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SIMULATED DROUGHT INFLUENCES OXIDATIVE STRESS IN *Zea mays* SEEDLINGS

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ABSTRACT
Drought is an abiotic factor that limits the productivity of crop plants survival and productivity. This study was conducted to evaluate the effects of simulated drought on the malondialdehyde (MDA) and antioxidant enzymes activity in Zea mays. Seedlings were grown for 8 weeks in nursery bags filled with sandy-loam soil in two categories. Category 1 which serves as a control and received 300 ml of water every three days throughout the experimental period . Category 2 received 300ml of water every 2 days for 5 weeks before subjecting them to simulated drought. Physiological and metabolic parameters which include biomass, relative water content, total chlorophyll, oxidative damage and antioxidant enzyme activity were evaluated after the treatments. Analysis of variance at 0.05 significance level was used as the statistical tool. The results showed that drought condition caused a significant decrease in biomass, total chlorophyll, relative water content (RWC) of the plant and a significant increase in MDA level, and activity of catalase peroxidase. It was concluded that drought made water absorption by plants difficult and also induced oxidative stress in plants.
Keywords: Catalase, drought, oxidative stress, seedlings, Zea mays

INTRODUCTION

Water is an important requirement for biochemical processes in all forms of life. Drought stress (also referred to as water or osmotic stress) limits the amount of water available for use in metabolic processes (Xiong *et al*., 2002). In extreme cases, drought stress may result in desiccation, which is the loss of most of the protoplasmic free water (Mundee *et al*., 2002). Desiccation results in cytoplasmic increase in concentrations of toxic ions which inhibits metabolic processes (Mundee *et al*., 2002). Like other abiotic stresses, drought stress therefore reduces the potential productivity of crops (Tester *et al*., 2005). However, the level of reduction varies with the degree of the interaction of drought stress with factors, such as genotype, developmental stage, as well as duration and severity of the stress (Salekdeh *et al*., 2007).

Maize is the third most important food crop worldwide (Frova *et al.,* 1999). It is used in more ways than any other cereal. Africa produces 6.5% in the world and the largest African producer is Nigeria with nearly 8 million tons (IITA, 2004).Therefore it is considered as a multi-purpose crop and has been put to a wider range such as human food, animal and poultry feed and for hundreds of industrial purposes. Maize grows over a wider geographical and environmental range than any other cereals . It is exposed to more hazards and it is a higher risk crop in general (Pandey *et al*., 2000).

Drought is an important climatic phenomenon, which after soil infertility, ranks as the second most severe limitation to maize production (Sallah *et al*., 2002). Maize is more susceptible to drought at its flowering stage than most other crop. In Nigeria, Maize production therefore, is of strategic importance for food security. All parts of the crop can be used for food and non-food products (IITA, 2004). In industrialized countries, maize is largely used as livestock feed and as a raw material for industrial products. It varies in colour of which yellow and white are the most important.

The main objective of this study is to assess the effects of drought on some biochemical parameters that induce oxidative stress using *Zea mays* .

MATERIALS AND METHODS

Plant Growth and Treatments

Seeds of *Zea mays* used for the study were collected from IITA, Ibadan, Nigeria. Maize genotype were planted in polythene bags containing sandy- loam soil to achieve 3 seeds per bag. Plants were watered regularly for 1 week to keep soils moist.After germination, the seedlings were thinned out to 1 seedling per nursery bag, and were arranged in a randomized block design. The study was carried out at the Biological garden of University of Lagos. Plants were grouped into 2 categories and replicated 10 times before subjecting them to 8 weeks of treatments.

Category 1 plants served as the control and received 300 ml of water every 3 days for the duration of the experiment. Category 2 plants received 300ml of water before subjecting to simulated drought . At the end of the treatment period, plants were harvested and physiological and biochemical parameters were evaluated.

One seedling was put in each bag representing each treatment and this was replicated six times..

Biomass Determination

Harvested plants were washed thoroughly in running tap water to remove attached soil particles and rinsed twice with distilled water. They were then placed in labeled paper bags and weighed after oven dried at 65 ^{o}C for 72 h. (Causton, 1994).

Relative Water Content (RWC)

The fourth leaf from top (fully expanded young leaf) of the plants representing each treatment
were harvested and weighed to determine their fresh weight (FW). The leaves were submerged separately in distilled water for 24 h in the dark. They were removed from the water after this period, mopped dry using an absorbent and weighed to determine their saturated weight (SW). The leaves were then placed in paper bags and dried in an oven at 65 ^{o}C for 72 h, following incubation in the oven, the weights were then taken to get the dry weight (DW).

The relative water content was calculated using the formula: RWC (%) = [(fresh weight - dry weight)/ (saturated weight - dry weight)] x100. (Turner, 1981).

Determination of Total Chlorophyll

Plant leaves (0.5g) were ground in 10ml 80% acetone in the dark. After centrifugation at 4000 g for 5 min, the absorbance of the supernatant was read at 645 and 663 nm according to Arnon DI (1949). The total chlorophyll content was calculated using the formula given by Machlachlan & Zalik (1963).

Lipid Peroxidation Measurement

Lipid peroxidation was measured by estimation of the malondialdehyde (MDA) content following a modified procedure of Wang and Jin (2005). Fresh leaves (0.5 g) were homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 g for 5 min. The supernatant (1ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450, 532 and 600 nm. The concentration of MDA was calculated as 6.45 (A532-A600) - 0.56 A450.

Determination of Catalase Activity

For enzyme analysis, fresh samples of leaves (100 mg each) were ground in a ceramic mortar and extracted with 5 ml of 100 mM potassium phosphate buffer pH 7.5, with 1% (w/v) polyvinylpyrrolidone. (Aebi ,1984) The homogenate was centrifuged at 12,000 rpm for 5 min. The supernatant was used for the estimation of antioxidant enzyme activities. Catalase (CAT) activity was determined according to Aebi (1984), by monitoring the decrease in the absorbance at 240nm as a consequence of H_20_2 disappearance.

Statistical Analysis

Means of three replicates as well as their standard errors (SE) were determined. The test of significance ($P< 0.05$) between the treatments was done using a one-way analysis of variance (ANOVA).

RESULTS

Treatments of *Z. mays* seedlings with drought inhibited the growth as indexed by dry weight biomass of the plant. The inhibitory effect was dependent and biomass accumulation significantly ($P<0.05$) decreased with increase in water deficit condition (Figure 1). While the control and the treated plants had a mean dry weight biomass of 23.07 ± 0.27 g and 12.03 ± 0.1g respectively.

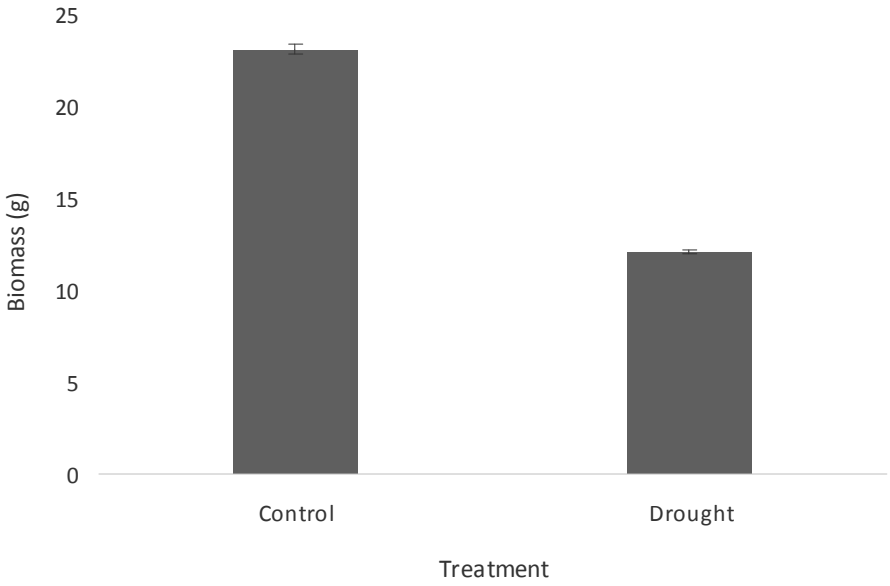

Fig.1: Dry weight of *Z. mays* seedling treated with water and exposed to drought. Error bars represents standard error (n=3).

Data showing the effect of drought on the relative water content of *Z. mays* is represented in Figure 2. It was observed that drought significantly (P< 0.05) reduced the relative water content of the plant. The control plants had a mean RWC of 78.01 ± 1.11% as against 42.55± 1.2 % observed for plants exposed to drought.

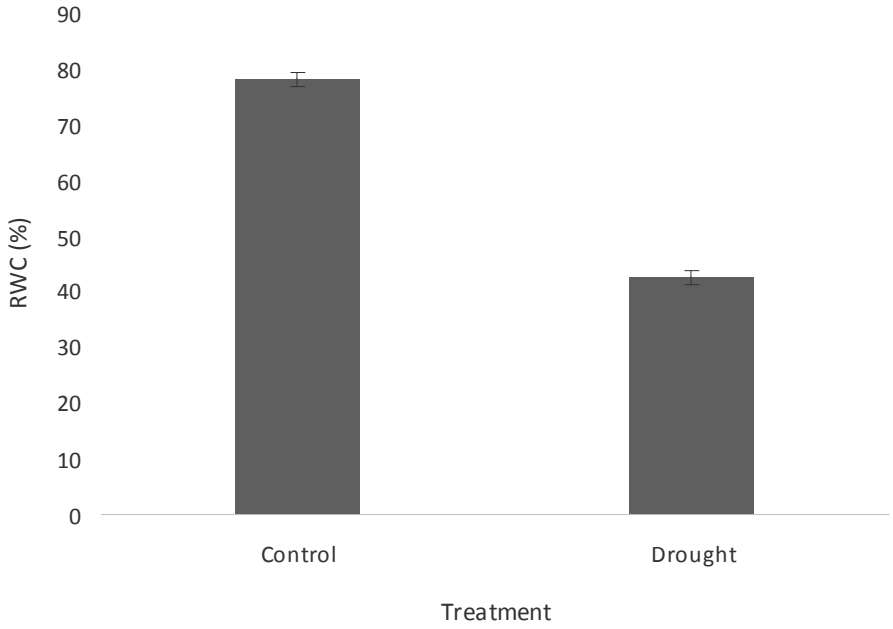

Fig.2: Relative water content of *Z. mays* leaves exposed to water and drought. Means and standard errors of 3 replicates are presented(n=3).

In this study, the total chlorophyll in *Z. mays* was quantified to know the effect of water stress treatment on the photosynthetic pigment level. It was observed that drought consistently reduced the amount of chlorophyll present in the leaves of the experimental plant. While the control plants had a mean value of 0.721 ± 0.012 mg/g fresh weight, seedlings treated with water as against plants exposed to drought with a value of 0.324 ± 0.01 mg/g fresh weight respectively (Figure 3).

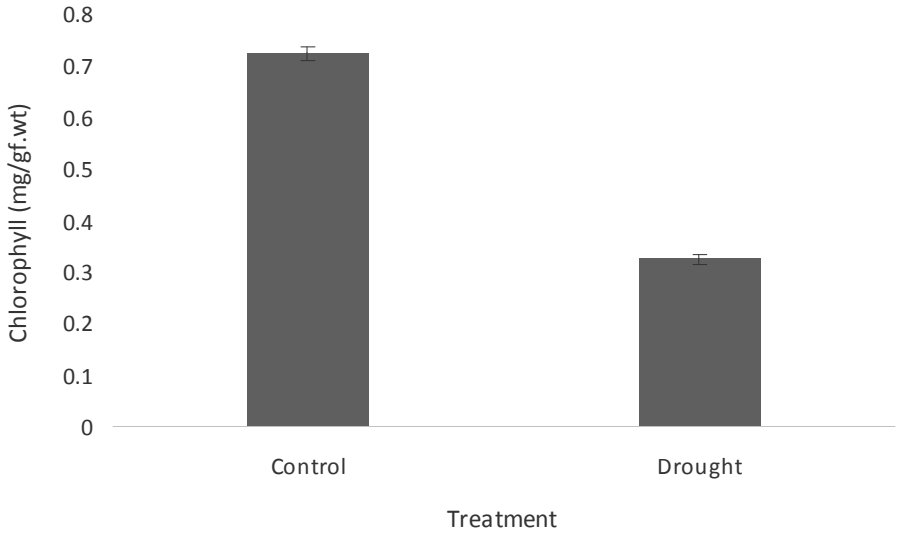

Fig. 3: The effect of drought on the total chlorophyll content in the leaves of *Z. mays* seedlings . Means and standard errors of 3 replicates are presented (n=3).

To determine the impact on lipid peroxidation, the malondialdehyde in the root tissue of *Z. mays* seedlings was measured. It was observed in this study that the plants exposed to drought caused lipid peroxidation as it led to an increase in MDA when plants were exposed to drought compared with the control (Figure 4). The control plants had a mean MDA content of 0.96 ± 0.04 mg/g fresh weight as against 1.31 ± 0.27 mg/g fresh weight observed for plants respectively.

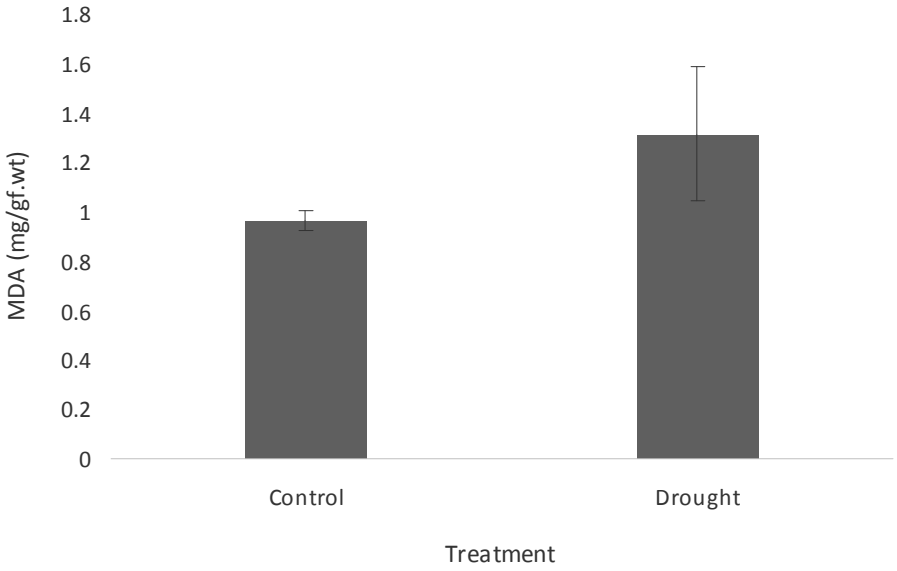

Fig.4: Malondialdehyde (MDA) content of *Z. mays* seedlings exposure to drought. Error bars represents standard errors (n=3).

Catalase activity was evaluated as a representative enzyme involved in antioxidant metabolism. The mean values observed in *Z. mays* grown under control and drought condition are as shown in figure 5. In this study, it was observed that foliar nitrogen supply had no influence on catalase activity under non-saline condition. It was however observed that salinity significantly increased catalase activity and the effect of foliar application of nitrogen was obvious.

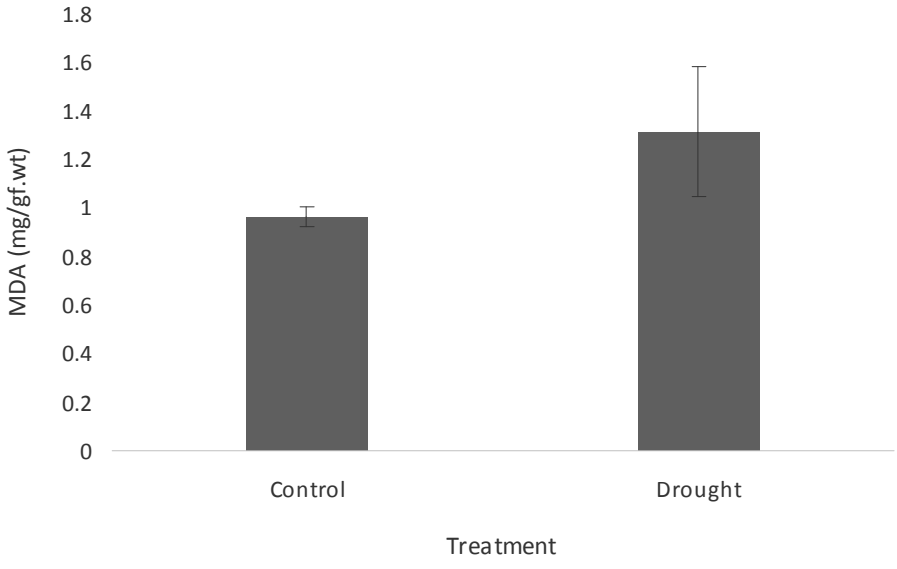

Fig.5: Catalase activity of *Z. mays* seedlings as affected by drought treatment. Means and standard errors of three replicates are presented.

DISCUSSION

The present study was conducted to assess the physiological and biochemical dysfunctions that could be associated with water stress in *Zea mays* seedlings. Drought affects plant growth in different parts of the plants. The low biomass accumulation observed in this study for plants exposed to drought could be due to the effect on photosynthesis which reduces stomata conductance (Yin *et al*., 2005). The result of this study indicated reduction in RWC under drought stress. This is in conformity with the result reported by Aranjuelo *et al*.(2011) in response to drought in the alfalfa plant. Drought usually disrupts the normal plant water relationship of the roots within the soil which consequently affect the amount of water absorbed by the plant thus drought resulted in low relative water content (RWC) in this study.

Drought affects water which make absorption of mineral nutrients and water by plant roots difficult. (Alvarez *et al*., 2009).

Generally, drought stress led to a significant reduction in total chlorophyll content. Inhibition of photosynthetic metabolism results in the diminished amount of photosynthetic assimilates available for sucrose and starch synthesis. Moreover, the activity of sucrose phosphate synthase is also greatly reduced by the water deficit and the ratio of starch/sucrose alters (Lawlor *et al*., 2002).This result confirmed previous observations by other researcher that drought led to reduced chlorophyll levels in plants under stress condition (Ghannoum, 2009: Lawlor *et al*., 2009; Lopes *et al*., 2011). This result suggests that drought causes a decline in the gross rate of carbon assimilation and the decrease of intercellular CO_2 concentration under drought stress.

With respect to the damage caused by drought to plants, drought induced considerable damage on the cellular membrane of *Z. mays*, as assessed by lipid peroxidation (Acworth and Bailey,1997). The capacity to avoid membrane damage during dehydration process is crucial for the maintenance of membrane integrity. Drought led to increased generation of reactive oxygen species (ROS) leading to photo oxidation and degradation of photosynthetic membrane proteins and associated pigments and lipids, and disorganization of thylakoid membranes (Reddy *et al*., 2004).

.Lastly, the severity of cellular damage was significantly reduced in plants that were treated with water while the MDA content was significantly increased.

It is observed that higher plants resist free radicals by increasing the activities of antioxidant enzymes after exposure to drought (Halliwell and Chirico, 1993), and this response reflects an adaptation of a plant to its environment (Yordanova *et al.,* 2004). In the present study, it was observed that the activities of catalase peroxidase increased significantly when *Z. mays* seedlings were exposed to drought. The increase in activity of these antioxidant enzymes as well as MDA content, emphasized the fact that drought stress induced oxidative stress in plants.

Conclusion

Drought is likely the most important environmental factor that adversely affects plant growth and development. Biochemical approaches together with physiological analysis, were used to analyze the response to drought in maize genotypes with response tolerances to dehydration.

The output of this study allowed the identification of oxidative stress responses with potential regulatory roles, thus providing a basis for the deeper understanding of drought stress response mechanisms.

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