Osmoregulatory appraisal of some osmoprotectantson hydrolytic activities of some enzymes on seeds oftwo water stressed cultivars of sorghum bicolor (Moench)

ABSTRACT

Enzymes play significant roles in metabolic processes of seeds. Therefore, this study evaluated osmo-regulatory potential of some osmoprotectantsonactivities of some hydrolytic enzymes in the seedsof two cultivars(SOSAT.C-88 and CV. LCIC 9702) of sorghum bicolor. Maturedseedsof the two cultivarswere harvested and prepared foralpha, beta, total amylase and proteinase activities assay. The osmoprotectantsproduced significant variations on the enzymes at 10 and 14 days (DA) of8weeks after treatments (WAT). Seeds of well-watered SOSAT.C-88 produced higher alpha (2.10 IU/ml), beta (1.70 IU/ml) and total amylase activities (3.30 IU/ml)at 14 days (DA). Higher alpha (2.01 IU/ml and total amylase activities (2.61 IU/ml) were recorded in theseeds of CV. LCIC 9702well-wateredat 14 days DA 8WAT. Furthermore,total amylase activities (3.87 IU/ml) were recorded in the seedsproduced by CV. LCIC 9702 well-watered at 14 days DA. Significant increase was noticed in beta (1.14 IU/ml) and alpha amylase (1.58 IU/ml) in the seedsof CV. LCIC 9702 treated with mycorrhiza.CV. LCIC 9702 wellwatered produced highest proteinase activities (1.57 U/ml)while least of the parameters were recorded inSOSAT.C-88 and CV. LCIC 9702droughted. In conclusion, the osmoprotectants had regulatory effects on the activities of hydrolytic enzymes therefore the use of the osmoprotectants in farming should be encouraged.

Keywords: Biochemical process, Alpha activities, Beta activities, Total amylase activities, Proteinase activities, Trehalse, proline, Coconut milk, Myccorhiza

1. INTRODUCTION

The need to increase crop production in other to meet the food demand of ever-increasing population in developing country such Nigeria is envisaged. According toZhu *et al.*(2004) and Muhammad (2018),yield of crops such as sorghum as one of the staple foods consumed in Nigeria is drastically decreasing due to unfavourableagro-climaticconditions such as water deficit. Erratic change being observed in the occurrence of weather parameters is a limiting factor for low crop production and increased rate of food insecurity in different parts of Nigeria(Haider, 2019; Lilianeet *al.*, 2020;Anie*t al.*,2021).Crop failure, low production efficiency and total extinction of many economic plants are other common incidences recorded among farmers and horticulturalists due to inability of such plants to cope with deleterious effects of drought(Fahad*et al.*, 2017; Temesgen2020; Seleiman*et al.*., 2021).

Water stress affectsnot only morphological charactersofplants but also physiological, biochemical, metabolic and nutritional compositionsof plants (Mangena2018). Seeds of many plants remain dormant due to lack of adequate supply of water needed for enzymatic-driven-hydrolysisused forreactivation of the metabolic activity of such seed embryo and emergence of radical and plumule(Ali *et al.*, 2017;El-Maarouf-Bouteau, 2022). Excessive dehydration can also impede synthesis of some hydrolytic enzymes de novo(Auber*et al.*, 2018)and their activation. Severe water deficit has ability to alter physiological process of some enzymes that can enhance effective germination, nutrient channelization and mass production of the seeds(Youyan*et al.*, 2012) (Kabbadj*et al.*, 2017; Riya and Jos 2021). Presence of such enzymes helps to break downstarches, fats, carbohydrates or proteins into simpler forms for energy generation and easy translocation to the growing points of the embryo. All these conversions are regulated by metabolic activity of some specific enzymes in a proper sequence usually in the presence of adequate water (Sergiloand Arnold 2008). In matured seeds but still attached to the stalk, water needed for such enzymatic action is gotten from stem via xylem as main conducting tissue, therefore, high dehydration caused by drought may limit quantity of water that can be absorbed from the soil and channeled to the seeds for such enzymatic or metabolic activities(Bogati and Walczak 2022).

For plants to be able to tolerate drought, they form pathways of biochemical mechanism which maintain osmotic adjustment and enhance enzyme driven metabolic activities. In many mesophytes, environmental stresses such as drought and low temperature initiate gene expression that induce production of osmoprotectants or antioxidants which have ability to scavenge oxidative effect of reactive oxygen species (ROS). Under natural conditions,these osmolyteshelp to accommodate osmotic pressure, avoid cellular injury within the affected

cells (Nezhadahmadi*et al.*,2013),stabilize protein and membrane structures of plants under dehydration and they maintain osmotic balance(Zhu2002).

Depending on severity of drought, studies have shown that not all plants have potential to synthesize adequateosmoprotectantsto cope with water deficit which could have negative implication onactivities of somehydrolases onseeds.

The seedsaccumulate mainly secondary metabolites such as carbohydrates, proteins, and lipids as stress coping strategies which later catabolized torelease energy (Ahanger*et al.*, 2018;Punia*et al.*, 2021). Onthisbasis, the present study was conducted to evaluate osmoregulatory effects of some osmoprotectantson activities of some hydrolytic enzymes on seed of two cultivars of Sorghum bicolor.

2. MATERIALS AND METHODS

2.1. Study Area: The experiment was carried out at teaching and research botanical garden of Lagos State University. The garden is located on latitude: 6.4668. $N6^0$ 28'0.760' and longitude; 3.19917 $E3^0$11'57.006'

Seed Sterilization: Seeds of two cultivars (CVS.SOSAT.C-88 and CV. LCIC 9702)of sorghumbicolor (*Pennisetumamericannum*) were surface sterilized using 10% bleach (sodium hypochlorite) in order to remove surface microbes.

2.2 Soil Analysis: Top soil was collected at 0-4 inches using soil probe as described by Vijay *et al.,* (2019). Soil samples were collected at 500m apart considering the heterogeneity of soils by factoring in variation in soil type, slope and land use. The soil samples were prepared, stored and labeled in plastic bags for analysis.The nutrient status of the soil was determined using the standard analytical methods described in Sachan and Deeksha (2018).Soil nutritional attributes such pH, Organic Carbon, Organic Matter, Available Phosphorous, exchangeable sodium, potassium, magnesium, calcium, iron, copper and zinc as well as soil properties (sand, clay and silt) were determinedat theDepartment of soil science, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

2.3 Land preparation and experimental design: Theexperimental field was cleared and marked out with wooden pegs. The experimental field was divided into two plots of 120 by 60. Each plot was subdivided into 6 sub plots, each of which was sub divided into 5, representing replicates of each treatment. The plot size was 2 × 2 meters, rows were drilled as 15-20 cm apart and 4 inches depth was used to give uniform germination and effective low weeding. The experiment was laid out in randomized complete blocked design of four replicates

2.4 Preparation and application of osmoprotectants: L-Prolineand trehalos were prepared by modifying method of Ojewumi and Kadiri (2021). Exactly 2g of L-proline and trehalose were measured separately and dissolved in 1liter of water. Twenty (20 %) (200 mL) of the proline and trehaloswere determined from L- proline and trehalose prepared. From each preparation, 50 mL of each preparation was measured using measuring cylinder and applied on the seedlings at three days intervals using foliar application method.

2.5 Coconut milk: Coconut milk was obtained from coconut fruits. Two hundred (200 mL) of coconut milk was extracted from edible part ofmatured coconut fruits, measured using measuring cylinder and diluted with 800 mL distilled water to determine 20% coconut milk. Fifty (50 mL) of the 20 % coconut milk was applied at three days interval using foliar application. Water served as control.

2.6 Osmoprotectants application: Five seeds of the two cultivars were planted usingplanting distance of 2 × 2m and were allowed to grow for four weeks after which they were sprayedusingwith 20% trehalose, 20% proline and 20% coconut milk.

2.7 ArbuscularMycorrhizal Fungi Inoculation; The seedlings were inoculated with 20 g arbuscularmycorrhizal fungi (AMF) into 1cm hole made directly beside the seedlings once throughout the period of the experiment.

2.8 Drought Application; The physiologically droughtedCV. SOSAT. C-88 and CV. LCIC 9702 were denied water two weeks (droughtedorControl I)while control II were well watered throughout the period of the study.

2.9 Determination of enzymes activities

At maturity, seeds of the plantswere harvested, dried used to determine alpha, beta and total amylaseactivities.

Total Amylase (α and β) Activity; Seedsof control and treatedSOSAT C – 88 and CV. LCIC 9702) were used for amylase activity essay. The enzyme extract was made by crushing separately 5 grams of treated and the controlled seeds with pestle and mortar using 20 mL 1/10 m sodium acetate buffer, pH 5.0 maintained at 5^0 C with crushed ice and the buffer extract was filtered. One milliliter of the filtrate was added to 1 mL of the 1 % soluble starch in 1/10M sodium acetate buffer pH 5.0 and the reaction mixture incubated in a water bath (27^0C) for 1 hour after which the enzyme action was terminated by adding 2 mL of 3, 5 - dinitrosalicyclic acid reagent(DNSA). The (DNSA)was prepared by dissolving one gram of 3, 5-dinitrosalicyclic acid in 20mL of 2m NaOH and mixed with 30 g sodium potassium tartrate in 50 mL distilled water. The coloured solution formed was made up to 10 mL with distilled water and cooled under running tap water. The amount of reducing sugar produced was measured by reading the optical density blank which contained 1 mL of boiled enzyme extract that was similarly treated. The amount of reducing sugar formed was calculated from the standard curve of various concentrations of maltose (Oliveira*et al.*, 2002).

α – amylase Activity: Five milliliters of the crude enzyme extracts were heated in water set at 70 ^{0}C for 15 minutes to denature β – amylase activity (Oliveira*et al.*, 2002). One milliliter of the heated enzyme extract was incubated with 1 mL of 1% soluble starch in 1/10M sodium acetate buffer (PH. 5.0) at 27^0C for 1 hour. The enzyme activity was terminated by adding 2 mL of DNSA reagent to the reaction mixture and the amount of simple sugar produced was measured as for total amylase activity.

Proteinase Activity: Enzyme extracts were prepared in a manner similar to those of amylase activity but 20 mL of 0.05M sodium phosphate buffer, PH 6.0 were used as the extracting

buffer. Proteinase activity was determined using the Lowry Folin-ciocalteu method ofKadiri, (1999, 2014). Two milliliters of 1 % soluble casein which was freshly prepared in 0.05m sodium phosphate buffer PH 6.0 were added to 1 mL of the crude enzyme extract and the resulting solution incubated in the water bath at 45^0C for 1hour. After one hour of incubation, the same volume of 10% trichloroacetic acid solution was added to the reaction mixture so as to precipitate the unhydrolysed casein formed during the reaction (Shakeel*et al.*, 2011). The suspension formed was filtered to one milliliter of the filtrate, 5 mL of 2% Na_2Co_3, 0.05 mL 2.7 % sodium potassium tartrate, 0.05 mL 1 % $CUSO_4$ and 3 mL of 0.2 M NaOH were added. At the end of 10 minutes, 0.5 mL of folinciocalteu reagent was added and the resulting mixture left to stay for 30 minutes at 30 ^{0}C with shaking at intervals. The optical density of the resulting solution was measured at 70nm against blank that had 1 mL of boiled enzyme extract which was exactly treated as above. Proteinase activity was calculated using a standard curve of different concentration of tyrosine (Shakeel*et al.*, 2011).

3. Statistical Analysis

Statistical analysis system (SAS 2013) package was used for the analysis of one-way Analysis ofVariance (ANOVA) and significance of difference between means using least significant difference (LS) at$p<0.05$

Table 1: Soil properties of the experimental location

Properties	Soil
Sand (%)	12.67
Clay (%)	6.53
Silt (%)	95.05
pH (1:25 water)	6.56
Organic Carbon (%)	3.26
Organic Matter (%)	3.45
Available Phosphorous (mg/kg)	10.56
Exchangeable Na^{+} (cent/kg)	0.58
Exchangeable K^{+} (cent/kg)	0.76
Exchangeable Mg^{2+} (cent/kg)	0.44
Exchangeable Ca^{2+} (cent/kg)	0.45
Exchangeable Fe^{2+} (mg/kg)	0.83
Exchangeable Cu^{2+} (mg/kg)	1.34
Exchangeable Zn^{2+} (mg/kg)	6.32

4. RESULTS

Soil properties of the experimental location

The soil on which this experiment was conducted was mainly silt (95.05%), with P^H 6.56 and some exchangeable minerals out of which zinc (6.32 mg/kg) among others was the major mineral (Table 1)

Effect of drought and drought ameliorative treatments on alpha, beta and total amylase activities of two cultivars (CV. LCIC 9702 and (CV. LCIC 9702)***P. americanum*** **at various weeks after treatments**

Effects of some osmoprotectants on activities of hydrolytic enzymes in the seeds of two cultivars of Sorghum bicolor is presented in table 2. Results revealed that there was no significant difference ($p>0.05$) in the alpha, beta and total amylase activities in the seeds of SOSAT.C-88 treated with 20% trehalose, proline and coconut milk compared with other treatments at 6days drought application (DA) 6 weeks after treatment (WAT). Meanwhile at 10 and 14 days DA of the same 6WAT, the treatments produced significant variations on the activities of the enzymes. Similar significant observation was noticed in the activities of the enzymes in the seeds of SOSAT.C-88 treated at 6, 10 and 14 days DA 8WAT. Seeds of well-watered SOSAT.C-88 produced higher alpha amylaseactivities (2.10 IU/ml). Beta amylase (1.70 IU/ml) and total amylase (3.80 mg maltose/h mg protein) were produced at 14 days DA. Least beta (0.60 IU/ml),alpha (1.01 IU/ml) and total amylase (1.61 IU/ml) were recorded in SOSAT.C-88 droughted.

Table 3 revealed that 20% of the treatments had no significant effects on alpha, beta and total amylase activities at 6 days DA of 8WAT.At 10 and 14-days DA of 8WAT, the treatments produced significant effects on the activities of the enzymes. Higher alpha amylase (2.01 IU/mland beta(2.61 IU/ml) were recorded in the seedsat 14 days DA in (CV. LCIC 9702) well-watered 8WAT. Total amylase activities (3.87 IU/ml) was recorded in the seeds produced byCV. LCIC 9702)well-wateredat 14 days DA.

Higher beta amylase (1,14 IU/ml) and alpha(1.58 mg maltose/h mg protein) were noticed in the seeds of (CV. LCIC 9702) treated with mycorrhiza after 14 days DA while least total amylase (2.09 IU/ml) was recorded in (CV. LCIC 9702)droughted.In addition,proteinase activities (1.56 U/ml) was significantly higher in the seeds produced by well watered SOSAT.C-88 while least proteinase activity (0.80 U/ml) was recorded in SOSAT.C-88 droughted (Table 4).

Effect of drought and drought ameliorative treatments proteinase activity of two cultivars SOSAT. C-88 and CV. LCIC 9702 of *P. americanum* seeds at various weeks aftertreatments

Table 5 revealed the effect of drought ameliorating treatments on proteinase activities in seeds produced by *P.americanum* (CV. LCIC 9702). There was no significant difference ($p > 0.05$) in proteinase activities in the seeds produced by (CV. LCIC 9702) treated with 20% trehalose and proline across the treatments on 6, 10 and 14daysDA at 6WAT as well as 6 and 10 days DA 8 WAT. The observation showed significant difference compared with control. Similar observations were recorded on 10 and 14 days DA at 10 WAT. CV. LCIC 9702 watered produced highest proteinase activities (1.57 U/ml) followed by proteinase activities of the plant treated with 20% proline while least proteinase activities (0.56 mg tyrosine/h mg protein) were recorded inCV. LCIC 9702droughted.

Table 2: Effect of drought and drought ameliorative treatments on alpha, beta and total amylase activities of *P. americanum*(CV. SOSAT. C-88) at various weeks after treatment

	Alpha amylase activities (IU/ml)						Beta amylase activities(IU/ml)						Total amylase activities					
	6 weeks			8 weeks			6 weeks			8 weeks			6 weeks			8 weeks		
	DA (Days)			DA (Days)			DA (Days)			DA (Days)			DA (Days)			DA (Days)		
	6days	10days	14days	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days
20 % trehalose	1.5c	1.58^{bc}	1.50^{c}	1.59^{cd}	1.71^{b}	1.75^{cd}	$1.^{12b}$	1.15^{b}	1.18^{b}	1.05^{a}	1.07^{cd}	1.09^{c}	2.64^{c}	2.71^{bc}	2.70^{c}	2.67^{b}	2.78^{c}	2.84^{c}
20 % proline	1.49c	1.50^{c}	1.55^{c}	1.52^{d}	1.55^{c}	1.62^{d}	1.09^{b}	1.11^{b}	1.14^{b}	1.29^{a}	1.02^{d}	1.04^{c}	2.58^{c}	2.61^{c}	2.69^{c}	2.81^{b}	2.57^{d}	2.66^{c}
20 % Coconut Milk	1.48c	1.68^{abc}	1.75^{ab}	1.75^{bc}	1.80^{b}	1.87^{cd}	1.21^{b}	1.25^{b}	1.27^{b}	1.09^{a}	1.12^{bc}	1.40^{b}	2.5c	2.92^{bc}	3.02^{b}	2.84^{b}	2.92^{c}	3.27^{b}
20 g Mycorrhiza	1.65ab	1.71^{ab}	1.80^{a}	1.87^{ab}	1.92^{a}	1.98^{ab}	1.26^{ab}	1.28^{ab}	1.31^{b}	1.18^{a}	1.19^{b}	1.20^{c}	2.91^{b}	2.99^{b}	3.07^{ab}	3.05^{ab}	3.11^{b}	3.18^{b}
Control 1(droughted)	0.98d	1.10^{d}	1.220^{d}	0.87^{e}	0.95^{d}	1.01^{e}	0.75^{c}	0.82^{c}	0.88^{c}	0.48^{b}	0.51^{e}	0.60^{d}	1.72^{d}	1.92^{d}	2.10^{d}	1.35^{c}	1.46^{e}	1.61^{d}
Control II (well-watered)	1.71a	1.82^{a}	1.85^{a}	1.96^{a}	1.98^{a}	2.10^{a}	1.47^{a}	1.49^{a}	1.50^{a}	1.46^{a}	1.50^{a}	1.70^{a}	3.18^{a}	3.31^{a}	3.35^{a}	3.42^{a}	3.48^{a}	3.80^{a}
LSD	0.06	0.08	0.04	0.15	0.19	0.37	0.05	0.08	0.08	0.10	0.12	0.10	0.08	0.14	0.20	0.22	0.26	0.27

Means followed by different superscripts across columns are significantly different at 5% probability level using lest significant difference (LSD), DA= Drought application

Table 3: Effect of drought and drought ameliorative treatments on alpha, beta and total amylase activities of *P. americanum* (CV. LCIC 9702) at various weeks after treatment

Treatments	Alpha amylase activities (IU/ml)						Beta amylase activities(IU/ml)						Total amylase activities					
	6 weeks			8 weeks			6 weeks			8 weeks			6 weeks			8 weeks		
	DA (Days)			DA (Days)			DA (Days)			DA (Days)			DA (Days)			DA (Days)		
	6days	10days	14days	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days
20 % trehalose	1.64^{c}	1.69^{c}	1.71^{b}	1.82^{c}	1.90^{bc}	1.96^{ab}	1.04^{ab}	1.13^{a}	1.13^{a}	1.14^{b}	1.26^{ab}	1.28^{b}	2.63^{b}	2.82^{b}	2.86^{b}	2.96^{c}	3.16^{b}	3.30^{ab}
20 % proline	1.62^{d}	1.46^{d}	1.51^{c}	1.83^{c}	1.70^{c}	1.78^{ab}	1.00^{ab}	1.07^{a}	1.07^{a}	1.05^{b}	1.08^{bc}	1.10^{bc}	2.40^{c}	2.53^{b}	2.60^{b}	2.70^{c}	2.78^{c}	2.88^{b}
20 % Coconut Milk	1.52cd	1.71^{b}	1.80^{b}	1.96^{c}	2.01^{bc}	1.91^{ab}	1.07^{ab}	1.13^{a}	1.13^{a}	1.14^{b}	1.46^{a}	1.56^{a}	2.59^{b}	2.84^{a}	2.96^{b}	3.8^{b}	3.47^{ab}	3.81^{a}
20 g Mycorrhiza	1.80b	1.87^{b}	1.94^{a}	2.14^{b}	2.25^{ab}	2.30^{a}	1.10^{a}	1.14^{a}	1.14^{a}	1.37^{a}	1.45^{a}	1.58^{a}	2.90^{a}	3.01^{a}	2.89^{b}	3.51^{b}	3.70^{ab}	3.86^{a}
Control 1(droughted)	0.95d	0.97^{e}	1.01^{d}	1.02^{d}	1.05^{d}	1.15^{b}	0.41^{c}	0.48^{b}	0.48^{b}	0.75^{c}	0.82^{c}	0.94^{c}	1.36^{d}	1.45^{c}	1.53^{c}	1.77^{d}	1.87^{d}	2.09^{c}
Control II (well-watered)	1.96a	1.93^{a}	2.00^{a}	2.50^{a}	2.34^{a}	2.61^{a}	0.98^{b}	1.02^{a}	1.02^{a}	1.10^{b}	1.18^{ab}	1.20^{b}	2.94^{a}	2.95^{a}	3.51^{a}	3.60^{a}	3.79^{a}	3.87^{a}
LSD	0.06	0.08	0.10	0.09	0.05	0.06	0.10	0.10	0.08	0.20	0.04	0.07	0.72	0.14	0.14	0.23	0.07	0.11

Means followed by different superscripts across columns are significantly different at 5% probability level using lest significant difference (LSD), DA= Drought application

Table 4: Effect of drought and drought ameliorative treatments proteinase activity of *P.americanum* (CV. SOSAT. C-88) at various weeks after treatment

Treatments	Proteinase Activity (U/ml)								
	DA (Days)								
	6weeks			8weeks			10weeks		
20 % trehalose	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days
20 % proline	0.51^{ab}	0.74^{ab}	0.86^{ab}	0.76^{ab}	0.94^{a}	1.03^{ab}	0.93^{bc}	1.26^{a}	1.33^{b}
20 % Coconut Milk	0.49^{ab}	0.57^{d}	0.60^{bc}	0.56^{bc}	0.62^{b}	1.04^{ab}	0.66^{bc}	0.73^{b}	0.80^{c}
20 g Mycorrhiza	0.55^{a}	0.59^{cd}	0.64^{bc}	0.62^{b}	0.66^{b}	0.73^{bc}	0.72^{abc}	0.79^{b}	0.90^{c}
Control 1(droughted)	0.57^{a}	0.68^{bc}	0.70ab	0.65^{b}	0.70^{b}	0.77^{bc}	0.77^{bc}	0.83^{b}	0.80^{cd}
Control II (well-watered)	0.30^{b}	0.38^{e}	0.40c	0.38^{c}	0.46^{c}	0.51^{c}	0.45^{c}	0.50^{b}	1.56^{a}
LSD	0.03	0.15	0.32	0.10	0.01	0.20	0.11	023	0.11

Means followed different superscripts across columns are significantly different at 5% probability level using lest significant difference (LSD). DA= Drought application

Table 5: Effect of drought and drought ameliorative treatments proteinase activity of *P.americanum* (CV. LCIC 9702) at various weeks after treatment

Treatments	Proteinase Activity (U/ml)								
	6 weeks			8week			10 weeks		
	DA (Days)								
	6 days	10 days	14 days	6 days	10 days	14 days	6 days	10 days	14 days
20 % trehalose	0.67^{bc}	0.71^{bc}	0.94^{b}	0.86^{b}	0.95^{c}	1.12^{c}	0.96^{b}	1.08^{b}	1.32^{b}
20 % proline	0.50^{d}	0.56^{c}	0.60^{c}	0.61^{c}	0.67^{d}	0.70^{e}	0.72^{d}	0.79^{c}	0.80^{c}
20 % Coconut Milk	0.58^{cd}	0.66^{c}	0.70^{c}	0.65^{c}	0.80^{cd}	0.92^{d}	0.74^{cd}	0.87^{c}	0.92^{c}
20 g Mycorrhiza	0.78^{b}	0.85^{b}	0.98^{ab}	0.93^{b}	1.24^{b}	1.36^{b}	0.91^{bc}	1.16^{ab}	1.25^{b}
Control 1(droughted)	0.29^{e}	0.36^{d}	0.39^{d}	0.38^{d}	0.40^{e}	0.48^{e}	0.42^{e}	0.51^{d}	0.56^{d}
Control II (well-watered)	0.91^{a}	1.05^{a}	1.15^{a}	1.21^{a}	1.45^{a}	1.66^{a}	1.16^{a}	1.31^{a}	1.57^{a}
LSD	0.10	0.05	0.13	0.09	0.05	0.20	0.12	0.16	0.07

Means followed by the different superscripts across columns are significantly different at 5% probability level using lest significant difference (LSD). DA= Drought application

DISCUSSION

Effectiveness of hydrolytic enzymes isassociated with several physiological processes including seed water imbibitions, catabolism, germination and maturation, response to environmental stimuli, metabolism,tuberization, lignin biosynthesis and water deficit (Oliveira *et al.*, 2002). The increase observed in the activities of enzymes such alpha, beta and total amylase as well as proteinase studied in the two cultivars of sorghum bicolor treated with mycorrhizaand trehalosemay indicate beneficial effects of mycorrhiza inabsorption of water and its translocation to seed in other toinitialize the synthesis of enzymes such as beta -amylase enzyme de novo and activation or reactivation of theirhydrolytic actions which precedes nutrient translocation or may explain osmoregulatorypotential of the treatments. Low level of enzymes activities recorded in sorghum bicolor droughted could suggest effect of low level of water as medium requiredbyamylase to break down the nutritional contents of the seeds suchasstarchwhich has accumulated in the seeds as physiological water deficit coping strategy. This response is may be due to the activity of alpha and beta amylase in the transformation of starch into carbohydrates and sucrose as influence by presence of water (Luma*et al.*, 2015).

Also, conversion of fat and oils to fatty acids and sugar,protein to amino acids and nitrogenare controlled by water driven hydrolytic activity of some specific enzymes in a proper sequence(Réhault-Godbert, 2019).

In viable seeds, for such seed to carry out physiological functions such as germination, metabolites (starch, proteins, fats or other polysaccharides) have to be hydrolysed and mobilized for the nourishment of the growing embryo and then seedling. The amelioration of drought stress exhibited bymycorrhizaand trehalose on the seedsmight have enhanced hydrolytic and catabolic action of the enzymes on the metabolites of the seeds (Ahmed and Waheed(2016).

Results of this study suggest beneficial effect of the osmoprotectantsonsorghum bicolor suffering from a biotic stresses during counteracting oxidative stress by modulating enzymes and that the treatments induced stress tolerance in the plant byimproving hydrolytic performance of enzymes which may decrease oxidative damage to the seeds (Liang *et al.,* 2007).

Studies have shown that increase in enzymatic activities is influenced by increase in temperature and that at high temperature enzymes such proteinase gets denatured. High enzyme activities recorded in well watered sorghum bicolor may be ascribed to the presence of adequate amount of water (Lee and Kim, 1995; Rezaei*et al.*, 2007; Devnarain*et al.*, 2016;Amit*et al.*, 2017). Rate of hydrolysis is usually high in cells with high water contents because, the water may serve as substrate for enzyme activation (Robinson, 2015; Amit*et al.*, 2017). According to Huang and Song (2013), in mature seeds, spores and pollen seeds, which have comparatively low hydrolysis level, enzyme activities arealways extremely feeble while in highly dehydrated tissue, enzymes activities is negligible. Lack of adequate water in the seeds may also impede activities of hydrolytic enzymes(Aubert*et al.*, 2018; Ali and Elozeiri, 2017) thereby acting as inhibitor because water is needed by both enzymes and plants.

Conclusion

This study established that the osmoprotectants have regulatory effects on the activities of hydrolytic enzymes therefore the use of the osmoprotectants in farming should be encouraged

REFERENCES

1. Ahanger MA, Alyemeni MN, Wijaya L, Alamri SA,Alam P, Ashraf M, Ahmad P. Potential of exogenously sourced kinetin in protecting Solanumlycopersicum from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate-glutathione cycle and glyoxalase system. PLoS ONE 2018, 13, e0202175.
2. Ahmed M and Waheed A. Effect of Silicon (Si) Application on Phoenix dactylifera L. Growth under Drought Stress Induced by Polyethylene Glycol (PEG) in Vitro, *American Journal of Plant Sciences,* 2016, 7; 1711-1728
3. Ali AS, and Elozeiri AA. Metabolic processes during seed germination. *Advances in seed biology*, 2017, 141-166
4. Ali AS, and Elozeiri AA. Metabolic Processes During Seed Germination. In (Ed.), Advances in Seed Biology. 2017, IntechOpen. https://doi.org/10.5772/intechopen.70653
5. Amit SK, Uddin M, Rahman R, Islam SM, and Khan MS. A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture & Food Security*, 2017, *6*(1), 1-22.
6. Ani KJ, Anyika VO, and Mutambara E. The impact of climate change on food and human security in Nigeria. *International Journal of Climate Change Strategies and Management*. 2021
7. Aubert MK, Coventry S, Shirley NJ, Betts NS, Würschum T, Burton RA, and Tucker MR. Differences in hydrolytic enzyme activity accompany natural variation in mature aleurone morphology in barley (Hordeumvulgare L.). *Scientific Reports*, 2018, *8*(1), 1-14
8. Bogati K, Walczak M. The Impact of Drought Stress on Soil Microbial Community, Enzyme Activities and Plants. Agronomy, 2022, 12, 189. https://doi.org/10.3390/ agronomy12010189
9. Devnarain N, Crampton B G, Chikwamba R, Becker JV and O'Kennedy MM. Physiological responses of selected African sorghum landraces to progressive water stress and re-watering. *South African Journal of Botany*, 2016, *103*, 61-69.
10. El-Maarouf-Bouteau, H. The seed and the metabolism regulation. *Biology*, 2022, *11*(2), 168.
11. Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A and Huang J. Crop production under drought and heat stress: plant responses and management options. *Frontiers in plant science*, 2017,1147
12. Haider H. Climate change in Nigeria: impacts and responses. 2019.
13. Huang H, Song S. Change in desiccation tolerance of maize embryos during development and germination at different water potential PEG-6000 in relation to oxidative process. Plant PhysiolBiochem. 2013, 68:61-70.
14. Kabbadj A, Makoudi B, Mouradi M, Pauly N, Frendo P, Ghoulam C. Physiological and biochemical responses involved in water deficit tolerance of nitrogen-fixing Viciafaba. PLoS One. 2017, 12(12):e0190284.
15. Kadiri M. 1999. Effect of Indole-3-acetic acid and coconut milk on the vegetativegrowth and yield of red pepper (*Capsicum annum L.*) *Global Journal of Pure and AppliedSciences*5, 313-316

16. Kadiri M. Studies on physiological amelioration of deleterious effects of drought on *Sorghum bicolor* (1) *Moench Direct Research Journal of Agriculture and Food Science* (DRJAFS), 2014, **2**: (3): 25- 27.
17. Lee SB.and Kim KJ. Effect of water activity on enzyme hydration and enzyme reaction rate in organic solvents. *Journal of fermentation and bioengineering*, 1995, *79*(5), 473-478.
18. Liang YC, Sun WC, Zhu YG. and Christie P.Mechanisms of Silicon-Mediated Alleviation of Abiotic Stresses in Higher Plants: A Review. *Environmental Pollution*,2007, 147, 422-428
19. Liliane TN and Charles MS. Factors affecting yield of crops. *Agronomy-climate change & food security*, 2020, 9.
20. Luma Castro de Souza GAN, Risely F A C, MyriamGalvão CFN, Tamires Borges de Oliveira IJM and Ricardo S O.. Application of multivariate analysis to evaluate the biochemical changes in sorghum (Sorghum bicolor L. Moench) after exposure to water stress and silicon applications, *African Journal of Biotechnology* 2015, 14(49); 3257-3263
21. Mangena P. Water Stress: Morphological and Anatomical Changes in Soybean (Glycine max L.) Plants. In (Ed.), Plant, Abiotic Stress and Responses to Climate Change. IntechOpen. 2018., https://doi.org/10.5772/intechopen.72899
22. Muhammad J I. Role of Osmolytes and Antioxidant Enzymes for Drought Tolerance 2018. Wheat,http://dx.doi.org/10.5772/intechopen.75926
23. Nezhadahmadi A, Prodhan ZH, Faruq G. Drought tolerance in wheat. The Scientific World Journal. 2013:1-12
24. OjewumiA W and Kadiri M. Physiological responses of photosynthetic and respiratory rates of some leafy vegetables to spent engine oil contamination, *Jewel Journal of Scientific Research (JJSR)*, 2021, **6:** (1&2):184-193
25. Oliveira Neto CF, Lobato AK S, Gonçalves-Vidigal, M C, Costa RCL, Santos Filho BG, Alves GAR, Maia WJMS, Cruz FJR, Neves, HKB, Onnerud H, Zhang L, Gellerstedt G. and Henriksson G. Polymerization of monolignols by redox shuttle-mediated enzymatic oxidation: a new model in lignin biosynthesis *International Plant Cell*, 2002, 14: 1953 – 1962
26. Punia H, Tokas J, Mor VS, Bhuker A, Malik A, Singh N, Satpal A AA, Hefft DI. Deciphering Reserve Mobilization, Antioxidant Potential, and Expression Analysis of Starch Synthesis in Sorghum Seedlings under Salt Stress. Plants2021,10, 2463.
27. Réhault-Godbert S, Guyot N, Nys Y. The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human Health. Nutrients. Mar 2019. 22;11(3):684.
28. Rezaei K, Jenab E. and Temelli F. Effects of water on enzyme performance with an emphasis on the reactions in supercritical fluids. *Critical reviews in biotechnology*, 2007, *27*(4), 183-195.

29. Riya J and Jos T P. Biostimulant priming in Oryza sativa: a novel approach to reprogram the functional biology under nutrient-defcient soil, Cereal Research Communications, 2021, https://doi.org/10.1007/s42976-021-00150-4
30. Robinson PK. Enzymes: principles and biotechnological applications. *Essays in biochemistry*, 2015, *59*, 1.
31. Sachan HK. and Deeksha K. Nutrient status and their relationship with soil properties of dalo (Colocasiaesculenta (L.) Schott) growing areas of Rewa district in Fiji, Indian J. Agric. Res., 2018, 52(6): 696-699
32. Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, RefayYandBattaglia ML. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants*, 2021, *10*(2), 259.
33. Sergilo, Sanchez and Arnold L Demain. Metabolic regulation and overproduction of primary metabolites, microbial biotechnology2008, 1(4): 283–319.
34. Shakeel A. A, Xiao-yu X, Long-chang, W, Muhammad FS, Chen Manand W L. Morphological, physiological and biochemical responses of plants to drought stress *African Journal of Agricultural Research* 2011, 6(9), pp. 2026-2032
35. Temesgen B. Major Challenging Constraints to Crop Production Farming System and Possible Breeding to Overcome the Constraints, International Journal of Research Studies in Agricultural Sciences (IJRSAS) 2020, 6, (7) 2454–6224
36. Vijay A, Dhotare V D, Guldekar S M, B and Sagar, N I., Evaluation of Soil Nutrient Index and their Relation with Soil Chemical Properties of Washim Road Farm of Dr.PDKV Akola, Maharashtra, India, International Journal of Current Microbiology and Applied Sciences, 2019, 8(9): 1773-1779
37. Youyan G, Wenhui Z, Jingfeng H, Jianyun Z and Hongyuan Y. Effects of water stress and seed mass on germination and antioxidative enzymes of Xanthocerassorbifolia, African Journal of Biotechnology, 2012. , 11(18):4187-4195
38. Zhu JK. Salt and drought stress signal transduction in plants. Annual Review of Plant Biology. 2002, 53:247-273
39. Zhu Y, Cao W, Dai T, Jiang D. A dynamic knowledge model for wheat target yield design and variety selection. The Journal of Applied Ecology 2004, 15(2):231-236