PHYSIOLOGICAL AND BIOLOGICAL RESPONSES OF *Sorghum bicolor* [L.] Moench. TO DROUGHT STRESS CONDITION.

BY

DAVID BAMIDELE SAMUEL

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CERTIFICATION

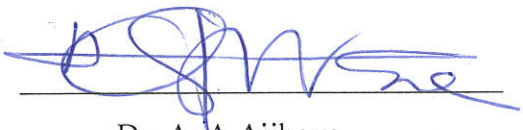
This is to certify that this final year project was carried out by David Bamidele Samuel with the matriculation number, PSB/14/2358, in the department of Plant Science and Biotechnology, Federal University Oye – Ekiti.

Mr. Iwuala E. N.

Supervisor

10/12/18

Date



Dr. A. A Ajiboye

Head of Department

19/03/19

Date

DEDICATION

This project is dedicated to Elohim whose amazing grace and mercy has brought me thus far in life. I would also dedicate this to my families Mr. and Mrs. David John and Mr. and Mrs. Oluwafemi Akintunde for their love and support.

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ABSTRACT

This study was conducted to ascertain drought tolerance in sorghum genotypes. Seedlings of locally cultivated sorghum were screened for drought tolerance by assessing percentage relative water content (RWC) after progressive water deficit. Plant biomass, root/shoot ratio, tolerance index, relative water content (RWC) were measured during harvest. Malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), chlorophyll and proline content were quantified. The four genotypes (DTSYN 11, EC – 3161, RJ – 2020 and BPCH – 6) recorded high RWC after subsequent evaluation for their physiological response to severe (10 days) water stress treatment. All four genotypes maintained RWC above 80% during severe stress treatment. MDA for EC – 3161 was 5% higher and BPCH – 6 were recorded within 5% less in comparison to their well – watered controls. while GSH, SOD, CAT had a significant difference in RJ – 2020, BPCH – 6 and DTSYN 11. Significant higher chlorophyll content was recorded for both RJ – 2020 and EC – 3161 during severe stress. The proline content significantly increased by 14 – fold and 16 – fold in RJ – 2020 and EC – 3161 respectively. In this study, RJ – 2020 and EC – 3161 of sorghum genotypes exhibited drought tolerance and these genotypes could be used for application in breeding program.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Drought stress is one of the most critical stress affecting plants. Drought can be defined in multiple ways, be it meteorological, hydrological, or socioeconomical context. When drought is defined in relation to crops or agriculture, it refers to shortage of water in the root zone that reduces yield. Under the climatic changing context, drought has been and is becoming one most sensitive problem constraining plant growth, terrestrial ecosystem productivity and in many regions and countries all over the world particularly in the arid and semi – arid areas (Ceccarelli *et al.*, 2004.).

Due to assessment, global surface average temperature will have increased in range from 1.1°C – 6.4°C by the end of this century which indicates warming above 3°C would eliminate thoroughly fixed carbon function of global terrestrial vegetation, shift a net carbon source. It is expected that with global warming water deficit would be escalated by increasing evapotranspiration, increasing the frequency and intensity of drought with an increase from 1% to 30% in extreme drought land area by 2100; which would offset the beneficial effect from the elevated CO_2 concentration thus limiting the structure and function of the terrestrial ecosystem (IIPC, 2007).

The yield potential of sorghum is evident from the fact that production of sorghum has been maintained despite a steady decline in its area of cultivation. In fact, the true yield potential of sorghum has rarely been realized, as it is grown mainly in areas of low rainfall and resource-poor agronomic conditions. Its ability to yield under such agronomic and adverse climate conditions is a proof of concept that sorghum is the crop of the future.

In the evolving global sequence of event, the world population is anticipated to rise from present 6.6 billion to 8.7–11.3 billion in 2050. The global demand for cereal production will also

increase by 60%. This task is challenging as the yield potential of cereal crops has reached its plateau, and there is reduction in cultivable land and availability of fresh water for irrigation. These problems are further exacerbated by global climate change – associated increase in the frequency of heat stress, droughts, and floods that negatively affect crop yields. Ability of crops to adapt and yield under such harsh environment will be crucial in determining the sustainability of food production in days to come. This will require a combination of adaptive agricultural strategy that includes new management and agronomic practices and further improvement in the genetic potential of productivity and abiotic stress resistance of crops. This also implies that lessons need be learned from plants that show high adaptability and tolerance to abiotic stresses.

Drought can occur at seedling, pre- flowering and post-flowering stages of development, and has the most adverse effect on yield. Drought stress at the seedling stage of development will severely affect plant establishment. If it occurs at flowering, or in the grain filling stages, it may cause reduced yields, or complete crop failure (Tesfamichael Abraha *et al.*, 2015). Researchers have classified drought as either pre- or post- flowering stress. The reactions of genotypes to these stresses are variable and controlled by different genetic mechanisms. Pre-anthesis moisture stress has effects on yield components such as stand count, tillering capacity, number of heads and number of seeds per head, while post-anthesis moisture stress affects transpiration efficiency, CO_2 fixation and carbohydrate translocation. The latter factors, in turn, results in low yields and premature plant senescence.

Plants deal with stress in three different ways, namely: escape, dehydration avoidance, and dehydration tolerance. Drought escape is defined as the ability of a plant to complete its life cycle before severe soil and plant water deficit develops. Escape mechanism involves rapid phenological development (early flowering and early maturity) and developmental plasticity

(variation in duration of growth period depending on the extent of water deficit). Dehydration avoidance is defined as the ability of plants to sustain high plant water status or cellular hydration under drought conditions. Crop plants avoid dehydration by augmenting seizure of soil moisture, by efficient root system and osmotic adjustment (OA), by limited crop water loss from transpiration and other non – stomata pathways such as through the plant cuticle, reduced absorption of radiation by radiation reflection, and leaf rolling/folding or drying. Dehydration tolerance is defined as the capacity to sustain or conserve plant function even in relatively low tissue water potential. Cellular water deficit stress tolerance in plants depends on modification of metabolism, production of organic compatible solutes (such as proline, sugars, polyols, betaine, etc.), and expression of genes involved in membrane integrity, cellular homeostasis (ionic-, osmotic-, and meta – bolic homeostasis), stress damage control, and repair.

Plants experience water stress either when the water supply to their roots becomes limiting or when the transpiration rate becomes intense. Water stress is primarily caused by the water deficit, i.e. drought. Drought stress tolerance is seen in almost all plants but its extent varies from species to species and even within species. Water deficit and salt stresses are global issues to ensure survival of agricultural crops and sustainable food production (Jaleel *et al.*, 2007). Tolerance to abiotic stresses is very complex, due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Razmjoo *et al.*, 2008).

Drought stress is considered to be a moderate loss of water, which leads to stomata closure and limitations of gas exchange. Desiccation is much more extensive loss of water, which can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme catalyzed reactions (Smirnoff, 1993; Jaleel *et al.*, 2007). Drought stress is

characterized by reduction of water content, diminished leaf water potential, turgor loss, closure of stomata decreases in cell enlargement and growth. Water stress inhibits cell enlargement more than cell division. It reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al.*, 2008; Farooq *et al.*, 2008). In plants, a better understanding of the morpho – anatomical and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions. The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and developmental stage (Chaves *et al.*, 2002; Jaleel *et al.*, 2008). The drought stress factors negatively affect growth and productivity, and plants have evolved different mechanisms to respond to such challenges. At the molecular level this involves induction of stress-responsive and stress-tolerance genes (Matsui *et al.*, 2008), often mediated by the phyto – hormone abscisic acid (ABA). ABA is referred to as the plant stress hormone because, in addition to its role in development, it plays a key role in responses to abiotic stress factors by regulating stomata closure to optimize transpiration, and by triggering the activation of many stress-related genes (Cutler *et al.*, 2010; Lindemose *et al.*, 2013).

It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. Drought stress affects cell elongation, cell expansion, and cell growth (Anjum *et al.,* 2003; Bhatt and Srinivasarao, 2005 Kusaka *et al.*, 2005; Shao *et al.,* 2008). In soybean, the shoot length was decreased under water deficit conditions (Specht *et al.,* 2001). The plant height was reduced up to 25% in water stressed citrus seedlings (Wu *et al.*, 2008). Stem length was significantly affected under water stress in potato (Heuer and Nadler, 1995) *Abelmoschus esculentus* (Sankar *et al.*, 2007) *Vigna unguiculata* (Manivannan *et al.*, 2007);

soybean (Zhang *et al.*, 2004) and *Petroselinum crispum* (parsley) (Petropoulos *et al.*, 2008). The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence in *A. esculentus* under water stress (Bhatt and Srinivasarao, 2005). Development of optimal leaf area is important to photosynthesis and dry matter yield. Water deficit stress resulted reduced leaf growth and leaf areas in many species of plant like Populus (Wullschleger *et al.*, 2005), soybean (Zhang *et al.*, 2004) and many other species (Farooq *et al.*, 2009). Significant inter-specific differences between two sympatric Populus species were found in total number of leaves, total leaf area and total leaf biomass under drought stress (Wullschleger *et al.*, 2005). The leaf growth was more sensitive to water stress in wheat than in maize (Sacks *et al.*, 1997) *Vigna unguiculata* (Manivannan *et al.*, 2007) and sunflower (Manivannan *et al.*, 2007).

The importance of root systems in acquiring water has long been recognized. The development of root system increases the water uptake and maintains requisite osmotic pressure through higher proline levels in *Phoenix dactylifera* (Djibril *et al.*, 2005). An increased root growth due to water stress was reported in sunflower (Tahir *et al.*, 2002) and *Catharanthus roseus* (Jaleel *et al.*, 2008).

Greater plant fresh and dry weights under water limited conditions are desirable characters. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al.*, 2009). Plant productivity under drought stress is strongly related to the processes of dry matter partitioning and temporal biomass distribution (Kage *et al.*, 2004). Diminished biomass due to water stress was observed in genotypes of sunflower (Tahir and Mehid, 2001). However, some genotypes showed better stress tolerance than the others. Mild water stress affected the shoot dry weight, while shoot dry weight was greater than root dry

weight loss under severe stress in sugar beet genotypes (Mohammadian *et al.*, 2005). Reduced biomass was seen in water stressed soybean (Specht *et al.*, 2001).

The interaction and adaptation of plants to environmental signals and stresses is complex and need to be analyzed in a network model (Pereira, 2007). The water stress occurs frequently and affects most plant habitats. Plants have developed several strategies to cope with this challenge. These strategies are either adaptation mechanisms which allow them to survive the adverse conditions or specific growth habits to avoid stress conditions (Zhu, 2001). Plants respond to environmental stresses such as drought, excessive salinity and low temperature through a wide variety of biochemical and physiological adaptive changes such as the accumulation of compatible solutes and synthesis of many regulatory proteins (Gong *et al.*, 2005).

1.1 OBJECTIVES

The overall objective of the present study is as follows;

- Responses of Sorghum bicolor [L.] Moench. Genotypes under stimulated drought stress condition.

However, to aid the achievement of the overall objective it is broken down further and they are;

- To compare the responses of sorghum genotypes to water deficit condition
- To study the physiological response of sorghum genotypes for limited water condition
- To establish the extent to which different regulatory responses in sorghum genotypes occurs as compared to DTSYN 11 a known drought tolerant line.

1.2 EXPERIMENTAL PLANT

1.2.1 **Sorghum** (*Sorghum bicolor* [L.] Moench.)

Sorghum of the family Poaceae and subfamily Panicoideae shares the tribe Andropogoneae with other major crops such as maize, sugarcane and millets. The Andropogoneae species are native to tropical and subtropical climates, and are characterized by C4 photosynthesis, high rates of carbon fixation, high water and nutrient use efficiency, high biomass productivity, adaptation to diverse environments, and have both annual and perennial life cycles (Monica *et al.*, 2007). However, many of these species are polyploids with large complex genomes. Sorghum, besides having all the advantageous characteristics, has a diploid genome that is already sequenced. Moreover, with its well-studied genetics, wide germplasm resource, lower level of gene duplication compared to other tropical cereals and amicability for genetic transformation, sorghum can be an ideal system particularly for grasses and plant genomics research as a whole (Messing and Rokhsar, 2009). Sorghum is the fifth most important cereal crop in the world after wheat, rice, maize, and barley. Known for its ability to survive harsh environments with prolonged drought period where other cereal crops cannot be grown successfully, sorghum is grown in arid and semiarid areas of the world. It is a staple food in parts of Africa, especially in Sudan and Asia and a major feed crop in the United States, Mexico, Australia, and South Africa (Krupa *et al.*, 2017). It has extensive variability such as grain sorghum, forage sorghum, and sweet stalk sorghum that provides food, feed, fodder, fiber, and fuel. Sorghum is produced by about 104 countries in the world. In 2009, Sorghum was grown on 43.74 million hectare of land worldwide with a yield of 14198 $Hgha^{-1}$ (FAO, 2009). Average area under sorghum cultivation in Asia has declined from 26.19 million hectare of land in the 1960s to 10.58 million hectares in 2008. However, yield increased from 6935 $Hgha^{-1}$ in the 1960s to 10377 Hg ha^{-1} in the late 2000s (FAO, 2009).

1.2.2 Taxonomic Classification of Sorghum

Kingdom – Plantae

Superdivision – Spermatophyta

Division – Magnoliophyta

Class – Liliopsida

Order – Poales

Family – Poaceae

Genus – Sorghum

Species – *Sorghum bicolor* (L.) Moench.

About 50% of the total area for the cultivation of cereal crops in Nigeria is occupied by sorghum locally known as guinea corn. The area is estimated at 6.86 million hectares extending north - wards from latitude 8^0N to latitude 14^0N (Aba, *et al.,* 2004). In 1978, the total production of sorghum in Nigeria was estimated at 4.8 million tonnes (Obilana, 1981). However, this estimate has risen to about 7.0 million tonnes annually (Obilana, 2005). Consequently, making Nigeria the highest sorghum producer in the West African sub-region, accounting for 71% of the regional total sorghum output. Although, the crop is environmentally-friendly as it is water-efficient, requires little or no fertilizers or pesticides and is biodegradable. Globally also, the country leads in sorghum production for human consumption and has risen from its fifth position in 1995 (FAO, 1995) to be the third largest producer of sorghum in the world after the USA and India where more than 90% of their sorghum harvest is used for animal feed (Obilana, 2005). Sorghum is adapted to a wide range of environmental conditions, particularly, drought. Hence, it is widely grown in different ecological zones of Nigeria (Showemimo *et al.*, 2000). It has a number of morphological and physiological characteristics that contribute to its adaptation to dry conditions. These include: an extensive root system, waxy bloom on the leaves that reduces

water loss, ability to stop growth in periods of drought and resume when conditions are favourable as well as tolerance to waterlogging (FAO, 1995). The crop equally grows on a wide range of soils: sand, loam, sandy loam, saline and alkaline soils with a pH range of 4.0 – 8.5 (Aba *et al.*, 2004).

The most common landraces of sorghum in Nigeria are: Kaura, Farafara and Guinea (Curtis,1967). They are variously tolerant to striga (a parasitic weed) in all the savanna zones and are agronomically alike. Sorghum improvement by breeding started in Nigeria in 1956, however, years of selection at the Institute of Agricultural Research (IAR), Samaru, Nigeria, have resulted in the development and release of sorghum varieties suited to specific ecological zones (Aba *et al.*, 2004).

Sorghum is the most amenable cereal grain to different processing technologies including: primary, secondary and tertiary methods (Obilana, 2005). Primary processing involves: fermentation, malting, wet & dry milling, boiling, roasting and popping. Secondary processing involves; brewing, beverage & drinks production, baking and confectionery making, steaming, extrusion (for pastes & noodles), while tertiary processing involves: composite flours (mixing of cereal/cereal flours, cereal/legume flours, cereal/cassava flours), bio – fortification and chemical fortification with additives. The different processing levels and their technologies are achieved using different agro-industrial equipment and machinery. These result in diversified end-products for foods, feeds, beverages, alcoholic and nonalcoholic drinks. Relative to other cereal grains, sorghum is the most widely cultivated and most widely utilized food and industrial raw material in Nigeria. The significant increases recorded in some of its malting and brewing properties using cysteine hydrochloride (Cyst.HCl) as extractant suggests that other such properties could equally be higher than those of barley malt if proper extraction procedures are adopted (Ogbonna, 2002). Sorghum is mainly eaten in the form of flour or paste. It has a high

caloric and nutritional value and is therefore recommended for infants, pregnant and lactating mothers, the elderly and the convalescents (Obilana, 2005).

The uses of sorghum in Nigeria can be grouped into two: traditional and industrial. The traditional uses include a variety of traditional foods, beverages and drinks while its non-food traditional uses include: thatching of roofs and fencing of compounds. Sorghum consumption for food is mainly in the form of flour or paste processed into two main dishes: "OGI" or "AKAMU", a thin porridge and "TUWO", a thick porridge. Other dishes that are sometimes made from sorghum include a number of deep fried snacks, steamed dumplings, etc. (Obilana, 1981). Of all the cereal crops, sorghum contributes about 50% of the calories in Nigeria generally and about 73% in the savanna regions of the country in particular (Simons, 1976). Sorghum foods are also high in minerals, vitamins and some essential amino acids which are further enhanced through bio – fortification thus, making them superior to other cereal foods. They contribute more energy and digestible protein in the diets of the majority of the people in the sub – Saharan regions than those obtained from root and tuber crops (Aba et al, 2004). In addition, its polyphenol (mostly tannin) contents are used as antioxidants just as the slow digestibility of sorghum starch and protein makes its foods useful in diabetic treatments.

CHAPTER TWO

2.0 MATERIALS AND METHOD

2.1 METHOD

Seeds of sorghum were purchased from Obalende local market, Obalende, Lagos in a single batch enough for the study. The genotypes (DTSYN 11, EC – 3161, RJ – 2020 and BPCH – 6) were sown in plastic bags of equal diameter containing sandy-loamy soil (1.5kg) to achieve three (3) seeds per bag in the screen house of Federal University Oye – Ekiti. The average temperature for Ekiti State at this time (August – October, 2018) was 28°C ± 4°C. After germination, the seedlings were thinned out to one (1) seedling per nursery bag of equal height (10cm) and were arranged in a randomized block design. Seedlings were watered and allowed to grow for two (2) weeks. Plants were grouped into two (2) categories, each representing a treatment and replicated three (3) times. Category one (1) served as the control and received 100ml of water every two (2) days throughout the experimental period, while category two (2) received 100ml of water every (2) days for six (6) weeks before subjecting them to ten (10) days of stimulated drought. The experiment lasted for twelve (12) weeks to vegetative state, and physiological parameters were taken.

The plants were harvested from the screen house and the following parameters were measured;

- Biomass determination
- Chlorophyll quantification
- Relative water content of the leaves
- Determination of lipid peroxidation
- Enzyme extraction and assays

2.2 BIOMASS DETERMINATION

Plants were uprooted carefully and washed thoroughly in a running tap water to remove soil particles. After rinsing with distilled water, they were placed in labelled paper bags and oven dried for 72hrs. The dried samples were weighed using a digital top loading weighing balance (Mettler AE 100) to determine the dry weight. Plants were also partitioned into root and shoot and their dry weight determined to evaluate root/shoot ratio (Guo *et al.*, 2010).

2.3 CHLOROPHYLL QUANTIFICATION

The extraction and estimation of chlorophyll content was done according to the method of Maclachlam and Zalik (1963). 3.0g of fresh leaves from the two treatments was grounded differently with mortar and pestle with little quantity of Sodium Potassium trioxocarbonate IV (Na_2CO_3) to keep the chlorophyll structure. Extraction was done with 25ml of 80% acetone (20% distil water + 100% acetone) and filtered through filter paper. Filtrates were centrifuged at 15000r/min for 20 minutes and the supernatant was used for spectrophotometer readings at 645nm and 633nm wavelength.

$$C_a = \frac{(12.3D663 - 0.86D645)V}{d \times 1000 \times W}$$

$$C_b = \frac{(19.3D645 - 3.6D663)V}{d \times 1000 \times W}$$

Where

C = concentration in mg/g fresh weight

a = chlorophyll a

b = chlorophyll b

D = optical density at wavelength indicated

V = volume of extract in ml

d = length of path in cm (breadth of the transparent part of the spectrophotometer cuvette)

W = fresh weight of leaves in grams

The total chlorophyll content = value of chlorophyll a + chlorophyll b

2.4 RELATIVE WATER CONTENT OF THE LEAVES

Weight of fresh leaves were taken and soaked in water for turbidity weight (absorb water into the leave) for 24 hours and thereafter oven dried. Dried weight was measured with the weighing balance; the relative water content was calculated as follows according to the method of Turner (1981):

$$\frac{Fresh\ weight - dry\ weight}{Turbidity\ weight - dry\ weight} \times \frac{100}{1}$$

2.5 DETERMINATION OF LIPID PEROXIDATION

Lipid peroxidation was estimated by measuring the formation of Malondialdehyde (MDA) with thiobarbituric acid (TBA), as described by Ali *et al.* (1995). The leaves and the roots were homogenized in 1% (w/v) trichloroacetic acid (TCA). After centrifugation, to 1ml of supernatant, 4 ml of 20% TCA containing 0.5% (w/v) TBA was added. The mixture was incubated at 95° C for 30 min. The absorbance was measured at 532 nm and 600nm and the concentration of MDA was calculated by using the 120 extinction coefficient of 155 mM per cm.

2.6 ENZYME EXTRACTION AND ASSAYS

Catalase

Catalase (CAT) activity was measured according to Aebi (1984). The assay mixture (3.0 ml) consisted of 100µl enzyme extract, 100µl H_2O_2 (300mM) and 2.8 ml 50mM phosphate buffer

with 2mM EDTA (pH 7.0). CAT activity was assayed by monitoring the decrease in the absorbance at 240nm as a consequence of H_2O_2 disappearance.

Superoxide dismutase

Superoxide dismutase (SOD) activity was determined by the method of Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm.

Glutathione content

The glutathione content (GSH) was estimated following the method of Anderson (1985). Fresh plant samples (0.3 g) were homogenized in 3 cm^3 of 5 % (m/v) sulfosalicylic acid at 4 °C. The homogenate was centrifuged at 10 000 *g* for 10 min. To 0.5 cm^3 of the supernatant, 0.5 cm^3 of a 0.1 M reaction buffer [0.5 mM Na_2EDTA and 50 mm^3 of 3 mM 5′dithio-bis-(2-nitrobenzoic acid); pH 7.0] was added. After 5 min, absorbance was read at 412 nm.

Proline

For calibration curve, stock solution of pure proline (0.1mg/1ml) was prepared and its serial dilutions were used. The content was expressed as mg Proline/g fresh weight and calculated according to formula by (Bates *et al.*, 1973):

Proline (mg.g−1 FW) = [(µg proline/ml × 4 ml toluene) / (0.5 g sample/2.5)] / 1000

2.7 STATISTICAL ANALYSIS

All statistical analyses were performed using one way analysis of variance, where all standard deviations and standard errors were calculated.

CHAPTER THREE

RESULTS

3.1 GROWTH PARAMETERS

3.1.1 Root/Shoot Weight Ratio and Tolerance Index (TI)

The results (Figure 1) showed an enhancement in the tolerance index as the drought stress increased. RJ – 2020 recorded the highest TI value than DTSYN 11, EC – 3161, BPCH – 6 at 10 days of drought stress respectively but the least TI value was recorded by DTSYN 11. Overall, it was shown that RJ – 2020 exhibited more drought tolerant than the susceptible genotype. The biomass root/shoot ratio in DTSYN 11 was significantly reduced for plant under drought stress in contrast to well – watered ones while RJ – 2020 genotype showed a significant ($P < 0.05$) increase in R/S ratio under drought condition was statistically significant at 10 days of stress.

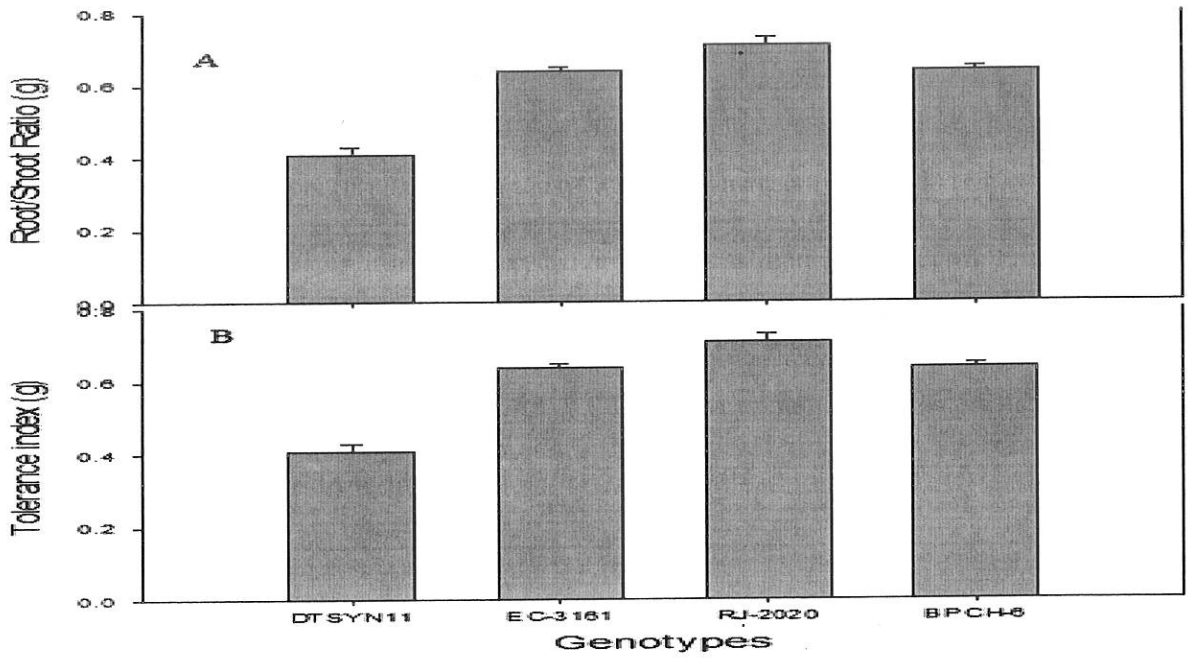

Figure 1: Effects of drought stress treatments on (A) Root/Shoot ratio and (B) Tolerance index of *S. bicolor* genotypes (DTSYN11, EC-3161, RJ-2020 and BPCH-6). Data mean ± SE

The biomass in DTSYN 11, EC – 3161, RJ – 2020, BPCH – 6 was significantly ($P < 0.05$) reduced for plants under drought stress in contrast to well-watered once (Figure 2) and the control genotypes showed a significant increase in biomass that was statistically significant at 10 day of stress.

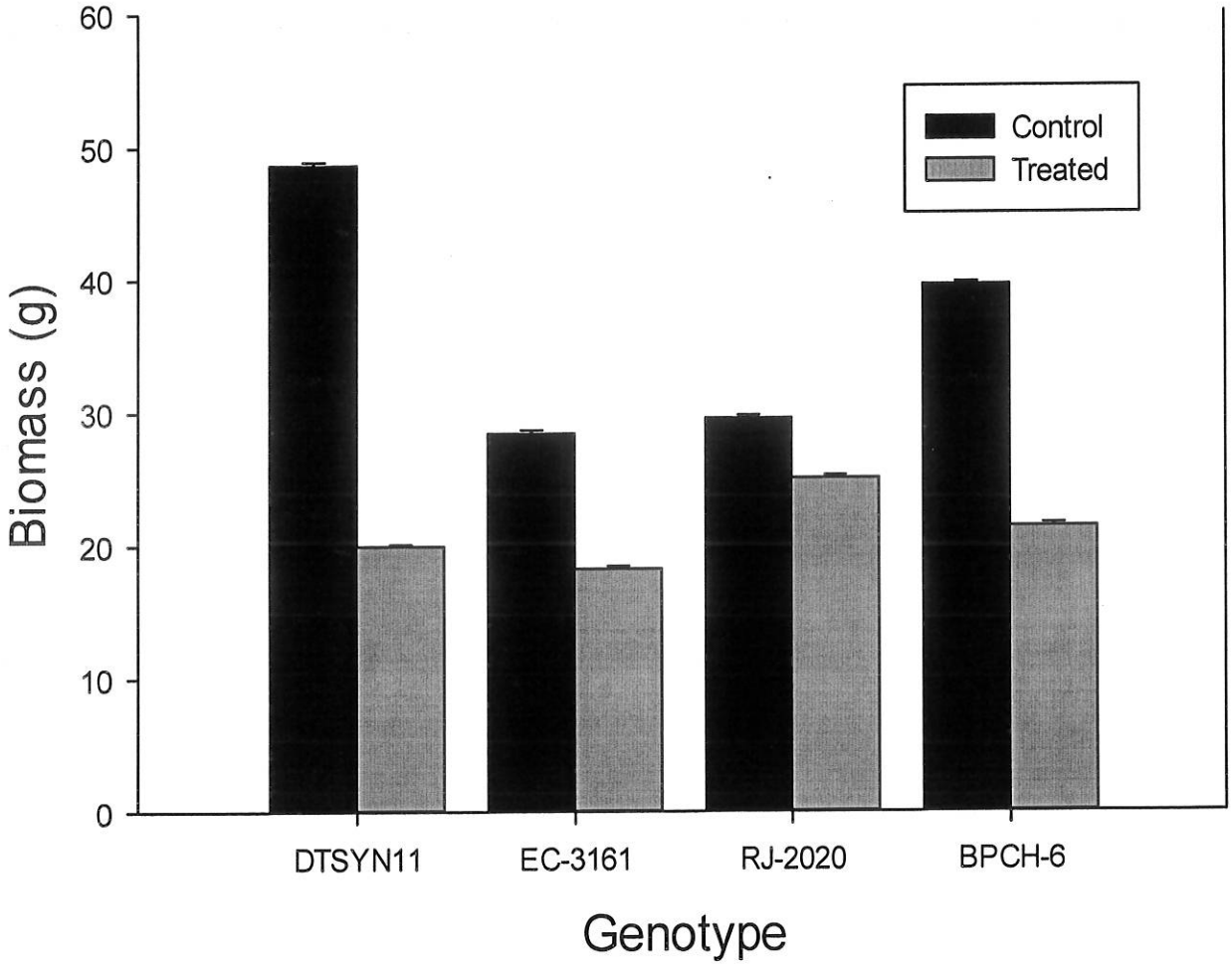

Figure 2: Effects of drought stress treatments on Biomass of *S. bicolor* genotypes (DTSYN11, EC-3161, RJ-2020 and BPCH-6) Data ± SE

3.1.2 Relative Water Content

Figure 3 showed the effect of drought on the relative water content in *S. bicolor*. The result indicated that drought caused a significant decrease in the RWC of the treated genotypes of *S. bicolor*. RJ – 2020 had a significant ($P < 0.05$) increase under drought condition of about 96% as compared to its controlled condition of 82%, DTSYN 11 had the same result under drought and controlled condition. While EC – 3161 and BPCH – 6 had a significant decrease as compared to the controlled with about 89.04% and 82.20% respectively.

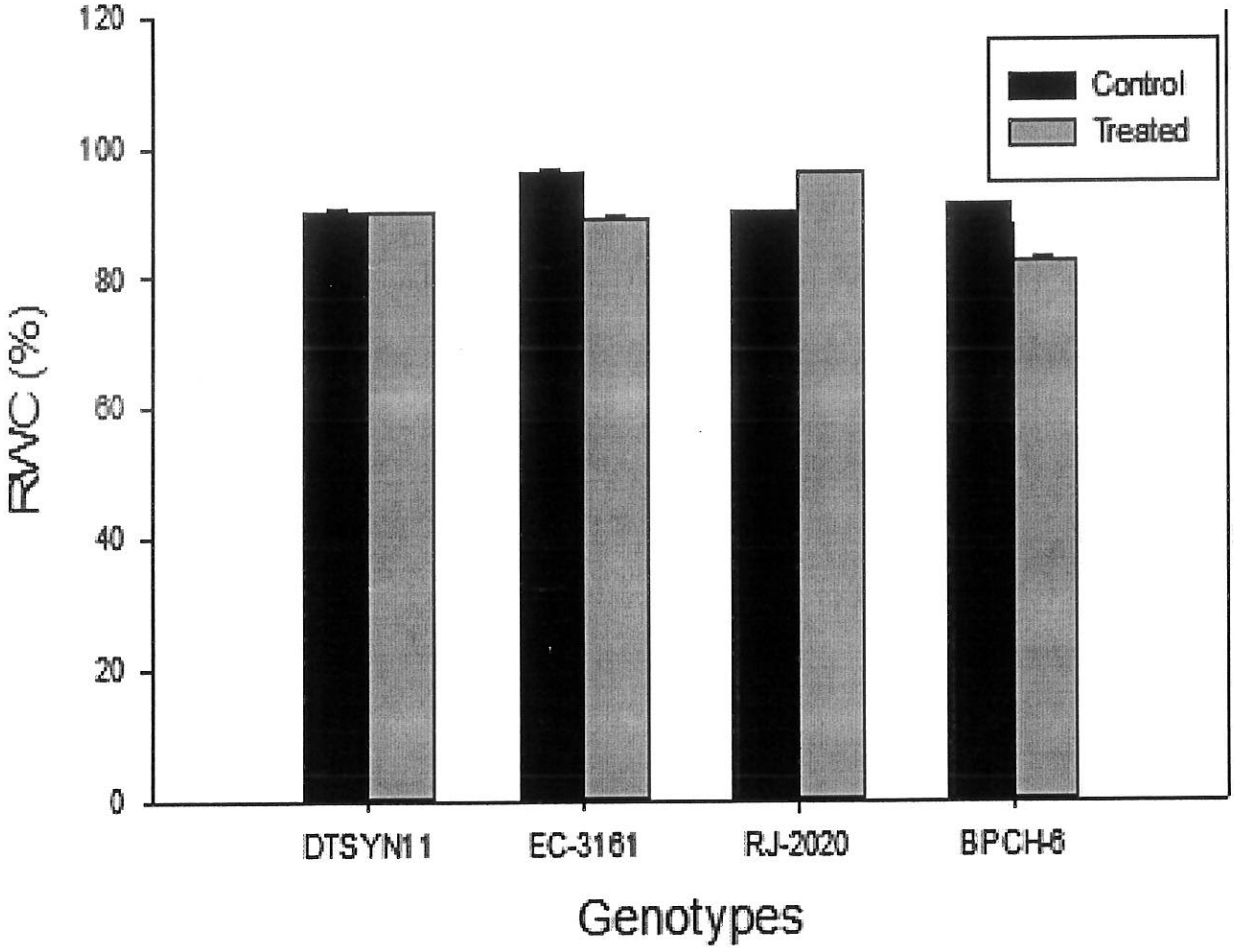

Figure 3: Effects of drought stress treatments on relative water contents (RWC) of *S. bicolor* genotype (DTSYN11, EC-3161, RJ-2020 and BPCH-6). Data ± SE

Table 1 showed significant differences ($P < 0.05$) recorded of total chlorophyll content (Tchl) of *S. bicolor* landraces for both control and treated plants. Tchl between sorghum landraces is at significant difference on 10 days of drought stress ranging from 7.05 mg.g^{-1} FW for DTSYN 11 and 7.37 mg.g^{-1} FW for BPCH – 6 in treated plant which was significantly low than 10.55 mg.g^{-1} FW and 17.06 mg.g^{-1} FW in control plants. RJ – 2020 and EC -3161 had a significantly high content of Tchl under drought treatment ranging from 33.72 mg.g^{-1} FW and 27.69 mg.g^{-1} FW as compared to 6.39 mg.g^{-1} FW and 7.28 mg.g^{-1} FW in control plants.

Proline content revealed a significant difference ($P < 0.05$) between *S. bicolor* landraces for both control and stressed plants. Proline content measured at 10th day ranged between 142.80 mg.g^{-1} FW for DTSYN, 202.88 mg.g^{-1} FW for BPCH – 6, 205.60 mg.g^{-1} FW for RJ – 2020 and 292.55 for EC – 3161 in plant treated with drought stress and 245.00 mg.g^{-1} FW, 264.53 mg.g^{-1} FW, 191.35 mg.g^{-1} FW and 225.78 mg.g^{-1} FW in control plant respectively. There was a remarkable increase in proline as indicated during drought stress for RJ – 2020 and EC – 3161. RJ – 2020 which recorded 205.60 mg.g^{-1} FW making an increase of 14 – fold in comparison to its value of control which is 191.35 mg.g^{-1} FW. EC – 3161 evidentially showed the highest content of proline with 292.55 mg.g^{-1} FW increase of 16 – fold in contrast to value of 225.78 mg.g^{-1} FW recorded in control plant

Table 1 Changes on total chlorophyll and proline content of *S. bicolor* genotypes under drought stress Data+ S.E

Plant	Genotype	Total Chlorophyll (mg g–1 FW)		Proline(mg g–1 FW)	
		Control	**Treated**	**Control**	**Treated**
Sorghum bicolor	DTSYN 11	10.55	7.05	245.00	142.80
	BPCH – 6	17.06	7.37	264.53	202.88
	RJ – 2020	6.39	33.72	191.35	205.60
	EC – 3161	7.28	27.69	225.78	292.55

3.1.3 Determination of Lipid Peroxidation

Figure 4 shows the effect of drought stress treatment on Malondialdehyde content (MDA) of *S. bicolor*. Under well – control condition DTSYN 11, RJ – 2020 and BPCH – 6 had a significant increase ($P < 0.05$) as compared to drought. EC – 3161 showed high concentration of MDA with a mean of 1.02 ± 0.12 µmol/ml as against 0.6 ± 0.02 µmol/ml under control.

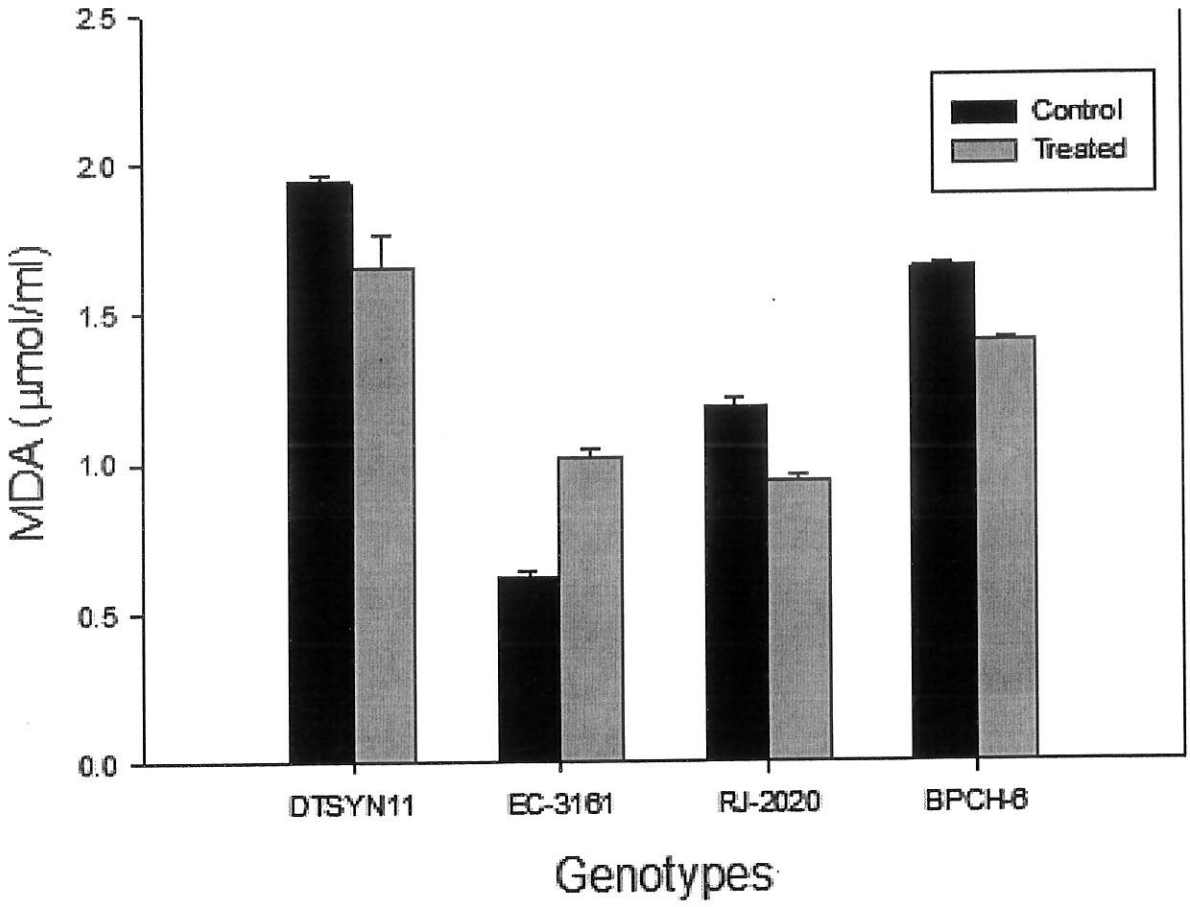

Figure 4: Effects of drought stress treatments on Malondialdehyde content (MDA) of *S. bicolor* genotypes (DTSYN11, EC-3161, RJ-2020 and BPCH-6) Data ± SE

3.1.4 Enzyme Extraction and Assay

Figure 5 shows drought stress treatments on Glutathione (GSH) of *S. bicolor* genotypes. Under well – control condition DTSYN 11, RJ – 2020 and BPCH – 6 had a significant decrease ($P < 0.05$) as compared to drought. EC – 3161 showed low concentration of GHS under drought with a mean of 4.70 ± 0.08 µmol/ml as against 12.90 ± 0.22 µmol/ml under control.

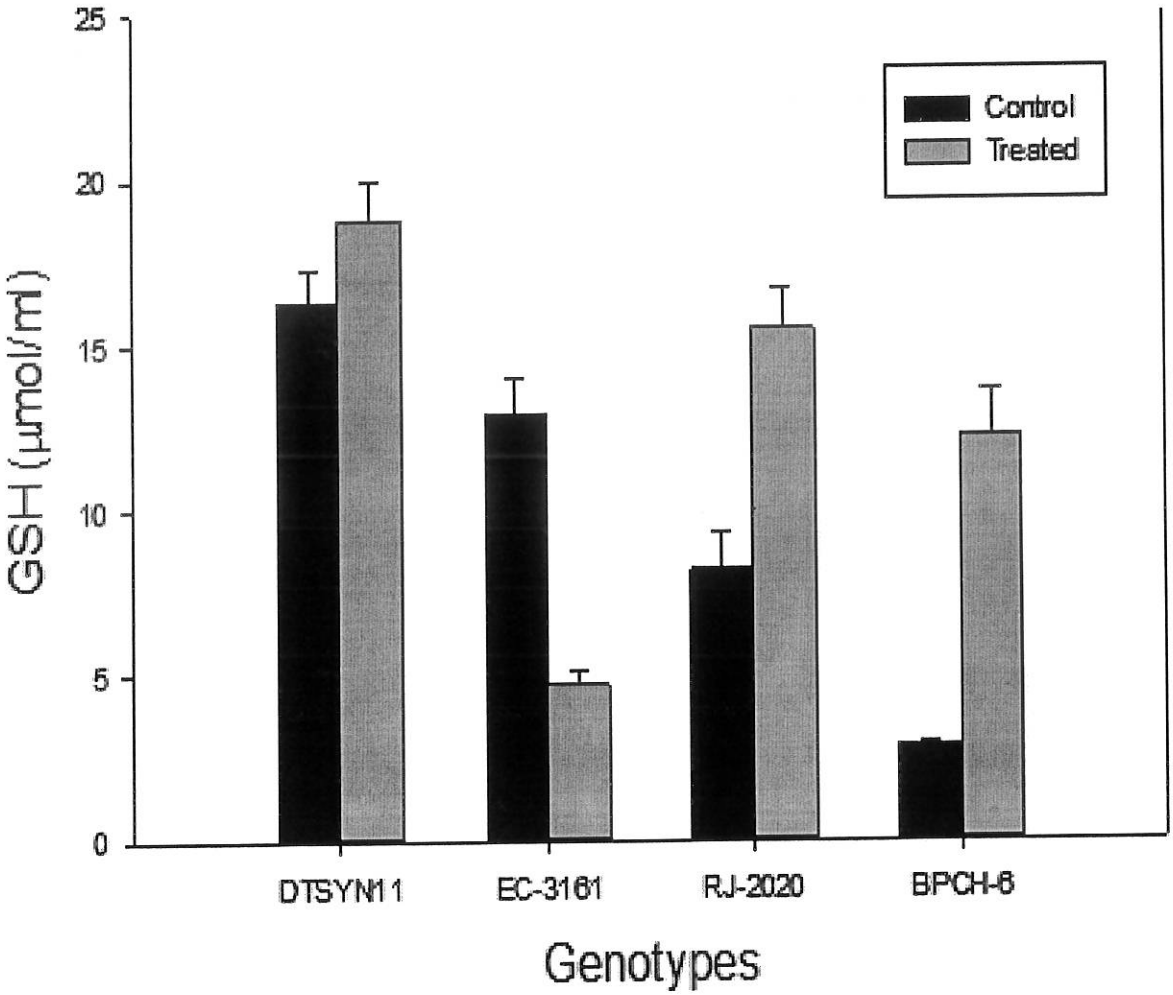

Figure 5: Effects of drought stress treatments on Glutathione (GSH) of *S. bicolor* genotypes (DTSYN11, EC-3161, RJ-2020 and BPCH-6). Data ± SE

Figure 6 shows drought stress treatments on Superoxide dismutase (SOD) of *S. bicolor* genotypes. Under well – control condition, DTSYN 11, RJ – 2020, EC – 3161 and BPCH – 6 had a significant decrease ($P < 0.05$) as compared to the treated under drought stress with a mean of 1.80 µmol/ml/min/mg, 0.97 µmol/ml/min/mg, 0.97 µmol/ml/min/mg and 1.04 µmol/ml/min/mg respectively.

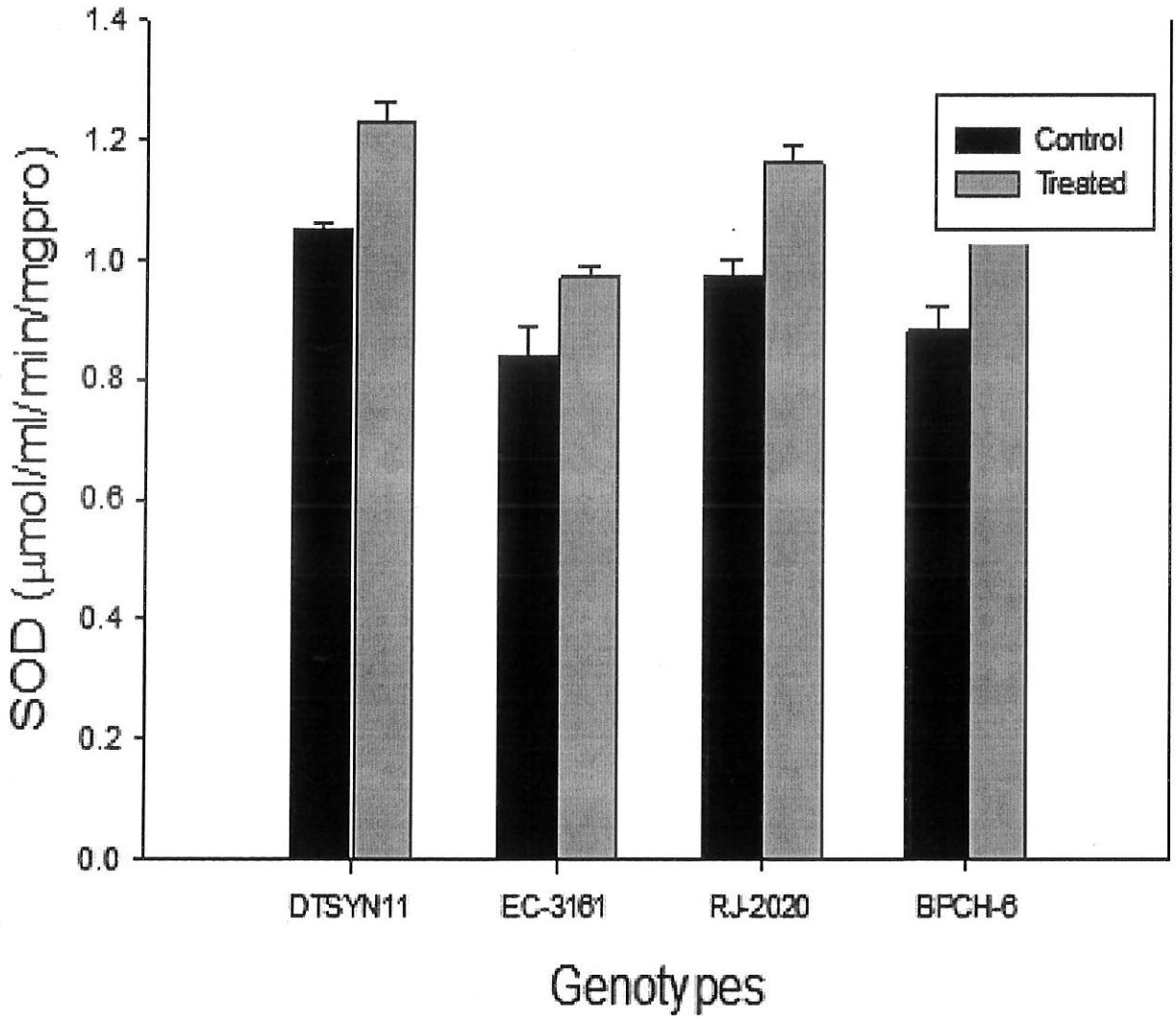

Figure 6: Effects of drought stress treatments on Superoxide dismutase (SOD) of *S. bicolor* genotypes (DTSYN11, EC-3161, RJ-2020 and BPCH-6). Data ± SE

Figure 7 shows drought stress treatments on Catalase (CAT) of *S. bicolor* genotypes. Under well – control condition DTSYN 11, RJ – 2020, EC – 3161 and BPCH – 6 had a significant decrease ($P < 0.05$) as compared to the treated under drought stress with a mean of 9.32 µmol/ml/min/mg, 4.20 µmol/ml/min/mg, 5.17 µmol/ml/min/mg and 4.19 µmol/ml/min/mg respectively.

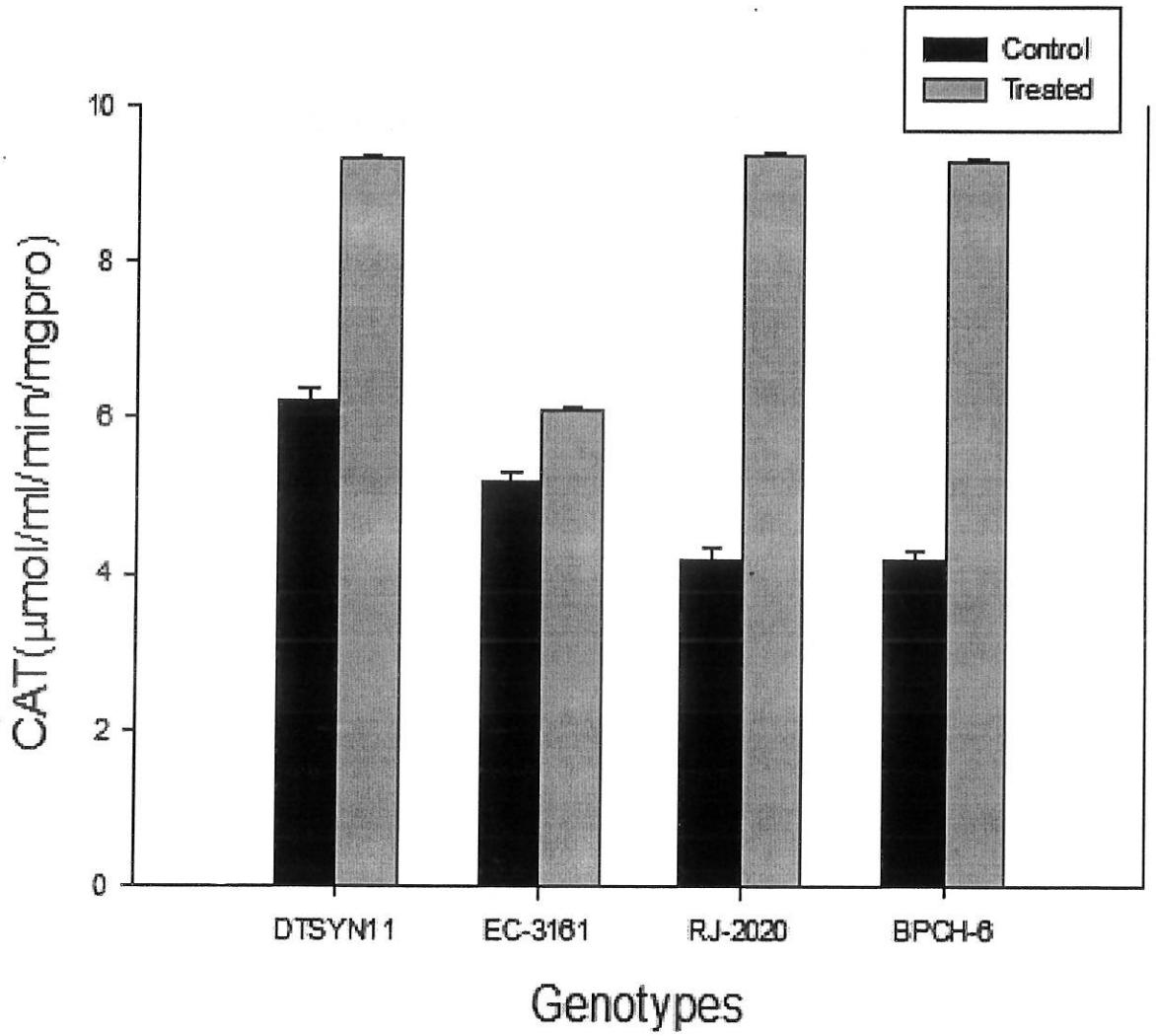

Figure 7: Effects of drought stress treatments on Catalase (CAT) of *S. bicolor* genotypes (DTSYN11, EC-3161, RJ-2020 and BPCH-6). Data ± SE

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The vegetative indices tolerance index was used to further confirm the information on the responsiveness to drought stress of DTSYN 11, RJ – 2020, EC – 3161 and BPCH – 6. RJ – 2020 showed more the tolerance to drought as compared to DTSYN 11, EC – 3161 and BPCH – 6 genotypes at 10 days of drought treatment. Furthermore, the resilience of RJ – 2020 to drought stress and its capacity to adapt under water – deficit condition was confirmed by TI (Mehmet *et al.*, 2005).

RWC for RJ – 2020 genotype was maintained at 96%, DTSYN 11 89.87%, EC – 3161 89.04% and BPCH – 6 82.20% during 10 days of drought stress treatments. This underscores the fact that adequate watering protects the plant against harshness of ion outflow and membrane disturbance due to the irreplaceable change in cell tissue. Therefore, RWC for all four sorghum genotypes were above the value of 76% across all treatment therefore this process enables the water potential of the soil to be greater than root tissues (Bray *et al.*, 2000). Furthermore, process of dehydration consequently affects damage of leakage ions due to irreversible changes within the cell membranes due to consequence from water drought (Ristic *et al.*, 1996; Triparthy *et al.*, 2000). Similarly, Lambers *et al.* (2008) reported that certain amount of water in tissues includes roots and in leaves range among 72% to 88%.

Xu *et al.* (2000) reported that in cereal crop reduction of 21% in total chlorophyll content in drought – resistant genotypes was recorded between drought stress and in control plants. Therefore, all landraces sustained significant contents of chlorophyll as compared to their controls at all days of treatments. Blum (2011) reported that plant height is a dominant character

affecting transpiration of plant, when sown in pot that has equal soil volume, thereby causing a large plant to require frequent application of water than in a small plant. Overall, under the consistent water deficit conditions, the four sorghum landraces investigated, showed that phenotypically plant height were influenced by the water use efficiency demand and duration of period of water stress observed. Also tall crops selectively grown by farmers in Africa after production of it grain could be utilized in fuel production (Maiti *et al.*, 2012). Content of chlorophyll maintained a significant difference between water stress and control plants in all the treatments, excluding DTSYN 11 at 10^{th} day which showed a reduction significantly of about 32.4 % during water stress condition. Chlorophyll plays a major role in photosynthesis which involves the ability to signal oxygen damaging radicals known to be produced readily by complexes during light splitting process of photosynthesis (McKersie and Leshem, 1994; Malkin and Niyogi, 2000; Santabarbara *et al.*, 2013). Takele (2010) investigated the reduction of chlorophyll content in drought – resistant sorghum at pre – flowering growth stage under dehydration. However, levels of chlorophyll were maintained under water deficit condition which indicates that no damage occurred in the apparatus of photosynthesis process in all the sorghum landraces due to the water stress condition.

Hare *et al.* (1998) reported that plants accumulate so much proline under multiple stress conditions which is due to induced synthesis and reduced degradation. Accumulation of proline under drought stress is a change capable of alleviating the effects of damage to membrane (Van Rensburg *et al.*, 1993; Bray *et al.*, 2000; Coruzzi and Last, 2000). However, accumulation content of solute proline under drought stress present in both RJ – 2020 and EC – 3161 underscore that these sorghum landraces have some measure of antioxidant activity which inhibits death of plant.

The more biochemical response of plant under stress id ROS, which causes oxidative stress and damage to membranes (Dietz, 2005). MDA is associated with an increase in concentration of MDA was induced significantly at 10 days of drought stress. The root showed significant increase in the concentration of MDA in DTSYN 11 than in RJ – 2020, EC – 3161 and BPCH – 6 genotypes. This suggests susceptibility to damage of cell membrane in the tolerant genotype as compared to the susceptible RJ – 2020, EC – 3161 and BPCH – 6. While in leaves a similar observation DTSYN 11 tolerant genotype had a greater concentration of MDA as compared to RJ – 2020, EC – 3161 and BPCH – 6. The high MDA content can be suggested to the high antioxidative activity present in the results of this study. The results shown here indicate that MDA was high in DTSYN 11 than in RJ – 2020, EC – 3161 and BPCH – 6. As similar observations were seen in the case of Lolium perenne where plants showed a significant decline in MDA due to the induction of activity in SOD (Lei *et al.*, 2006, Odjegba and Adeola, 2012). MDA is associated with increase in antioxidant enzymes causing oxidative stress tolerance to be enhanced. The activities of antioxidant enzymes displayed a consistent increase in all genotypes as drought stress increases. The concentration of CAT in leaves and root showed significant increase in tolerant genotypes DTSYN 11, RJ – 2020, EC – 3161 and BPCH – 6 under 10 days drought stress confirming that DTSYN 11, RJ – 2020, EC – 3161 and BPCH – 6 possess a desirable mechanism of safeguarding membrane from ROS effects. This in conformity with an increase of CAT in tolerant varieties as described by Sairam and Tyagi (2004). GSH activity in roots increased significantly in DTSYN 11, RJ – 2020 and BPCH – 6 in contrast to EC – 3161 while GSH activity in leaves of DTSYN 11, RJ – 2020 and BPCH – 6 showed a progressive significantly increase as compared to EC – 3161. In leaves similar observation as the root confirmed the increase of GSH activity in both genotypes. The higher activity of CAT and GSH observed in DTSYN 11, RJ – 2020 and BPCH – 6 indicates that this genotype has a high ability

for combating ROS produced by drought as compared with EC – 3161 genotypes. Therefore, the concentration of MDA and high accumulation of antioxidant enzymes as stress days progressed suggest that DTSYN 11 genotype has a certain measure of osmotic protection and influence of antioxidant activity leading to death of plant.

4.2 CONCLUSION

Screening of four selected Nigerian sorghum landraces at vegetative state has revealed that they have significant tolerance against drought as they managed to maintain high RWC as compared to a previously known tolerant drought line, DTSYN 11. All parameters investigated in the present study indicates that the selected landraces could be used as tolerant breeding lines under drought condition. The landraces identified in this study will be used as a drought tolerant parent for breeding programs related to drought tolerance.

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