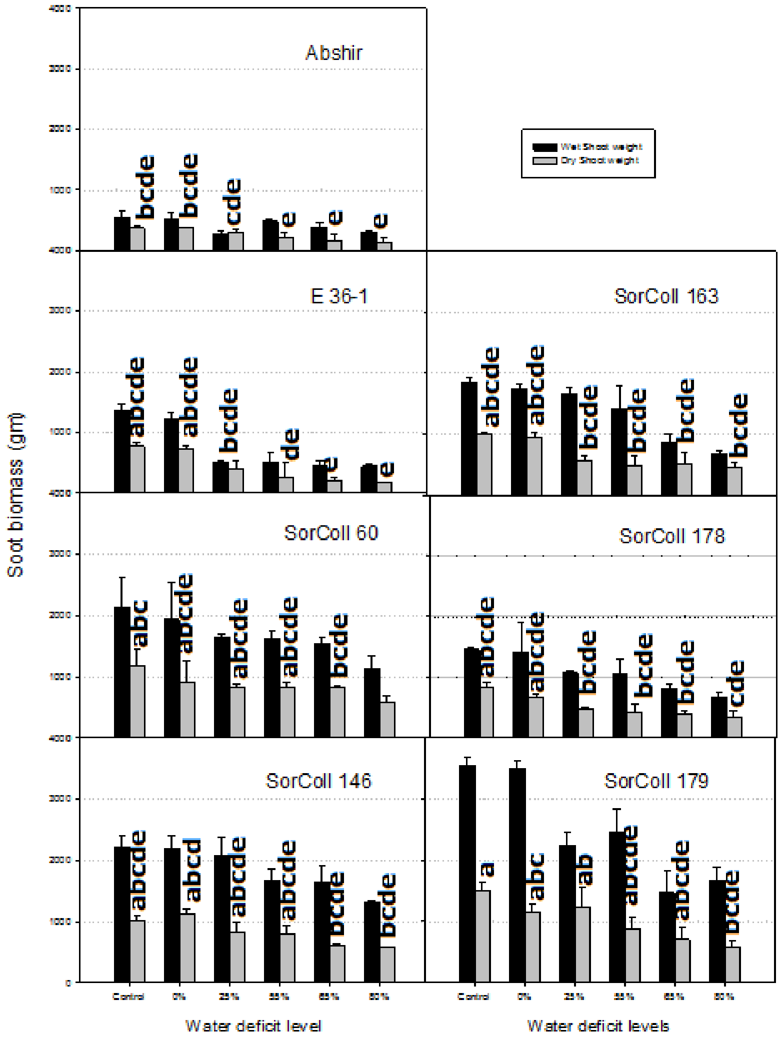

Figure 12 **Wet and dry shoot biomass production of sorghum plants grown at different water regimes. Means that shared the same letter are not significantly ($p < 0.05$) different.**

4.1.1.2 Root biomass

Highest root biomass was recorded in SorColl 179 at the mild stress levels and Abshir and E 36-1 demonstrated least root biomass value at control condition. In our study, sorghum is found to involve partitioning of dry matter among organs in combating drought (Figure 13). Significant difference ($P<0.01$) in dry root biomass allocation was recorded at different water deficit levels. Root biomass of E 36-1 increased at severe drought stress levels. Root biomass of SorColl 163 increased at mild stress level but decreased at the sever water deficit levels, 65% and 80% (Figure 13 and Table 10). SorColl 178 and Abshir had relatively stable root biomass in all water regimes. In case of the root biomass of SorColl 60 and SorColl 179, it went diminishing with increasing water deficit levels. SorColl 146 demonstrated a decrease in wet biomass and almost stable in dry root biomass with increasing water deficit stress. Highest difference dry and wet root biomass was demonstrated in early treatments and SorColl 179 had largest difference in dry and wet root biomass.

Table 10 **Mean dry root biomass measured during harvest**

variety	control	Imposed to pds	25% wdsl	55%wdsl	65%wdsl	80%wdsl
Abshir	96.5c	68.3c	34.4c	47c	41.3c	43c
E-36	91.1c	84.1c	55c	60.2c	75.9c	161.5abc
SorColl60	158.6abc	185abc	367.7ab	91.1c	60.9c	94.8c
SorColl 146	148.4abc	131.4bc	135.4abc	141.6abc	82.1c	152.5abc
SorColl 163	156.3abc	129.3bc	232.9abc	141.4abc	121.3c	71.2c
SorColl 178	167.5abc	147.2abc	96.6c	131.4bc	127.9bc	125.2bc
SorColl 179	374.7a	179.3abc	132.7abc	164.5abc	54.1c	87.8c

Means that shared the same letter are not significantly ($p < 0.05$) different.

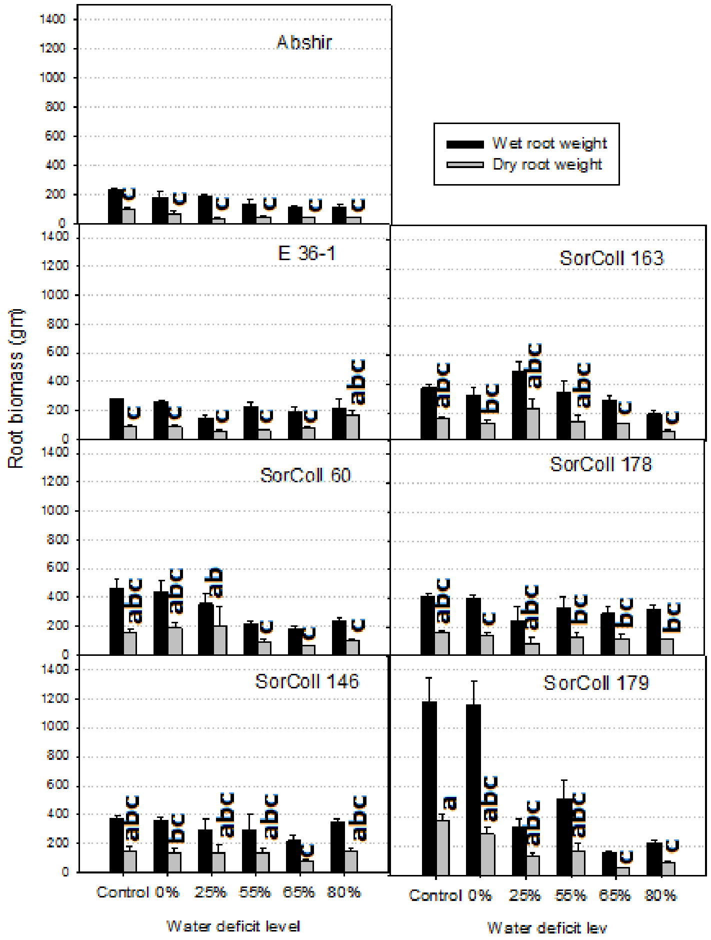

Figure 13 **Wet and dry root biomass production of sorghum plants grown at different water regimes. Means that shared the same letter are not significantly ($p < 0.05$) different.**

4.1.1.3 Root-Shoot Biomass ratio

In our field study, drought stress effect was responded differently by the two systems as well as sorghum varieties at different stress levels. Shoot system was relatively more affected than root system (Figure 12 and 13). The changes in root to shoot dry mass ratio (R/S) is one of the mechanisms involved in the adaptation of plants to drought (Turner, 1997) and used as a means of evaluation for plant species or variety adaptation to drought. Sorghum varieties response in root to shoot ratio to drought increased with increasing water deficit levels except SorColl 179. Varieties E 36-1, SorColl 178 and SorColl 163 had increased in root-shoot biomass ratio better than others indicating relatively a higher dry mater partitioning to roots than shoots in response to the water deficit stress (Figure 14). The genotype SorColl 179 with relatively highest root biomass responded least in dry mater partitioning towards root in response to the water deficit regimes (Figure 14).

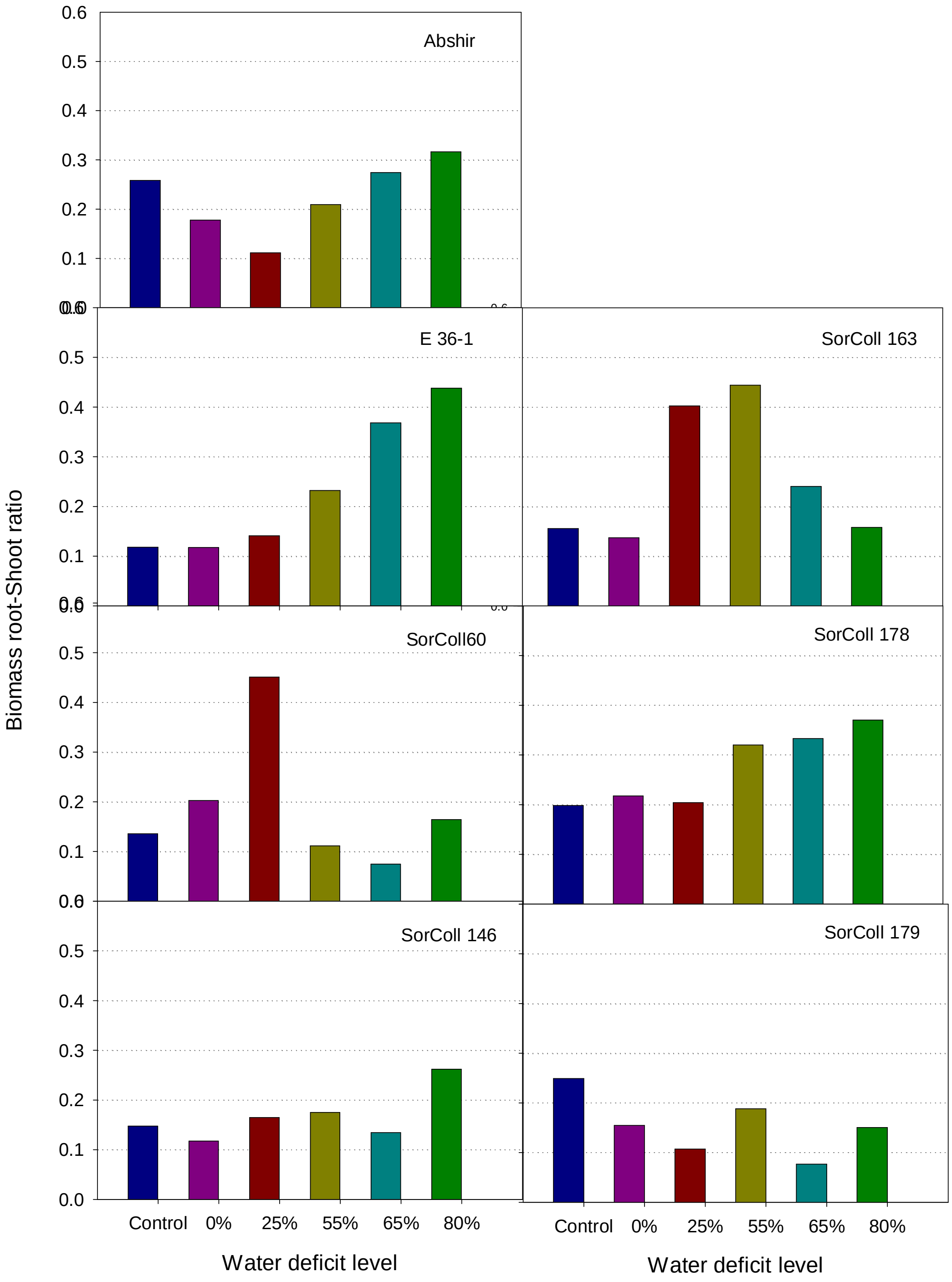

Figure 14 Root-shoot biomass partitioning of sorghum plants grown at different water regimes.

4.1.1.4 Root Length Density

Like in the root biomass, the sorghum plants demonstrated different RLD values and distribution under non stress condition. Total RLD value in cm/cm^3 of the varieties in an increasing order was revealed as E 36-1 (1.25), SorColl 163 (1.31), Abshir (1.36), SorColl 178 (1.38), SorColl 146 (1.48), SorColl 60 (1.83) and SorColl 179 (2.47) (Figure 15). In all sorghum varieties under non stress condition /full irrigation/, RLD distribution to depths was greater than 80cm and skewed (greater than 50%) to the first two upper soil depths, 0-40cm in all genotypes. The highest RLD proportion at 0-41cm depth was read in Abshir (71.9%) and SorColl 178 (71.3%) and the genotype with almost uniform distribution was SorColl 179 (55.4%) (Figure 15). RLD distribution as a result of terminal drought stress was different among varieties grown in different water regimes at all depths except at the first (0-20cm).

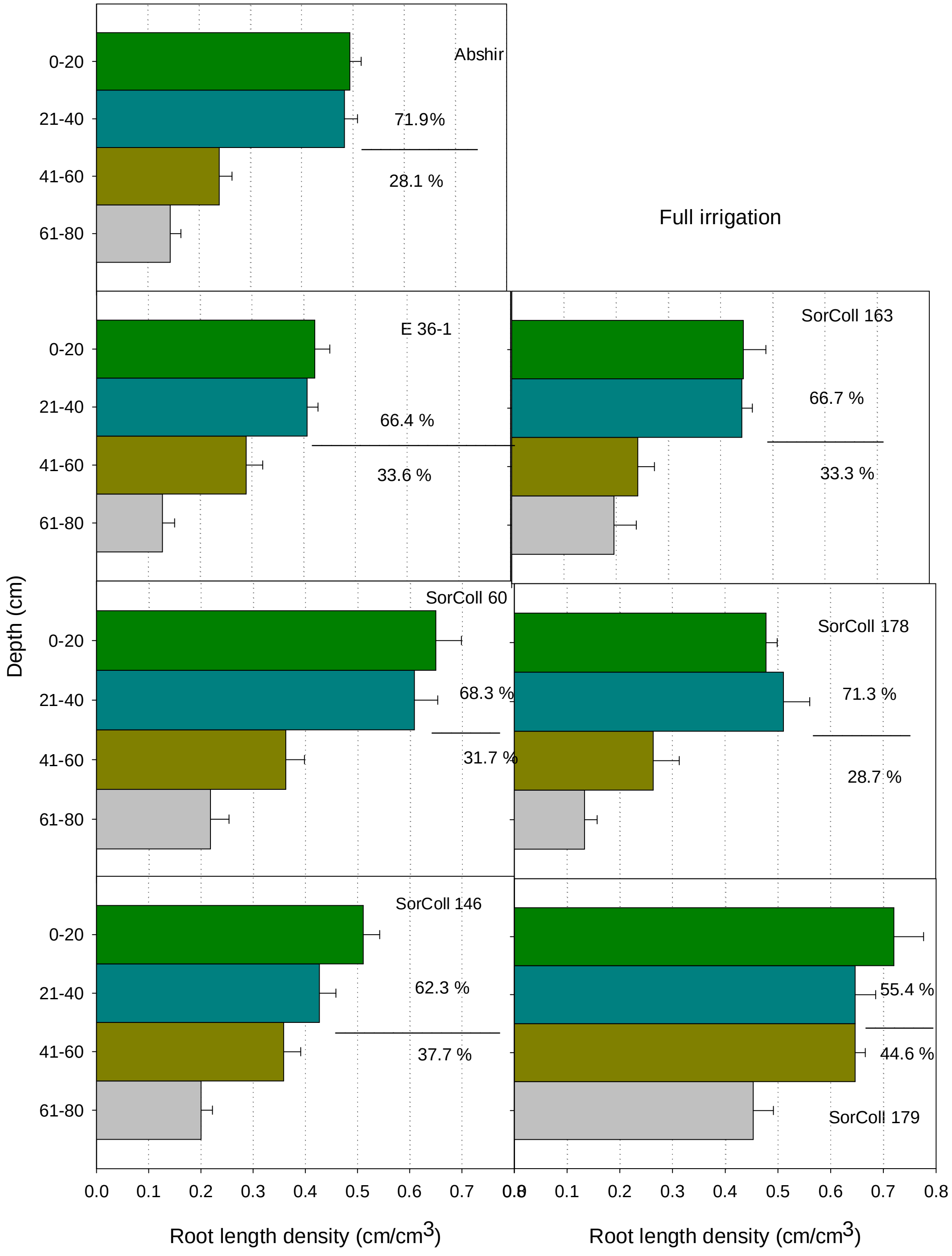

Figure 15 **Root length density of sorghum varieties grown at full irrigation/control/. Data were the mean of three replicates with S.E shown by vertical bars.**

The total RLD value increased in all sorghum varieties except SorColl 179 (2.37) as a result of exposure only to progressive drought stress (0% deficit during pre-flowering). The highest RLD value was recorded by SorColl 179 followed by SorColl 60 (1.98) and SorColl 178 (1.62) (Figure 16). The highest total RLD increase was recorded by SorColl 163 (1.57). The distribution of the roots in the two major depths remained with higher proportion in the upper irrespective of the RLD increase in the lower depth (41-80$_{cm}$) and a decrease in the upper depth due to the progressive drought stress imposed (Figure 16). SorColl 60, SorColl 163 and SorColl 178 had developed almost similar RLD values in all the four depths whereas the other four have got a deceasing RLD value with increasing depth (Figure 16).

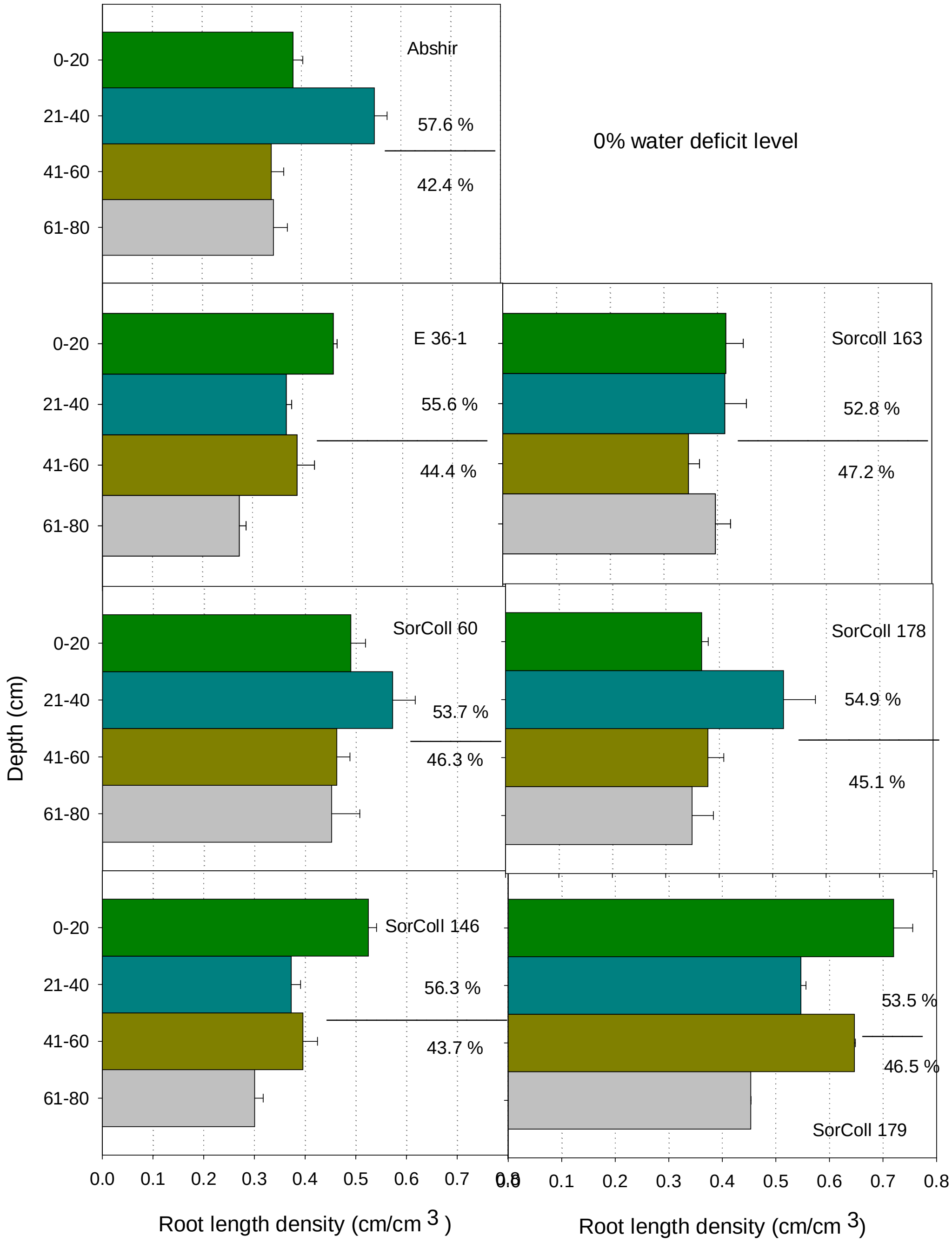

Figure 16 Root length density of sorghum varieties grown at full irrigation and finally imposed to post-flowering progressive drought.

Data were the mean of three replicates with S.E shown by vertical bars.

In line with those stressed only during post flowering, total RLD value increased in the sorghum varieties grown under 25% deficit irrigation except SorColl 179 (Figure 17). However, the increase from plants in 25% was revealed only in Abshir, SorColl 60 and SorColl 178 (Figure 17). The distribution in RLD between the two major depth categories (above and below 40cm) was continued with higher proportion in the upper 40 cm soil depth except in SorColl 146 with 51 % at lower depth.

When irrigation was diminished by 25% total available water in the soil, SorColl 163 responded with increase in RLD in the first three depths and slightly decreased at the last depth (61-80). Conversely, E 36-1 responded with increment in RLD in the lower depth except 41-60cm and the surface RLD didn’t show any change. SorColl 146 increased in RLD at the lower three depths but a slight decrease on the surface. On the other hand, SorColl 178 showed a slight increase on the surface but little response at the lower depth (41-80cm). Like SorColl 146, Abshir and SorColl 60 responded negatively in the depth 0-40, like SorColl 178 they had little reaction in RLD at the lower depth, 41-80cm. The SorColl 179 responded to 25% water deficit stress with significant diminishing RLD value.

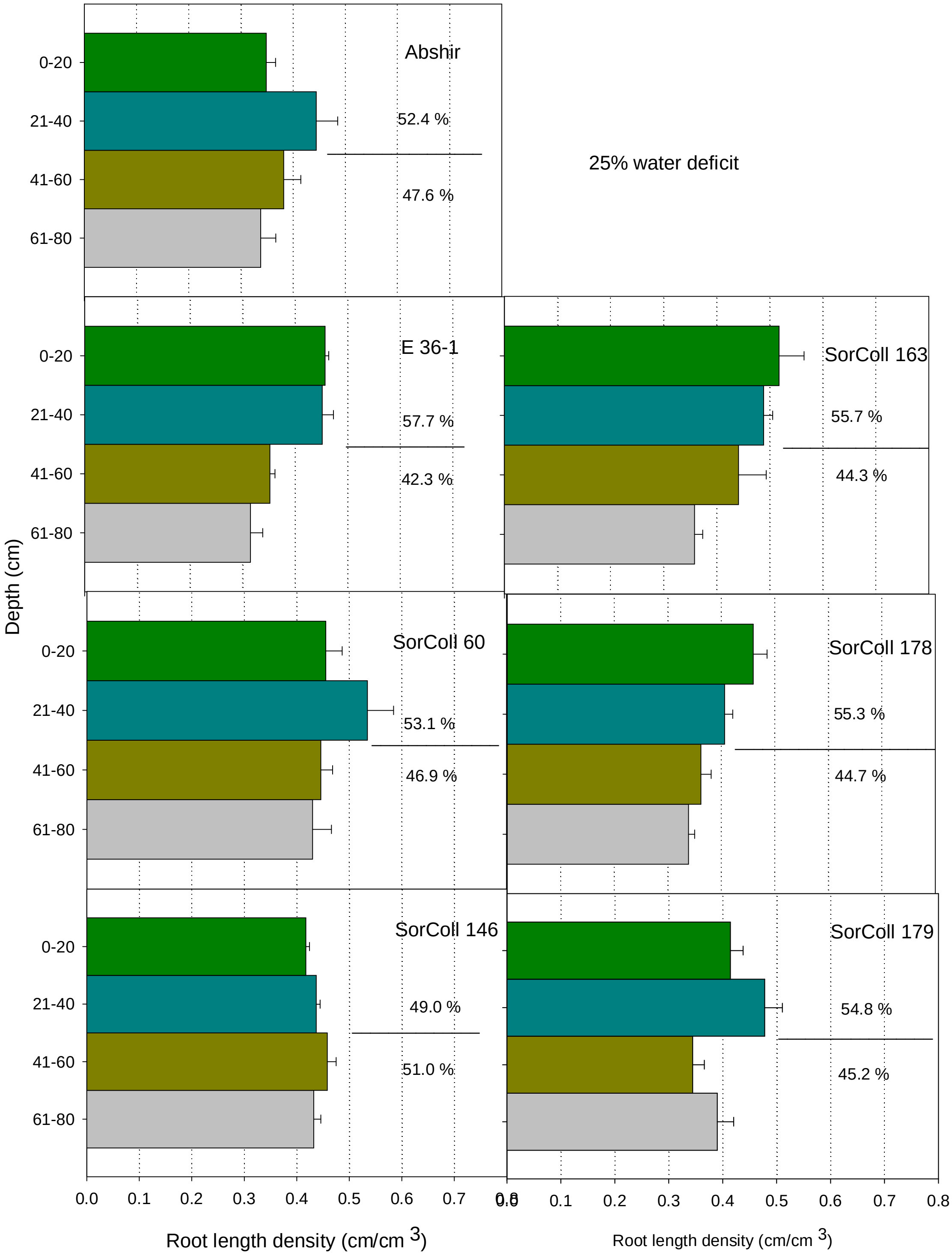

Figure 17 **Root length density of sorghum varieties grown at 25% water deficit condition. Data were the mean of three replicates with S.E shown by vertical bars.**

Total RLD value was found increasing in the varieties grown at 55% water deficit level in comparison to control plants except SorColl 179 (1.74) and SorColl 60 (1.54) with lower values than control plants (Figure 18) but SorColl 60 had more RLD (53.3 %) at the lower depth. E 36-1 and SorColl 146 depicted positive response in the three lower depths (21-80 cm) with slight decrease at the surface.

SorColl 163 and SorColl 178 increased at 41-60 cm while the former remained unchanged at 41-60cm, the later decreased in RLD at depth 0-41cm.

Abshir remained unchanged at depth 41-80 and positive response was recorded in RLD at 0-20cm and negative response at depth 21-40cm.

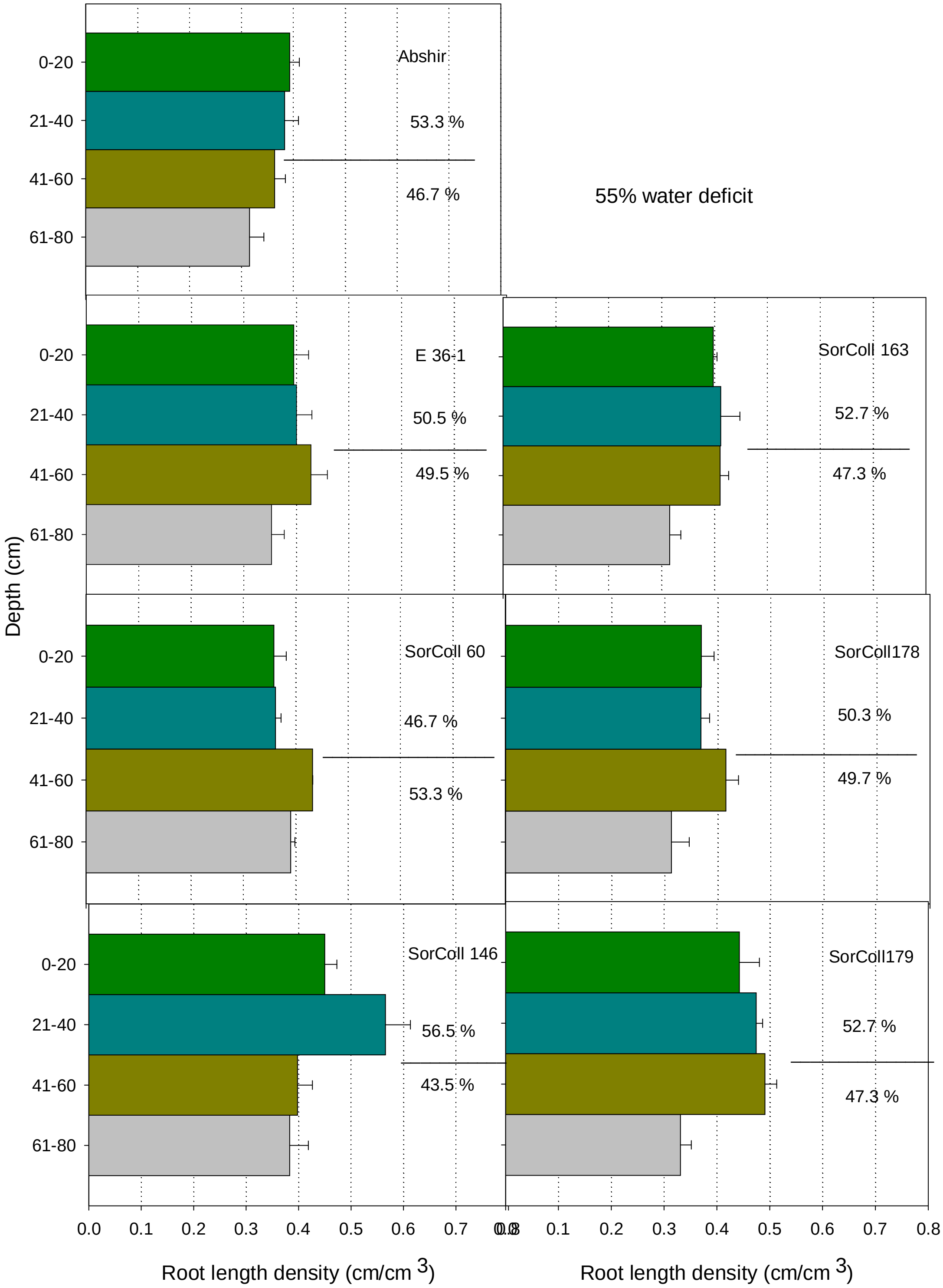

Figure 18 **Root length density of sorghum varieties grown at 55% water deficit condition. Data are the mean of three replicates with S.E shown by vertical bars.**

Total RLD value in the sorghum varieties grown under 65% deficit irrigation before flowering was higher than control plants except SorColl 179 (Figure 19). However, the increase in RLD in this water regime was less than plants in 0% and 25% water deficit levels.

The distribution of RLD between the two major depths was continued with higher proportion in the upper 40 cm soil depth except in SorColl 146 (50.7 %) and SorColl 178 (50.4 %) with higher at lower depth.

In the plots irrigated with 65% water deficit, SorColl 163, Abshir and SorColl 60 positively responded in RLD value, but they had lower RLD value than plants at 0%. RLD value in E 36-1 and SorColl 146 decreased at 65% water deficit condition except at depth 21-40 and 61-80, respectively.

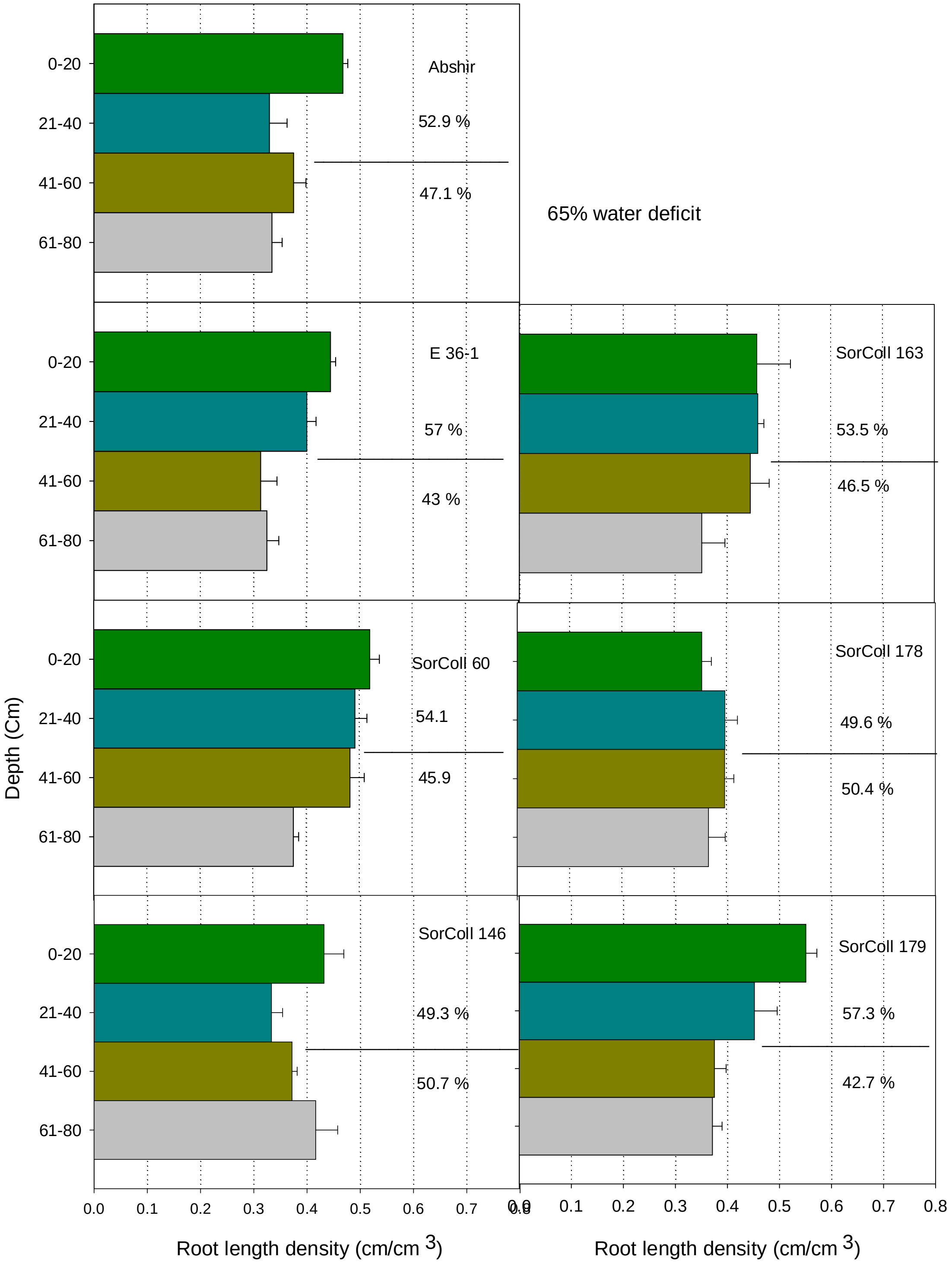

Figure 19 **Root length density of sorghum varieties grown at 65% water deficit condition. Data were the mean of three replicates with S.E shown by vertical bars.**

In sorghum plants grown at 80% during pre-flowering, progressive drought stress after anthesis resulted in total RLD value increase except SorColl 146 and SorColl 179 in relation to the plants from non stressed condition (Figure 20). The increase in RLD of the varieties other than the two was greater than that of plants in 55% and 65% water deficit levels. RLD value of E 36-1 and SorColl 146 grown under most severe water regime were severely affected by the progressive stress over others. Abshir (52.5 %) and SorColl 60 (50.4 %) attained higher RLD proportions at the lower depths but others had below 50% RLD but with improvement. The lower depths RLD per cent of SorColl 178 (48.6 %) SorColl 146 (47 %) and E 36-1(46.6 %) improved next to Abshir and SorColl 60 at 80% water deficit condition. SorColl 146 and SorColl 163 responded to 80% water deficit level positively at the lowest depth, 61-80cm relative to that of control plants.

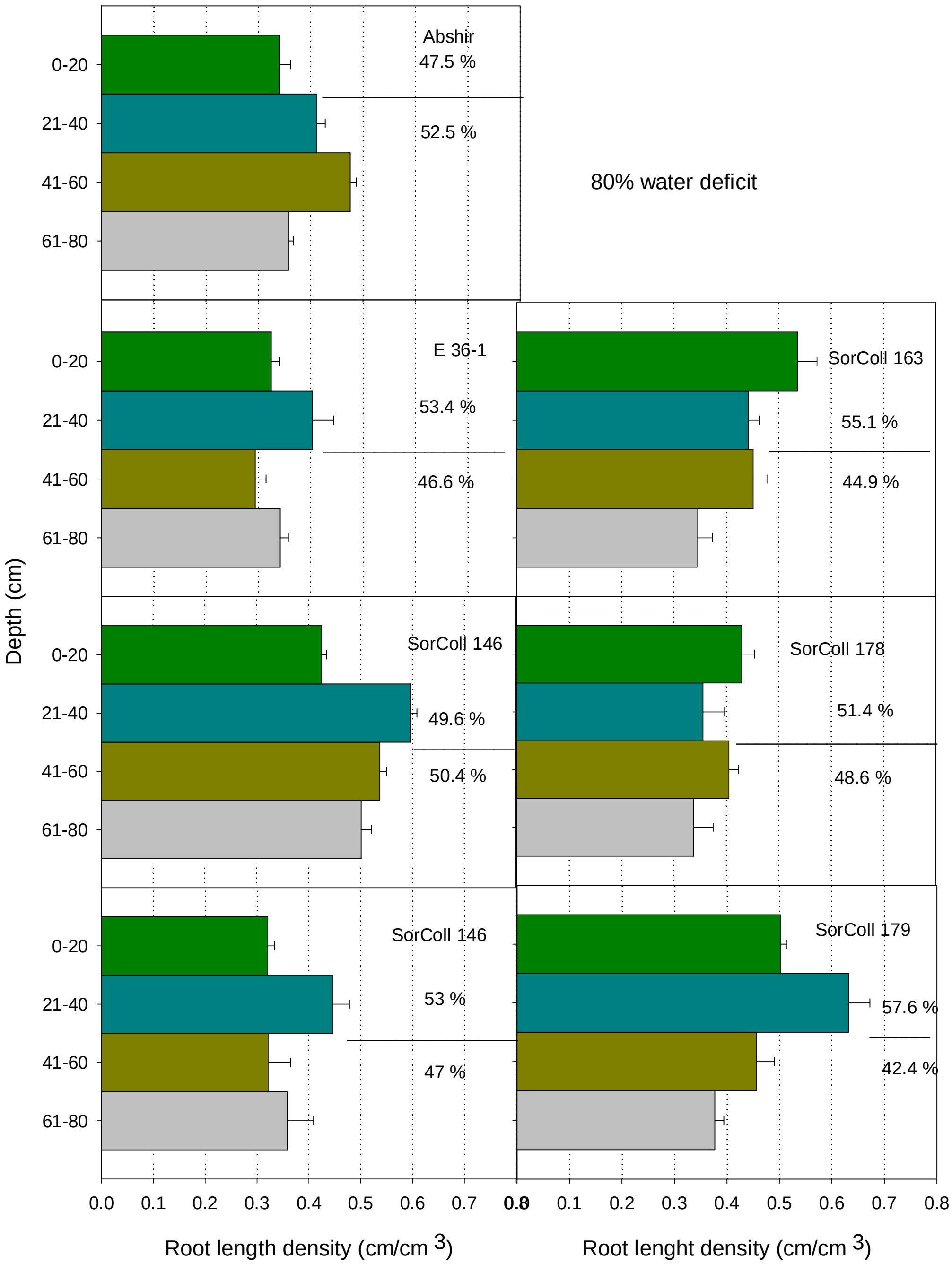

Figure 20 Root length density of sorghum varieties grown at 80% water deficit condition. Data are the mean of three replicates with S.E shown by vertical bars.

Drought tolerance index increased with increasing drought stress (Figure 21) except that of SorColl 179. The DTI value in Abshir, SorColl 178 and SorColl 60 exceeded (above 1.4) over others followed by another group that include SorColl 163, E 36-1 and SorColl 146 (1.1-1.4). Abshir is uniquely with higher DTI that increased with increasing drought effect; on the contrary SorColl 179 demonstrated least index value and also declined with increasing drought stress (Figure 21). The varieties with stay green trait are clustered together with the intermediate group.

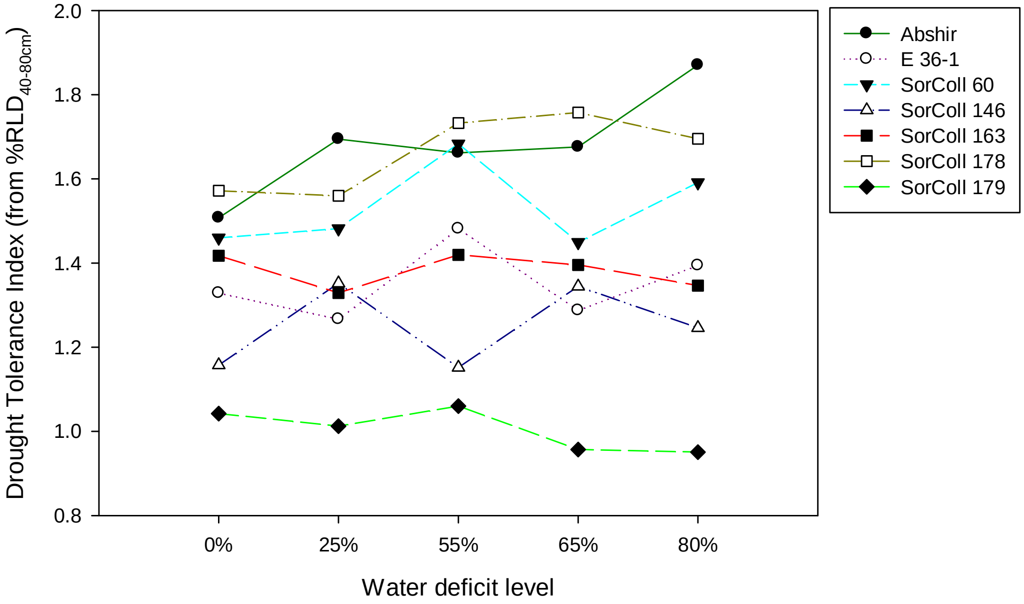

Figure 21 **Drought Tolerance Index (DTI) from % RLD$_{40\text{-}80cm}$**

4.1.2 Physiological parameters

Photosynthesis is often used as a criterion of dehydration tolerance or general “drought resistance.” It became popular more recently in the evaluation of transgenic plants and as instrumentation became better and easier to use. Variation in dehydration tolerance has been identified by genotypic physiological performance under different water deficit conditions.

4.1.2.1 Assimilation rate (A_N)

At non stress condition, assimilation rate (A_N) of all sorghum varieties was measured within the range 47-52μmole CO_2/m^2s except for SorColl 178 with lower value (43.4 μmole CO_2/m^2s). The A_N was significantly ($P< 0.01$) decreased with different degrees among the varieties with increasing drought stress. Drought stress had a significant ($P<0.01$) interaction with varietal assimilation rate responses. At 25% deficit SorColl 146 (48μmole CO_2/m^2s) SorColl 163 (47.4 μmole CO_2/m^2s) and SorColl 60 (46.2 μmole CO_2/m^2s) attained the highest assimilation rate over other genotypes. Whereas, E 36-1 (12 μmole CO_2/m^2s), SorColl 179 (18 μmole CO_2/m^2s) and SorColl 178 (24 μmole CO_2/m^2s) scored the least (Figure 22). At 55% water deficit level, SorColl 146 (48.4 μmole CO_2/m^2s), E 36-1(43.4 μmole CO_2/m^2s) and SorColl 163 (40 μmole CO_2/m^2s) maintained the rate of assimilation better, while others demonstrated a sharp decrease down below (20 μmole CO_2/m^2s). At 65% and 80 % levels, sorghum varieties were severely affected in $A_{N,}$ where insignificantly lowered to the range of 11-14 μmole CO_2/m^2s and below 1 μmole CO_2/m^2s value at 65% and 80% water deficit stress levels respectively.

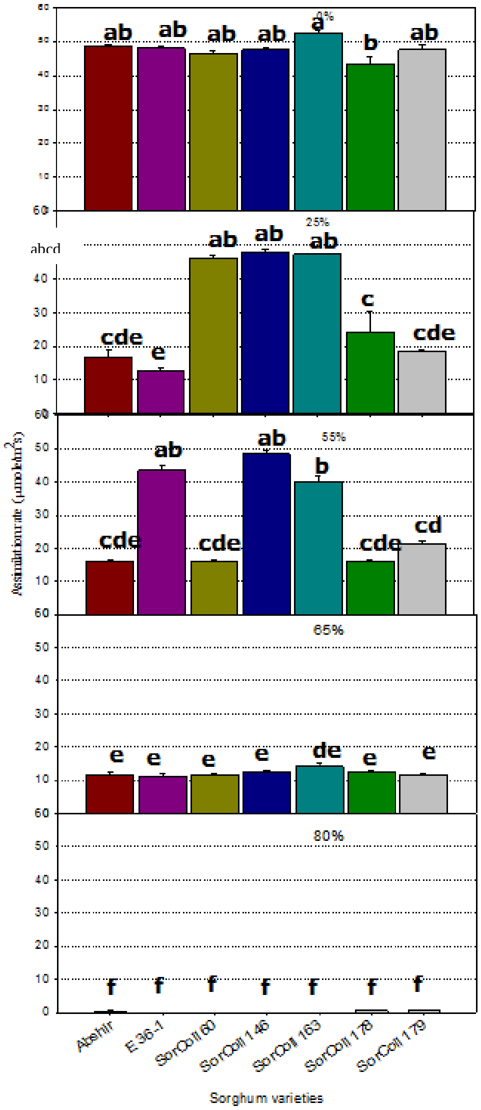

Figure 22 **Assimilation rate response of sorghum varieties to drought stress**

Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.2.2 Transpiration rate (E)

At non drought stress condition, the highest Transpiration rate (E) was recorded in SorColl 179 (2.3 mmole/m^{2}s), SorColl 178 (2.3 mmole/m^{2}s), and SorColl 60 (2.13 mmole/m^{2}s), whereas, SorColl 163 (1.1 mmole/m^{2}s) and Abshir (1.2 mmole/m^{2}s) had relatively lowest transpiration rate. SorColl 146 and E 36-1 had intermediate and equal value (1.85 m mole/m^{2}s).

Varieties had significantly ($P< 0.01$) different transpiration rate responses to the drought stress imposed. Unlike rate of assimilation, E was found increased at 25% level drought stress in Abshir (3.8 mmole/m^{2}s) SorColl 178 (3.2 mmole/m^{2}s), SorColl 146 (3 mmole/m^{2}s), others with 2.5 mmole/m^{2}s except SorColl 179 which decreased to 2 mmole/m^{2}s. At 55% water deficit level, E values diminished again except that of SorColl 163 (3.8 mmole/m^{2}s), SorColl 146 (3.1 mmole/m^{2}s) and SorColl 179 (3.1 mmole/m^{2}s) that increased (Figure 23). At both 65 % and 80% water deficit levels, E further decreased down to 0.4-1.4 mmole/m^{2}s and 0.1-1.4 mmole/m^{2}s respectively in all sorghum varieties with slightly higher values in SorColl 179 (2 mmole/m^{2}s) and SorColl 163 (1.6-2 mmole/m^{2}s) (Figure 23).

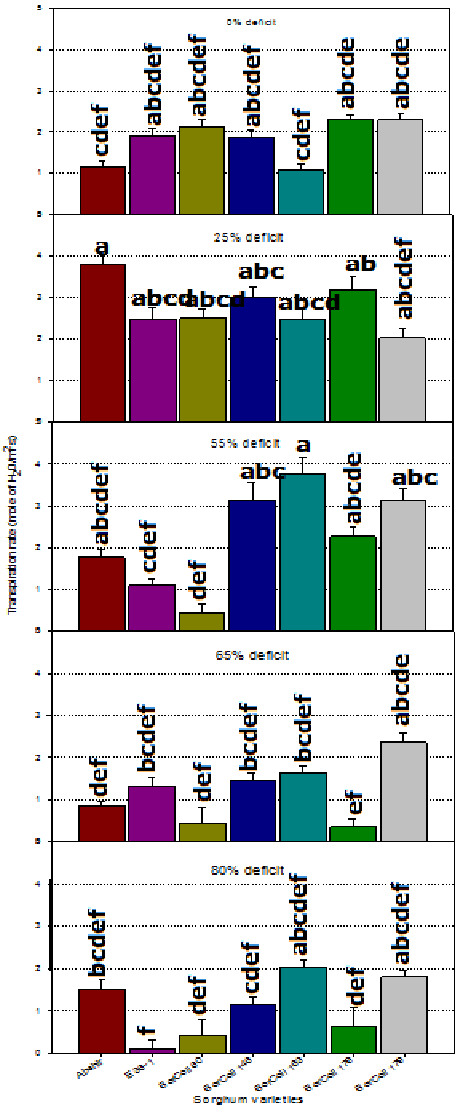

Figure 23 Transpiration rate. Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.2.3 Intercellular Carbondioxide concentration (C_i)

Although Sorghum varieties had different intercellular carbondioxide concentrations (C_i) at both stressed and non stressed conditions, they were not statistically different ($P>0.05$) for both treatments, varietal responses and their interactions (Figure 24). However, SorColl 163 (451.9 mmole CO_2/mole air), SorColl 60 (402.2 mmole CO_2/mole air), E 36-1 (380.1 mmole CO_2/mole air) had relatively highest values (Figure 24). On the other hand, Sorcoll 146 (358.6 mmole CO_2/mole air), Abshir (333.3 mmole CO_2/mole air) as well as SorColl 178 and SorColl 179 (312.6 mmole CO_2/mole air) had lower values. The sorghum varieties demonstrated an increase in C_i at 25% except Sorcoll 163. SorColl 163 had lower C_i at all drought stress levels than at non stress condition. In case of Abshir as well as E 36-1 continued lowering up to 55% drought stress but had higher C_i in the last two most severe drought stress conditions (65% and 80%) than that of under non stressed conditions. Other varieties continued having higher intercellular carbon dioxide concentration in the remaining different drought stress levels.

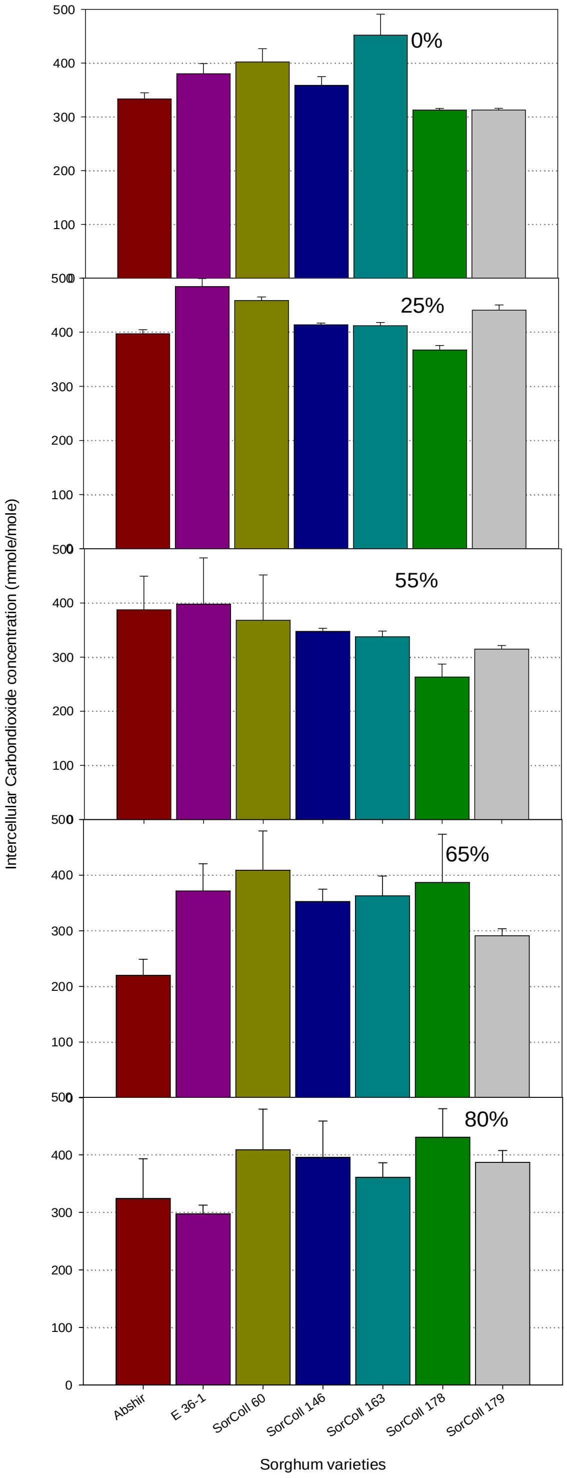

Figure 24 Intercellular CO_2 concentration

4.1.1 Yield and its attributes

Yield is a complex polygenic trait which is highly influenced by the environment and season. A basic knowledge on the association of various components of yield with biomass and grain yield is a pre-requisite for initiating successful breeding program.

4.1.1.1 Days to 50 per cent flowering

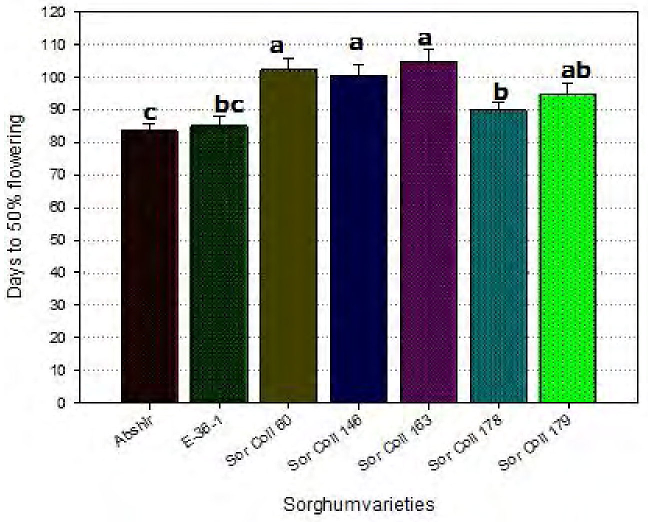

Figure 25 Days to 50% flowering of sorghum genotypes.
Means that shared the same letter are not significantly ($p < 0.05$) different.

Our study revealed that sorghum varieties showed heterogeneous days (late, intermediate and early maturing) to reach 50% anthesis unlike Duncan (1981), considered only sorghum plants that required least number of days to reach 50% anthesis. SorColl 163, SorColl 146 and SorColl 60 took 105, 101 and 102 days for 50% flowering (Figure 25). Sorghum is known to be sensitive to water stress during flowering and early grain filling stages as compared to vegetative growth period.

4.1.1.2 Grain number per panicle

SorColl 179 had the highest seed count per panicle, whereas Abshir had the least at control. The double water stress imposition to the genotype one during vegetative and the other progressive stress at post-flowering (seed filling and maturation) stages reduced seed number per head and grain yield. Sorghum plants under non-stressed conditions had higher number of grains per panicle than that of under stress conditions (Figure 26) and as a result the grain weight was also found decreasing with increasing drought stress significantly (P<0.01) among varieties and treatment and their interaction was also significantly (P=0.001) different.

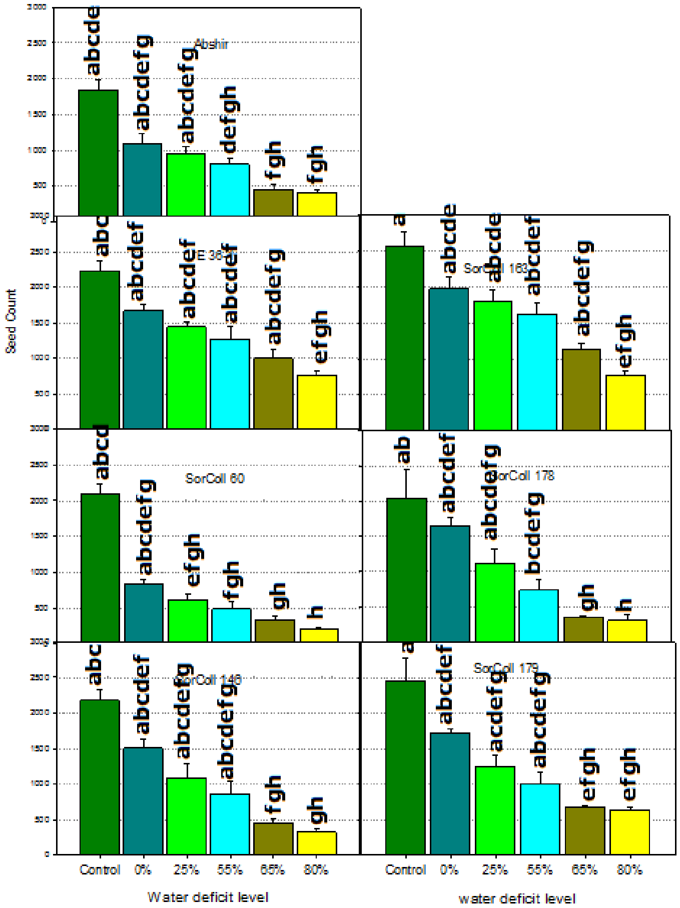

Figure 26 Sorghum seed count per panicle grown at different water deficit levels

4.1.1.3 Panicle with seed dry weight (g plant-1)

The sorghum varieties also exhibited significant differences among themselves and treatment and their interactions (P< 0.00) at all levels. The panicle weight is more determined by the seed weight. SorColl 146 followed by SorColl 60 scored the maximum panicle weight under the non-stressed condition (Figure 27). SorColl 163, E-36-1 and Abshir maintained panicle dry weight best over the other genotypes compared to controls.

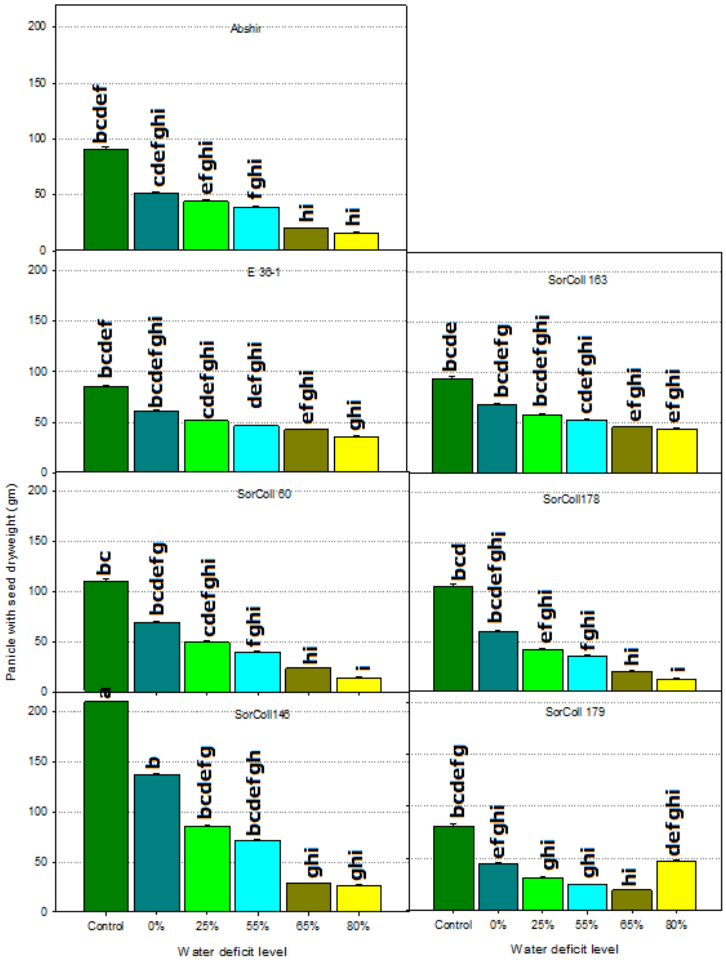

Figure 27 Sorghum panicle with seed dry weight grown at different water deficit levels

Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.1.4 Seed weight per panicle

Yield stability in terms of seeds per panicle in the most severe water deficit stress levels was observed in the check E 36-1 followed by SorColl 163, whereas SorColl 178 performed least in the same conditions (Figure 28).

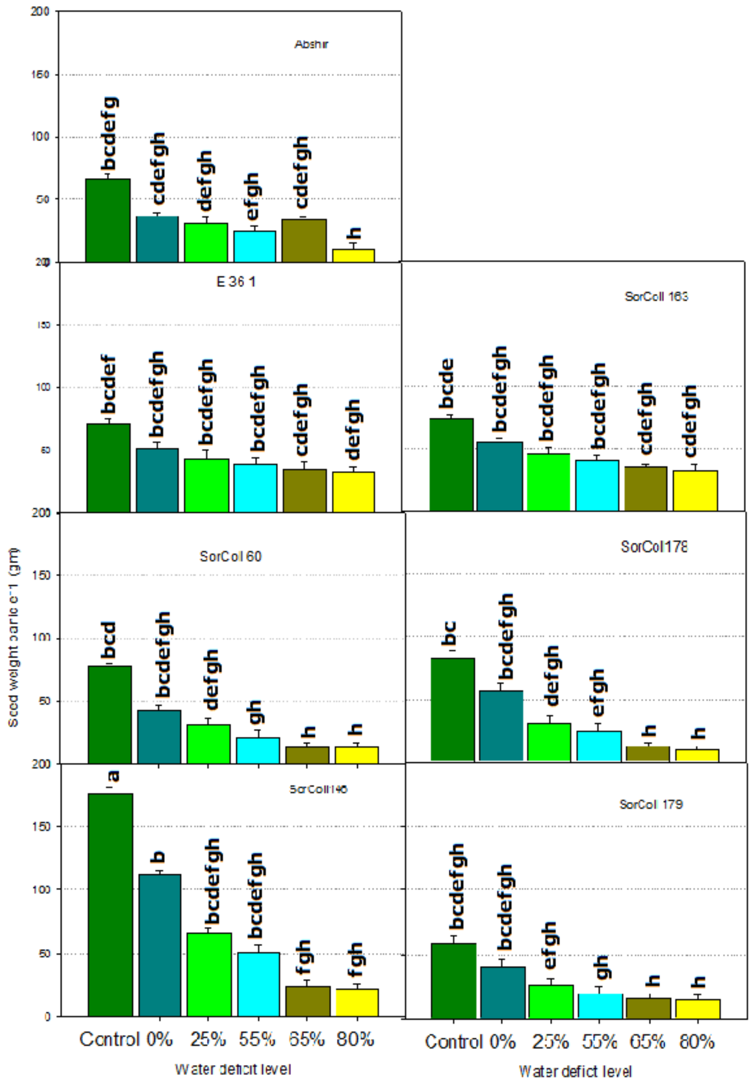

Figure 28 Sorghum seed weight per panicle grown at different water deficit levels.
Means that shared the same letter are not significantly ($p < 0.05$) different.

Table 11 Mean dry panicle with seed weight (gm) of sorghum varieties during harvest

variety	control	Imposed to pds	25% wdsl	55%wdsl	65%wdsl	80%wdsl
Abshir	91bcdef	51.1cdefghi	44.2defghi	38.9efghi	19.9hi	16i
E-36	95.3bcde	61.2cdefghi	51.7cdefghi	46.7defghi	42.7efghi	35.4efghi
SorColl60	110bc	65.3cdefghi	49.3cdefghi	39.7efghi	23.4hi	14i
SorColl 146	176.5a	137.5ab	86bcdefghi	71.7cdefghi	28.3ghi	26.2ghi
SorColl 163	93.7bcde	68.5cdefghi	58.2cdefghi	52.8cdefghi	45.7defghi	44defghi
SorColl 178	105.7bcd	69.7cdefghi	42efghi	35.5efghi	20hi	12.7i
SorColl 179	81.2bcdefgh	44.8defghi	31fghi	24.3ghi	19.6hi	47.2defghi

Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.1.5 1000-seed weight

In our study, 100 seed weight was found to be least affected by treatments and variety* treatment interaction was not significantly different (P>0.05). In some panicles like E 36-1 and SorColl 60 the remnant seeds were found larger and heavier than the seeds from plants grown in the less severed conditions (Figure 29).

Table 12 1000 seed weight (gm) of sorghum varieties under different wdls

variety	control	Imposed to pds	25% wdsl	55%wdsl	65%wdsl	80%wdsl
Abshir	36.6efgh	32.4gh	31.7gh	31.2ghi	30.8ghi	26.8ghij
E-36	36.6efgh	30.7ghi	30.1ghij	30.5ghij	34fgh	32gh
SorColl60	38.3defg	31.4ghi	31.4ghi	31.5gh	34.8fgh	29.3ghij
SorColl 146	81a	74.4ab	60bc	59.6bc	53.9cd	49.7cdef
SorColl 163	29.7ghij	28.3ghij	25.8ghij	25.3ghij	24ghij	23.7ghij
SorColl 178	51.2cde	35.1efgh	29.4ghij	33.3fgh	32.2gh	22.7ghij
SorColl 179	22.8ghij	22.9ghij	20.2hij	19.6hij	13.5j	14.3ij

Means that shared the same letter are not significantly (p < 0.05) different.

Hence, E 36-1, SorColl 60, SorColl 163, Abshir and lastly SorColl 179 were superior in 1000 seed weight maintenance. On the other hand, SorColl 146 and SorColl 178 showed less stability in 1000seeds weight under drought conditions.

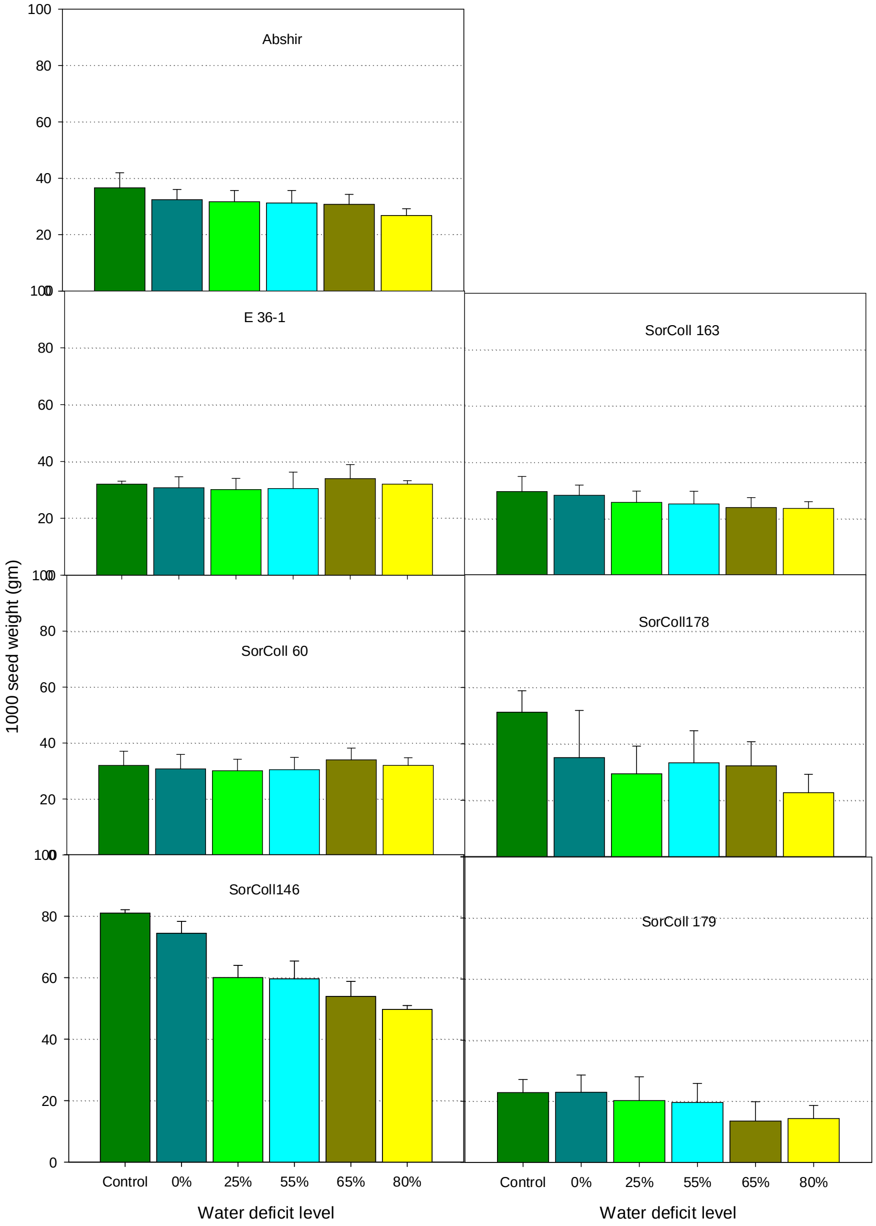

Figure 29 Sorghum 1000 seed weight grown at different water deficit levels

4.1.1.6 Yeild of sorghum varieties

Yield is a complex trait influenced by many traits and the environment. Yield estimation from field plots is found most of the time overestimating it, for research sites are uniform and highly protected and extrapolate from a few plant numbers. The yield both per panicle and in Kg/ha was significantly decreasing with increasing the severity of drought (Figure 27, 28 and Table 13). The varieties with high yield at optimum conditions were SorColl 146 (6757 kg/ha), SorColl 178 (6429.6Kg/ha) and Sorcoll 60 (6079Kg/ha) respectively (Table 13). On the other hand, E 36-1, SorColl 163 had intermediate yield but relatively had the most stable yield in the most severe wdsl.

Table 13 **Yield performance (kg/ha) of sorghum varieties imposed to different water deficit stress levels (wdsl)**

variety	**control**	**Imposed to pds**	**25% wdsl**	**55%wdsl**	**65%wdsl**	**80%wdsl**
Abshir	5250bcdefg	2833.7cdefgh	2348.9cdefg	1936.7efg	2631.5cdefgh	855.6h
E-36	5587bcdef	4005.6bcdefgh	3349.6bcdefgh	2994.4bcdefgh	2670.4cdefgh	2494.1defg
SorColl60	6079.6bcd	3538.9bcdefgh	2359.3defg	1607.4gh	1005.9h	985.2h
SorColl 146	6757.4 a	4881.9b	2566.7cdefgh	3992.6bcdefgh	1840.7fgh	1724.1fgh
SorColl 163	5781.5bcde	4290.7bcdefgh	3564.8bcdefgh	3175.9bcdefgh	2696.3cdefgh	2488.9defg
SorColl 178	6429.6bc	4446.3bcdefgh	2346.3defgh	1879.6defgh	959.3h	1114.8h
SorColl 179	4537bcdefgh	3033.3bcdefgh	1970.4efgh	1477.8gh	1218.5h	738.9h

Means that shared the same letter are not significantly ($p < 0.05$) different.

Coefficient of correlation for traits indicated that yield was positively correlated with plant height; third leaf area and SPADR after exposure to progressive stress, dry shoot and root biomass, RLD at 41-80cm, assimilation rate and all yield traits (Table 14). Yield was significantly positively correlated with seed weight per panicle and dry shoot biomass, but negatively correlated with senescence rate after grain filling and RLD at 0-40cm.

Table 14 Correlation coefficients of selected traits post progressive drought stress plant height, third leaf area and SPADR, senescence rate after grin filling, dry shoot biomass, dry root biomass, RLD at 0-40cm, RLD at 41-80cm assimilation rate, total seed weight per panicle, seed count per panicle, 000 seed weight, yield (Kg/ha) under wdsl.

	PHT Pos	**LA Pos**	**SPAD post**	**Sen ASF**	**DShBM**	**DRtBM**	**RLD$_{41-80cm}$**	**RLD$_{0-40cm}$**	**Assim**	**SWtPP**	**SCPP**	**ThSWt**	**Yld**
PHTPos	1												
LAPos	0.256	1											
SPADpost	0.914	0.626	1										
Sen ASF	-0.846	-0.732	-0.99	1									
DShBM	0.98	0.444	0.977	-0.936	1								
DRtBM	0.977	0.045	0.807	-0.714	0.915	1							
RLD$_{41-80cm}$	0.902	0.648	0.97*	-0.993	0.97	0.79	1						
RLD$_{0-40cm}$	-0.631	-0.911	-0.892	0.947	-0.774	-0.453	-0.904	1					
Assim	0.903	0.647	0.96*	-0.993	0.971	0.791	0.89**	-0.903	1				
SWtPP	0.618	0.918	0.884	-0.942	0.763	0.438	0.897	-0.87*	0.896	1			
SCPP	0.903	0.647	0.95*	-0.993	0.971	0.791	0.89**	-0.903	0.88**	0.9	1		
ThSWt	0.917	0.62	0.98**	-0.988	0.978	0.812	0.99*	-0.888	0.99*	0.9	0.98*	1	
YldKgHa	0.993	0.369	0.956	-0.903	0.997*	0.945	0.947	-0.719	0.948	0.7*	0.95	0.958	1

Key: ** significant at 0.01 level of probability
* significant at 0.05 level of probability

PHpost=plant height, LApost =third leaf area and SPADRpost after progressive drought stress, SeneASF= senescence rate after grin filling, DrSHBM=dry shoot biomass, DrRtBM=dry root biomass, RLD$_{0-40cm,\ 41-80cm}$ =Root Length density at 0-40cm, at 41-80cm Assmi=Assimilation rate, SWtPP=seed weight per panicle, SCPP=seed count per panicle, ThWt=1000 seed weight and YldKgHa=Yield in Kg/ha.

4.1 Responses to interrupted drought stress at different reproductive stages

Analysis of variance depicted that the traits had significant difference ($P<0.05$) at both water treatment and variety except water treatment in dry shoot biomass, which had no significant difference. However, none of the traits considered under this experiment had significant ($P>0.05$) treatment*varietal interaction. Therefore, mean separation tables are not presented.

4.1.1 Phenology

The sorghum varieties had different heights measured during booting stage before drought was imposed (Figure 30). After drought was imposed there was no variation in time to show the leaf of booting, but panicle exertion dates were delayed from controls (from observation). Time to flowering and duration to complete the duration was almost the same. Whereas the time of grain filling started at the same time with controls but the early stage of grain filling (soft dough) was shorter than controls. In case of the varieties exposed to stress at maturity, moisture was retained longer in the non stressed than stressed forms but yield was harvested at the same time. This depicts drought imposed at vegetative stage had great contribution to delayed harvest and the booting stage is the most important in delaying harvest time of sorghum varieties.

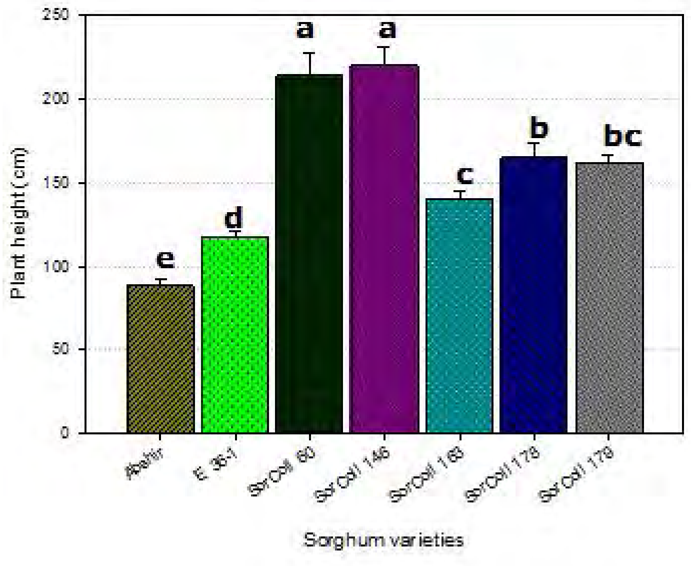

Figure 30 Height of sorghum plants before exposure to drought at booting stage.

Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.1.1 Shoot and root biomass responses

The drought imposition at different phases of the reproductive stage had a negative impact on the biomass of the sorghum genotypes. However, different responses have been observed among the sorghum varieties. SorColl 163 and E 36-1 were found highly resistant to the short period drought stresses except imposition at the maturity stage. The most susceptible landraces were SorColl 179 and SorColl 178 (Figure 31).

Drought stress at maturity stage had caused the highest dry shoot biomass loss (4-30%) followed by booting stage (3-14%). The least shoot biomass loss was recorded in sorghum varieties stressed at flowering stage (1.66 - 12%) (Figure 31). Similarly, roots exposed to drought at the maturity stage also decreased highest (12-37%) followed by those imposed at grain filling stage

(4-25% weight loss) (Figure 32). The least root biomass loss was recorded in plants exposed at booting stage.

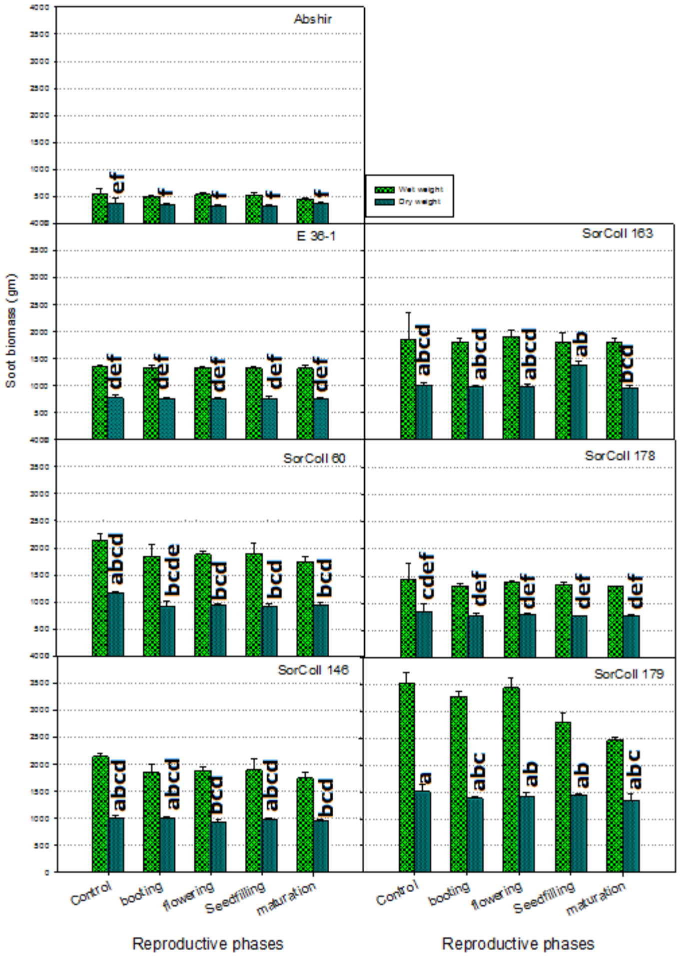

Figure 31 Shoot biomass response to short period drought exposure at different phases of reproductive stage. Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.1.2 Root biomass

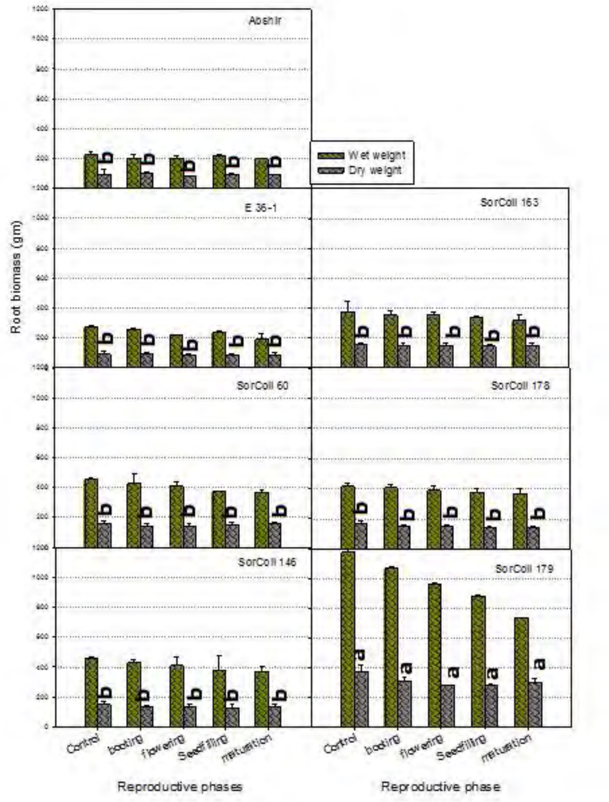

Figure 32 Root biomass response to short period drought exposure at different phases of reproductive stage. Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.2 Yield and yield attributes response

4.1.2.1 Effect of drought stress at periods of booting and anthesis

Our study revealed that all drought interruptions at different phases of reproductive stage influenced the sorghum production significantly ($P<0.01$) from control plants. However, drought at booting stage was accountable for the 8-26 % loss of production from control due to the decrease in panicle size (Figure 33) and increase in the unfilled sites of the panicle (Figure 34B). The sorghum genotype with higher root biomass, SorColl 179 and SorColl 163 had least panicle size reduction due to the drought effect during booting. Abshir and SorColl 146 demonstrated higher panicle reduction. The control plants had 4% (in E 36-1) to 18% (in SorColl 146) loss of grains per panicle due to any physiological and mechanical disorders. When moisture was held at booting (before flowering) for about 10 days, loss in grain examined as unfilled glumes was increased significantly ($P=000$) in E 36-1 (11.8%), SorColl 163 (20.8%), Abshir (22.6%), SorColl 179 (28.2%), SorColl 60 (34%), SorColl 178 (38.7%) and SorColl 146 (40%) in an increasing order (Figure 34A). Unfilled glumes were also found significantly increased over control plants as a result of short period, 15 days, drought imposition at post-flowering. Seed count loss (unfilled glumes) was examined in E 36-1 (8.6%) Abshir (15.7%), SorColl 163 (18.2%), SorColl 178 (21.5%), SorColl 179 (25.5%), SorColl 60 (32.1%) and SorColl 146 (32.4%) in an increasing order. The unfilled sites as a result of drought in both periods (before and soon after flowering) had a tight glume. SorColl 60, E 36-1 and Abshir were least affected, whereas SorColl 179, SorColl 178 and SorColl 146 were more affected with regard to an average unfilled glumes per panicle exceeding their control. Seed free small glumes were more pronounced in the booting than anthesis and accountable for the 10% and 20% production loss respectively. The unfilled sites of sorghum varieties stressed in the later phases of reproduction were below 10% and were insignificantly different (P value 0.264) and (see also Figure 34B). 1000 seed weight loss from that of control plants in both booting and anthesis drought effect had least influence 2-18% loss (Figure 35A), and hence negligible contribution to the yield loss.

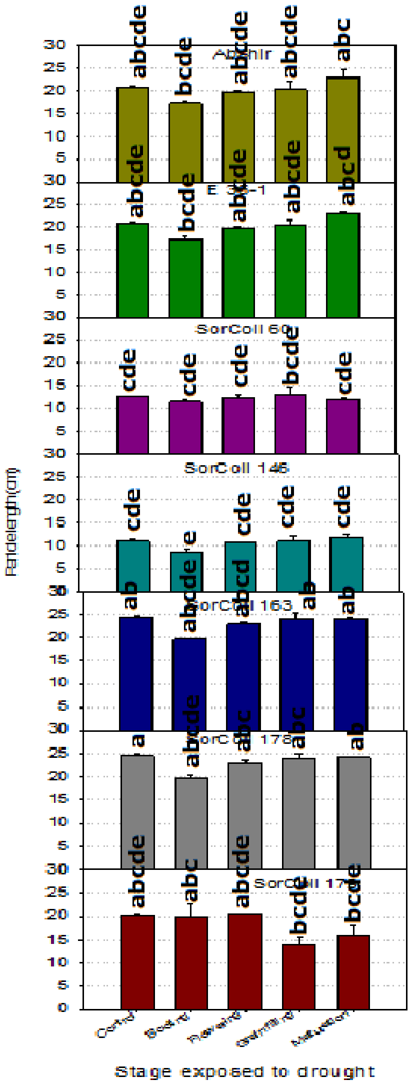

Figure 33 Panicle size of sorghum varieties grown under interrupted irrigation at different reproductive stages.

Means that shared the same letter are not significantly (p < 0.05) different.

4.1.2.2 Drought imposed at periods of seed setting and maturity

The post-flowering drought imposition just for about 15 days during grain filling and 45 days during maturation was also accountable for significant yield loss (Figure 33). There was slight panicle size variation except in SorColl 179 from control plants due to drought effect imposed at seed filling and maturity periods (Figure 31). Results from this study have further showed a reduction in a seed weight per panicle (production) from controls due to negative effect of drought on seed weight and seed count per panicle (Figure 32 and 32). Seed count reduction was due to seed fall leaving open glumes unlike that of the early two phases. Of all the genotypes, SorColl 146 was not affected in seed count followed by the E 36-1 and 163 (Figure 32B). In general, E 36-1 and SorColl 163 were found least affected among others both in seed count per panicle and 1000 seed weight (Figure 32 A and 32).

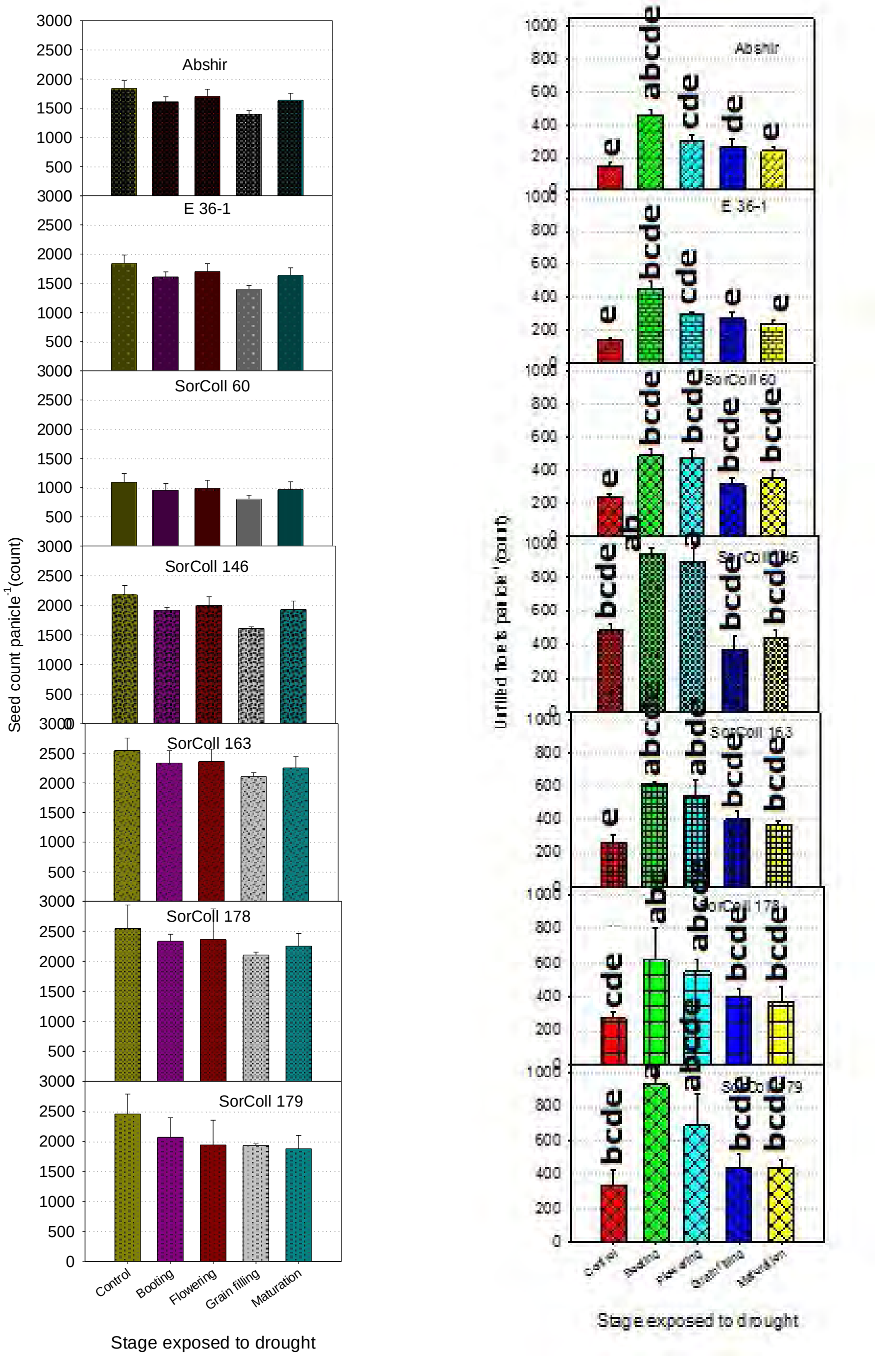

Figure 34 **Seed count (A) and unfilled florets (B) per panicle of sorghum varieties interrupted irrigating at different reproductive stages.**

Means that shared the same letter are not significantly ($p < 0.05$) different.

High number of unfilled glumes as a result of short drought during booting had less contribution in weight loss than that of unfilled formed during filling to maturity, this may be due to the higher sink to reduced number of seeds increased the 1000seed weight as it is shown in our result with least weight of 1000 seeds from sorghum plants imposed to drought during seed setting to seed filling (Figure 35a). Yield was found most affected with plants exposed to drought at seed filling to maturity as it was also longer period than others.

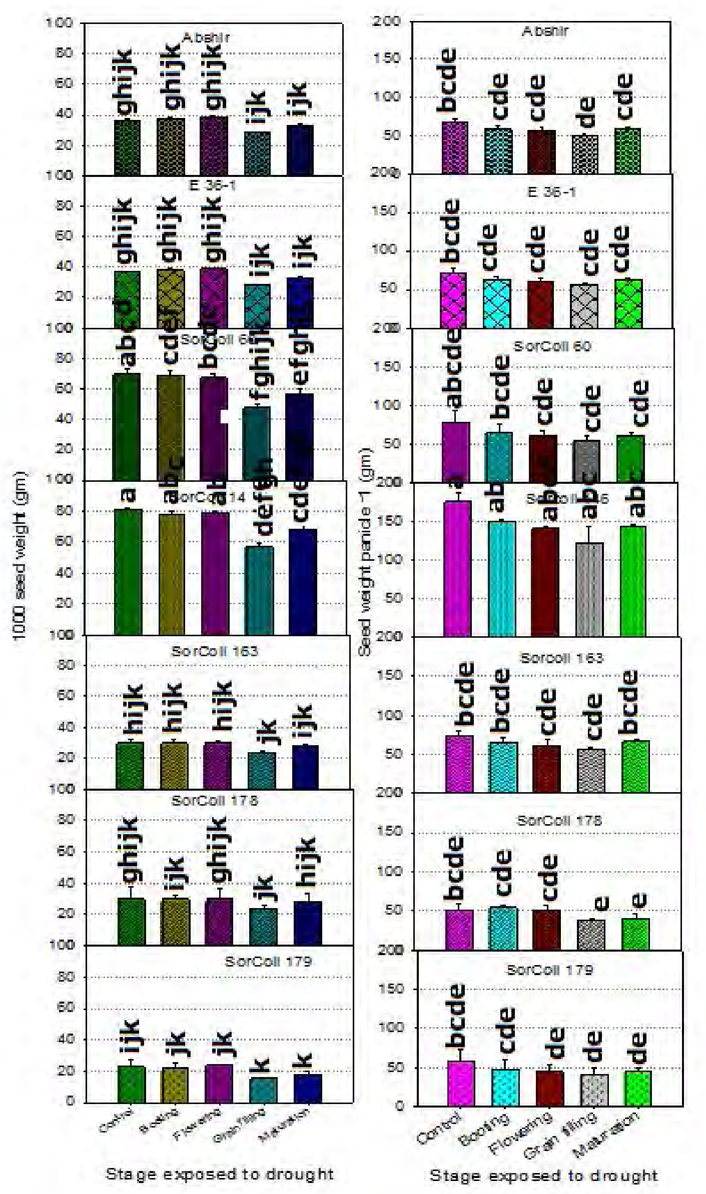

Figure 35 100 seed weight (A) and seed weight per panicle (B) of sorghum varieties treated with interrupted irrigation at different reproductive stages.

Means that shared the same letter are not significantly ($p < 0.05$) different.

4.1.2.3 Yield response

The overall yield response of the varieties is presented in Table 14, indicating that the short period drought effect had remarkable yield loss (up to 40%) but was less than the experiment II (Table 13). As indicated in Table 13, yield loss was observed in all sorghum varieties imposed to the different phases of the reproductive stage. The longer period exposure to drought starting seed filling was the main cause for yield loss followed by the drought imposed at seed setting. The effect of short drought during booting was more severe than that of flowering phase.

Table 15 Yield performance (Kg/ha) of sorghum varieties exposed to short drought stress at different reproductive stages

variety	control	booting	flowering	seed filling	maturity
Abshir	5250cde	4362.8 cde	4523.6cde	3822.5 cde	3321.9e
E-36	5587cde	4688.4cde	4971.8cde	4197.1cde	3822.8cde
SorColl60	6079.6bcde	5054.3 cde	4978.6cde	4253.9 cde	3468.9c
SorColl 146	6757a	4485.9cde	5573.35cde	4423.44cde	3498.4c
SorColl 163	5781.5cde	5106.1 cde	5060cde	4269 cde	3730.5de
SorColl 178	6429.6bcde	4484.7cde	5401.7cde	4340.5cde	3615.4e
SorColl 179	4537cde	4006.3 cde	3680.7de	3233.7e	2470.5e

Means that shared the same letter are not significantly ($p < 0.05$) different.

Correlation coefficient indicated that yield was found positively correlated with all traits of biomass and yield except unfilled glumes (Table 16). Seed weight per panicle and thousand seed weight were significantly positively correlated with yield. Thousand seed weight was the only yield trait that was correlated positively with unfilled glumes (Table 16).

Table 16 **Correlation coefficients of yield traits panicle length, panicle width, total seed weight per panicle, seed count per panicle, panicle with seed weight, 1000 seed weight, unfilled glumes, yield (Kg/ha) for short period drought imposed at different reproductive stages**

Traits	**Dry shoot BM wt**	**Dry root BM wt**	**Panicle length**	**Seed wt pp**	**Seed count**	**1000 wt**	**unfill**	**Yield (kg/hr)**
DshtBM	1							
DrtBM	0.571	1						
panlgth	0.376	0.345	1					
Seedwt pp	0.543	0.684	0.148	1				
Seed count pp	0.231	0.777	0.359	0.842	1			
Seedwt pp	0.329	0.604	-0.283	0.894*	0.715	1		
unfill	-0.449	-0.654	-0.883*	-0.175	-0.444	0.143	1	
Yield (Kg/hr)	0.543	0.684	0.149	0.98**	0.842	0.893*	-0.18	1

** significant at 0.01 level of probability

* significant at 0.05 level of probability

4.3 Sorghum seedlings response to water deficit stress

Analysis of varience for the means of traits from the green house demonstrated that physiological performance at variety level and their interaction with treatment was not significant except assimilation rate at treatment level (annex 2). Biomass responses were also significantly different only at treatments. Proline from leaf, stem and root were significant at the variety, treatment and their interaction levels but Proline from the root had no significant difference at varietal level. Moreover, chl a and b were not significant at treatment and variety interaction as well as varietal level, respectively (annex 2).

4.3.1 Morphological study

4.3.1.1 Seedling height

The sorghum seedlings at the age of two months had demonstrated height variation at non stress condition /control/. SorColl 178 had the longest height (32cm) followed by B 35 and SorColl 179 with same average height (30cm), while SorColl 60 had the shortest height (23cm) followed by Abshir (25cm). All sorghum varieties have shown a similar trend of decreasing in height with increasing drought stress. Relatively, SorColl 146 and SorColl 163 followed by SorColl 60 were least affected (16.26 %, 18.55% and 26.1% loss in height respectively) in the more severe (65%) water deficit stress (Table 17). Uniquely, SorColl 60 maintained growth in height at mild water deficit conditions. Whereas, SorColl 179, SorColl 178, B 36-1 and B 35 were found severely affected by the mild water deficit stresses (Table 17).

Table 17 **Drought effect on height of sorghum seedlings**

Sorghum varieties	Plant height (cm)				Height loss (in %)		
	Control	25%	55%	65%	25%	55%	65%
Abshir	24.78	23.67	17.78	16.33	4.48	28.25	34.08
B 35	29.89	25.78	23.11	18.56	13.75	22.68	37.92
E 36-1	27.22	25.56	18.67	16.00	6.12	31.43	41.22
SorColl 60	22.67	23.89	24.14	16.75	-5.39	-6.51	26.10
SorColl 146	26.78	27.78	22.50	22.43	-3.73	15.98	16.24
SorColl 163	27.01	25.00	24.33	22.00	7.44	9.91	18.55
SorColl 178	31.44	25.00	19.89	19.78	20.49	36.75	37.10
SorColl 179	30.00	20.00	19.44	19.11	33.33	35.19	36.30

4.3.1.2 Seedling Biomass response

The negative effect of mild drought stress (25%) to dry shoot biomass of sorghum seedlings was SorColl 146, SorColl 163, SorColl 60, E36-1, B 35 and SorColl 179 in a decreasing order (Figure 36). On the other hand, only Abshir (30.5, 7%) and SorColl 178 (21, 2%) dry shoot biomass respectively, were negatively affected by the mild drought stress (25%).

Seedlings of sorghum varieties were found decreasing in root biomass with increasing drought stress except SorColl 60, SorColl 146 and SorColl163 (Figure 37). Landraces SorColl 60 and SorColl 146 had positive root biomass response (17%) and (10%) dry biomass and SorColl 163 increased by 6% dry weight to mild (25%) drought stress, while others were negatively responded. The least affected next to SorColl 60 and SorColl 146 was SorColl 163 (1.13% loss at 25%) and B 35 next. Other varieties SorColl 179, E 36-1 SorColl178, Abshir and B 35 were negatively affected by the drought stress in root biomass in a decreasing order. In general, relatively roots were more negatively affected by the stress than that of shoots compared to their control plants (Figures 35 and 36) under green house.

In the last drought stress levels (55% and 65%) both the shoot and root biomass was severely affected by the drought stress. At 55 %, shoot biomass loss was above 67% in SorColl 163 to 93% Abshir and all lost above 90% dry shoot biomass. At 65% water deficit stress seedlings were more severely affected, 85% wet shoot biomass loss was observed in B 35 to 96.5% loss in SorColl 179 and all varieties lost dry shoot biomass above 94% except B 35 by 70.4%. In case of the root biomass at the most severe water deficit stress (65%) together with the shoot biomass, the sorghum seedlings were found severely affected (almost all above 90%) except B 35 with relatively lower (70%) loss in dry biomass of the shoot (Figures 35 and 36). Except SorColl 178 in 55% and SorColl 146 at 65% roots were more severely stressed than shoots.

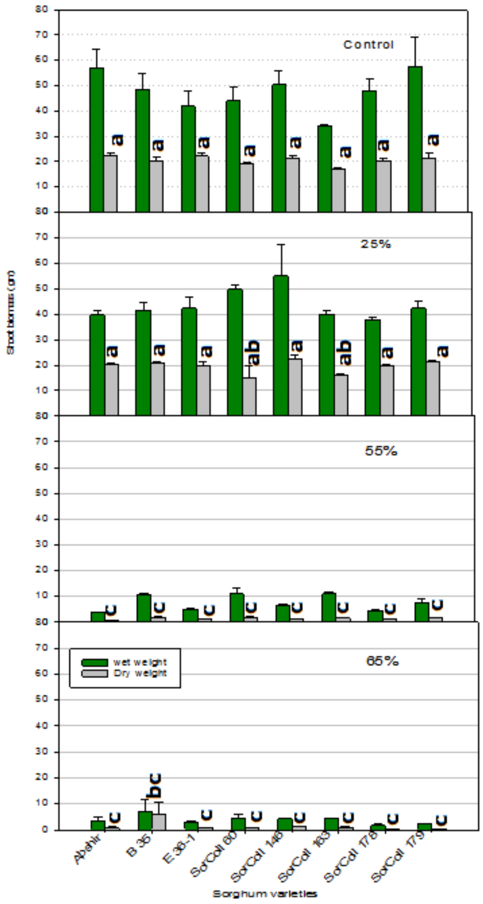

Figure 36 Sorghum seedling shoot biomass responses to different levels of drought stress

Means that shared the same letter are not significantly ($p < 0.05$) different.

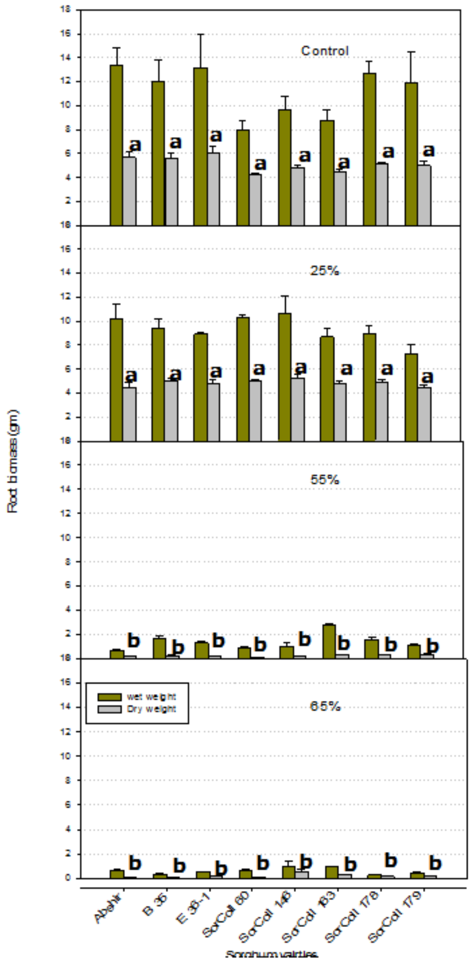

Figure 37 Sorghum seedling root biomass responses to different levels of drought stress

Biomass partitioning/ Root shoot ratio.

Means that shared the same letter are not significantly ($p < 0.05$) different.

The sorghum varieties at seedling stage responded differently in dry root-shoot biomass ratio to the different water regimes. A decrease in root-shoot biomass ratio from the controls was recorded in all except SorColl 60, SorColl 163, SorColl 146 at 25%, SorColl 178 at 55% and SorColl 146, SorColl 178, SorColl 179 and SorColl 163 at 65% (Figure 38) with increasing drought stress .

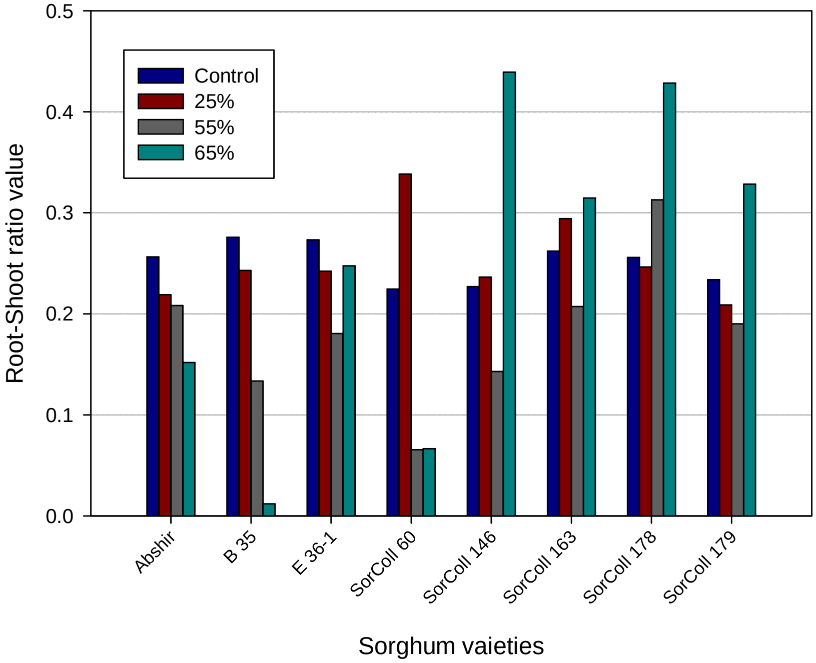

Figure 38 Sorghum seedling root-shoot ratio from different water deficit levels

4.3.1.3 Third leaf area

The sorghum varieties had variations in third leaf area in the non stress condition (Table 18). The SorColl 60, Abshir and SorColl 146 had the widest area of third leaf, whereas SorColl 178 and SorColl 179 had narrower area. Unlike height, the third leaf area response to the different regimes was not a linear decrease with increasing drought stress. The response of the third leaf area was with the trend of an inverted parabolic shape up on exposure of the sorghum varieties to different water regimes, the 25% water deficit condition with higher third leaf area (Table 18).

Table 18 **Effects of water stress on 3rd leaf area of sorghum seedlings**

Variety	3rd LA (cm^3) at water regime				LA change from control (in %) (% decrease (-) or increase in LA)		
	control	25%	55%	65%	25%	55%	65%
Abshir	16.41	13.31	12.18	6.91	-18.89	-25.82	-57.88
B 35	10.44	11.41	8.08	5.68	9.34	-22.63	-45.63
E 36-1	11.49	10.55	10.33	6.65	-8.16	-10.12	-42.11
SorColl 60	17.51	22.50	12.61	6.56	28.48	-27.98	-62.53
SorColl 146	14.48	15.18	8.95	6.19	4.84	-38.17	-57.25
SorColl 163	10.66	11.33	9.48	6.88	6.21	-11.14	-35.52
SorColl 178	9.06	13.04	6.80	6.81	43.86	-24.97	-24.83
SorColl 179	8.58	14.15	9.06	6.25	65.01	5.69	-27.11

4.3.2 Physiological responses

4.3.2.1 SPAD chlorophyll value

The sorghum varieties had SPAD reading difference at the control treatment (Table 19). Abshir, B 35 and SorColl 179 had relatively higher chlorophyll content (37, 34, 32 respectively) in no stress condition while SorColl 163, SorColl 178 and SorColl 60 showed relatively lower value (23, 27 and 28 respectively) (Figure 39). At the 25% water deficit level, all varieties scored an increased SPAD value, Abshir had still higher value followed by SorColl 146 and SorColl 163 (40, 35, 34) respectively. Chlorophyll content in all sorghum varieties decreased in the severe water stress regimes (Figure 39 and Table 19). At 55% water deficit level, varieties could be grouped into two based on SPAD values as B35, SorColl 163, Abshir and SorColl 179 with higher value ranging between 25-30 and SorColl 178, E 36-1 and SorColl 146 with lower value around 20 (Figure 39).

Table 19 SPADR for sorghum varieties at different wdsl

variety	control	25% wdsl	55%wdsl	65%wdsl
Abshir	37.2ab	39.8a	26.4cdefg	11.4ij
B 35	33abcd	29.8abcdef	27.4cdef	9.4j
E 36-1	28.3abcdef	34.1abc	20.4efghij	14.9ghij
SorColl60	28.1abcdef	31.2cdef	26.1cdefgh	13.8hij
SorColl 146	28.1abcdef	35.1c	19.2fghij	12.3ij
SorColl 163	22.9abcdefghi	33.8c	26.2cdefg	13.7ij
SorColl 178	27bcdefg	29.9cdef	20.8defghij	10.7ij
SorColl 179	31cdef	31.9cde	26.4cdefg	8.7j

Means that shared the same letter are not significantly ($p < 0.05$) different.

Under most severe drought conditions, SPADR value decreased down to 10-15 where E 36-1 and SorColl 163 were least affected and SorColl 179 and B 35 were highly affected.

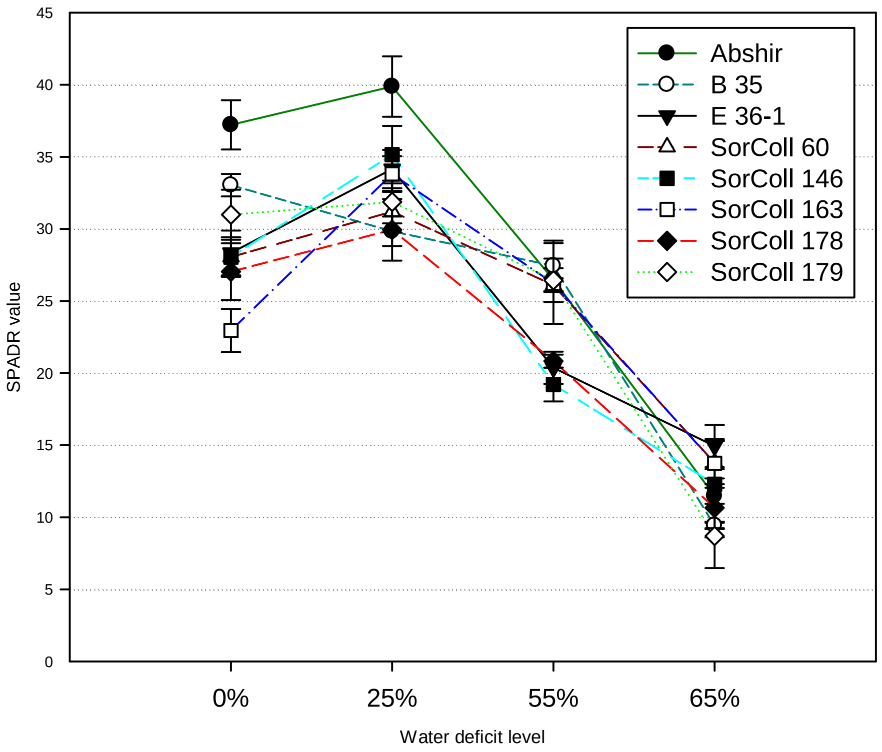

Figure 39 Effects of water stress on chlorophyll content from SPADR

4.3.2.2 Assimilation rate (A_N)

Assimilation rate (A_N) was recorded highest in E 36-1 (34.15 µmole CO_2/m^2s) over other varieties (20-29 µmole CO_2/m^2s) under non stressed condition (Figure 40). The A_N was decreased with increasing drought stress in the sorghum seedlings except in SorColl 178 (at 25%) and SorColl 179 (at 25% and 55%). These varieties showed an increased A_N. On the contrary, SorColl 163 and SorColl 146 demonstrated a higher decrease in rate of assimilation relative to the control plants, followed by B 35 at 25% water deficit. B 35 and Abshir recorded the highest loss in A_N followed by E 36-1 at 55% water deficit. Similarly, at 65% water deficit level, E 36-1, Abshir, B 35 recorded the highest loss in A_N followed by SorColl 163 and SorColl 178 due to drought effect.

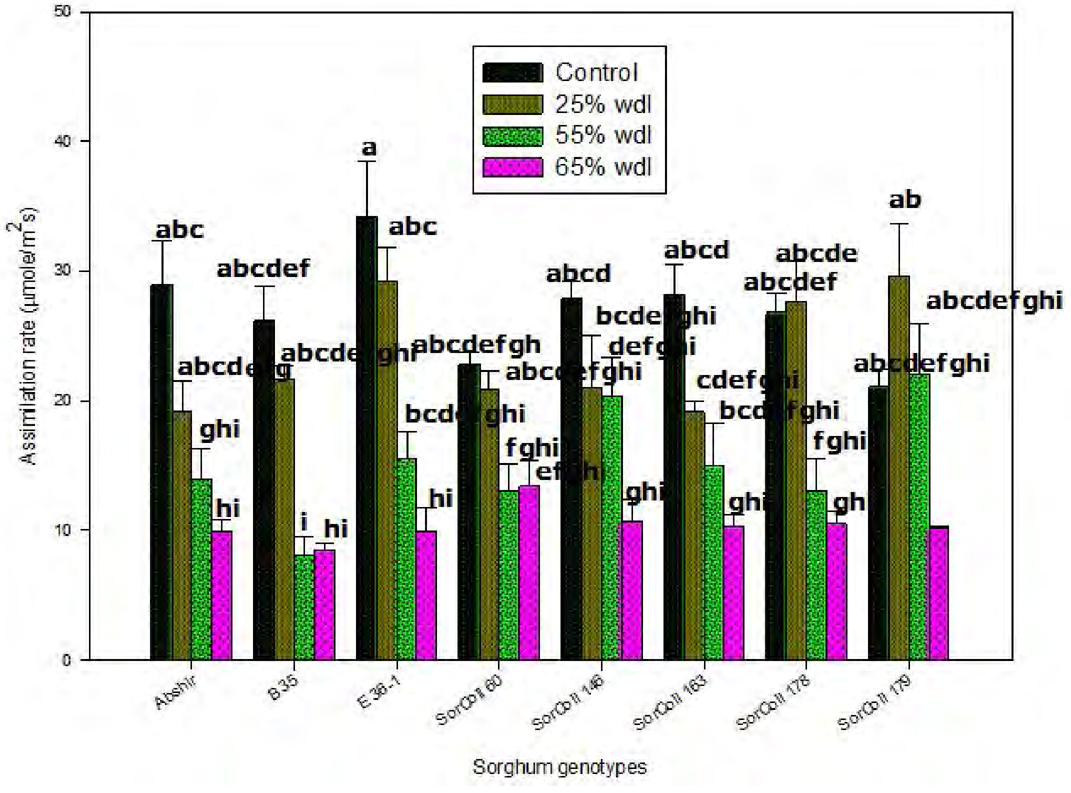

Figure 40 Sorghum seedling assimilation rate responses

4.3.2.3 Transpiration rate (E)

Transpiration rate (E) was recorded highest in E 36-1 (2.76 mmole/m^{2}s) and Abshir (2.72 mmole/m^{2}s) under non stressed condition, while SorColl 146 had the least value (1.85 mmole/m^{2}s). E was also reduced with increasing drought stress in the sorghum seedlings except in SorColl 146, SorColl 179 and SorColl 60 at 25% (Figure 41). However, E in SorColl 163 was highly reduced followed by SorColl 178, Abshir, B 35, E 36-1.

Sorghum seedlings of Abshir, B 35, SorColl 178 and E 36-1 controlled rate of transpiration highest relative to control plants at both 55% and 65% water deficit levels (Figure 41). SorColl 163, SorColl 179, SorColl 146, and SorColl 60 were relatively able to minimize E better than others relative to control plants at 55% and 65% (Figure 41).

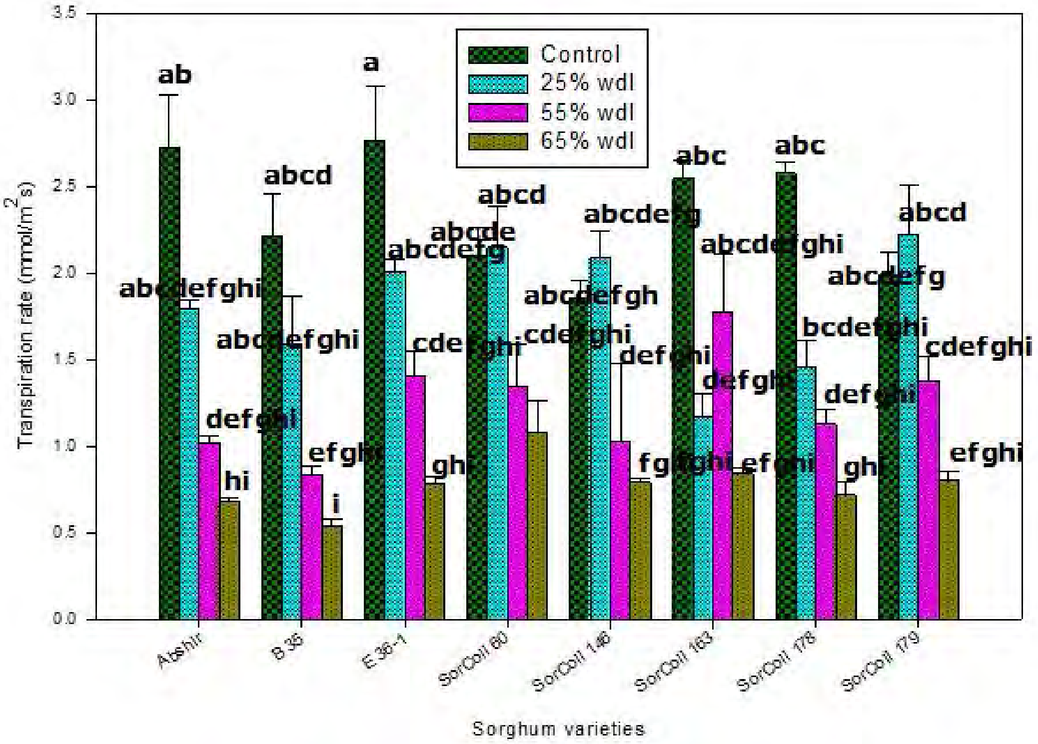

Figure 41 **Sorghum seedling transpiration rate response**

4.3.2.4 Stomata conductance (g_s)

The sorghum seedlings demonstrated a decrease in stomata conductance (g_s) with increasing drought stress effect except SorColl 146, SorColl 60 and SorColl 179, which increased at 25% wdl and decreased thereafter (Figure 40).

At 25%, the highest reduction in g_s was measured in SorColl 163 followed by SorColl 178, Abshir and E 36-1. In B 35, it was pari with control plants. At 55%, g_s in Abshir decreased highest followed by B 35 SorColl 178, SorColl 146, E 36-1, SorColl 179 and SorColl 60. SorColl 163 had no difference in g_s with control plants at this water stress level. At 65% a decrease n stomata conductance was observed in all varieties with highest decrease in Abshir (Figure 42).

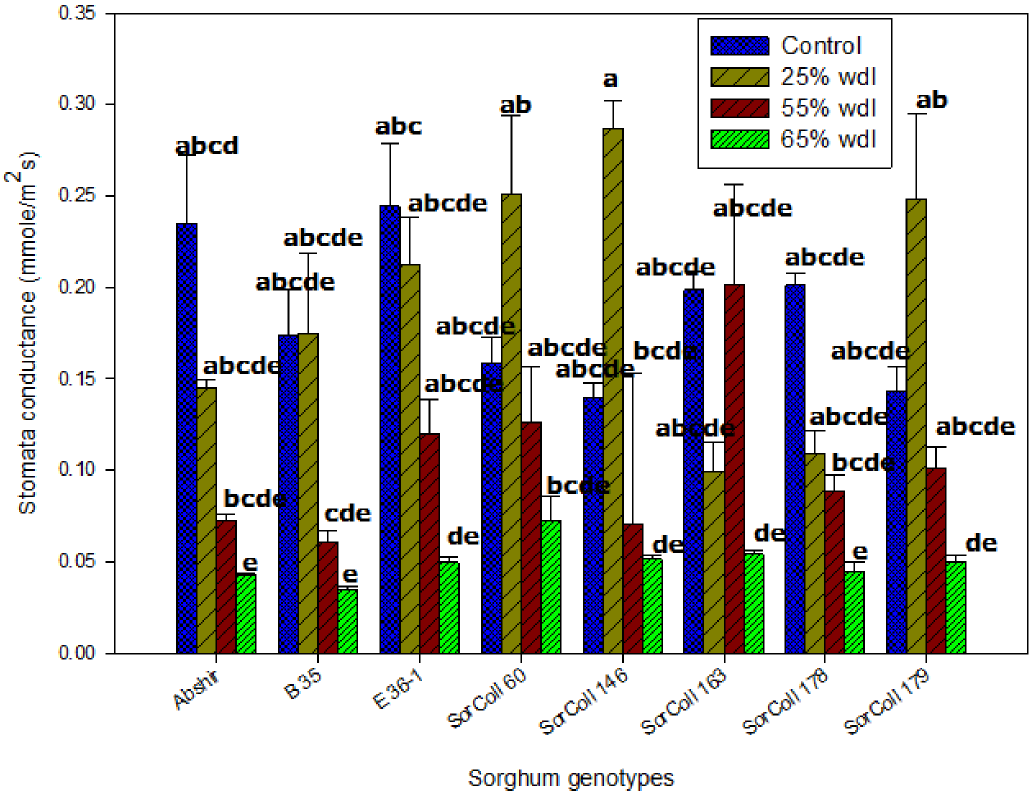

Figure 42 **Sorghum seedling stomata conductance response**

4.3.2.5 Intercellular Carbondioxide concentration (C_i)

The sorghum seedlings response in C_i was not significantly ($P > 0.05$) different, but C_i decreased in the stressed than control plants and some reversed C_i at more severe drought stress levels (Figure 41). Seedlings C_i concentration is diminished least at 55% water deficit level. At 25% wdl, C_i decreased in SorColl 178, B35, SorColl 163, SorColl 179, E36-1, Abshir and SorColl 60, where least decreased in the last. At 55%, C_i in SorColl 146 was reduced followed by SorColl 179, SorColl 178, SorColl 163, Abshir, SorColl 60, E36-1 and B 35 in a decreasing order. At 65%, B 35 loss in C_i was recorded the highest followed by SorColl 178, SorColl 60, Abshir, SorColl 179, SorColl 163 and E 36-1 in a decreasing order (Figure 43). The decrease in Ci with increasing drought was reverted in SorColl 146 and E 36-1 in the severe water deficit levels.

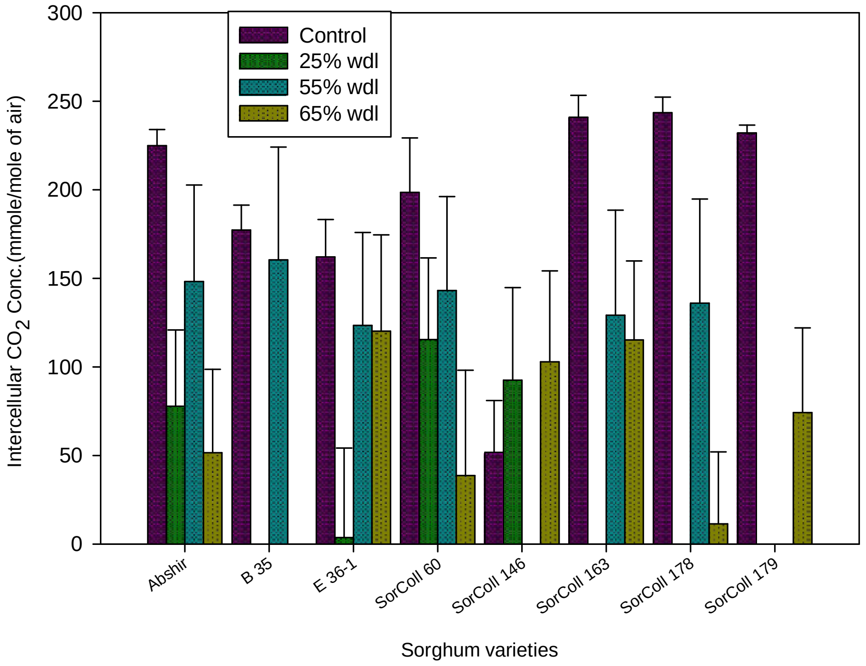

Figure 43 **Sorghum seedlings intercelular CO_2 concentrtion varation**

4.3.3 Seedling biochemical assay

4.3.3.1 Chlorophyll a and b response

i. Chlorophyll a content

Sorghum varieties had statistically insignificant ($P>0.05$) difference in Chl a content at non stressed condition ranging from 10.04 to 3.33 µgm/gm (Figure 44). The highest chl a content from leaf extract was noticed in SorColl 179 (10.04µgm/gm) followed by SorColl 146 (7.56 µgm/gm), B 35 (7.38 µgm/gm) and SorColl 163 (7.02 µgm/gm). SorColl 178 (3.33 µgm/gm), however, followed by SorColl 60 (5.25 µgm/gm) and E 36-1 (6.43 µgm/gm) had the least chl a content at the same condition. The chlorophyll a content was found maximum at 25% drought stress and decreased thereafter in all varieties up to 65% except SorColl 146 and SorColl 179 demonstrated a trend of gentle decrease with increasing drought stress (Figure 42). At the 25% water stress condition, B 35 (11.88 µgm/gm) and SorColl 163 (11.52 µgm/gm) recorded the highest while SorColl 146 (6.3 µgm/gm) and SorColl 178 (7.1 µgm/gm) shown relatively the least chl a content. At 55% Abshir (11.02 µgm/gm), E 36-1 (10.72 µgm/gm) and SorColl 60 (8.87 µgm/gm) SorColl 163 (6.55 µgm/gm) accumulated relatively the highest chl a in a decreasing order, whereas the lowest content was observed in SorColl178 (4.02 µgm/gm) followed by SorColl146 (6.26 µgm/gm) and SorColl 179 (6.34 µgm/gm). In general, all varieties had statistically in significant ($P>0.05$) chl a content but had above the controls at all drought levels except for a sharp decrease in SorColl 179 and SorColl 146 (at 25% and 55%), and B 35 (at 65%) which revealed below that of control plants (Figure 44).

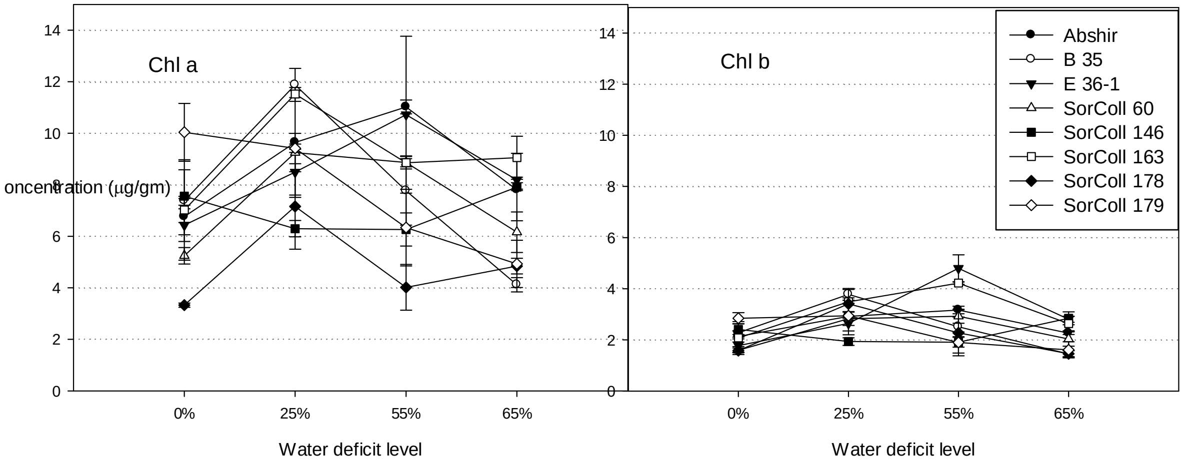

Figure 44 Sorghum seedling chlorophyll a and b content response to different water deficit levels

ii. Chlorophyll b response

The value of Chl b content was significantly ($P<0.05$) different among varieties (Table 20). It was found to be less than that of chl a by three folds, i.e. the ratio of chl a to chl b was within the range of 3:1 to 3.5:1 except for the SorColl 178 around 2:1 up to 55% (Figure 45). The trend of chl b content measured in the varieties at different water regimes was the same as that of chl a in that most demonstrated a gentle increase in concentration but with relatively lower concentration at 65% than 25% and 55%. SorColl 179, SorColl 146 and B 35 which had the highest concentration in chl a content under non stress were also found with higher chl b content (2.84 μgm/gm, 2.41 μgm/gm and 2.28 μgm/gm), respectively, under the same condition. SorColl 163 and E 36-1 demonstrated a more sharp increment in chl b content up to 55% water deficit condition over the other varieties (Figure 45). Abshir and SorColl 60 followed the same trend at 55% and 65% water regimes but with lower concentration. Other genotypes: SorColl

178, SorColl 179 and B 35 indicated a sharp decrease in chl b concentration both at 55% and 65%, where the chl b concentration at 65% was lower than that of the control plants. SorColl 146 uniquely decreased in chl a and chl b content in both 25% and 55% but both pigments increased again above the control plants at 65%. Therefore, our result showed that mild water stress increased the concentration of chlorophyll a and b but severe drought stress, 65%, caused majority to decrease but above that of control plants.

Chlorophyll b content assay grouped the varieties into three at all water deficit levels. At 25%, B 35, SorColl 163 and SorColl 178 scored highest, where as SorColl 146 had the least content and others Abshir, SorColl 60, E 36-1 and SorColl 179 were intermediate. At 55%, E 36-1 and SorColl 163 recorded the highest whereas B 35, SorColl 179, SorColl 178 and SorColl 146 had least content while Abshir and SorColl 60 were intermediates.

Table 20 Chlorophyll b concentration (μgm/gm) of sorghum varieties grown at different wdsl

variety	control	25% wdsl	55%wdsl	65%wdsl
Abshir	2.1bc	2.9abc	3.2abc	2.3abc
B 35	2.3abc	3.8abc	4.8a	1.5c
E 36-1	1.8bc	2.6abc	2.5abc	2.8abc
SorColl60	1.6	2.8abc	2.9abc	2bc
SorColl 146	2.4abc	1.9bc	1.9bc	2.8abc
SorColl 163	2.1bc	3.5abc	4.2ab	2.6abc
SorColl 178	1.6c	3.4abc	2.3abc	1.5c
SorColl 179	2.8abc	2.9abc	1.9bc	1.6c

Means that shared the same letter are not significantly ($p < 0.05$) different.

At 65%, E 36-1, SorColl 163 and SorColl 146 had the highest chl b content whereas, SorColl 178 SorColl 179 and B 35 recorded the least where Abshir and SorColl 60 were intermediates (Table 20). SorColl 146 differently responded to drought effect in that chl a and b content decreased in mild and increased in severe drought level.

4.3.3.2 Proline concentration

Many plant species naturally accumulate proline and proteins as major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play adaptive roles in mediating osmotic adjustment and protecting sub cellular structures in stressed plants. Different approaches have been contemplated to increase the concentrations of proline like compounds in plants grown under stressed conditions to increase their stress tolerance.

The results on free proline content revealed significant differences among the varieties at the last two water deficit stresses. A lower increase in proline has been obtained under mild drought stress and thereafter increased progressively with increasing level of drought stress.

i. Leaf proline concentration

A non significunt differences in leaf proline content has been observed under non stressed condition among the sorghum varieties. Proline content increment in leaf has been observed in all varieties with increasing drought stress (Figure 45 and Table 21). However, an increase in proline content was examined from the leaves of sorghum varieties grown under 25% wdl, which were also significantly ($P>0.05$) different among theselves. However, the increment was highest at 65% wdsl, which entails slow induction of proline concentration triggered by mild drought stress.

Table 21 **Proline concentrations from leaves of sorghum grown at different wdsl**

variety	control	25% wdsl	55%wdsl	65%wdsl
Abshir	4.00c	5.30c	11.00bc	10.80bc
B 35	3.90c	6.70c	7.30c	6.20c
E-36	3.30c	9.40bc	11.90bc	36.10a
SorColl60	3.80c	5,00c	5.50c	6.20c
SorColl 146	4.80c	5.30c	7.60c	5.30c
SorColl 163	5.30c	6.40c	17.60bc	26.20ab
SorColl 178	4.10c	5.80c	7.20c	5.20c
SorColl 179	2.60c	6.50c	6.40c	7.50c

Means that shared the same letter are not significantly ($p < 0.05$) different.

E 36-1 (9.36 μgm/gm), SorColl 163 (6.43 μgm/gm), SorColl 179 and Abshir (6.5μg/gm) had higher proline content than other varietiesgrown under 25%.

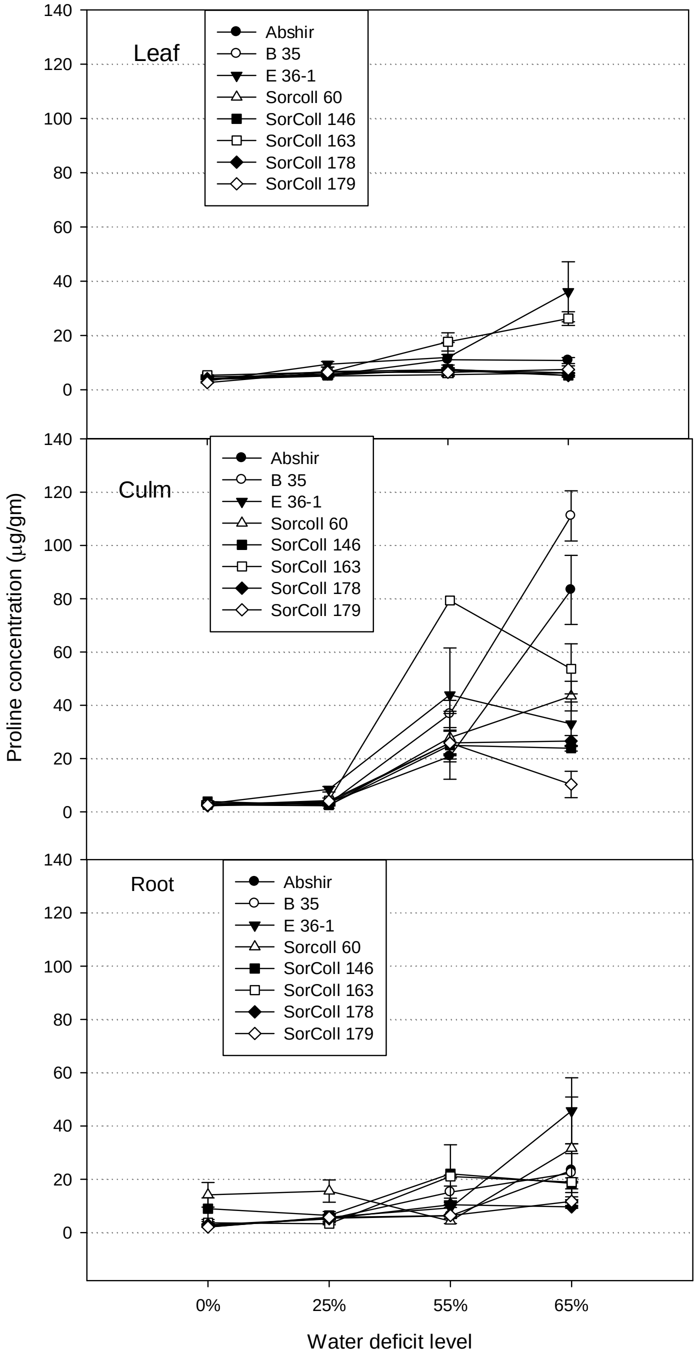

Figure 45 Sorghum seedlings response in proline concentration in leaf, stem and root plant organs at different water deficit levels

Abshir (10.76, 11.05µg/gm), E 36-1 (11.93, 36.10 µgm/gm) and SorColl 163 (17.62, 26.21µgm/gm) demeostrated a significant ($P<0.05$) increase, whereas SorColl 60 (5.26, 6.24µg/gm), SorColl 146 (7.57, 5.26µg/gm) and 178 (7.17, 5.24µg/gm) recorded the least content over other varieties as a result of the response to the last two severe stress conditions (Figure 45 and Table 21).

ii. Stem proline concentration

Sorghum varieties like from the leaf extract, had significant ($P<0.05$) difference in proline content among themselves as a result of water eficit treatment. Stem extracts had lower proline content in controls than leaf extract SorColl 146 (3.89µgm/gm), abshir (3.17µgm/gm) E 36-1 (3.02µgm/gm), SorColl 60 (2.62µgm/gm), SorColl 163 (2.57µgm/gm), SorColl 179 (2.48 µgm/gm), B 35 (2.38µgm/gm) and SorColl 178 (2.24µgm/gm) (Figure 45).

Table 22 Proline concentrations (µgm/gm) from stems of sorghum grown at different wdsl

variety	control	25%wdsl	55%wdsl	65%wdsl
Abshir	3.20c	3.50c	20.80bc	83.30ab
B 35	2.40c	3.10c	36.70bc	111.10a
E-36	3.00c	8.50c	43.90abc	33.00bc
SorColl60	2.60c	2.30c	27.80bc	43.50abc
SorColl 146	3.90c	2.50c	25.0bc	23.90bc
SorColl 163	2.60c	4.20c	79.30ab	53.70abc
SorColl 178	2.20c	4.00c	25.90bc	26.60bc
SorColl 179	2.50c	3.50c	25.80bc	10.30c

Means that shared the same letter are not significantly ($p < 0.05$) different.

Like the leaf extract, the proline concentration from the stem of varieties grown at 25% drought stress also revealed no significant ($P<0.05$) difference with varieties grown under no stress (Table 22). E 36-1 (8.48μgm/gm), SorColl 163 (4.21μgm/gm) and SorColl 179 (4.05μgm/gm) had the highest proline content over other genotypes. However, unlike the extract from the leaf, the proline content from the stem extract of all varieties shown a sharp increase at 55% and 65% drought stress levels. Slight lowering but still above 55% was demonstrated at 65% except B 35, Abshir and SorColl 60 which kept increasing in proline concentration with drought stress. SorColl 163 (79.29μgm/gm) had the highest concentration of proline followed by E 36-1 (43.88μgm/gm) and B 35 (36.74μgm/gm) at 55% drought stress. At 65% proline concentration increased sharply in B 35 (111.07μgm/gm) followed by Abshir (83.29μgm/gm) and SorColl 163 (53.67μgm/gm). SorColl 179 (10.27μgm/gm), SorColl 146 (23.86μgm/gm) and SorColl 178 (26.57μgm/gm) recorded the least proline content at 65% drought stress. There was both continual increase like in B 35, Abshir, SorColl 60 and SorColl 178 and a decrease like in SorColl 179, E 36-1, 163 and SorColl 146 in proline content of the varietiesgrown at 65% compared with that of 55% (Figure 45 and Table 22).

iii. Root proline concentration

Similarly, root extract from the varieties in the control had low proline contents: SorColl 60 (14.17) SorColl 146 (8.95), SorColl 163 (3.69), SorColl 178 (2.93), B 35 (2.69), Abshir (2.29), E 36-1 (2.10) and SorColl (2.07). Like the other two organs, the poline content in the root extract increased with increasing drought stress significantly ($P<0.05$) (Table 23). The proline content values in varieties grown at 25% and no stress condition were not significantly different (Table 23). Significant increament in proline content have been recorded in all varieties up on exposure to 55% and 65% drought stress levels. At 55% moisture level, SorColl 146 (22.07µgm/gm), SorColl 163 (21.07µgm/gm) and B 35 (15.19µgm/gm) revealed relatively higher proline content over the other genotypes. whereas, Abshir, SorColl 179 (6.4µgm/gm), E 36-1 (9.29µgm/gm) and SorColl 178 (10.45µgm/gm) recorded the least free proline content (Figure 45).

At 65% moisture level, E 36-1 (45µgm/gm), SorColl 60 (31.62µgm/gm) and B 35 (22.33µgm/gm) recorded the highest proline content over the other genotypes. On the other hand, SorColl 178 (9.64µgm/gm), SorColl 179 (11.71µgm/gm) and SorColl 146 (18.48µgm/gm) had gentle slope increment in proline content with incresing drought stress and recorded the least free proline content at 65% moisture level. Insignificant variation in proline content was observed between treatmwents 55% and 65%. A decrease in proline content from roots at 65% relative to 55% was observed only in varieties SorColl 178, SorColl 146 and SorColl 163 (Figure 45).

Table 23 Proline concentrations (µgm/gm) from roots of sorghum grown at different wdsl

variety	control	25%wdsl	55%wdsl	65%wdsl
Abshir	2.3b	5.4b	6.3ab	23.4ab
B 35	2.7b	5.1b	15.2ab	22.3ab
E-36	2.1b	5.8b	9.3ab	45.7a
SorColl60	14.2ab	15.6ab	4.3b	31.6ab
SorColl 146	9ab	6.5ab	22.1ab	18.5ab
SorColl 163	3.7b	3.3b	21.1ab	18.9ab
SorColl 178	2.9b	5.7b	10.5ab	9.6ab
SorColl 179	2.1b	5.2b	6.4ab	11.7ab

Means that shared the same letter are not significantly ($p < 0.05$) different.

Of the three plant organs considered the stem part recorded highest proline content in severely stressed plants (Table 22). Leaf proline at control and mild stress had recorded relatively slightly higher but increased insignificantly ($P<0.05$) with increasing drought. This may be due to the fact that proline is a multiporpose amino acide, which may be needed always with limited concentration in the leaf, chemical industry of the plant. Root free proline content was determined between the leaf and stem under the 55% and 65% drought conditions. At mild drought stress majority of the sorghum plants had proline with higher content at the root part (Figure 45 and Table 23).

Correlation coefficient of the seedling traits indicated that proline from leaf, stem and root were negatively correlated with chla, chlb, dry shoot and root biomass and all physiological traits (Table 24). On the other hand, dry shoot and root biomass was positively correlated with chla, chlb, SPADR and all physiological traits. Moreover, all related traits (biomass-biomass, proline-proline and physiological traits) had positive correlations among themselves. Assimilation rate was significantly positively correlated with dry shoot and root biomass, conductance and transpiration. Prolines from all organs were significantly negatively correlated with physiological traits except ci (Table 24).

Table 24 **Correlation coefficient of proline from leaf, stem and root, chla, chlb, dry shoot and root biomass, SPADR, A, cond, internal Ci and transpiration**

	DshtBM	**DrtBM**	**prolf**	**prols**	**prolr**	**chla**	**chlb**	**SPAD**	**photo**	**cond**	**Ci**	**Tran**
DshtBM	1											
DrtBM	0.98*	1										
prolf	-0.899	-0.905	1									
prols	-0.974*	-0.98*	0.959*	1								
prolr	-0.833	-0.839	0.971*	0.935	1							
chla	0.348	0.343	-0.263	-0.433	-0.436	1						
chlb	-0.043	-0.043	-0.061	-0.122	-0.294	0.864	1					
SPAD	0.819	0.823	-0.901	-0.923	-0.968*	0.646	0.472	1				
Assimi	0.962*	0.966*	-0.978*	-0.974*	-0.912	0.220	-0.072	0.845	1			
cond	0.951*	0.954*	-0.965*	-0.996*	-0.959*	0.471	0.193	0.952*	0.963*	1		
Ci	0.086	0.098	-0.344	-0.101	-0.208	-0.774	-0.632	-0.043	0.312	0.092	1	
Tran	0.920	0.926	-0.985*	-0.945	-0.916	0.136	-0.107	0.823	0.992*	0.938	0.42	1

* signifies significant different at $P \leq 0.05$

5 DISCUSSION

5.1 Plant growth response

5.1.1 Plant height

Growth results from the interaction of all the processes within the plant and is expressed as increment in dry mass, volume, length or area of cells (Lambers *et al.*, 1998). All the processes responsible for the plant growth are influenced both by endogenous and exogenous factors. The different responses /performance/ to the environmental factors can be considered as means of selection for the resistant types under stress conditions. Maintaining plant growth or showing relatively better performance is associated with tolerance of the plants to stress conditions. It has been described in several studies that plant height is often influenced by many environmental factors including moisture stress and soil fertility that affect the photosynthate allocation for cell elongation and expansion.

In our study, loss in plant height increased with increasing severity of water deficit stress levels both at field under terminal drought stress and seedling stage. Similarly, height of sorghum, maize and sunflower studied for biogas source were reduced (Schittenhelm, 2010) with increasing drought. Plant height loss before exposure to progressive stress in E 36-1 was increased from 8% at 25%wdl to 14 % at 80% wdl followed by SorColl 163 (4% at 0%wdl to 14% at 80% wdl) were least affected. SorColl 179 and SorColl 146 followed by Abshir were relatively highly affected in height over others at 65% and 80% wdl. Pre-flowering susceptible varieties shown unexerted or small panicles at the time of blooming due to drought stress, the effect was more pronounced at the severe water deficit stress levels. Pre-flowering water stress levels also delayed maturity in all varieties

(Figure1A), for example SorColl 163 and SorColl 178 demonstrated heterogeneous date of flowering among plants in different water deficit levels. Researchers suggest that radiation interception during stem elongation has been associated to the effect of photoperiod to the final number of grains in wheat (Gonzalez *et al*., 2005) which means that a longer stem elongation phase results in greater crop growth during this phase, higher spike dry matter at anthesis and subsequently more grains being filled. This is experimentally proved that stem elongation phase altered the duration of reproductive phase, which was associated with changes in the number of fertile florets and grains as a result of modifications in spike dry matter at anthesis (Gonzalez- *et al*., 2005). Moreover, physical positions for markers in QTL regions projected on the sorghum genome suggest that genes of known plant height in addition to the maturity gene had a major confounding impact on the expression of yield and stay green QTL (Sabadin *et al*., 2013). On the other hand, plant height has been explained to be negatively related to the HI (Royo *et al*., 2007), but it has been shown a minimum value below which yield limitation is evident (Slafer *et al*., 2005). In general, the last two water regimes were set to be below the threshold sorghum varieties can normally function, with 0.50 (50%) plant allowable soil moisture depletion (FAO, 2002), reduced plant height significantly before progressive water stress was applied. Pre-flowering drought stress effect was found more pronounced on culm height than the other two components followed by the peduncle height (Figure 1). Panicle height was relatively resistant to pre-flowering drought stress over the other parts of the culm (Figure 1).

Progressive water deficit stress imposed after flowering stage affected sorghum varieties differently. Abshir, E 36-1, SorColl 163 and SorColl 179 were relatively negatively affected less than SorColl178, SorColl 60 and SorColl 146, probably the former group are more efficient in residual moisture utilization. In addition to other traits, probably the drought escaping (Abshir), stay green (E 36-1 and SorColl 163) and large root biomass (SorCall 179) might have contributed in maintaining the height growth under post-flowering drought stress. Among others, SorColl 163 avoided reduction in height due to post-flowering drought stress most over other genotypes, depicting a possible continuation of photosynthesis that could help in biomass production satisfying the sink, grain filling. This is best indication for the existence of the stay green trait among the experimental sorghum genotypes. This supports the idea that greenness only cannot be regarded as post-flowering tolerance unless grain filling is achieved having functional leaves (Borrell and Hammer, 2000; Yang *et al*, 2001). In general, post-flowering drought stress was not found significantly reducing sorghum plant height, which is in line with the result of Mastrorilli *et al*. (1999) who concluded that most sorghum plants had high water demand during the first weeks, and irrigation should be emphasized in early stages and any time in which soil water is below wilting point.

It is also good to emphasize from our result of this section that post-flowering progressive drought stress demonstrated mild influence on plant height unlike that of the different water regimes, but the former drought type was found aggravating reduction in culm girth more than that of height (data not presented) in which susceptible forms were seen logging down in the absence of stalk borer.

The panicle height in Abshir and E 36-1 demonstrated a reduction in length in relative to that of SorColl 163 and SorColl 178 booted before the imposition of post-flowering drought stress, as both are early maturing and moisture loss could be attached to such panicle size reduction. In SorColl 60, SorColl 146 and SorColl 178 vegetative culm height values were relatively highly reduced over others (Table 4), probably due to faster translocation of stem reserves to the sink, ensuring seed filling and the photosynthesis rate in these varieties must have slowed or ceased earlier than others.

Especially, in case of SorColl 178 it was observed with significant reduction in girth and was prone to logging. This is the feature of sorghum varieties that lack stay green trait or can be attached to the trait described by Thomas and Howarth (2000) called cosmetic stay green because it confirms for faster rate of des-functioning of photosynthetic pigments.

The water deficit stress effect on plant height was higher at seedling stage under greenhouse condition than the field terminal drought stress. As compared with control, the shoot height of the sorghum varieties was reduced to16-41% at 65% water deficit condition. 80% water deficit was a limiting water regime for seedling establishment during transplanting in the green house, which was tolerable after establishment in the field. Mastrorilli *et al.* (1999) recommended emphasis in irrigation to be given any time in which soil water is below wilting point. The drought effect was found to be expressed in the vegetative and reproductive stages. The sorghum seedlings had different heights at non stress condition /control/ indicates that genetic variation in height existed among the varieties (Table 7). The SorColl 178 had the longest height (31.44cm) followed by SorColl 179 and B 35 (29.89, 30cm) and SorColl 60 had the shortest height (22.67cm) followed by Abshir (24.78cm). This height category did not coincide to the field study, where shorter height was to early maturing and longer height to late maturing sorghum varieties (Figure 1). This indicates significant height growth that brings significant variation among varieties based on days to maturity occurred in late growing season. The sorghum varieties have shown a sharp decrease in height with increasing level of water deficit (Table 7), except SorColl 60 and SorColl 146, which continued to increase their height at 25% and including at 55% for the former. Other studies also revealed a similar sharp decrease in height with increasing water stress. Drought stress study in three forage sorghum cultivars at 30%, 50% and 70% field capacity, in a pot experiment exhibited a decreasing values in plant height, leaf area, leaf water potential (LWP) and relative water content (RWC) with increasing drought stress maintaining the same ranking (Sher *et al.*, 2013). This is because water limitation results in decrease in osmotic potential of the soil which in turn reduces the turgor pressure and water potential of the cell sap (Sanchez-Diaz and Kramer, 1971). Drought conditions influence the root/shoot elongation process by affecting turgidity of the cells (Marcinska et al., 2013). Cellular growth is one of the most sensitive plant processes to water stress and is reduced long before photosynthesis or stomata conductance (Boyer, 1982). Water stress not only restricted the elongation of root/shoot cells but also the production of dry mass because growth and turgor pressure of the cells are directly related to each other (Boyer , 1982).

5.1.2 3rd Leaf area

Development of optimal leaf area is important to photosynthesis and dry matter yield. Our field study in sorghum varieties revealed a decrease in third leaf area with increasing water deficit stress, which supports findings of many authors who identified reduced leaf growth and in turn the leaf areas in many species of plants like in sorghum (Habyarimana, *et al*., 2004; Borrell *et al*., 2000 a and b) and many other species due to water deficit stress. SorColl 146, Abshir and SorColl 163 demonstrated least variation in both periods of leaf area measurement and maintained better during post-flowering drought stress (Figure 2). These sorghum varieties had intermediate sized leaf and relatively thicker (from my observation). The other sorghum varieties studied were also highly conservative in leaf area reduction, showing a gentle decrease in third leaf area with increasing water deficit stress visa vise that of control plants. This is in agreement with the result of Borrell *et al*., (2000 a and b) in sorghum varieties that maintained green leaf area at maturity (GLAM) under drought which were characterized with stay green trait. Leaf area response to pre-flowering drought stress existed in landraces with larger (SorColl 179) and smaller (SorColl 178) third leaf area. The leaf growth was more sensitive to progressive water stress in late maturing than early ones. Leaf area and total biomass reduction in sorghum was found induced by leaf extension reduction due to water deficit and the former was also found highly correlated with a reduction in cumulative transpiration (Habyarimana *et al*., 2004). The massive translocation of food to the reproductive part for seed filling must have resulted in a decrease rate of leaf cells division and elongation besides the progressive water deficit stress. So, withholding water later in post-flowering in all the treatments aggravated leaf area reduction except in those with stay green property. Therefore, the early exposure of the plants to water deficit levels was less important for leaf area maintenance but the plants with stay green nature were in advantage. The leaf area reduction together with senescence rate had gentle slope depicting high resistance to drought stress; this indicates the landraces are with trait to delayed senescence. Even a tendency of increasing leaf area was seen at severe wdsls, may be the plants grown under severe drought stress could respond faster to the increasing relative humidity in the last measurements. SorColl 163 was the most responsive variety among others. Studies revealed that leaf area maintenance under drought, besides maturity and plant height, to have direct relation with yield (Habyarimana *et al*., 2004). Variation in third leaf area has been considered as one of selection traits for tolerant types to environmental stresses (Borrell *et al*., 2000a and b; Sher *et al*., 2013).

Unlike the sorghum plants response to terminal drought, seedlings attained inverted parabolic shape upon exposure of the sorghum varieties to increasing level of drought stress, the 25% water deficit condition being with higher third leaf area (except E 36-1 and Abshir). The better performance of the sorghum varieties in leaf size response at 25% water deficit than the field capacity, it indicates that loss in turgor pressure is maintained well. Therefore, sorghum varieties are less responsive to low water deficit stress in leaf area. However, this increase in the 3rd leaf area at 25% water deficit was not involved in increase in photosynthetic rate as the plant height in the same water regime was smaller than that of the varieties in the control (Figure 22). This is in accordance with the finding of Fraser *et al*. (2009) for the leaf area of blue bunch wheatgrass (*Pseudoroegneria spicata)* with both water deficit and temperature stress conditions but a parabolic stomata density have been observed in their study. During water stress, total leaf area was also found decreased significantly in *Eragrotis curvula* (Colom and Vazzana, 2001) and in

Sorghum (Yadav *et al*., 2005). It was reported that the smaller size of the leaves resulted due to the smaller size of cells caused by drought conditions (Nilsen and Orcut, 1996). Researches revealed that the number of leaves per plant, individual leaf size and leaf longevity reduced by decreasing soil water potential, according to Reddy *et al.*, (2003) this is because leaf area expansion depends on leaf turgor, temperature and assimilates supply for growth, which is found to be all affected by drought. Bunce (1989) has also reported that reduction in leaf area, leaf growth and development being accountable for the largest reduction in photosynthesis. The smaller cell size of leaves, which contributed to smaller leaf area, in response to drought was observed at 55% water deficit condition, which must have brought a reduction in the potential for carbon gain of the sorghum varieties. The maximum and insignificant reduction in leaf area among the varieties was recorded at 65% water deficit condition and leaf area was reduced significantly under 65% water stress. Therefore, seedlings were less tolerant to drought stress in leaf area than that of field grown.

5.2 Biophysical properties

5.2.1 Soil Plant Analytical Development Reading (SPADR)

The portable chlorophyll meter (or SPAD meter) is a simple diagnostic tool that measures the greenness or relative chlorophyll content of leaves (Fontes and de Araujo, 2006), hence it has been used extensively to check for early senescence or /and nitrogen status (Rao *et al*., 2003) of leaves. The sorghum varieties had insignificant variation at early time of measurement and relatively SorColl 178 and SorColl 179 had the least SPAD reading values. E 36-1, Abshir and SorColl 146 followed by SorColl 163 had higher

SPAD reading, all above 50, value in most of the measurement times. Rao *et al*. (2003) reported that SPAD values in sorghum were ranged from 33 to 50 per cent at 50% flowering which could be used as a surrogate for leaf and stem nitrogen percentage in post rainy season. The progressive drought stress imposed at post-flowering caused a decrease in SPAD reading with lower value of the above two varieties and a sharp decrease recorded in SorColl 60 all had values above SPAD value 44. This supports the correlation made by Xu *et al*. (2000), under severe post-flowering drought conditions a stay green line B 35 had a SPAD values above 40 and an average total chlorophyll concentration of 0.085 mg/cm in their top leaves, which confirms the selected landraces studied are with a potential stay green trait. The sorghum varieties studied had almost insignificant change in SPAD reading with the drought effect. Studies, however, indicated severe stress factors, such as drought to susceptible crop plants strongly impair leaf expansion (Royo *et al.*, 2004) and thus plant growth (Villegas *et al.*, 2001) and further yield (Araus *et al.*, 2002). Based on results from SPADR, under such plant status, Araus *et al*. (2002) recommended total green biomass evaluation as potentially powerful method at a critical plant stage (i.e., anthesis) or its change over time than SPAD reading.

Seedlings of sorghum varieties had SPAD reading difference at non stress condition like field experiment. Unlike filed condition, 25% water deficit condition favored the varieties better than the controls and all increased in SPAD value. The genotype Abshir had maintained the higher value followed by SorColl 146 and SorColl 163 (40, 35 and 34, respectively) at 25% water deficit level. The increase in the mild stress condition can be ascribed among others to the sensitive nature of sorghum varieties to flooding where drainage from the pots of control plants would have been less after irrigation (maintained the field capacity longer period) due to small perforations. Unlike field condition, mid season severe water stress regimes decreased SPAD reading of all varieties. In this case, our result is in line with the finding of Jangpromma *et al*. (2010) where drought significantly reduced both SPADCMR (33.06 to 27.36) and chlorophyll content (6.64 to 3.78 gm/ cm^{-3}) for chlorophyll content in Sugarcane *(Saccharum officinarum* L.) in 20 days exposure to drought. Such a decrease in absorbing pigments by degrading have been attached with a mechanism of avoidance to reactive oxygen species mainly driven by excess energy absorption in the photosynthetic apparatus (Esfandiari *et al*. 2008; Mafakheri *et al*. 2010; Marcinska *et al*., 2013). At severe water deficit condition (55%), the SPAD values were found grouped in to two , B 35, SorColl 163, Abshir and SorColl 179 with relatively higher values (between 25 and 30) and the second group, SorColl 178, E 36-1 and SorColl 146 had lower value (reading around 20). In the most severe water deficit condition all decreased down to 10-15 SPAD value where E 36-1 and SorColl 163 were least affected and SorColl 179 and B 35 were highly affected. Peanut genotypes study for WUE demonstrated that those with high SCMR were found with higher WUE under drought conditions (Songsri *et al*., 2009). Chlorophyll content (SPAD) study on the other hand, revealed that seedlings of drought susceptible form of wheat was with slightly higher than that of resistant form on early days of drought treatment but the resistant form maintained higher level late days of treatment (Marcinska *et al*., 2013). A decrease in total chlorophyll with severe drought stress as in the 65% in other studies has been implicated to a lowered capacity for light harvesting. In our experiment, irrespective of the reduction in SPADCMR value with increasing drought stress in the green house, sorghum varieties at seedling stage didn’t show a visually discernible senescence except total death of seedling at 80% water deficit at early stage. SPADCMR is most used in agricultural practices in field condition.

B 35, SorColl 163, Abshir at both conditions and SorColl 146 only at field condition performed better in SPADR under severe drought condition with significance level ($P<0.05$).

Therefore, like the senescence rate, SPADR is not a sensitive parameter to screen drought adaptability at seedling and green house condition. Fontes and de Araujo (2006) criticized visual ratings of plant canopy that requires to be more evaluated and improved, as the quantitative results from using a SPAD meter are believed to provide good measure of the N requirement of plants under cultivation as long as appropriate SPAD critical values are established. Nemeskeri, *et al*., (2010) also found out that SPAD being the best indicator of water supply related with the density of stomata and for indentifying drought tolerant ones and used for apple varieties grafted on various stocks drought tolerant selection in agricultural practice.

5.2.2 Leaf senescence rate

Although leaf senescence is an adaptation mechanism to enhance photosynthate partitioning during grain filling, it is accelerated by drought leading to a decrease in canopy size, loss in photosynthesis and finally reduced yields (Dhindsa *et al*., 1981; Chen *et al*., 1991; Kipp *et al*., 2014). Selecting genotypes that delay leaf senescence under drying soil is becoming a core in crop production enhancement under post-flowering drought stress (Rosenow *et al*., 1996). This is evidenced by the possibility to enhance drought tolerance by delaying drought induced leaf senescence in many crops including in sorghum (Borrell *et al*., 2000 a and b; Borrell and Hammer, 2000); wheat (Rivero *et al*., 2007) and helped to maintain high water contents and retain with minimal reduced level of photosynthetic activity during drought. Besides, the newly invented physiological instrument such as Green sicker, the rate of green down as a result of drought has been commonly determined by means of visual rating (Xu *et al*., 2000). A visual senescence/ stay green rating was found to be a reliable indicator of leaf senescence (Xu *et al.*, 2000) and suggested as a useful method to sorghum breeders. Visual rating in our study indicated different responses in senescence rate to the different water regimes exposed (Figure 10). The senescence rate, however, was found to increase with increasing both the level of water deficit regimes during pre-flowering and subsequent exposure to the progressive water deficit stress during post-flowering in all genotypes. E 36-1, SorCol1 179, E 36-1, and SorColl 163 demonstrated lower senescence rate but within the range of 35-65% before grain filling evaluation time (Figure 10), while SorColl 60 and SorColl 178 were more severely senesced, 65% and 74.8% respectively, relative to other genotypes. According to Xu *et al.* (2000) our result seems to underestimate the stay green trait of the varieties under severe post-flowering drought conditions than the authors correlated highly susceptible sorghum lines with a stay green rating above 3.5 out of 5, which in our case means less than 30% senescence rate, correspondingly had a SPAD value of 27, probably this can be due to the additional exposure to pre-flowering drought stress and time of measurement soon after seed filling, which could be coincided with the time of nutrient remobilization for the grain filling, a major role of leaf senescence (Munne-Bosch and Alegre, 2004) and a reproductive sink accelerates drought-induced leaf senescence (Khanna-Chopra and Sinha, 1988). SorColl 163 maintained greenness even better than the check E 36-1, followed by SorColl 179 and SorColl 146 under both pre-flowering levels of water deficit and later post-flowering progressive drought stress. The benefits of stay green phenotypes have been described in sorghum (Borrell *et al.*, 2000 a, b), where the rate of leaf senescence was shown to be negatively correlated with yield under conditions of late (pre- and post-flowering) water deficits.

The response of the varieties in maintaining the green leaf to drought goes in line with that of 3rd leaf area and SPAD reading evaluation. Most probably, leaf physiological role in SorColl 163 and the larger root biomass in SorColl 179 must have played in delaying the leaf senescence rate better than others, as the role of stomata closure was limited in sorghum varieties under study. This result is in agreement with that of Stout and Simpson (1978), who indicated the existence of open stomata in both irrigated and non-irrigated plants using leaf diffusive resistance measurements so that under the imposed water stress conditions stomata closure was not affecting the transpiration requirement. On the other hand, the greater leaf senescence of the landraces SorColl 178 (38%), SorCol1146 (28.2%) and SorColl 60 (27.7%) at non-stress or mild condition may entail the existence of a drought avoidance mechanism to decrease rate of transpiration.

Like the result in SPAD reading, senescence records of early maturing varieties Abshir and E 36-1 with the late maturing varieties underestimated their capacity to stay their leaves alive up to the time of measurement after post-flowering progressive drought stress. This calls attention to be taken when both senescence rate by visual means and SPAD reading are used as means of screening purpose among populations with different maturing periods.

Dealing with pre-flowering and post-flowering drought stress responses in developing drought tolerant varieties is so vital as keeping their leaves alive under post-flowering drought stress are found susceptible to pre-flowering drought stress as in B 35 (un published green house study. This supports Rivero *et al.* (2007) who explained that food production can be ensured under water limited lands when drought tolerant crops are able to grow under any restricted water regimes without diminution of yield. So, to satisfy the demand of crop production increment under unreliable precipitation, which is most common in the arid and semi arid regions, the varieties with stay green trait must be capable of maintaining growth under any period of restricted water regimes. The suppression of drought-induced leaf senescence using transgenic plants resulted in outstanding drought tolerance as shown by Rivero *et al.* (2007), among other responses, vigorous growth after a long drought period that killed the control plants (Rivero *et al.*, 2007), they maintained high water contents and retained photosynthetic activity with minimal reduced level during the drought. Visual stay green ratings were found a reliable indication of leaf senescence in our study and it was also recommended to breeders as a useful means of evaluating genotypes for post-flowering drought tolerance (Xu *et al.*, 2000).

However, senescence at seedling stage under green house condition was not visually detectible while photosynthetic rate was extremely negatively affected by drought stress and hence, not applicable to use it for screening at this stage. This is because seedling stage is the pick time for carbon and nitrogen capture (Thomas and Ougam, 2014).

5.3 Dry matter accumulation and partitioning

5.3.1 Total biomass production

It is common phenomenon that stress factors, such as drought, strongly impair leaf expansion (Royo *et al*., 2004) and thus plant growth (Villegas *et al*., 2001). Therefore, the potentially powerful traits for evaluation in severe effect of drought are total green biomass at a critical plant stage (i.e., anthesis) or its change over time (Farooq *et al*., 2009). The destructive method of measuring biomass, a direct and feasible measurement, has been commonly used in studying crop productivity (Sher *et al*., 2013). The direct measurement, in our study, also revealed a significant difference in total dry weight per plant among the varieties at all levels of water regimes, which supports the outcome of researches done by others (Schittenhelm, 2010; Sher *et al*., 2013). The maximum total dry weight plant^{-1} was recorded in SorColl 179 (1679.37 gm) followed by SorColl 60 (1327.80 gm) at full irrigation (Figure 6), which supports the finding of Narayanan *et al*. (2013) in sorghum. The lowest total biomass was recorded in Abshir (470.31 gm dry plant^{-1}) at the same water regime. However, the varieties demonstrated a progressive weight loss with increasing water deficit levels. At mild stresses (25% and 55%), SorColl 163 and SorColl 146 recorded the least percent weight loss and it was most stable. At 80% water deficit level, the maximum per cent weight loss from the controls was demonstrated in SorColl 179 (60%) followed by early maturing (Abshir and E 36-1). SorColl 146 had the lowest percent weight (36%) loss as compared to other genotypes. Better biomass acquisition under drought condition, was implied stable photosynthetic and respiration balance (Flexas *et al*., 2006). Wheat plants biomass increase has been associated with increase in photosynthetic rate and this in turn was higher at grain filling stage, which helped for yield increment (Reynolds *et al.*, 2005). Similarly, higher total biomass was accompanied with higher gains in yield potential of maize (Tollenaar and Lee, 2006).

Above ground dry mass yield in our study was found associated with plant height. However, yield was not directly associated with plant height under drought stress condition. Our result supports many studies which confirm sorghum varieties with different genetic culm height whose leaves photosynthetic activity vary in drought conditions and those with stay-green trait and that mature early have got a yield advantage over others (Ludlow and Muchow, 1990; Borrell *et al.*, 2000a and Borrell *et al.*, 2001; Thomas and Ougam, 2014). These authors indicated that genotypes with stay green trait could get a yield advantage as far as carbohydrate-rich produce is concerned.

5.3.2 Shoot biomass

The field study demonstrated variation in both biomass among sorghum varieties at control and stressed conditions. Above ground total biomass measurement at harvest time indicated an increase in biomass loss with increasing water deficit levels in all sorghum genotypes, which is in line with other findings (Olufayo, 1997). Least shoot biomass record in the controls was found in the early maturing varieties (Abshir and E 36-1) followed by SorColl 178. SorColl 179, SorColl 146, SorColl 60 followed by SorColl 163 had shoot biomass in a decreasing order. Shoot biomass loss from the control plants is recorded the highest (greater than 50%) in E 36-1, SorColl 163 and SorColl 178 in the severe water deficit regimes. This can be attached to a strategy to enhance root biomass reduction in the first two and an adverse effect to the third because the first two have been recording best performing over other varieties in the previous parameters under the same drought condition. A study in tropical sorghum landraces by Habyarimana *et al*. (2004) in Mediterranean climate revealed that sorghum can be a potential crop for irrigation practice in that yields higher in total aboveground dry biomass (33–51 t ha^{-1}) than under rain-fed conditions (20–29 t ha^{-1}).

5.3.3 Root biomass

Water deficit is primarily placed as the cause of root death under field conditions (Smucker *et al*., 1991). Therefore, maintaining root growth is very important for a plant drought tolerance (Huang *et al*., 1997). In our field study, sorghum varieties responded in root biomass differently to the different deficit levels and progressive water stress. Root biomass value for E 36-1 increased at severe drought stress levels and root biomass in SorColl 163 increased at medium stress level but decreased at the severe water deficit levels at 65% and 80%. While, SorColl 178 and Abshir had relatively stable root biomass in all water regimes. In case of landraces SorColl 60 and SorColl 179, root biomass diminished with increasing water deficit levels. The dry and wet root biomass had less variability in response to drought in some sorghum genotypes. SorColl 146 demonstrated a decrease in wet biomass and almost stable in dry root biomass with increasing water deficit stress. The highest difference between dry and wet root biomass was demonstrated in mild drought treatments and SorColl 179 had the largest difference in dry and wet root biomass over others. A drought susceptible cultivar had a large number of roots per plant in non stress condition and a low root penetration index compared with drought-resistant (Babu *et al*., 2001).

Although root biomass is always found to decrease with increasing drought stress, it better explains the performance of crops in drought condition when compared with the shoot biomass. The root weight loss from the control plants is relatively smaller than that of shoot in conformity to the work of Sacks *et al*., (1997) where root growth was not significantly reduced under water deficits in maize and wheat. Many researchers also found root biomass to be a good indicator of drought avoidance and greater root biomass was positively correlated with higher root penetration index in crop (Babu *et al*., 2001).

Like the leaf area and chlorophyll content, some sorghum seedlings improved in shoot biomass production in relation to controls except Abshir (7%) and SorColl 178 (2%) which slightly were negatively affected by the mild drought stress (25%). This may be due to pot soils saturation to field capacity temporarily imposed an over saturation situation during watering as sorghum plants are adapted to drier regions (Subudhi *et al*., 2000). Seedlings of sorghum varieties were found decreasing in root biomass with increasing drought stress except SorColl 60, SorColl 146 and SorColl163. SorColl 60 (17%) and SorColl 146 (10%) had positive root biomass response and SorColl 163 increased by 6% dry weight to mild (25%) drought stress, while others was negatively responded. Our result partially support finding of Zhang *et al*. (2009) that indicated biomass increase in root as a result of drought effect in seedlings of forage triticale. However, at the last two water regimes (55% and 65%) both the shoot and root biomass were insignificantly severely affected. Other findings also showed that the root growth in peanut was increased initially, but in severe stresses it was reduced because of the severe drought stress (Songsri *et al*., 2009). Similarly, studies in Jabeen *et al*. (2008) water deficit conditions (60% of field capacity) imposed for a period of 15 days to 12-day-old plants had a significant detrimental effect on the growth of 7 maize cultivars. These results and some earlier studies in rice and wheat reports demonstrated exposure to progressive drought stress during early growth stage suppressed the shoot dry matter production, root development and water uptake (Banoc *et al*., 2000; Lopez- Bucio *et al*., 2003). Under drought conditions with lower cell water potential, the expansion of developing cells is limited and the growth is reduced. Cellular growth is one of the most sensitive plant processes to water stress and is reduced long before photosynthesis or stomata conductance (, 1973).

Drought stress imposed late in the maturity stage of sorghum varieties caused shoot biomass loss (4-30%) followed by booting stage (3-14%). The drought imposition at different phases of the reproductive stage had a weaker negative impact on the biomass performance of the sorghum genotypes (see also Figure 11 and 35). However, different responses were observed among the sorghum varieties. SorColl 163 and E 36-1 were highly resistant to the short period drought stresses except for the maturity stage imposition, whereas the most susceptible landraces were SorColl 179 and SorColl 178 (Figure 31). The least shoot biomass loss was recorded in sorghum stressed at flowering stage (1.66 - 12%) (Figure 31). Similarly, roots exposed to drought at the maturity stage also decreased highest (12-37%) followed by those imposed at grain filling stage (4-25% weight loss) (Figure 32). The least root biomass loss was recorded in plants exposed at booting stage. The last could be technical problem, for roots in the dry soils in the late season are so fragile and dead, made difficulties during sample collection at field condition.

5.3.4 Root-Shoot Biomass ratio

In our field study, sorghum plants were observed to involve partitioning of dry matter between the two systems in combating drought (Figure 8). Drought stress reduced more shoot system than root system in comparison to control plants. Biomass partitioning in four alfalfa genotypes submitted to progressive drought was also recorded (Erice *et al.*, 2010). The changes in root to shoot dry mass ratio is one of the indicator mechanisms involved in the adaptation of plants to drought (Turner, 1997; Erice *et al.*, 2010). Hence, our field study indicated that E 36-1, SorColl 163, SorColl 178 and SorColl 146 demonstrated increased root to shoot ratio, partitioning more biomass to the root. SorColl 60 and Abshir responded positively only to the mild and sever water deficit stresses respectively. The sorghum genotype SorColl 179 with large root biomass responded least in dry mater partitioning to the water deficit regimes. This is because the vigorous root endowed, an adaptation for the seasonal rainfall harvest (Palta *et al.*, 2011) couldn't made it in stored soil water because over absorption under such condition exposes it to the risk of soil water depletion.

The sorghum varieties at seedling stage responded differently in dry root-shoot biomass ratio to the different water regimes. Unlike, the result from the field, a decrease in root-shoot biomass ratio from the controls was recorded by the majority except SorColl 163, SorColl 146 and SorColl 178 with increasing drought stress. This indicates the drought effect was indiscriminately inhibited both shoot and root biomass production. Therefore, the adverse effect of the drought at seedling stage in the green house was a limiting factor for the dry mater partitioning between the two systems. A common adverse effect of low water potential or water deficit stress was also recorded due to the reduction in fresh and dry biomass production in different crops such as grasses (Ashraf and Yasmin, 1995), maize (Abrecht and Carberry, 1993) and wheat (Ashraf *et al*., 1998).

5.3.5 Root Length Density

Root length density measurement was recorded in the field experiment, because pots used were not convenient in the greenhouse. Sorghum varieties in our study were endowed with long depth penetration (beyond 80 cm) both in the control and stressed conditions indicating that all had a mechanism of taping water from a longer depth. This may help to enhance drought resistance by improving the ability of the crop to extract water from the soil (Wright *et al*., 1983). Rapid growth of functional roots into unoccupied regions of soil increases the competitive and tolerant traits of plants due to their greater acquisition rates of biogeochemical resources (Eissenstat and Caldwell, 1989). Deep rooting, root length density (RLD) and root distribution/ architecture have been identified as drought adaptive traits (Taiz and Zeiger, 2010) that can be used as selection criteria for drought resistance. Deep rooting and higher RLD are described as the second mechanism of the two important strategies for adaptation to water deficit, which is an escape mechanism from water stress (Tari *et al*., 2013).

In all sorghum varieties under non stress condition /full irrigation/, RLD distribution was found to depths greater than 80cm and skewed (greater than 50%) to the first two upper soil depths, 0-40cm in all genotypes. Similar root distribution patterns were reported for maize, sweet sorghum and sorghum-sudangrass hybrids (Schittenhelm and Schroetter, 2014). The highest RLD proportion at 0-41cm depth is read in Abshir (71.9%) and

SorColl 178 (71.3%) and the genotype with almost uniform distribution was SorColl 179 (55.4%) (Figure 15). With increasing drought stress, the RLD distribution has tried to amend by increasing root RLD in the two lower soil depths. The sorghum genotype with the highest biomass and RLD at full irrigation decreased in RLD with increasing drought stress. The RLD of sorghum varieties decreased with increasing soil depth but the rate varied among genotypes. This is in line with the finding of Schittenhelm and Schroetter (2014). Drought tolerance index (DTI) increased with increasing drought stress except for SorColl 179. The DTI value in Abshire, SorColl 178 and SorColl 60 exceeded (above 1.4) over others followed by another group that included SorColl 163, E 36-1 and SorColl 146 (1.1-1.4). Abshir had with higher DTI that increased with increasing drought effect; similarly, higher and increasing DTI value under severe drought stress have been found in drought resistant peanut varieties (Songsri *et al.*, 2008). Having high root length density under drying soils increases the number of contact points between root and soil, which is crucial for water uptake. On the contrary, SorColl 179 demonstrated least index value and also declined with increasing drought stress. Our result is, therefore, a composite of sorghum plants with positively and negatively responsive as well as non responsive forms in root length density to drought stress, which is against the finding of Schittenhelm and Schroetter (2014) who generalized sorghum crop with a higher water uptake capacity (positively responsive in DTI) from sweet sorghum and sorghum-sudangrass hybrids response to drought effect. The varieties with stay green trait were clustered together under the intermediate group, which entails lack of strong linkage between both traits. Whereas, exploitation of the residual moisture in soils during post-flowering drought stress is so vital for the delay of senescence and securing grain filling as in the legumes (Songsri *et al*., 2008) which demand higher root contact. Therefore, stay green did not directly associate with higher density of roots trait but the later might be important to maintain the characteristic physiology of the leaves that help leaves to stay alive longer, as a result roots and other sink organs could be furnished with photosynthate. Similar result was observed in stay green rice under deficit condition (Hoang and Kobata, 2009). Root study on rain fed lowland rice by Henry *et al*. (2011) also revealed greater drought resistance association with deep root growth and the highest drought response index in yield (less reduction in yield by drought stress). So far, targeted approaches to drought tolerance have largely been emphasized on shoot parameters, particularly those associated with photosynthesis and recently stay green trait, rather than on root traits such as soil moisture capture for transpiration, root architecture, and improvement of effective use of water, which are now increasingly considered as important traits for yield improvement in C4 plants under drought stress (Lopes *et al*., 2011). The root study and stay green trait should also be researched together than separately to the detail, because the efficiency in photosythate utilization by roots is guaranteed when effective root density and distribution existed when the plant depends on residual moisture.

5.4 Physiological responses

5.4.1 Assimilation rate (A_N)

The relationships between stomata resistance, leaf water potential, leaf temperature, and environmental factors such as temperature and humidity are important to rate of photosynthesis in plants growing in arid or semiarid conditions. Photosynthesis is often used as a criterion of dehydration tolerance or in general drought resistance. It became popular when evaluation of transgenic plants and as instrumentation became better and easier to use. Variation in dehydration tolerance has been identified by genotypic physiological performance under different water deficit conditions and at a whole plant level the effects of drought is usually perceived as a decrease in growth and photosynthetic carbon assimilation (Zlatev and Lidon, 2012).

Assimilation rate of the sorghum varieties was similar (47-52μmole CO_2/m^2s) except for SorColl 78 with lower value (43.4 μmole CO_2/m^2s) at non stress condition. Sorghum varieties at both seedling and late season stage study demonstrated a decrease in assimilation rate with increasing drought stress but with different degrees.

At 25% wdl, SorColl 146 (48μmole CO_2/m^2s) SorColl 163 (47.4 μmole CO_2/m^2s) and SorColl 60 (46.2 μmole CO_2/m^2s) recorded highest over other genotypes. Similarly, seedling of SorColl 178 (at 25%) and SorColl 179 did not show a reduction assimilation rate (A_N). A decline in the photosynthetic rate under drought stress conditions could be attributed either to a decrease in stomata conductance and/or to non-stomata limitations (Cornic and Massacci, 1996). Both Stomata and non stomata limitations to photosynthesis have been observed in lilies (Zhang *et al.*, 2012), chickpea cultivars (Mafakheri *et al.*, 2010) due to drought stress. Most researchers agree that the stomata closure and the resulting CO_2 deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano, 2002; Berhanu Amsalu *et al.*, 2012). The down regulation of the photochemistry due to drought at early vegetative growth stages has been regarded as the main photoprotective/ regulative mechanisms in sweet sorghum Zegada-Lizarazu and Monti (2013).

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At 65% and 80 % levels, assimilation rate was highly insignificantly affected by the wdl and progressive drought effect in the field study (11-14 and below 1 μmole CO_2/m^2s) and at 65% for sorghum seedlings. The effect of drought stress at 65% on sorghum seedlings was beyond regulation mechanism as the biomass was found highly reduced. Such decrease in leaf photosynthetic rate occurs as the relative water content and leaf water potential decreases due to drought (Lawlor and Cornic, 2002) and ultimately becomes the cause for the decrease in the amount of biomass (Reynolds *et al*., 2005) and grain yield (Reynolds *et al*., 2005; Gholamin and Khayatnezhad, 2011). Photosynthetic rate reductions due to increasing drought stress have been observed in a variety of plant species including sorghum genotypes. Photochemical efficiency of PSII in five test varieties of sorghum was severely reduced by multiple abiotic stresses, where PEPcase activity was more susceptible to water stress (Jagtap *et al*., 1998). Maize cultivars under water deficit conditions were also found with reduced photosynthetic capacity (A_N) (Ashrafi *et al*., 2007). However, sorghum is more drought-resistant species than maize as the corn was found losing much more water before the stomata were fully closed under the same condition (Singh and Singh, 1995). Evaluation of Tea (*Camellia sinensis* L.) response to moisture stress in some selected physiological and biochemical parameters (Damayanthi *et al*., 2010) revealed that the drought tolerant cultivars maintained a high water status with substantial rate of photosynthesis as a result of osmotic adjustments, elevated total soluble sugars, where these parameters were recommended to be used in drought screening studies. Complete failure in establishment of transplanted sorghum seedlings at 80% FC can be due to metabolic impairment as suggested by (Flexas and Medrano, 2002). The photosynthetic systems of C4 plants like sorghum are as sensitive to drought induced inhibition as they are in C3 plants (Ripley *et al*., 2010) even more susceptible (Ghannoum, 2009; Ibrahim *et al*., 2008). So, only drought stresses that did not cause total sorghum failure were found important in screening the genotypes.

5.4.2 Transpiration rate (E)

Individual leaves have been recommended in cereals, such as maize, which are characterized by tall height and more heterogeneous canopies for canopy temperature measurement Araus *et al*., 2008). Stomata play a key role in plant adaptation to changing environmental conditions as they control both water losses and CO_2 uptake. Our result from the gas exchange analyser indicated that transpiration rate (E) in sorghum varieties at non stress conditions had variations and values were clustered in to higher in SorColl 179, SorColl 178 and SorColl 60 (2.13-2.3 mmole/m^2s); SorColl 146 E and 36-1 (1.85 mmole/m^2s) recorded intermediate as well as SorColl 163 and Abshir (1.1-1.2 mmole/m^2s) had lower transpiration rate (Figure 23). The slight variation can be ascribed to technical barriers but it is easy to understand the existence of genetic variations among sorghum varieties.

Like the rate of assimilation, slight increase in drought to 25% deficit caused an increase in the rate of transpiration (2.5 -3.8 mmole/m^2s) in all except SorColl 179 which decreased to 2 mmole/m^2s. Similarly, at seedling stage rate of transpiration of SorColl 146, SorColl 179 and SorColl 60 increased at 25% FC greenhouse condition. This positive response may be explained as the varieties were less sensitive in rate of transpiration under mild drought stress due to higher demand of CO_2 by the plants for more food synthesis. Although inhibitions at the chloroplast level have also been proposed, stomata effects are usually considered to be the first and major limitations to CO_2 fixation at mild to moderate drought stress (Flexas and Medrano, 2002).

E was also recorded least both in the two severe water deficit levels (Figure 23 and 40). However, sorghum varieties studied at field were clustered in to two with above that of control but below that of 25% (Abshir, SorColl 163) and others decreased transpiration rate with increasing drought stress (E 36-1, SorColl 60, SorColl 178, SorColl 146). Sorghum seedlings decreased transpiration rate with increasing drought. Abshir, B 35, SorColl 178 and E 36-1 controlled rate of transpiration highest over other varieties at both 55% and 65% water deficit levels (Figure 41).

The role of stomata closure and the resulting CO_2 deficit in the chloroplasts as main cause of decreased photosynthesis under drought stresses has been identified (Flexas and Medrano, 2002; Zhang *et al*., 2012), which supports partly our result for the second group and sorghum seedlings response. Such small decline in stomata conductance under mild stress may have protective effects against stress, by allowing plant water saving and improving plant water-use efficiency (Chaves *et al*., 2009). This was also reported in terminal drought stressed pearl millet (*Pennisetum glaucum* (L.) R. Br.), abscisic acid was the cause for the decline of transpiration rate (Kholova *et al*., 2010).

On the other side, the decrease in photosynthesis in presence of transpiration rate can be either due to the limitation of mesophyll conductance ($g_{m)}$, where g_m can also vary at least as fast as stomata conductance (Flexas *et al*., 2007) and other physiological or metabolic limitation (Lawlor and Tezara, 2009). The relative effects of stomata and metabolic limitations are species dependent and they are also influenced by growth and experimental conditions (Lawlor and Tezara, 2009). However, photosynthetic electron flow through PSII was found insensitive to water deficit in field grown Cotton (*Gossypium hirsutum*) (Sinder *et al*., 2014). Therefore, our result revealed that reduced leaf diffusive capacity which caused reduction in photosynthetic rate in sorghum varieties was either due to stomata closure or reduced mesophyll conductance to CO_2 (gm). In general, in all sorghum varieties transpiration rate (g_s) was not zero, hence reduced CO_2 diffusion from the atmosphere to the site of carboxylation was the main cause for decreased photosynthesis under most severe water-stress conditions, in line to the findings of other researchers (Flexas *et al*., 2004; Grassi and Magnani, 2005; Chaves *et al*., 2009; Erismann *et al*., 2008). Similarly, Ackerson and Krieg (1977) leaf resistances were minimal and stomata were no longer sensitive to bulk leaf water status even when leaf water potentials approached -27 bars during reproductive growth in sorghum. On the contrary, the conservative use of water early in the cropping cycle is the most critical component of drought tolerance in chickpea, explained partly by a lower canopy conductance, resulted in more water available in the soil profile during reproduction leading to higher reproductive success (Zaman *et al*., 2011).

The structure of the leaf was also found to be involved in controlling water status in sorghum genotypes. Shawesh *et al*. (1985) found higher stomata frequency for drought tolerant varieties than susceptible varieties in sorghum. They also observed higher number of stomata on abaxial surface than on adaxial surface positively correlated with grain yield. A new functional role of venation architecture and small leaf size in drought tolerance has been found (Scoffoni *et al*., 2011), where both simulations with a spatially explicit model and experimentally using 10 plant species ranging strongly in drought tolerance confirmed that smaller leaves with higher major vein density were more tolerant of major vein embolism, thereby increase leaf hydraulic conductance (K_{leaf}) represents the capacity of the transport system to deliver water, allowing stomata to remain open for photosynthesis. These relationships were independent of other aspects of physiological and morphological drought tolerance (Nagai and Makino, 2009). Stem hydraulic conductance in corn (*Zea mays* L.) was involved in balancing the evaporative demand and water absorption (Li *et al*., 2009).

5.4.3 Intercellular Carbondioxide concentration (C_i)

Sorghum varieties had different intercellular carbondioxide concentrations (C_i) at non stress conditions. They demonstrated an increase in C_i at 25% except SorColl 163. SorColl 163 had lower C_i at all drought stress levels than the control. In case of Abshir as well as E 36-1 continued lowering up to 55% drought stress but had higher C_i than the control in the last two most severe drought stress conditions (65% and 80%). Thus, the faster and complete stomata closure was shown by SorColl 163 followed by Abshir and E 36-1. This could have a significant advantage in coping with a sudden water shortage under field conditions. Similar studies revealed that such performance of enhanced stomata response has been explained by ABA, where varieties with a higher rate of ABA synthesis upon exposure to osmotic stress and the sensitivity of the stomata to ABA appeared greater. A decrease in $C_{i,}$ however, below ambient will lower A_N and the pools of Calvin cycle intermediates, which can affect the activity of Rubisco and other enzymes (Long and Bernacchi, 2003). Therefore, only stomata regulation, or adjustments that facilitate CO_2 diffusion while minimizing water loss, has been found enhancing the drought tolerance of plants subjected to temporal or sustained water deficits.

Other varieties continued having higher intercellular carbon dioxide concentration in the remaining different severe drought stress levels. This is in agreement with the findings of Flexas and Medrano (2002), where sub stomata CO_2 concentration decreases as g becomes smaller, but increases again at small g. The increase in *C*i at severe water deficit level as in Abshir at late season and SorColl 146 at seedling stages as well as E 36-1 in both stages with lowering photosynthesis rate indicates the predominance of non-stomata limitations to photosynthesis (Flexas *et al.*, 2008; Mafakheri *et al.*, 2010). Under 80% wdl in the field and 65% in green house water deficit stress, increasing intercellular CO_2 was associated with extreme lowering of assimilation rate. Irrespective of the higher SPAD reading value at these water regimes, synthesis of food was impaired indicating that the photosynthetic pigments were not functional.

5.4.4 Stomata conductance (g_s)

The loss in g_s at mild drought stress with subsequent relax in g_s was seen in SorColl 163, which may not cause a decline in assimilation because the CO_2 concentrating mechanism in C_4 and reusing the CO_2 from photorespiration may saturate C4 photosynthesis (Ghannoum, 2009). Whereas, under severe drought condition (55% and 65%), Abshir, B 35, E 36-1 and SorColl 178 demonstrated a significant decrease in g_s, seem to be with better adaptation in controlling the stomata in response to the drought stress. However, such plants with C_4 losing their water-use advantage even under conditions of mild drought is mainly a consequence of metabolic limitations to CO_2 assimilation (Ripley *et* *al.*, 2010), which can be a regulation role in C3 drought-induced decrease in photosynthesis. Three decades back, both stomata and non stomata limitations were recognized being the causes for the decline of photosynthesis during drought stress (Flexes *et al*. 2008, Flexas and Medrano, 2002; Ackerson and Krieg, 1977)). In our study, the sharp decrease in biomass and plant height confirms the severe effect of the drought stress at seedling stage in the green house. This depicts drought stress effect at seedling stage was more severe than that of late vegetative and maturation stages under field condition. This calls attention to be given in drought stress study for sorghum.

5.5 Yield responses to drought stress

Grain yield or attributes of grain yield are among the most important trait in screening drought tolerant cultivars. In fact, selecting for other drought traits without considering yield will not be useful (Schaffert *et al.*, 2010) in the production enhancement program. Moreover, green house pot experiments are also always recommended for further check up at field because yield is a complex polygenic trait which is highly influenced by the environment and season. Yield response of forage sorghum cultivars to three levels of soil moisture under green house condition was recommended by Sher *et al*. (2013) for further research at field plot for actual grains assessment for the selected cultivars with resilience yield response to drought.

5.5.1 Days to 50 per cent flowering

Our study revealed that non-senescent varieties require both intermediate and least number of days to reach 50% anthesis in sorghum unlike Duncan (1981) has found only least number of days to reach 50% anthesis. SorColl 163, SorColl 146 and SorColl 60 need 105, 101 and 102 days to 50% flowering. However, with the exposure to different water deficit levels, the time of vegetative stage increased. In interrupted drought stress level at reproductive stage, there was a significant difference in the time of flowering among sorghum genotypes. Abshir and E 36-1 are found early maturing with lower number of days to flowering followed by SorColl 163 and SorColl 179, which can be a potential candidate with drought escape strategy in areas with terminal drought. Sorghum is known to be sensitive to water stress during flowering and early grain filling stages as compared to vegetative growth period. As the number of days from 50 per cent flowering to physiological maturity increases under normal condition, the yield also increases. Such increase in yield is due to increased number of days for dry matter accumulation in the grain, which is highly preferred trait by farmers. On the other hand, late maturing sorghum plants are more prone to drought condition especially to post-flowering drought. Therefore, it is more advantageous to identify stay green trait in late maturing or intermediate type in our region than early maturing germplasm, like B 35 and E 36-1, which are already gifted to escape late-season drought stress and also lack higher grain particle density. This case was also examined in barley, early maturing landraces are blamed by Ethiopian house wives for the low water intake during kneading (Yemane Gebre-Egziabher, 2001).

5.5.2 Yield attributes response to terminal drought

5.5.2.1 Panicle dry weight (g plant^{-1})

The sorghum varieties also exhibited significant differences among themselves at all levels because the panicle weight is more determined by the seed weight. SorColl 146 followed by SorColl 60 scored the maximum panicle weight under the non-stressed condition. SorColl 163, E-36-1 and Abshir maintained panicle dry weight best over the other varieties under severe water stress.

5.5.2.2 Grain number and weight per panicle

The water stress imposed to the varieties both during vegetative and the progressive stress in post-flowering (seed filling and maturation) stages reduced seed number per head and grain yield in the sorghum genotypes. Under non-stress conditions, all sorghum plants demonstrated high number of grain per panicle. Yield stability in terms of seeds count per panicle in the most severe water deficit stress levels was observed in the check E 36-1 followed by SorColl 163 and SorColl 60. Similarly under post-flowering water stress, tolerance to moisture stress in sorghum is manifested by a stay green phenotype and normal grain filling (Xu *et al.*, 2000). SorColl 178 performed least in grain weight per panicle due to smaller sized and few counts of grain under the same drought condition, indicating that it is more susceptible than others as also indicated in other parameters measured. As recorded in the work of Saeedipour and Moradi (2011) where severe water stress resulted in more deleterious effect on grain yield than the mild in wheat cultivars of susceptible and tolerant forms led to a smaller kernels and lesser aerial biomass at maturity but the loss was more severe in susceptible. The above three have been characterized in the previous studies vigor in lower rate of senescence, higher third leaf area and SPADR value, all are important factors in prolonging greenness of leaves. Such trait is important as it improves genotype adaptation to post-flowering drought stress, particularly in environments where the crop depends on stored soil moisture to fill the grains (Rosenow *et al.*, 1977). This is because severe stress that occurs during grain filling may result in drastic reduction of photosynthetic rate followed by leaves desfunctioning, hence non-senescent forms with high stem and leaves food reserve play great advantage during this time (Borrell and Hammer, 2000; Yang *et al*, 2001).

The stay-green trait has recently been characterized in a few cultivars such as B35 and E-36-1 where the Quantitative Trait Loci (QTL) has been mapped (Rosenow and Clark, 1981; Hirut Kebede *et al.*, 2001; Haussmann *et al*., 2002). One of these varieties namely, E36-1 is a high yielding breeding line assigned to the Guinea-Caudatum race, an Ethiopian sorghum that has been used as a source of stay green (Haussmann *et al.*, 2002).

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5.5.2.3 1000-seed weight

Significant differences in grain yield were observed among the cultivars of sorghum under field study with irrigation. In our study, 100 seed weight was least affected by the terminal drought. In some panicles like SorColl 60 the remnant seeds were found heavier than the seeds from plants grown in the less severed conditions. Hence, E 36-1, SorColl 60, SorColl 163, Abshir and lastly SorColl 179 are superior in 100 seed weight maintenance. On the other hand, SorColl 146 and SorColl 178 showed less stability in 1000 seeds weight under drought conditions. High seed weight varieties in the breeding program could result in higher grain yields. Water deficit at maturity affects seed weight

(Olufayo, 1997) and hence yield reduction. In general, the seed weight component of grain yield was influenced by water stress if stress occurs during grain filling stage. Under field conditions generally, the grain yield reduction due to moisture stress was mainly through both low seed weight and grain number per panicle is found to be associated with relatively susceptible varieties. Similarly, resilience to drought in yield response is seen in drought adapted sorghum varieties (Sher *et al.*, 2013), whereas a higher yield response factor to water supply, meaning a stronger yield decrease under water deficit in less efficient cultivars. In general, higher yield (Kg/ha) at optimal condition is found in varieties whith relatively lower stay green trait and demands genetic work to have high yielders with the desirable stay green trait.

5.5.3 Yield response to interrupted drought in the reproductive stage

The short period drought effect on the sorghum varieties in the field condition at different phases of reproduction depended on when the stress was imposed and different ways of yield loss have been observed in our study. Different yield losses were also recorded from different development stages drought imposition to grain sorghum at green house condition by Castro-Nava *et al*. (2012).

5.5.3.1 Responses to drought imposed at booting and anthesis

Our study revealed that all drought interruptions at different phases of reproductive stage influenced the sorghum production. However, drought at booting stage was accounted for the 8-26 % loss of production from control due to the decrease in panicle size (Figure 33) and increase in the unfilled sites of the panicle (Figure 34B). The sorghum genotype with higher root biomass and Sorcoll 163 with succulent roots had least panicle size reduction due to the drought effect during booting. Abshir and Sorcoll 146 demonstrated higher panicle size reduction. Unfilled glumes examined from panicles from plants that were stressed had small and tight indicating abortion in the development of sex cells. Similarly, the reduction in yield due to drought imposed at the anthesis was the reduction of filled sites and the nature of the glume was similar to that of booting stage. The reduction in unfilled sites during anthesis was ascribed to a consequence of fewer pollen grains and poor pollen viability (Prasad and Staggenborg, 2008). These were accountable for the 10% and 20% production loss respectively due to reduction in number of seeds per panicle from that of control. Hence, drought stress in both stages caused the same effect that is seed free small and tight glumes space formation, which is more pronounced in the anthesis. In our study, it was found out that E 36-1, SorColl 163 and Abshir were with relatively lower unfilled glumes with respect to control in both drought phases. The drought effect at the booting and flowering stages, however, favored maximum photosynthate partitioning to the seeds remained and showed increased size and slightly improved 1000 seed weight over control (Figure 34A). It is found out in our research that 1000 grain weight in sorghum increased as grain number per panicle decreased due grain number decrease in booting and anthesis phases. This supports the results found by Kiniry (1988) and Maman *et al*. (2004) in sorghum.

5.5.3.2 Responses to drought imposed at Seed Filling and Maturity

Drought during grain filling accelerates senescence due to lower photosynthesis but initiates translocation of carbohydrates from culm to seeds and shortens grain filling period (Blum, 1998). Moreover, drought stress affects seed quality through its impact on nutrient uptake; assimilate supply, partitioning and remobilization of nutrients (Prasad and Staggenborg, 2008), and ultimately reduce harvest index.

Results from this study showed a reduction in a seed weight per panicle (production) from that of control due to negative effect of drought on seed weight and seed count per panicle (Figure 34 and 34) imposed later in the seed filling and maturity. The seed weight per panicle loss due to drought was found to be the major cause for the production loss in seed filling and maturity phases of reproductive stage. Liu *et al*. (2011) early milk grain stage of rice, identified flag leaves producing excessive reactive oxygen species, which resulted in membrane damage as a result of lack of functional protective system. The yield loss due to drought in the late reproductive stage was also as a result of seed count loss but much less than the early phase drought effect. The unfilled glumes were widely opened unlike that of the early two phase of reproduction. SorColl 146 was not affected in seed count followed by the E 36-1 and 163 (Figure 34B). In general, of all the landraces and checks E 36-1 and SorColl 163 and SorColl 60 were found least affected among others both in seed count per panicle and 1000 seed weight (Figure 34A and 34). Abshir and SorColl 146 were intermediates. Seeds from the susceptible varieties (SorColl 179 and SorColl 178) were not fully filled and looked shrunken and chaffy grains in addition to the higher count of open spaced glume. Lizana, *et al*. (2006) obtained the contrasting rate of abscission of the reproductive organs under drought stress clearly consistent with differences among susceptible and tolerant common bean (*Phaseolus vulgaris*). Among the production traits, kernel weight has been recommended to get more emphasis in sorghum breeding program (Maman *et al*., 2004). Similarly, Kernel weight was found an important factor determining grain yield and nutritional quality in sorghum (Yang *et al*., 2009). However, our finding came up with the seed count reduction in the early reproductive phases and kernel weight loss in the late phases as the primary causes for production loss. This confirms the higher production recorded in stay green varieties succeeded to maintain panicle size and fertilization at early and dry mater partitioning later that helped to have more seed count, which is the major yield attribute in cereals (Saeedipour and Moradi, 2011).

Drought stress at grain-filling period had reduced 1000-grain weight significantly than that of other stages of reproduction, which indicates that kernel size and weight increment depended most on the plant's ability to accumulate dry matter during the seed filling stage. The 1000-seed weight and number of grains per panicle were equivalently negatively affected at grain filling stage.

A higher reduction in grain dry weight and count was observed for plants that were stressed at grain filling, a reduction of 21-38%**,** while plants that were stressed at maturity time showed a reduction of 10-23% (Figure 34A and 33B). Following the grain filling stage, grain count and weight was reduced in plants stressed during booting and flowering stages, respectively.

Failure in the translocation of photosynthates and carbohydrate reserves from the leaves and stem to the grains due to limited moisture in the plants with lower production might have been the cause for abortion from seed filling through to maturity. This was not the case in E 36-1 and SorColl 163 as the later is also identified with stay green trait from its phonological study. Moreover, the insignificance reduction of 1000 seeds weight in both varieties can be ascribed to the water status in the plants enough to manufacture food and that may permit metabolism translocation to continue in the kernel despite the water deficit condition in the soil.

5.6 Sorghum seedling biochemical responses to drought stress

5.6.1 Chlorophyll content

During photosynthesis, antenna pigments absorb solar radiation and transfer the resulting excitation energy by triplet-triplet means in to the reaction centre pigments, which finally bring about the photochemical process. The chlorophylls, Chl *a* and Chl *b*, are the most important components of reaction centers which are also inter-convertible with other antenna pigments during light stress (Krause and Weis, 1991). The amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments, and therefore low concentrations of chlorophyll can directly limit photosynthetic potential and hence primary production (Filella *et al.*, 1995). In this case, pigmentation can be directly related to stress physiology, as chlorophyll a and b concentrations change with the effect of abiotic stresses and during senescence (Larscher, 1995; Penuelas and Filella, 1998). However, low chlorophyll content does not mean lower performance as studied by Li *et al*. (2013) in mutant rice. Therefore, quantifying proportions and evaluating functions of photosynthetic pigments can provide important information about relationships between plants and their environment.

Chlorophyll a content

It is under debate whether drought mainly limits photosynthesis through stomata closure or through metabolic impairment (Lawson *et al*., 2003; Anjum *et al.*, 2003). Many studies confirmed that both stomata and non-stomata limitations are generally accepted to be the main determinant of reduced photosynthesis under drought stress (Farooq *et al*., 2009). However, the limitation of photosynthesis under drought through metabolic impairment is more complex phenomenon than stomata limitation (Reddy *et al*., 2004) and different plant responses have been reported with regard to the effect of drought to chlorophyll concentration.

Sorghum landraces and checks in our study responded by an increase in both chlorophyll a and b concentration to mild drought stress and a decrease with increasing deficit level above control. Similarly, an increase in chlorophyll contents in severe drought stress was also reported in corn cultivars (Valentovic *et al.*, 2006 and Jabeen *et al*., 2008). Menhsa *et al*. (2006) also found out that sesame subjected to drought stress increased in leaf chlorophyll content and then remained unchanged. For such positive responses in chlorophyll concentration different reasons have been attached besides the limitation of photosynthesis due to lack of water. The absorption spectrum of photosynthetic pigments in response to water stress reveals that maximum light is absorbed in two distinct regions (from wave length 400-500 nm and 600 to 700 nm) (Taiz and Zieger, 2010). The absorption of light in both these regions increase with the increase in water stress, however, the peaks of maximum absorption of chlorophyll ‘a’ would shift to shorter wavelengths, which in turn drops the efficiency of photosystem II (decrease in electron transport) assumed to be a cause for the overall growth depression although the chlorophyll contents (Chlorophyll ‘a’) increased in drought stress. It has also been reported that under stress conditions the internal concentration of CO_2 become high which decrease the carboxylation efficiency which in turn decrease the photosystem II activity (decrease in electron transport) (Ramanjulu *et al.*, 1998) or decrease in photoposphorylation (Yordanov *et al.*, 2000) in response to water deficit stress. Taiz and Zeiger (2010) expressed that water losses cause to increase contraction of cells that result in increasing cell solution concentration, which may be, mild stress that increase the concentration of chlorophyll per unit leaf area. The authors, described that adding severe stress would stop making chlorophyll, which is true to the case of sorghum plants grown at 65% FC, with little synthesis of food.

Unlike our result, many researches came up with reduced photosynthetic pigment contents as in chickpea (*Cicer arietinum)* (Mafakheri *et al*., 2010), wheat (*Triticum aestivum)* cultivars by Nyachiro *et al*. (2001), and *Catharanthus roseus* (Jaleel *et al*., 2008). Gholamin and Khayatnezhad (2011) also found out that the amount of chlorophyll and quantum yield (FV/Fm) ultimately the amount of grain yield of maize plants reduced due to a negative effect of the drought stress on the chlorophyll parameters. Similarly, Reddy *et al*. (2004) found limitation in photosynthesis of sunflower exposed to drought mainly through reduced photosynthetic pigment contents. Authors with such findings have been associating the reduction in main pigments with their conversion in to carotenoids which are also involved in regulation of photosynthetic inhibition as a result of drought stress. A decrease of total chlorophyll with drought stress implied to a lowering capacity for light harvesting (Oraki *et al*., 2012) in that the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Mafakheri *et al.*, 2010). Others like Dhindsa *et al*. (1981) and Chen *et al*. (1991) have associated the increased electrolyte leakage, an indicator for cellular metabolic disorder, to reductions in chlorophyll concentrations with severe drought effect.

At 55% Abshir, E 36-1, SorColl 163 and SorColl 60 accumulated relatively the highest chl a. SorColl 163 and E 36-1 demonstrated a more sharp increment in chl b content over the other varieties at the same water regime, while SorColl 178, SorColl 179 and B 35 indicated a sharp decrease in chl b concentration both at 55% and 65%. Therefore, majority of the landraces responded positively in the same trend to drought effect in chl content, where SorColl 163 and E 36-1 had the highest content over others under severe drought stress. However, sorghum response to drought in chl content was not a sensitive means of screening.

5.6.2 Proline content

Many plant species naturally accumulate proline and protein as major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play adaptive roles in mediating osmotic adjustment and protecting sub cellular structures in stressed plants. Different approaches have been contemplated to increase the concentrations of proline like compounds in plants grown under stress conditions to increase their stress tolerance.

5.6.2.1 Proline content from leaf extract

In all varieties proline increment has been observed from leaf extract with increasing drought stress. E 36-1 (1-14μg/gmFW), SorColl 163 (2.5-8.2 μg/gmFW) and Abshir (2-4.5 μg/gmFW) demeostrated a significant increase over other genotypes. A slight or no difference have been shown between varieties from the control and 25%, which entails slow induction of proline concentration in no stress and 25% wdl. Our result is in line with the work of Rahdari *et al.* (2012) where proline concentration was found increasing with increasing drought stress and free proline did not accumulate significantly in leaves of sorghum [*Sorghum bicolor* (L.) Moench until plants were severely stressed (Jabeen *et al.*, 2008). Waldren *et al.*, (1974) found proline to be less important indicator for irrigation scheduling in maize because it increased after the critical time to irrigate for maximum yield. Similarly, a significant increase in proline was not observed on the proline levels until the 4th day of water restriction in *Glycine max* cv. Sambaiba (Lobato *et al.*, 2008) and concluded that the plants had inefficient osmotic adjustment but high sensitivity to carbon metabolism. A drought study on bean (*Vicia faba* L.) by El-Tayeb, (2006), however**,** revealed an increase in proline and soluble sugars as well as CAT and POX activity with much higher accumulation in the resistant form and had better protection against drought-induced oxidative stress. Abshir, E 36-1 and SorColl 163 had the highest but SorColl 60 and SorColl 179 demonstrated least response in proline production with increasing drought stress over other genotypes.

5.6.2.2 Proline content from culm extract

The varieties like from the leaf extract, the proline concentration from the stem also demonstrated a slight or no change at 25% drought stress with that of varieties under no stress. However, unlike the extract from the leaf, the proline content from the stem extract of all varieties shown a sharp increase at both 55% and 65% drought stress. Slight lowering but still above 55% was demonstrated at 65% except B 35, Abshir and SorColl 60 which kept increasing in proline concentration with drought stress. SorColl 163 demonstrated the highest concentration of proline followed by E 36-1and B 35 at 55% drought stress. At 65% proline concentration increased sharply in B 35 followed by Abshir and SorColl 163.

SorColl 179, SorColl 178 and SorColl 146 recorded the least proline content at 65% drought stress. Significant difference was not observed except B 35, similarly observed by Sivaramakrishan *et al*., (1988), where proline levels increased as leaf water potential and relative water content fell, and there was no apparent difference among the different sorghum lines with change in plant water status.

5.6.2.3 Proline content from root extract

Like the other two organs, the proline content in the root extract was found with no significance difference in the varieties grown both at 25% and control levels of moisture. SorColl 60 followed by SorColl 146 demonstrated the highest free proline content in no stress level of moisture. Slight increament in proline content have been recorded in all varieties up on exposure to 55% and 65% drought stress levels. At 55% moisture level, SorColl 146, SorColl 163 and B 35 revealed relatively higher proline content over the other varieties whereas, Abshir, SorColl 178, E 36-1 and SorColl 179 recorded the least free proline content.

At 65% moisture level, root extract from E 36-1 and SorColl 60 recorded the highest proline content over the other genotypes, on the other hand, SorColl 178 and SorColl 179 demonstrated a gentle slope increment in proline content with increasing drought stress and recorded the least free proline content at 65% moisture level.

In conclusion, proline content in the most severe drought stresses was highest in Abshir, SorColl 163, E 36-1, B 35 and SorColl 60 from extracts of two or one organ of the sorghum plants. This finding indicates that the stay green sorghum plants besides other means also involved proline in drought tolerance at seedling stage. Similarly, drought resistant sorghum (*Sorghum bicolor* L. Moench) lines by Sivaramakrishan *et al*., (1988) also revealed that the, rice seedlings (Roy *et al*., 2009) accumulated high levels of proline, while the susceptible lines showed no significant proline accumulation.

In generall, our research demonstrated an increase in proline concentration in all the three plant organs with increasing drought but it was delayed response. The delayed accumulation of compatible solutes in droughted sweet sorghum plants was explained by Zegada-Lizarazu and Monti (2013) to have a more preponderant role and a means for recovery from drought stress in late growth stage, while the down regulation of the photochemistry as the main photoprotective/ regulative mechanisms for drought effect at early vegetative growth stage. Similarly, many prevoius studies also reported proline content response to water deficit stress for sorghum (Waldren *et al*., 1974, Blum and

Ebercon, 1976, Bhaskaran, *et al*., 1985, Jafar *et al*., 2004; Yadav *et al*., 2005) *Gossypium hirsutum* (Ronde *et al*., 1999), wheat (Hamada, 2000, Bowne, 2012) and in salt-stressed *Catharanthus roseus* (Jaleel *et al*., 2007), maize (Jabeen *et al*., 2008), rice (Mostajeran and Rahimi-Eichi, 2009). Counter checking with the activity of proline oxidase for proline accumulation in bhendi varieties demonstrated an increase in proline with increasing drought (Fujita *et al*., 2003; Sankar, *et al*., 2007). Other studies also found out that abiotic stresses other than water deficit stress such as salt stress in rice (Lin *et al*., 2002) caused an increase in proline content. Proline accumulation with increasing drought has been explained as a result of both induction of proline biosynthesis and/or inhibition of its oxidation (Hong *et al*., 2000,). The accumulation of proline in drought condition as a result of activation of proline synthesis through the glutamate pathway involving g-glutamyl kinase, glutamyl phosphate reductase and pyrroline-5-carboxylate reductase activities (Fujita *et al*., 2003; Sankar, *et al*., 2007 and Shukla *et al*., 2012). The inhibition of proline dehydrogenase (an enzyme from proline degradation) and proline oxidase activities are other mechanisms that also cause proline accumulation (Sankar, *et al*., 2007) in resistant forms of *Abelmoschus esculentum* varieties under water deficit conditions. Both forms of proline accumulations are faster in fast drought than in slow drought (Sanada *et al*., 1995). The PDH gene expression is regulated by the level of free proline pool (Wang and Brandriss, 1986**).** Arabidopsis gene expression is also induced by the excess proline in plants; however, this induction does not occur under stress conditions (Peng *et al*., 1996)**.** Therefore, a mechanism must exist to prevent induction of PDH gene expression by proline in plants under osmotic stress. The exact nature of this regulatory system is not fully understood.

Drought tolerance is positively correlated with high accumulation of proline in many crops like wheat and barley (Nayyar and Walia, 2003), rice (Hsu *et al*., 2003). Transgenic tobacco plant also showed high drought resistance by having high proline content (Kishor *et al*., 1995). Proline accumulation is thought to be a key adaptation under severe water deficit conditions (Akram *et al*., 2007). The role of proline has been described as a protective agent against reactive oxygen species (Hare *et al*., 1999; Reddy *et al*., 2004), in chlorophyll stability (Ashraf *et al*., 1995), in protecting and stabilizing membranes and enzymes (Rudolph *et al*. 1986; Thomas and James (1999), in osmotic adjustment (Voetberg and Sharp, 1991; Reddy *et al*., 2004; Jabeen *et al*., 2008), a source of respiratory energy in the recovering plant (Blum and Ebercon, 1976; Chandrashekar and Sandhayarani, 1996), as factors that affected transpiration demand were also affected free proline accumulation (Waldren *et al*. (1974), and helps in stress tolerance of plants (Bartels and Nelson, 1994).

Our study revealed that of the three plant organs, the stem extract from sorghum varieties grown in severe drought stress, 55% and 65%, recorded the highest proline content. Similarly, the exposure of maize plants to 24 h osmotic stress also increased in proline and sugar accumulation in all studied organs of the maize cultivars (Valentovic *et al*., 2006).

Leaf proline content at non stress and mild drought stress had recorded relatively higher but increased insignificantly with increasing drought. In case of free proline in root, intermediate content was recorded with increasing drought stress following that of stem.

The sharp increase in the stem extract may be attributed, at least in part, to the fact that the translocations of proline from the root to the target organs increased proline content in the stem in severe water deficit stresses. Similar results have been found in Sankar, *et al*. (2007), who explained indirectly based on proline oxidase activity the great of drought stress. The apparent discrepancy in roots between low Proline content and high P5CS/P5CR transcripts levels is explained by Verbruggen and Hermans (2008) due to proline export via xylem to the shoot. The sorghum varieties in our study demonstrated a sharp increase and significant difference among the varieties in the stem than the other two organs under severe water deficit stresses. This is in agreement with the finding of Mostajeran and Rahimi-Eichi (2009) where a significant variation in proline content among rice cultivars from leaves was detected.

5.2 CONCLUSION AND RECOMMENDATION

5.2.1 Conclusion

- ✓ The differences in phenotypic traits at control and responses exhibited to different water deficit levels indicates that sorghum germplasm in northern Ethiopia is a potential source for traits involved in sorghum production improvement under drought condition.
- ✓ Better performance in Culm height was in E 36-1, SorColl 163 and Sorcoll 60.
- ✓ SorColl 60, Abshir and Coll 163 maintained third leaf area better under drought condition
- ✓ SPADR value for the sorghum varieties studied was less affected by terminal drought. E 36-1, Abshir and SorColl 146 and SorColl 163 had higher SPAD reading in most of the drought stress levels
- ✓ Like third leaf area, senescence rating was found sensitive to both pre-flowering and post-flowering drought stress.
- ✓ Delaying the onset of leaf senescence and reducing its rate (i.e., two components of the stay-green trait) offered an effective strategy for the increase in grain production.
- ✓ Post grain filling senescence rating revealed: SorColl 163 (35.6-48.5%), SorCol1146 (48.4-51.5%), E 36-1 (45.9-57.0%), which are potential with delaying senescence rate at terminal drought.
- ✓ SorColl 179 and (1679.37 gm) and SorColl 60 (1327.80gm) had maximum total dry weight plant^{-1} under full irrigation. Biomass was found an important factor for screening resistant forms in drought condition. At 80% wdl, SorColl 179 (60%),

SorColl 146 and SorColl 163 had the lowest percent weight (36%) loss as compared to other genotypes.

- ✓ Root biomass of E 36-1 and SorColl 163 increased at severe and mild drought stress levels respectively. In contrast, root biomass of SorColl 60 and SorColl 179 went diminishing with increasing water deficit levels. Therefore, based on the interest of projects, they can be helpful for further study.
- ✓ E 36-1, SorColl 178 and SorColl 163 demonstrated a sharp increase in root-shoot biomass ratio indicating relatively a higher dry matter partitioning to roots than shoots in response to the water deficit stress, whereas, SorColl 179 with least in dry matter partitioning towards root in response to the water deficit regimes.
- ✓ E 36-1 (1.25), SorColl 163 (1.31), Abshir (1.36) were also the varieties with highest total RLD value in cm/cm^3 indicating higher chance of water contact per unit soil volume. Improvement in RLD distribution was examined in most sorghum varieties with increasing drought, 70-60% upper surface root proportion at non stress condition to 55-47.5% at lower depth and 80%wdl.
- ✓ Different responses have also been recorded with increasing drought stress, positively and negatively responding as well as non- responsive forms. Abshire, SorColl 178 and SorColl 60 had the highest DTI calculated. Abshir DTI was supreme with higher DTI that increased with increasing drought effect but (SOrColl 179) recorded least IDT value. Robust root biomass recorded in SorColl 179 at full irrigation was found to be negatively affected by drought conditions in our study. However, SorColl 178 was almost non responsive.

- ✓ Higher RLD but positive response to drought in DTI was found positively associated with sorghum plants with stay green trait. Moreover, large biomass in root system under full irrigation was not found associated with high drought tolerance and production under drought conditions.
- ✓ Assimilation rate decreased with increasing drought stress. SorColl 146 (48.4 μmole CO_2/m^2s), E 36-1(43.4 μmole CO_2/m^2s) and SorColl 163 (40 μmole CO_2/m^2s) had better performance at the tolerable lower deficit level, 55%.
- ✓ A_N in sorghum varieties was severely affected (11-14 and below 1 μmole CO_2/m^2s values respectively) by 65% and 80% drought stress levels.
- ✓ Field study for terminal drought depicted that highest yield in terms of seeds per panicle was observed in the check E 36-1 followed by SorColl 163 and SorColl 60 whereas SorColl 178 performed least under most severe wdl.
- ✓ The study on interrupted drought in different phases of the reproductive stage revealed insignificant biomass changes but all short period drought imposed at different intervals caused yield loss due to poor panicle development, seed set abortion, poor grain filling (small seed size) and seed fall due to dehydration (lower seed count) were accountable during booting, flowering, seed filling and maturity phases respectively. Loss in seed count had least contribution in E 36-1, SorColl 146 and SorColl 163.
- ✓ According to the results of the field research, higher dry biomass, lower senescence rate, higher third leaf area and photosynthetic rate contributed to sorghum varieties terminal drought stress tolerance and found positively correlated with yield.

- ✓ Sorghum seedlings establishment at green house condition was impossible to seedlings at 80% FC.
- ✓ Seedlings of Sorghum varieties were found decreasing in height, biomass, SPADR, assimilation rate but increasing in Chlorophyll a and b and proline content with increasing drought stress.
- ✓ However, seedling stage is also with potential source for tolerant forms to early stage rainfall delay. Sorghum varieties SorColl 146, SorColl163, SorColl 60 relatively performed better, whereas SorColl1 179, E 36-1, SorColl178, Abshir, and B 35 were negatively affected by the drought stress. Root shoot ratio of SorColl 146, SorColl 178, SorColl 179 and SorColl 163 were higher than others at the most severe drought stress (65%). Third Leaf area in SorColl 179, SorColl 163, and E 36- SorColl 178 was less affected over other genotypes. SPADR for varieties B 35, SorColl 163, Abshir and SorColl 179 were higher than others at 55% FC, but all dropped to lower SPADR values (10-15). Unlike to measurements taken at maturity at field condition, assimilation rate of SorColl 163 and SorColl 146 including the checks were affected highest at seedling and green house condition.
- ✓ Increase in Chl a and Chl b content measured from leaf extract of sorghum exposed to the different water regimes was accompanied with lowered photosynthetic rate, used as a regulatory mechanism than increasing dry matter.
- ✓ Chl a was recorded the highest in B 35, SorColl 163 and SorColl 60 but SorColl 146 and SorColl 179 had least content and with relatively sharp decrease in Chl a.

On the other hand, Chl b content in SorColl 179, SorColl 146 and B 35 was highest but SorColl 146, SorColl 163, E 36-1 decreased sharply up to 55% FC.

- ✓ The change in chlorophyll a and b proportion due to stress was not important factor for screening because the ratio of chlorophyll a to chlorophyll b content was almost same (3:1) in all water deficit stress levels.
- ✓ Proline at non drought stress condition had low concentration (about 2-5 μgm/gm) from the three organs and insignificant variation among the varieties except SorColl 60 and SorColl 146 in root extract.
- ✓ Proline content increased with increasing drought stress in the three organs, it increased sharply in the stem followed by the root.
- ✓ E 36-1, SorColl 163, B 35 and Abshir demonstrated the highest proline content in the organs at the severe drought stress levels, indicating that all the check varieties had active response.
- ✓ Therefore, among the grain sorghum plants studied SorColl 163 had a stay green trait enabled to give high yield under terminal drought stress. The SorColl 146 has been performing in many traits next to SorColl 163 but had reduced yield yield proportion due to the drought effect from controls, which indicated the functionality of the pigments was relatively impaired due to drought effect, hence can be categorized as a genotype with cosmetic stay green trait. Together with SorColl 60 which had inconsistent responses but better biomass yield than Sorcoll 146 under severe drought stress are alternative potential sources for sorghum production.

5.2.2 Recommendation

Sorghum plants studied were able to give yield even under stress condition far below plant allowable water, hence they can be a candidate crop for combating climate change effect on crop production.

The onset and progress of senescence are phenological metrics that show climate change sensitivity, indicating that understanding stay-green can contribute to the design of appropriate crop types for future environments.

Landrace SorColl 163 is found to be with a stay green tait and the yield attributes recorded were better than the checks. Therefore, this landrace can be takenover for further genetic and molecular studies after which it may be used in production enhancement programmes in areas prone to terminal drought stress.

Other landraces reseached SorColl 146 and SorColl 60 performed better in many pysiological traits next to SorColl 163 under the same drought condition. However, their yield was found highly diminished under the severe drought stress. Since they better perform than SorColl 163 under full irrigation, they can be the source for the high yield trait in breeding programmes.

SorColl 179 and SorColl 60 with maximum total dry weight plant^{-1} under full irrigation, are potential sources for those projects interested in biomass yield (bio-fuel, animal feed and human non food use) besides grain yield, under water is non limiting condition.

The robust root biomass recorded in SorColl 179 can still be used to benefit in stored seasonal moisture, like in areas that cultivate sorghum post rainy season in farmlands dominated by clay soil.

Interruptions with drought during reproductive stage was found to be an important factor in production loss in the sorghum varieties studied. Early pre-flowering and maturity period insignificantly reduced yield to all varieties. Drought during reproduction caused panicle size reduction, abortion during gametes development (absence of fertilization, failure in fertilization or and failure in seed set, retarded grain filling and small sized (1000 seed weight loss), abortion of seed during maturity, which make up causes for yield loss. Stay green varieties performed in the last two drought periods better over others. Higher yield loss is accountable to seed number reduction due to both drought beginning and last maturity phase.

Biochemical assessment involving proline and chlorophyll a and b on sorghum seedlings grown under greenhouse condition entailed increase with increasing drought. Increase or decrease in chl a and b but with lowered photosynthetic rate was sorghum plants responses to drought. Therefore, quantifying greenness using spectrophotomere alone can be a weak indicator for the actual performance of plants under drought stress. Therefore, it demands further investigation.

Proline content increase was not sharp at mild stress. Therefore, has less contribution in screening drought tolerant sorghum plants, or probably had less contribution in drought tolerance of seedlings. Stem part provided better proline information for screening sorghum plants than leaves and roots at severe drought stress. Using mixed pant organs extracts for proline analyses blends much information and therefore each plant organ separately must contribute information during analysis.

The identification of tolerant and susceptible sorghum varieties could be better accomplished by applying drought stress at the more susceptible stage of development.

Selection for drought tolerant varieties is very complex, but must be always done involving many phenotypic traits, based on our study it is recommend that photosynthetic rate, biomass, leaf area, senescence rate and yield and its components are effective screening parameters.

As the rainfall in our country is not reliable, drought may occur either at seedling or mid season or pre- or post-flowering time, multi traits investigation at different growth stages gives holistic impression in identifying and developing better parental lines resistant to drought stress.

In general, at field study complete failure in yield was not recorded irrespective of the severe water deficit stress exposure, which depicts grain sorghum a promising crop to feed people in the drought prone areas and multidisciplinary researches must be strengthen to overcome the prevailing and future food scarcity being aggravated by population size increase and climate change.

During our field study, protection of birds of prey incurred much expense especially for weaver birds (*Quelea quela*), which are among the main challenges of the farmers including the research station, necessitates long lasting innovation to avoid.

6 Reference

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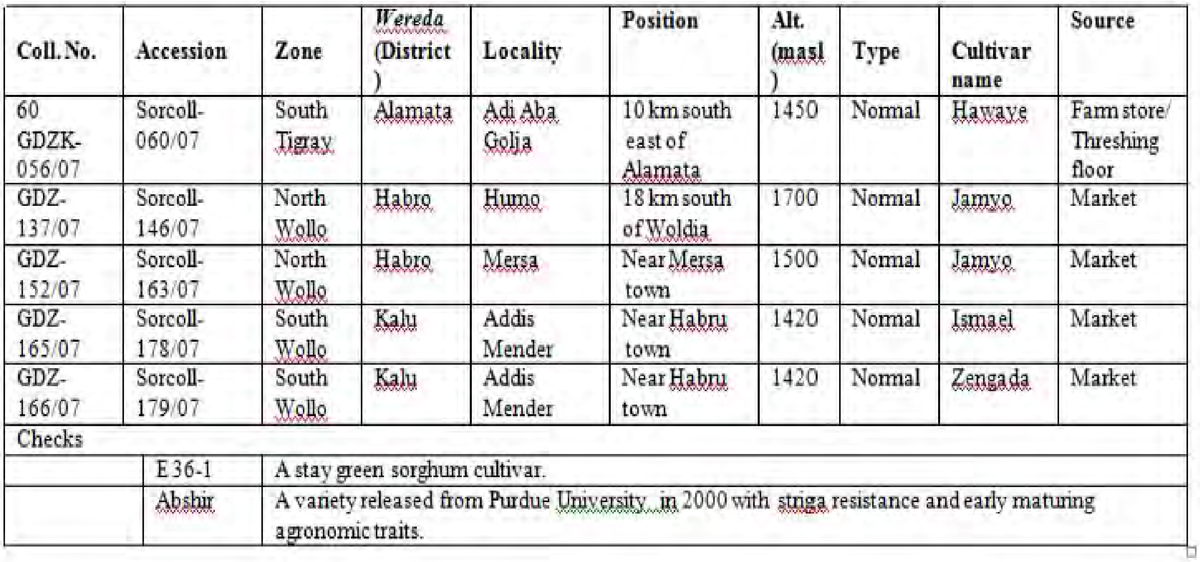
7 Index

Annex 4 List of experimental sorghum landraces and cultivars



Coll. No.	Accession	Zone	*Wereda* (District)	Locality	Position	Alt. (masl)	Type	Cultivar name	Source
60 GDZK-056/07	Sorcoll-060/07	South Tigray	Alamata	Adi Aba Golja	10 km south east of Alamata	1450	Normal	Hawaye	Farm store/ Threshing floor
GDZ-137/07	Sorcoll-146/07	North Wollo	Habro	Humo	18 km south of Woldia	1700	Normal	Jamyo	Market
GDZ-152/07	Sorcoll-163/07	North Wollo	Habro	Mersa	Near Mersa town	1500	Normal	Jamyo	Market
GDZ-165/07	Sorcoll-178/07	South Wollo	Kalu	Addis Mender	Near Habru town	1420	Normal	Ismael	Market
GDZ-166/07	Sorcoll-179/07	South Wollo	Kalu	Addis Mender	Near Habru town	1420	Normal	Zengada	Market
Checks									
	E36-1	A stay green sorghum cultivar.							
	Abshir	A variety released from Purdue University in 2000 with striga resistance and early maturing agronomic traits.							

Annex 5 Experiment I Statistical output

Table 1B Analysis of variance for the water treatment, variety and their interaction for sorghum traits

Traits	water treatment	variety	Wt*variety
PH pre pds	7701.76**	1458.71**	423.33ns
PH Pos pds	13656.61**	205693.98**	1349.23ns*
LA Pre pds	7138.44*	38548.70.54**	3369.14*
LA Pos pds	5125.47ns	16969.09**	3692.15ns
SPAD pre pds	106.09**	45.14**	9.34ns
SPAD post pds	195.76**	54.91**	9.15ns
Sen before SF	1571.56**	263.67**	52.44ns
Sen after SF	3402.28**	822.57**	148.04**
DShBM	948783.34**	1264520.83**	33495.38ns
DRtBM	22447.39*	28514.63**	11897.89**
$RLD_{61\text{-}80cm}$	0.053*	.052**	0.014ns
$LRLD_{41\text{-}60cm}$	0.024ns	0.052**	0.013ns
$RLD_{21\text{-}40cm}$	0.011ns	0.047**	0.019*
$RLD_{0\text{-}20cm}$	0.064ns	0.028ns	0.009ns
$RLD_{41\text{-}80cm}$	0.116ns	0.19**	0.033ns
$RLD_{0\text{-}40cm}$	0.116ns	0.142**	0.042ns
Assimi rate	7077.82**	408.26**	261.58**
ci	22651.75ns	10939.38ns	7229.5ns
Transp rate	10.5**	2.69**	1.62**
SWtPP	13648.69**	651.85**	58.54**
SCPP	6629347.35**	2004884.41**	9481.00ns
PSWt	20787.10**	5656.38**	1153.30**
1000 SWt	976.9**	4161.63**	105.84*
Yield (Kg/ha)	82566110.3**	28140400.5**	4588722**

df of water treatment, variety and water treatment*variety are 5, 6 and 30 respectively
** significant at 0.01 level of probability
* significant at 0.05 level of probability
ns stands for non significant

Table 2B Mean performance of sorghum traits plant height, third leaf area and SPADR before and after exposure to progressive stress, senescence rate before and after grin filling under water deficit stress level.

Treatment	PH pre-pds	PH post pds	LA pre pds	LA post pds	SPAD pre pds	SPAD post pds	Senesce rate before SF	Senesce rate after SF
control	183.59a	238.67a	485.72a	416.37a	57.10a	57.10a	14.50d	21.65c
Imposed to pdf	179.22a	221.57ab	454.66b	406.23a	56.14a	52.29b	27.59c	51.22ab
25% wds	170.94ab	205.28b	438.86b	372.40a	55.52ab	51.64b	30.99bc	48.86b
55% wds	154.79bc	202.7b	436.26b	384.61a	52.90bc	49.32cd	34.43ab	49.053b
65% wds	143.55bc	175.41c	445.10b	395.15a	51.22c	48.34d	38.42a	56.20a
80% wds	137.51c	173.5c	450.84b	399.15a	53.25bc	51.36bc	36.11ab	54.57ab
Grand mean	161.60	202.86	451.91	395.65	54.36	51.68	30.32	46.93
CV%	18.76	15.14	9.58	13.75	5.60	7.06	21.39	16.70
Treatment	PH pre- pds	PH post pds	LA pre pds	LA post pds	SPAD pre pds	SPAD post pds	Senesce rate before SF	Senesce rate after SF

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Table 2B continued Mean performance of sorghum traits dry shoot biomass, dry root biomass, RLD at 0-20cm, 21-40cm, 41-60cm, 61-80cm, RLD at 0-41cm, RLD at 41-80cm under water deficit stress level.

Treatment	**Dry shoot BM wt**	**Dry root BM wt**	**RLD at 61-80cm**	**RLD at 41-60cm**	**RLD at 21-40cm**	**RLD at 0-20cm**	**RLD at 41-80cm**	**RLD at 0-40cm**
control	953.42a	170.46a	0.538a	0.499a	0.363a	0.374a	1.034a	0.596a
Imposed to pds	844.25ab	132.09ab	0.481ab	0.477a	0.422a	0.372a	0.958ab	0.790a
25% wds	660.82bc	150.66ab	0.439bc	0.463a	0.419a	0.368a	0.902ab	0.771a
55% wds	556.16cd	111.04bc	0.401c	0.424a	0.399a	0.362a	0.870ab	0.762a
65% wds	482.63cd	105.14bc	0.461bc	0.409a	0.395a	0.343ab	0.824b	0.758a
80% wds	406.75d	80.50c	0.411c	0.470a	0.420a	0.232b	0.880ab	0.793a
Grand mean	650.67	124.98	0.45	0.46	0.40	0.34	0.91	0.75
CV%	40.10	59.20	26.90	26.81	24.81	33.36	21.66	23.18

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Table 2B continued **Mean performance of sorghum traits assimilation rate, internal CO_2 concentration, transpiration rate, total seed weight per panicle, seed count per panicle, panicle with seed weight, 1000 seed weight, yield (Kg/ha) under water deficit stress level.**

Treatment	Assimi rate	ci	Transp rate	Seed wt per panicle	Seed count per panicle	Panicle +seed weight	1000 seed wt	Yield (Kg/ha)
control	47.66a	364.46a	1.82b	86.91a	2054.44a	110.95a	46.20a	6759.30a
Imposed to Pds	-	-	-	56.18b	1489.38b	71.15b	39.31b	4369.90b
25% wds	30.58b	424.86a	2.79a	38.70c	1180.67c	51.77c	34.68bc	3010.30c
55% wds	28.96b	348.05a	2.23b	31.32c	969.76c	44.22c	32.51c	2435.90c
65% wds	12.28c	341.92a	1.21c	21.06d	625.81d	28.51d	29.40c	1638.10d
80% wds	1.48d	372.11a	1.10c	19.11d	487.52d	27.91d	28.99c	1485.90d
Grand mean	24.192	370.28	1.83	42.21	1134.60	55.75	35.18	3283.23
CV%	11.18	36.41	40.53	29.24	27.86	26.06	28.02	29.24

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Table 3B Mean performance of sorghum traits plant height, third leaf area and SPADR before and after exposure to progressive stress, senescence rate before and after grin filling among varieties.

Variety	PH pre-pds	PH post pds	LA pre pds	LA post pds	SPAD pre pds	SPAD post pds	Senesce rate before SF	Senesce rate after SF
Abshir	124.14d	135.63c	429.67cd	400.31a	54.08bcd	52.97a	30.72b	50.03b
E 36-1	144.22bc	155.68d	402.34d	406.41a	56.71a	53.22a	27.58bc	43.04c
SorColl 60	188.37a	237.36a	450.76bc	431.13a	55.63ab	51.73ab	30.35b	52.37b
SorColl 146	186.12a	248.60a	477.88b	409.82a	53.69bcd	50.04bc	29.20bc	44.64c
SorColl 163	154.10b	193.21b	459.24bc	422.95a	55.17abc	52.94a	30.34b	39.79c
SorColl 178	196.43a	211.64bc	404.73d	407.66a	53.15cd	52.26ab	38.07a	58.03a
SorColl 179	137.81cd	237.91a	535.24a	407.49a	52.07d	48.57c	25.98c	40.57c
Grand mean	161.599	202.86	451.409	412.253	54.36	51.67	30.32	46.93
CV%	15.00	15.46	11.33	13.2	5.6	7.05	21.53	23.17

Table 3B continued Mean performance of sorghum traits dry shoot biomass, dry root biomass, RLD at 0-20cm, 21-40cm, 41-60cm, 61-80cm, RLD at 0-41cm, RLD at 41-80cm among varieties.

Variety	Dry shoot BM wt	Dry root BM wt	RLD at 61-80cm	RLD at 41-60cm	RLD at 21-40cm	RLD at 0-20cm	RLD at 41-80cm	RLD at 0-40cm
Abshir	262.97f	55.08c	0.404b	0.434b	0.363c	0.306b	0.838c	0.67c
E 36-1	419.13ef	87.96bc	0.424b	0.399b	0.360c	0.316b	0.823c	0.68c
SorColl 60	851.94ab	159.69a	0.484ab	0.528a	0.452ab	0.394a	1.01ab	0.85ab
SorColl 146	824.74bc	131.89ab	0.443b	0.431b	0.391bc	0.347ab	0.87c	0.74bc
SorColl 163	663.94cd	142.09a	0.461b	0.442b	0.389bc	0.327ab	0.90c	0.72c
SorColl 178	521.05de	132.62ab	0.409b	0.427b	0.371c	0.305b	0.084c	0.68c
SorColl 179	1010.95a	165.54a	0.558a	0.538a	0.495a	0.397a	1.10a	0.89a
Grand mean	650.67	124.98	0.45	0.46	0.40	0.34	0.91	0.75
CV%	40.08	59.21	26.92	26.81	24.81	33.36	21.66	23.18

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Table 3B continued Mean performance of sorghum traits assimilation rate, internal CO_2 concentration, transpiration rate, total seed weight per panicle, seed count per panicle, panicle with seed weight, 1000 seed weight, yield (Kg/ha) among varieties

Variety	Assimi rate	ci	Transp rate	Seed wt per panicle	Seed count per panicle	Panicle+ seed wt	1000 seed wt	Yield (Kg/ ha)
Abshir	18.69c	332.42a	1.82bc	30.64d	925.80c	43.52cd	31.59c	2383.5d
E 36-1	23.27b	390.82a	1.39cd	45.18bc	1396.0ab	53.81bc	32.54c	3513.8bc
SorColl 60	23.84b	409.36a	1.19d	32.88d	592.40e	50.29bcd	45.78b	2557.2d
SorColl 146	31.30a	373.61a	2.13ab	75.03a	1067.2cd	93.25a	63.09a	5835.5a
SorColl 163	30.62a	384.53a	2.20ab	47.14b	1639.30a	60.47b	26.12d	3666.4b
SorColl 178	19.40c	352.14a	1.75bc	36.00cd	1033.60cd	47.58cd	32.61c	2800.0cd
SorColl 179	19.95c	349.07a	2.33a	28.61d	1287.9bc	41.6d	15.5e	2225.6d
Grand mean	23.86	370.28	1.83	42.11	1134.6	55.79	35.32	3283.14
CV%	8.5	29.02	34.56	35.00	40.08	10.10	21.23	35.15

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Annex 6 Experiment II statistical output

Table 4B Ananysis of variance for the water treatment imposed at different stages of reproduction, variety and their interaction

character	Wt Trt	variety	Wt Trt * variety
Dry shoot BM wt	28564.10ns	1594802.53**	20900.51ns
Dry root BM wt	1910.50*	82655.39**	552.70ns
Panicle length	34.65**	296.82**	6.23ns
Seed weight pp	3574.89**	13106.45**	176.37ns
seed count pp	608723.33**	2425793.89**	257130.37ns
1000 seed weight	974.81**	4595.54**	61.31ns
unfilled	627407.91**	528659.36**	39221.78ns
Yield (Kg/ha)	21625839.38**	79285929.7**	1066930.2 ns

df of water treatment, variety and water treatment*variety are 4, 6 and 24 respectively
** significant at 0.01 level of probability
* significant at 0.05 level of probability
ns stands for non significant

Table 5B Mean performance of sorghum traits panicle length, panicle width, total seed weight per panicle, seed count per panicle, panicle with seed weight, 1000 seed weight, unfilled glumes, yield (Kg/ha) to short period drought stress imposed at different reproductive stages

Treatment at	Dry shoot BM wt	Dry root BM wt	Panicle length	Seed weight pp	Seed count pp	1000 seed weight	unfill	Yield (kg/ha)
control	953.42a	170.46a	16.86a	86.2a	2054.44a	46.20a	254.86c	6759.3a
Booting	880.12a	152.24b	13.64c	68.17b	1778.39bc	43.87a	688.32b	5301.9b
Flowering	879.71a	148.12b	16.29ab	74.05b	1912.13ab	39.47b	519.91b	5760.1b
seed filling	935.44a	146.66b	15.86b	61.74c	1607.09c	33.76c	508.8c	4802.3c
Maturity	874.81a	152.75b	16.57a	52.20d	1746.28bc	29.88d	320.95c	4060.2d
Grand mean	904.69	154.05	15.84	68.47	1819.67	38.64	458.57	5336.76
CV%	21.64	12.65	9.50	12.78	10.15	3.90	23.01	34.78

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Table 6B Mean performance of sorghum traits panicle length, panicle width, total seed weight per panicle, seed count per panicle, panicle with seed weight, 1000 seed weight, unfilled glumes, yield (Kg/ha) for each variety

Variety	Dry shoot BM wt	Dry root BM wt	Panicle length	Seed weight pp	Seed count pp	1000 seed weight	unfill	Yield (kg/ha)
Abshir	350.14d	93.36c	20.23b	54.72b	1598.7bc	31.43d	287.16cd	4256.2b
E 36-1	762.35c	89.28c	14.57d	59.83b	1861.1ab	28.64de	224.95d	4653.4b
SorColl 60	974.08b	151.17b	12.23a	61.29b	1160.6c	56.65b	410.02bc	4767.1b
SorColl 146	973.53b	134.07b	10.67ef	134.37a	1927.8ab	67.13a	763.27a	10451.1b
SorColl 163	1061.95b	149.40b	23.05a	61.58b	2241.9a	25.74e	440.23b	4789.4b
SorColl 178	792.93c	149.71b	13.5de	62.4b	1621.8bc	41.58c	447.35b	4854.4b
SorColl 179	1417.87a	311.33a	16.67c	46.1b	2325.9a	19.27f	637.01a	3585.7b
Grand mean	904.69	154.05	15.85	68.61	1819.69	38.63	458.57	5336.76
CV%	17.67	22.80	14.53	34.70	41.64	18.37	45.51	34.78

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Annex 7 Experiment III Statistical output

Table 7B Analysis of variance for the water treatment, variety and their interaction for sorghum traits at seedling stage

Character	Water treatment	variety	Wt Trt * variety
Dry shoot BM wt	2768.99**	15.16ns	8.14ns
Dry Root BM wt	182.7**	0.31ns	0.38ns
Proline from leaf	361.51**	195.41**	90.16**
Proline from stem	12505.67**	1239.34*	1094.67**
Proline from root	1555.37**	168.19ns	108.1**
Chla	33.79*	18.93**	10.42ns
Chlb	6**	1.42ns	1.52**
SPADR	2083.29**	41.46*	31.73*
Assimi rate	1435.79*	44.47ns	59.33ns
Cond	0.11ns	0.003ns	0.008ns
Ci	125137ns	12623.96ns	19999.27ns
Transp	10.48ns	0.24ns	0.32ns

df of water treatment, variety and water treatment*variety are 3, 7 and 21 respectively
** significant at 0.01 level of probability
* significant at 0.05 level of probability
ns stands for non significant

Table 8B Mean performance of sorghum traits proline from leaf, stem and root, chla, chlb, dry shoot and root biomass, SPADR, assimilation rate, conductance, internal CO_2 concentration and transpiration to water treatments at green house condition

Treat ment	Dry Shoot BM wt	Dry Root BM wt	Prol leaf	Prol stem	Prol root	Chl a	Chlb	SPAD R	Assimi rate	Cond	Ci	Trans rate
control	20.44a	5.13a	3.69c	2.8b	4.86c	6.72b	2.09b	29.46b	27.53a	0.19a	177.8a	2.34a
25% wds	19.45a	4.81a	6.831bc	3.95b	6.57bc	9.2a	2.99a	33.22a	22.95ab	0.19a	3a	1.79ab
55% wds	1.33b	0.23b	9.32ab	35.65a	11.89b	7.7ab	2.96a	24.12c	14.16bc	0.1ab	97a	1.22b
65% wds	1.37b	0.17b	12.9a	48.15a	22.72a	6.67b	2.14b	11.86d	10.77c	0.05b	71.2a	0.83b
Grand mean	10.65	2.59	3.75	10.88	6.27	7.57	2.55	24.67	18.85	0.13	87.25	1.55
CV%	25.06	23.85	91.80	80.64	68.14	35.03	26.36	18.03	81.57	141.2	442.62	100.69

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Table 9B Mean performance of sorghum traits proline from leaf, stem and root, chla, chlb, dry shoot and root biomass, SPADR, assimilation rate, conductance, internal CO_2 concentration and transpiration for each variety

Variety	Dry Shoot BM wt	Dry Root BM wt	Prol leaf	Prol stem	Prol root	Chl a	Chlb	SPADR	Assmi	Cond	Ci	Transp rate
Abshir	11.01ab	2.60a	7.77b	27.70abc	9.33a	8.81a	2.62ab	28.7a	18.58a	0.12a	125.62a	1.56a
B 35	12.20a	2.74a	6.03b	38.32a	11.34a	7.79a	2.51ab	24.93b	16.10a	0.11a	47.55a	1.29a
E 36-1	10.92ab	2.80a	15.16a	22.10abc	15.72a	8.46a	3.01a	24.45b	22.21a	0.15a	102.36a	1.74a
SorColl 60	9.008b	2.37a	5.14b	19.05bc	16.43a	7.38a	2.34b	24.78a	17.55a	0.15a	123.95a	1.67a
SorColl 146	11.43ab	2.68a	5.78b	13.81c	13.99a	7.10a	2.28b	23.67b	17.95a	0.14a	55.58a	1.44a
SorColl 163	9.00b	2.37a	13.89a	34.94ab	11.75a	8.54a	3.11a	24.18c	18.58a	0.14a	113.61a	1.59a
SorColl 178	10.33ab	2.63a	5.58b	14.53c	7.054a	4.84b	2.18b	22.11b	19.53a	0.11a	59.88a	1.47a
SorColl 179	11.23ab	2.48a	5.74b	10.66c	6.48a	7.68a	2.32b	24.5b	20.73a	0.14a	70.12a	1.6a
Gtotal	10.65	2.58	3.74	10.88	6.27	7.58	2.55	24.66	18.90	0.13	87.33	1.55
CV%	30.21	21.55	71.32	96.84	99.17	32.84	31.91	15.23	42.45	75.47	219.20	47.56

Means that shared the same letter in a column of the same trait are not significantly ($p < 0.05$) different.

Declaration

I, the undersigned, declare that this Dissertation is my original work and it has not been presented in other Universities, Colleges or Institutes for a degree or other purposes. All sources of the materials used have been duly acknowledged.

Name:_________________________ Signature: _____________ Date: _____________

This work has been done under my supervision.

Name:_________________________ Signature: _____________ Date: _____________