

**ADDIS ABABA UNIVERSITY**  
**SCHOOL OF GRADUATE STUDIES**  
**DEPARTEMENT OF BIOLOGY**



**EVALUATION OF POST FLOWERING DROUGHT RESISTANCE  
(STAY-GREEN) PROPERTY OF ETHIOPIAN SORGHUM  
ACCESSIONS COLLECTED FROM THE DROUGHT PRONE  
NORTHERN PART OF ETHIOPIA**

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## ABSTRACT

Drought is the major constraint limiting production of crops especially in the Sub-Saharan Africa. Therefore, developing crops that have the mechanism to cope with such a drought prone production environments is vital. Sorghum is known for its post-flowering drought resistance ability termed as ‘stay-green’ which enables the crop to maintain photosynthetically active green leaves under progressively depleting soil moisture. In the present study, 165 sorghum landraces, which were collected from drought prone areas of Central Tigray, South Tigray, North Wello and South Wello, were evaluated for the stay-green property. The planting time was adjusted so that the plants could be exposed to post-flowering drought stress. Augmented experimental design with 5 blocks was used. Based on visual rating, 56 accessions were selected and further data was recorded on them. Twelve accessions were identified that scored within the top 20 in at least three of the four traits which are positively correlated (relative water content, chlorophyll content, proportion of green leaves remaining 30 days after flowering, green leaf area at flowering). Most of the accessions in this grouping had higher proportion of green leaves remaining 15 days after flowering and nitrogen content at anthesis. Nineteen accessions, in addition to showing these traits also had higher extent of tillering, indicating the potential for dual purpose sorghum. The cluster analysis grouped the accessions into four clusters. Based on the cluster mean, Cluster 4 and Cluster 2 showed superiority in most of the traits. Members of Cluster 1 had higher proportion of green leaves remaining 30 days after flowering and the least proportion of green leaves remaining 15 days after flowering; indicating that the accessions included in this cluster showed better performance through a mechanism of slower rate of senescence or type B stay-green

rather than delayed on set of senescence. The cluster groups showed a clear pattern of association according to their collection area; however, there were two distinct clusters (Cluster 3 and Cluster 4). The present result proposed 56 candidate stay-green sorghum accessions. The result also showed that these candidate materials differ in the mechanism by which they attained the stay-green syndrome, indicating the possibility of generating stay-green materials that would be different genetically and in their physiological responses as well.

**Key words:** drought, drought resistance, post-flowering, sorghum, stay-green

# 1. INTRODUCTION

Drought occurs in all continents of the world. It occurred in the grain-producing regions of the United States and the former Soviet Union and affected greatly the international food supply and demand (Meshcherskay and Blazherich, 1997). However, in recent decades most droughts, which devastated human populations and the environment, occurred in Africa. To many African states, drought is a persistent and recurrent problem. Drought has been identified as the main cause of food insecurity, for example in the Amhara Region of Ethiopia (Ramakrishna and Assefa Demeke, 2002).

Drought is a major factor for crop loss especially in the semi-arid tropics. Due to human population growth increased production of crops is required more than ever. Sorghum is better adapted to water limiting environments compared to most other crops (Mullet *et al.*, 2001; Sanchez *et al.*, 2002). This attribute is of great importance as the demand for food and water supplies increases due to world population growth (Gleick, 2003). In the semi arid environments, sorghum is the most important crop well known for its drought resistance ability, especially of the stay-green property (post-flowering drought resistance property). This property of sorghum has been reported to have no yield penalty under optimal conditions, improving yield under post-flowering drought stress conditions. Hence many breeding programs are trying to incorporate this valuable trait. For stay-green property only three known sources of stay green are being used in breeding programs, these are B-35 and E-36 which have Ethiopian origin and KS-19 which has Nigerian origin. A major undertaking at present is to identify molecular markers

associated with stay-green property, for which there is a need to identify more stay-green materials.

Ethiopia is home for diversity of sorghum landraces. From this diversity many economically valuable traits have been found for international usage. The post-flowering drought resistant trait named as stay-green has also been found in Ethiopian sorghum accessions. For example, B-35 has already been identified as a stay-green material derived from Ethiopian line and is now used in breeding programs (Borrell *et al.*, 1999); this probably indicating that Ethiopia has production environments in which this trait is likely to develop.

Landraces, also known as traditional varieties, are cultivated forms of a crop species, which have evolved over generations of selections by farmers (Harlan, 1992). Landraces are characterized by high genetic heterogeneity, good adaptation to local environmental conditions and low productivity. They are also noted as sources of useful genes required for further increment and maintenance of the productivity of modern varieties (Harlan, 1992).

In landraces, a series of beneficial selection has occurred as a result of environmental stresses. The screening of such landraces is an important part of the agricultural research and development process. Hence, these landraces are potential evaluation materials for positive out come in the search for more stay-green materials.

The limited numbers of sources of stay-green sorghum materials currently in use in sorghum breeding programs contrasts with its importance in improving adaptation to post-flowering drought stress and with the effort being invested in identifying molecular markers to transfer it to new lines more effectively. A search for additional source of stay green, which may be different genetically or physiologically, would thus be worthwhile. The objective of this project is thus to evaluate Ethiopian sorghum accessions for stay-green property.

### **1.1. OBJECTIVES OF THE STUDY**

**1. General Objectives:** To evaluate Ethiopian *sorghum bicolor* [L] Moench accessions for post flowering drought resistance property (stay-green).

**2. Specific Objectives:**

- a) To see the variation among accessions for the stay-green property.
- b) To identify candidate stay-green materials for further study.

## 2. LITERATURE REVIEW

### 2.1. *Sorghum bicolor* [L] Moench

#### 2.1.1. Classification and botanical description

*Sorghum bicolor* [L] Moench (Sorghum) belongs to the grass family (Poaceae). In 1794, Moench distinguished the genus *Sorghum* from the genus *Holcus* and in 1805 Person suggested the name *Sorghum vulgare* for *Holcus sorghum* [L.]. In 1961, Clayton proposed the name *Sorghum bicolor* [L.] Moench as the correct name for cultivated sorghum and this is currently the accepted one.

Like most angiosperm lineages, sorghum is thought to be around 200 million years old (Paterson *et al.*, 2003). Paterson *et al.* (2003) also reported that sorghum, maize, rice and wheat diverged from a common ancestor 50-70 million years ago. The main races of cultivated sorghum which are distinguished by grain and panicle morphology are: bicolor, vulgare, caudatum, kafir, guinea and dura (Deu *et al.*, 1994). Race kafir is a southern African form, but the other four races are grown in Ethiopia. Common names of sorghum vary from continent to country levels. In Ethiopia it has various names based on the different ethnic language like, Mashela (Amharic), Mishinga and/or Bobee (Oromigna), Missinga (Somali), etc.

Sorghum is a cane like grass, up to 6m tall with large branched clusters of grains. The individual grains are small about 3-4 mm in diameter. They vary in color from pale yellow through reddish brown to dark brown depending on the cultivar. Most cultivars

are annuals, few are perennials. The leaves look much like those of maize, they sometimes roll over.

### **2.1.2. Origin and geographic distribution**

It is believed that sorghum originated in Ethiopia between 5000 and 7000 years ago (ICRISAT, 2005). From there it was dispersed along the trade and shipping routes around the African continent, and through Middle East to India at least 3000 years ago. It then spread along the Silk Route in to China. Sorghum was first taken to North America in the 1700-1800's through slave trade from West Africa. It was re-introduced in Africa in the late 19<sup>th</sup> century for commercial cultivation and spread to South America and Australia. Sorghum is now widely found in the dry areas of Africa, Asia (India and China), the Americas and Australia (Balole and Legwaila, 2005).

The domestication of grain sorghum of the dura race may have occurred before 2000 BC in Ethiopia (Doggett, 1988), but an estimate of 1000 BC is now more widely accepted (Balole and Legwaila, 2006). However, early archaeological evidences for the crop is lacking in Africa while such evidence is much strong in Arabia and India (Rowley-Conway *et al.*, 1999; Young and Thompson, 1999). Remains of grains of wild sorghum, presumably collected from natural stands for food or irregularly cultivated have been found in an early Neolithic site in Southern Egypt that has been dated as 8000 years old (Wasylikava and Dahlberg,1999). Archaeological work at Aksum in Northern Ethiopia found sorghum grain deposits of 6<sup>th</sup> and 7<sup>th</sup> century AD, but not in pre-Aksumite deposit

that dated to the 5<sup>th</sup> century BC, while kernels of teff, oat, barley and wheat and other crops were found in the early deposits (Boardman, 1999).

### **2.1.3. Production**

Sorghum is quantitatively the world's fifth most important cereal grain, after wheat, maize, rice and barley. World annual sorghum production is over 60 million tones, of which Africa produces about 20 million tones (FAO, 2003). This makes Sorghum, quantitatively the second most important cereal grain in Africa after maize. Its production takes place across the continent, with the northern African countries of Nigeria, Sudan, Ethiopia and Burkina Faso accounting for nearly 70% of Africa's production. (FAO, 2003)

In Ethiopia, sorghum is a major crop both in terms of production and area cultivated. About 1.4 million hectares of land is put to sorghum production every year which contributes 1.7 million metric tones to the national grain production (CSA, 2004).

### **2.1.4. Adaptation**

Sorghum is better adapted to water-limiting environments compared to most other crops (Mullet *et al.*, 2001; Sancheze *et al.*, 2002). It is well known for its capacity to tolerate conditions of limited moisture and be productive during periods of extended drought, circumstances that would impede production of most other grains. It possesses a variety of anatomical, morphological and physiological features that enable it to survive in water

limited environments (Jordan, *et al.*, 1983). For example, under the same stressed environment the adaptation and yield stability of sorghum is more enhanced than that of maize (Berenji and Dahlberg, 2004).

This makes sorghum an important crop in arid or semi-arid environments, where it may not be economic or productive to grow other cereals. Hence, this attribute is of great importance as the demand for food and water supplies increases due to world population growth (Gleick, 2003).

#### **2.1.5. Genetic resources**

A major collection of sorghum germplasm is maintained by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The collection extends to over 36,000 accessions from all the major sorghum growing regions of the world (90 countries). Large germplasm collections of sorghum are also held in the United States (Southern Regional Plant Introduction Station, Griffin, Georgia, 30,100 accessions and National Seed Storage Laboratory, Fort Collins, Colorado, 10,500 accessions) and China (Institute of Crop Germplasm Resources (CAAS), Beijing, 15,300 accessions). In tropical Africa large germplasm collections of sorghum are held in Zimbabwe (SADC/ICRISAT Sorghum and Millet Improvement Program, Matopos, 12,340 accessions), Ethiopia (Institute of Biodiversity Conservation (IBC), Addis Ababa, 7260 accessions, Kenya (National Gene Bank of Kenya, Crop Plant Genetic Resources Center, KARI, Kikuyu, 3410 accessions) and Uganda (Serere Agricultural and Animal Production Research Institute, Serere, 2635 accessions) (Balole and Legwaila, 2006).

Plant genetic resource includes landraces, advanced varieties, wild relatives of domesticated plants, wild species, genetic stock and cloned genes (Chang, 1992). Landraces, also known as traditional varieties are cultivated forms of a crop species, which have evolved over generations of selections by farmers (Harlan, 1992). Landraces are characterized by high genetic heterogeneity, good adaptation to local environmental conditions and low productivity. They are also noted as sources of useful genes required for further increment and maintenance of the productivity of modern varieties (Harlan, 1992).

The local community has accumulated the information as a stock within the plant varieties selected and already in use. Sometimes this selection may have occurred hundreds or even thousands of years ago, but the varieties still retain some residual of their then-existing beneficial traits. Thus, a landrace may be conceptualized as an organism in which a series of beneficial selections have occurred in response to environmental stresses which is influenced by farmers' decision making processes, through their selection criteria (Awegechew Teshome *et al.*, 1999). The screening of such landraces is an important part of the agricultural research and development process; that is this stock information provides immediately identifiable innovation for use in agriculture (Evenson *et al.*, 1998).

Traditional farmers have themselves survived by a process of observing this naturally produced information and the consequent selection and use of the traits and characteristics that have aided survivability of the crop. In this way, traditional plant

varieties (landraces) are transformed into the accumulated history of the information, which nature has generated and that farmers have observed and used. Even though germplasms are major sources of crop improvement elsewhere in the world, their exploitation in different crops is still minimal.

In order to make any genetic advances in yield, to bring stability in tolerance to different biotic and abiotic stresses or to effect change in crop characteristics and meet certain consumer requirements, a breeder usually depends on the variability that is present in the germplasm collections.

#### **2.1.6. Sorghum in Ethiopia**

There is high genetic diversity in sorghum in Ethiopia with several pockets of geographical isolation. The current number of indigenous sorghum germplasms contained in the gene bank stands at about 7260 (Balole and Legwaila, 2006) and represents a wide array of diversity in the major sorghum growing areas of Ethiopia. This could serve as valuable genetic base for breeding and improvement of the crop in the country and the world at large (Brhane Gebrekidane, 1973). Highly desirable genetic characteristics were identified in some of the Ethiopian materials and were utilized in breeding programs extensively elsewhere in the world (Melaku Werede, 1998). The wealth of genetic variability in the Ethiopian germplasm has been and will continue to be useful sources of economically important traits like stay-green property.

The wide agro-climatic conditions in Ethiopia have contributed to the present biological resource of the country. Sorghum exists in tremendous diversity throughout the growing areas, which contains pockets of geographical isolation. Ethiopian sorghum germplasm is also noted worldwide as a source for useful genes such as high lysin content, cold tolerance, good grain quality and disease and insect resistance (Dogget, 1988; Yilma, 1991). For example, one of the stay-green materials named B-35 is derived from Ethiopian sorghum.

Based on their adaptation zones within the country, cultivated sorghums are grouped into highland, intermediate and lowland sorghum (Alemayehu Bekele, 2003). This classification has been made largely based on altitude, length of growing period and amount and distribution of rainfall (Yilma Kebede and Abebe Menkir, 1987). Intermediate zone sorghum grows at an altitude range of 1600-1900 masl (meter above sea level) and those of low lands grow in areas of altitude less than 1600 masl.

The progenitors (wild sorghum (*Sorghum bicolor* spp *Verticilliflorum* (L) Moench)) of cultivated sorghum are widely distributed across many African countries including Ethiopia. It was further noted that wild sorghum in Ethiopia is rapidly disappearing, largely because more and more of its habitat is used for agriculture, grazing, human settlement and construction (Amsalu Ayana *et al.*, 2000).

### **2.1.7. Sorghum utilization**

Sorghum is among one of the most versatile crop. It is an important part of the diets of many people in the world, mainly those living in the drier areas of Africa and India (Datke *et al.*, 2003). It is the dietary staple of more than 500 million people in more than 30 countries, making it the world's fifth most important crop for human consumption after rice, wheat, maize and potatoes (Miller, 1996).

Besides its use as food for humans it is used as animal feed, as a raw material for the production of alcoholic drinks, inks and biodegradable packaging materials (Balole and Legwaila, 2005).

Physically the flowering panicles are used as brushes, brooms and whisks and the canes are used for weaving fences, mats, and in the building of wattle houses. In Africa the straw of the traditionally tall sorghums is used to make palisades in villages or around homesteads while the plant residues are important sources of fuel for cooking. The stems of wild varieties are used to make baskets and fish traps. Dye extracted from sorghum is used in West Africa to color leather red (Balole and Legwaila, 2005).

## **2.2. Drought Stress**

Plants seldom exist under just the right conditions. But, what so ever the case will be plants do have optimal environmental condition in which they function appropriately (i.e. they under go normal growth and development). When there exist a significant deviation from this condition of optimality we say a stressful condition is prevailing. This stress

exhibits itself by reducing or adversely changing the plant growth and development (i.e. its normal function will be altered).

Water is important to the plant as a solvent, as a cooling agent, as a reagent, and for maintaining structure by keeping the pressure inside cells high enough so that they are fully expanded (i.e., turgid). When the plant wilts, its turgor approaches zero, the cells begin to collapse, membranes suffer damage and protein such as key enzymes can be denatured as their structure is altered. Cells can recover after drought stress. However, the damage must be repaired, and this takes time. If the damage is too great, the cells die.

Drought is one of the most serious global problems for agriculture. Drought stress affects plant growth, survival and productivity (Boyer, 1982). It is the primary cause of crop loss worldwide and it is perhaps the most prevalent abiotic stress. Bray *et al.* (2000) reported that drought reduces yield by as much as 50%. The effect of drought is more pronounced in the semi arid tropics (SAT), where rainfall is generally low and erratic in distribution.

Droughts occur in all continents of the world. It occurred in the grain-producing regions of the United States and the former Soviet Union and affected greatly the international food supply and demand (Meshcherskay and Blazherich, 1997). However, in recent decades the most severe droughts, which devastated human populations and the environment, occurred in Africa, perhaps giving the impression that drought is principally an African problem. To many African states, drought is a persistent and recurrent problem.

The impact of recurrent drought has resulted in food insecurity in many parts of the world especially in Africa. Moreover, the alarming trends of population growth make the problem sever. The world's population is expected to consume twice as much food in the next 50 years as it has in the past 10,000 years and in order to meet this need, global grain production must increase 40 percent by 2020 (Dupont, 2000). Therefore, understanding the mechanisms of drought resistance in sorghum is fundamental to meeting this challenge.

### **2.3. Coping with Drought Stress**

Crop plants have evolved various mechanisms to cope with the drought stress patterns under which they had naturally evolved or were domesticated. Thus, landraces are well adapted to local environmental conditions, and have evolved a range of morphological, phenological, and physiological mechanisms to efficiently utilize the available production environment. This local adaptation becomes a dominant force and presents a major difficulty in evaluating drought resistance of a wide range of genetic material at any one location (Ashraf and Karim, 1991).

From an ecological perspective, survival and perpetuation of the genome plays a dominant role in the adaptation of a given landrace to a region. Consequently, these landraces usually have low yield potential, despite their superior local adaptation (Fischer and Maurer, 1978). Also, many of these landraces do not fit into present-day agriculture,

where cropping systems and crop management practices are radically different from the conditions under which they had evolved or were domesticated. Thus, it is important from the crop improvement perspective to identify the specific physiological attributes contributing to their adaptability to the moisture deficit patterns of their native environment. This would assist breeders to selectively combine some of these physiological attributes into the high yielding cultivars required for present-day production systems (Rosielle and Hamblin, 1981).

In sorghum a property termed 'stay-green' has been identified that enables this crop to perform well in areas characterized by post flowering drought stress. Efforts are underway to evaluate sorghum germplasms for this specific property. Many traits associated with drought stress resistance have yield penalties; but one of the most important features of this stay-green trait is, it does not have yield penalty under optimal conditions, as discussed below.

#### **2.4. Drought Adaptation in Sorghum**

Sorghum has a reputation as a drought resistant cereal. It is a grass of the steppes and savannas of Africa (Kimber, 2000), and its adaptation to drought is a consequence of its evolution in these arid zones. Sorghum is a repository of drought resistance mechanisms, and has developed biochemical, physiological, and morphological characteristics such as C<sub>4</sub> photosynthesis, deep roots, and thick leaf wax that enable growth in hot and dry environments (Doggett, 1988). Sorghum genotypes may also

adapt to temperate or tropical climates, high or low altitudes, and too much or little water (Maunder, 2001; Blum, 2004).

#### **2.4.1. Breeding strategies**

Two main strategies have been employed by breeders attempting to improve drought resistance of sorghum :( 1) direct selection for yield in drought environments and (2) indirect selection for traits known to be associated with yield under drought.

##### **2.4.1.1. Direct selection**

Sorghum is generally grown in regions that commonly experience water deficit. As a result, most breeding programs practice direct selection for yield under drought to a greater or lesser extent. However Borrell *et al.* (2006) indicated, as there is little published work to indicate that selection for drought resistant genes has contributed to any genetic gain in yield when direct selection for yield has been practiced. Problems of low heritability and G X E (genotype by environment) interaction suggest that most progress can be attributed to improvement in general adaptation rather than specific adaptation to drought environments.

Recently, new tools have emerged with the potential to enhance the capacity of sorghum breeders to directly select for yield under drought. One such tool that addresses the G X E issue is the combining of simulation modeling (Hammer and Muchow, 1994) and long-term weather data to calculate a water stress index for specific environments, grouped by

pattern analysis (Chapman *et al.*, 2000). This approach not only identifies particular environment types, but also indicates how frequently these environments occur in the target population of environments (concept introduced by Chapman *et al.*, 2000). Podlich and Cooper (1998) suggested that genetic gain could be enhanced by using this information to weigh the data from particular tests, depending on how frequently the environment of the particular tests occurred in the particular target population of environments. Such an approach could also be used to more successfully select for specific adaptation to a particular pattern of water stress. Although this relatively new tool has significant potential, there is no indication at this time of its effect on the genetic gain in sorghum drought resistance breeding programs (Borrell, *et al.*, 2006).

#### **2.4.1.2. Indirect selection**

Indirect selection involves the selection for a trait with high heritability to generate a correlated genetic response with the trait of primary concern (in this case drought resistance). Before indirect selection can be practiced confidently, it is necessary to identify candidate trait linked to drought resistance, identify genetic variation for the candidate trait, confirm that the traits actually result in enhanced yield under drought conditions common in the target population environments of the breeding program, and develop screening methodologies to select for the traits (Borrell, *et al.*, 2006).

The selection of candidate traits can be assisted by considering the environment types and their frequency of occurrence in the target population environment, as suggested by Chapman *et al.* (2000). While genetic variation exists for many candidate drought

resistance traits that have been proposed, such as root architecture (Jordan and Miller, 1980), osmotic adjustment (Shackel *et al.*,1982), stay-green (Rosenow,1977), and transpiration efficiency (Hammer *et al.*,1997), few are used as selection criteria in applied breeding programs, either because of a lack of large-scale selection methodologies or because the association between the trait and yield under drought has not been well established.

Stay-green trait is a clear example of the successful use of indirect selection for post-anthesis drought resistance breeding. For example in Australia, much of sorghum is grown in clay soil with farming system that utilizes stored soil moisture; practical experience indicated that post-anthesis drought stress was common as confirmed and quantified by Chapman *et al.* (2000).

It is well known that sorghum plants that remain alive or senesce more slowly when faced with post-anthesis drought are less prone to lodging (Rosenow, 1977; Henzell *et al.*, 1984). Source of genetic variation for non-senescence or stay-green have been identified and a screening method based on visually rating leaf senescence in trials subjected to post-anthesis drought has been developed (Rosenow, 1977). However, the association of stay-green with post-anthesis drought resistance in terms of grain yield was less clear. Some research indicated that sorghum plants with low source to sink ratio are more likely to senesce under moisture stress conditions (Henzell and Gillieron, 1973; Rosenow *et al.*, 1983), suggesting a yield cost may be associated with stay-green. However, Henzell *et al.* (1992) demonstrated in large breeding population that selection

for stay-green derived from the germplasm line B35 results in improved yield under drought stress. This point was supported by Borrell *et al.* (1999) in study of 160 recombinant inbred lines that varied in the B35 source of stay-green, grown under terminal drought in Southern India. Physiological studies using small number of genotypes also found a positive association between stay-green and yield under postanthesis drought (Borrell *et al.*, 2000b).

The strongest evidence for the value of stay-green trait comes from a retrospective analysis of 15 years of sorghum breeding trials by Queensland Department of Primary Industries that sampled 48 environments, indicating that stay-green was positively associated with a best linear unbiased estimate of hybrid grain yield (Jordan *et al.*, 2003). Further analysis of this dataset showed that these environments could be classified into two groups on the basis of G X E interactions. Stay-green was positively associated with yield in the group that contained environments in which post-anthesis drought stress was likely to have occurred. However, stay-green was not associated either positively or negatively with grain yield in the other group, which consisted of environments in which post-anthesis drought was less frequent (Jordan, 1999). Importantly, this suggests that stay-green is not associated with low grain yield in high-yield environments.

Although phenotypic selection for stay-green is feasible (and to date is the only selection methodology used in breeding programs), it relies on the use of replicated trials in an endeavor to sample appropriate post-anthesis stress environments, the environments in which stay-green is expressed. Selection efficiency is limited because heritability of stay-

green is too low for early generation screening. Molecular marker technology offers substantial efficiency by allowing genotypes to be screened for stay-green in early generations, and to be screened in the absence of post-anthesis drought. Because of the importance of this trait, four independent mapping studies were conducted to develop markers linked to QTLs from B35 source of stay-green (Tunistra *et al.*, 1997; Crasta *et al.*, 1999; Tao *et al.*, 2000; Xu *et al.*, 2000). Where the studies could be compared, it was apparent that some of the stay-green QTLs were identified consistently while others were not. So, further work is required to determine which of the QTLs are responsible for the components of the trait that enhance grain yield in post-anthesis drought and to determine the physiological basis of these functional differences.

#### **2.4.2. Stay-green**

Sorghum is better adapted to water limiting environments compared with most other crops (Dogget, 1988). This attribute is of great importance as the demand for food and water supplies increases due to world population growth (Gleick, 2003).

Plants lose green leaf through a process of senescence in which many components of plants, including the green pigment chlorophyll, are broken down. Stay-green results when the plant's normal process of senescence is disrupted. There are a number of possible ways in which such a disruption may arise: for example the signals that would ordinarily induce senescence may be inhibited, or the enzymes responsible for senescence may be dysfunctional. Whatever the mechanism, the wide range of different types of stay green that result have found an increasing number of important use in agriculture. Stay-

green varieties have proved to be high yielding and provide benefits in harsh environments such as drought. The highest recorded yield per hectare in maize was achieved using stay-green variety. Stay-green varieties of sorghum out perform conventional varieties under drought conditions (Paul *et al.*, 2001).

So, stay-green is a general term given to a variant in which senescence is delayed compared with a standard reference genotype. Thomas and Smart (1993) showed how a stay-green phenotype could arise in one of four fundamentally distinct ways. In type A stay-green, senescence is initiated late but then proceeds at a normal rate. Assuming a tight correlation between net carbon out put and chlorophyll content (an assumption by no means justified in practice). In type B stay-green initiate senescence on schedule, but there after senesce comparatively slowly. In type C stay green behavior; chlorophyll may be retained more or less indefinitely but measures of physiological function such as photosynthetic capacity show that senescence is proceeding normally beneath the cosmetic surface of retained pigmentation. Another way of ensuring unlimited color retention is to kill the leaf by, for example freezing, boiling or drying. Stay-greens of the frozen spinach or herbarium specimen kind are referred to as type D (Thomas and Smart, 1993). Classifying stay green in this way is useful for understanding the kinds of modified genes or physiological processes underlying the phenotype, but in practice, particular stay greens can be combinations of two or more different functional types.

Positive correlation of stay-green trait with high grain yield has been found in many crops. In durum wheat, a stay green mutant has been associated with increased leaf area, rate and duration of grain filling and photosynthetic competence (*Spano et al.*, 2003).

During grain maturity in wheat, green and viable leaves significantly contribute photosynthates to developing grain (Thorne, 1982). Since there is a strong association between the duration of photosynthetically active leaf area and grain yield (Rawson *et al.*, 1983), selection for stay-green is expected to have significant implication in productivity of crops (for example wheat) particularly under harsh environments (Reynolds, *et al.*, 1999).

Sorghum genotypes exhibiting stay-green continue to fill their grain normally under drought (Rosenow and Clarck, 1981) and display increased resistance to charcoal rot (Rosenow, 1984) and lodging (Henzell *et al.*, 1984; Woodfin *et al.*, 1988). Cytokinins (McBee, 1984) and basal stem sugars (Ducan, 1984) are higher in stay-green than senescent genotypes. Greater functional leaf area during grain filling in stay-green types is associated with increased accumulation of soluble sugars; thereby reducing their dependence on stored assimilates from the stem to fill grain (Ducan *et al.*, 1981; McBee *et al.*, 1983). Higher concentration of stem sugars also improve the digestible energy content of the straw, making stay-green a valuable trait for both grain and fodder production in dual-purpose sorghums (Van Oosterom *et al.*, 1996). Longer than normal maintenance of photosynthesis in stay-green types may enhance yield in crops for which carbohydrate is a main harvest component (Thomas and Smart, 1993). Positive association between stay-green and yield have been reported for field grown sorghum in the United States (Rosenow *et al.*, 1983), Australia (Henzell, *et al.*, 1992).

Stay-green has been classified into five types (Thomas and Smart, 1993; Thomas and Howarth, 2000). The first two classes are functionally stay-green and may occur after alteration of genes involved in the onset of senescence (Type A) and the regulation of its rate of progress (Type B). Stay-green in the next two classes (Type C and D), however, is cosmetic. Type C stay-green is characterized by retention of chlorophyll, but not photosynthetic capacity. Killing a leaf by freezing, boiling, or drying is another way to ensure unlimited color retention, and stay-greens of the herbarium specimen kind are classified Type D. Type E stay-greens are those with greater leaf greenness, and delayed leaf senescence in these types is simply because the initial benchmark of leaf greenness is higher and chlorophyll has further to fall. Genetic variation for type A and B (Borrell *et al.*, 2000b) and Type E (Borrell *et al.*, 2001) has been reported for grain sorghum.

The stay-green phenomenon can be examined at the cell, leaf, or whole plant level (Borrell *et al.*, 2001). At the cell level, the retention of chloroplast protein such as LHCP2, OEC33, and Rubisco until late senescence has been reported in sorghum (De Villiers *et al.*, 1993) indicating that photosynthesis may be maintained for longer during senescence in these genotypes. Genes associated with Type A stay-green are to be specifically activated at the initiation of senescence. Initiation of senescence is the point at which all the various transduction pathways converge, invoking the stay-green syndrome through environmental and internal cues (Thomas and Smart, 1993). Genes involved in Type B stay-green are more likely to encode for senescence related activities, such as catabolic enzymes, to show increased expression during remobilization (Thomas and Smart, 1993). Type B stay-green has also been observed in some maize varieties. In the stay-green variety FS854, levels of

both chlorophyll and phosphoenolpyruvate carboxylase began to decline at the normal time, but the rate of decrease was less compared with other varieties, so that it retained more reduced N and NO<sub>3</sub> reductase activity in its leaves, as well as carboxylating enzymes and chlorophyll (Crafts-Brandner, *et al*, 1984a,b).

At the leaf level, longevity of photosynthetic apparatus is intimately related to nitrogen (N) status (Thomas and Rogers, 1990). For sorghum grown under post-anthesis drought, Borrell and Hammer (2000) found that specific leaf nitrogen and leaf area index were highly correlated at maturity, suggesting that retention of green leaf area during latter half of the grain filling period is dependent on specific leaf nitrogen and, that if specific leaf nitrogen falls below a critical level, the leaves will senesce.

At the whole plant level stay-green can be viewed as a consequence of the balance between N demand by the grain and N supply during grain filling (Borrell and Hammer, 2000). Matching N supply from age related senescence and N uptake during grain filling with grain N demand, Borrell and Hammer (2000) found that shortfall in N supply for grain filling was greater in the senescent than stay-green hybrids, resulting in more accelerated leaf senescence in the former. They hypothesized that increased N uptake by stay-green hybrids is a result of greater biomass accumulation during grain filling in response to increased sink demand (higher grain numbers), which, in turn, is the result of increased radiation use efficiency and transpiration efficiency due to higher specific leaf nitrogen. Delayed leaf senescence resulting from higher specific leaf nitrogen should, in turn, allow more carbon and nitrogen to be allocated to the roots of stay-green hybrids during grain

filling, thereby maintaining a greater capacity to extract N from the soil compared with senescent hybrids.

It is still not clear whether stay-green genotypes use more water or use water more efficiently. Retaining green leaf area under drought should result in comparable transpiration loss, unless water saving occur due to higher transpiration efficiency in some stay-green hybrids grown under rain-out shelters.

## **2.5. Few General Points Regarding Ethiopian Agriculture**

Agriculture remains by far the most important sector in the Ethiopian economy for the following reasons: (i) it directly supports about 85% of the population in terms of employment and livelihood; (ii) it contributes about 50% of the country's gross domestic product (GDP); (iii) it generates about 88% of the export earnings; and (iv) it supplies around 73% of the raw material requirement of agro-based domestic industries (MEDAC 1999). It is also the major source of food for the population and hence the prime contributing sector to food security. In addition, agriculture is expected to play a key role in generating surplus capital to speed up the country's overall socio-economic development (MEDAC 1999).

Ethiopia has a total land area of about 112.3 million hectares. Of this, about 16.4 million hectares are suitable for producing annual and perennial crops. Of the estimated arable land, about eight million hectares is used annually for rainfed crops. The country has a population of about 70 million (not a recent estimate) with a growth rate of about 3.3%.

At the present growth rate the population is expected to increase to about 129.1 million by the year 2030.

Small-scale farmers who are dependent on low input and low output rainfed mixed farming with traditional technologies dominate the agricultural sector. The present government of Ethiopia has given top priority to this sector and has taken steps to increase its productivity. However, various problems are holding this back. The major causes of underproduction are drought, which often causes famine, and floods. This climate related disaster make the nation dependent on food aid.

Hence, understanding of the drought resistance mechanisms of crops which are seen to perform well in drought prone areas is crucial to overcome the challenge of underproduction in the present day world. One such mechanism termed ‘stay-green’ property is identified in sorghum. This study evaluates the Ethiopian sorghums collected from drought areas for this property.

### 3. MATERIALS AND METHODS

#### 3.1. Weather Condition during the Experimental Season.

The rainfall was highest in July which is after planting. Comparable rainfall but lower than that of July was observed in August and September; however, the lowest rain was observed in October followed by no rain in November (Table 1). Flowering for all the collection materials was observed at the last week of September. As a result the experimental season was promising for the evaluation of the sorghum accessions under post-anthesis drought stress conditions for selecting candidate materials.

**Table 1.** Meteorological record of monthly rainfall and its distribution for the experimental season at the experimental site (Dera)

Month	Rainfall (mm) (monthly)	Rain days
January	18.2	2
February	37.1	4
April	26.1	3
May	50.0	3
June	77.6	11
July	115.4	13
August	103.9	12
September	104.8	14
October	6.3	2
November	0	0

### **3.2. Seed Sample Collection**

From 24 December 2006 to 4 January 2007 a field trip was organized to Northern part of Ethiopia for seed sample collection.

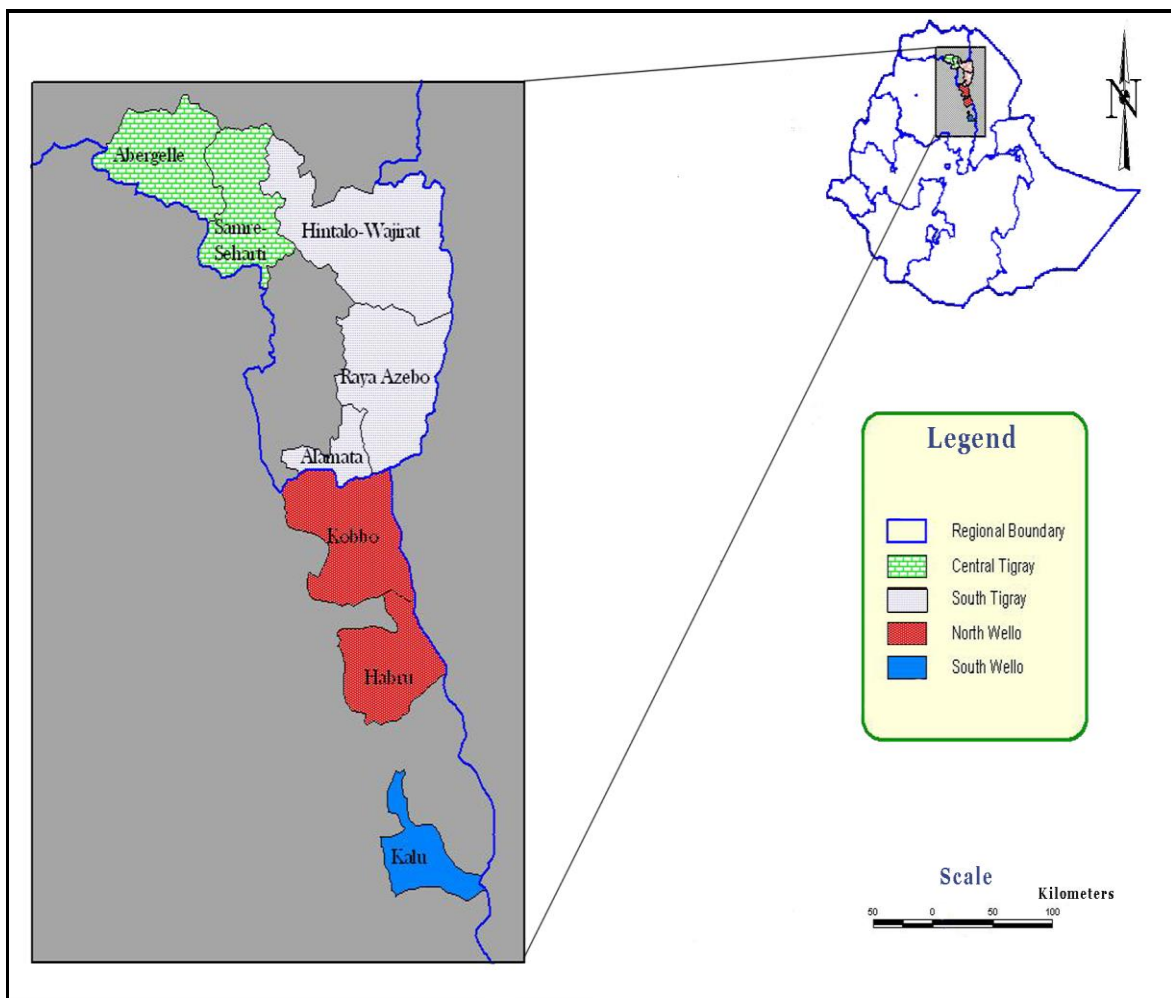
Prior to sample collection, identification of the most important drought prone production pockets that are likely to show greatest diversity of forms was done. For this, different stakeholders, particularly research centers and sub-centers and the bureau of agriculture and rural development at district (Wereda) level were approached. They not only assisted in the identification of the likely potential diversity targets in their respective mandate areas, but also they gave local guidance service in the course of the collection so that as many diverse types as possible hopefully with minimum number of duplication is acquired.

A total of 165 accessions were collected from Central Tigray (37), South Tigray (69), North Wello (42) and South Wello (17) (Appendix 1).

Standard collection method followed by Ethiopian Institute of Biodiversity Conservation (IBC) was used. Efforts were also made to address both ecological and genetic diversity by making frequent sampling (every 5-10 km distance) particularly from predetermined likely hot spots of genetic diversity. The collection was done from farmer's field, threshing ground, farm stores and from markets. Random sampling technique (for seed) supplemented with biased sampling (for panicle) was employed as deemed necessary. Passport data were completed for each sampling as per the IBC format. This format also

enables to make preliminary (informal) observation of the production constraints, usage of the crop and historical trends in seed delivery system.

Accordingly, 165 accessions collected from drought prone areas of Northern Ethiopia along with two adapted varieties (Seredo and Meko) and two known sources of stay-green (B-35 and E-36) as controls (standard checks) were used.



**Figure 1.** Map showing areas from which the sorghum accessions were collected.

### **3.3. Field Preparation and Experimental Design**

The experiment was conducted under rainfed lowland conditions during 2007/2008 cropping season at Dera (Sub-center of Kulumsa Agricultural Research Center). The augmented experimental design introduced by Federer (1956, 1961, and 1991) was used for the experiment. An augmented experimental design (AED) is usually useful for testing a large number of genotypes in early generations when valid statistical analyses are needed particularly when seed supplies are too limited to permit replication. The basic concept of augmented design construction is to establish a standard replicated design using checks for which sufficient seeds are available. Each replicate forms a complete block, incomplete block, or cell, depending on the standard design. Additional unassigned plots are created within each replicate and unreplicated entries for which there are insufficient seeds. The entries are assigned to these plots in the form of an incomplete block design. In this experiment there were five (5) blocks; the area of each block was  $253.125\text{m}^2$  (4.5m x 56.25m). The space between blocks was 2m. And the plot area of a single row is  $3.375\text{m}^2$  (4.5m x 0.75m).

The checks were randomly allotted to each plot within the blocks. Each of the accessions was planted in a single row of 4.5m long using the recommended row to row and plant to plant spacing. Two seeds were planted per hill and finally thinned to one plant per hill after two weeks of emergence.

A common border of a variety with modest resource requirement separating each plot was alternatively planted every other row to make border effects constant across the genotypes (accessions and checks).

Planting was done on June 23 and DAP fertilizer was applied on the same day as side dressing and other agricultural practices were done as per recommended for sorghum cultivation.

### **3.4. Data Collection**

#### **3.4.1. Green leaf area, number and visual rating of stay-green**

In order to minimize effect due to intra accession competition, plants to be used for plant data collection were selected from spots with full stand (avoiding those that are immediately adjacent to a missing hill from any side). Anthesis was defined as the time when 50% of the anther had extruded from 50% of 10 tagged panicles in each plot. Physiological maturity was determined by assessing black layer in 10 tagged plants, beginning with the grain in the upper most quartile. Physiological maturity was defined as the time at which basal grains in 50% of the tagged panicles attained black layer (Borrell, *et al.*, 2000a)

According to Van Oosterom *et al.* (1996) and Borrell *et al.* (2000a) green leaf area duration during grain filling appears to be a product of different combinations of three distinct factors: green leaf area at flowering, time and on set of senescence and subsequent rate of senescence. And hence, green leaf area was measured using CI, 202

Area Meter (CID, Inc. USA). At flowering the fourth leaf was cut from three stands for each accession and total leaf area was determined for each and the mean was taken as representative for comparison.

Number of green leaves only on the main stalk was counted starting from flowering. The numbers of fully expanded leaves were measured at 15 days interval. A leaf was considered senesced when more than 50% of its area had senesced. Then, at physiological maturity visual ratings of stay-green expression were recorded on a scale of 1-5 based on the degree of leaf and plant death at physiological maturity on a plot basis. Score 1 indicates very few or no senesced leaves where as 5 indicates complete leaf and plant death as used by Hirut Kebede *et al.* (2001).

### 3.4.2. Leaf relative water content and chlorophyll content

Relative water content of the leaves was determined at physiological maturity. Fixed size of leaf (1cm<sup>2</sup>) was sampled from the leaves and fresh weight was measured on the field, then leaves were suspended in distilled water for 24 hours at 25<sup>0</sup>C under fluorescent light. The sample was blotted dry and weighed prior to oven drying at 80<sup>0</sup>C for at least 15 hours, and then relative water content was determined by the method of Barrs and

Weatherley using the following

$$\left( \frac{FW - DW}{TW - DW} \right) \times 100\%$$

formula

Where FW = Fresh weight, DW= dry weight, TW=turgid weight)

Relative water content at mid day was determined for the whole samples and on ten randomly selected accessions relative water content at dawn was determined. This was to avoid the sampling bias that would be created due to the variation in light intensity when sampling the leaves for relative water content at dawn determination.

At physiological maturity Chlorophyll index reading were taken with SPAD-502 Chlorophyll Meter (Spectrum Technology Inc.). These values provide an indication of the relative amount of total chlorophyll present in plant leaves. The values were calculated according to the amount of light transmitted by the leaf area ( $2 \times 3$  mm) in two wavelength regions in which the absorption of chlorophyll is different. Higher SPAD values represent higher total chlorophyll contents.

#### **3.4.3. Leaf nitrogen analysis**

Leaf nitrogen concentration was determined at flowering. The nitrogen analysis was done at the Laboratory of Water Works Design and Supervision Enterprise. To cross check the accuracy duplicate of three randomly taken samples was provided together with the samples.

#### **3.4.4. Tillering and plant height**

Tillering (basal tillers) was scored at flowering and plant height was also determined at maturity.

After visual rating of senescence at physiological maturity only those accessions that performed well (i.e. those who scored 1 and 2) were considered for the subsequent data collection. The twelve characters scored and the abbreviations used are given in Table 2.

**Table 2. List of characters studied in the experiment**

Character	Abbreviations
Green leaf number at flowering	GLNAF
Proportion of green leaves remaining 15 days after flowering	R15DAF
Proportion of green leaves remaining 30 days after flowering	R30DAF
Maximum green leaf area at flowering	GLAAF
Chlorophyll content (SPAD reading value)	SPAD
Relative water content	RWC
Nitrogen content	Nc
Plant height	PH
Extent of tillering	TL
Thousand seed weight	TSW
Grain yield per panicle	GYPP
Visual rating of Senescence	VR
GYPP	Grain yield per panicle

#### 4. DATA ANALYSIS

The analysis was done as per the procedure for standard augmented design. Since all new selections did not occur together in a block, it was impossible to compare them directly. However, it was possible to get a reasonable estimate of block effect as the checks occurred together in a block. This estimate was used to adjust the treatment means. The adjustment index is calculated as:

$$A_j = \frac{1(B_j - M)}{C}$$

Where  $B_j$  = Block total

$M$  = Sum of check means ( $C = \sum C_i / 5$ )

$C$  = Number of checks

(The adjustment index is calculated for each block)

Then, the adjusted mean of the new selections was obtained by subtracting the adjustment index of each block from the observed means of the new selections.

$$Y_{ij} = O_{ij} - A_j$$

The analysis of variance was calculated just like random complete block design (RCBD) with  $C$  checks as treatments,  $B$  blocks and a total of  $CXB$  observations. Due to the imbalance created owing to the occurrence of new entries in a block, different standard errors were used in comparing different terms. The formulas used to compare new entries in the same and different block were,

- Between test treatments in the same block=

$$\sqrt{2MSE}$$

- Between two test treatments not in the same block=

$$\sqrt{2MSE(1 + 1/C)}$$

- Where MSE is the mean square error from analysis of variance of the checks and C is the number of checks

The analysis of variance for the checks was done using the SAS SOFTWARE VERSION 2001(The Proc ANOVA procedure was used). For the Multivariate analysis (cluster analysis and principal component) the SAS SOFTWARE VERSION 2001 was used. The Proc cluster and Prin Comp procedure were used respectively. The SAS procedure was first run and the appropriate number of clusters was determined from the Pseudo F and  $t^2$  values. The appropriate number of clusters was determined by observing the point at which the Pseudo F value showed a sudden positive change and  $t^2$  value is lower.

The data set contained 56 entries, and 9 quantitative traits for each entry. The traits included height, number of green leaves at flowering, proportion of green leaves remaining 15 and 30 days after flowering, extent of tillering, green leaf area at flowering, chlorophyll content, relative water content and leaf nitrogen content. Three clustering methods were used- average linkage, centroid and Ward's minimum variance. In the first two clustering methods, the pseudo F reached its peak at cluster 4. The pseudo  $t^2$  also reached its minimum at cluster 4. Therefore, it seems that the two methods agree on the

number of clusters (while the Ward's minimum variance showed no clear pattern). Looking at the second candidate was a bit difficult because if we go to cluster 6 the pseudo F is smaller than that of cluster 4. Hence, we may decide that the entries could be classified in to 4 clusters based on 9 characteristics.

The SAS program was run using 7 numbers of clusters then it was adjusted to 4 numbers of clusters and re-run. The data were standardized before running the analysis by subtracting the mean and dividing by the standard deviation.

## 5. RESULT

### 5.1. The Stay-green Evaluation

At physiological maturity senescence and stay-green accessions could be easily differentiated by visual observation as captured in Figure 2, thus only those accessions that have performed well up to physiological maturity were selected based on the visual rating of senescence.



Sorcoll-008/07

Sorcoll-035/07

**Figure 2.** Senescent (Left) and potential stay-green (Right) accessions (These photographs were taken on the same day at physiological maturity).

Table 3 presents the error mean square, least significant difference, critical value at t error degrees of freedom and alpha from analysis of variance for augmented randomized complete block design for 9 traits. Based on the error mean square from the analysis of variance, Table 4 presents the estimated standard errors calculated for pair wise comparison of entries belonging to the same block and different block. Accordingly, the means of all traits and the block accessions are occurring in is attached in Appendix 3 and

Appendix 4, respectively. Appendix 2 presents pair wise mean comparison among 10 accessions for GLNAF as a sample. Sorcoll-160/07, Sorcoll-047/07, Sorcoll-056/07, Sorcoll-168/07 and Sorcoll-119/07 showed significant variation with Sorcoll-137/07 and Sorcoll-039/07. Only Sorcoll-160/07 and Sorcoll-168/07 showed significant variation with Sorcoll-156/07.

**Table 3.** Error mean square, least significant difference, critical value at t error degrees of freedom and alpha from analysis of variance for augmented randomized complete block design for 9 traits

<b>Parameter</b>	<b>Error Mean Square</b>	<b>Least Significant Difference (LSD)</b>
NGLAF	1.155996	1.4816
R15DAF	0.001281	0.0493
R30DAF	0.029643	0.2373
TL	0.282343	0.7322
GLAAF	1744.045	57.548
SPAD	1.568347	1.7257
PH	483.4213	30.298
RWC	189.274	18.958
NC	0.054437	0.3215
<b>Alpha=0.05; Error degrees of freedom=12; Critical value at t= 2.17881</b>		

**Table 4.** Estimated Standard error of differences calculated for pair wise multiple comparisons among accessions within and different blocks for 9 traits

<b>Parameter</b>	<b>Within block</b>	<b>Between blocks</b>
NGLAF	1.52052359	1.699997
R15DAF	0.0506162	0.056591
R30DAF	0.24348717	0.272227
TL	0.75145592	0.840153
GLAAF	59.0600542	66.03115
SPAD	1.77107143	1.980118
PH	31.0940927	34.76425
RWC	19.45631	21.75282
NC	0.3299606	0.368907

Appendix 3 presents means of 9 traits as obtained from each of the experimental materials. Sorcoll-160/07 has the highest and third lowest green leaves number at flowering and nitrogen content respectively. Sorcoll-39/07 has the lowest green leaves number and relative water content. Sorcoll-176/07 has the highest proportion of green leaves remaining 15DAF (R15DAF) while Sorcoll-56/07 has the lowest and the second lowest R15DAF and R30DAF. Sorcoll-159/07 has the highest and the lowest R30DAF and plant height respectively. Sorcoll-032/07 has the lowest R30DAF. Sorcoll-180/07 has the highest, second highest and third highest green leaves area at flowering, tillering and relative water content, respectively. Sorcoll-028/07 has the lowest and third highest green leaf area at flowering and tillering, respectively. Sorcoll-015 has the highest and second lowest plant height and extent of tillering, respectively. Sorcoll-035 and Sorcoll-009/07 have the highest and lowest SPAD reading respectively. Sorcoll-009/07 has the highest and second lowest nitrogen content and relative water content. Sorcoll-101/07 has the lowest nitrogen content.

Table 5 shows the correlation among the 9 measured traits. Green leaf area at flowering has shown positive significant correlation with SPAD chlorophyll index reading (SPAD) and relative water content at 0.01 confidence interval. SPAD reading and proportion of green leaves remaining 30 days after flowering (R30DAF) have shown significant positive correlation at 0.05 confidence interval. Similarly relative water content (RWC) showed significant positive correlation with proportion of green leaves remaining 30 days after flowering. Plant height showed significant negative correlation with proportion of

green leaves remaining 15 days after flowering, extent of tillering, green leaf area at flowering, SPAD chlorophyll index reading and relative water content.

Table 6 shows the top 20 ranking accessions for each of the traits measured. For the traits that showed significant positive correlation (R30DAF, GLAAF, SPAD and RWC), 12 accessions have occurred overlapping in at least three of the above mentioned correlated traits (Table 7). Most of the accessions in this group have also shown higher proportion of green leaves remaining 15 days after flowering and nitrogen content at anthesis.

Accession Sorcoll-015/07, Sorcoll-028/07 and Sorcoll-035/07 have been observed to occur overlapping in proportion of green leaves remaining 15 days after flowering and leaf nitrogen content at anthesis. Accession Sorcoll-160/07 occurred in both proportion of green leaves remaining 15 days after flowering and 30 days after flowering. While accession Sorcoll-027/07 observed to have higher proportion of green leaves remaining 15 days after flowering.

Accession Sorcoll-062/07 and Sorcoll-155/07 had higher proportion of green leaves remaining 15 days after flowering, SPAD chlorophyll index reading and leaf nitrogen content at anthesis.

**Table 5.** Correlation coefficients of 9 traits used

	NGLAF	R15DAF	R30DAF	TL	GLAAF	SPAD	PH	RWC	NC
NGLAF	1.000								
R15DAF	-0.141	1.000							
R30DAF	0.103	0.196	1.000						
TL	-0.191	0.111	-0.068	1.000					
GLAAF	0.222	-0.059	0.239	0.034	1.000				
SPAD	0.208	0.081	0.288*	0.037	0.406**	1.000			
PH	0.142	-0.109	-0.060	-0.299*	-0.145	-0.138	1.000		
RWC	0.099	-0.037	0.295*	-0.020	0.386**	0.245	-0.281*	1.000	
NC	-0.206	0.178	-0.268*	0.202	-0.259	-0.129	-0.055	0.004	1.000

\* 0.05, \*\* 0.01

**Table 6.** Summary of the top 20 selected accessions in their descending ranking order

GLNAF	R15DAF	R30DAF	GLAAF	PH	TL	SPAD	RWC	NC
Sorcoll-160/07	Sorcoll-176/07	Sorcoll-159/07	Sorcoll-180/07	Sorcoll-015/07	Sorcoll-179/07	Sorcoll-035/07	Sorcoll-068/07	Sorcoll-009/07
Sorcoll-047/07	Sorcoll-027/07	Sorcoll-089/07	Sorcoll-080/07	Sorcoll-101/07	Sorcoll-180/07	Sorcoll-141/07	Sorcoll-086/07	Sorcoll-178/07
Sorcoll-056/07	Sorcoll-015/07	Sorcoll-026/07	Sorcoll-087/07	Sorcoll-080/07	Sorcoll-028/07	Sorcoll-146/07	Sorcoll-180/07	Sorcoll-053/07
Sorcoll-168/07	Sorcoll-011/07	Sorcoll-087/07	Sorcoll-179/07	Sorcoll-012/07	Sorcoll-027/07	Sorcoll-040/07	Sorcoll-090/07	Sorcoll-029/07
Sorcoll-119/07	Sorcoll-026/07	Sorcoll-163/07	Sorcoll-176/07	Sorcoll-013/07	Sorcoll-032/07	Sorcoll-124/07	Sorcoll-070/07	Sorcoll-012/07
Sorcoll-054/07	Sorcoll-028/07	Sorcoll-146/07	Sorcoll-060/07	Sorcoll-124/07	Sorcoll-076/07	Sorcoll-026/07	Sorcoll-159/07	Sorcoll-155/07
Sorcoll-062/07	Sorcoll-012/07	Sorcoll-180/07	Sorcoll-085/07	Sorcoll-029/07	Sorcoll-009/07	Sorcoll-062/07	Sorcoll-176/07	Sorcoll-062/07
Sorcoll-026/07	Sorcoll-160/07	Sorcoll-081/07	Sorcoll-091/07	Sorcoll-091/07	Sorcoll-011/07	Sorcoll-180/07	Sorcoll-146/07	Sorcoll-011/07
Sorcoll-159/07	Sorcoll-159/07	Sorcoll-179/07	Sorcoll-168/07	Sorcoll-056/07	Sorcoll-029/07	Sorcoll-081/07	Sorcoll-032/07	Sorcoll-026/07
Sorcoll-124/07	Sorcoll-089/07	Sorcoll-141/07	Sorcoll-101/07	Sorcoll-089/07	Sorcoll-035/07	Sorcoll-060/07	Sorcoll-085/07	Sorcoll-085/07
Sorcoll-141/07	Sorcoll-101/07	Sorcoll-090/07	Sorcoll-146/07	Sorcoll-141/07	Sorcoll-053/07	Sorcoll-179/07	Sorcoll-163/07	Sorcoll-124/07
Sorcoll-089/07	Sorcoll-180/07	Sorcoll-091/07	Sorcoll-054/07	Sorcoll-045/07	Sorcoll-124/07	Sorcoll-070/07	Sorcoll-081/07	Sorcoll-040/07

Table6. Continued.....								
Sorcoll-090/07	Sorcoll-163/07	Sorcoll-139/07	Sorcoll-139/07	Sorcoll-127/07	Sorcoll-142/07	Sorcoll-032/07	Sorcoll-178/07	Sorcoll-060/07
Sorcoll-091/07	Sorcoll-155/07	Sorcoll-142/07	Sorcoll-063/07	Sorcoll-081/07	Sorcoll-012/07	Sorcoll-159/07	Sorcoll-124/07	Sorcoll-156/07
Sorcoll-101/07	Sorcoll-162/07	Sorcoll-178/07	Sorcoll-062/07	Sorcoll-138/07	Sorcoll-156/07	Sorcoll-138/07	Sorcoll-060/07	Sorcoll-028/07
Sorcoll-081/07	Sorcoll-062/07	Sorcoll-160/07	Sorcoll-141/07	Sorcoll-046/07	Sorcoll-159/07	Sorcoll-127/07	Sorcoll-076/07	Sorcoll-015/07
Sorcoll-011/07	Sorcoll-141/07	Sorcoll-086/07	Sorcoll-142/07	Sorcoll-139/07	Sorcoll-162/07	Sorcoll-164/07	Sorcoll-089/07	Sorcoll-035/07
Sorcoll-180/07	Sorcoll-035/07	Sorcoll-035/07	Sorcoll-163/07	Sorcoll-146/07	Sorcoll-178/07	Sorcoll-090/07	Sorcoll-127/07	Sorcoll-076/07
Sorcoll-127/07	Sorcoll-127/07	Sorcoll-101/07	Sorcoll-090/07	Sorcoll-076/07	Sorcoll-119/07	Sorcoll-155/07	Sorcoll-087/07	Sorcoll-013/07
Sorcoll-139/07	Sorcoll-142/07	Sorcoll-085/07	Sorcoll-127/07	Sorcoll-027/07	Sorcoll-085/07	Sorcoll-178/07	Sorcoll-179/07	Sorcoll-032/07

**Table 7.** Accessions that ranked within the top 20 in at least three of the correlated traits (R30DAF, GLAAF, SPAD and RWC)

<b>In at least three</b>
<b>Accessions</b>
Sorcoll-060/07
Sorcoll-085/07
Sorcoll-087/07
Sorcoll-090/07
Sorcoll-127/07
Sorcoll-141/07
Sorcoll-146/07
Sorcoll-159/07
Sorcoll-163/07
Sorcoll-178/07
Sorcoll-179/07
Sorcoll-180/07

## **6.2. Principal Component and Cluster Analysis**

The principal component analysis showing the factor scores of each character among the 56 sorghum accessions, eigen values and percentage total variance accounted for by three principal components is presented in Table 8. The three principal components accounted for about 55.13% of total variance with the first principal component only taking 27.07%. The relative discriminating power of principal axes as indicated by the eigen values was high (2.71) for axis 1 and low (1.11) for axis 3. The first principal component that accounted for the highest proportion (27.07%) of total variation was mostly correlated with green leaf area at flowering, proportion of green leaves remaining 30 days after flowering, SPAD chlorophyll index reading and relative water content. Characters that were mostly correlated with the second principal component were proportion of green leaves remaining 15 days after flowering, extent of tillering, and leaf nitrogen content at anthesis. The third

principal component was dominated by traits such as proportion of green leaves remaining 15 days and 30 days after flowering and plant height.

The average linkage cluster (Table 9) analysis grouped those accessions which are in a near geographical proximity of their collection area. Out of the 28 accessions in cluster 1 most accessions were collections from Central and Southern Tigray while only four accessions were from North Wello. Central Tigray and South Tigray are in a near geographic proximity and with greater possibility of seed exchange than North Wello as a result they have contributed many accessions to this cluster showing the similarity among the collection materials from these areas.

Out of the 23 accessions in cluster 2, South Tigray and North Wello contributed 21 accessions, whereas Central Tigray and South Wello contributed one accession each. This shows that North Wello collections share much similarity with those collections from South Tigray. The only accession from South Wello grouped itself with this cluster, showing its similarity with collections from North Wello.

Distinct clusters which only contain collections from Central Tigray and South Tigray have been grouped in Cluster 3. This shows that there are accessions which are distinct from the accessions of their collection area. Cluster 4 also contained only collections from south Tigray, showing their distinctiveness from accessions of their collection area and also their proximities.

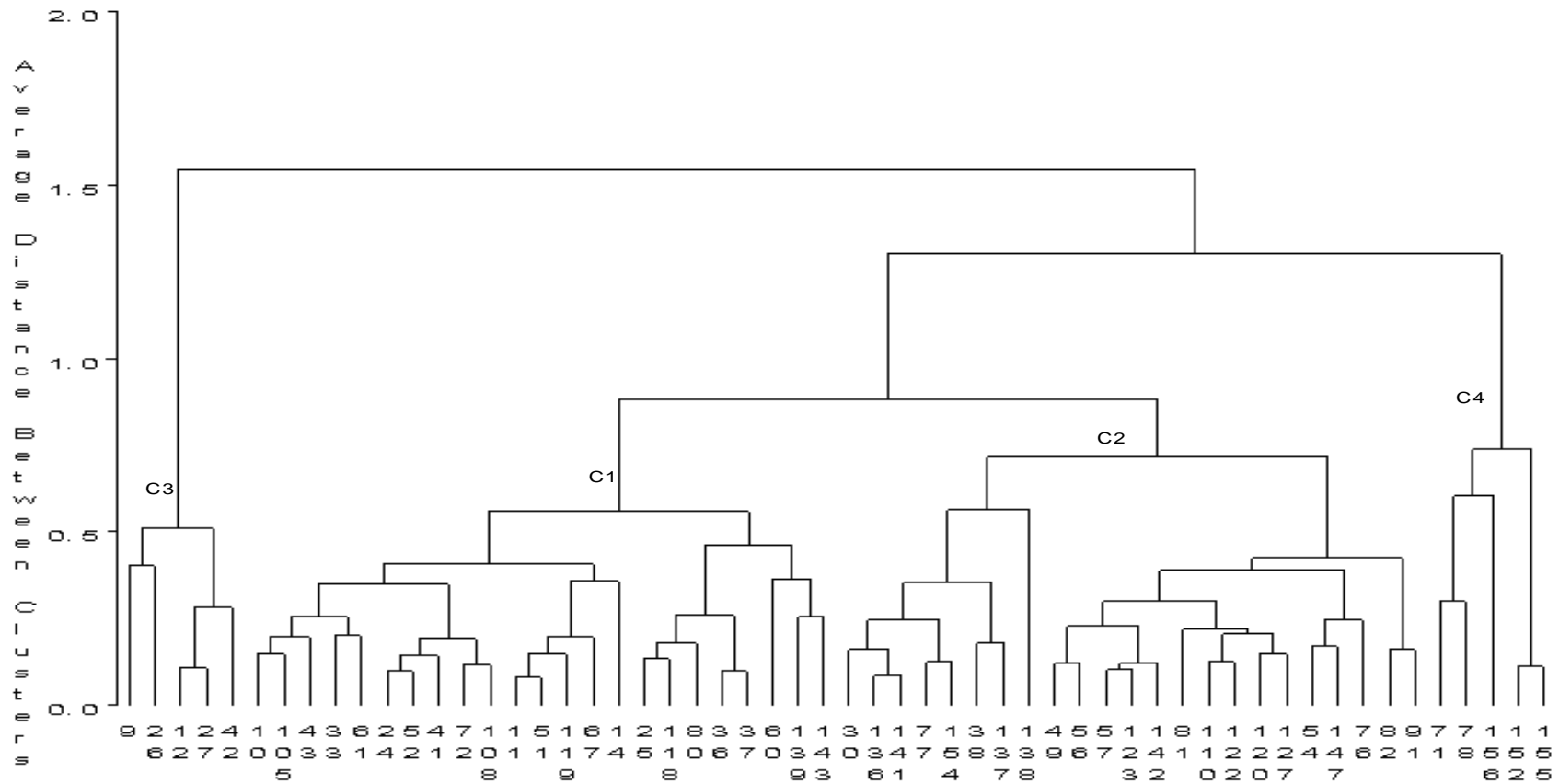
If we also try to see the contribution of each collection area to the selected candidate materials; South Tigray has contributed 26 accessions, Central Tigray 13 accessions, North Wello 16 accessions and South Wello 1 accession.

**Table 8.** Principal component analysis showing the contribution (factor score) of each character among the 56 sorghum accessions, eigen values and percentage total variance accounted for by the first four principal components

Characters	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>
NGLAF	0.18	-0.43	-0.11
R15DAF	0.05	0.36	0.71
R30DAF	0.37	-0.05	0.50
TL	0.02	0.51	-0.24
GLAAF	0.45	-0.08	-0.23
SPAD	0.37	-0.02	0.05
PH	-0.24	-0.48	0.27
RWC	0.37	0.09	-0.22
NC	-0.24	0.40	-0.06
Eigen values	2.71	1.70	1.11
% Variance	27.07	16.99	11.07
% Cumulative	27.07	44.06	55.13

**Table 9.** Average linkage cluster analysis, origin of accessions and accessions belonging to each of the four clusters based on 9 traits.

Clusters	No. of Accessions	Origin	Accessions
<b>C1</b>	<b>23</b>	<b>8</b> Central Tigray	Sorcoll-011/07, Sorcoll-012/07, Sorcoll-015/07, Sorcoll-026/07, Sorcoll-027/07, Sorcoll-035/07, Sorcoll-038/07, Sorcoll-039/07
		<b>10</b> South Tigray	Sorcoll-045/07, Sorcoll-047/07, Sorcoll-053/07, Sorcoll-056/07, Sorcoll-068/07, Sorcoll-070/07, Sorcoll-076/07, Sorcoll-081/07, Sorcoll-089/07, Sorcoll-119/07
		<b>5</b> North Wello	Sorcoll-124/07, Sorcoll-137/07, Sorcoll-138/07, Sorcoll-160/07, Sorcoll-164/07
<b>C2</b>	<b>23</b>	<b>1</b> Central Tigray	Sorcoll-032/07
		<b>10</b> South Tigray	Sorcoll-040/07, Sorcoll-054/07, Sorcoll-060/07, Sorcoll-062/07, Sorcoll-063/07, Sorcoll-085/07, Sorcoll-086/07, Sorcoll-090/07, Sorcoll-091/07, Sorcoll-101/07,
		<b>11</b> North Wello	Sorcoll-127/07, Sorcoll-139/07, Sorcoll-141/07, Sorcoll-142/07, Sorcoll-146/07, Sorcoll-155/07, Sorcoll-156/07, Sorcoll-159/07, Sorcoll-162/07, Sorcoll-163/07, Sorcoll-168/07
		<b>1</b> South Wello	Sorcoll-178/07
<b>C3</b>	<b>5</b>	<b>4</b> Central Tigray	Sorcoll-009/07, Sorcoll-013/07, Sorcoll-028/07, Sorcoll-029/07
		<b>1</b> South Tigray	Sorcoll-046/07
<b>C4</b>	<b>5</b>	<b>5</b> South Tigray	Sorcoll-080/07, Sorcoll-087/07, Sorcoll-176/07, Sorcoll-179, Sorcoll-180



**Figure 3.** Dendrogram showing the genetic similarity among the 56 sorghum accessions based on nine traits.

### **6.3. Grouping of Candidate Accessions on the Basis of Stay- green Traits**

The 56 candidate stay green sorghum accessions have been grouped into 4 clusters. On the basis of combined mean of the accessions in their respective cluster, Cluster 1 accessions had a higher proportion of green leaves remaining 30 days after flowering (slower rate of senescence) followed by accessions from Cluster 4. The four Clusters have a relatively comparable proportion of green leaves remaining 15 days after flowering (delayed onset of senescence), with Cluster 4 having the highest value and Cluster 1 the least. Cluster 3 had the higher leaf nitrogen content at flowering, second higher value for number of green leaves remaining 15 days after flowering and extent of tillering (Table 10).

Cluster 4 accessions had a higher green leaf area at flowering, mid day relative water content followed by Cluster 2 accessions for the respectively mentioned traits. Whereas Cluster 2 accessions had higher SPAD Chlorophyll index reading values which in turn Cluster 4 accessions had the second place. Cluster 4 accessions had also the higher extent of tillering.

Cluster 1 accessions with higher proportion of green leaves remaining 30 days after flowering had also second higher value for nitrogen content at flowering.

**Table 10.** Cluster mean for each traits.

Cluster	NGLAF	R15DAF	R30DAF	TL	GLAAF	SPAD	PH	RWC	NGLAF
1	11.05	0.957	1.615	1.615	482.8	38.25	249.9	36.64	3.011
2	11.09	0.961	0.625	1.527	590.5	40.55	222.8	41.87	2.972
3	10.2	0.982	0.518	2.435	340.4	26.3	209.4	28.59	3.253
4	10.47	0.983	0.66	3.081	708.9	39.62	217.5	48.8	2.92

#### **6.4. Further Descriptive Results**

Thousand seed weigh and grain yield per panicle have also been recorded. Accordingly, Sorcoll-026/07(32,75.88), Sorcoll-029/07 (38,98.36), Sorcoll-045/07 (36,72.74), Sorcoll-046/07 (31,66.56), Sorcoll-178/07 (36,65.5) are accessions which have occurred on top ten for both thousand seed weight and grain yield per panicle (values indicated in the bracket are thousand seed weight (in grams) and grain yield per panicle (in grams) respectively). Whereas Sorcoll-09/07(31), Sorcoll-015/07 (38), Sorcoll-035/07 (35), Sorcoll-080/07 (30), Sorcoll-081/07 (30) were the top five for thousand seed weight. Sorcoll-013/07 (55.6), Sorcoll-038/07 (56.6), Sorcoll-162/07 (71.92), Sorcoll-163/07 (87.6), Sorcoll-176/07 (82.2) were the top five for grain yield per panicle.

## 7. DISCUSSION

### 7.1. Stay-green Ratings of the Accessions

The long term goal of this project is to identify more stay-green materials, understand the physiological basis of the sorghum stay-green property and to identify the genes that contribute to this trait in different sorghum genotypes. This preliminary evaluation of the Ethiopian sorghum cultivars collected from drought prone areas of the Northern part was intended to identify candidate stay-green materials for future study.

Xu *et al.* (2000) indicated that visual stay-green ratings were a reliable indicator of leaf senescence and should be useful to sorghum breeders in evaluating progeny when breeding for drought tolerance. Likewise, in this experiment visual ratings was done at physiological maturity in a 1-5 scale and those scored 1 and 2 have been promoted for further data recordings (Appendix 5). Visual rating was used in evaluating the germplasm for post-flowering drought tolerance and in transferring the stay green trait to elite lines, for example, conversion lines such as B35 (Rosenow, 1994).

Drought stress during the post-flowering period accelerates senescence in many plant species by driving many physiological processes in the same direction as normal senescence (Nooden, 1988). Senescence is normally characterized by chlorophyll loss and a progressive decline in photosynthetic capacity. Early onset of senescence affects assimilation and grain filling in crop plants. The rate of senescence determines the maintenance of quality flowers, fruits, and vegetables. Therefore, any defense mechanism that postpones the onset of senescence and keeps leaves green will benefit the crop.

In the present study accessions such as Sorcoll-011/07, Sorcoll-015/07, Sorcoll-026/07, Sorcoll-027/07, Sorcoll-028/07, Sorcoll-176/07 displayed higher proportion of green leaves remaining 15 days after flowering. According to the classification of stay-green by Thomas and Smart (1993), they may have probably shown Type A behavior. Genes associated with Type A stay-green are likely to be involved in the timing of the initiation of senescence. Accessions such as Sorcoll-087/07, Sorcoll-089/07, Sorcoll-146/07, Sorcoll-159/07, Sorcoll-163/07, Sorcoll-180 have displayed higher proportion of green leaves remaining 30 days after flowering. According to Thomas and Smart (1993), they may have probably shown Type B behavior. Genes involved in Type B stay-green are more likely to encode for senescent related activities such as catabolic enzymes. Thomas and Smart (1993) emphasized that the stay-green character in one genetic line may have a superficial resemblance to the character in another, yet the common phenotype may arise from very different underlying physiological and biochemical mechanisms. Accessions such as Sorcoll-026-07, Sorcoll-035-07, Sorcoll-101/07, Sorcoll-141/07, Sorcoll-159/07, Sorcoll-160/07, Sorcoll-163/07, and Sorcoll-180/07, displayed higher proportion of green leaves remaining 15 days and 30 days after flowering; and hence according to Thomas and Smart (1993), they may have probably shown both Type A and Type B behavior. This shows that even if the common end in this sorghum accessions is phenotypically similar, they have followed different physiological mechanisms. This is in line with the works of Thomas and Smart (1993) and Borrell *et al.* (2000a). Borrell *et al.* (2000a) found that KS 19 hybrids displayed delayed onset and reduced rate of senescence, yet B35 hybrids displayed delayed onset. This showed that a common stay-green phenotype

may arise from very different underlying physiological mechanisms indicating that there is more than one way for a plant to stay-green.

Borrell and Hammer (2000) indicated that stay-green hybrids exceeded senescent hybrids in leaf nitrogen concentration at anthesis (3.11 vs. 2.83). In our study, only ten out of the 56 accessions showed leaf nitrogen concentration less than or equal to 2.83. Whereas, the rest had values greater than 2.83. Out of the accessions that had higher leaf nitrogen concentration 28 accessions have values more than 3.00. Borrell and Hammer (2000) indicated that leaf nitrogen concentration at anthesis was correlated with onset ( $r=0.751^{**}$ ,  $N=27$ ) and rate ( $r=-0.783^{**}$ ,  $N=27$ ) of leaf senescence under terminal water deficit. In our experiment similar trend was recorded for rate ( $r=-0.268^*$ ) of leaf senescence, but not with proportion of green leaves remaining 15 days after flowering ( $r=0.178$ ).

During grain filling period, there are two sources of nitrogen for grain growth: concurrently absorbed nitrogen from the soil and remobilized nitrogen from vegetative tissue (Ta and Weiland, 1992). Rajcan and Tollenaar (1999) reported that the proportion of nitrogen derived from the soil during grain filling in maize (*Zea mays* L.) was 60% for Pioneer 3902 (stay-green hybrid) and 40% for Pride 5 (senescent hybrid). Under conditions of abiotic stress such as drought or nitrogen deficiency, remobilization of nitrogen from vegetative tissues becomes particularly important for grain growth (Ta and Weiland, 1992). Delayed remobilization of nitrogen from leaves maintains photosynthesis capacity for long, possibly resulting in higher grain yield. According to

Borrell *et al.* (2001) leaf nitrogen concentration of stay-green hybrids is higher than senescent hybrids in both higher and lower soil nitrogen conditions; and hence, delayed senescence resulting from this should, in turn, allow more carbon and nitrogen to be allocated to the roots of stay-green hybrids during grain filling, thereby maintaining a greater capacity to extract nitrogen from the soil compared with senescent hybrids. The selected candidate materials may probably reward materials which drive most proportion of nitrogen from the soil during grain filling.

According to Xu *et al.* (2000) under severe post-flowering drought conditions, the stay-green lines, such as B35 have a SPAD values above 40 and extremely susceptible lines, such as Tx700 have a SPAD value of about 14 and essentially be without chlorophyll in leaves. The candidate materials selected in this study showed a SPAD reading value up to 60 (Sorcoll-068/07); 23 accessions had a SPAD values greater than 40, 26 accessions greater than 30, 6 accessions greater than 25, and 1 accession less than 20. The results show these materials have potential stay-green property.

Relative water content at dawn of the 10 randomly selected accessions of the candidate materials was higher ranging from 58.32%-99.11% (Appendix 6) indicating that their stalk transporting system continued to function under severe drought condition. In a study conducted by Xu *et al.* (2000) non stay-green lines had 38% relative water content. In this evaluation relative water content at mid day of 31 accessions was less than or equal to 38%, the rest had more than 38%. This indicated that the candidate accessions do have higher relative water content at dawn.

## 7.2. Grouping of Candidate Accessions on the Basis of Stay- green Traits

If selection is to be made between cluster groups for future breeding exercise on various traits the combined mean table of the four similarity clusters would be of use.

Cluster 1 which has the highest proportion of green leaves remaining 30 days after flowering and the lowest proportion of green leaves remaining 15 days after flowering. According to Thomas and Smart (1993) this cluster constitutes accessions that prevail Type B. Our findings suggest the fact that if further evaluation for this specific trait is needed, this cluster (cluster 1) would be of better preference.

Cluster 4 probably constitutes accessions with more stay-green property since it has higher values for most of the traits. It had the higher proportion of green leaves remaining 15 days after flowering, green leaf area at flowering, mid day relative water content and extent of tillering. It also had second higher value for proportion of green leaves remaining 30 days after flowering, SPAD chlorophyll index reading. Cluster 2 may also take the second position in this respect following Cluster 4.

Van Oosterom *et al.* (1996) stated stay-green as a valuable trait for both grain and fodder production in dual sorghums. This is because greater functional leaf area during grain filling in stay-green types is associated with increased accumulation of soluble sugars, thereby reducing their dependence on stored assimilates from the stem to fill grain (McBee *et al.*, 1983). Hence, the higher concentration of stem sugar in turn improves the digestible energy content of the straw, making stay-green a valuable trait for both grain

and fodder production. Therefore, accessions in Cluster 3 and 4 which have a higher extent of tillering, could potentially contribute to dual purpose sorghum.

### **7.3. Relative Contribution of Each Collection Area for Candidate Materials**

Since the sample sizes from each collection area are different it will be difficult to with certainty about the relative favorability of the collection areas to develop the stay-green property, but in this particular experiment South Tigray and North Wello take the first and second place, respectively, in contributing more candidate materials. Here, these two zones had also similar position in the original number of sample. Therefore, to see the clear variation in relative favorability of the collection areas to develop the stay-green trait comparable and extensive sampling from each collection areas is needed.

For those interested to carry on this study back up seed of these accessions is available at Holeta Agricultural Research Center. The collection data book of the accessions is compiled and available at Addis Ababa University, Ecophysiology Laboratory.

## **8. CONCLUSIONS AND RECOMMENDATIONS**

Variation was observed among sorghum accessions for post-flowering drought resistance property (stay-green). A total of fifty-six potential candidate materials were selected that attained the stay-green syndrome in various ways. Hence, the present result suggests that these selected candidate materials have the potential to give out stay-green materials which may be different physiologically.

Candidate materials collected from the same area which grouped themselves in one cluster may be showing their genetic similarity. Some candidate materials grouped themselves in a different cluster showing their distinctiveness from those accessions in their collection area.

For future it is recommended that, these sorghum accessions need to go through extensive study to come up with best materials. In the present study the selected materials had showed higher SPAD Chlorophyll reading at physiological maturity but there is a need to see their performance across their developmental stages. Further measurements on total green leaf area both at flowering and maturity and observing the rate of loss of leaf area between flowering and maturity is also essential. Measurements on above ground biomass both at flowering and maturity should be done by first dividing them in to main stem and tillers, then dividing each of those components into green leaf, dead leaf, stem and panicle. Investigating the yield potential of candidate materials under fully irrigated condition and comparing it with their yield under post-flowering drought stress condition is essential.

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## 10. APPENDICES

### Appendix 1. List of the collection materials and their origin

No.	Acc. No.	Origin	No.	Acc. No.	Origin	No.	Acc. No.	Origin
1	Sorcoll-001/07	Central Tigray	56	Sorcoll-062/07	South Tigray	111	Sorcoll-128/07	North Wello
2	Sorcoll-002/07	Central Tigray	57	Sorcoll-063/07	South Tigray	112	Sorcoll-130/07	North Wello
3	Sorcoll-003/07	Central Tigray	58	Sorcoll-065/07	South Tigray	113	Sorcoll-131/07	North Wello
4	Sorcoll-004/07	Central Tigray	59	Sorcoll-066/07	South Tigray	114	Sorcoll-132/07	North Wello
5	Sorcoll-005/07	Central Tigray	60	Sorcoll-068/07	South Tigray	115	Sorcoll-134/07	North Wello
6	Sorcoll-006/07	Central Tigray	61	Sorcoll-070/07	South Tigray	116	Sorcoll-135/07	North Wello
7	Sorcoll-007/07	Central Tigray	62	Sorcoll-071/07	South Tigray	117	Sorcoll-136/07	North Wello
8	Sorcoll-008/07	Central Tigray	63	Sorcoll-072/07	South Tigray	118	Sorcoll-137/07	North Wello
9	Sorcoll-009/07	Central Tigray	64	Sorcoll-073/07	South Tigray	119	Sorcoll-138/07	North Wello
10	Sorcoll-011/07	Central Tigray	65	Sorcoll-074/07	South Tigray	120	Sorcoll-139/07	North Wello
11	Sorcoll-012/07	Central Tigray	66	Sorcoll-075/07	South Tigray	121	Sorcoll-140/07	North Wello
12	Sorcoll-013/07	Central Tigray	67	Sorcoll-076/07	South Tigray	122	Sorcoll-141/07	North Wello
13	Sorcoll-014/07	Central Tigray	68	Sorcoll-077/07	South Tigray	123	Sorcoll-142/07	North Wello
14	Sorcoll-015/07	Central Tigray	69	Sorcoll-078/07	South Tigray	124	Sorcoll-143/07	North Wello
15	Sorcoll-016/07	Central Tigray	70	Sorcoll-079/07	South Tigray	125	Sorcoll-144/07	North Wello
16	Sorcoll-017/07	Central Tigray	71	Sorcoll-080/07	South Tigray	126	Sorcoll-145/07	North Wello
17	Sorcoll-018/07	Central Tigray	72	Sorcoll-081/07	South Tigray	127	Sorcoll-146/07	North Wello

18	Sorcoll-019/07	Central Tigray	73	Sorcoll-082/07	South Tigray	128	Sorcoll-147/07	North Wello
19	Sorcoll-020/07	Central Tigray	74	Sorcoll-083/07	South Tigray	129	Sorcoll-148/07	North Wello
20	Sorcoll-021/07	Central Tigray	75	Sorcoll-084/07	South Tigray	130	Sorcoll-149/07	North Wello
21	Sorcoll-022/07	Central Tigray	76	Sorcoll-085/07	South Tigray	131	Sorcoll-150/07	North Wello
22	Sorcoll-023/07	Central Tigray	77	Sorcoll-086/07	South Tigray	132	Sorcoll-151/07	North Wello
23	Sorcoll-024/07	Central Tigray	78	Sorcoll-087/07	South Tigray	133	Sorcoll-152/07	North Wello
24	Sorcoll-026/07	Central Tigray	79	Sorcoll-088/07	South Tigray	134	Sorcoll-153/07	North Wello
25	Sorcoll-027/07	Central Tigray	80	Sorcoll-089/07	South Tigray	135	Sorcoll-154/07	North Wello
26	Sorcoll-028/07	Central Tigray	81	Sorcoll-090/07	South Tigray	136	Sorcoll-155/07	North Wello
27	Sorcoll-029/07	Central Tigray	82	Sorcoll-091/07	South Tigray	137	Sorcoll-156/07	North Wello
28	Sorcoll-030/07	Central Tigray	83	Sorcoll-093/07	South Tigray	138	Sorcoll-159/07	North Wello
29	Sorcoll-031/07	Central Tigray	84	Sorcoll-094/07	South Tigray	139	Sorcoll-160/07	North Wello
30	Sorcoll-032/07	Central Tigray	85	Sorcoll-095/07	South Tigray	140	Sorcoll-161/07	North Wello
31	Sorcoll-033/07	Central Tigray	86	Sorcoll-096/07	South Tigray	141	Sorcoll-162/07	North Wello
32	Sorcoll-034/07	Central Tigray	87	Sorcoll-097/07	South Tigray	142	Sorcoll-163/07	North Wello
33	Sorcoll-035/07	Central Tigray	88	Sorcoll-098/07	South Tigray	143	Sorcoll-164/07	North Wello
34	Sorcoll-036/07	Central Tigray	89	Sorcoll-099/07	South Tigray	144	Sorcoll-165/07	North Wello
35	Sorcoll-037/07	Central Tigray	90	Sorcoll-100/07	South Tigray	145	Sorcoll-166/07	North Wello
36	Sorcoll-038/07	Central Tigray	91	Sorcoll-101/07	South Tigray	146	Sorcoll-167/07	North Wello
37	Sorcoll-039/07	Central Tigray	92	Sorcoll-102/07	South Tigray	147	Sorcoll-168/07	North Wello
38	Sorcoll-040/07	South Tigray	93	Sorcoll-103/07	South Tigray	148	Sorcoll-169/07	North Wello
39	Sorcoll-041/07	South Tigray	94	Sorcoll-104/07	South Tigray	149	Sorcoll-173/07	South Wello
40	Sorcoll-044/07	South Tigray	95	Sorcoll-107/07	South Tigray	150	Sorcoll-174/07	South Wello

41	Sorcoll-045/07	South Tigray		96	Sorcoll-108/07	South Tigray		151	Sorcoll-175/07	South Wello
42	Sorcoll-046/07	South Tigray		97	Sorcoll-110/07	South Tigray		152	Sorcoll-176/07	South Wello
43	Sorcoll-047/07	South Tigray		98	Sorcoll-111/07	South Tigray		153	Sorcoll-177/07	South Wello
44	Sorcoll-048/07	South Tigray		99	Sorcoll-112/07	South Tigray		154	Sorcoll-178/07	South Wello
45	Sorcoll-049/07	South Tigray		100	Sorcoll-113/07	South Tigray		155	Sorcoll-179/07	South Wello
46	Sorcoll-050/07	South Tigray		101	Sorcoll-114/07	South Tigray		156	Sorcoll-180/07	South Wello
47	Sorcoll-051/07	South Tigray		102	Sorcoll-115/07	South Tigray		157	Sorcoll-181/07	South Wello
48	Sorcoll-052/07	South Tigray		103	Sorcoll-116/07	South Tigray		158	Sorcoll-182/07	South Wello
49	Sorcoll-054/07	South Tigray		104	Sorcoll-117/07	South Tigray		159	Sorcoll-183/07	South Wello
50	Sorcoll-055/07	South Tigray		105	Sorcoll-119/07	South Tigray		160	Sorcoll-184/07	South Wello
51	Sorcoll-056/07	South Tigray		106	Sorcoll-120/07	South Tigray		161	Sorcoll-185/07	South Wello
52	Sorcoll-053/07	South Tigray		107	Sorcoll-123/07	North Wello		162	Sorcoll-188/07	South Wello
53	Sorcoll-058/07	South Tigray		108	Sorcoll-124/07	North Wello		163	Sorcoll-189/07	South Wello
54	Sorcoll-060/07	South Tigray		109	Sorcoll-125/07	North Wello		164	Sorcoll-190/07	South Wello
55	Sorcoll-061/07	South Tigray		110	Sorcoll-127/07	North Wello		165	Sorcoll-194/07	South Wello

**Appendix 2.** Pair wise mean comparison of number of green leaves at flowering using estimated standard error and critical value at t=2.17881(from analysis of variance of the checks)

	Sorcoll-160/07	Sorcoll-047/07	Sorcoll-056/07	Sorcoll-168/07	Sorcoll-119/07	Sorcoll-087/07	Sorcoll-009/07	Sorcoll-156/07	Sorcoll-137/07	Sorcoll-039/07
Sorcoll-160/07	0.0000									
Sorcoll-047/07	0.4897	0.0000								
Sorcoll-056/07	0.4897	0.0000	0.0000							
Sorcoll-168/07	0.6577	0.0985	0.0985	0.0000						
Sorcoll-119/07	0.6843	0.1946	0.1946	0.0961	0.0000					
Sorcoll-087/07	2.4990**	2.0093	2.0093	1.9108	1.8147	0.0000				
Sorcoll-009/07	2.5010**	2.0113	2.0113	1.9127	1.8167	0.0020	0.0000			
Sorcoll-156/07	2.8499**	2.0593	2.0593	2.1922**	1.8647	0.0500	0.0480	0.0000		
Sorcoll-137/07	2.8412**	2.3515**	2.3515**	2.2529**	2.4115**	0.3422	0.3402	0.2922	0.0000	
Sorcoll-039/07	3.6270**	3.5076**	3.5076**	3.0387**	2.9427**	1.1279	1.1260	1.0779	0.7858	0.0000

### Appendix 3. Means of ten characters taken

Accession No.	GLNAF	R15DAF	R13DAF	GLAAF	PH	TL	SPAD	RWC	NC	GYPP
Sorcoll-009/07	9.2475	0.9787	0.5587	336.39	208.26	2.6035	18.652	22.863	3.538 <sup>H</sup>	45.5
Sorcoll-011/07	11.581	1.0406	0.4462	495.58	227.26	2.6035	32.185	31.465	3.278	43.2
Sorcoll-012/07	10.581	1.0129	0.4883	520.25	287.26	2.2635	29.252	32.039	3.318	50.1
Sorcoll-013/07	11.248	0.9529	0.489	345.35	284.01	0.9335	25.452	42.55	3.138	49.4
Sorcoll-015/07	10.914	1.0431	0.565	504.35	319.26 <sup>H</sup>	0.2635	29.818	32.983	3.168	55.8
Sorcoll-026/07	12.248	1.0384	0.8301	476.41	257.26	0.9335	47.218	30.892	3.278	-
Sorcoll-027/07	10.248	1.0459	0.4066	452.35	261.26	3.6035	34.818	29.875	2.858	74.2
Sorcoll-028/07	9.5808	1.0143	0.5741	289.31 <sup>L</sup>	245.26	4.2635	32.385	26.225	3.178	-
Sorcoll-029/07	10.914	0.982	0.5345	347.28	280.26	2.6035	25.718 <sup>L</sup>	26.73	3.348	89.1
Sorcoll-032/07	10.914	0.9209	0.3512 <sup>L</sup>	542.63	168.26	3.6035	43.885	51.86	3.138	49.9
Sorcoll-035/07	11.248	0.9825	0.6668	509.14	222.26	2.6035	58.018 <sup>H</sup>	34.877	3.168	45.9
Sorcoll-038/07	10	0.9133	0.4999	427.84	239.91	1.7685	35.968	28.239	2.5005	56.2
Sorcoll-039/07	7.3333 <sup>L</sup>	0.9273	0.6362	424.23	226.91	1.7685	36.202	22.686	2.7805	58.4
Sorcoll-040/07	10.667	0.95	0.5624	579.1	154.91	1.7685	47.602	37.729	3.2405	54.4
Sorcoll-045/07	10.333	0.9484	0.5805	475.82	274.91	0.7685	36.102	32.906	3.0405	74.1
Sorcoll-046/07	10	0.98	0.4332	383.85	265.91	1.7685	29.302	24.56	3.0605	54.5
Sorcoll-047/07	12.667	0.8789	0.4736	526.49	242.91	0.7685	39.868	26.597	2.9905	-

Sorcoll-054/07	12.333	0.9027	0.5674	615.74	241.91	1.0985	36.802	24.361 <sup>L</sup>	2.6705	-
Sorcoll-056/07	12.667	0.7474 <sup>L</sup>	0.3946	525.81	277.91	1.7685	33.768	33.559	2.9805	-
Sorcoll-053/07	9.6667	0.9793	0.655	466.81	253.91	2.4385	37.335	34.833	3.4105	52.6
Sorcoll-060/07	10.333	0.9806	0.4192	645.76	209.91	0.7685	44.668	46.701	3.2405	-
Sorcoll-062/07	12.333	0.9838	0.4053	601.51	244.91	1.7685	47.135	29.648	3.2805	-
Sorcoll-063/07	10.333	0.9161	0.645	601.94	219.91	0.7685	36.102	40.032	2.8305	-
Sorcoll-068/07	10.667	0.8875	0.4374	428.19	206.91	0.0985 <sup>L</sup>	33.135	60.057 <sup>H</sup>	2.9405	49.0
Sorcoll-070/07	11	0.9515	0.6059	492	213.91	1.0985	44.102	54.866	3.0005	50.6
Sorcoll-076/07	11.251	0.8784	0.5261	543.25	262.41	3.261	27.243	46.694	3.158	43.3
Sorcoll-080/07	10.918	0.9357	0.5422	710.85	296.41	0.931	38.477	42.189	2.808	58.5
Sorcoll-081/07	11.584	0.9682	0.7124	481.85	269.41	0.931	45.043	48.861	2.918	54.2
Sorcoll-085/07	10.918	0.9052	0.6643	639.04	237.41	1.931	33.743	50.778	3.278	-
Sorcoll-086/07	10.251	0.9641	0.675	548.64	213.41	1.261	33.043	59.888	3.088	32.9
Sorcoll-087/07	9.2508	0.9242	0.82	696	253.41	0.601	32.377	45.194	2.848	47.7
Sorcoll-089/07	11.918	0.9971	0.8323	439.61	276.41	0.601	36.51	46.231	2.928	-
Sorcoll-090/07	11.918	0.9132	0.6925	589.12	251.41	0.601	42.477	58.073	2.688	-
Sorcoll-091/07	11.918	0.9132	0.6925	637.69	278.41	0.931	41.71	36.555	2.698	56.4
Sorcoll-101/07	11.918	0.9971	0.6645	631.4	299.41	1.261	41.177	26.418	2.318 <sup>L</sup>	-
Sorcoll-119/07	12.336	0.9027	0.52	506.69	246.78	1.936	31.143	31.867	3.013	-
Sorcoll-124/07	12.003	0.9556	0.5345	494.66	280.78	2.266	47.477	46.962	3.243	-

Sorcoll-127/07	11.336	0.9824	0.6541	587.45	273.78	0.936	43.31	45.417	3.093	64.7
Sorcoll-137/07	8.6692	0.9769	0.5862	436.41	251.78	1.596	41.677	32.427	2.813	38.2
Sorcoll-138/07	11.003	0.9818	0.6134	534.63	268.78	1.266	43.543	31.698	3.113	-
Sorcoll-139/07	11.336	0.953	0.6835	611.75	263.78	0.936	38.843	36.78	2.833	-
Sorcoll-141/07	12.003	0.9833	0.7011	599.64	275.78	0.936	53.677	36.027	2.783	-
Sorcoll-142/07	11.336	0.9824	0.6835	591.13	230.78	2.266	32.41	40.297	2.833	-
Sorcoll-146/07	10.669	0.9813	0.7575	623.83	263.78	1.596	47.977	53.056	2.883	-
Sorcoll-155/07	9.4992	0.9963	0.579	543.86	187.66	1.431	41.993	43.078	3.2905	-
Sorcoll-156/07	9.1658	0.9598	0.491	577.4	178.66	2.101	35.86	30.667	3.2105	35.9
Sorcoll-159/07	12.166	0.9971	0.8905 <sup>H</sup>	518.41	112.66 <sup>L</sup>	2.101	43.627	53.654	2.8505	-
Sorcoll-160/07	13.499 <sup>H</sup>	0.9974	0.6791	482.42	175.66	0.771	31.16	37.869	2.5405	-
Sorcoll-162/07	9.4992	0.9963	0.5439	535.15	187.66	2.101	34.86	37.407	2.8705	68.1
Sorcoll-163/07	11.166	0.9969	0.7613	589.64	213.66	1.431	33.427	49.88	2.8805	52.8
Sorcoll-164/07	10.833	0.966	0.6616	458.79	203.66	1.771	42.993	34.136	2.8205	45.8
Sorcoll-168/07	12.499	0.9705	0.6001	634.19	202.66	1.431	36.46	26.531	2.8805	-
Sorcoll-176/07	10.499	1.0919 <sup>H</sup>	0.4921	681.16	163.16	1.101	36.327	53.398	3.0705	91.5
Sorcoll-178/07	10.499	0.9649	0.6826	536.15	214.66	2.101	41.76	48.28	3.4705	47.8
Sorcoll-179/07	10.166	0.9638	0.705	688.26	157.66	7.431 <sup>H</sup>	44.127	43.567	2.9205	59.4
Sorcoll-180/07	11.499	0.997	0.7392	768.05 <sup>H</sup>	216.66	5.431	46.793	59.647	2.9505	-

NB: - **H**=Highest, **L**=Lowest

**Appendix 4.** Accessions and the block they are occurring in

<b>Accessions</b>	<b>Block</b>	<b>Accessions</b>	<b>Block</b>	<b>Block</b>	<b>Block</b>	<b>Accessions</b>	<b>Block</b>
Sorcoll-009/07	1	Sorcoll-045/07	2	Sorcoll-085/07	3	Sorcoll-142/07	4
Sorcoll-011/07	1	Sorcoll-046/07	2	Sorcoll-086/07	3	Sorcoll-146/07	4
Sorcoll-012/07	1	Sorcoll-047/07	2	Sorcoll-087/07	3	Sorcoll-155/07	5
Sorcoll-013/07	1	Sorcoll-054/07	2	Sorcoll-089/07	3	Sorcoll-156/07	5
Sorcoll-015/07	1	Sorcoll-056/07	2	Sorcoll-090/07	3	Sorcoll-159/07	5
Sorcoll-026/07	1	Sorcoll-053/07	2	Sorcoll-091/07	3	Sorcoll-160/07	5
Sorcoll-027/07	1	Sorcoll-060/07	2	Sorcoll-101/07	3	Sorcoll-162/07	5
Sorcoll-028/07	1	Sorcoll-062/07	2	Sorcoll-119/07	4	Sorcoll-163/07	5
Sorcoll-029/07	1	Sorcoll-063/07	2	Sorcoll-124/07	4	Sorcoll-164/07	5
Sorcoll-032/07	1	Sorcoll-068/07	2	Sorcoll-127/07	4	Sorcoll-168/07	5
Sorcoll-035/07	1	Sorcoll-070/07	2	Sorcoll-137/07	4	Sorcoll-176/07	5
Sorcoll-038/07	2	Sorcoll-076/07	3	Sorcoll-138/07	4	Sorcoll-178/07	5
Sorcoll-039/07	2	Sorcoll-080/07	3	Sorcoll-139/07	4	Sorcoll-179/07	5
Sorcoll-040/07	2	Sorcoll-081/07	3	Sorcoll-141/07	4	Sorcoll-180/07	5

**Appendix 5.** Accessions selected based their visual rating of senescence with their visual rating

<b>Accessions</b>	<b>Visual Rating</b>	<b>Accessions</b>	<b>Visual Rating</b>	<b>Accessions</b>	<b>Visual Rating</b>	<b>Accessions</b>	<b>Visual Rating</b>
Sorcoll-009/07	2	Sorcoll-045/07	2	Sorcoll-085/07	1	Sorcoll-142/07	1
Sorcoll-011/07	2	Sorcoll-046/07	2	Sorcoll-086/07	1	Sorcoll-146/07	1
Sorcoll-012/07	2	Sorcoll-047/07	1	Sorcoll-087/07	1	Sorcoll-155/07	2
Sorcoll-013/07	2	Sorcoll-054/07	2	Sorcoll-089/07	1	Sorcoll-156/07	2
Sorcoll-015/07	2	Sorcoll-056/07	1	Sorcoll-090/07	1	Sorcoll-159/07	1
Sorcoll-026/07	1	Sorcoll-053/07	1	Sorcoll-091/07	2	Sorcoll-160/07	2
Sorcoll-027/07	2	Sorcoll-060/07	2	Sorcoll-101/07	1	Sorcoll-162/07	2
Sorcoll-028/07	2	Sorcoll-062/07	1	Sorcoll-119/07	2	Sorcoll-163/07	2
Sorcoll-029/07	2	Sorcoll-063/07	2	Sorcoll-124/07	1	Sorcoll-164/07	2
Sorcoll-032/07	1	Sorcoll-068/07	2	Sorcoll-127/07	1	Sorcoll-168/07	2
Sorcoll-035/07	1	Sorcoll-070/07	1	Sorcoll-137/07	2	Sorcoll-176/07	2
Sorcoll-038/07	2	Sorcoll-076/07	2	Sorcoll-138/07	1	Sorcoll-178/07	2
Sorcoll-039/07	2	Sorcoll-080/07	1	Sorcoll-139/07	1	Sorcoll-179/07	1
Sorcoll-040/07	1	Sorcoll-081/07	1	Sorcoll-141/07	1	Sorcoll-180/07	1

**Appendix 6.** Relative water content at dawn of the 10 randomly selected candidate materials

ACCESSION No.	RWC (%)
Sorcoll-026/07	75.29976
Sorcoll-035/07	99.11504
Sorcoll-040/07	97.34345
Sorcoll-053/07	67.61488
Sorcoll-070/07	74.79167
Sorcoll-086/07	58.3196
Sorcoll-087/07	84.75336
Sorcoll-142/07	87.97251
Sorcoll-146/07	75.29412
Sorcoll-179/07	84.39201
Sorcoll-180/07	76.42857



Photo by Zelalem

**Appendix 7.** Sample candidate stay-green sorghum accessions (above) and senescent sorghum accessions (below).

## DECLARATION

I, the under signed, declare that this thesis is my original work. It never has been submitted in any institution and that all sources of materials used for the thesis have been dully acknowledged.

Name: Zelalem Mengiste

Signature: \_\_\_\_\_

Date of submission: \_\_\_\_\_

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