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Osmoregulatory Appraisal of Some Osmoprotectants on Hydrolytic Activities of Some Enzymes on Seeds of Two Water Stressed Cultivars of *Sorghum bicolor* (Moench)

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Enzymes play significant roles in metabolic processes of seeds. Therefore, this study evaluated osmoregulatory 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*. Matured 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 weeks 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 8WAT. 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 (1.14 IU/ml) and alpha amylase (1.58 IU/ml) in the seeds

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of CV. LCIC 9702 treated with mycorrhiza. CV. LCIC 9702 well watered produced highest proteinase activities (1.57 U/ml) while least of the parameters were recorded in 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 countries like Nigeria is envisaged. According to Zhu *et al.* [1] and Muhammad [2], yield of crops such as sorghum as one of the staple foods consumed in Nigeria is drastically decreasing due to unfavourable agro-climatic conditions such as water deficit. Erratic changes being observed in the occurrence of weather parameters is a limiting factor responsible for low crop production and increased rate of food insecurity in different parts of Nigeria [3,4,5]. 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 [6-8].

Water stress affects not only morphological characters of plants but also physiological, biochemical and nutritional compositions of plants [9]. Seeds of many plants remain dormant due to lack of adequate supply of water needed for enzymatic- driven- hydrolysis used for reactivation of metabolic activity of such seeds, embryo and emergence of radicle and plumule [10,11]. Excessive dehydration can also impede synthesis of some hydrolytic enzymes de novo [12] and their activation. Severe water deficit has ability to alter physiological processes of some enzymes that can enhance effective nutrient channelization and utilization, germination and mass production of the seeds [13,14,15]. Presence of such enzymes helps to break down starches, fats, carbohydrates or proteins into simpler forms for energy generation and maximum translocation of the metabolites 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 [16]. 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 [17].

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 stress such as drought and low temperature initiate gene expression that induces the 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 [18], stabilize protein and membrane structures of plants under dehydration and maintain their osmotic balance [19]. Depending on the severity of drought, studies have shown that not all plants have potential to synthesize adequate osmoprotectants to ameliorate negative effects of drought on activities of hydrolases on seeds.

The seeds accumulate mainly secondary metabolites such as carbohydrates, proteins, and lipids as stress coping strategies which later release energy for seed germination and other physiological activities [20,21]. On this basis, the present study was conducted to evaluate osmoregulatory effects of some osmoprotectants on activities of some hydrolytic enzymes on seeds of two cultivars of Sorghum bicolor.

2. MATERIALS AND METHODS

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.

Soil Analysis: Top soil was collected at 0-4 inches using soil probe as described by Vijay *et al.,* [22]. 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 [23]. 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 the Department of soil science, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

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

Preparation and application of osmoprotectants: L-Proline and trehalos were prepared by modifying method of Ojewumi and Kadiri [24]. Exactly 2g 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 preparation was measured using measuring cylinder and applied on the seedlings at three days intervals using foliar application method.

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.

Osmoprotectants 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 using with 20 % trehalose, 20 % proline and 20 % coconut milk.

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

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

Determination of enzymes activities: At maturity, seeds of the plants were harvested, dried and 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 5grams 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 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 [25].

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 [25]. 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, [26,27]. 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 [28]. 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 was 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 1mL of boiled enzyme extract which was exactly treated as above. Proteinase activity was calculated using a standard curve of different concentration of tyrosine [28].

Statistical analysis: Statistical analysis system (SAS 2013) package was used for the analysis of one-way Analysis of Variance (ANOVA) and significance of difference between means using least significant difference (LS) at p< 0.0.

3. 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 6 days 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 amylase activities (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 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

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/ml and beta (2.61 IU/ml) were recorded in the seeds at 14 days DA in (CV. LCIC 9702) well-watered CV.LCIC 9702 8WAT. 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 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 after treatments: 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 14 days DA at 6 WAT 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 in CV. LCIC 9702 droughted.

4. 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 [25]. 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 mycorrhiza and trehalose may indicate beneficial effects of mycorrhiza in the absorption of water by seeds to initialize synthesis of enzymes such as beta - amylase enzyme de novo and activation or reactivation of their hydrolytic actions as precursors of nutrient translocation or may explain osmoregulatory potential of the treatments. Low level of the enzymes activities recorded in sorghum bicolor (droughted) could suggest effect of low level of water as medium required by amylase to break down the nutritional contents of the seeds into products such as starch which has accumulated in the seeds as physiological water deficit coping strategy. This response 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 [29]. Also, conversion of fat and oils to fatty acids and sugar, protein to amino acids and nitrogen is controlled by water driven hydrolytic activity of some specific enzymes in a proper sequence [30].

For viable seeds to carry out physiological functions such as germination, metabolites formation, the seeds have to be hydrolysed and their nutrients mobilized for nourishment cotyledon and then seedling. The amelioration of drought stress exhibited by mycorrhiza and trehalose on the seeds might have enhanced hydrolytic and catabolic action of the enzymes on the metabolites of the seeds [31].

Results of this study suggest beneficial effects of the osmoprotectants on sorghum bicolor suffering from a biotic stress by modulating enzymes. Also, the treatments might have induced water stress tolerance of the seeds by improving hydrolytic performance of enzymes and reducing oxidative damage of the seeds [32].

Studies have shown that increase in enzymatic activities is influenced by inceasing 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 [33-36]. Rate of hydrolysis is usually high in cells with high water contents because the water may serve as substrate for enzyme activation [37,36]. According to Huang and Song [38], in mature seeds, spores or pollens, with comparatively low hydrolysis level, enzyme activities are always extremely feeble. Lack of adequate water in the seeds may also impede activities of hydrolytic enzymes [12,39] thereby acting as inhibitor because water is needed by both enzymes and plants.

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)		
	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	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)		
	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	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

5. 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

COMPETING INTERESTS

Author has declared that no competing interests exist.

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