RESPONSE OF SELECTED GRAIN SORGHUM LINES TO WATER STRESS

BY

LANGAT ZEDDY CHEPKURUI

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School of Science,

University of Eldoret, Kenya

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DECLARATION

DECLARATION BY THE CANDIDATE

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Langat Zeddy Chepkurui

SC/PGB/04/07

Sign ……..…… Date……

DECLARATION BY SUPERVISORS

This thesis has been submitted for examination with our approval as University supervisors.

PROF. AUGUSTINO O. ONKWARE
Rongo University College,
P.O. Box 103-40404,
RONGO, Kenya

Sign ...…….. Date……

DR. BEATRICE A. WERE
Department of Biological Science,
University of Eldoret,
P.O. Box 1125, Eldoret, Kenya.

Sign...…….. Date……

PROF. SAMUEL O. GUDU
Rongo University College,
P.O. Box 103-40404
RONGO, Kenya

Sign...…….. Date……

DEDICATION

To my mother Leah.

ABSTRACT

Sorghum (*S. bicolor* (L.) Moench) is an important cereal crop in drier areas of the world where drought is a major cause of low yields. Drought can occur at any stage of growth of sorghum in the field. It is characterized by low rainfall and high evapotranspiration rate that leads to water stress in plants. The objective of this study was to determine response of twelve selected sorghum lines to water stress at various stages of growth and development. Screening was carried out on seed imbibition rates, activity of starch degrading enzymes, germination, selected isozymes, and assessment of pre- and post-flowering water stress tolerance in the green house using potted and field grown plants. The sorghum lines which showed the highest imbibition rates and high percentage germination under water stress was MCSR I10, a short duration to total germination under water stress was recorded in MCSR T30 and Gadam. The lines with the longest seedling radicle lengths were MCSR T28, MCSR O2 and MCSR I10. The lines with the highest starch degrading enzyme activity included MCSR O2 and Gadam. The sorghum lines with shortest time to panicle emergence under the pre-flowering water stress included MCSR G2 and MCSR C1, and lines with the lowest percentage panicle weight reduction under water stress included MCSR N4, MCSR G2 and MCSR T28. Under the post-flowering water stress, the lines with the highest total chlorophyll concentration included MCSR D1b, MCSR C1, MCSR G2 and MCSR N4; lines with the lowest percentage in leaf senescence in the field under post flowering stress were MCSR T30, MCSR T28, MCSR I10 and MCSR D1b and sorghum lines with the highest grain yield in the field were MCSR F14a, Gadam, MCSR T28 and MCSR A11. The sorghum lines identified as tolerant to moisture stress during germination included MCSR O2, MCSR I10 and Gadam. The lines which had normal growth, with early panicle emergence and low number of nodal tillers under pre-flowering water stress included MCSR N4, MCSR G2 and MCSRT28. The identified sorghum lines Gadam, MCSR T28 and MCSR G2 can be adopted for planting in dry areas. The lines recommended for use to develop drought tolerant high yielding varieties includes MCSR C1, MCSR T30, MCSRD1b, MCSR N4 and MCSR F14a. These lines need to be tested extensively in the dry areas of the country to confirm their potential for high productivity in arid and semi-arid lands.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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'Success lies not at some high place along the way, but in having made the

journey, step by step..."

CHAPTER ONE

INTRODUCTION

1.1 Background.

Cultivated grain sorghum [*Sorghum bicolor* (L.) Moench] is an important staple food crop for millions of people worldwide. Sorghum is supposedly a native to Sub-Saharan Africa from where it later spread to Asia and the America (Ayana and Bekele, 1998). Sorghum is also used as feed for livestock and as a raw material for the manufacture of bio-fuel (Casa et al., 2008). Sorghum has various uses in Kenya. It is dehulled to form rice-like product which is marketed as 'super mtama' (Yetneberk and Mitaru, 2007). The by-products from this process include bran, germ and grits which are then used as animal feeds. The grain is milled to form flour which is put to various uses that includes making of "uji" locally, biscuits, brewed for beer 'senator keg' or to make baby foods by Irangi hill and Proctor and Allan companies in Kenya. Milled flour can also be made into composites to make "chapatti" (mix with wheat flour) and "ugali" (mix with cassava flour) (Yetneberk and Mitaru, 2007). However, there is limited knowledge on whether water stress affects germination, growth and production in sorghum that is held by Kenyan farmers.

Sorghum is one of the crops that are suitable for cultivation in most ecological zones (FAO, 1995) due to its adaptation to drier conditions. It is grown from latitude 35°S to 45°N, altitude 0 to 2000 meters above sea level and in areas receiving rainfall between 300 to 2000 millimeters per annum (Saxena and O'Toole, 2002). Sorghum landraces have been cultivated in Africa for a long time, and this has led to extensive genetic diversity for drought tolerance for both cultivated and wild forms. There are

five cultivated races of sorghum that includes bicolor, caudatum, durra, guinea and kafir (Fiquiredo et al., 2008).

Drought is a meteorological and an environmental condition characterized by absence of, or low rainfall accompanied by high evapotranspiration (Pinheiro et al., 2005). Drought may occur at any stage of sorghum plant growth and this period may be long enough to deplete soil moisture and injure plants (Kramer and Boyer, 1997). It is a serious agronomic problem that causes substantial crop yield losses (Saxena and O'Toole, 2002; Li-Ping et al., 2006). In grain sorghum, drought causes losses of about 1.8 tons per hectare per year in East Africa (Wortman et al., 2006). These losses are expected to increase in the future because of climate change which has been predicted to result in increased atmospheric temperatures accompanied by lower rainfall in many parts of the world (Atkin and macherel, 2008; FAO, 2008). This will result in ecosystem alteration and failure of drought-sensitive crops (Schafleitner et al., 2009).

The arid and semi arid land comprises 25% of the total land of our planet and is about 85% of Kenyan land (Earthtrends, 2003). Four tenths of the world's agricultural land lies in these regions. Sorghum is in cultivation under 40 million hectares in the world, that yields60 million tonnes of grain. Africa produces 20 million tonnes from 14 million hectares are in Africa (FAO, 1995). Sorghum grain comes second to maize in importance in Africa. (FAO, 1995). Grain sorghum production in Africa is low because of low soil fertility, drought as a result of erratic and inadequate rainfall, negligible production inputs, continued use of unimproved cultivars and high prevalence of diseases and pests (FAO, 1995). In east Africa, sorghum is cultivated under 7 million hectares per year (Mutisya et al., 2010). In Kenya the area under

sorghum production totals 123 000 ha and most of this lies in the medium and low altitude areas of Nyanza, Western, Eastern-Central, Coast and Rift Valley (Wortman et al., 2006) (Figure 1). These areas are drought prone with a combination of warm mean temperatures (> 20°C) and low mean monthly rainfall (< 120 mm) (Wortman et al., 2006). Grain sorghum production has been on the decline with 220 metric tones in the 1980 to 130 metric tones in 2009 as indicated in data by UN department of Agriculture (2011). These losses from drought has been reported to total 38 000 Mg per year (70% loss) (Pocket, 2009). Sorghum production is normally carried out by small holder farmers under rain fed farming in areas which are too dry for other cereals like maize (FAO, 1995).

Kenya is 80%-85% sub-humid to arid and these areas will likely become drier with low and erratic rainfall as predicted by Climate change models (Atkin et al., 2008; FAO, 2008). Therefore, drought remains a major factor contributing to low grain sorghum yields.

Sorghum production can be improved by a combination of genetic improvements in cultivars and agronomic practices that includes irrigation (Dwivedi et al., 2007). Irrigation in Kenya is limited mainly to research and government projects. These are capital intensive and way too expensive for the larger sorghum small scale growers. Therefore genetic improvement is the most appropriate method for improving sorghum yields in arid and semi arid areas.

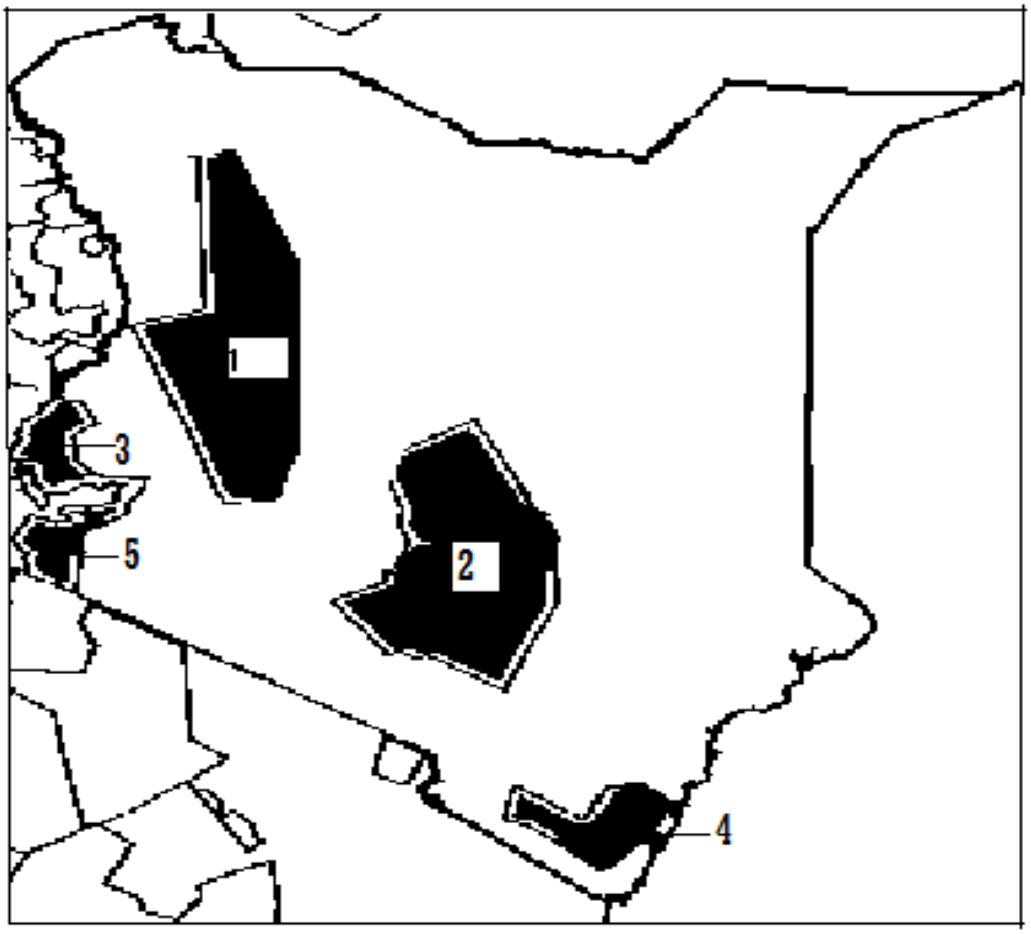
Drought tolerance in sorghum varies with the genotype and involves different mechanisms of tolerance (Xiong et al., 2006). The drought tolerance mechanisms includes normal germination under low soil moisture content, normal growth under the pre-flowering water stress, short period to maturity, deep root depth, maintaining

large green leaf area and normal grain filling under the post-flowering water stress (Borrel et al., 2000). Several studies have revealed positive correlation between drought tolerance and sorghum yields (Borrel et al., 2000: Jordan et al., 2003). Some sorghum genotypes have been identified and confirmed to be drought tolerant (E 36-1 and B35 lines) (Kebede etal., 2001) and that they have been successfully used to develop commercial cultivars (Henzel et al., 2001). The drought tolerant cultivars have been reported to yield more biomass between flowering and maturity than their senescent counterparts under water stress conditions (Borrel et al., 2000).

Drought tolerance traits have been incorporated into high yielding genetic backgrounds of chickpea and groundnut (Saxena and O'Toole, 2002). Research findings have also reported that hybrids yields better under water stress than their parental genotypes (Okiyo et al., 2010). This can also be replicated in grain sorghum for better production.

1.2 Statement of the problem.

Drought is a major cause of low production in cultivated sorghum in Kenya. Sorghum production in Kenya is carried out under rain fed farming by resource poor small scale farmers in the drier areas of arid and semi arid lands (Saxena and O'Toole, 2002). These areas receive low annual rainfall of less than 750 mm per annum which is often unevenly distributed, erratic and unreliable; and are expected to be warmer and drier as predicted by current global climate change models (Wortman et al., 2006). Sorghum yields in these areas are quite low (0.6 – 1.5 mg ha^{-1} yr^{-1}) as compared to yields in well watered environments (4.3 mg ha^{-1} yr^{-1} in USA) (FAO, 2008).



Key: 1-Rift Valley, 2-Eastern-Central, 3-Western, 4-Coast, 5-Nyanza,

Figure 1: Sorghum growing areas in Kenya: Adopted from Wortman et al., (2006)

Sorghum is a popular crop in the dry agro-ecological zones, but drought has kept the production low. Drought is a serious agronomic problem which can occur during germination, vegetative or reproductive stages of sorghum growth causing substantial grain losses. Production of sorghum can be improved by genetic improvement of cultivars or by irrigation. Irrigation is a capital intensive practice and this makes it unsuitable for small scale sorghum growers because it is expensive. Genetic improvement is the most appropriate method for improving sorghum production among the small holder farmers. This is because to date there are no known drought tolerant varieties recommended for various agro-ecological zones in Kenya due to

limited research on drought tolerance of Kenyan sorghum. It is also not known which stage of water stress is detrimental and which varieties are less affected.

The Kenyan farmers have diverse sorghum germplasm which has not been assessed for drought tolerance. Drought tolerant sorghum genotypes can be used to develop high yielding drought tolerant cultivars which may be deployed in the semi-arid agro-ecological zones to improve food production and to sustain food security. In addition, this will be useful in the conservation and development of improved sorghum varieties.

1.3 Objectives of the study.

1.3.1 General Objective.

The general objective of this study was to screen for drought tolerance among selected sorghum lines for use in breeding programs in order to produce drought tolerant varieties for arid and semi-arid lands (ASALs).

1.3.2 Specific Objectives.

i) To determine seed imbibition rates, selected enzymes activity and germination percentage during germination under water stress in sorghum lines.

ii) To determine plant height reduction, low percentage reduction in number of days to panicle emergence, high number of nodal tillers and high shoot dry weights of sorghum lines under the pre-flowering water stress in sorghum.

iii) To determine leaf pigment concentration, senescence and grain yield of sorghum lines under post-flowering water stress.

1.3.3. Study Hypothesis.

Sorghum genotypes express significant variations in water stress tolerance at various stages of growth.

1.4 Justification.

Grain sorghum is a staple food crop which is grown in drier areas due to its tolerance to water stress (Saxena and O'Toole, 2002) that varies from one genotype to another. This attribute is of great importance as demand for food and water supplies increases (Sanchez et al., 2002: Balota et al., 2008) due to impending drought as predicted in climate change models (FAO, 2008). This can be an added advantage in selecting sorghum lines that show high tolerance to water stress. The identified drought tolerant varieties will be a low input technology that would be readily acceptable to the resource poor, rain fed, small hold farmers. This will further increase grain sorghum yields and improve food security.

It has also been reported that demand for sorghum, one of the cereals, is growing and is expected to double between 1995 and 2020 (Dwivedi et al., 2007) because the human population have grown faster than agricultural production. Demand for livestock feeds is also expected to double by 2020 with an increase of 40% in developing countries of which Kenya is included (Dwivedi et al., 2007). It is paramount to note that there is a growing market for grain sorghum in Eastern and Horn of Africa because it is becoming an important industrial crop for the manufacture of beer (EABL, 2010) and in generation of biofuel. There is then an urgent need to develop sorghum germplasm with improved drought tolerance and high yields to meet this demand for sorghum grains.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of Sorghum.

Sorghum is a genus with many species and sub-species and belongs to the family *Poaceae* , tribe Andropogoneae, sub-tribe Sorghinae (Clayton and Renvoize, 1986). The many subspecies are divided into four groups; grain sorghum, grass sorghums, sweet sorghums and broom corn (Rai et al., 1999). Races of cultivated sorghum include guinea, caudatum, kaffir and durra; other sorghum races include Verticilliflorum and Drumondii that constitute some of the weeds (De Wet, 1976).

2.2 Plant Description.

Cultivated sorghum is an annual with culms that may grow to a height of 5 m, often branched with many tillers (Dillon et al., 2007). They have panicles that measure 8 cm to 40 cm long and are loose or compact with sessile spikelet that measure 4 mm to 6 mm long. Mature glumes of spikelet could be red or reddish brown, straw coloured or yellowish, and sometimes flushed with dark red or reddish brown (IBPGR and ICRISAT, 1993).

2.3 Uses of Sorghum.

Sorghum grains have a range of uses that includes human consumption, beer brewing and animal feed and in Bio-ethanol for energy production (Dwivedi et al, 2007). More than 35% of sorghum is grown directly for human consumption. It is also used primarily for production of alcohol and industrial starch. Sorghum stems are used in fencing, thatching, making baskets, brushes, brooms, fuel, extraction of dye and medical uses (FAO, 1995; Awika and Rooney, 2004).

Sorghum flour can be processed into leavened or unleavened flat bread, porridges and side dishes, steamed food and rice-like boiled products, alcoholic beverages that are malted or distilled and foods such as popped grains (Noah and Waithaka, 2005). In West Africa, unfermented sorghum grains are generally used for the preparation of porridge, and couscous (Ghebru et al., 2002). Malted sorghum is used in the processing of local beers, infant porridge and non-fermented beverages (Dicko et al., 2006).

Sorghum is an important crop for animal feeds, which is the major reason for its production in USA and other industrialized countries (Rai et al., 1999). It may also be used for silage and pasture but may first require to be processed to hay in order to reduce prussic acid poisoning in animals. Sorghum stems may be used to make wallboard, fences, biodegradable packaging materials and solvents. Dried stalks are used as fuel, and also for extraction of dye which is used to colour leather (Rai et al., 1999; FAO, 1995).

2.4 Sorghum growing areas.

Sorghum is cultivated in many countries of tropical, sub-tropical and warm temperate regions of the world and mostly in the arid and semi-arid tropics (De Wet et al., 1976 and Dillon et al., 2007). In Kenya, sorghum is grown in the drought prone areas of Eastern, Nyanza and Coast provinces (Noah and waithaka, 2005). Sorghum performs well in areas with altitude range between 500 m to 1700 m above sea level with a minimum rainfall between 300 mm –750 mm per annum (Noah and Waithaka, 2005).

2.5 Effect of drought on sorghum.

Plants become water stressed when the available moisture in the soil is low (Saxena and O'Toole, 2002) due to failure to rain and high evapotranspiration. When soil loses more water through evapotranspiration than it gets from precipitation it suffers from water deficit. If water loss exceeds absorption, the plant experiences water stress. This disturbs the metabolic processes in the plant and to maintain these processes, water stressed plants need to intensify the water absorption and or to reduce the water loss.

Sorghum is one of the most drought tolerant cereals (Dogget and Rao, 1988) because of its well developed and finely branched rooting system for efficient water absorption from the soil. It also has stomata that close rapidly in response to water stress (Teare et al., 1973) thus reducing water loss by transpiration. The plant has the ability to reduce growth and metabolic activities to minimum during water stress and resume growth soon after when conditions become favourable. Sorghum plants can grow new shoots which form seed in case the main tiller is destroyed. It also has a rich genetic diversity for water stress tolerance (Blum, 1979; Wortman et al., 2006).

Responses to water stress in sorghum vary from one variety to another. Some sorghum varieties are more susceptible to water stress during early vegetative phase than others but are tolerant during post flowering stage. Some sorghum varieties are more susceptible to water stress at the period of panicle development prior to flowering than others but are tolerant to water stress during other stages of growth (Rosnow and Clark, 1981). However, drought tolerance in sorghum has been classified into two forms namely pre-flowering and post-flowering drought tolerance (Tuinstra et al., 1998).

Water deficit affects every aspect of plant growth, including the anatomy, morphology, physiology and biochemistry (Kramer, 1983). The magnitude of injury caused by water stress depends on severity of stress and the stage of plant growth. The critical stages of sorghum growth can be divided into three stages: germination and seedling establishment, vegetative growth, and the reproductive stage (Kramer, 1983).

Non-uniform seed germination that results from low moisture availability in the soil can lead to non-synchronized and low percentage of seedling emergence, which subsequently leads to poor crop stand establishment (Sharma et al., 2004; Gholami et al., 2010) and poor yields. Uptake of water by dry seeds occurs in three phases. It starts with a rapid uptake followed by a plateau phase then uptake until germination is completed at radicle emergence (Bewley, 1997). Dry seeds imbibe moisture from the soil and only get committed to germination when the critical threshold hydration level is reached (Ramagopal, 1990). Inadequate soil moisture will make seeds fail to attain the critical hydration level and thus fail to germinate at the expected time. The seeds which attain the critical hydration level will germinate but fail to emerge due to death out of desiccation caused by inadequate soil moisture supply (Pestsova et al., 2008).

When plants are subjected to water stress, they respond by altering physiological and biochemical processes (Shinozaki and Yamaguchi, 2007). At the whole plant scale, gradual soil water depletion would lead to reduction of cell division and expansion, solute accumulation in cells, changes in water relations, abscisic acid (ABA) synthesis, reduced and impaired photosynthesis, cessation of shoot growth, reduced root growth, induction of leaf senescence and plant death (Shinozaki and Yamaguchi, 2007).

2.6 Effects of water stress on plant cell functions.

One of the injuries caused by water stress to the photosynthetic apparatus is the reduced rate of formation of chlorophyll a/b protein and retarded accumulation of chlorophyll b (Efoeglu et al., 2009). Most of the chlorophyll loss occurs in the mesophyll cells during water stress. The loss consists of the lamellae, which forms the light harvesting site containing chlorophyll a and b, and is a major component of chloroplast membranes (Kramer and Boyer, 1997).

2.7 Biochemical response to water stress.

Water stress stimulates the hydrolysis of starch and proteins resulting in accumulation of sugars and amino acids in the cells (Neto et al, 2009) which help maintain cell osmotic potential and protect cells from dehydration. Water stress also stimulates proline synthesis from glutamate by loss of feed-back inhibition, decreased rate of proline oxidation and decrease in proline incorporation into protein (Rizhsky et al., 2004). Proline acts as an osmoprotectant protecting cellular organelles from destruction. It also acts as a sink for energy to regulate redox potentials by scavenging on hydroxyl radicals which then leads to reduced acidity in the cell (Porcel and Ruiz-Lozano, 2004). There is also an increased amount in other organic solutes like glycine betaine, mannitol, trehalose, fructans, sorbitol and inositol or ononitol that also play a role in osmoprotection of cell organelles and scavenging of hydroxyl radicals to maintain cell integrity. Production of heat shock proteins and molecular chaperones also occurs (Salekdeh et al., 2009).

Plants exposed to water stress react by closing leaf stomata in order to reduce water loss through transpiration (Borrel et al., 2000). However, stomatal closure also limits carbon (IV) oxide diffusion into the leaf and its subsequent fixation, which leads to

insufficient sink for electrons generated by the electron-transport-system. The immediate acceptor of electrons in such conditions is oxygen which leads to the generation of reactive oxygen species (ROS) including super oxide radical (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2). The ROS species are either scavenged by enzymes or removed by the non enzymatic detoxification mechanisms. The non-enzymatic antioxidants include glutathione, ascorbic acid, α-tocopheral carotenoids and phenolic compounds (Porcel and Ruiz-Lozano, 2004).

Carotenoids are multifunctional compounds that serve as structural components of light harvesting complexes and act as accessory pigments for light harvesting (Dellapenna., 1999). They transfer some of the light they absorb to chlorophylls for photosynthesis (Demmig-Adams et al. 1996). They also act as components of photo protection by harmlessly dissipating excess light energy under stress conditions which they absorb as heat and scavenge reactive oxygen species formed within the chloroplast (Young, 1991;; Younis et al., 2000).

2.8 Gene expression under drought stress.

Plant cellular water-deficit occurs under conditions of reduced soil moisture supply. This initiates changes in gene expression within a cell (Kotchoni and Bartels, 2003). Seed germination determines plant population which directly contributes to total yields in crop production. The factors affecting seed germination includes temperature, moisture, soil crusting, genetic factors and seed size among others.

Under normal conditions, germination is regulated by the interaction between gibberellins (GAs) and abscicic acid (ABA) (Holdsworth et al., 2008). These hormones regulate the synthesis of hydrolytic enzymes which are expected to break

down the stored organic reserves to glucose in preparation for germination. The stored starch reserves are degraded by hydrolytic enzymes that include α-amylase, β-amylase and α-glucosidase (Nomura et al., 2007). The products are then re-mobilized to the developing embryo axis to be used in respiration and synthesis of seedling tissues for growth (Ramagopal, 1990). Seed germination under water stress takes place under altered nutrient reserve mobilization pathways that result in accumulation of the osmoprotectant glycine betaine, late embryogenesis abundant proteins (LEA) and detoxification enzymes (Pestsova et al., 2008). Inadequate moisture availability during seed germination causes failure to attain the critical hydration level that is necessary for the synthesis of hydrolytic enzymes. This interferes with germination of seeds by limiting the hydrolysis of stored reserves and also impairs their translocation from storage sites to the developing embryo axis (Pestsova et al., 2008).

Plants growing in the field under water stress show changes in gene expression which may lead to adaptive mechanisms for tolerance or injury in plants (Hanson and Hitz, 1982). Several genes with diverse functions are induced or repressed by drought stress (Shinozaki et al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2005). The adaptive mechanisms of the cell involve activation and increase in expression of stress-induced genes and in phytohormones such as ABA. The resulting gene products which include compartible solutes, protective proteins, and increase in levels of anti-oxidants accumulate in the cell to protect cell organelles and function at the cellular level (Shinozaki and Yamaguchi, 2007). These may confer adaptation to water-deficit stress.

Water stress may also induce down or up-regulation of some genes which deal with generated ROS under water stress. The ROS cause injury to the cell membrane due to

lipid peroxidation (Chen and Dai, 1994) and also cause protein degradation, enzyme inactivation, pigment bleaching and disruption of nucleic acids especially deoxyribonucleic acid (He et al., 2005).

The genes that code for the synthesis of antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), ascorbate Peroxidase (APOX), glutathione reductase and catalase (CAT) are up-regulated. These enzymes also scavenge the ROS (Cosgrove, 1997). Peroxidase enzyme is also involved in synthesis of cell wall polymers, lignin and suberin. The cellular acid phosphatase is activated in order to release soluble phosphate from insoluble compounds in the cells because delivery of phosphate to the cells during water stress is impaired (Sharma et al., 2004). The catalase enzyme activity is increased under water stress due to its role in scavenging for ROS whose generation is increased in plant cells under water stress (Sharma et al., 2004). Peroxidase enzyme activity is also increased in plant cells subjected to water stress to scavenge on ROS and to increase formation of cell polymers to protect cell walls from damage of ROS (Cosgrove, 1997). Acid phosphatase enzyme activity is increased to release phosphate from insoluble compounds in the cell for use when delivery of phosphate is impaired under water stress (Sharma et al, 2004).

At maturity, plants enter senescence stage and during this period, materials used to build up leaves during vegetative growth stage are re-mobilized and transported into the developing seed (He et al., 2005). Visual leaf senescence includes loss of chlorophyll pigments in yellowing, desiccation and eventual death. At cellular and molecular level, there is chloroplast disintegration, a decline in photosynthesis and loss of proteins and nucleic acids (He et al., 2005). However, leaf senescence initiation and progression can be modified by environmental factors such as drought

and temperature; and internal factors such as plant growth hormones (He et al., 2005). Absciscic acid accelerates leaf senescence while Cytokinins delays it and reduces expression of stress related genes. (Li et al., 2006).

Genetic variation in the timing and rate of leaf senescence occur. (He et al., 2005). Plants with delayed leaf senescence under drought have been termed stay-green. They maintain a higher green leaf area and photosynthetic capability under water stress than the senescent types (Dillon et al., 2007).The physiological components of stay-green includes green leaf area, time of onset of senescence and rate of senescence which are independently inherited (Borrel et al., 2000). The stay-green trait is known to be controlled by dominant action of major genes (Borrel et al., 2008) which are both constitutive and adaptive and are located in four genomic regions *stg1*, *stg2*, *stg3* and *stg4* (Borrel et al., 2008). Phenotypic stay-green characteristic is often as a result of combination of two or more of these genes (Thomas and Howarth, 2000). These plants also show increased levels of Cytokinins in their cells under drought stress (Harris et al., 2007).

2.9 Mechanisms of drought tolerance in plants.

Drought tolerance in plants is as a result of complex mechanisms and adaptations that are dependent on the genome of a plant (Xiong et al., 2006). Plants have varied mechanisms that may be manifested as that of drought escape in which the plant has a short life cycle, drought avoidance whereby the plant grow deeper roots, deposit leaf wax or close stomata; or drought tolerance where the plant produces osmolytes and antioxidants (Yue et al., 2005). Drought tolerance is reported to be controlled by either constitutive or adaptive genes (Tuberosa and Salvi, 2006). The former is under the control of a few major genes while the latter is controlled by many adaptive genes

expressed depending on the timing and severity of moisture stress (Nguyen et al., 2005). Adaptive gene control that confer water stress tolerance involves the activation of stress induced genes that leads to transient increase in concentration of phytohormones, the accumulation of compartible solutes and protective proteins in the cells of water stressed plants (Yue et al., 2005).

Moisture availability during the vegetative stage determines the crop stand, tillering, number of heads, and number of seeds per head (Squire, 1993), hence determine crop yields. Mechanisms of water stress tolerance enable the plant to either avoid stress or to tolerate the stress. Pre-flowering drought tolerant sorghum plants show normal panicle development, good seed set and typical leaf morphology when water stressed during vegetative stage whereas susceptible ones experience leaf rolling, unusual leaf erectness, delayed flowering, floret abortion, reduced seed set and panicle size, and reduced plant height (Tuinstra et al., 1998).

The pre-flowering water stress tolerant plants reduce water loss by closing stomata to counter drought stress (Heschel and Riginos, 2005). Pre-flowering leaf photosynthetic rate of sorghum positively correlate to biomass and grain production under both well-watered and water-limited conditions (Mckey et al., 2003).

Post-flowering tolerant plants have traits that includes, greater root depth and extension, small plant and organ size, cuticular wax, non senescence (stay green) and efficient remobilization of stem reserves (Borrell et al., 2008). A plant which avoids water stress may have a short life cycle that is completed within the wet season, enabling the plant to avoid the severe end of season (post-flowering) water stress (Mckey et al., 2003). Such plants uses less water because of the short growing period and often have relatively small leaf area. Plant and leaf size have a major control over

plant and crop water use. Small plants with small leaf area use relatively less water and are expected to be less water demanding than larger plants or plants with greater leaf area (Borrell et al., 2000).

Leaf surface properties such as the composition of cuticular or epicuticular wax (glaucouseness) affect the rate of transpiration. Plant glaucousness reduces cuticular conductance and reflect incoming radiation which help in reducing the leaf temperature and this reduces the rate of transpiration (Saxena et al, 2002). This trait is controlled by constitutive gene expression. However, in sorghum, the epicuticular wax may increase if leaf water deficit persists for a long time due to expression of an adaptive gene *Bm;* but its effect is quite small when compared to the constitutive expression (Saxena et al, 2002).

Root traits that include root depth and extension are important plant adaptations to water stress. Extensive and deep root system absorbs more water for plant use (Tuberosa and salvi, 2006). Root development may be modified by soil conditions especially moisture status. (Blum, 1989). Soils with low soil moisture have high penetration resistance that impedes root growth (Saxena et al, 2002). However an adaptive response on inhibition of lateral root elongation under water stress has been reported (Xiong et al., 2006). This response is beneficial to plants under water stress since the available photosynthates from the limited photosynthesis under water stress is used to promote growth of primary roots to grow to deeper depths of soil profile where water is more available. This will alleviate water stress (Xiong et al., 2006).

Post-flowering water stress tolerance is the ability to resist premature plant senescence, retain green leaf area, fill grain normally, and resist lodging under conditions of post-flowering water stress (Xu et al., 2000). This trait is also termed

stay-green or non-senescence. Stay-green alleles in sorghum have been mapped to four major loci; stg1, stg2, stg3 and stg4 (Harris et al., 2007). Post-flowering drought sensitive plants have accelerated leaf and stalk senescence and reduced seed weight. Post-anthesis drought tolerant varieties stay green and have normal grain filling when stressed from pollination to maturity (Burows et al., 2006).

Plant stem organic reserves are made up of carbohydrates and nitrogen accumulated during the vegetative stage of growth in all cereal plants (Salem et al., 2007). These are converted into soluble forms and mobilized for grain filling when transient photosynthesis is impaired during water stress. Plants with more stem organic reserves are often more tolerant to water stress because they fill grains normally under water stress. (Mcintyre et al 2007 and Delphine et al., 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sorghum seed.

The sorghum seed was obtained from the collection held at the Department of Biological Science, Moi University under the BIOEARN project. Advanced grain sorghum lines were used in the project experiments. These included MCSR A11, MCSR C1, MCSR C26, Gadam, MCSR D1 brown, MCSR F14a, MCSR G2, MCSR I10, MCSR N4, MCSR O2, MCSR T30 and MCSR T28 (Table 1; Figure 2). These lines included local varieties and some lines from ICRISAT standards.

Table 1: Sorghum seeds used in the experiments for drought tolerance.

Variety	Source	Seed Colour	Plant height to flag leaf (cm)	Useful characteristics
MCSR A11	Tanzania	White	107	High yielding, good panicle exertion, medium height
MCSR C1	ICRISAT	White	95	Stay green, high yielding, medium height, early maturing.
MCSR C26	ICRISAT	Cream white	125	Aluminium standard, medium height
MCSR D1b	International material	Purple red	70	Short variety, stay-green, good panicle extension
MCSR F14a	Ukambani	Cream	121	High yielding, good panicle extension, early maturing
MCSR G2	Tanzania	White	93	Medium height, high yielding, early maturing
Gadam	International material	White	100	Medium height, early maturing, high yielding
MCSR I10	Ukambani	Cream red	132	Stay green, high yielding, good panicle extension
MCSR N4	Ndiwa Homa Bay	Brown	125	Stay green, good panicle exertion, high yielding
MCSR O2	KARI	Cream	120	High yielding, phosphorus efficient, good panicle extension
MCSR T28	Tanzania	Cream with black specks	105	Large grains, easy threshability, good panicle exertion early maturing, drought standard
MCSR T30	Tanzania	Cream brown	120	Stay green, Drought standard

Source: BIOEARN records, Moi University 2008.

MCSR I10

MCSR O2

Gadam

MCSR C1

MCSR A11

MCSR C26

MCSR G2

MCSR N4

MCSR D1b

MCSR F14a

MCSR T28

Figure 2: The seed of selected sorghum lines that were used in drought experiments. (Source: Author, 2010)

3.2. Experimental sites.

Sorghum germination and greenhouse experiments were carried out in 2008-2010 at Chepkoilel Biotechnology Laboratory and in the greenhouse at Chepkoilel campus (which is located at latitude 00^0 35.77' N; longitude 35^0 1744; Elevation 2548 m; NW 255 km. This area receives annual rainfall of 1124 mm in one season from March to September (Jaetzold and Schmidt, 1983).

Sorghum field experiments were carried out in 2009-2010 at Kiboko KARI Research Station which is situated E 37° 80' S 2˚ 30'; Elevation 1000m. It is classified as Agro-ecological zone (iv) with bimodal pattern of rainfall with main rains falling in March – May and short rains in mid-October – mid December. It receives between 300 – 655 millimeters of rainfall per annum. It experiences diurnal temperatures of maxima 24.7˚C and minima 13.7˚C. (KARI, 2012).

3.2. Effect of water stress on imbibition rates in sorghum seeds.

Clean grains were surface sterilized by placing them in 10 % sodium hypochlorite for 10 minutes then rinsed in distilled water eight times. The seeds were placed in plastic dishes (25 ml capacity) following the procedure outlined in Suriyong et al (2002). They were then soaked in 5 ml solutions of graded mannitol with varied concentrations that corresponded to osmotic potentials of 0.00, -0.40, -0.80 and -1.43 Mega Pascals (MPa) which indicates water stress levels (Salisbury and Ross, 1992). The values of water stress level were calculated as described in Salisbury and Ross (1992) from the equation:

$$\Psi_\pi = -\frac{miRT}{10} \quad \text{..... (i)}$$

(Salisbury and Ross, 1992)

Where;

Ψ_π = Osmotic potential (Mega Pascals (MPa))

m = Molal concentration (Moles per 1000 ml solvent)

i= Ionization level of solute (1)

R = Universal gas constant (8.3145 J $mol^{-1}K^{-1}$)

T = Temperature (Absolute scale °C + 273)

Mannitol is poorly metabolized in most seed plants (Salisbury and Ross, 1992) and can be used for osmotic stress experiments. One molar stock solution was prepared by dissolving 182.17g of mannitol in 1000 ml distilled water. Serial dilutions were prepared to make working solutions that corresponded to solute potentials of -1.43, -0.80 and-0.40 MPa with three replications. The grain weight was recorded over a period of six hours at one hour intervals. Every time, the samples were blotted dry with paper towels and immediately weighed and recorded and returned into the respective mannitol solutions.

Rate of imbibitions were calculated as described by Schneider (1998) using the following equation;

$$IR = \frac{W_t - W_0}{W_0} \times \frac{1}{t} \quad \text{..... (ii)}$$

(Modified from Schneider, 1998)

Where:

IR=imbibition rate (water (g) /seed weight (g)/ hour)

t = time in hours

W_t = weight of seed (g) at time t

W_0 = weight of seed (g) at previous measurement

3.3 Effect of water stress on germination in sorghum seedlings.

The grains that were used for imbibition tests were then germinated in an incubator at 24°C by placing them in petri dishes lined with paper towels (Swagel et al., 1997) and moistened with the 10 ml of graded mannitol solutions to give osmotic potentials equivalent to 0.00, -0.40, -0.80 and -1.43 MPa. Successful germination was based on 1cm radicle emergence (Ellis and Roberts 1980). Germination was assessed and recorded every 24 hours until no more germination was observed. The percentage of germination was calculated as:

$$\%\ germination = \frac{N_g}{N_t} \times 100 \quad \text{..... (iii)}$$

(Ellis and Roberts, 1980).

Where: N_g= Number of germinated grains in a dish

N_t = Total number of grains in the dish.

The mean germination time was calculated as described in Ellis and Roberts (1980) calculated as:

$$MGT = \sum SNGI\ X\ \frac{IT}{TNSG}$$

……(iv)

(Ellis and Roberts, 1980)

Where:

$MGT =$ Mean germination time (hours)

$SNGI =$ Seed number germinated in the interval

$IT =$ Interval time (24 hours)

$TNSG$=Total number of seed germinated.

3.4 Effect of water stress on the activity of starch remobilization enzymes in the germinating sorghum seeds.

Effect of water stress on starch remobilizing enzymes was assessed by absorption spectroscopy (Dubois et al., 1956). A standard curve was developed by recording absorbance of standard starch solutions at 620 nm using a spectrophotometer (Biomate^{TM} 3 series). A stock solution of 1% starch solution was made by dissolving 10g starch in 1000 ml distilled water. Serial starch solution dilutions of 0.01 to 0.08% were made. Standard starch solutions were made by mixing 1ml of each serial starch solution with 1ml of 1% iodine solution. A blank was made by mixing 1ml of distilled water added to 1ml of 1% iodine and used to zero the absorbance at 620 nm. The absorbances of the standard starch solutions were determined at 620 nm and the readings used to construct a standard absorption curve (Appendix I and II).

The germinating seedlings were selected randomly and weighed after 72 hours of germination in three replicates per treatment. The enzyme extraction was carried out among the uniformly germinated seedlings based on radicle length as described by Witham et al (1971). The uniformly germinated seedlings at similar stage of development were ground to a fine mixture with addition of 5 ml 0.2 M borate (pH 8.8) buffer using a pestle and mortar. The mixture was transferred into a centrifuge tube and topped up to ten milliliters (10ml) using the borate buffer and centrifuged at 10,000 revolutions per minute for 10 minutes. Six milliliters (6ml) of the supernatant was transferred into a clean test tube.

The enzyme extract was mixed with the 0.1% starch solution and the enzyme-starch solutions were incubated for 10 minutes at 26°C in the incubator. The reaction was stopped by adding1ml of 1% iodine solution. The absorbance was measured at 620 nm in the spectrophotometer. The amount of starch in the samples was estimated using standard calibration curve.

3.5 Effect of water stress on radicle growth in young sorghum seedling.

Three young seedlings for every replication were randomly selected from the incubator at 96 hours of incubation and their radicle lengths measured and recorded.

3.5.1 Effect of water stress on catalase, Peroxidase and acid phosphatase variation in six day old sorghum seedlings.

The enzymes catalase, peroxidase and acid phosphatase were selected for assay by electrophoresis (Murphy et al., 1996; valejos, 1983) for six day old seedlings.

3.5.1.1 Enzyme extraction.

Crude enzyme extracts were obtained by grinding eight uniformly grown seedlings

with radicle of about 1millimetre. Whole seedlings were ground in a microfuge tube using a pestle in 100 µl pre-chilled buffer (100 mM Tris HCl of pH 7.8, 10 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 5% PVP-10 and 1.5% glycerol) as described by Tanksley and Orton (1983). The crude homogenates were maintained on ice before loading into the starch gel.

3.5.2 Preparation of starch gel.

The starch gel was prepared as described by Tanksley and Orton (1983) and Murphy et al. (1996). Forty-four grams of hydrolyzed potato starch (Sigma, S5651) was suspended in 400 ml (11%) of gel buffer containing 15 mM Tris base [2-amino-2-(hydroxymethyl)-propane-1,3-diol,(Tris)] and 3 mM citric acid of pH 7.8 in a 1-litre Erlenmeyer flask and heated with continuous swirling on hot plate until a clear, vigorously boiling solution was obtained. Air bubbles were removed by suction pump for about 15-20 seconds. The cooked starch mixture was poured onto an acrylic gel mould in which electrode strips were sealed with masking tape. Air bubbles were quickly removed from the gel soon after pouring onto the mold using a pasteur pipette. The starch gel was allowed to cool and set for 60 minutes at room temperature (23°C) and then covered with a cling film to prevent evaporation of the gel water and stored at room temperature overnight.

3.5.3 Sample loading onto the starch gel.

The starch gel was kept in a refrigerator at 4^0C for one hour before loading to maintain low temperature which discourage denaturation of enzymes. A slit was cut 3.5 cm from the cathodal end of the gel using a sterilized surgical blade. Eighteen paper wicks each measuring 6 x 12 mm were cut from sterilized Whatman No. 2 filter papers and used to take up crude enzyme extract. The excess crude extracts from the

wicks was removed by pressing the wicks between Whatman No.1 filter papers prior to insertion into the gel. The wicks carrying crude extracts were then sequentially inserted in the slot using sterile forceps.

3.5.4 Electrophoresis.

Electrophoresis was carried out as decribed by Murphy et al. (1996). The edges of gel mold were unsealed to expose the gel in the electrode strips. The gel mold was then mounted onto the electrode trays containing 0.3 M boric acid (pH 8.6) as electrode buffer. An ice bag was placed onto the gel to cool the gel during electrophoresis. The electrodes were connected to the stabilised direct current power supply (Griffin Model) initially set at 200 V for 20 minutes to transfer the enzymes from the wicks into the gel matrix. The sample wicks were removed, the cathode and anode portion of the gel pressed together and then covered with a cling film. The power supply was increased to 300V. The electrophoresis was continued until the borate front had migrated about 9 cm from the sample origin.

The gel was removed from the electrode tray buffer and rectangular slabs were prepared with the anodal and cathodal parts starting from the origin of migration. The starch gel was removed from the gel mold and placed on an acrylic slicing board, and a wire drawn horizontally through the gel to cut a 1 mm slices which were transferred to staining boxes.

3.5.5 Staining for enzyme activity.

Each gel slice was stained for a different enzyme system using different substrates. The following three systems were stained: catalase, peroxidase and acid phosphatase following the procedures described by Vallejos (1983).

3.5.5.1 Catalase.

Catalase staining solution was prepared by mixing 6 ml of 0.06M sodium thiosulphate with 14 ml of 3% hydrogen peroxide. The solution was poured onto the gel, followed by incubation at room temperature for 30 seconds and the solution poured off. Then, 20 ml of 0.09 M potassium iodide was mixed with 0.5 ml of glacial acetic acid and then poured onto the gel. The resulting clear bands against a blue background were recorded by photography using a digital camera as soon as they became evident and stable.

3.5.5.2 Peroxidase.

Fifty (50) milligrams of 3-amino-9 ethylcarbazole was dissolved in 3 ml of N, N-dimethyl formamide. The solution was added to 25 ml of 50 mM sodium acetate (pH 4.5), and 1 ml of 3% hydrogen peroxide was added just before incubation at room temperature. Areas of peroxidase activity were identified as bright red bands on the gel after 60 minutes. The result was recorded by taking a photograph by using a digital camera.

3.5.5.3 Acid Phosphatase.

Acid phosphatase staining solution was prepared by dissolving 50 mg of Fast Garnet GBC salt in 50 ml of 50 mM sodium acetate (pH 5.5) buffer containing 0.5 ml of 1M magnesium chloride. Thirty (30) milligrams of α-naphythyl acid phosphate was weighed and dissolved in 3ml of 50% acetone and added to the buffer. The gels were incubated in the dark at 30°C for 5 hours until purple or red bands appeared. The solution was poured off and the gel rinsed with tap water and fixed in 50 % glycerol.

The zymograms were recorded by placing the stained starch gels against a light box and taking photographs.

3.5.5.3 Alpha (α) – amylase.

Alpha (α)-amylase staining solution was prepared by mixing 100 ml of 50 mM sodium acetate (pH 5.6) and 2 ml of 1 M calcium chloride. The gel was flooded with the staining solution and incubated at 30˚C for 1 hour. The solution was discarded and the gel thoroughly rinsed using distilled water. Staining solution containing 10 mM iodine and 14 mM potassium chloride was poured onto the rinsed gel. Zones of enzyme activity appeared blue or brown. The gel was rinsed and the zymogram recorded by photography.

3.6 Greenhouse Experiments

The greenhouse water stress experiments were carried out using seed from the 8 selected sorghum lines used in the germination experiments in the laboratory. The clean seeds were sown in 45 kg of soil in polythene bags in the green house as . The soil was mixed with DAP fertilizer to supply P at the rate of 9 kg ha^{-1} and nitrogen at 20 kg ha^{-1}. Five (5) grains were sown in each bag and later thinned to one plant at three-leaf stage. The treatments were made up of the control (well watered throughout growth period) post-anthesis stress (well watered up to anthesis, then stressed), pre-anthesis stress (stressed up to anthesis and then well-watered up to maturity) and stressed throughout the entire growth period. Each treatment was replicated five times for each sorghum genotype.

The experiment consisted of randomized complete block design (RCBD). Two water treatment levels were imposed between the 3-leaf stage and anthesis; 1) the control in

which 2 litres of water were supplied per bag and 2) pre-anthesis stress where 1 litre of water was supplied per bag on the 14th day. From anthesis to maturity, 4 treatments were imposed on the sorghum. Half of the well-watered bags continued to receive 2 litres of water on every 7th day; whereas the other half was supplied with 1 litre of water per bag on every 14th day. Half of the formerly stressed bags received 2 litres of water per bag every 7th day, and the other half continued to receive 1 litre of water per bag on every 14th day.

3.6.1. Effects of water stress on quantitative characters in sorghum plants.

Potted plants grown in the greenhouse were used to assess the effect of water stress on quantitative characters. These included; 1. Days to panicle emergence, 2. Number of nodal tillers, 3. Length and width of third leaf from top of plant, 4. Number of dead leaves, 5. Plant height at maturity, 6. Chlorophyll concentration and carotenoids concentration in flag leaf, 7. Panicle lengths and widths and 8. Shoot dry weights.

3.6.2 Effect of water stress on pigment concentration in sorghum flag leaf post flowering stage.

Leaf discs were collected from flag leaves of the greenhouse grown sorghum plants as described by Witham et al (1971) by means of a 2.38 cm cork borer. The leaf discs were ground in 2 ml of 80% acetone using mortar and pestle under diffuse light. A pinch of calcium carbonate ($CaCo_3$) was added to the extraction solvent to raise pH and to prevent loss of the magnesium atom from chlorophyll molecules during extraction.

The extract was transferred into a graduated centrifuge tube and topped up to 11 ml with more of the 80% acetone. The extract was transferred into a centrifuge tube and

centrifuged at 10,000 rpm for 10 minutes to clarify the extract. The clear supernatant was transferred into a 10 mm path length glass cuvette and the absorbance was determined against a blank containing 80% acetone at 480, 510, 645, 652, 663 and 750 nm in a spectrophotometer (Biomate^{TM} 3 series). The working absorbance at all wavelengths was calculated by subtracting the absorbance at 750 nm from each reading (Witham et al., 1971).

The chlorophyll concentration (mg cm^{-2}) was calculated from the formulas described by Witham et al (1971) as;

$$Chlorophyll\ a = \frac{12.7A_{663} - 2.69\,A_{645}}{AR \times 1000} \times V \quad \text{..... (v)}$$

$$Chlorophyll\ b = \frac{22.9A_{645} - 4.68A_{663}}{AR \times 1000} \times V \quad \text{..... (vi)}$$

$$Total\ Chlorophyll = \frac{A652}{34.5\ x\ AR}\ x\ V \quad \text{.....(vii)}$$

$$Total\ Carotenoids = 7.6\ (A480 - 1.49A510) \quad \text{.....(viii)}$$

(Witham et al., 1971)

Where;

A = Absorbance

V = final volume of 80% acetone-chlorophyll extract (11 cm^3)

AR = area of leaf disc in cm^2 (4.5cm^2)

3.6.3 Effect of water stress on shoot weight and panicle size.

The panicles and all shoots were harvested at physiological maturity (when seed reaches formation of black layer). The shoots were chopped up into small pieces and air dried for two weeks to 12 % moisture content. Shoot dry weight was determined using a weighing balance and recorded. The panicle lengths and widths from main tillers were measured using a ruler (cm) and recorded.

3.7 Field experiments.

Sorghum seed from 11 sorghum lines as used in the laboratory experiments were sown in the field at Kiboko KARI/ICRISAT research station in randomized complete block design (RCBD) during the dry season in blocks measuring 3 metres by 2 metres at spacing of 60 cm between rows. The plants were irrigated to field capacity by overhead irrigation and thinned at three-leaf stage to plant spacing of 25 cm between plants. DAP fertilizer was applied to meet the rates 100kg nitrogen per hectare, 50 kg Phosphorus per hectare. Shoot fly and stem borer were controlled by applying insecticide and fungal infection was also controlled by applying fungicide as appropriate. Weeding was carried out by manually. Irrigation was continued until 50% flowering and then completely withdrawn in order to subject the plants to post flowering water stress. Data was collected on plant height, days to panicle emergence, leaf death and grain yield. The plant height was determined taking measurement from soil level to flag leaf. Days to panicle emergence were counted from planting date to the day the panicle emerged from the flag leaf. The leaves which had died and turned brown were counted and entered as number of dead leaves. The panicles were dried, threshed and winnowed. Grain from panicles were weighed in grams and recorded as grain yield.

3.8 Data analysis.

3.8.1 Seed Imbibition rates.

The seed imbibition rates data were log-transformed and then subjected to Analysis of Variance (ANOVA) with variety, water stress level and duration of imbibition being sources of variation. Means were separated by Duncan's multiple range test (P=0.05).

3.8.2 Germination data.

All count data were log-transformed and the percentages were arcsine transformed to standardize before statistical analysis. Data obtained from percent germination, mean germination time, starch concentration and radicle lengths were subjected to analysis of variance with variety and level of water stress as sources of variation and means separated by Duncan's multiple range test (P=0.05).

3.8.3 Green house data.

The values obtained on plant height, days to panicle emergence, number of nodal tillers, were first arcsine transformed to standardize them then subjected to analysis of variance with variety and water stress level being sources of variation. The numbers of dead leaves were first converted to percentage leaf senescence, then logarithm transformed and subjected to analysis of variance.

The flag leaf chlorophyll and carotenoids concentrations in flag leaves were arcsine transformed, then subjected to analysis of variance with variety and treatment being sources of variation.

The number of dead leaves was assessed from start of post-flowering water stress on daily basis for one week and thereafter on weekly basis. The values were used to

calculate percentage leaf senescence in the post flowering stressed plot. The data on plant height collected were used to calculate height reduced by subtracting the height at a water stress level from that of a well watered control experiment.

The data on panicle lengths, panicle widths, panicle dry weight and shoot dry weights were presented along with the percentage reduction from mean of control at the pre-flowering, post-flowering and the continuous water stress levels. The resulting ratio values were arcsine transformed to standardize the data and subjected to analysis of variance.

3.8.4 Field data.

The data collected on leaf senescence and yields were subjected to one sample T-tests with variation being a source of stress and to cluster analysis using the hierarchical distance based on un-weighted paired group method using arithmetic averages (UPGMA) and used to derive a Dendogram based on taxonomic distance-similarity (Panchen, 1992).

CHAPTER FOUR

RESULTS

4.1: Effect of osmotic stress on seed imbibition rates in sorghum lines.

The results showed a significant variation in seed imbibition rates among sorghum lines with level of water stress (p=0.05). The results showed decreasing rates of imbibition with increase in water stress intensity. The highest water stress level of -1.43 MPa had the greatest negative effect on seed imbibition rates and -0.04 MPa water stress level had the least effects when compared to the control (Table 2).

Under the 0.00 MPa (control), the sorghum lines with the fastest rate of seed imbibition were MCSR T30, MCSR I10 and MCSR C26; and MCSR T28, MCSR F14a, Gadam and MCSRN4 had the slowest rate of imbibition. Under the -0.04 MPa, the sorghum lines which had the fastest rates of seed imbibition were MCSR I10 and MCSR C26; whereas MCSR G2, MCSR F14a, MCSR T28 and MCSR D1b had the slowest rate of seed imbibition. Under -0.08 MPa, the sorghum lines which had the highest rates of seed imbibition were MCSR I10 and MCSR C26, MCSR G2 and MCSR O2; whereas MCSR F14a, MCSR D1b, Gadam and MCSR N4 had the lowest rates of seed imbibition. Under the -1.43 MPa, the sorghum lines which had the fastest rate of seed imbibition were MCSR C26, MCSR I10, MCSR G2 and MCSR O2; and the lines which had the slowest rates of seed imbibition were MCSR F14a, MCSR N4 and MCSR D1b.

The sorghum lines which had the highest rates of seed imbibition were MCSR I10, MCSR C26, and MCSRT30; whereas MCSR F14a, MCSRT28 and MCSR N4 had the slowest rates of seed imbibition.

Table 2: Rate of imbibition in seeds of 11 sorghum lines under osmotic stress levels.

	Water stress level			
sorghum line	Ψ=0.00 MPa	Ψ=-0.04 MPa	Ψ=-0.08 MPa	Ψ=-1.43 MPa
MCSR A11	31^{cd}	28^{bc}	27^{ab}	26^{cd}
MCSR C26	37^{b}	38^{a}	34^{a}	35^{a}
MCSR D1B	29^{cde}	25^{bc}	24^{b}	23^{cd}
MCSR F14a	25^{e}	24^{c}	22^{b}	21de
MCSR G2	34^{bc}	23^{c}	29^{ab}	28^{c}
Gadam	28de	26^{bc}	25^{ab}	24de
MCSR I10	42^{a}	38^{a}	34^{a}	32^{b}
MCSR N4	28de	25^{bc}	24^{ab}	22de
MCSR O2	34^{bc}	29^{b}	28^{ab}	26^{cd}
MCSR T28	25^{e}	24^{c}	26^{ab}	23de
MCSR T30	44^{a}	26^{bc}	25^{b}	24de

Means in a column followed by the same letter do not differ significantly at $p \leq 0.05$.

4.1. Effect of water stress on Seed imbibition rates at ψ=0.00 MPa in sorghum.

There was a gradual decrease in rates of seed imbibition from the first hour to the sixth hour (Table 3). The sorghum line with the highest seed imbibition rate at 0.00 MPa during the first hour was MCSR T30; and MCSR I10 had the lowest seed imbibition rate. In the second hour, MCSR I10 had the highest seed imbibition rate, and MCSR A11 had the lowest rate of seed imbibition. In the third hour, MCSR I10 had the highest seed imbibition rate; and MCSR F14a and MCSR N4 had the lowest rates of seed imbibition. During the fourth hour, MCSR C26 had the highest rate of seed imbibition whereas MCSR D1b and MCSR O2 had the lowest rates of seed imbibition. In the fifth hour, MCSR C26 had the highest seed imbibition rate; and MCSR G2 had the lowest rates of seed imbibition. In the sixth hour, MCSR N4 had highest seed imbibition rate; and MCSR G2 had the lowest. The sorghum line that had the highest rate of seed imbibition over the six hours was MCSR T30 and MCSR I10. The lines which had the lowest rates of seed imbibition included MCSR T28, MCSR N4 and Gadam. The plateau phase was reached after 4 hours of imbibition.

Table 3: Seed imbibition rates at Ψ=0.00 MPa of 11 sorghum lines.

Sorghum Line	DURATION OF IMBIBITION (HOURS) 1	2	3	4	5	6	MEAN
MCSR A11	142^{b}	18^{b}	14^{a-d}	4^{bc}	3^{abc}	3^{b-e}	31^{cd}
MCSR C26	157^{b}	28^{ab}	20^{abc}	10^{a}	6^{a}	1^{f}	37^{b}
MCSR D1b	117^{c}	30^{ab}	21^{ab}	3^{c}	3^{abc}	3^{c-f}	29^{cde}
MCSR F14a	112^{c}	20^{ab}	10^{d}	5^{bc}	2^{bc}	2^{def}	25^{e}
MCSR G2	155^{b}	24^{ab}	15^{a-d}	5^{bc}	1^{c}	1^{f}	34^{bc}
Gadam	115^{c}	27^{ab}	15^{a-d}	6^{bc}	3^{abc}	3^{c-f}	28de
MCSR I10	183^{a}	34^{a}	2^{a}	5^{bc}	5^{abc}	4^{bcd}	42^{a}
MCSR N4	104^{c}	30^{ab}	11^{d}	8^{ab}	3^{abc}	1^{b}	27de
MCSR O2	158^{b}	22^{ab}	13^{cd}	3^{c}	3^{abc}	5^{a}	34^{bc}
MCSR T28	106^{c}	19^{ab}	14^{bcd}	5^{bc}	4^{abc}	2^{ef}	25^{e}
MCSR T30	202^{a}	32^{ab}	16^{a-d}	5^{bc}	6^{ab}	4^{bc}	44^{a}

Means in a column followed by same letter do not differ significantly at $p \leq 0.05$.

4.1.3 Effect of water stress on seed imbibition rates at Ψ = - 0.40 MPa in sorghum.

The seed imbibition rates decreased over time in all sorghum lines under water stress (Table 4). The rates in the first one hour were high for all the lines. The imbibition rates gradually decreased over time with the sixth hour having the lowest rate of seed imbibition. The results also showed significant difference in the seed imbibition rates among the sorghum lines. During the first hour of imbibition, MCSR C26 and MCSR I10 had the highest seed imbibition rates whereas MCSR G2 had the lowest seed imbibition rates. MCSR G2 and MCSR I10 had the highest seed imbibition rate; and Gadam and MCSR A11 had the lowest rates of imbibition during the second hour. During the third hour, seed imbibition rate was the highest in MCSR I10; and MCSR T28 had the lowest rate. In the fourth hour, the highest value in seed imbibition rate was recorded in the varieties MCSR C26 while MCSR F14a had the lowest value. In the fifth hour, MCSR N4 was faster in imbibition rate and the line with the lowest rate was MCSR D1b. In the sixth hour, MCSR I10 had the highest value while MCSR N4

had the lowest. MCSR C26 and MCSR I10 showed the highest imbibition rates over the six hours while MCSR G2 and Gadam had the lowest rates.

Table 4: Effect of water stress on seed imbibition rates at Ψ = -0.40 MPa in sorghum.

SORGHUM LINES	DURATION OF IMBIBITION (HOURS)						
	1	2	3	4	5	6	MEANS
MCSR A11	124^{cd}	20^{c}	12^{bc}	7^{bcd}	4^{a}	$2.^{bc}$	28^{bc}
MCSR C26	165^{a}	28^{abc}	18^{ab}	10^{a}	5^{a}	3^{abc}	38^{a}
MCSR D1b	92^{ef}	33^{abc}	14^{bc}	7^{bcd}	3^{a}	2^{bc}	25^{bc}
MCSR F14a	96^{ef}	24^{c}	12^{bc}	6^{d}	4^{a}	2^{bc}	24^{c}
MCSR G2	71^{f}	39^{a}	16^{ab}	6^{d}	4^{a}	3^{abc}	23^{c}
Gadam	95^{ef}	26^{c}	18^{ab}	9^{bcd}	4^{a}	3^{abc}	26^{bc}
MCSR I10	153^{ab}	38^{ab}	21^{a}	9^{abc}	5^{a}	4^{a}	38^{a}
MCSR N4	97^{ef}	20^{c}	15^{ab}	9^{ab}	5^{a}	2^{bc}	25^{c}
MCSR O2	130^{bc}	24^{c}	13^{bc}	7^{cd}	3^{a}	3^{abc}	30^{b}
MCSR T28	101^{de}	23^{c}	9^{c}	$8.^{bcd}$	4^{a}	2^{bc}	24^{c}
MCSR T30	105^{cde}	24^{c}	15^{ab}	$7.^{bcd}$	4^{a}	3^{abc}	26^{bc}

Means in a column followed by same letter do not differ significantly at $p \leq 0.05$.

4.1.4 Effect of water stress on seed imbibition rates at Ψ = - 0.80 MPa in sorghum.

The sorghum seed imbibition rates showed a gradual decrease over time (Table 5) of at -0.80 MPa. In the first one hour, MCSR O2 showed the highest rate of seed imbibition while MCSR F14a had the lowest seed imbibition rate. During the second hour, MCSR I10 was fast in uptake of water while MCSR T28 was the slowest. In the third hour, MCSR C26 and MCSR I10 had highest rates of seed imbibition while Gadam had the slowest. The sorghum line MCSR I10 showed the highest rate of seed imbibition in the fourth hour, and MCSR D1b had the lowest rate. In the fifth hour, MCSR O2 showed the highest seed imbibition rate while MCSR C26 was the lowest. In the sixth hour, MCSR T28 showed the highest rate of seed imbibition while MCSR

G2 showed the lowest rate. The sorghum lines which showed the highest rates of seed imbibition over the six hours were MCSR C26 and MCSR I10; and MCSR F14a and MCSR D1B had the lowest.

Table 5: Effect of water stress on seed imbibition rates at Ψ = -0.80 MPa in sorghum.

	DURATION OF IMBIBITION(HOURS)						
Sorghum Lines	1	2	3	4	5	6	MEANS
MCSR A11	109^{ab}	31^{b}	10^{b}	5^{cd}	4^{ab}	2^{b}	27^{ab}
MCSR C26	146^{ab}	29^{bc}	19^{a}	5^{cd}	2^{b}	2^{b}	34^{ab}
MCSR D1b	95^{ab}	27^{bc}	11^{b}	4^{d}	4^{ab}	2^{ab}	24^{b}
MCSR F14a	78^{b}	32^{b}	11^{b}	6^{cd}	4^{ab}	2^{ab}	22^{b}
MCSR G2	114^{ab}	32^{b}	12^{b}	10^{ab}	3^{ab}	1^{b}	29^{ab}
Gadam	118^{ab}	30^{bc}	10^{b}	6^{cd}	3^{ab}	3^{ab}	25^{ab}
MCSR I10	131^{ab}	40^{a}	19^{a}	11^{a}	4^{ab}	2^{ab}	35^{ab}
MCSR N4	94^{ab}	34^{ab}	16^{ab}	8^{bc}	3^{ab}	2^{ab}	24^{ab}
MCSR O2	165^{a}	34^{ab}	16^{ab}	8^{bcd}	6^{a}	3^{ab}	28^{a}
MCSR T28	109^{ab}	22^{c}	12^{b}	5^{cd}	3^{ab}	4^{a}	26^{ab}
MCSR T30	92^{ab}	30^{bc}	13^{b}	8^{bc}	5^{ab}	2^{ab}	25^{b}

Means in a column followed by same letter do not differ significantly at $p \leq 0.05$.

4.1.5 Effect of water stress on seed imbibition rates at Ψ = -1.43 Mpa in sorghum.

There was significant difference (p = 0.05) in rate of water uptake among the sorghum lines with a gradual decrease over time (Table 6). MCSR C26 had the highest seed imbibition rate during the first hour, and MCSR N4 had the lowest seed imbibition rate. During the second hour, high rates of seed imbibition were shown in lines MCSR I10 and MCSR O2; and the lowest rate was recorded by MCSR T28. In the third hour, the highest imbibition rates were recorded in MCSR I10 and MCSR N4 while MCSR T28 recorded the lowest seed imbibition rate. MCSR C26 had the highest imbibition

rate in the fourth hour; and MCSR D1B recorded the lowest seed imbibition rate. In the fifth hour, MCSR T30 showed the highest seed imbibition rate; and MCSR T28 had the lowest rate. MCSR I10 showed the highest rate of imbibition in the sixth hour while MCSR D1b recorded the lowest rate. The lines which showed higher seed imbibition rates over the six hours included MCSR C26, MCSR I10 and MCSR G2 and MCSR F14a and MCSR N4 recorded the lowest.

Table 6: Effect of water stress on seed imbibition rates at Ψ=-1.43 MPa in sorghum.

	DURATION OF IMBIBITION (HOURS)						
Sorghum line	1	2	3	4	5	6	MEAN
MCSR A11	112^{b}	21^{def}	10^{cde}	8^{abc}	3^{b}	2^{cd}	26^{cd}
MCSR C26	141^{a}	25^{cde}	15^{bc}	12^{a}	4^{b}	3^{ab}	35^{a}
MCSR D1b	112^{b}	27^{cde}	14^{bcd}	2^{c}	2^{b}	1^{e}	23^{cd}
MCSR F14a	93^{cd}	24^{cdef}	12^{bcd}	4^{bc}	4^{b}	2^{de}	21^{de}
MCSR G2	101^{bc}	37^{b}	13^{bcd}	8^{abc}	3^{b}	3^{bc}	28^{c}
Gadam	98^{bcd}	45^{a}	12^{bcd}	11^{ab}	3^{b}	3^{bc}	25^{de}
MCSR I10	107^{bc}	45^{a}	28^{a}	6^{abc}	4^{b}	6^{a}	33^{b}
MCSR N4	73^{e}	29^{bc}	19^{b}	6^{bc}	6^{b}	2^{cde}	22^{e}
MCSR O2	101^{bc}	35^{b}	11^{cde}	6^{bc}	3^{b}	3^{cd}	26^{cd}
MCSR T28	104^{bc}	17^{f}	5^{e}	7^{abc}	2^{b}	2^{cd}	23^{de}
MCSR T30	83^{de}	29^{bcd}	9^{de}	6^{abc}	2^{a}	2^{cde}	24^{de}

Means in a column followed by same letter do not differ significantly at $p \leq 0.05$.

4.2 Effect of water stress on germination of sorghum seeds.

There was significant differences (p = 0.05) in percent germination among sorghum lines under water stress.

At Ψ = -1.43MPa, highest percent seed germination was recorded in varieties MCSR I10, MCSR O2, MCSR T28, MCSR T30, MCSR F14a and MCSR A11 (Figure 3). The lowest seed germination was recorded MCSR N4. All lines except MCSR N4 recorded more than 70% germination.

The sorghum lines MCSR T30, MCSR A11, MCSR F14a, and MCSR O2 attained the highest germination at the water potential of -0.08 MPa; and MCSR N4and MCSR D1b had the lowest germination percentage.

The sorghum lines which attained the highest germination percentage at Ψ = -0.40MPa were MCSR T30, MCSR O2, MCSR F14a, and MCSR A1; and MCSR N4 and MCSR D1b had the lowest germination percentage.

Under the control (Ψ = 0.00 MPa), MCSR I10, MCSR O2, MCSR F14a ,MCSR A11 and MCSR T30 had the highest germination percentage while MCSR N4 had the lowest.

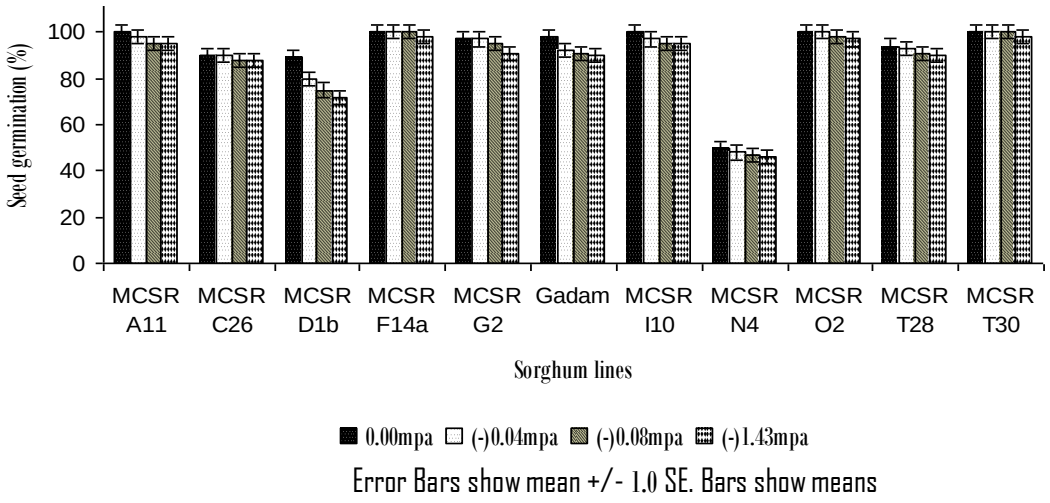


Figure 3: Seed germination (%) for selected sorghum lines under water stress.

4.3. Effect of water stress on germination time in seed of sorghum lines.

There was no significant difference in the time taken to attain maximum germination at p=0.05 among sorghum lines with levels of water stress.

However the sorghum lines which took more time to reach maximum germination at the highest water stress level (Ψ = -1.43MPa) were MCSR I10, MCSR G2 and MCSR

N4 (Figure 4). The lines which took the shortest time to reach maximum germination were MCSR T30 and Gadam.

At Ψ = -0.80 MPa, MCSR D1b took the longest time to attain maximum germination while MCSR O2 and MCSR T30 took the shortest time to maximum germination.

At Ψ = -0.4 MPa, the lines which took the longest time to attain maximum germination were MCSR D1b, MCSR G2, MCSR N4 and MCSR O2 and those which took the shortest time to attain maximum germination were MCSR C26, MCSR I10, MCSR T28 and MCSR T30.

At Ψ = 0.00MPa, MCSR A11 and MCSR C26 took a longer time to attain maximum germination whilst lines MCSR T30 and MCSR Gadam took a shorter time to reach maximum germination.

The lines which had fast germination at all water stress levels were MCSR T30 and Gadam.

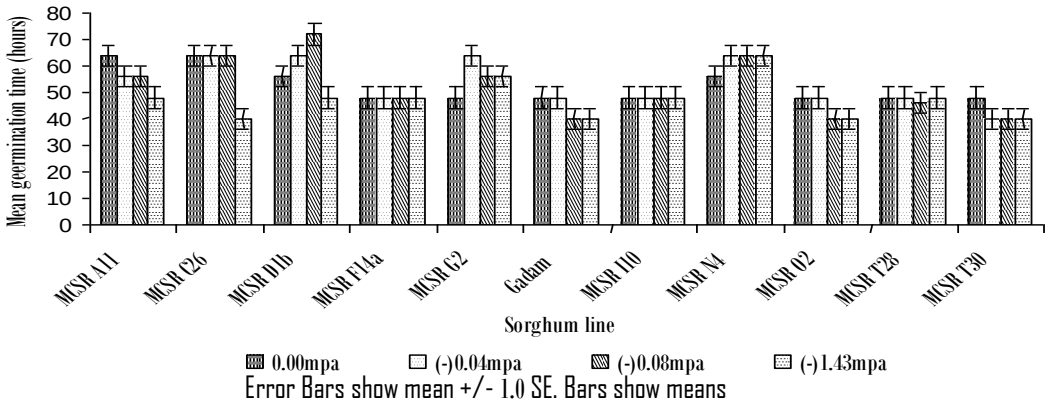


Figure 4: Effect of water stress on seed germination time (MGT) in sorghum.

4.4. Effect of water stress on seedling radicle length for selected sorghum lines

Seedling radicle lengths in sorghum were significantly different ($p \leq 0.05$) among the lines with the level of water stress (Figure 5). The lines MCSR A11, MCSR F14a, MCSR O2, MCSR T28 and MCSR T30 had significantly longer seedling radicles than Gadam, MCSR G2, MCSR I10 and MCSR N4.

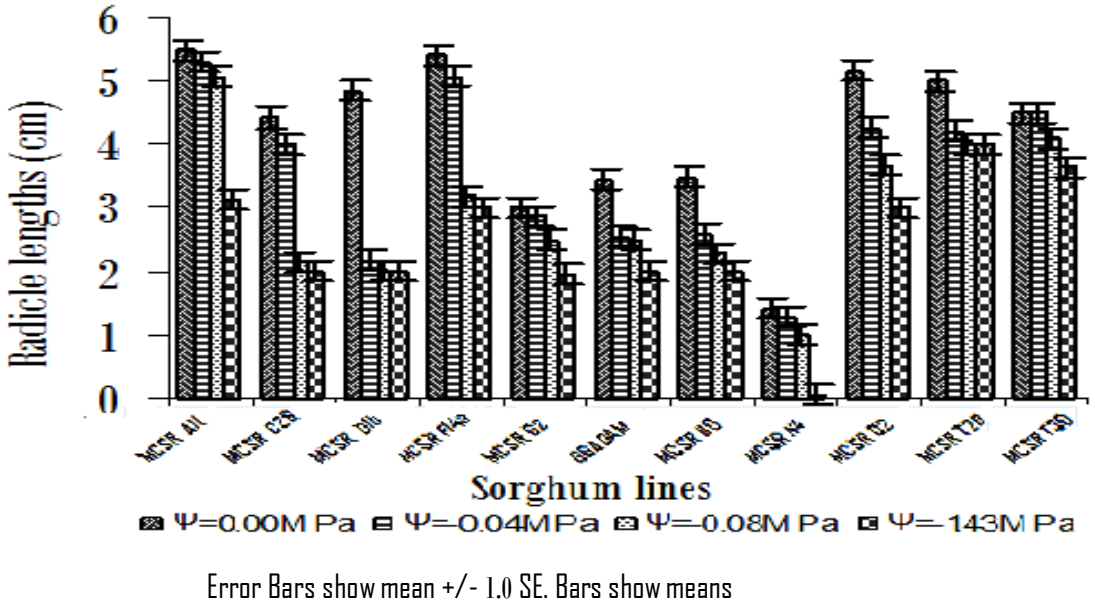


Figure 5: Seedling radicle lengths in sorghum.

4.5. Effect of water stress on starch degrading enzymes in germinating sorghum.

There was significant difference ($p \leq 0.05$) in starch degradation potential of germinating seed among sorghum lines. The water stress also significantly reduced starch degradation potential in sorghum lines.

The starch degradation activity was highest at Ψ = -0.8 MPa and lowest at Ψ = -1.43MPa (Figure 6). The sorghum lines which had low starch degradation activity at Ψ = 0.00MPa, -0.40MPa, -0.80MPa and -1.43MPa were MCSR F14a, Gadam, and MCSR D1b. MCSR I10 line showed the highest starch degradation activity under

water stress of Ψ = 0.00 to -0.08 MPa. Gadam and MCSR O2 at had high starch degradation potential at Ψ = -1.43 MPa.

4.6. Correlations of various attributes related to germination in sorghum.

There was positive correlation between seed imbibition rate and potential for starch degradation in germinating seeds of sorghum at p = 0.01 (Table 7). Percentage seed

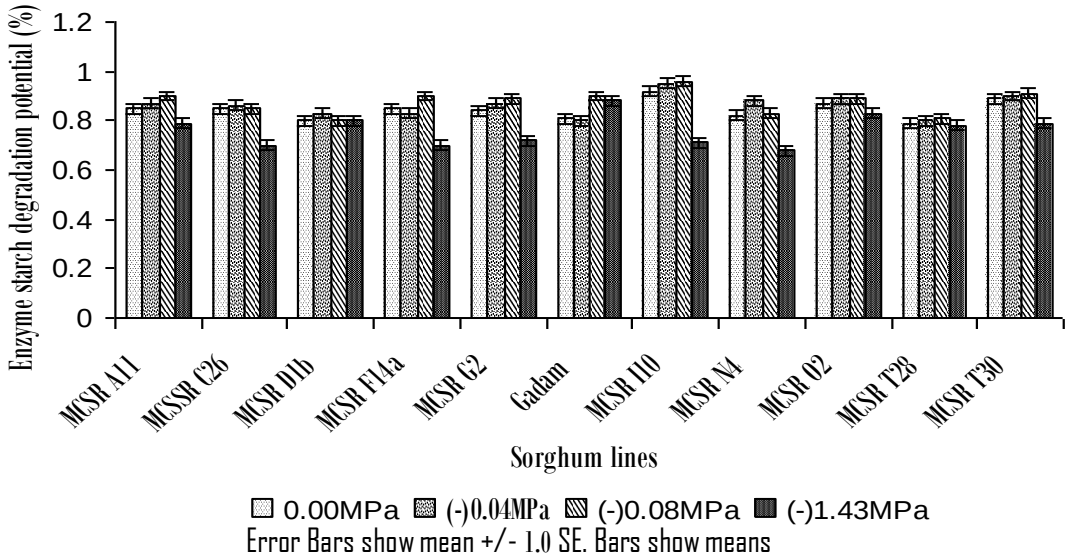


Figure 6: Effect of water stress on then activity of starch degrading enzymes (%).

germination positively correlated with percentage starch concentration at p = 0.05. Seedling radicle length negatively correlated with mean germination time at p = 0.05. The seedling radicle length was also positively correlated with percent seed germination at p = 0.01. The total mean germination time negatively correlated with percentage total seed germination at p = 0.01.

Table 7: Pearson's correlation coefficients and significance.

	%Starch concentration degraded	Seedling radicle length(cm)	Seed imbibition rate (g water/g seed/hour)	Mean germination time	% Germination
%Starch concentration	1				
Seedling radicle length	0.282 (0.064)	1			
Seed imbibition rate	0.403** (0.007)	0.118 (0.446)	1		
Mean germination time	-0.274 (0.072)	-0.302* (0.046)	-0.110 (0.477)	1	
% Total germination	0.325* (0.031)	0.684** (0.000)	0.195 (0.205)	-0.412**(0.006)	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed)
() in brackets are values on significance levels

4.7. Effect of water stress on six day old sorghum seedling's enzymes.

4.7.1 Acid phosphatase isozyme.

The zymogram showed monomorphic bands which varied in intensity of staining depending on the level of water stress and the sorghum lines (Figure 7). Higher levels of water stress caused increased band intensity in staining. MCSR A11 at 0.00 MPa (Lane1) and -1.43 MPa (4) had darkly stained band 3 and lightly stained bands 1, 2, 4 and 5. MCSR N4 had darkly stained band 3 at -1.43MPa (Lane 5) and -0.80 MPa (Lane 6) while at -0.40 MPa (Lane 7) and 0.00 MPa (Lane 8), the bands were light stained. MCSR C26 had intensely stained bands 1, 2 and 3 at -1.43 MPa (Lane12), -0.80 MPa (Lane11), and -0.40 MPa (Lane10), while at 0.00 MPa (Lane 9), it had lightly stained bands. MCSR G2 had dark stained bands 1, 2, 3, 4 and 5 at levels of water stress. -1.43 MPa (Lane13), -0.80MPa (Lane14) and -0.4MPa (Lane15) while under control (Lane16), it had bands 3 being darker than the other bands.

4.7.2 Peroxidase isozyme.

Peroxidase zymogram showed monomorphic bands. There were three anodic and two cathodic bands (Figure 8). Anodic band 1 stained uniformly dark in all the genotypes. However, the band staining intensity of anodic band 2 and 3 varied among the genotypes and with the level of water stress. The cathodic bands also stained with varied intensities among the genotypes and with the levels of water stress.

In MCSR G2, anodic band 2 and 3 stained darker at -1.43 MPa (Lane1) than in -0.80 MPa (Lane3). The cathodic bands 1 and 2 stained at equal intensities in MCSR G2 at the -1.43 MPa and -0.8 MPa. MCSR D1b had anodic band 2 and 3 that were lightly stained at -0.80 MPa (Lane 12) but heavily stained at -1.43 MPa (Lane 7). It also had cathodic band 2 stained heavily at -1.43 MPa (Lane 7) and lightly stained at -0.80 MPa (Lane12).

Gadam (Lanes 5 and 6) had the anodic bands staining uniformly dark while cathodic band 2 stained darker at -1.43 MPa (Lane 6) than -0.8 MPa (Lane 5).

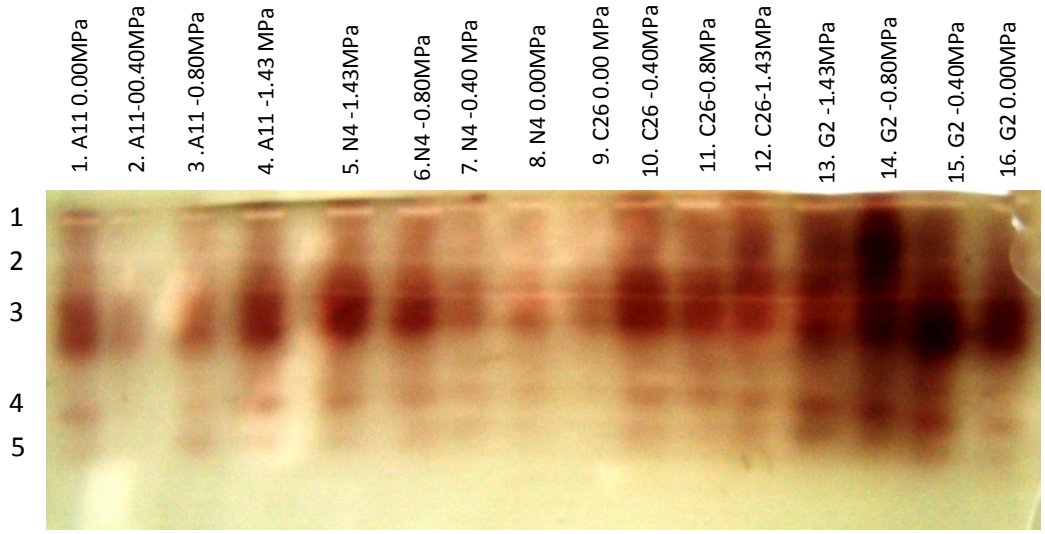


Figure 7: Zymogram of Acid Phosphatase in six day old sorghum seedlings at different levels of water stress.

In MCSR O2 (Lanes 8 and 10) , the anodic bands stained dark uniformly but cathodic band 1 was darker at -1.43 MPa (Lane 8) than at -0.80 MPa (Lane 10) while cathodic band 2 stained darker at -0.08 MPa (Lane 10) than at -1.43 MPa (Lane 8). MCSR A11 (Lanes 9 and 11) had uniformly dark stained anodic bands and light stained cathodic bands.

MCSR F14a at -1.43 Mpa (Lane 17) had a dark stained anodic band 3 while the rest of the bands showed equally stain intensity at both levels of stress (Lanes 15 and 17).

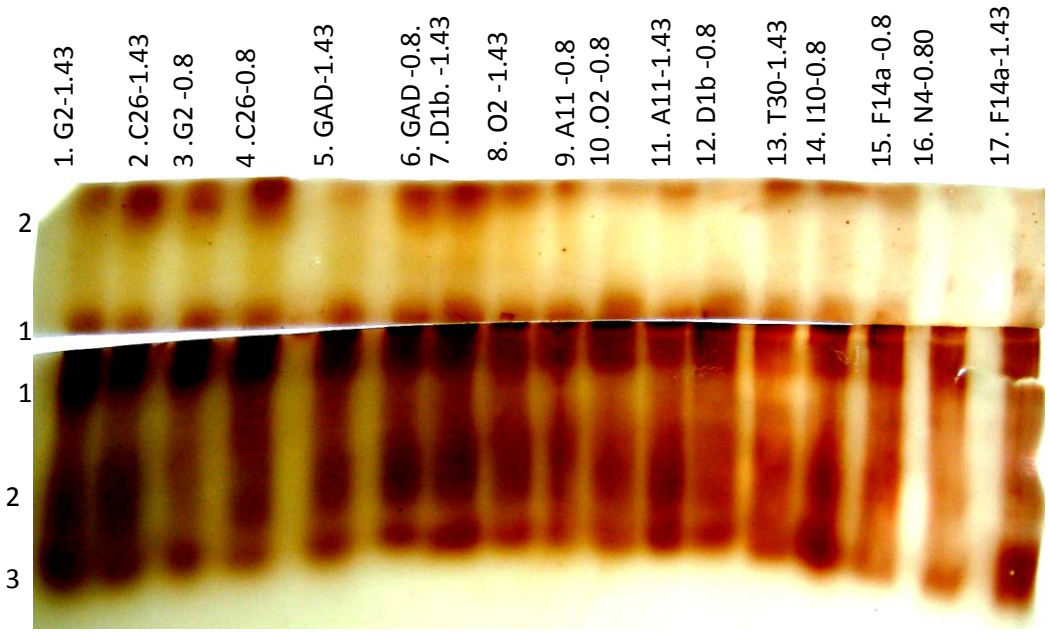


Figure 8: Peroxidase zymogram in six day old sorghum seedlings.

4.8.1 Effect of water stress on number of days to panicle emergence in sorghum.

There was a significant difference in days to panicle emergence among sorghum lines and with the levels of water stress ($p \leq 0.05$). Interaction between sorghum lines and

level of treatment was significant. MCSR T28 and MCSR C1 flowered earlier than other varieties while MCSR D1b and MCSR N4 were late to flower under the control (Figure 9). Under the pre-flowering stress, MCSR G2 and MCSR C1 flowered the earliest and MCSR N4 flowered last. MCSR T28 flowered earlier than the other lines and MCSR N4 took more days to flowering under the post-flowering stress. MCSR C1 and MCSR G2 flowered earlier while MCSR N4 and MCSR D1b were late to flower under continuous water stress. From these results the early maturing varieties included MCSR C1, MCSR G2, and MCSR T28 at all levels of water stress. The late varieties were MCSR N4, MCSR D1b and MCSR C26. Pre-flowering and continuous stresses significantly delayed panicle emergence.

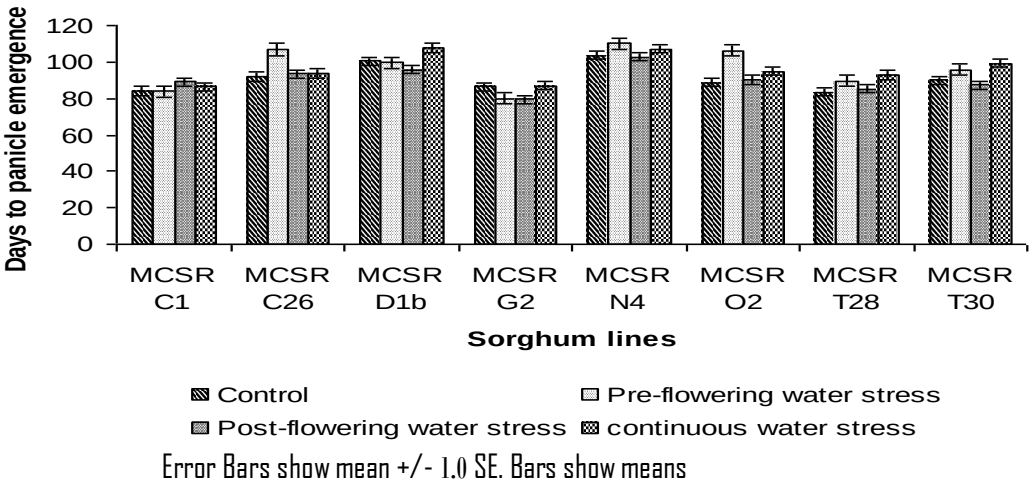


Figure 9: Days to panicle emergence sorghum under varying levels of water stress.

4.8.2 Effect of water stress on plant height in sorghum.

The difference in plant height was significant ($p \leq 0.05$) among the lines. The result showed general reduction in plant height at all levels of water stress with continuous stress being more severe.

Pre-flowering water stress only slightly reduced the height in MCSR D1b, MCSR G2, MCSR C1, and MCSR T28 when compared to the control (Figure 10). It caused higher plant height reduction in MCSR C26, MCSR N4 and MCSR O2 when compared to the control.

Post-flowering water stress caused significant height reduction in MCSR C26 and MCSR N4 when compared to the control. It led to low height reduction in MCSR G2, MCSR T28, MCSR T30 and MCSR C1 when compared to the control.

Continuous stress led to the higher plant height reduction in MCSR C26 and in MCSR O2 than in other varieties and the lines with lowest height reduction were MCSR G2, MCSR T28, MCSR C1 and MCSR T30 respectively.

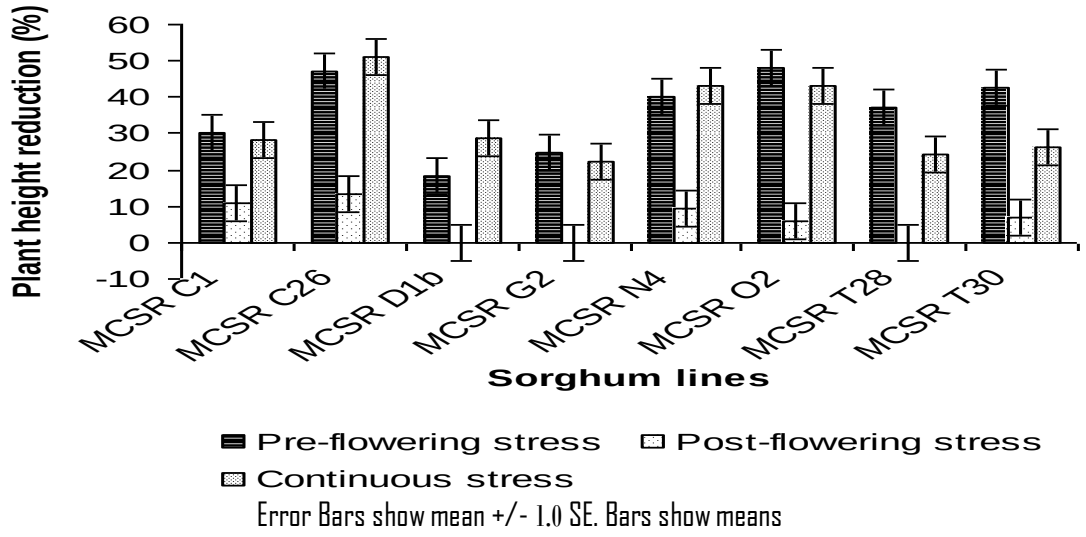


Figure 10: Percentage of plant height reduced by water stress.

4.8.3 The effect of water stress on length of third leaf in sorghum plants.

The length of third leaf from apex of plant was significantly different ($p \leq 0.05$) under water stress.Continuous water stress was more severe in causing a reduction in lengths of third leaf when compared to the control (Table 8). Under the well watered

control regime, the longest third leaf length was recorded in MCSR C26, MCSR D1b and MCSR T30. The shortest leaf length was recorded in lines MCSR N4, MCSR G2, MCSR C1 and MCSR T28 under the well watered control.

The sorghum lines which showed the longest third leaf length under the pre-flowering water stress included MCSR C26, MCSR D1b, MCSR O2 and MCSR T30 while lines MCSR N4, MCSR G2, MCSR C1 and MCSR T28 showed the shortest leaf lengths. The highest percent leaf length reduction under this stress was recorded in lines MCSR C26, MCSR D1b, MCSR O2 and MCSR T30. The lines which recorded the lowest percentage decrease in leaf length included MCSR N4, MCSR T30 and MCSR O2.

Under the post-flowering water stress, the lines with the longest third leaf included MCSR C26, MCSR T30 and MCSR T28 while lines which recorded the shortest were MCSR N4, MCSR G2, MCSR C1 and MCSR T28. Post flowering water stress regime caused the highest percentage third leaf length reduction in lines MCSR C26, MCSR D1b and MCSR O2 while lines MCSR T30, MCSR N4, MCSR C1 and MCSR T28 recorded the lowest percentage leaf length reduction.

The sorghum lines which showed the longest third leaf lengths were MCSR D1b, MCSR T30 and MCSR O2 while the lines which showed shortest third leaf lengths included MCSR N4, MCSR G2 and MCSR C1. The continuous stress treatment caused the highest reduction in leaf length in MCSR C26 followed by MCSR D1b and MCSR O2 when compared to the control. The sorghum line least affected by continuous water stress was MCSR N4 followed by MCSR G2 , MCSR T28 and MCSR T30 when compared to the control. The lines MCSR C26, MCSR D1b and

MCSR T30 recorded the longest third leaf lengths whereas lines MCSR N4, MCSR G2 and MCSR C1 recorded the shortest line mean lengths.

4.8.4 Effect of water stress on widths of third leaf in sorghum plants.

The results showed significant difference in widths of third leaves with the levels of water stress at $p \leq 0.05$.

The sorghum lines MCSR C1 followed by MCSR D1b recorded the highest third leaf reduction under the pre-flowering water stress (Table 9). However, width of third leaf

Table 8: Third leaf length (cm) and percentage reduction under water stress in sorghum plants.

Sorghum line	Control	Pre-flowering water stress	% reduction	Post-flowering water stress	% reduction	Continuous water stress	% reduction	MEAN
MCSR C1	64.70	60.60	6.34	64.60	0.15	59.30	8.35	62.30
MCSR C26	82.30	74.40	9.60	81.40	1.09	62.50	24.06	75.15
MCSR D1b	82.20	72.90	11.31	68.90	16.18	68.00	17.27	73.00
MCSR G2	60.80	58.20	4.28	56.60	6.91	57.00	6.25	58.15
MCSR N4	47.20	54.45	-12.71	49.20	-4.24	56.90	-17.80	51.94
MCSR O2	73.20	71.10	2.87	67.50	7.79	62.60	14.48	68.60
MCSR T28	66.30	62.30	6.03	66.20	0.15	61.70	6.94	64.12
MCSR T30	72.70	71.80	1.24	76.20	-4.81	67.70	6.88	72.10

Means on third leaf length at $p \leq 0.05$

in other lines were least affected under the same level of water stress.

The post-flowering water stress regime caused reduction in third leaf width in MCSR D1b when compared to the control but least affected widths in other sorghum lines.

Continuous water stress led to a highest reduction of width of third leaf in MCSR D1b, followed by MCSR G2 and MCSR O2. The sorghum line MCSR C1 leaf width was not affected whilst MCSR N4 had the lowest leaf width reduced.

4.8.5 Effect of water stress on leaf senescence under water stress in sorghum.

There was a significant difference in the percentage of dead leaves on weekly basis among the sorghum lines during post flowering water stress (Figure 10). The first one week of stress caused high leaf senescence in MCSR T30 and MCSR O2 when compared to the control while MCSR C1, MCSR D1b, MCSR T28 and MCSR N4 showed the lowest percent leaf senescence (Figure 11). The second week of post-flowering stress led to high percent leaf senescence in MCSR T30 and MCSR C26

Table 9: The effect of water stress on third leaf width (cm) in sorghum plants.

Sorghum line	Control	Pre-flowering water stress	% width reduction	Post-flowering water stress	% width reduction	Continuous stress	% width reduction	MEAN
MCSR C1	6.64	6.34	4.52	7.30	-9.94	6.80	-2.41	6.77
MCSR C26	6.86	6.80	0.87	7.60	-10.79	6.48	5.54	6.94
MCSR D1b	8.22	7.92	3.65	7.98	2.92	6.82	17.03	7.74
MCSR G2	7.08	7.20	-1.69	7.20	-1.69	6.08	14.12	6.89
MCSR N4	7.06	7.38	-4.49	7.06	0.00	6.94	1.70	7.11
MCSR O2	6.50	7.10	-9.23	6.90	-6.15	5.70	12.31	6.55
MCSR T28	6.86	7.60	-10.79	7.20	-4.96	6.44	6.12	7.05
MCSR T30	6.66	6.90	-3.60	7.72	-15.92	6.26	6.01	6.89

Means on third leaf width at $p \leq 0.05$

while causing low leaf senescence in MCSR C1, MCSR G2 and MCSR N4. By the third week of post-flowering water stress, MCSR T30 and MCSR N4 had the highest percentage leaf senescence. The varieties with the lowest leaf senescence percentage at post flowering stress included MCSR D1b, MCSR G2 and MCSR C26.

The sorghum lines which showed faster rate of leave senescence when subjected to post-flowering water stress included MCSR T30, MCSR O2 and MCSR C26. The

lines with the slowest rate of senescence included MCSR C1, MCSR D1b and MCSR G2.

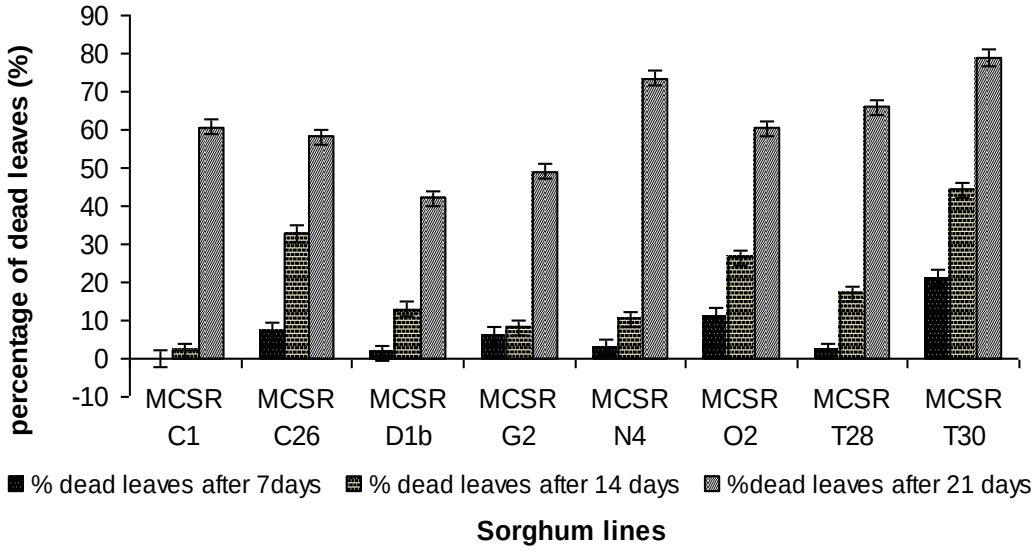


Figure 11: Percent dead leaves in block under the pos-flowering regime in sorghum plants

4.8.6 Effect of water stress on number of nodal tillers in sorghum lines.

There was a significant difference ($p \leq 0.05$) in number of nodal tillers among sorghum varieties and with the levels of water stress.

The line MCSR T30 followed by MCSR C26 and MCSR O2 showed the highest number of nodal tillers while MCSR G2 followed by MCSR T28, MCSR C1 and MCSR D1b recorded the lowest number of nodal tillers under the control (Figure 12). MCSR C26, MCSR T30, MCSR T28 and MCSR O2 recorded the highest number of nodal tillers under the pre-flowering water stress while MCSR G2, MCSR C1 and MCSR D1b recorded the lowest. MCSR C26, MCSR T30 and MCSR O2 recorded the highest number of nodal tillers under the post-flowering water stress while MCSR C1 and MCSR T28 recorded the lowest number of nodal tillers. Under continuous water stress, the number of nodal tillers was highest in MCSR C26,

MCSR T30 and MCSR N4 but lowest in MCSR T28, MCSR G2 and MCSR C1. Pre-flowering water stress and continuous stress induced the highest Number of nodal tillering in sorghum lines.

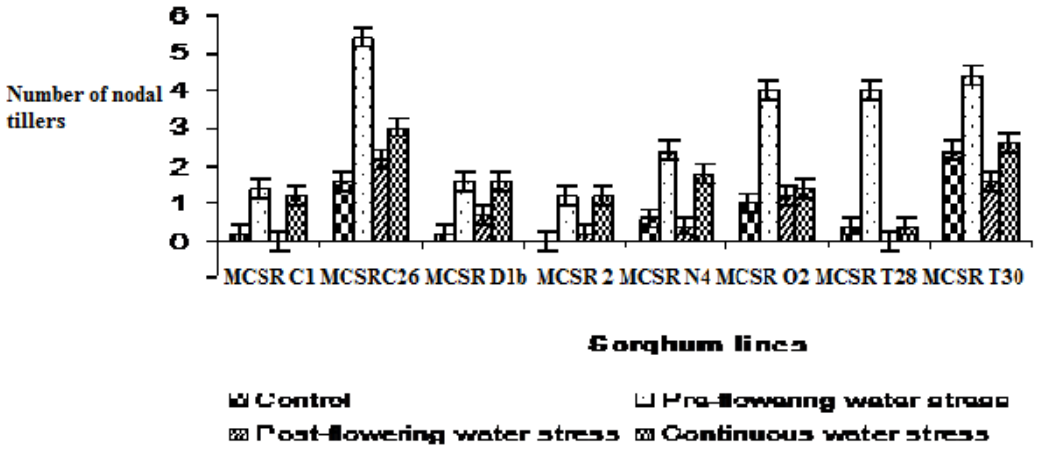


Figure 12: Nodal tillering in sorghum lines under varied levels of water stress.

4.8.7 Effect of water stress on chlorophyll a concentration in sorghum flag leaf.

The concentration of chlorophyll a content in sorghum flag leaves was significantly different ($p \leq 0.05$) with the levels of water stress.

Continuous water stress caused the highest decrease in chlorophyll a concentration in flag leaves of water stressed sorghum plants than in other water stress levels (Table 10). MCSR G2, MCSR O2 and MCSR N4 showed the high chlorophyll a concentration in sorghum flag leaf while MCSR T30, MCSR T28 and MCSR D1b showed low concentrations under the well watered control. MCSR D1b, MCSR C1, MCSR N4 and MCSR G2 recorded the highest chlorophyll a concentration under the pre-flowering water stress while MCSR O2, MCSR T28 and MCSR C26 recorded the lowest in sorghum flag leaf. Under the post flowering stress, MCSR D1b, MCSR C1, MCSR G2 and MCSR N4 showed the highest chlorophyll a concentration and MCSR O2 had the lowest chlorophyll a concentration in sorghum flag leaf. Under the

Continuous water stress, MCSR C1, MCSR D1b and MCSR O2 had the highest chlorophyll a concentration and MCSR T30 had the lowest. The sorghum lines with the highest genotypic mean chlorophyll a concentration included MSCR G2, MCSR C1, MCSR D1b and MCSR N4 while the lines with low chlorophyll a concentration included MCSR T30, MCSR T28, MCSR C26 and MCSR O2.

Table 10: chlorophyll a concentration (µg cm^{-2}) in sorghum flag leaf.

Sorghum lines	Control	Pre-flowering stress	Post-flowering stress	Continuous stress	MEAN
MCSR C1	12.05^{c}	12.73^{a}	11.63^{ab}	12.07^{a}	12.12^{ab}
MCSR C26	13.08^{c}	8.15^{bc}	7.44^{bc}	4.92^{b}	8.40^{cd}
MCSR D1b	11.74^{c}	12.77^{a}	13.75^{a}	8.13^{ab}	11.60^{ab}
MCSR G2	24.72^{a}	10.64^{ab}	10.25^{abc}	6.63^{ab}	13.05^{a}
MCSR N4	18.88^{b}	10.89^{ab}	9.52^{abc}	5.92^{ab}	11.32^{ab}
MCSR O2	22.65^{ab}	4.89^{c}	6.00^{c}	7.04^{ab}	10.15^{bc}
MCSR T28	8.38^{c}	7.39^{bc}	7.25^{bc}	6.97^{ab}	7.50^{d}
MCSR T30	8.33^{c}	8.17^{bc}	7.75^{bc}	3.49^{b}	6.93^{d}

Means in the same column followed by same letter do not differ significantly at $p \leq 0.05$.

4.8.8 Effect of water stress on chlorophyll b in sorghum flag leaves.

The chlorophyll b concentration in sorghum flag leaf was significantly different ($p \leq 0.05$) with the levels of water stress.

Continuous water stress led to a higher reduction of chlorophyll b concentration in sorghum flag leaves when compared to other levels of water stress (Table 11). Under the control, MCSR G2, followed by MCSR N4 and MCSRO2 had the highest chlorophyll b concentration while MCSR T28 followed by MCSR T30and MCSR C1 had the lowest. MCSR O2 recorded the highest chlorophyll b concentration followed by MCSR C1 and MCSR N4 while MCSR T28, followed by MCSR C26 and MCSR

T30, had the lowest chlorophyll b concentration under the pre- flowering stress. Under the post-flowering water stress, chlorophyll b content in MCSR D1b followed by MCSR C1 and MCSR N4 was the highest while MCSR O2 followed by MCSR T28 and MCSR T30 had the lowest chlorophyll b content in sorghum flag leaf. MCSR C1, MCST T30 and MCSR D1b recorded the highest chlorophyll b concentration while MCSR C26, MCSR N4 and MCSR G2 recording the lowest concentration under the continuous water stress regime. The genotype means showed MCSR G2 having the highest chlorophyll b content followed by lines MCSR D1b, MCSR N4 and MCSR C1 respectively. The lines with the lowest chlorophyll b included MCSR T28, MCSR C26 and MCSR T30.

4.8.9 Effect of water stress on chlorophyll a/b ratio in sorghum flag leaf.

The results showed no significant difference ($P \leq 0.05$) in chlorophyll a/b ratio with the level water stress in sorghum plants.

However, the lines which showed the highest ratios of chlorophyll a/b included

Table 11: Effect of water stress on Chlorophyll b concentration (µg cm^{-2}) in sorghum flag leaves.

Sorghum line	Control	Pre-flowering water stress	Post-flowering water stress	Continuous water stress	MEAN
MCSR C1	3.88^{b}	3.77^{a}	3.53^{ab}	3.19^{a}	3.59^{ab}
MCSR C26	4.30^{ab}	2.45^{a}	2.64^{bc}	1.35^{a}	2.68^{b}
MCSR D1b	3.99^{b}	3.51^{a}	4.68^{a}	2.66^{a}	3.71^{ab}
MCSR G2	8.51^{a}	3.41^{a}	3.56^{ab}	1.91^{a}	4.35^{a}
MCSR N4	7.14^{ab}	3.13^{a}	2.94^{bc}	1.57^{a}	3.70^{ab}
MCSR O2	5.89^{ab}	3.97^{a}	2.08^{c}	2.37^{a}	3.58^{ab}
MCSR T28	3.09^{b}	2.28^{a}	2.09^{c}	1.98^{a}	2.36^{b}
MCSR T30	3.23^{b}	2.50^{a}	2.52^{bc}	2.85^{a}	2.77^{b}

Means in a column followed by the same letter do not differ significantly at $p \leq 0.05$.

MCSR O2, followed by MCSR G2, MCSR N4 and MCSR C1 (Table 12). MCSR T30 and MCSR T28 recorded the lowest chlorophyll a/b ratios. Under the pre-flowering water stress, MCSR D1b followed by MCSR N4, MCSR C1 and MCSR C26 recorded high ratios of chlorophyll a/b while MCSR O2 had the lowest. Line MCSR T28 followed by MCSR C1 and MCSR N4 had high ratios of chlorophyll a/b; while MCSR C26 had the lowest. Under the continuous water stress, MCSR N4, MCSR C26 and MCSR C1 had the highest chlorophyll a/b ratio in that order while MCSR T30 had the lowest. The overall means showed that MCSR N4, MCSR C1 and MCSR C26 and MCSR T28 had high chlorophyll a/b ratios while MCSR T30 recorded the lowest.

4.8.10. The effect of water stress on total chlorophyll concentration in sorghum.

Total chlorophyll concentration in flag leaves of sorghum varied significantly ($p \leq 0.05$) with the level of water stress.

Water stress reduced the quantity of total chlorophyll concentration in sorghum flag

Table 12: Effect of water stress on chlorophyll a/b ratio in sorghum flag leaves.

Sorghum lines	Control	Pre-flowering water stress	Post-flowering water stress	Continuous water stress	MEAN
MCSR C1	3.04^{ab}	3.39^{a}	3.27^{ab}	3.77^{a}	3.37^{a}
MCSR C26	2.99^{ab}	3.29^{a}	2.80^{b}	3.78^{a}	3.22^{a}
MCSR D1b	2.94^{ab}	3.64^{a}	2.96^{ab}	3.04^{a}	3.15^{a}
MCSR G2	3.37^{ab}	3.12^{a}	2.87^{b}	3.45^{a}	3.20^{a}
MCSR N4	3.08^{ab}	3.47^{a}	3.16^{ab}	3.83^{a}	3.38^{a}
MCSR O2	3.84^{a}	1.93^{b}	2.89^{b}	2.92^{a}	2.90^{a}
MCSR T28	2.71^{b}	3.28^{a}	3.49^{a}	3.38^{a}	3.22^{a}
MCSR T30	2.58^{a}	3.28^{b}	3.07^{ab}	2.36^{a}	2.82^{a}

Means with same letter in a column do not differ significantly at $p \leq 0.05$.

leaves with the continuous water stress being more severe (Table 13). Under the well watered control, MCSR G2 posted the highest total chlorophyll concentration, followed by MCSR O2, MCSR N4 and MCSR C1 while MCSR T30 followed by MCSRT28 and MCSR D1b recorded the lowest in sorghum flag leaf. MCSR C1 followed by MCSR D1b, MCSR G2 and MCSR N4 recorded the highest total chlorophyll concentration while MCSR O2 and MCSR T28 recorded the lower total chlorophyll concentration under the pre-flowering water stress in sorghum flag leaf. Under the post flowering water stress, MCSR D1b, MCSR C1, MCSR G2 and MCSR N4 had the highest, while MCSR O2, MCSR T28 and MCSR C26 recorded the lowest chlorophyll concentration in sorghum flag leaf. MCSR C1 followed by MCSR D1b, MCSR O2 and MCSR T28 had the highest total chlorophyll concentration while MCSR T30,MCSR C26 and MCSR N4 showed the lowest under the continuous water stress regime. The sorghum line which recorded the highest genotype mean was MCSR G2, followed by MCSR C1, MCSR D1b and MCSR N4 respectfully. MCSR

Table 13: Effect of water stress on total chlorophyll concentration (µg/cm^2) in sorghum flag leaf.

Sorghum lines	Control	Pre-flowering water stress	Post-flowering water stress	Continuous water stress	MEAN
MCSR C1	20.35^{cd}	18.94^{a}	17.29^{ab}	17.57^{a}	18.54^{ab}
MCSR C26	19.49^{d}	12.28^{b}	11.37^{bc}	7.24^{b}	12.58^{c}
MCSR D1b	17.72^{d}	18.49^{a}	18.77^{a}	12.40^{ab}	16.84^{ab}
MCSR G2	36.78^{a}	16.07^{ab}	15.60^{abc}	9.84^{ab}	19.57^{a}
MCSR N4	28.28^{bc}	16.03^{ab}	14.02^{abc}	8.65^{b}	16.74^{ab}
MCSR O2	31.91^{ab}	12.30^{b}	9.08^{c}	10.58^{ab}	15.97^{b}
MCSR T28	13.26^{d}	11.11^{b}	10.68^{bc}	10.17^{ab}	11.30^{c}
MCSR T30	13.04^{a}	12.25^{b}	11.70^{abc}	6.43^{b}	10.85^{c}

Means in the same column followed by the same letter do not differ significantly at $p \leq 0.05$

T30, MCSR T28 and MCSR C26 recorded the lowest mean total chlorophyll concentration in sorghum flag leaf.

4.8.11 Effect of water stress on carotenoids concentration in sorghum flag leaf.

The carotenoids concentration in sorghum flag leaf did not vary significantly ($p \leq 0.05$) with the levels of water stress.

However, water stresses reduced the flag leaf carotenoids concentration at all levels with the continuous water stress regime being more severe (Table 14). Lines MCSR N4 followed by MCSR G2, MCSR T28 and MCSR O2 posted the highest total carotenoids concentration under the well watered control while MCSR T30, MCSR C1 and MCSR C26 recorded the lowest concentration in sorghum flag leaf.

The sorghum Line MCSR O2 followed by MCSR G2, MCSR N4 and MCSR D1b showed the highest carotenoids concentration in sorghum flag leaf under the pre-flowering water regime. The lowest carotenoids concentration at this water stress level was recorded in MCSR C26, MCSR T28 and MCSR T30 respectively.

Under the post-flowering water stress regime, line MCSR D1b followed by MCSR N4, MCSR C1 and MCSR T30 recorded the highest flag leaf carotenoids concentration while lines MCSR T28,MCSR O2 and MCSR C26 recorded the lowest flag leaf carotenoids concentrations in sorghum flag leaf.

Under the continuous water stress regime, line MCSR C1 followed by MCSR O2, MCSR T30 and MCSR T28 posted the highest carotenoids concentration while MCSR C26, MCSR N4, MCSR D1b and MCSR G2 recorded the lowest carotenoids content under the continuous water stress regime.

Sorghum lines MCSR N4, MCSR G2, MCSRD1b and MCSR O2 showed high genotype means while MCSR C26, MCSR T30, MCSR T28 and MCSR C1 showed low genotype means.

4.8.12.1 Effect of water stress on lengths (cm) of panicles in sorghum lines.

There were significant difference in panicle lengths among the sorghum lines under both control and water stress conditions at $p \leq 0.05$.

Table 14: Effect of water stress on carotenoids concentration (µg cm^{-2}) in sorghum flag leaves.

	TOTAL CAROTENOID CONCENTRATION (µg cm^{-2})				
Sorghum lines	Control	Pre-flowering water stress	Post-flowering water stress	Continuous water stress	MEAN
MCSR C1	7.13	8.64	8.89	9.32	8.50
MCSR C26	9.68	7.29	6.80	3.10	6.72
MCSR D1b	12.27	9.51	16.73	4.49	10.75
MCSR G2	19.84	12.40	8.08	5.01	11.33
MCSR N4	27.60	10.80	11.45	4.47	13.58
MCSR O2	13.85	13.43	5.30	8.58	10.29
MCSR T28	15.43	7.93	5.09	5.37	8.49
MCSR T30	6.67	8.01	8.59	6.41	7.42

Means on total carotenoids concentration at $p \leq 0.05$

The panicle lengths were reduced by water stresses with the continuous water stress being more severe. The longest panicles were recorded in line MCSR D1b followed by MCSR C1 and MCSR G2; and the shortest panicles were recorded in MCSR N4, MCSR C26 and MCSR O2 under the well watered control (Table 15).

MCSR T30 had the longest panicle, followed by MCSR D1b, MCSR C1 and MCSR G2 under the pre-flowering water stress regime. The shortest panicles under the same treatment were MCSR N4, MCSR O2 and MCSR C26. The lines with the highest percentage panicle reduction were MCSR T30, MCSR D1b, MCSR C26 and MCSR

O2. The lines which had the lowest panicle length reduction were MCSR G2, MCSR N4 and MCSR C1 at the same level of water stress.

Under the post flowering stress MCSR D1b, MCSR C1, MCSR G2 and MCSR T30 had the longest panicles while MCSR N4, MCSR O2 and MCSR C26 had the shortest panicles. The highest percentage panicle length reductions were recorded in MCSR O2, MCSR C26, MCSR T28 and MCSR T30. The sorghum line with the lowest panicle length reductions under the same water regime were MCSR N4, MCSR C1, MCSR G2 and MCSR D1b respectively.

The sorghum lines with the longest panicles under the continuous water stress were MCSR C1, MCSR D1b and MCSR G2 while MCSR T30 followed by MCSR N4 and MCSR O2 recorded the shortest panicle length. Continuous water stress caused the greatest reduction on panicle length percentage in MCSR T30, MCSR C26, MCSR D1b and MCSR O2; and MCSR N4, MCSR C1 and MCSR G2 had the lowest length reduction.

The longest genotype panicles were recorded for MCSR D1b, MCSR C1 and MCSR G2. The shortest genotype panicles were recorded for MCSR N4, MCSR C26 and MCSR T30.

4.8.13 Effect of water stress on panicle widths (cm) in sorghum lines.

The panicle widths were significantly different ($p \leq 0.05$) among the sorghum lines with the level of water stress.

Water stresses reduced sorghum panicle widths with the continuous stress being more severe (Table 16). Continuous water stress caused the most reduction in panicle

Table 15: Effects of water stress on Panicle lengths (cm) sorghum lines.

Sorghum line	Control	Pre-flowering water stress	% length reduced	Pos-flowering water stress	%length reduced	Continuous water stress	% length reduced	MEAN
MCSR C1	19.9	18.2	8.5	22.0	-10.6	19.6	1.5	19.9
MCSR C26	17.1	13.6	20.5	16.3	4.7	11.5	32.8	14.6
MCSR D1b	26.2	19.7	24.8	26.8	-2.3	18.2	30.5	22.7
MCSR G2	18.1	17.9	1.1	19.3	-6.3	16.8	7.2	18.0
MCSR N4	8.8	8.6	2.3	12.4	-40.9	10.4	-18.2	10.1
MCSR O2	17.1	13.8	19.3	16.1	5.9	14.0	18.1	15.3
MCSR T28	17.5	14.6	16.6	17.3	1.1	14.5	17.1	16.0
MCSR T30	18.3	13.6	25.7	18.1	1.1	9.8	46.5	15.0
MEAN	17.9	15.0	14.8	18.5	-5.9	14.4	16.9	16.4

Means on third leaf width at $p \leq 0.05$

widths. The post-flowering water stress had the smallest effect on panicle widths. Under the well watered control, sorghum lines with the widest panicle width were MCSR C1, MCSR C26, MCSR G2 and MCSR N4; and MCSR D1b, MCSR O2, MCSR T28 and MCSR T30 had the narrowest panicles.

MCSR C1, MCSR N4 and MCSR G2 had the widest panicle width under the pre-flowering water stress, and MCSR O2, MCSR C26 and MCSR D1b had the narrowest. The sorghum lines with the highest width reduction under the pre-flowering water regime were MCSR C26, MCSR T30 and MCSR T28. The lines which had the lowest percentage panicle width reduction included MCSR D1b, MCSR N4 and MCSR C1.

The sorghum lines MCSR C1, MCSR N4 and MCSR G2 had the widest panicles under the post-flowering water regime, and lines MCSR D1b, MCSR C26 and MCSR O2 had the narrowest panicles. Post-flowering water stress regime caused the highest

percentage reduction of panicle widths of lines MCSR C26, MCSR T30 and MCSR G2, and causing the lowest reduction in lines MCSR O2, MCSR N4 and MCSR C1.

Under the continuous water stress, the lines MCSR G2, MCSR T28 and MCSR C1 had the widest panicles, and lines MCSR D1b, MCSR C26 and MCSR T30 had the narrowest panicle width. The width reduction was highest in lines MCSR C26, MCSR T30 and MCSR N4, and MCSR G2, MCSR O2 and MCSR T28 had the lowest width reduction.

The sorghum lines with the widest panicles were MCSR C1, MCSR G2 and MCSR N4. The lines with the narrowest panicles were MCSR D1b and MCSR O2.

Table16: Effect of water stress on panicle widths sorghum lines.

Sorghum line	Control	pre-flowering water stress	% width reduction	post-flowering water stress	% width reduction	continuous water stress	% width reduction	MEAN
MCSR C1	9.2	6.6	28.3	8.4	8.7	4.4	52.2	7.2
MCSR C26	9.2	3.5	62.0	5.8	37.0	2.8	69.6	5.3
MCSR D1b	5.2	4.0	23.1	4.3	17.3	2.4	53.9	4.0
MCSR G2	8.7	6.2	28.7	7.1	18.4	6.2	28.7	7.1
MCSR N4	8.7	6.4	26.4	8.0	8.1	3.9	55.2	6.8
MCSR O2	5.4	3.3	38.9	5.1	5.6	3.5	35.2	4.3
MCSR T28	7.8	4.3	44.9	6.5	16.7	4.6	41.0	5.8
MCSR T30	7.8	4.2	46.2	6.3	19.2	2.9	62.8	5.3
MEAN	7.2	4.8	37.3	6.4	16.4	3.8	49.8	5.6

Means on third leaf width at $p \leq 0.05$

4.8.14 Effect of water stress on panicle weights in sorghum

Panicle weight was significantly different ($p \leq 0.05$) among sorghum lines with the levels of water stress.

Water stress caused a general decrease in panicle weights (Table 17). The continuous water stress had the greatest negative effect on panicle weights; whereas the post-flowering water stress had the least effect. The lines with the heaviest panicles under the well watered control included MCSR N4, MCSR G2 and MCSR C1, and the lines with the lightest panicles included MCSR D1b, MCSR O2 and MCSR T28.

Under the pre-flowering water stress regime, lines MCSR N4, MCSR G2 and MCSR T30 recorded the heaviest panicles, and MCSR D1b, MCSR O2 and MCSR T28 recording the lightest panicles. The sorghum lines with the most panicle weight reduction were MCSR O2, MCSR C1and MCSR D1b under the pre-flowering water stress; and the lines with the least panicle weight reduction included MCSR N4, MCSR G2 and MCSR T30.

The sorghum lines MCSR C1, MCSR G2 and MCSR T28 had the heaviest panicles whereas lines MCSR D1b, MCSR O2 and MCSR C26 had the lightest panicles under the post-flowering water stress. The sorghum lines which showed the highest percentage of panicle weight reduction under this water regime were MCSR C26 MCSR N4 and MCSR O2 whereas MCSR C1, MCSR T28 and MCSR G2 had the lowest reduction. Under the continuous water stress the sorghum lines with the heaviest panicles were MCSR G2, MCSR T28 and MCSR C1, whereas the lines MCSR C26, MCSR T30 and MCSR D1b had the lowest reduction. Percentage weight reduction was highest in lines MCSR C26, MCSR T30 and MCSR N4.The least percentage panicle weight reduction was recorded in lines MCSR G2, MCSR T28 and MCSR O2 under the continuous water stress.

The lines with the heaviest panicles were MCSR G2, MCSR N4 and MCSR C1 whereas the lines with the lightest panicles were MCSR D1b, MCSR O2 and MCSR C26 respectively.

Table 17: Dry panicle weights (g) and percent panicle weight reduced under water stress in sorghum.

Sorghum line	Control	Pre-flowering water stress	% panicle weight reduction	post-flowering water stress	% panicle weight reduction	continuous water stress	% panicle weight reduction	MEAN
MCSR C1	87.3	59.0	32.4	86.7	0.7	31.1	64.4	66.0
MCSR C26	83.5	59.8	28.4	37.2	55.5	14.4	82.8	48.7
MCSR D1b	32.9	22.7	31.0	25.5	22.5	14.8	55.0	24.0
MCSR G2	93.9	84.9	9.6	83.3	11.3	53.1	43.5	78.8
MCSR N4	96.7	98.8	-2.2	52.3	45.9	26.8	72.3	68.7
MCSR O2	57.9	37.0	36.1	36.6	36.8	20.8	64.1	38.1
MCSR T28	74.1	56.8	23.4	67.4	9.0	33.4	54.9	57.9
MCSR T30	80.4	65.2	18.9	57.4	28.6	14.5	82.0	54.4
MEAN	75.8	60.5	22.2	55.8	26.3	26.1	64.9	54.6

Means on third leaf width at $p \leq 0.05$

4.8.15 Effect of water stress on shoot dry weights in sorghum lines.

There was significant difference in shoot dry weight among sorghum lines with the level of water stress at $p \leq 0.05$.

The water stresses caused a reduction in shoot dry weights (Table 18). Continuous water stress had the greatest negative effect on shoot dry weights, whereas the pre-flowering stress had the least effect on shoot biomass. The sorghum lines which had the highest shoot dry weights under the well watered control were MCSR T30, MCSR C26 and MCSR G2. The lines which had low shoot dry weights under the control were MCSR D1b, MCSR O2 and MCSR T28. Under the pre-flowering water stress,

the lines which recorded the highest shoot dry weights included MCSR C26, MCSR T30 and MCSR N4, whereas the lines MCSR D1b, MCSR C1 and MCSR O2 showed the lowest shoot dry weights.

The percentage shoot dry weight loss under the pre-flowering water stress was highest in MCSR C1, MCSR D1b and MCSR T30. The sorghum lines with the lowest shoot dry weight reduction were MCSR N4, MCSR C26 and MCSR G2. The sorghum lines with the highest shoot dry weight under the post flowering water stress regime were MCSR C1 MCSR G2 and MCSRT30. The lines with the lowest shoot dry weights were MCSR D1b, MCSR O2 and MCSR C26. The post-flowering water stress caused the highest shoot dry weight loss in MCSR O2, MCSR C26 and MCSR N4. The lowest shoot dry weight loss was recorded in MCSR C1, MCSR T28 and MCSR D1b.

Under the continuous water stress, the sorghum lines with the highest shoot dry weights were MCSR G2, MCSRT30; whereas MCSR C1, and MCSR C26, MCSR D1b and MCSR O2 had the lowest shoot dry weights. The percentage of shoot dry weight reduction was highest in MCSR C26, MCSR T30 and MCSR O2; whereas MCSR D1b, MCSR G2 and MCSR C1 had the lowest dry shoot weight loss under the continuous water regime. The lines with the highest shoot dry weights were MCSR T30 and MCSR G2; whereas the lowest shoot dry weights were recorded in MCSR D1b and MCSR O2.

4.9. Effect of post-flowering water stress on field grown sorghum plants

4.9.1 Leaf senescence under water stress in field grown sorghum.

Percentage leaf senescence varied significantly ($p=0.05$) among the sorghum lines under the post-flowering water stress (Figure 13). The lines MCSR A11, MCSR O2

and MCSR G2 recorded the most leaf senescence. The lines MCSR T30, MCSR T28, MCSR I10 and MCSR D1b recorded the least percentage of leaf senescence.

Table 18: shoot dry weights (g) and percentage shoot weight reduction in sorghum.

Sorghum line	Control	Pre-flowering water stress	% weight reduced	Post-flowering water stress	% weight reduced	Continuous water stress	% weight reduced	MEAN
MCSR C1	175.2	133.1	24.0	135.7	22.6	85.7	51.1	132.4
MCSR C26	186.1	178.5	4.1	111.4	40.1	66.3	64.4	135.6
MCSR D1b	122.5	95.2	22.3	86.8	29.1	68.5	44.1	93.2
MCSR G2	179.8	169.8	5.6	132.0	26.6	99.6	44.6	145.3
MCSR N4	173.6	170.7	1.7	114.4	34.1	83.3	52.0	135.5
MCSR O2	152.3	142.4	6.5	90.9	40.3	70.5	53.7	114.1
MCSR T28	168.5	155.3	7.8	119.9	28.8	77.7	53.9	130.2
MCSR T30	200.4	174.3	13.0	130.1	35.1	86.0	57.1	147.7
MEAN	169.8	152.4	10.6	115.2	32.1	79.7	52.6	129.3

Means on shoot dry weights and shoot weight reduction (%) at $p \leq 0.05$

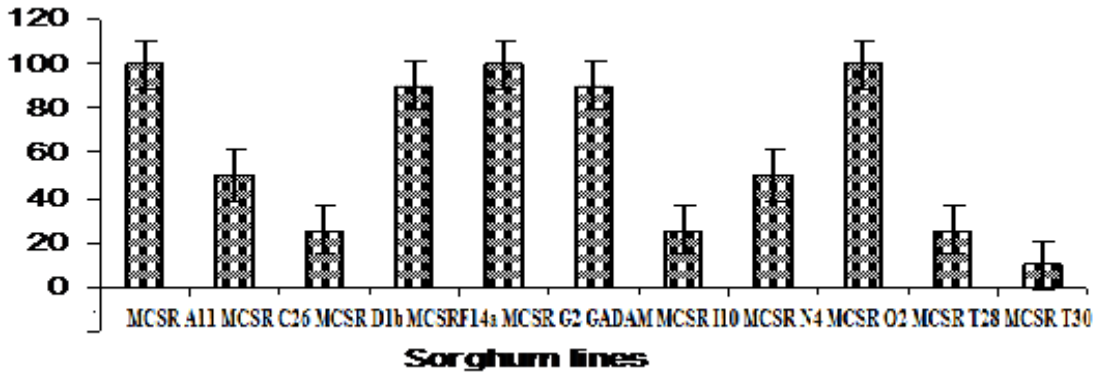


Figure 13: Effect of post-flowering water stress on leaf senescence in sorghum lines.

4.9.2 Effect of post-flowering water stress on yield in field grown sorghum.

The yields under post-flowering water stress were significantly different (p=0.05) among the sorghum lines (Figure 14). The sorghum lines which recorded the highest yields MCSR F14a, Gadam, MCSR T28 and MCSR A11; whereas the lines MCSR D1b, MCSR I1O, MCSR O2 and MCSR C26 recorded the lowest yield.

4.9.3 Grouping of the selected sorghum lines using field data.

The sorghum lines were grouped into four major clusters according to average linkage distance similarity (Figure 15). The cluster 1 consisted of MCSR G2, MCSR A11, MCSR O2 and MCSR F14a, cluster 2 consisted of Gadam. The two clusters consisted of lines which showed high leaf senescence but with higher grain yields.

The cluster 3 consisted of lines MCSR D1b, MCSR T28, MCSR I10 and MCSR C26, whereas cluster 4 consisted of MCSR N4. These two clusters consisted of lines which showed lower leaf senescence and low yields under the post flowering water stress in the field.

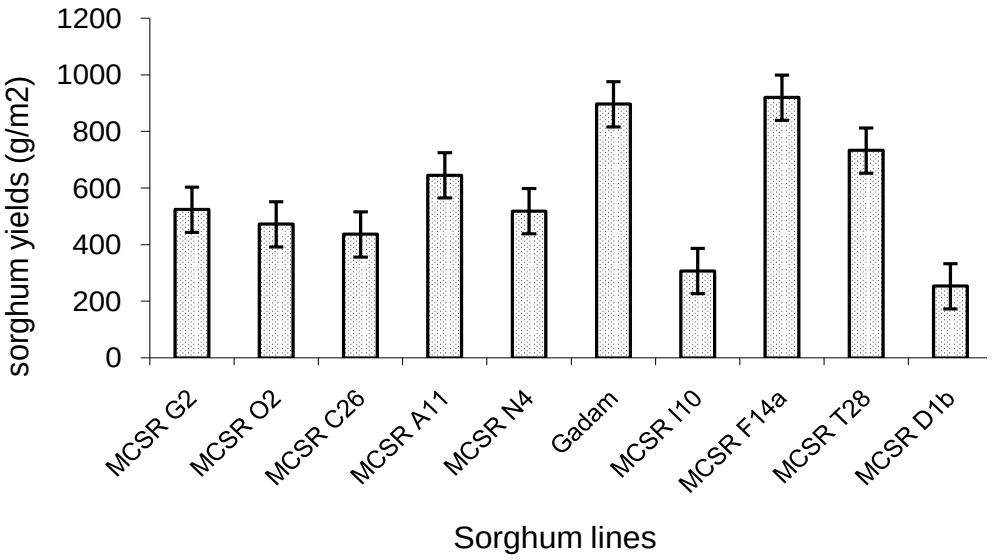


Figure 14: Effect of post-flowering water stress on sorghum yield (gm^{-2}).

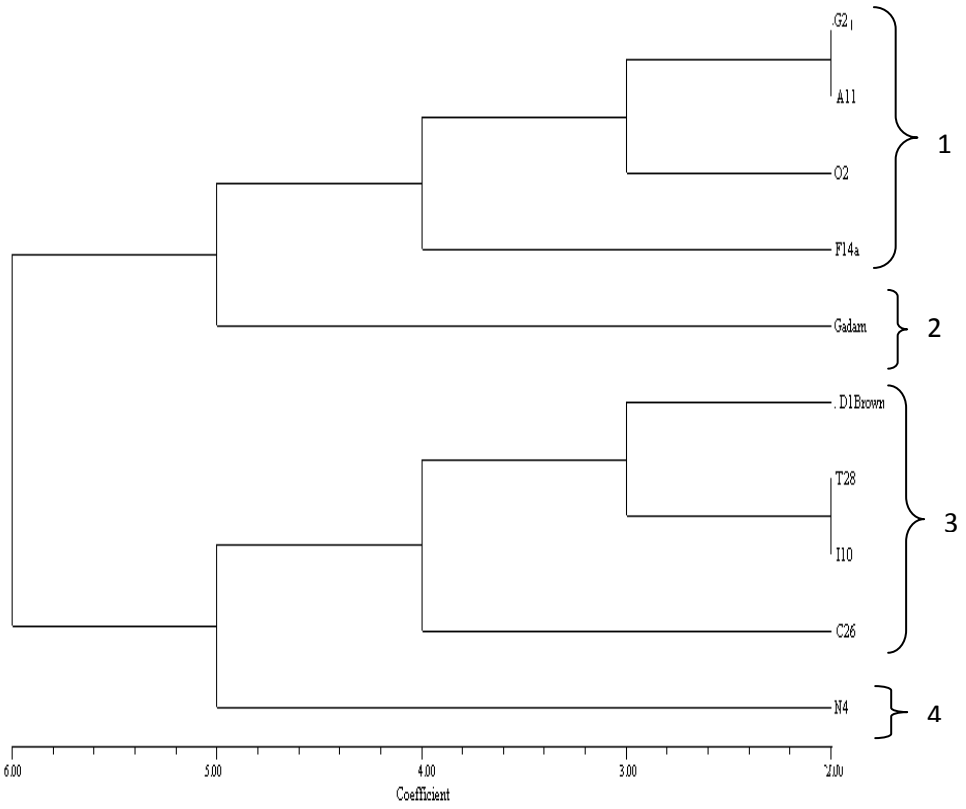


Figure 15: Dendogram grouping on selected sorghum relative to response to post-anthesis water stress.

CHAPTER FIVE

DISCUSSION

5.1 Seed imbibition rates under water stress.

Seed imbibition rates under water stress significantly differed among the selected sorghum lines. These results agree with that of Bogumila et al (2006). He showed that seed imbibition rates under water stress were significantly different. He also stated that, the difference in seed imbibition rates were genome dependent. It is then possible to select for water stress tolerance in sorghum at the seed imbibition stage. Good imbibition rates under water stress leads to high germination which contributes directly to yields in sorghum.

5.2 Germination of sorghum seeds under water stress.

The results on germination estimates showed variation among the selected sorghum lines under water stress in the laboratory. Seed germination decreasesd with increase in level of water stress. These results agree with that of Swagel et al (1997). More negative water potential make cells in the germinating seed to be lowered and this leads non-committance of a seed to germination because the critical hydration point is not reached subsequently leading to lower germination percentage.

The mean germination time under water stress was significantly different among the sorghum varieties. These results agree with that of Patane et al., (2008). Radhoune (2007) also reported variation in germination rates in pearl millet under osmotic stress; and that selection for high germination rates in the laboratory led to better crop field emergence and establishment.

Bijagare et al (1994) also reported germination percentage variations in seeds under water stress. Habibi et al., (2004) and McGrath et al., (2008) reported a possibility to select for water stress tolerance in the laboratory that possibly led to better germination, establishment and performance in the field. Hadas (1977) also stated that germination estimates in the laboratory correlated well with germination estimates under field conditions. It is then possible to select sorghum genotypes that carry tolerance to low moisture content in the field by selecting those with higher germination rates under moisture stress in the laboratory. The sorghum lines which attained maximum germination in a short length of time were MCSR T30 and Gadam.

Seedling radicle length was significantly different among sorghum lines under water stress. The sorghum lines with long embryonic roots access deep soil layers moisture of germination bed (Sima et al., 2009) that is necessary for emergence. Selecting for seedlings that have longer radicle would lead to better germination, establishment and good crop cover for better yields.

The sorghum lines which had longer radicle under water stress were MCSR T28, MCSR O2, MCSR T30, MCSR A11 and MCSR F14a.

Isozyme banding showed increase in activity of Peroxidase which agrees with those of Foyer and Noctor (2003). El-aref (2002) also reported increased activity of peroxidase in drought tolerant beans and wheat and that Peroxidase banding variations were used in selecting maize explants for drought tolerance. Therefore variations in acid phosphatase and peroxidase isozymes under water stress can be used to select sorghum varieties that carry water stress tolerance. The lines recommended for better isozyme activity under water stress included MCSR G2, MCSR C26 and MCSR A11.

5.2. Effect of water stress on growth of sorghum plants in the greenhouse.

The sorghum lines which showed normal growth under pre-flowering water stress were MCSR C1, MCSR G2 and MCSR T28. These lines were early to flower, of medium to short heights, low number of nodal tillers, high pigment concentration, low leaf senescence, longer panicles of good widths but low percent reduction in size and weights under pre-flowering water stress. The lines had near normal panicle development and grain filling after post flowering stage passed.

MCSR D1b would have been a good material for pre-flowering water stress tolerance since it is short in height with high chlorophyll concentration and low leaf senescence; however, it is late maturing with low panicle weights and low shoots dry weights. It uses the available water for vegetative growth which is soon depleted before it flowers. MCSR N4 is another line with good pre-flowering green leaf retention and good pigment concentration, low leaf size reduction under pre-flowering water stress, but is tall and late maturing making it to use available water for vegetative growth under the pre-flowering water stress. Pre-flowering drought tolerant sorghum germplasm have been reported by other authors (Tuinstra et al., 1998; Xu et al., 2000).Therefore, it is possible to select sorghum lines tolerant to water stress during pre-flowering stage.

The sorghum lines which might be useful in dry areas where rains may fail soon after sorghum germination will be MCSR C1, MCSR G2, MCSRT28, MCSR D1b and MCSR N4. These lines can also be used in breeding to improve drought tolerance in sorghum varieties

Post-flowering water stress caused significant increase in percentage of leaf senescence. These results were similar to those reported in maize by Laurer (2003).

In the greenhouse, leaf senescence progressed slowly over time and showed low leaf senescence in lines MCSR C1, MCSR N4, MCSR D1b and MCSR G2 under the post-flowering water stress. The sorghum lines which show low leaf senescence under the post-flowering water stress have been described as stay-green (Harris et al., 2007; Borrel et al., 2008). They have either reduced rate of normal senescence, delayed senescence or have high concentration of chlorophyll.

The sorghum lines which had higher total chlorophyll concentration under the pos-flowering stress included MCSR C1, MCSR D1b, MCSR G2, and MCSR N4. They also had higher carotenoid concentration under the post-flowering water stress as compared to the other lines. Ristick and Cass (1991) reported that some maize genotypes that were tolerant to water stress had lower degree of thylakoid degradation and pigment loss under water stress. Similar results were also reported by Margues da Silva and Arrabaca (2004). Sharma and Hall (1991) reported a decrease in carotenoids and chlorophyll pigment levels in sorghum under pre-flowering and post-flowering water stress. Campos (1998) also reported significant decrease in chlorophyll a/b ratios in *Vigna* under severe water stress. The reduction in carotenoids and chlorophyll concentration under the post-flowering stress is linked to a decrease in synthesis and/ or an increase in degradation of carotenoids (Neto et al., 2009) which protect chlorophyll from photo oxidation. It is then possible to select sorghum lines with high pigment concentration which could possibly confer drought tolerance in post-flowering growth stage.

Panicle weight in MCSR N4 was low because this sorghum line have tall plants and are late maturing and uses up the available water to develop vegetative parts like leaves and stem at expense of grain filling. MCSR T30 is also important because it has high shoot dry weight under the post-flowering water stress with medium pigment concentration. It can be useful in breeding programs to introgress the stay-green trait into high yielding varieties.

Under post-flowering water stress in the field, the sorghum lines which showed low leaf senescence included MCSR T30, MCSR T28, MCSR I10 and MCSR D1b. The lines which had higher yields under field post flowering conditions included MCSR F14a and Gadam. These lines were early maturing, and therefore used the available moisture early in the season before it was depleted. MCSR N4 and MCSR I10 are tall plants and also late maturing. Such plants have been reported to use up water to add more height and number of leaves and water become depleted from the soil before they complete grain filling (Saxena and O'Toole, 2002).

The clustering analysis placed the sorghum lines into four clusters. Cluster 1 constituted MCSR G2, MCSR A11, MCSR O2, and MCSR F14a; and cluster 2 consisted of MCSR Gadam. These lines had leaf senescence of more than 50 % and had fairly higher yields. The cluster 3 was made up of the lines MCSR D1b, MCSRT28, MCSR I10 MCSR C26 and cluster 4 consisted of MCSR N4. These lines had leaf senescence of $\leq$ 50% under the post-flowering water stress indicating that they were stay green sorghum lines.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions.

The sorghum varieties which showed good imbibition rates and germination included MCSR A11, Gadam and MCSR O2. The sorghum lines which were identified as early maturing included MCSR G2, MCSR T28, MCSR C1 and Gadam. The sorghum lines which recorded the lowest panicle weight reduction under pre-flowering water stress included MCSR N4, MCSR G2 and MCSR T28. The post-flowering drought tolerant lines which showed low percentage leaf senescence, high pigment concentration and high grain yields included MCSR T30, MCSR C1, MCSR T28, MCSR N4, MCSR I10, MCSR N4, and MCSR D1b. These lines were also stay-green.

The identified drought tolerant grain sorghum lines can be used in breeding for drought tolerant high yielding varieties includes MCSR C1, MCSR T30 and MCSR D1b. The lines which can be adopted for drier areas include MCSR T28, MCSR G2, Gadam, MCSR N4 and MCSR F14a. These will improve food production in the dry areas and improve food security in the country.

6.2 Recommendations.

The identified lines need to be tested extensively in dry areas to verify their production. There is a need to carry out molecular research in drought tolerance along with the use of morpho-physiological and chemical methods.

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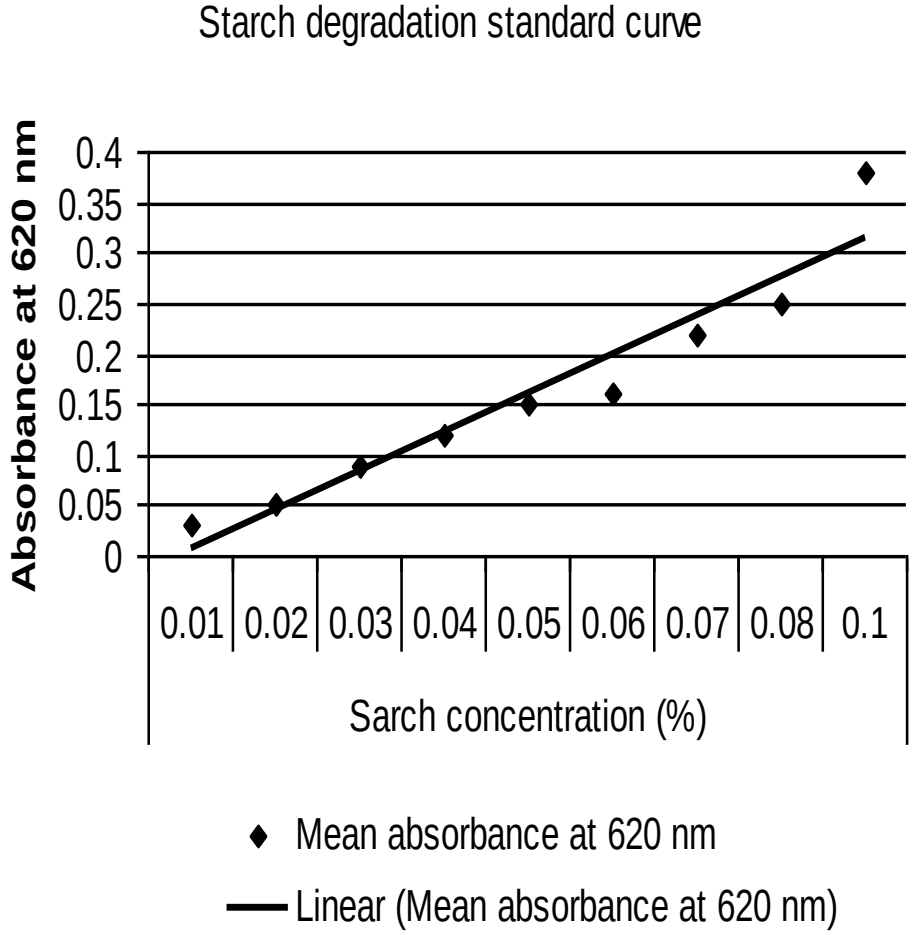
APPENDICES

Appendix I: starch degradation standard curve



Appendix II: ANOVA on seed imbibition rates (water (g) / unit seed weight (g) / hour) in sorghum

Source of variation	d.f.	s.s.	m.s.	F
Treatment	3	0.008	0.003	
Genotype	10	0.104	0.010	< 0.001
Time	5	1.156	0.231	< 0.001
Genotype. Treatment	30	0.029	0.001	< 0.001
Genotype. Time	50	0.085	0.002	< 0.001
Treatment. Time	15	0.047	0.003	< 0.001
Genotype.Treatment.Time	150	0.105	0.001	< 0.001

Analysis of variance on sorghum seed imbibition rates at p=0.05

Appendix III: ANOVA on percentage seed germination

Source of variation	d.f.	s.s.	m.s.	F pr.
Treatment	3	0.08007	0.02669	
Block.Replications	8	0.24155	0.03019	
Genotype	10	11.30251	1.13025	<.001
Genotype.Treatment	30	1.30356	0.04345	0.224
Residual	80	2.80836	0.0351	
Total	131	15.73605		

Appendix IV: ANOVA on time taken to total germination

Source of variation	d.f.	s.s.	m.s.	F
Genotype	10	0.3764	0.03764	0.15
Treatment	3	0.01697	0.00566	0.878
Genotype*treatment	30	0.3797	0.01266	0.981
Residual	88	2.19732	0.02497	
Total	131	2.97039		

Appendix V: ANOVA on seedling radicle lengths under water stress.

Source of variation	d.f.	s.s.	m.s.	F
Genotype. Treatment	33	219.0904	6.6391	
Block .Replication	56	11.4973	1.991	
Genotype	10	716.2238	71.6224	< .001
Genotype .Treatment	33	496.9959	15.0605	< .001
Residual	557(1)	451.9575	0.8114	
Total	659 (1)	1994.016		

Appendix VI: ANOVA on starch concentration (%)/seed weight (g) sorghum lines.

source of variation	d.f.	s.s.	m.s.	F
Treatment	3	2.1872	0.7291	
Block.Replication	12	1.5248	0.1271	
Genotype	10	4.4972	0.4497	0.003
Genotype.Treatment	30	6.6037	0.2201	0.099
Residual	120	18.7043	0.1559	
Total	175	33.5172		

Appendix VII: ANOVA on days to panicle emergence in sorghum plant.

source of variation	d.f	s.s	m.s.	F
Treatment	3	1214.19	404.73	
Block.replication	16	1023.14	63.95	
Genotype	7	8197.32	1171.05	< .001
Genotype.treatment	21	2019.47	96.17	< .001
Residual	111(1)	3224.24	29.05	
Total	158(1)	15458.43		

Appendix VIII: ANOVA on plant height (cm) at maturity

Source of variation	d.f	s.s.	m.s.	v.r.	F
Treatment	3	61206.3	20402.1		
Block.Replication	16	5623.2	351.5	0.95	
Genotype	7	60351.8	8621.7	23.36	< .001
Genotype.Treatment	21	11709.4	557.6	1.51	0.088
Residual	111(1)	40969.3	369.1		
Total	158(1)	179775			

Appendix IX: ANOVA on third leaf (from the tip) length reduction in sorghum

Source of variation	d.f	s.s	ms	F
Treatment	3	928.28	309.43	
Block.Replication	16	1102.29	68.89	
Genotype	7	9047.61	1292.52	< 0.001
Genotype.Treatment	21	2028.23	96.58	0.146
Residual	111(1)	7789.75	70.18	
Total	158(1)	20687.42		

Appendix X: ANOVA on third leaf width

source of variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	19.097	6.3657		
block.replication	16	12.8596	0.8037	1.11	
Genotype	7	16.766	2.395	3.31	0.003
genotype.treatment	21	13.0493	0.6214	0.86	0.644
Residual	111(1)	80.4029	0.7244		
Total	158(1)	142.0873			

Appendix XI: ANOVA on number of nodal tillers in sorghum.

Source of variation	d.f	s.s	m.s.	v.r.	F
Treatment	3	136.136	45.379		
Block.Replication	16	25.016	1.564	1.21	
Genotype	7	116.075	16.582	12.87	< .001
Genotype.Treatment	21	43.775	2.085	1.62	0.048
Residual	111(1)	142.999	1.288		
Total	158(1)	462.767			

Appendix XII: ANOVA on chlorophyll a concentration in sorghum flag leaf.

Source Of Variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	846.358	282.119		
Block .Replication	8	64.913	8.114	1.11	
Genotype	7	434.672	62.096	8.50	< .001
Genotype. Treatment	21	839.786	39.990	5.47	< .001
Residual	56	409.040	7.304		
Total	95	2594.769			

Appendix XIII: ANOVA on chlorophyll b content in sorghum flag leaf.

Source of variation	d.f	s.s.	m.s.	v.r.	F
Treatment	3	99.62	33.21		
Block.Replication	8	11.88	1.48	0.69	
Genotype	7	37.34	5.34	2.48	0.027
Genotype.Treatment	21	78.23	3.73	1.73	0.045
Residual	56	120.45	2.15		
Total	95	347.52			

Appendix XIV: ANOVA on chlorophyll a:b ratio in sorghum flag leaf

Source of variation	d.f.	s.s	m.s.	v.r.	F
Treatment	3	1.01	0.34		
Block.Replication	8	3.03	0.38	0.92	
Genotype	7	3.44	0.49	1.20	0.32
Genotype.Treatment	21	12.37	0.59	1.44	0.14
Residual	56	22.97	0.41		
Total	95	42.82			

Appendix XV: ANOVA on total chlorophyll concentration in sorghum flag leaf.

Source of variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	1947.44	649.15		
Block.Replication	8	121.93	15.24	0.97	
Genotype	7	921.41	131.63	8.34	< .001
Genotype.Treatment	21	1391.12	66.24	4.2	< .001
Residual	56	883.39	15.77		
Total	95	5265.3			

Appendix XVI: ANOVA on carotenoids concentration (µg cm^{-2}) in sorghum flag leaf.

Source of variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	829.33	276.44		
Block.Replication	8	393.87	49.23	1.09	
Genotype	7	434.37	62.05	1.37	0.235
Genotype.Treatment	21	1100.00	52.38	1.16	0.321
Residual	56	22531.05	45.2		
Total	95	5288.61			

Appendix XVII: ANOVA on panicle lengths in sorghum lines

Source of variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	514.761	171.587		
Block.replication	16	95.369	5.961	0.94	
Genotype	7	2041.802	291.686	46.07	< .001
genotype.treatment	21	301.225	14.344	2.27	0.003
Residual	112	709.083	6.331		
Total	159	3662.24			

Appendix XVII: ANOVA on panicle width in sorghum

Source of variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	360.105	120.035		
Block.Replication	16	36.225	2.264	2.12	
Genotype	7	204.073	29.153	27.26	< .001
Genotype.Treatment	21	71.558	3.408	3.19	< .001
Residual	112	119.775	1.069		
Total	159	791.736			

Appendix VVIII: ANOVA on dry panicle weights in sorghum

Source of variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	51967.2	17322.4		
Block.Replication	16	14017.8	876.1	3.7	
Genotype	7	43415.5	6202.2	26.23	< .001
Genotype.Treatment	21	16321.5	777.2	3.29	< .001
Residual	112	26486.2	236.5		
Total	159	152208.2			

Appendix XIX: ANOVA on shoot dry weights in sorghum

source of variation	d.f.	s.s.	m.s.	v.r.	F
Treatment	3	193484.6	64494.9		
Block.Replication	16	8295.7	518.5	0.92	
Genotype	7	44317	63310	11.21	< .001
Genotype.Treatment	21	19059.7	907.6	1.61	0.04
Residual	112	63225.8	564.5		
Total	159	328382.7			

Appendix XX: watering regime in the greenhouse