EFFECT OF DROUGHT STRESS TOLERANCE IN MAIZE (*Zea mays* L.) AND PEARL MILLET (*Pennisetum glaucum* L.)

BY

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A FINALYEAR PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE, FACULTY OF BASIC AND APPLIED SCIENCES IN PARTIAL FULFILMENTOF THE REQUIRMENS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN PLANT SCIENCE AND BIOTECHNOLOGY, FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE

OCTOBER, 2015.

CERTIFICATION

This is to certify that this final year project was carried out under my supervision by Alozie, Precious with the matriculation number, BTH/11/0247, in the department of Plant science and Biotechnology, Federal university Oye-Ekiti

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DEDICATION

This project is dedicated to the Almighty God in whose amazing grace has kept me throughout the period of this work. I would also dedicate this to my late father Chief Mr Modestus Alozie, my mum, Mrs. Alozie and my entire family for their love and support

ACKNOWLEDGEMENT

My profound gratitude goes to God for guiding me through this entire Bachelors programme. I would wish to thank the Head of department and members of staff of the Department of Plant Science and Biotechnology, Federal University Oye-Ekiti for their selfless contribution towards the successful conclusion of this programme.

My gratitude goes to my supervisor, Mr Emmanuel Iwuala, for his kind support and encouragement, and also for guiding me all through this program. My special thanks goes to Mr. Akinloye, the chief technician of Botany department Obafemi Awolowo University (O.A.U) for his technical support and permission granted me to use his laboratory. I would also like to thank Mr. Awoyemi and Mr. Fayemiro, the lab technicians of Plant Science and Biotechnology department, Federal University Oye-Ekit for their advice and for putting me through some of my work, I also acknowledge Mr. Adeleke, the lab technician of Microbiology department, Federal university Oye-Ekit

My special thanks goes to my mother Mrs. Alozie, Hon. Emeka Anohu and Hon. Yemi Adaramodu, for their financial help to me throughout this program.

Finally I would like to thank my co-student Uche Jennifer, Omotunde Mercy, Olaiya Aderonke, Omodara Pelumi and Olanbiwonu Temitayo for all their love and support.

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ABSTRACT

This investigation is to determine the physiological basis of water deficit stress in pearl millet, (*Pennisetum glaucum)* and maize (*Zea mays).* Crops were grouped into two categories, each representing a treatment and replicated 3 times. Category (1) which serve as the control received 100ml of water every 2 days throughout the experimental period. Category (2) received 100ml of water every 2 days for 6 weeks before subjecting them to simulated drought. Physiological parameters evaluated after the treatment include biomass, relative water content of leaf, leaf area, chlorophyll level and leaf epidermal quantification. Analysis of variance was also found to be significant for genotypes, treatments and their interactions at 0.05% level. The results showed drought caused decrease in biomass, chlorophyll, relative water content (RWC), leaf area, and adaxial epidermal surface of *Zea mays* exposed to drought, while in *Pennisetum glaucum,* showed a significant increase in root/shoot ratio, biomass, and abaxial epidermal surface under drought condition. Therefore, the comparative analysis of these two genotypes under well-watered and water deficit stress condition revealed that *P. glaucum* was superior over *Z. mays* indicating that such relationships can be positively attributed to drought tolerance.

CHAPTER ONE

1.0 INTRODUCTION AND LITERTURE REVIEW

In coming decades, the combination of the rising world population and climate change will place new demands on agriculture globally. The emission of carbon iv oxide (CO_2) and other greenhouse gases will result in the warming of the climate across the world, and this will have significant negative effect on agricultural productivity due to increase in incidence of extreme weather events, including heat waves and drought. (Geber *et al.,* 1990)

Drought stress is one of the main causes for crop yield reduction in the majority of agricultural regions of the world. In many researches, drought tolerance in plants has been studied in relation to regulatory mechanism of osmotic stress (Ashraf and Foolad, 2007)

Plant would respond to water stress by dramatically complex mechanisms from genetic molecular express, biochemical metabolism, through individual plant physiological processes to ecosystem levels which may mainly include four (4) aspect; (1) drought escape via completing plant life cycle before severe water deficit e.g. earlier flowering in annual species before the onset of severe drought (Geber *et al.,* 1990). (2) Drought avoidance via enhancing capacity of getting water, e.g. developing root systems or conserving it such as reduction of stomata and leaf area/canopy cover. (Schulze, 1986; Jackson *et al.,* 2000) (3) Drought tolerance mainly via improving osmotic adjustment ability and increasing cell wall elasticity to maintain tissue integrity (Morgan, 1984) (4) drought resistance via altering metabolic path for life survive under severe stress e.g. increased antioxidant metabolism (Bartolie *et al.,* 1999; Penuelas *et al.,* 2004)

Drought, as an abiotic stress, is multidimensional in nature, and it affects plants at various levels of their organization. Under prolonged drought, many plants will dehydrate and die. Various plant physiological processes are altered by plant water stress and are likely to affect

growth. For example, photosynthesis is reduced and rate of translocation is declined. Greenway *et al.,* (1969) suggested that disturbance in mineral nutrition is partly responsible for reduced growth in plants experiencing water stress. Water stress in plants reduces the plant-cell's water potential and turgor, which elevate the solutes' concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases leading to growth inhibition and reproductive failure. This is followed by accumulation of Abscisic acid (ABA) and compatible osmolytes like proline, which causes wilting (Lisar *et al.,* 2012). Drought not only affects plant water relations through the reduction of water content, turgor and total water, it also affects stomatal closure, limits gaseous exchange, reduces transpiration and arrests carbon assimilation (photosynthesis) rates. Many studies on plant responses to water stress were carried out by investigators concerned with agricultural production, environment and resources, macroscopic physics of soils, plant and atmospheric water (Denmead and Shaw, 1960; Slatyer, 1967; Loomis, *et al.*, 1971; Fischer, 1973; Hsiao, 1973).

Drought in many cases actually causes specific changes similar to changes induced by nutrient deficiencies. For example, free amino acids and sugars accumulate during water stress as in potassium deficiency and ribonuclease increases when either water or potassium is deficient (Gates and Bonner, 1959). However,, it is essential to examine water stress effects within the reference framework of stress severity and time courses, only then can we hope to unravel the causal relations and the sequence of complex events which constitute plant responses to water stress. Plants adapt themselves to drought conditions by various physiological, biochemical, anatomical, and morphological changes, including transitions in gene expression. The physiology plants' response to drought at the whole plant level is highly complex and involves deleterious and/or adaptive changes. This complexity is due to some factors such as plant species and variety, the dynamics, duration and intensity of soil water depletion, changes in water demand from the atmosphere, environmental conditions, as well as plant growth and the

phonological state in which water deficit is developed. Plants' strategies to cope with drought normally involve a mixture of stress avoidance and tolerance strategies. Early responses of plants to drought stress usually help the plant to survive for some time. The acclimation of the plant to drought is indicated by the accumulation of certain new metabolites associated with the structural capabilities to improve plant functioning under drought stress. The main aspect of plant responses to water involve the maintenance of homeostasis (ionic balance and osmotic adjustment), counter action to resulted damages and their quick repair such as scavenging of ROS and decrease oxidative stress and the regulation of recovery of growth. Under limited water conditions, plants respond differently and show a wide range of drought tolerance mechanism both in terms of morphology and physiology.

Drought stress can trigger overproduction of reactive oxygen species (ROS) in plants eventually resulting in oxidative stress (Reddy *et al.*, 2004). Drought stress, like other abiotic stresses, can also lead to oxidative stress through the increase in reactive oxygen species (ROS) such as superoxide (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO) which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Apel and Hirt, 2004; Mudgal *et al.*, 2010; Nojavan and Khorshidi, 2006). To able to endure oxidative damage under conditions which favours increased oxidative stress such as drought, plants must possess efficient antioxidant system. Plant cells have evolved a (glutathione, ascorbate and carotenoids) as well as ROS- scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate proxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) (Apel and Hirt, 2004; Bhardwaj *et al.*, 2007). Growth and development of plants is reduced due to oxidative stress (Azevedo *et al.*, 1998; Noctor and Foyer; 1998; Zhu *et al.*, 1999).

The growth and development of plants is directly regulated by plants hormones (Shakirova *et al.*, 2003). Plant hormones influence the plants in multifarious ways affecting a number of physiological or biochemical processes in plants subjected to biotic and abiotic stresses (Hildmann *et al.*, 1992; McConn *et al.*, 1997; Reymond and Farmer, 1998). The stimuli in response to plant hormone are complex phenomena. These processes in7volve a signalling system that extends across the organs and organelles and also within an individual cell (Tiryaki, 2004). This integrated networking system performs activities across different organs of plant by detecting and transmitting signals in their own specific way (Klumpp and Krieglstein, 2002). There are number of plant hormones such as Indo acetic acid (AA), gibberellin (GA) cytokinnins (CK), abscisic acid (ABA), ethylene (ACC) that are involved in signalling system. Apart from these, brassindosteroids (BRS), jasmonic acid (JA) and salicylic acid (SA) are also potential molecules that are involved in signal transduction (Aberg, 1981; Raskin, 1992, Gunes *et al.*, 2006). These hormones activate a range of signal transduction pathways (Gunes *et al.*, 2006)

Water deficit is one of the major abiotic factor limiting crop productivity in semi-arid tropics and climate change is likely to make drought stresses even more severe in the future, under drought, the leaf gas exchange is reduced and this leads to lower biomass accumulation and grain yield. Previous work in several crops shows genetic differences in how leaf-gas exchange responds to water stress, with certain genotypes being capable of sustaining plant transpiration until the soil becomes fairly dry whereas others react with a decline in transpiration when the soil is relatively wet, this has been documented in maize (Ray and Sinclai, 1997) and Soya bean (Hufstetler *et al,* 2007)

Stomata are portals for gas exchange between the leaf mesophyll cells and the environment, they occupy between 0.5% and 5% of the leaves epidermal and are most abundant at the bottom or abaxial surface. Amphistomatous leaves such as leaves have stomata on both sides. The

pattern of the eoidermal cells and abaxial/adaxial polarity of the maize leaf is established in a meristem and is subsequently maintained throughout leaf development (Juarez *et al.*, (2010). The development of epidermal cell structure and stomatal density on the upper and lower surface of maize leaves has effect on photosynthesis on each surface.

1.1 OBJECTIVES

The over-all objective of the present study is as follows;

- To compare the responses of maize and pearl millet to water deficit condition.
- To study the physiological responses of pearl millet as an alternative crop to maize for limited water conditions.
- To establish the extent to which different regulatory responses vary between pearl millet and maize

1.2 EXPERIMENTAL PLANTS

1.2.1 Maize (*Zea mays* L)

Maize, (*Zea mays*) belong to the family Poaceae, the centre of origin of maize is said to be from the Mesoamerican region, Mexico highland. Maize can grow in wide range of environment, showing high diversity of morphological and physiological traits. Maize is the 3rd most important food crop worldwide (Frova *et al.*, 1999) It is used in many way than any other cereals therefore it is considered as a multipurpose crop and has been put to a wider range of uses such as for human food, poultry feed, and for hundreds of industrial purposes. Typically, it is grown in areas where the annual mean temperature is greater than 18°c. Worldwide production of maize is 785 million tons with the largest producer, the Unites states producing 42%, Africa produces 6.5% and the largest African producer is Nigeria with nearly 8 million tons followed by South Africa. Maize is more sensitive to drought. It is exposed o more hazards and it is a higher risk crop in general (Misovic, 1985). Ribaut *et al,* (1997) reported that maize susceptible to drought at flowering stage than any other crop while Mangombe *et al,* (1996) found that varieties of maize exposed to unpredictable drought stress during the growing season produce low quality yield. The development of maize genotypes with high and stable yields under drought is important since access to drought adapted

genotypes maybe the only alternative to many small scale farmers, consequently, improved tolerance to drought is an important breeding objective of (IITA, 2004). A characteristics of maize under environmental stress is an increase in the anthesis-silking interval (Bolanos and Edmemedes, 1993).

About fifty (50) varieties exist and consist of different colours, textures, grain shape and sizes. White, yellow and red are the most common types. The white and yellow varieties are preferred by most people depending on the region.

1.2.2 Taxonomic Classification of Maize

Kingdom	Plantae
Family	Poaceae
Subfamily	Panicoideae
Order	Poales
Tribe	Andropogoneae
Genus	*Zea*
Specie	*Z. mays*

Maize is a short day plant, with 12.5hrs/day as suggested. Photoperiods greater than this may increase the total number of leaves produced prior to initiation of tasselling, and may increase the time taken from emergence to tassel initiation (Birch, 1997).

Maize is well adapted to a wide range of soil with a pH within 5.0 to 8.0, maize does not thrive well in acidic soil and it is moderately sensitive to salinity, which reduces the uptake of nutrient and reduces total dry matter production but low soil water is more of a problem to maize. It is important to plant maize seeds at an even depth of 2 to 5 cm into firm, moist soil to ensure good seed-to-soil contact for moisture uptake and subsequent germination.

1.2.3 Pearl Millet *(Pennisetum glaucum)*

Pearl millet (*Pennisetum glaucum* L) is a hardy cereal crop belonging to the family Poaceae, grown mostly in marginal environments in the arid and semi-arid tropical regions of Asia and Africa (FAO, 2007) There are other different varieties of millet which include Proso millet *Panicum miliaceum,* Kodo millet *Paspalum scrobiculatumh,* Finger millet *Eleusine coracana,* Foxtail millet, *Setaria italic,* Little millet *Panicum sumatrense*.

It is grown primarily for grain production but is also valued for its fodder, the importance of which has been rising in the recent years. It is known as 'yadi' in Marghi language of north-eastern Nigeria making it the most important and probably having the greatest potential among the millet varieties, it is robust, quick growing cereal crop with large stems and leaves. It is a staple food for millions of people and the 6th most important cereal annually cultivated as rain-fed crop (FAO, 2007). The crop is well adapted to some extent to growing areas characterized by drought, low fertility and high temperatures (Izge, 2006). Because of its tolerance to difficult growing conditions, it can be grown in where other cereals such as maize or wheat would not survive. India is the largest producer, with 9-10 million ha in area and 7-8million tons of growing production. In Africa, the largest pearl millet growing countries are Senegal, Mali, Burkina Faso, Niger, Nigeria and Sudan. Pearl millet is sensitive to photoperiodism as reported by Clerget *et al.,* (2004) which is a way that has evolved to trigger an escape mechanism, since it appears that the time of flowering is closely related to the end of the raining season. In other words, pearl millet flowers on time to ensure that it can complete its maturation cycle with the remaining soil moisture. During the vegetative growth, root growth is very profuse, with the ability to match it's rooting to water availability in a very plastic manner, leading to a highly varying root growth to shoot growth ratio, depending on the intensity of water limitation (Squire *et al.,* 1987). Being a C_4 plant, pearl millet has a high transpiration efficiency which is

a major strategy to maximize carbon fixation as long as water is available, therefore, stomata movement adapt in such a way that the transpiration rate is kept as high as possible (Squire, 1979). Pearl millet breeding in Nigeria has concentrated on the development of open pollinated varieties. Pearl millet is an excellent forage crop because its low hdrocyanic content, it is more digestible when fed green to animals rather than chaffed straw (Chopra, 2001). It is also used in making popular fried cake known as 'masa' likewise consumed as a beverage called 'fura' in Hausa language. The grain fodder is rich in protein, calcium, phosphorous and other minerals with oxalyic acids in safe limit.

1.2.4 Taxonomic Classification of Pearl millet

Kingdom	Plantae
Order	Poales
Family	Poaceae
Subfamily	Panicoideae
Genus	*Pennisetum*
Species	*P. glaucum*

CHAPTER TWO

2.0 MATERIALS AND METHOD

2.1 METHOD

Seeds of *Zea mays* and *Pennisetun glaucum* were purchased from International Institute of Tropical Agriculture, Ibadan and Plateau Agricultural Development Program respectively in a single batch enough for the study, both genotypes were sown in polythene 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 was 28°C high and 24°C low. After germination, the seedlings were thinned out to one (1) seedling per nursery bag of equal height (10cm) and were arranged in a randomised 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) serves 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 two (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
- Leaf area measurement
- Chlorophyll quantification
- Relative water content of the leaves
- Leaf epidermal analysis

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 LEAF AREA

The leaf area was determined by comparing the weight of leaf traces with a standard paper of known weight described by (Eze, 1965)

$$\text{Leaf area} = \frac{\text{specimen weight x standard area}}{\text{Standard area weight}}$$

2.4 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 grinded 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 663nm 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 light 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

2.5 RELATIVE WATER CONTENT OF THE LEAVES

Weight of fresh leaves were taken and soaked in water for turbidity weight 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):

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

2.6 LEAF EPIDERMAL ANALYSIS

Fresh samples of the two groups of plants were collected and taken to the laboratory. The adaxial and abaxial layer were peeled separately and place in bleach inside Petri dishes to allow full discoloration and transferred to another Petri dish containing distil water then placed in water to avoid drying out. These layers was then placed on a glass slide one to two drops of alcohol was added to remove excess water, stained with safrannin, one to two drops of glycerol was added for clearer view, cover with a cover slip and view under a light microscope. Check for the epidermal cells and stomata number, the stomata index was calculated as:

$$\frac{S}{S+E} \times \frac{100}{1}$$

S = stomata number

_ = Epidermal cell number

CHAPTER THREE

RESULTS

3.1 GROWTH PARAMETERS

3.1.1 Dry Weight and Root/Shoot Weight Ratio

Drought stress significantly reduced the control whole plant dry weight of *Z. mays* and increased the treated condition of *P. glaucum.* The control condition in maize had a mean dry weight of 25.5±1.2g which was significantly higher than 6.86± 0.22g of the treated drought condition. For millet it was however observed in *P.glacum* that the treat drought condition was 9.56±1.23g which is significantly (P > 0.05) higher with 5.5±0.34g of the control.

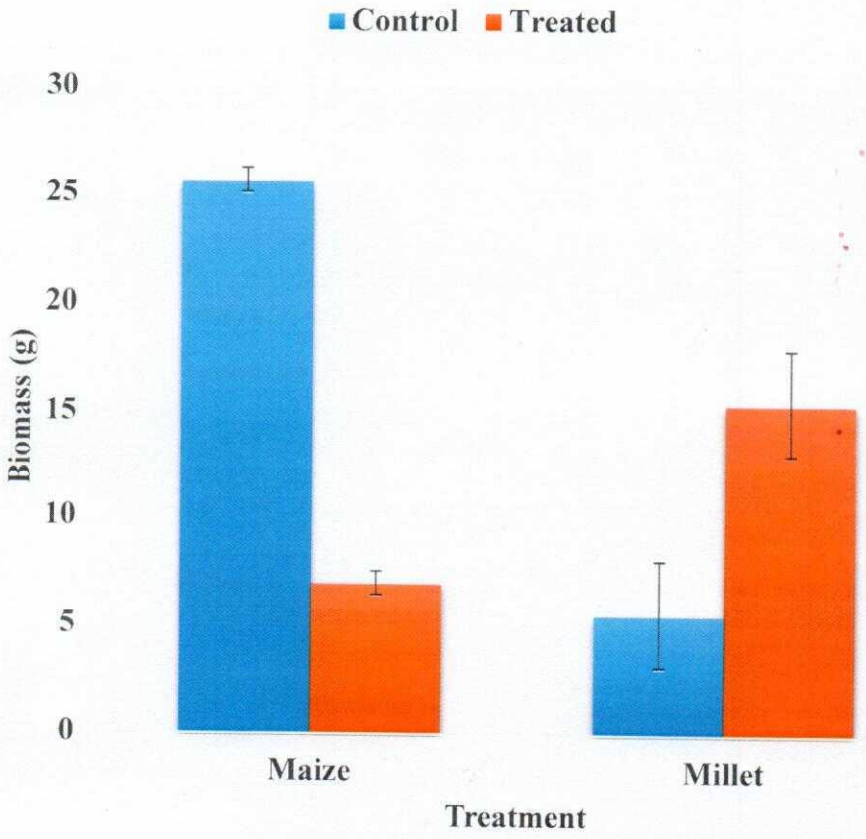


Figure 1: Total dry weight of *Z. mays* and *P. glaucum* affected by simulated drought application. Data are means, + SE of three (3) replicates.

Figure 2 showed the effect of drought on root/shoot ratio on *Z. mays* and *P. glaucum*. It was observed in this study that the drought treatment favoured root development in both genotypes. For *Z. mays*, the treated mean value of 0.117± 0.03g was significantly ($P < 0.05$) higher than the control 0.0633 ± 0.02g, while for *P.glaucum* 0.113± 0.01g significantly ($P < 0.05$) higher than the control 0.26± 0.2g respectively.

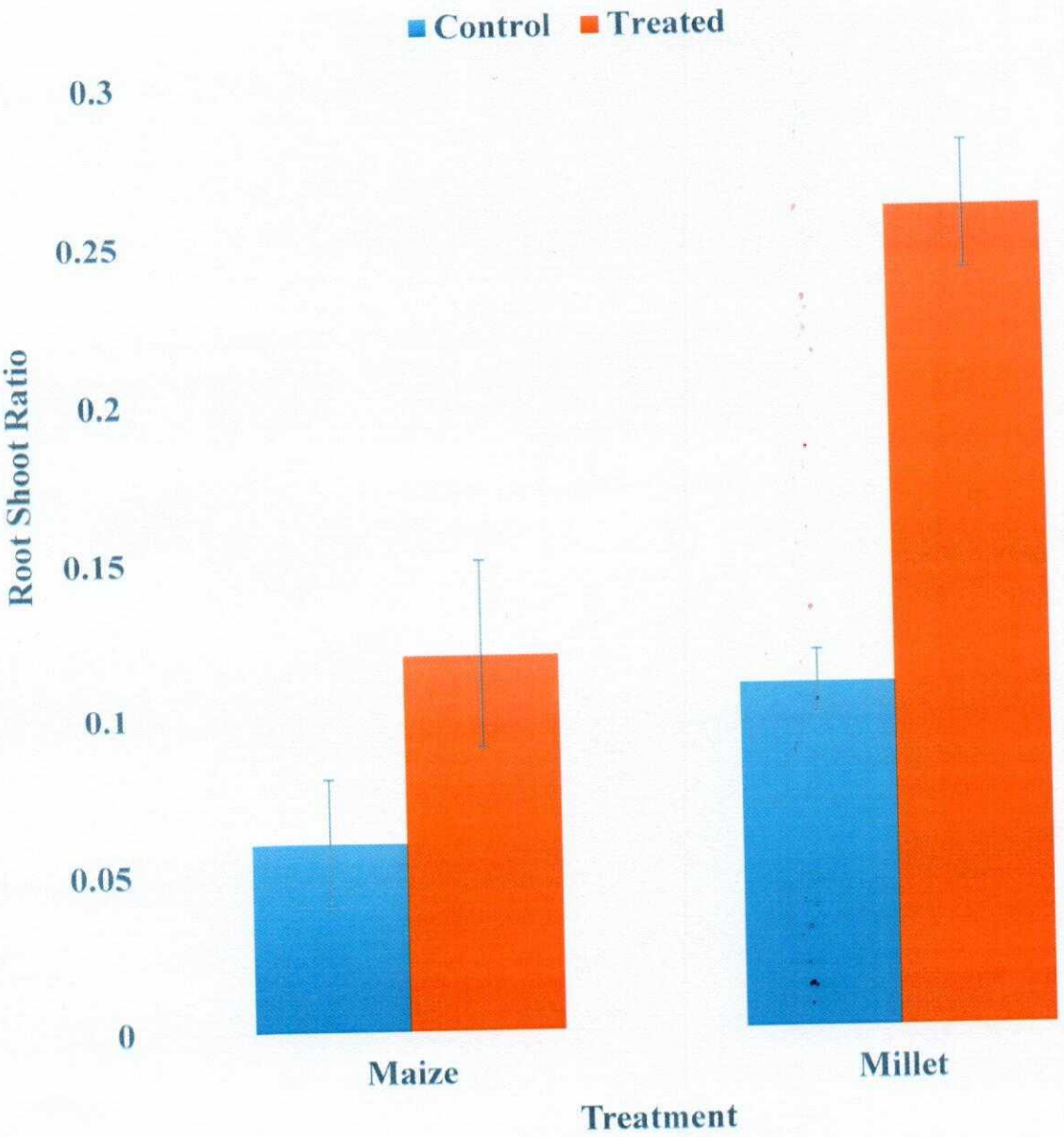


Figure 2: Root/Shoot ratio of *Z. mays* and *P. glaucum* affected by simulated drought application. Data are Means + SE of three (3) replicates

3.1.2 Total Chlorophyll Content

Figure 3 showed the effect of drought on the chlorophyll content of *Z. mays* and *P. glaucum*. In control plant for *Z. mays* the control mean value 0.34 ± 0.13g was significantly ($P < 0.5$) higher than the treated mean value of 0.11± 0.03g for *P. glaucum* there was no recorded significant difference.

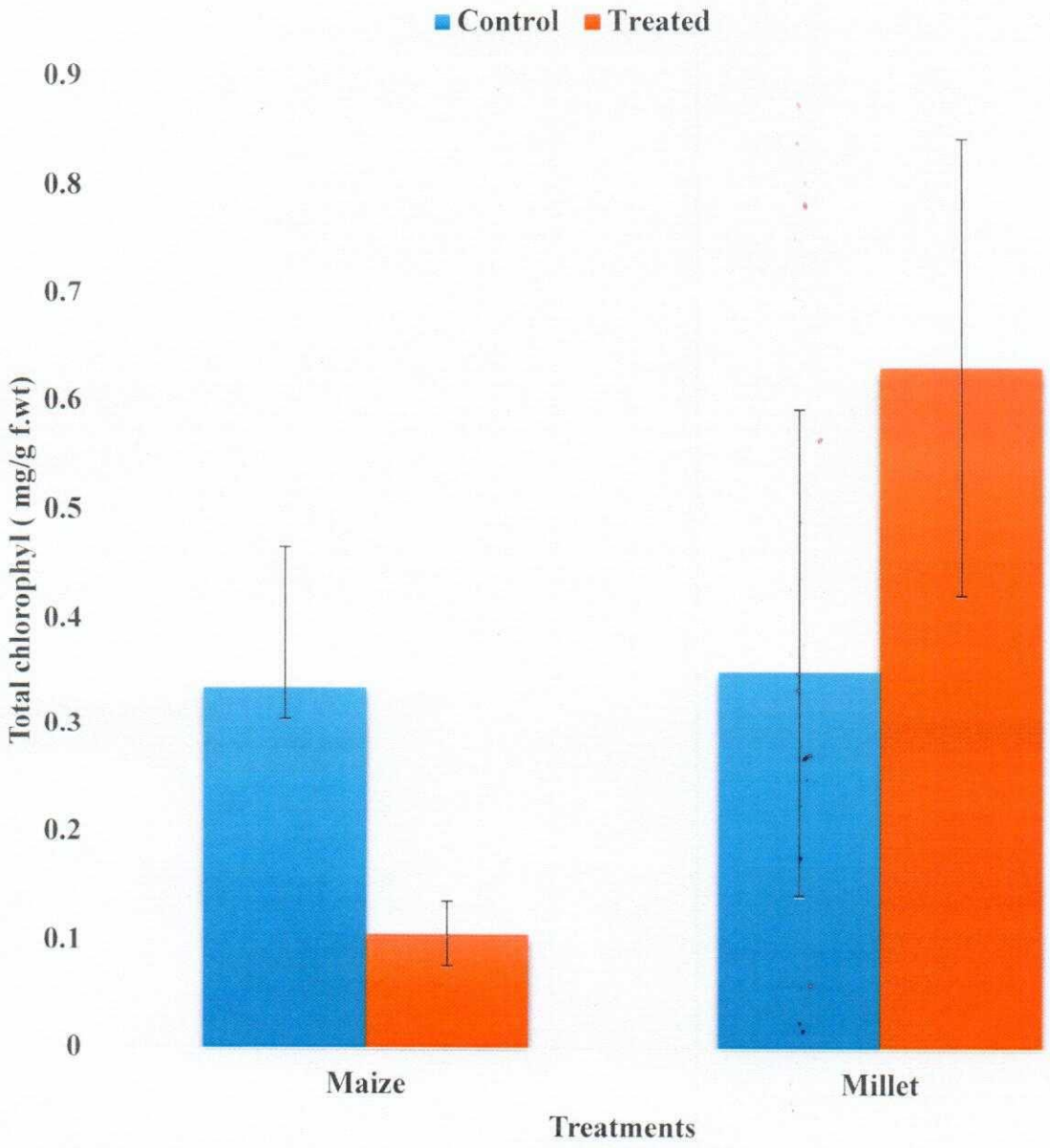


Figure 3: Total chlorophyll content of *Z. mays* and *P. glaucum* affected by simulated drought application. Data are means + SE of three (3) replicates

3.1.3 Relative Water Content

Figure 4 showed the effect of drought on the relative water content in *Z. mays* and *P. glaucum*. The result indicated that drought caused a significant ($P < 0.05$) increase in the RWC of *Z. mays* as compare to the *P. glaucum* where no significant difference was recorded.

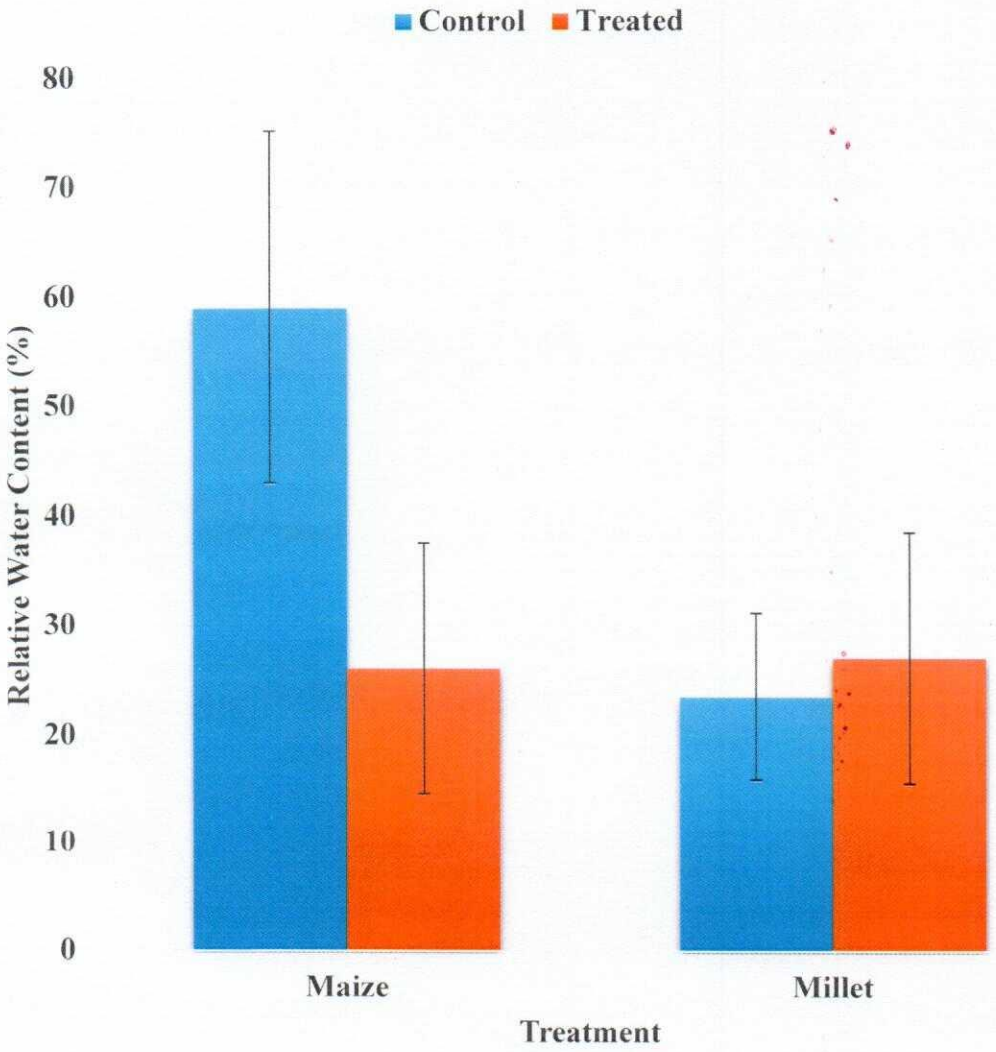


Figure 4: Relative water content of *Z. mays* and *P. glaucum* affected by simulated drought application. Data are means + SE of three (3) replicates

3.1.4 Leaf Area

Drought stress led to a significant reduction in leaf area of *Z. mays* and *P. glaucum*. *Z. mays* genotype under well-watered, control had increased leaf area with mean value of 53.3± 16.1cm^2 while the treated had mean value of 36.0± 6.45cm^2. While *P. glaucum* exposed to drought had a significant increase compared to the control.

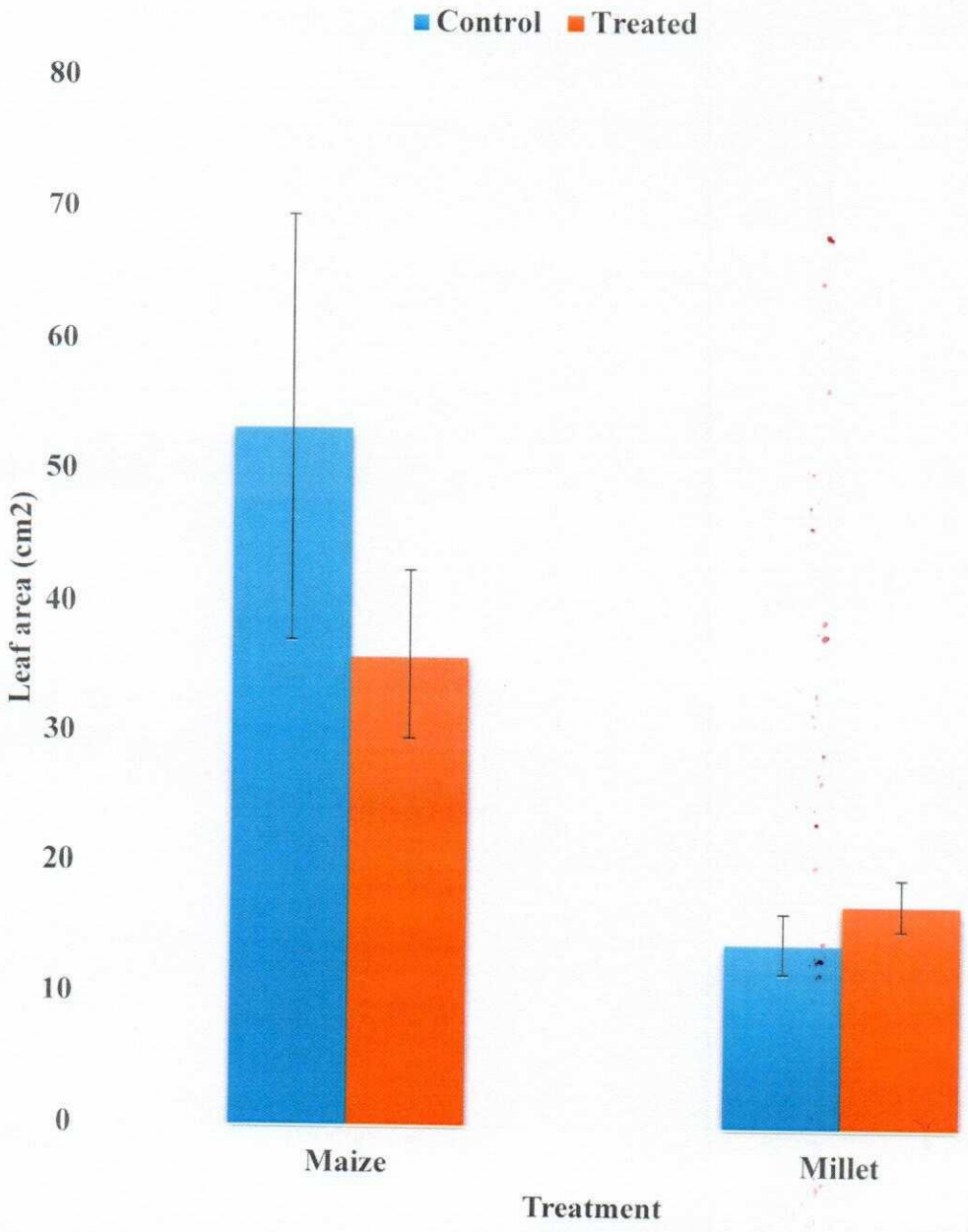


Figure 5: Leaf area of *Z. mays* and *P. glaucum* affected by simulated drought application. Data are means + SE of three (3) replicates

3.1.5 Leaf Epidermal

Figure 6, under well-watered control condition the mean stomata index for *Zea mays* is 46.15% is significantly ($P < 0.05$) higher than the treated drought condition 31.22% respectively. However, *P. glaucum* for the treated was significantly higher ($P < 0.05$) than the control 38.87%, 44.34% respectively.

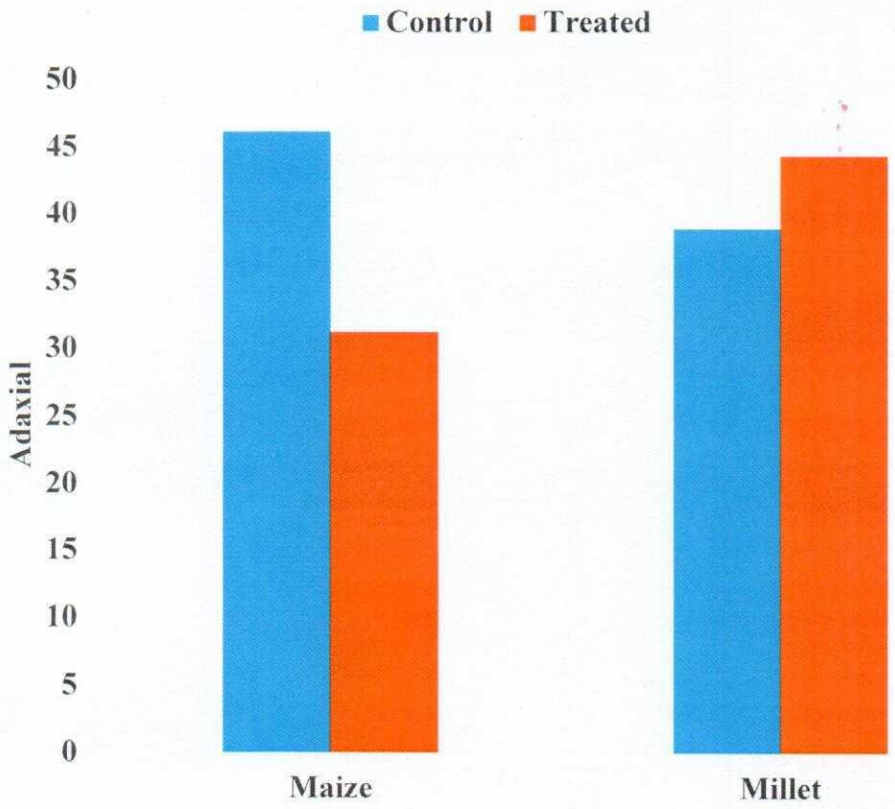


Figure 6: Comparative column chart of adaxial stomata index % of maize and millet genotype

Figure 7, under well-watered control condition the mean stomata index for *Zea mays* is 30.55% is significantly ($P < 0.05$) lower than the treated drought condition 42.32% respectively. However, *P. glaucum* for the treated was significantly higher ($P < 0.05$) than the control 37.41%, 29.3% respectively

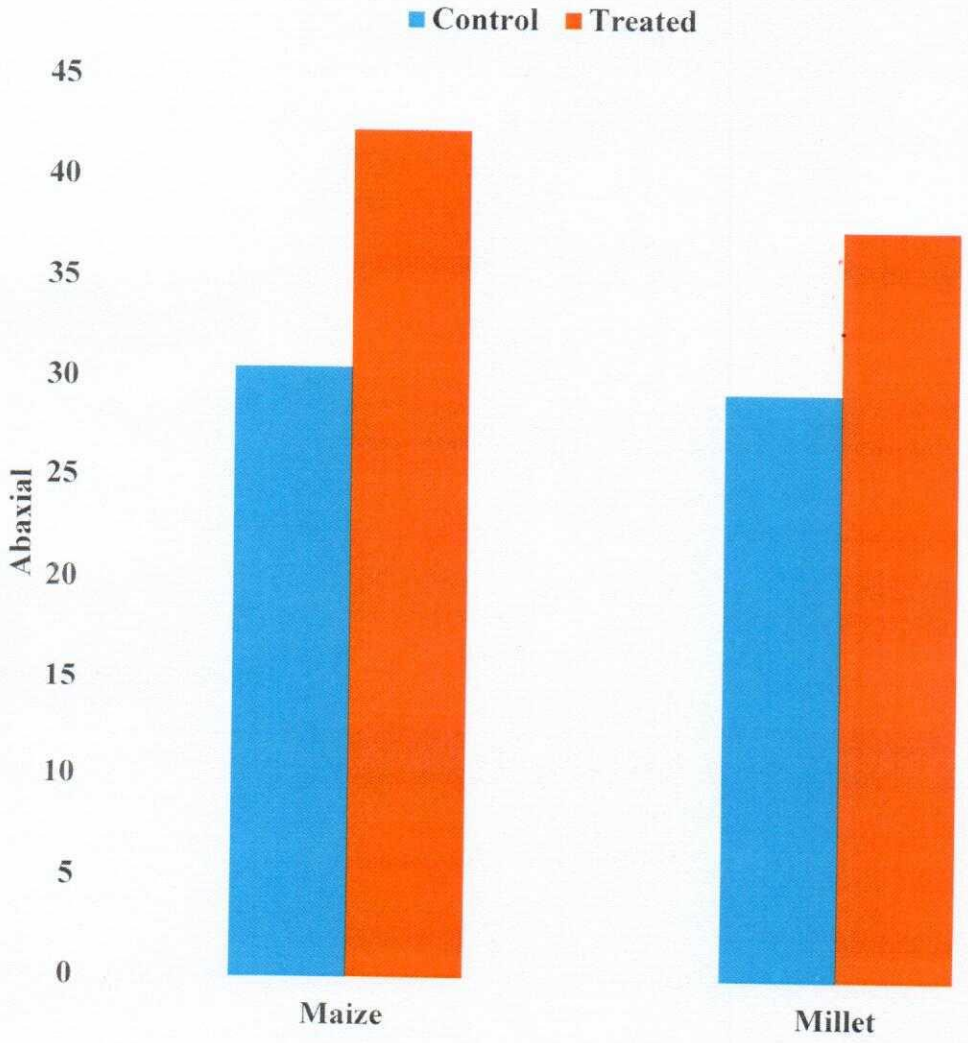
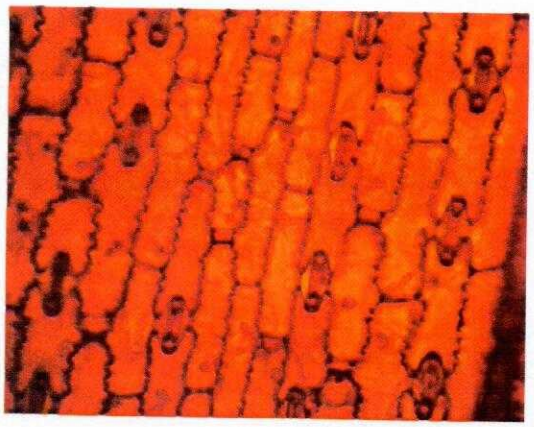


Figure 7: Comparative column chart of abaxial stomata index % of maize and millet genotype

TABLE 1: A COMPARATIVE QUANTITATIVE FOLIAR EPIDERMAL FEATURES OF SPECIES OF *Zea mays* AND *Pennisetum glaucum*

SPECIE	EPIDERMAL CELL NUMBER	STOMATA CELL NUMBER	STOMATA INDEX %
***Zea mays* (control)**			
Adaxial	2921	2143	46.15
Abaxial	4310	1894	30.53
***Zea mays* (treated)**			
Adaxial	3142	1452	31.22
Abaxial	2128	1823	42.32
***Penisetum glaucum* (control)**			
Adaxial	2113	1667	38.87
Abaxial	4027	1344	29.3
***Penisetum glaucum* (treated)**			
Adaxial	1786	1423	44.34
Abaxial	3243	1943	37.41



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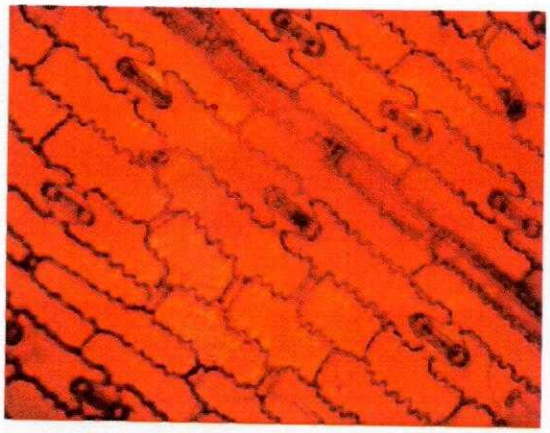

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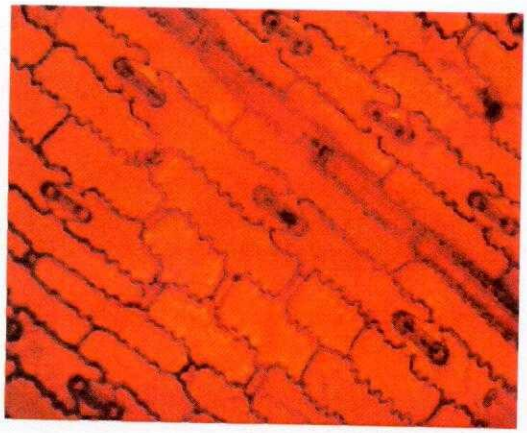

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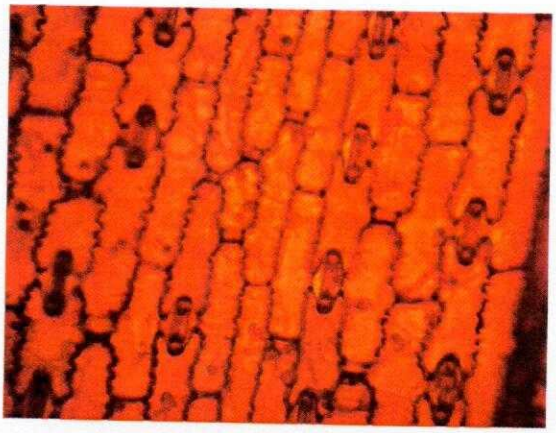
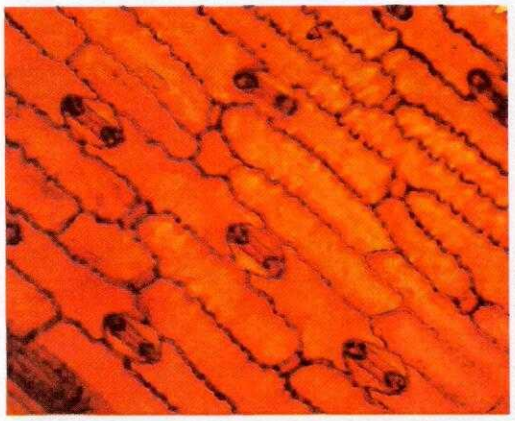


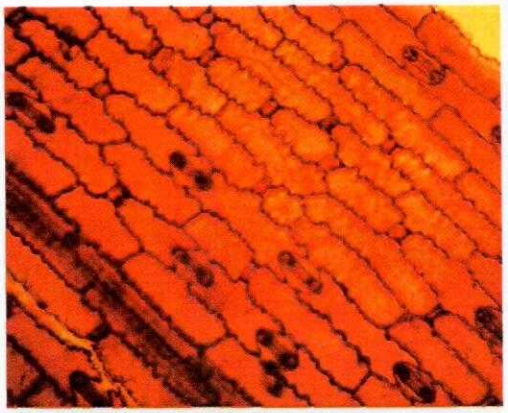
4.

Plates 1 and 2 shows the photographicmicrograph of the adaxial layer of *Zea mays (*control).

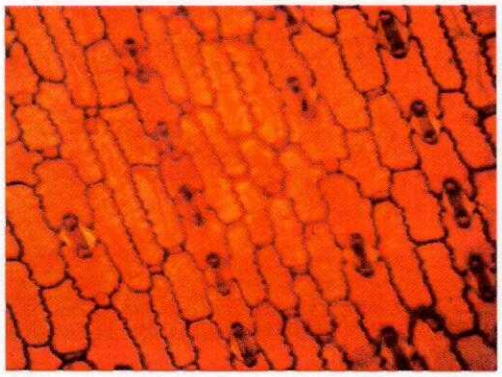
Plates 3 and 4 shows the photographicmicrograph of the adaxial layer of *Zea mays (*treated).



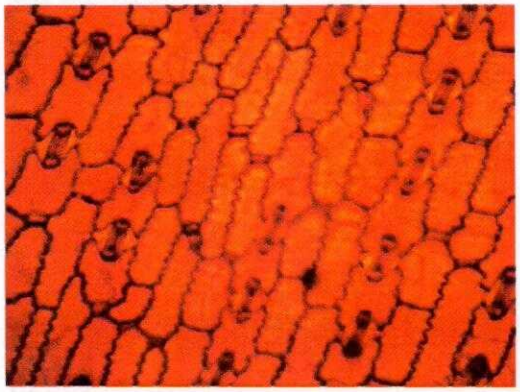
5.



6.



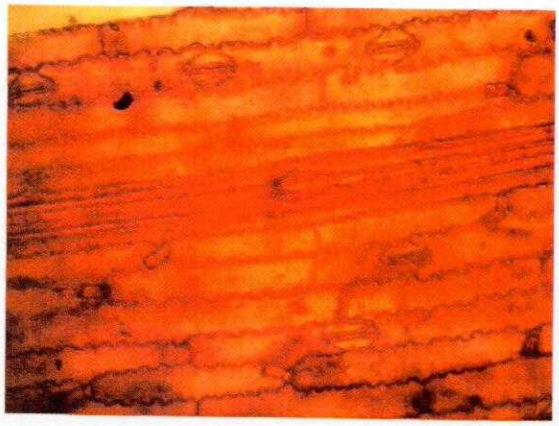
7.



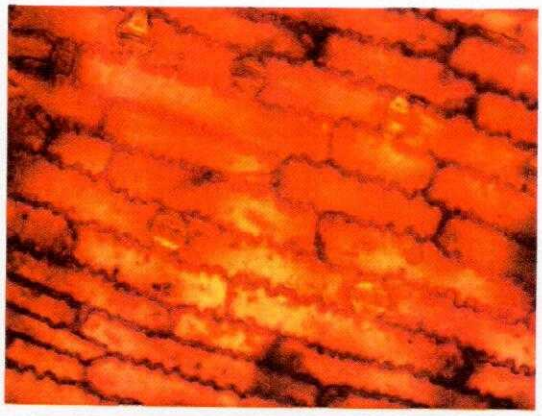
8.

Plates 5 and 6 shows the photographicmicrograph of the abaxial layer of *Zea mays (*control).

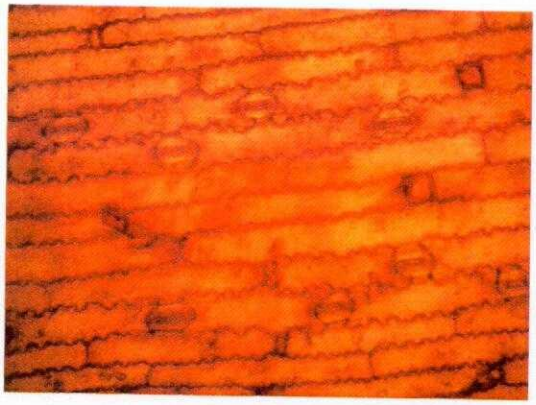
Plate 7 and 8 shows the photographicmicrograph of the abaxial layer of *Zea mays (*treated).



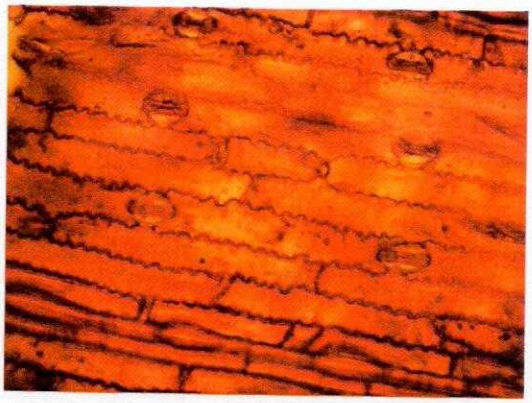
13.



14.



15.

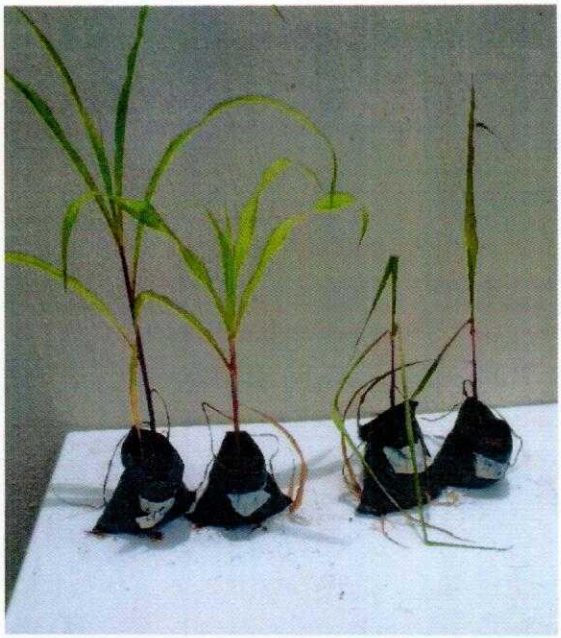


16.

Plates 13 and 14 shows the photographicmicrograph of the abaxial layer of *Pennisetum glaucum (*control).

Plates 15 and 16 shows the photographicmicrograph of the abaxial layer of *Pennisetum glaucum (*treated).

17.



18.

19.

Plate 17 and 18 represents the control and treated plant of *Zea mays.*

Plates 19 represents the control and treated plant of *Pennisetum glaucum*

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISSCUSION

Drought like other environmental stresses affect some physiological and metabolic processes within a plant (Spollen *et al.,* 1993). Drought result in considerable decrease in whole dry weight of *Zea mays* and *Pennisetum glaucum* as a model crop. In this study, the total dry weight of *Zea mays* showed a significant decrease in the treated plant compared to the control while in the *Penisetum glaucum* there was a significant increase in whole plant dry weight in contrast to the control. The reduction in the whole plant dry weight could be attributed to drought effect to photosynthesis as it affects the gas exchange parameters such as carbon assimilation rate and stomata conductance (Yin *et al.,* 2005). Previous studies reported that root/shoot weight ratio were increased and shoot growth were reduced by drought stress (Alvarez *et al.,* 2009). Drought stress also increased the root/shoot ratio in (figure 2) of both genotypes, the reason might be that plant grown under drought stress improved water use efficiency by increasing the proportion of water absorbing root biomass relatively to water loosing shoot biomass (Lei *et al.,* 2006).

Photosynthetic pigment such as chlorophyll-a and chlorophyll-b are the main components of photosystem driving dry matter production. Drought stress lead to a significant reduction in total chlorophyll content as observed in *Zea mays* while there was a slight increase in chlorophyll content of the crop exposed to drought in *Pennisetum glaucum.* This result confirm present observation by other researchers that drought stress could increase chlorophyll levels in millet species under stress condition (Hayat *et al.,* 2007; Cornic and Massaci, 1990).

The relative water content is used to indicate the extent of dehydration. Changes in leaf water content is one of the responses of whole plant to drought stress (Matin *et al.,* 1989). The result

of this study indicated reduction in *Zea mays* genotype exposed to drought stress while the *Pennisetum glaucum* experienced no significant difference. This agreed with the result earlier reported in maize (Levent Tuna *et al.*, 2007) and wheat (Agarwal *et al.*, 2005).

Drought stress led to a reduction in leaf area of *Zea mays* crop exposed to drought in contrast to *Pennisetum glaucum* crop exposed to drought as reported by Stone *et al.* (2001) where there was a reduction in response to deficit irrigation in maize and sorghum.

The adaxial surface of the *Zea mays* always had more stomata than the abaxial surface due to high transpiration rate with increasing number of stomata (Lake *et al.*, 2002). The amphistomaotus nature of maize leaves means that they have the capacity to open and close their stomata on both sides independently, with transpiration rate being more sensitive to changes in stomata aperture on the surface. In table 1, the adaxial surface showed increase in stomata index in *Zea mays.* This result is in agreement with Lake *et al.* (2002) that plant with higher stomata densities generally have high conductance and photosynthetic rate while the stomata index in *Pennisetum glaucum* showed more increased stomata index in the abaxial surface which indicates that the millet crop has a sunken stomata when transpiration rate is high as observed by Watling *et al.* (2001).

These results show that *Zea mays* and *Pennisetun glaucum* response to various physiological responses as induced by drought.

4.2 CONCLUSION

In conclusion this study showed the effects of drought stress on the growth of maize crop as well as the drought tolerance ability of millet crop. Thus, the increase in incidence of extreme weather effect such as drought that could result from global warming can be resolved through biotechnological approaches by producing crop plant that has high tolerance to drought.

4.3 RECOMMENDATION

From the result of this research work I recommend that *Pennisetum glaucum* should be substituted for *Zea mays* in situations or areas where there is little or no availability of water. I also recommend that through biotechnological techniques creation of drought tolerant specie of *Zea mays* should be created and made available to farmers in these areas of low rain fall.

REFRENCES

Aberg, B. (1981). Plant growth regulators (XLI): Monosubstituted benzoic acid, *Sweden Journal of Agricultural Research* **11:**93-105

Agarwal S, Sairam R.K, Srivatava G.C. and Meena R.C., (2005) Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicyclic acid in wheat genotypes. *Biologia Plantarum* **49:** 541-550.

Alvarez S, Navarro N, Banon S. and Sanchez-Blanco M.J., (2009). Regulated deficit irrigation in potted *Dianthus* plants: effects of severe and moderate water stress on growth and physiological responses. *Scientia Horticulture* **122:** 579-585

Apel, K. and Hirt, H. (2004). Receive oxygen species: Metabolism, oxidative stress and signal transduction. *Annual Review of plant physiology* **55:** 373-399.

Ashraf, M. and Foolad, M.R. (2007). Roles of glycinebetaine and proline in improving plant biotic stress resistance. *Environmental Experimental Botany* **59**: 206-216

Azevedo, R.A., Alas, R.M., Smith, R.J. and Lea, P.J (1998). Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation in the leaves and roots of wild type and a catalase deficient mutant of barley, *physiologia Planatarum* **104:** 208 -292

Bartoli C.G., Simontacchi M., Tambuss E., Batrano J. and Drought (1999) and watering-dependent oxidative stress effect on antioxidant content in *Triticum aestiyum* L. leaves. *Journal Experimental Botany* **50:** 375-385.

Birch, C.J., Robertson, M.J., Humpherys, E., and Hutchins, N, (2003). Agronomy of maize in Australia- in review and prospect. In C.J Birch, S.R Wilson eds, *versatile maize- golden opportunities; 5th Australian maize conference, city of golf club, Toowoomba.*

Bolanous, J. and Edmedes, G.O. (1993) Eight cycles of selection for drought tolerance in low land tropical maize. Responses in grain yield, biomass, and radiation utilization. *Field Crops Research* **31:** 253-258

Chopra, V. L. (2001). *Breeding Field Crops.* Oxford IBH Publishing Company Ltd. England

Clegert B., Dingkuhm M., Chanterau J. Vaksman M., (2004). Does panicle initiationin tropical sorghum depend on day-to-day change in photoperiod? *Field crop Research* **88:** 21-37.

Cornic G. and Massacci A. (1996) Leaf Photosynthesis under drought stress In: Baker N.R edition, Photosynthese and the Environment, Kluwer Academic Publishers, Dordrechi 347-366Pp.

Eze J. M. O. (1965) studies on growth regulation, salt uptake and translocation PhD. Thesis. University of Durham, England pp 31-33

Denmead, O. and Shaw, R. (1960). The effects of soil moisture stress at different stages of growth on the development and yield of corn. *Agronomy journal*. **52**:272-274.

FAO (2007). Annual Publication. Rome, Italy: Food and Agricultural Organization

Farnham, D.E., Benson, G.O., Pearce, R.B. (2003). Corn perspective and culture. Chap1. In: PJ White, LA Johnson, eds. Corn. *Chemistry and technology,* Edition 2nd. American Association of Cerial chemicals, inc. St. Paul, Minesota, USA, PP1-33

Frova, C., Krajewski, P., Fronzo, N.D., Villa M., and Sari-Gorla (1999). Genetic analysis for drought tolerance in maize and molecular marker. I. Yield component. *Theoretical Applied Genetics* **99:** 218-288

Fischer, R. (1973). The effects of water stress at various stages of development of yield processes in wheat, In: Plant responses to climatic factor. *UNESCO*, Paris. pp 233-240.ame, B.R., Drayton, P.R., Bangall, Frame,

Gates, C. and Bonner. (1959).The response of young tomato plant to a brief period of water shortage. IV. Effects of water stress on RNA metabolism of tomato leaves. *Plant physiology*. **34**:49-55.

Gerber, M.A. and Dawson, T.E. (1990) Genetic variation and co-variation between leaf-gas exchange, morphology and development in *Polygonum arenastrum,* An annual plant. *Oecologia.* **85:** 153-158

Greenway, H., Hughes, P. and Klepper, M. (1969). Effects of water deficit on phosphorus of tomato plants. *Plant physiology* **22**:199-207.

Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G and Cicek, N. (2006). Salicy;ic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mayz* L.) grown under salinity. *Journal of Plant Physiology, **164:*** 728-736

Guo J. Yang Y. Wang G, Yang L, Sun X. (2010) Ecophysiological responses o *Abies fabri* seedlings to Drought stress and nitrogen supply. *Physiologia Plantarum* **139:** 335-347.

Harlan, J.R. and de Wet, J.M.J. (1977). Pathways of genetic transfer from Tripsacum to *Zea mays. Proceedings of the National Academy of Sciences* **74:** 3494-3497

Hayat S., Ali B. and Ahmad A., (2007) Salicylic acid, Biosynthesis, metabolism and physiological role in plants. In: Hayat S., and Ahmad edition: salicylic acid; *A plant hormone.* Springer Netherland 1-4

Hildmann, T., Ebneth, M.H., Pena-cortes, J.J., Sanchez-Serrant, L., Willmitzer, L. and Prat, S. (1992). General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding. *Plant Cell,* **4:** 1157_1170.

Hsiao T.C. (1973). Plant response to water stress. *Annual Review Plant Physiology*, **24:** 519-570.

IITA, (2004) Nigeria's Maize Industry: Statistical Handbook. 45pp.

Izge A. U. (2006) Combining ability and heterosis of grain yield component among pearl millet *(Pennisetum glaucum* L) in breds PhD Thesis, Federal University of Technology, Yola, Nigeria pp: 148.

Jackson R.B., Sperry J.S., Dawson T.E., Root water uptake and transport using physiological processes in global prediction. *Trends. Plant science* **5:** 482-488

John Wiley and Sons, New Loomis, T., Williams, W. and Hall, A. (1971). Agricultural productivity. *Annual Review on plant physiology.* **22:** 431-468.

Juarez M. T, Twigg R. W, Timmemans M.C.P. (2004) Specification of adaxial cell fate during maize leaf development. *Development* **131:** 4533-4544.

Kramer P.J., (1980). Drought stress and origin of adaptation. In N.C. Turner, and P.J. Kramer (ed.) Adaptation of Plants to Water and High Temperature Stress

Klumpp, S. and Krieglstein, J. (2002). Phosphorylation and dephosphorylation of histidine resides in protein. *Europe Journal of Biochemistry,* **269:** 1067-1071.

Lake J.A, Quick W.P, Woodward F.I. (2002). Long-distance CO_2 signalling in plants. *Journal of Experimental Botany,* **53:** 183-193

Levent Tuna A., Kaya C, Dikkilitas M, Yokas I, Burun B, Altunlu H, (2007) Comparative effects of various salicylic acid derivatives on key growth parameters and some enymes activities in salinity stressed maize (*Zea mays* L) plants. *Pakistan Journal of Botany* **39** (3)**:** 787-798.

Lei Y.B, Yin C.Y., Li C.Y. (2006) Differences in some morphological, physiological and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii. Phsiologia Plantarum* **127:** 182-191

Loomis, T., Williams, W. and Hall, A. (1971). Agricultural productivity. *Annual Review on plant physiology.* **22**: 431-468.

Machlachlan S., Zalik S., (1963) Plastid structure of chlorophyll concerntration and free amino acid composition of a chlorophyll mutant of barley. *Canadian Journal of Botany* **41:** 1053-1062.

Matin M.A., Brown J.H., Ferguson H., (Leaf water potention, relative water content, and diffusive resistance as screening technique for drought resistance in barley. *Agronomy Journal* **81:** 100-105

McConn, M., Creelman, R.A., Bell, E., Mullet, J.E and Browse, J. (1997). Jasmonate is essential for insect defense in *Arabdosis, Proceedings of National Acedemics Science, U.S.A.,* **94:** 5473-5477

Misovic, M.S. (1985). Maize breeding methodologies for environmental stress. In breeding strategies for maize production in the tropics. Florence and Bergam pp: 207-227

Morgan J.M. (1984) Osmoregulation and water stress in higher plant. Annual Revision. *Plant Physiology* **35:** 299-319

Mudgal, V., Madaaan, N. and Mudgal, A. (2010). Biochemical mechanisms of salt tolerance in plants: *A review: International Journal of Botany,* **6:** 136-143

Noctor, G. and Foyer, C. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Animal Review of Physiological and Plant Molecular Biology,* **49:**249-279

Nojavan, A.M. and Khorshidi, M. (2006). An investigation of vanillin imposed oxidative stress in corn (*Zea mays* L.) and the activities of antioxidative enzymes. *Pakistan Journal of Biological Science,* **9:** 34-38.

Penuelas J., Munnne-Bosch S, llusia J. and Filella I., (2004) Leaf reflectance and photo and antioxidant protection in field-grown summer stressed *Phillyrea angustifolia.* Optical signals of oxidative stress. *New Phytology* **162:** 115-124

Raskin, I. (1992). Salicylate, a new plant hormone. *Plant Physiology* ***99*:** 799-803

Reddy, A.R., Chaitanya, K.V. and Vivekanandan, M. (2004). Drought-induced response of photosynthesis and antioxidant metabolism in higher plants. *Plant physiology,* **161:**1189-1202

Reymond, P. and Farmer, E.E (1998). Jasmonate and salicylate as global signals for defence gene expression. *Current Opinion in Plant Biology,* **1:** 404-411

Ribaut, J.M, Jiang, C., Gonzalez-de-leon, D. and Edmeades, G.O. Identification of quantitative trait loci under drought conditions in tropical maize 2. Yield component and marker assisted selection strategies. *Theoretical Applied Genetics* **92**(7): 905-940

Schulze, E.D. (1986) Carbon dioxide and water vapour exchange in response to drought in the atmosphere and soil. Annual Revision. *Plant Physiology.* **37:** 247-274.

Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova, M.V. and Farkhutdinova, D.R. (2003). Changes in hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Science,* **164:**317-322.

Stone, P.J., Wilson, D.R. and Jamieson P.D., (2001) Water deficit effect on sweet corn, II. Canopy development. *Australian Journal of Agricultural Research.* **52:** 115-156

Spollen, W.G., Sharp R.E., Saab I.N. and Wu, Y. (1993)Regulation of cell expansion in roots and shoots at low water potentials, In: Smith J.A.C and H. Griffiths. Water deficit plant responses from cell to community. Bios Scientific Publishers, Oxford. 37-52

Squire, G. R. (1979). The response of the stomata of pearl millet (*Pennisetum typhoid's)* to atmospheric humidity. *Journal of Experimental Botany* **30:** 925-933.

Squire, G.R., Ong, C.K. and Montecito, J. L. (1987) crop growth in semi-arid environments, in *proceedings of the International Pearl Millet workshop,* 7-11, eds Witcombe J. R., Beckman S. R., editors. (Patancherú; ICRISAT ;) 219-231

Slatyer, R. (1957). Significance of the permanent wilting percentage in studies of plant and soil- Water relations. *Botany Review*. **23:** 585-636.

Turner N.C., (1981) Techniques and experimental approaches for the measurement of plants water status. *Plant and Soil* **58:** 339-336.

Tiryaki, I. (2004). Hormones signalling pathways in plants: The role of jasmonic acid in plant cell signalling. *Turkish Journal of Agriculture,* **28:** 291-299.

Watling, J.R., Press, M.C. and Quick, W.P. (2000). Elevated CO_2 induces biochemical and ultrastructural changes in leaves of the C_4 cereal sorghum. *Plant physiology* **123:**1143-1152

Yin C., Peng Y., Zang R, Zhu Y., Li C., (2005) Adaptive Responses of *Populus kangdingensis* to drought stress. *Physologia plantarum,* **123:** 445-451

Zhu, Y.L., Pilon-Smith, E.A.H., Jouanin, L. and Terry, N. (1999) over expression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. *Plant Physiology,* **199:**1073-1079

APPENDIX

BIOMASS

MAIZE

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**43**	**25.57**	**12.9**	**1.2**
2	**21.5**			
3	**12.2**			
Treated				
1	**6.1**	**6.9**	**0.95**	**0.22**
2	**6.3**			
3	**8.2**			

Table showing mean, standard deviation and standard error for biomass of maize.

BIOMASS

PEARL MILLET

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**13.9**	**5.5**	**5.95**	**1.23**
2	**1.6**			
3	**1**			
Treated				
1	**13.1**	**5.23**	**4.24**	**0.34**
2	**1.6**			
3	**1**			

Table showing mean, standard deviation and standard error for biomass of pearl millet.

ROOT/SHOOT RATIO

MAIZE

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**0.02**	**0.063**	**0.031**	**0.02**
2	**0.09**			
3	**0.08**			
Treated				
1	**0.12**	**0.117**	**0.0287**	**0.03**
2	**0.15**			
3	**0.08**			

Table showing mean, standard deviation and standard error for root/shoot ratio of maize.

ROOT/SHOOT RATIO

PEARL MILLET

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**0.03**	**0.26**	**0.291**	**0.01**
2	**0.67**			
3	**0.08**			
Treated				
1	**0.06**	**0.1133**	**0.0386**	**0.02**
2	**0.13**			
3	**0.15**			

Table showing mean, standard deviation and standard error for root/shoot ratio of pearl millet.

TOTAL CHLOROPHYLL CONTENT

MAIZE

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**0.0222**	**0.3351**	**0.2234**	**0.03**
2	**0.4541**			
3	**0.5291**			
Treated				
1	**0.1492**	**0.1057**	**0.0597**	**0.13**
2	**0.1467**			
3	**0.0213**			

Table showing mean, standard deviation and standard error for total chlorophyll content of pearl millet.

TOTAL CHLOROPHYLL CONTENT

MILLET

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**0.9381**	**0.3508**	**0.4177**	**0.21**
2	**0.1123**			
3	**0.0019**			
Treated				
1	**0.1095**	**0.6306**	**0.3691**	**0.24**
2	**0.9173**			
3	**0.8649**			

Table showing mean, standard deviation and standard error for total chlorophyll content of pearl millet.

RELATIVE WATER CONTENT

MAIZE

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**70.5**	**58.5**	**27.86**	**11.5**
2	**85**			
3	**20**			
Treated				
1	**55**	**43.133**	**19.9**	**16.1**
2	**59.3**			
3	**15.1**			

Table showing mean, standard deviation and standard error for relative water content of maize.

RELATIVE WATER CONTENT

PEARL MILLET

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**42**	**23.4**	**13.23**	**11.5**
2	**15.8**			
3	**12.4**			
Treated				
1	**53.3**	**25.27**	**19.85**	**7.65**
2	**12.4**			
3	**10.1**			

Table showing mean, standard deviation and standard error for relative water content of pearl millet.

LEAF AREA

MAIZE

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**80.46**	**53.31**	**27.801**	**6.45**
2	**64.37**			
3	**15.11**			
Treated				
1	**26.44**	**36.023**	**11.189**	**1.96**
2	**51.72**			
3	**29.91**			

Table showing mean, standard deviation and standard error for leaf area of maize.

LEAF AREA

PEARL MILLET

Replicates	Values	Mean	Standard deviation	Standard error
Control				
1	**19.54**	**13.973**	**4.0119**	**6.45**
2	**10.24**			
3	**12.14**			
Treated				
1	**13.05**	**16.553**	**3.407**	**1.96**
2	**15.44**			
3	**21.17**			

Table showing mean, standard deviation and standard error for leaf area of pearl millet.

Smith, A.J. (2001): Poultry. The Tropical Agriculturalist, Revised Edition; CTA/Macmillan Publishing Company. 1-78.

SOFIA. 2007. The state of world fisheries and aquaculture 2006. FAO fisheries and aquaculture department, Rome, 2007, pp. 1-180.

Soltan, M.A.; Ibrahim, M.K.; Fatma, A.H.; and Fath El-Bab, A.F. (2001). Effect of partial and total replacement of fish meal by soybean meal on growth and proximate analysis of Nile tilapia (*Oreochromis niloticus*). Egypt. *J. Nutr. & Feeds.* **4** (Special Issue): 799-812.

Soltan, M.A. (2005a). Partial and total replacement of soybean meal by raw and heat treated linseed meal tilapia diets. Egypt. *J. Nutr. & Feeds* (Special Issue). **8** (1): 1091-1109.

Soltan, M.A. (2005b). Potential of using raw and processed canola seed meal as an alternative fish meal protein source in diets for Nile tilapia (*Oreochromis niloticus*). Egypt. *J. Nutr. & Feeds* (Special Issue). **8** (1): 1111-1128.

Tacon, A.G.J. (2001). Fish meal and fish oil: Globalsupply, Demand and Outlook, Report to international food policy research institute, Washington, D.C.

Tacon, A.J. and Forster, I.P. (2001). Global trends and challenges to aquaculture and aquafeed development in the new millennium. http;//www.seaweb.org/AAAS/trends html. Accessed on August, 2015.

Tacon, A.J. and Metian, M. (2009). Fishing for feed of fishing for food – Lenfes Ocean Program. *Ambio*, **38**(6): 294 – 302.

Tacon, A.J. (2011). The role of rendered products in aquaculture. http://cdn. harmonyapp.com/assets/4f26fba7dabe9

d49a7063c1c/9_albert_tacon_rendered_ products_in_aquaculture2.pdf. Assessed August, 2015.

Verdin, J. (2012). Governance of West African fisheries: Experiences from the West Africa regional fisheries program. 20pp.

Verreth, J.H.; Eding, G.M.; Rao, F.H. and Segner, H. (1993). A review of feeding practices, growth and nutritional physiology in larvae of the catfishes *Clarias gariepinus* and *Clarias batrachus*, j. *world aquacult. soc.* **24**: 135-144.

Waldron, K.W.; Parker, M.L.; and Smith, A.C. (2003). Plant cell wall and food quality. A review. *J. Sc. Food Technol.* **2**: 109 – 110.

Watanabe, T.H.; Aoki, V.; Viyakarn, M.; Maita Y.; Yamagata, S.S.; and Takeuchi, T. (1995). Combined use of alternative protein sources as a, partial replacement for fish meal in a newly developed soft-dry pellet for yellowtail. *Suisan Zoshoku.* **43**: 511-520.

Yang, S.D.; Liou, C.H.; and Liu, F.G. (2002). Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture.* **213**: 363-372.

Yuangso, B. And Masumoto, T. (2012). Replacing moringa leaf (*Moringa oleifera*) partially by protein replacement in soybean meal of fancy Carp (*Cyprinus carpio*); 2012. Available: http://rdo.psu.ac.th/sjstweb/Ar-Press/55-Aug/25.pdf 3rd, Oct.