Osmoregulatory appraisal of some osmoprotectants on hydrolytic activities of some enzymes on seeds of two 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 osmoprotectants on activities of some hydrolytic enzymes in the seeds of two cultivars (SOSAT.C-88 and CV. LCIC 9702) of sorghum bicolor. Maturated seeds of the two cultivars were harvested and prepared for alpha, beta, total amylase and proteinase activities assay. The osmoprotectants produced significant variations on the enzymes at 10 and 14 days (DA) of 8 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 the seeds of CV. LCIC 9702 well-watered at 14 days DA 8WAP. Furthermore, total amylase activities (3.87 IU/ml) were recorded in the seeds produced by CV. LCIC 9702 well-watered at 14 days DA. Significant increase was noticed in beta amylase (1.14 IU/ml) and (1.58 IU/ml) in the seeds of CV. LCIC 9702 treated with mycorrhiza. CV. LCIC 9702) watered produced highest proteinase activities (1.57 U/ml) while least of the parameters were recorded SOSAT.C-88 and (CV. LCIC 9702) droughted. 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 to Zhu *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 unfavourable of aggro-climatic situations 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. 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 (Temesgen 2020).

Water stress affects not only morphological characters plants but also physiological, biochemical and metabolic and nutritional compositions of plants (Mangena 2018). Seeds of many plants remain dormant due to lack of adequate supply of water needed for enzymatic-driven- hydrolysis used for reactivation of the metabolic activity of such seed embryo and emergence of radical and plumule (Ali *et al.*, 2017). Excessive dehydration can also impede synthesis of some hydrolytic enzymes de novo 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 down starches, 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 (Sergio and Arnold 2008). In matured seeds but still attached to the mother plant, 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 meophytes, 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 osmolytes help to accommodate osmotic pressure, avoid cellular injury within the affected

cells (Nezhadahmadi *et a*. 2013), stabilize protein and membrane structures of plants under dehydration and their maintain osmotic balance (Zhu 2002).

Depending on severity of drought, studies have shown that not all plants have potential to synthesize adequate osmorptectants to cope with water deficit which could have negative implication on activities hydrolytic activities of some enzymes on seeds where plants accumulate mainly secondary metabolites such as carbohydrates, proteins, and lipids as stress coping strategies which later break down by the catabolic action of some enzymes to to release energy (Ahanger *et al.*, 2018) and Punia *et al.*, 2021). On the basis of this, the present study was conducted to evaluate osmoregulatory effects of some osmoprotectants on activities of 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 sorghum bicolor (*Pennisetum americannum*) 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 500 m 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 determined at Department of soil science, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

2.3 Land preparation and experimental design: The experimental 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-Proline and trehalos were prepared by modifying method of Ojewumi and Kadiri (2021). Exactly 2 g of L-proline and trehalose were measured separately and dissolved in 1liter of water. Twenty (20 %) (200 mL) of the proline and trehalos were determined from L- proline and trehalose prepared. From each preparation, 50 mL of each preparations 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 of matured 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 Planting/posmoprotects application: Five seeds of the two cultivars were planted using planting distance of 2 × 2 m and were allowed to grow for four weeks after which they were sprayed with using with 20 % trehalose, 20 % proline and 20 % coconut milk.

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

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

2.9 Determination of enzymes activities

At maturity, seeds of the plants were harvested, dried used to determine alpha, beta and total amylase activities.

Total Amylase (α and β) Activity; Seeds of control and treated SOSAT 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 reagen (DNSA). The (DNSA was prepared by dissolving one gram of 3, 5-dinitrosalicyclic acid in 20 mL of 2 m 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 27^0 C 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 of Kadiri, (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 450C 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 70 nm 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

The data obtained were subjected to Analysis of Variance (ANOVA) and separation of means using least significant difference at $p \leq 0.05$

list 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

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 planting**

Effects of some osmoprotectants on activities of hydrolytic enzymes in the seeds of two cultivars of Sorghum bicolor is presented in table 1. 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 6 days drought application (DA) 6 weeks after planting (WAP). Meanwhile at 10 and 14 days DA of the same 6WAP, 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 8WAP. Seeds of well-watered SOSAT.C-88 produced higher activities of alpha amylase (2.10 IU/ml). beta amylase (1.70 IU/ml) and total amylase (3.80 mg maltose/h mg protein) at 14 days DA. Least β-amylase (0.60 IU/ml) α-amylase (1.01 IU/ml) and total amylase (1.61 IU/ml) were recorded in SOSAT.C-88 droughted (Table 1).

Table 2 revealed that 20 % of the treatments had no significant effects on alpha, beta and total amylase activities at 6 days DA of 8WAP. The observation showed significant increase compared with other treatments studied. At 10 and 14-days DA of 8WAP, the treatments produced significant effects on in the activities of the enzymes. Higher alpha amylase (2.01 IU/ml and (2.61 IU/ml) were recorded in the seeds at 14 days DA in (CV. LCIC 9702) well-watered 8WAP. Total amylase activities (3.87 IU/ml) was recorded in the seeds produced by (CV. LCIC 9702) well-watered at 14 days DA.

Higher beta amylase (1,14 IU/ml) and (1.58 mg maltose/h mg protein) were noticed in the seeds of (CV. LCIC 9702) treated with mycorrhiza after 14 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 3).

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

Table 4 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% rehalose and proline across the treatments on 6, 10 and 14DA 6 of WAP as well as 6 and 10 of 8 WAP. The observation showed significant difference ($p < 0.05$) compared with control. Similar observations were recorded on 10 and 14 days DA 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 (CV. LCIC 9702) droughted (Table 4).

Table 1: 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 planting

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

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

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

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 is associated 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 α- amylase, β-amylase, total amylase and proteinase studied in the two cultivarts of sorghum bicolor treated with mycorrhiza and trehalose may indicate beneficial effects of mycorrhiza in absorption of water and its translocation to seed in other to initialize the synthesis of enzymes such as beta -amylase enzyme de novo and activation or reactivation of their hydrolytic actions which precedes nutrient translocation or may explain osmoregulatory potential of the treatments. Low level of enzymes activities recorded in sorghum bicolor droughted could suggest effect of low level of water as medium required for amylase to break down the nutritional contents of the seeds such starch as a major components to simple Sugar which have accumulated in the seeds as physiological coping strategies to withstand water deficit. This response is probably due to the primary activity of α and β- amylase enzymes, in the transformation of starch into carbohydrates and sucrose (Luma *et al.,* 2015). yet absence of water is needed to activate hydrolytic actions of these enzymes.

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

In a viable seeds, for such seed to carry out physiological function such as germination, several metabolites such as 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 by exhibited mycorrizal and trehalose on the seeds might have enhanced the

activity of enzymes on the hydrolytic and catabolic action of the enzmes on the metabolites of the seeds (Ahmed and Wahee (2016).

Results of this study suggest beneficial effect of the osmoprotectants onsorghum bicolor suffering from a biotic stresses during counteracting oxidative stress by modulating enzymes and that the ameliorative treatments induced stress tolerance in the plant by increasing not only the antioxidant enzymes activity, but the performance of hydrolytic enzymes activities which in turn 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 amount of enzmes activities recored in well watered sorghum bicolor may be ascribed to the presence of adequate amount of water. Rate of hydrolysis is usually high in cells with high water contents because, the water may serve as substrate for enzyme activation. According to Huang and Song (2013), in mature seeds , spores and pollen seeds, which have comparatively low, hydrolysis level, enzyme activities is always extremely feeble while in highly dehydrated tissue , enzymes activities is negligible.. Lack of adequate water in the seeds may also impede activities of hydrollytic enzymes 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

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