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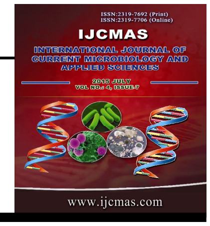

Original Research Article

Amelioration of polyethylene Glycol Simulated Water Deficit Stress by Benzyl Adenine in Pearl Millet Seedling

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A B S T R A C T

Keywords

Biochemical components, Peroxidase, Polyphenol oxidase, Pearl millet, *Pennisetum americanum* L.

The effect of benzyladenine (BA) on polyethylene glycol-6000 induced water deficit stress on different biochemical constituents such as chlorophyll, protein, free amino acids, proline, total carbohydrates, anti oxidant enzymes *viz*, peroxidase and polyphenoloxidase activities in 15 days old seedlings of pearl millet cultivars GHB-538 and GHB-732 were studied. Cultivar GHB-538 exhibited highest enzyme activities under the stress conditions. The cultivar GHB-732 found to have comparatively lower enzyme activities additionally; it also seemed lagging behind with respect to other biochemical components such as true protein, free amino acid, proline, total carbohydrates, total soluble sugars, reducing sugars and chlorophyll content. The overall results proved that cultivar GHB-538 as a more suitable option for cultivation in water deficient environments.

Introduction

Pearl millet (*Pennisetum americanum* L.) is one of the most important cereal crops cultivated all over the world for grain and fodder. It belongs to family gramineae. The genus comprises over 140 species, with chromosome numbers in multiples of x = 5, 7, 8 and 9 and ploidy ranging from diploid to octaploid levels (Brunken *et al.*, 1977). The crop is subjected to soil moisture deficits of varying degree and duration, which occasionally result in substantial loss of yield. Therefore, to develop the drought tolerant cultivars of pearl millet assumed considerable importance. The key factor affecting growth and yield is the availability of moisture during the cropping season. Therefore, improvement for drought tolerance in pearl millet is vital to stabilize the yield to previously unsuited regions and seasons.

A major effect of drought is reduction in photosynthesis, which arises by a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in food production (Farooq *et al.*, 2009). Beneficial effects of benzyl adenine under moisture deficit stress conditions have been reported to increase chlorophyll content, photosynthesis and rubisco activity (Dong *et al.*, 1995). The role of benzyl adenine (BA) is apparently on regulation of some physiological and metabolic activities in crop plants. The research on drought resistance has been very slow and its success through plant breeding seems very difficult in near future. Attempts were, therefore, made in the present experiment on to study the effects of benzyl adenine and PEG induced stress on chlorophyll content, protein, free amino acid, proline, total carbohydrates in pearl millet cultivars anti oxidant enzymes *viz*, peroxidase and polyphenoloxidase in pearl millet cultivars. Prolonged water stress leads to several problems such as decreased water flux, closing of stomata and reduction in photosynthetic CO_2 fixation and decrease in photosynthesis (Bohnert, 2007). BA has been shown increase plant height, leaf area; chlorophyll and pigment content also the proline, carbohydrates, soluble protein, total amino acid and mineral content (Ashraf *et al.*, 2001). Chlorophyll content and relative water content also increases in groundnut by application of BA (Dhruve and Vakharia, 2008).

Materials and Methods

Two pearl millet cvs., GHB-538 (resistant) and GHB-732 (susceptible) were examined for water stress tolerance and susceptibility in a pot with three replications in completely randomized design pattern. Fifteen days old seedlings were subjected to 10, 15 and 20% PEG-6000 induced water deficit stress. Prior to this induction of stress seeds were treated with 25 ppm BA for one hour. A sum of eight treatments (T_1 – Control, T_2 - PEG-6000 (10%), T_3 - PEG- 6000 (15%), T_4 - PEG- 6000 (20%), T_5 - Seed treatment with BA (25 ppm) for 1h, T_6 - Seed treatment with BA (25 ppm) + PEG (10%), T_7 - Seed treatment with BA (25 ppm) + PEG (15%), T_8 - Seed treatment with BA (25 ppm) + PEG (20%),) were applied for present experiment. The seedlings were analysed for biochemical constituents such as true protein (Lowry *et al.*, 1951), free amino acid (Vijayalakshmi *et al.*, 2000), proline (Bates *et al.*, 1973), total carbohydrates (Sadasivam and Manickam, 1996), total soluble sugars (Dubois *et al.*, 1956), reducing sugars (Somgyi, 1952), anti oxidant enzymes i.e. peroxidase (Deshmukh and Dhumal, 2005) and polyphenoloxidase (Sadasivam and Manickam, 1996), chlorophyll content (Radhika and Thind, 2013).

Results and Discussion

Moisture content is an important quality parameter in terms of storage of pearl millet grains or definite periods before it is rendered fit for use. The moisture content showed significantly higher mean values with treatment T_5 which was at par with treatment T_1 i.e. control in resistant CV. GHB-538 (Table 1). In seedlings of susceptible cultivar GHB-732 also showed higher moisture content with treatment T_5 (86.33 %) which was at par with treatment T_1 (85.30 %), T_6 (84.47 %), T_7 (84.33 %) and T_2 (84.22 %). With enhanced water deficit stress, moisture content was declined. However the moisture content was higher in water deficit stress treatment along with BA soaking treatment. It can be concluded from the above results that the benzyladenine can gave positive response to accumulate the moisture content in pearl millet seedlings under water deficit stress condition (Table 1). Similarly, Noorka and silva (2012) also reported that water deficit stress played a key role in reducing the moisture content in wheat. The result of Aly and latif (2011) also suggested the moisture content may be exhibited a significant decrease in response to water deficit stress treatments in wheat cultivars.

Protein content pearl millet cultivars, PEG stress treatments and combinations of PEG and BA stress treatment are depicted in figure 1S. Among the treatments true protein was significantly the highest in treatment T_5 (0.29%) in GHB-538. The combined effect of BA-PEG stress treatments resulted in significantly higher protein concentration in Treatment T_6 (0.26%) in GHB-538. A similar trend was also recorded for susceptible CV. GHB-732. However the protein concentration found higher in GHB-538 cultivar as compared to GHB-732, coinciding with the results obtained by Radhika and Thind (2013).

Delayed leaf senescence was observed in rice due to BA treatment, where the proteolysis is extended by BA revealed by Swider *et al.* (2004). Total free amino acids content in pearl millet cultivars, PEG stress treatments and combinations of PEG and BA stress treatment are presented in figure 2S. Among the treatments total FAA were significantly highest in treatment T_8 (0.208%) in GHB-538. Treatments, T_3 and T_5 remained at par to each other. The rate of FAA was increasing with increasing in concentration of PEG. The combined effect of PEG stress and BA- PEG stress treatments showed significantly higher degree of FAA concentration in treatment T_8 (0.208 %) in GHB-538. Surprisingly, in case of susceptible cultivar (GHB-732) also, the FAA seemed rising with rise in PEG concentrations. Interestingly, the all BA soaking treatments found related with higher accumulation of FAA in both the varieties. However the FAA concentration found higher in cv. GHB-538 as compared to GHB-732.

The FAA may be seem to stimulate the proline accumulation for deciding tolerance in given crop species under water deficit stress stated by Reddy *et al.* (2004) and Bano and Yasmeen, (2010). Among the treatments proline content was significantly higher in treatment T_8 (1.62%) which was at par with T_7 (1.56 %) in GHB-538 (Fig. 3S). The effect of PEG stress and combined BA-PEG stress treatments showed a significantly higher proline concentration in Treatment T_8 in GHB-538. Similar trend was also recorded for GHB-732. However the proline concentration was higher in GHB-538 as compared to GHB-732.

Cytokinins may partially ameliorate the negative effect of water stress by simulating osmotic adjustment (Gill and Tuteja, 2010). Similar effect found in BA application. Thus the drought tolerant cultivar accumulated more proline content and showed higher osmotic adjustment capacity. However, the excessive proline accumulation by drought tolerant cultivars may be a result of increased synthesis or decreased degradation (Aly and Latif, 2011).

Mostajeran and Rahimi-Eichi (2009) revealed that the sugars have been shown to protect the cells during drought**.** Among the treatments total carbohydrates content was significantly higher in treatment T_8 (1.21%) in GHB-538 which was at par with treatment T_6, T_4, T_5 and T_3 (Table 2). The amount of total carbohydrates found increasing with increase in concentration of PEG stress. The effect of PEG stress and combined BA- PEG stress treatments showed significantly higher total carbohydrates concentration in treatment T_8 in GHB-538. GHB-732 also exhibited similar pattern, but the total carbohydrates concentration was higher in GHB-538.

Recently it has been claimed that, under drought stress condition, even sugar flux may be signal for metabolic regulation (Patil and Patil, 2007). Among the treatments total soluble sugars content was significantly the highest in treatment T_8 (0.65%) in GHB-538 (Table 3). The amount of total soluble sugars increased with increased PEG stress. The effect of PEG stress and combined BA-PEG stress treatments showed marked rise in soluble sugars concentration in Treatment T_8 in GHB-538 as well as in cultivar GHB-732; however the total soluble sugars concentration was higher in GHB-538.

The reducing sugar percent of the pearl millet seedlings obtained from various treatments (Table 4) showed significant differences. However, the reducing sugars percent found maximum T_8 (1.13%) in GHB-538 cultivar. In case of GHB-732 cultivar, the reducing sugars were recorded higher in treatment T_8 (0.93%). The combined effect of PEG and BA showed significant differences in both the cultivars. However GHB-538 showed higher reducing sugars content as compared to GHB-732. Treatments T_6, T_4, T_3 and T_5 remained at par to each other. Similar results have also been demonstrated with wheat showed by Radhika and Thind (2013).

Peroxidase plays an important role in fine regulation of ROS concentration in the cell by activating and deactivating H_2O_2.POX; which is among the major enzymes that scavenge H_2O_2. In chloroplasts it is produced through dismutation of O_2 catalysed by SOD (10). Similar studies have already been conducted on groundnut, where the accumulation of peroxidase is known to protect the plant membrane from free radicals (5). In this study, the activity was increased and higher in treatment T_8 (2.00 $\Delta A\ min^{-1}g^{-1}$ fw.) which was at par with treatment T_6 and T_7 in seedlings of GHB-538 (Fig. 4). In seedlings of GHB-732 similar results were observed where maximum and minimum activities of POX was found for treatment T_8 (1.78 $\Delta A\ min^{-1}g^{-1}$ fw.) and Treatment T_1 (1.14 $\Delta A\ min^{-1}g^{-1}$ fw.).

The PPO is a mixture of monophenol oxidase and catechol oxidase enzymes that is present in nearly all plant tissues. PPO is a part of plant anti oxidative system (8). The PPO activities in case of our cultivars seemed significantly lowered in treatment T_1 for GHB-538 (0.119 $\Delta A\ min^{-1}g^{-1}$ fw) and GHB-732 (0.106 $\Delta A\ min^{-1}g^{-1}$ fw) cultivars (Fig. 5). With rise in PEG concentration the polyphenol oxidase activity also increased in T_8 of both the cultivars. The higher values were recorded in cultivar GHB-538 as compared to GHB-732.

Mean Chlorophyll content was the highest in treatment T_5 (1.47 mg/g) and lowest in treatment T_4 (0.82 mg/g fw.) for both the cultivars. Among all the treatments, the rise in concentration of PEG stresses the chlorophyll content was decreased (Table 5) with highly significant differences. Effect of BA was examined in combination with different intensities of PEG stresses. Here also the chlorophyll content was declined with increased PEG concentration. Treatment T_1 and T_7 were recorded at par to each other in GHB-538. In case of GHB-732 highest chlorophyll content was recorded with treatment T_5 i.e. BA soaked seeds. Treatment T_1 and T_6 were recorded at par each other for this cultivar.

The decreased chlorophyll content under water stress may be due to decreased synthesis and increased degradation of chlorophyll content in leaves of pearl millet under water stress stated by Ali *et al.* (2011). Previous reports have demonstrated increased chlorophyll content due to BA application where the role of BA may be in reducing the chlorophyll breakdown increased the cell division, cell elongation, increasing the chlorophyll content and delaying the leaf senescence.

Table.1 Combined effects of PEG-induced water deficit stress and benzyladenine (BA) on changes in moisture content in 15 days old seedlings of pearl millet

	Moisture (%)	
Treatment	**GHB-538**	**GHB-732**
T_1 (Control)	80.33	85.30
T_2 (PEG 10%)	77.10	84.22
T_3 (PEG 15%)	76.17	82.77
T_4 (PEG 20%)	73.63	81.67
T_5 (BA Soaked)	81.50	86.33
T_6 (BA+ PEG 10%)	78.63	84.47
T_7 (BA+ PEG 15%)	78.03	84.33
T_8 (BA+ PEG 20%)	77.67	83.33
S.Em±	0.65	0.72
C.D at 5%	1.94	2.16
C.V%	1.44	1.49

Table.2 Combined effects of PEG-induced water deficit stress and benzyladenine (BA) on changes in total carbohydrates content in 15 days old seedlings of pearl millet

	Total Carbohydrates (%)	
Treatment	**GHB-538**	**GHB-732**
T_1 (Control)	0.70	0.51
T_2 (PEG 10%)	0.84	0.65
T_3 (PEG 15%)	0.94	0.70
T_4 (PEG 20%)	1.00	0.82
T_5 (BA Soaked)	0.97	0.76
T_6 (BA+ PEG 10%)	1.03	0.82
T_7 (BA+ PEG 15%)	1.11	0.91
T_8 (BA+ PEG 20%)	1.21	0.99
S.Em±	0.026	0.017
C.D at 5%	0.077	0.052
C.V%	4.55	3.94

Table.3 Combined impact of PEG-induced water deficit stress and benzyladenine (BA) on changes in total soluble sugars content in 15 days old seedlings of pearl millet

	Total Soluble Sugars (%)	
Treatment	**GHB-538**	**GHB-732**
T_1 (Control)	0.20	0.17
T_2 (PEG 10%)	0.31	0.20
T_3 (PEG 15%)	0.43	0.32
T_4 (PEG 20%)	0.56	0.41
T_5 (BA Soaked)	0.39	0.31
T_6 (BA+ PEG 10%)	0.50	0.46
T_7 (BA+ PEG 15%)	0.60	0.51
T_8 (BA+ PEG 20%)	0.65	0.63
S.Em±	0.010	0.005
C.D at 5%	0.029	0.016
C.V%	3.66	2.49

Table.4 Combined impact of PEG-induced water deficit stress and benzyladenine (BA) reducing sugars content in 15 days old seedlings of pearl millet

	Reducing sugars (%)	
Treatment	**GHB-538**	**GHB-732**
T_1 (Control)	0.57	0.41
T_2 (PEG 10%)	0.69	0.53
T_3 (PEG 15%)	0.81	0.65
T_4 (PEG 20%)	0.92	0.72
T_5 (BA Soaked)	0.83	0.63
T_6 (BA+ PEG 10%)	0.94	0.71
T_7 (BA+ PEG 15%)	1.03	0.83
T_8 (BA+ PEG 20%)	1.13	0.93
S.Em±	0.02	0.02
C.D at 5%	0.06	0.06
C.V%	3.92	4.77

Table.5 Combined effects of PEG-induced water deficit stress and benzyl adenine (BA) on changes in chlorophyll a content in 15 days old seedlings of pearl millets

	Chlorophyll a (mg/g)	
Treatment	**GHB-538**	**GHB-732**
T_1 (Control)	1.28	0.95
T_2 (PEG 10%)	1.08	0.67
T_3 (PEG 15%)	0.89	0.59
T_4 (PEG 20%)	0.82	0.43
T_5 (BA Soaked)	1.47	1.36
T_6 (BA+ PEG 10%)	1.27	0.89
T_7 (BA+ PEG 15%)	1.17	0.71
T_8 (BA+ PEG 20%)	1.06	0.56
S.Em$\pm$	0.02	0.03
C.D at 5%	0.07	0.10
C.V%	3.47	7.29

Table.6 Combined effects of PEG-induced water deficit stress and benzyl adenine (BA) on changes in chlorophyll b content in 15 days old seedlings of pearl millet

	Chlorophyll b (mg/g)	
Treatment	**GHB-538**	**GHB-732**
T_1 (Control)	0.98	0.92
T_2 (PEG 10%)	0.81	0.55
T_3 (PEG 15%)	0.57	0.36
T_4 (PEG 20%)	0.32	0.28
T_5 (BA Soaked)	1.18	0.99
T_6 (BA+ PEG 10%)	1.09	0.93
T_7 (BA+ PEG 15%)	1.03	0.76
T_8 (BA+ PEG 20%)	0.93	0.49
S.Em$\pm$	0.05	0.03
C.D at 5%	0.14	0.10
C.V%	9.05	8.90

Table.7 Combined effects of PEG-induced water deficit stress and benzyl adenine (BA) on changes in chlorophyll b content in 15 days old seedlings of pearl millet

	Total Chlorophyll (mg/g)	
Treatment	**GHB-538**	**GHB-732**
T_1 (Control)	2.26	1.87
T_2 (PEG 10%)	1.89	1.22
T_3 (PEG 15%)	1.47	0.95
T_4 (PEG 20%)	1.14	0.71
T_5 (BA Soaked)	2.65	2.35
T_6 (BA+ PEG 10%)	2.36	1.83
T_7 (BA+ PEG 15%)	2.20	1.47
T_8 (BA+ PEG 20%)	1.99	1.05
S.Em$\pm$	0.05	0.05
C.D at 5%	0.14	0.15
C.V%	4.16	6.12

Fig.1 True protein content in pearl millet seedlings under PEG induced water deficit and BA application

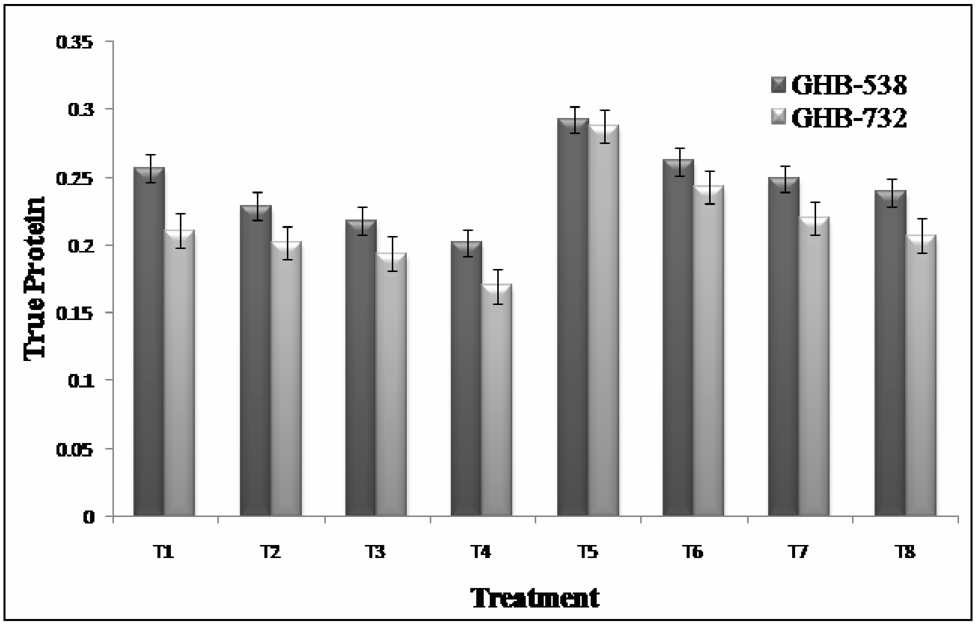


Fig.2 Free amino acids content in pearl millet seedlings under PEG induced water deficit and BA application

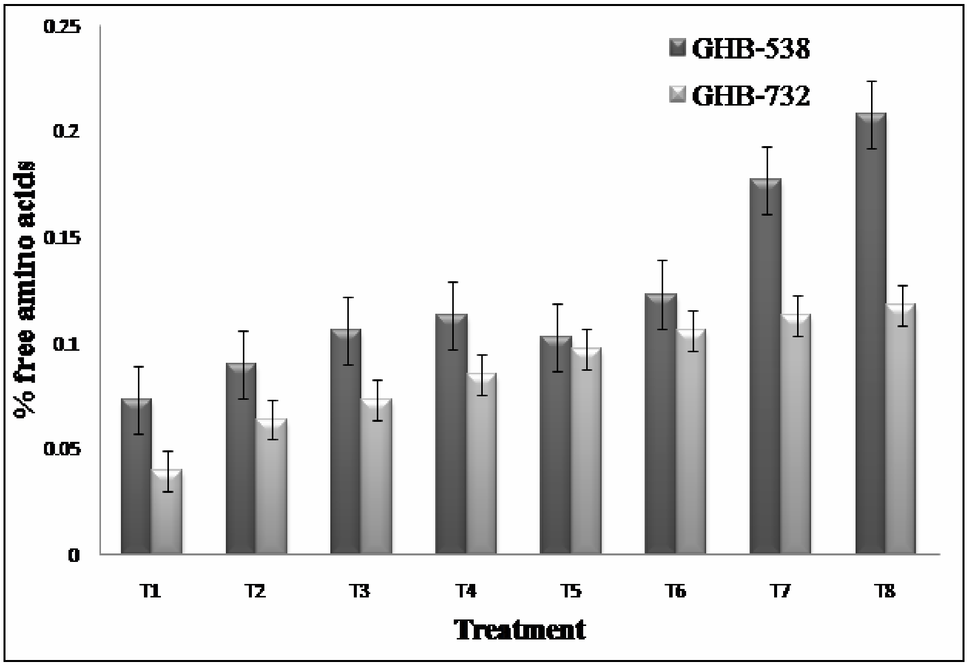


Fig.3 Proline content in pearl millet seedlings under PEG induced water deficit and BA application

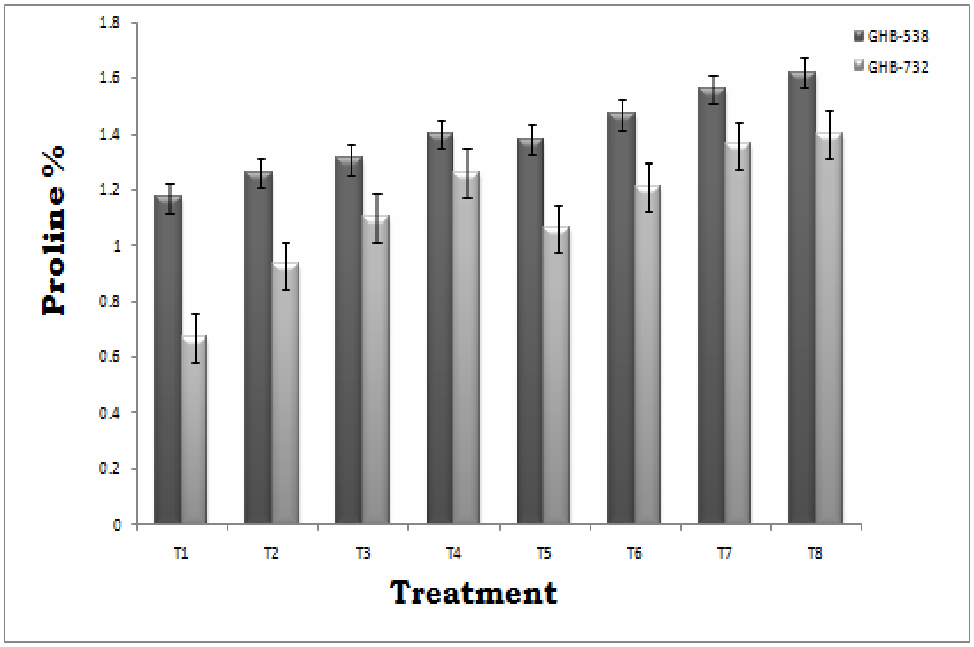


Fig.4 Peroxidase activity in pearl millet seedlings under PEG induced water deficit and BA application

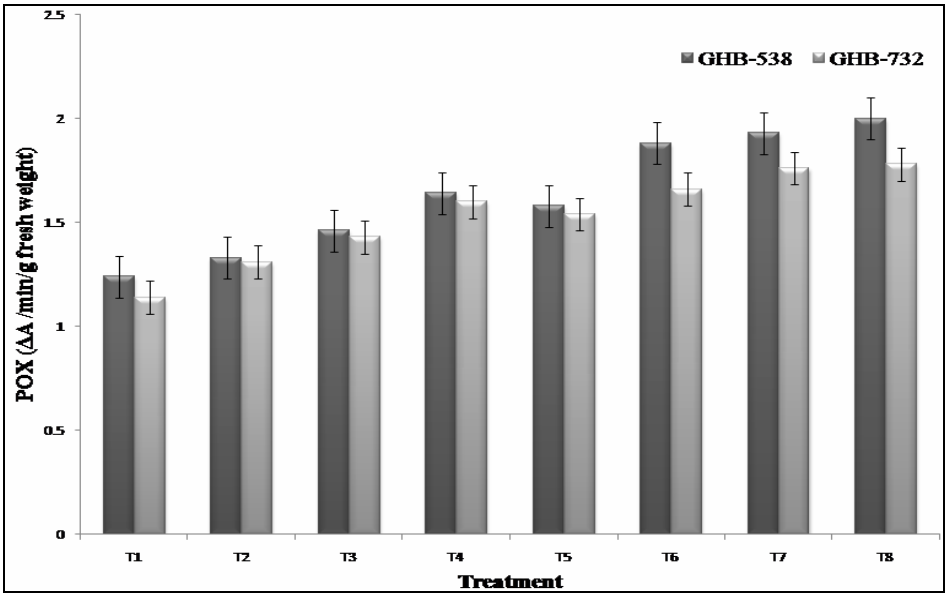
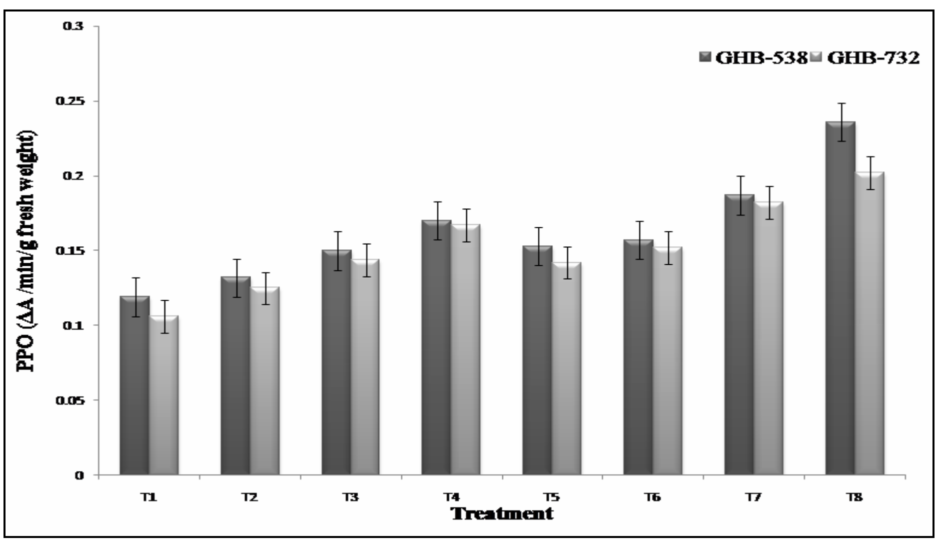


Fig.5 Polyphenol oxidase activity in pearl millet seedlings under PEG induced water deficit and BA application



Among the treatments chlorophyll b content was significantly highest (Table 6) in treatment T_5 (1.18 mg/g) in GHB-538 which was at par with treatment T_6. The rate of chlorophyll b content declined with increased concentrations of PEG stress. The effect of PEG stress and combined BA- PEG stress treatments resulted in significantly higher chlorophyll b concentration in treatment T_5 in GHB-538. In case of seedlings of GHB-732 also higher chlorophyll b content was recorded in treatment T_5 (0.99 mg/g) which was at par with treatment T_1 (0.92 mg/g) and T_6 (0.93

mg/g). However the chlorophyll b concentration was higher in GHB-538 as compared to GHB-732.

Dhruve and Vakharia (2008) stated that upon imposition of drought the chlorophyll content declines; but the effect of drought remains greater with the progress of growth stage. In groundnut, BA soaked seeds followed by water stress treatment at different growth stages are known to prevent the loss of total chlorophyll as compared to water deficit stress. These results also seemed applicable in case of our study as well, where the total chlorophyll content was significantly higher in treatment T_5 (2.65 mg/g) in GHB-538 (Table 7). The rate of total chlorophyll content declined with increase in concentration of PEG stress. The effect of PEG stress and combined BA- PEG stress treatments yielded significantly higher total chlorophyll concentration in treatment T_5 in GHB-538. In case of GHB-732, total chlorophyll content was higher in treatment T_5 (2.35 mg/g). Treatment T_1, T_6, T_8 and T_3 remained at par to each other. However the total chlorophyll concentration was comparatively higher in GHB-538.

Overall findings in this study concluded that both GHB-538 and GHB-732 show better performance under water deficit stress upon BA treatment. However, the pearl millet cultivar GHB-538 has a superior adaptability to water deficit stress when treated with BA-PEG combination.

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