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Effect on Enzyme Activity in Pearl Millet (*Pennisetum glaucum* L.) Seedlings in Response to Sulphate Based Salinity

Dr. Piyush Ukani*, Dr. Jaydeep Talaviya
Department of BIochemistry, College of Agriculture,
Junagadh Agricultural University, Junagadh, Gujarat, 362001

Abstract— The seeds of thirteen varieties of Pearl millet (*Pennisetum glaucum* (L.)) were evaluated for enzyme activity in response to sulphate base salinity. Sterilized seeds were safely maintain in filter paper lined Petri dishes and irrigated with water or salt solution (00, 40, 80, 120 m.eq/L). Practice was maintained for the filter paper kept moist by periodic additions of salt solution or water for the required treatments. The research carried out on sulphate dominant salinity. Enzymatic study protease and peroxidase activity increased with increase in salinity, while in case alfa amylase activity, it decreased with increasing salinity level. There was significant interacting effect between salinity treatments and varieties of pearl millet with respect to enzyme activities.

Keywords — *Salt Stress; Pearl Millet; A-Amylase; Peroxidase; Protease*

1. Introduction

Pearl millet (*Pennisetum glaucum* (L.)) is a monocot with cross pollinating crop in the family poaceae and sub family penicedae, having comparatively small diploid genome (2n = 2x = 14) with DNA content of 1C of 2.36 pg (Budak *et al*., 2003) [1], with a genome size of 2350 Mb. Pearl millet are C4 species with high photosynthetic efficiency and known as bajra. It is also known as *bulrush millet, cat tail, spiked millet*.

Salt stress is due to accumulation of high concentrations of salt in soil. The major salts are sodium chloride (NaCl), sodium sulphate (Na_2So_4), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), potassium chloride (KCl). These salts not only lead to excess salinity in soil but also affect crop growth severely. In Asia alone, 21.5 million ha are affected of which 12 million ha are saline 9.5 million are alkaline/sodic (Anon., 2004) [2]. Agricultural lands that have been heavily irrigated are extremely saline.

2. Materials and Methods

2.1. Estimation of α--amylase (*E.C. 3.2.1.1*)

The total enzyme activity of α--amylase (α-1, 4 glucan 4-glucanohydrolase *EC 3.2.1.1*) was determined by the starch degrading method Sadasivam and Manickam (1992) [3].

The seedling tissue (0.1-0.5 g) was weighed accurately and ground in mortar and pestle. Five ml sodium phosphate buffer 0.1M P^H 7.0 was added for extraction. It was transferred to centrifuge tube. The extra ction procedure was repeated times, under ice cold condition. The tube was collected and volume was made up to 10 ml. From this aliquots were used for enzyme assay.

One ml 1 % starch (as substrate) was taken in a tube. To this 0.75 ml phosphate buffer was added followed by 0.25 ml enzyme extract to start the reaction. The test tube was incubated in water bath for 15 min. At the end of incubation period 0.5 ml DNSA reagent was added to the tube for termination of the reaction. The tubes were put for 5 minutes in a boiling water bath. After cooling, 1 ml 40 % Na-K tartarate and 6.5 ml distilled water were added in the tubes. The colour of the reaction product between DNSA and maltose resulting from starch hydrolysis was measured in spectrophotometer at 530 nm.

The blank was prepared by using boiled enzyme. The reference curve was prepared with maltose in the range 200-1000 µg. The amylase activity was expressed as mg maltose released per gram fresh weight per 20 min.

2.2. Estimation of Protease (E.C. *3.4.21.25*) Activity

Protease activity was estimated by the method described by Nayak *et al.,* (1979)[4]. Protease enzyme degraded proteins to yield peptide which was calorimetrically estimated.

One g seedling tissue was weight accurately and ground with 2 ml phosphate buffer (0.1M P^H 7.2) using mortar and pestle under ice cold condition. The extract was decanted to centrifuge tube. This procedure was repeated 2-3 times and volume of extract was made to 10.0 ml with Phosphate buffer. The extract was centrifuged for 15 minutes at -10 ^OC, The supernatant collected and used for assay.

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Suitable aliquots (0.2-0.5 ml) were taken in test tubes. To this, 2.5-2.8 ml Phosphate buffer and 1.0 ml of casein was added. The tubes were incubated in water bath 37 ^{O}C for 1 h. The reaction was terminated by addition of 0.5 ml, 10 % TCA. The protein precipitate was removed by centrifugation at 4000 rpm for 20 min. 0.5 ml supernatant was used for assay of TCA soluble peptide by Folin Lowery method as described earlier.

In case of blank, TCA was added to the tube prior to the addition of enzyme. The reference curve was prepared by using 10 mg/100 ml of BSA. The absorbance was recorded at 750 nm in spectrophotometer and activity was expressed as mg peptides released per gram fresh weight per 20 min.

2.3. Estimation of peroxidase (*E.C. 1.11.1.7*)

The enzyme activity of Peroxidase (H_2O_2 Oxidoreducatse *E.C.1.11.1.7*) was estimated method suggested by Thimmaiah (1999) [5].

One gram weight the sample and homogenized it with using extract on buffer i.e. phosphate buffer p^{H} 7.2 0.1 M. Filter this mixture with the filter paper. Use filtrate as enzyme source. To 3.5 ml phosphate buffer in a clean dry cuvette, 0.3 ml enzyme extract and 0.1 ml freshly prepared Ortho dianisidine solution containing hydrogen peroxide were added. Now place the Cuvette in the spectrometer set at 460 nm immediately start the stop watch, read the initial absorbance and then at every 30 second interval take reading. Distilled water was used for the blank. Enzyme activity was expressed as unit is change in O.D min^{-1} g^{-1} fresh tissue.

3. Results and Discussions

3.1. α--amylase activity

The data on α--amylase activity of seedlings of different pearl millet varieties treated with salts treatments Sulphate dominant salinity which recorded at 1st day and 4th days after germination is presented in higher glycine betaines in treatment like T_4 i.e 120 m eq /L and however, the same was lower in control of GHB-1126.

The α--amylase activity in seedlings of pearl millet varieties at 1st day after germination is presented in Table 1 and Fig.1(a), 1(b). The mean α--amylase activity was varied from 4.65 to 7.10 (mg maltose released g^{-1} fr. wt. 20min^{-1}) among pearl millet varieties.

Table 1: Effect on alpha-amylase activity (maltose released, mg g^{-1} fresh weight per 20 min.) of pearl millet seedlings in response to sulphate dominant salt stress.

Sr. No.	Varieties	1st day				Mean (Vx)	4th day				Mean (Vx)
		T1	T2	T3	T4		T1	T2	T3	T4	
1	GHB-1132	8.33	6.81	6.02	5.32	**6.62**	16.57	15.05	14.26	13.56	**14.86**
2	GHB-538	8.00	5.78	5.27	4.47	**5.88**	16.24	14.02	13.51	12.71	**14.12**
3	GHB-719	5.59	5.24	4.33	3.45	**4.65**	13.83	13.48	12.57	11.69	**12.89**
4	GHB-577	7.03	5.99	5.11	4.19	**5.58**	15.27	14.23	13.35	12.43	**13.82**
5	GHB-1126	6.76	6.33	5.29	4.16	**5.64**	15.00	14.57	13.53	12.40	**13.88**
6	GHB-744	9.34	7.12	6.78	5.17	**7.10**	17.58	15.36	15.02	13.41	**15.34**
7	GHB-1138	7.33	6.81	6.06	5.35	**6.39**	15.57	15.05	14.30	13.59	**14.63**
8	GHB-905	8.12	6.99	5.13	4.22	**6.11**	16.36	15.23	13.37	12.46	**14.35**
9	GHB-935	7.10	6.35	5.32	4.16	**5.73**	15.34	14.59	13.56	12.40	**13.97**
10	GHB-757	7.17	6.62	5.61	4.86	**6.06**	15.41	14.86	13.85	13.10	**14.30**
11	GHB-557	8.32	7.79	6.69	5.50	**7.08**	16.56	16.03	14.97	13.71	**15.32**
12	GHB-1120	7.42	7.21	6.30	5.36	**6.57**	15.66	15.45	14.54	13.60	**14.81**
13	GHB-732	8.93	6.45	5.30	4.50	**6.30**	17.17	14.69	13.54	12.74	**14.54**
	Mean (Tx)	**7.65**	**6.57**	**5.63**	**4.67**		**15.89**	**14.81**	**13.87**	**12.91**	

	S.Em.±	C.D. at 5%	CV %	S.Em.±	C.D. at 5%	CV %
V	0.040	0.114	2.29	0.042	0.115	0.98
T	0.025	0.063		0.022	0.061	
V X T	0.081	0.229		0.085	0.230	

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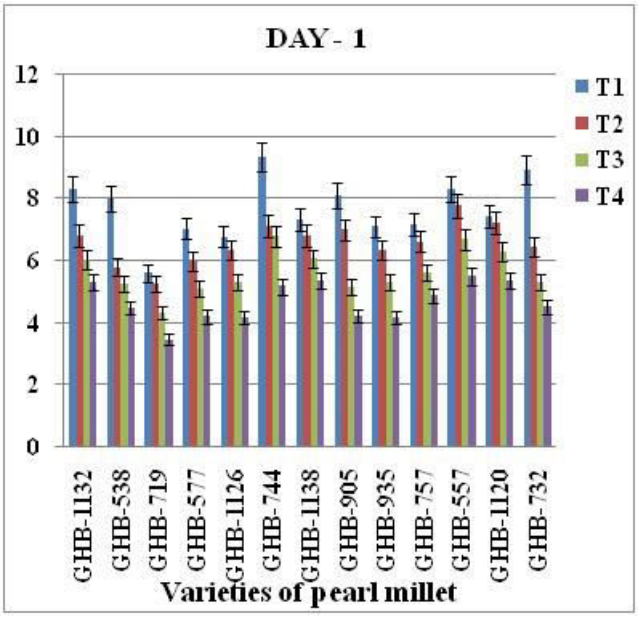

Fig.1(a)

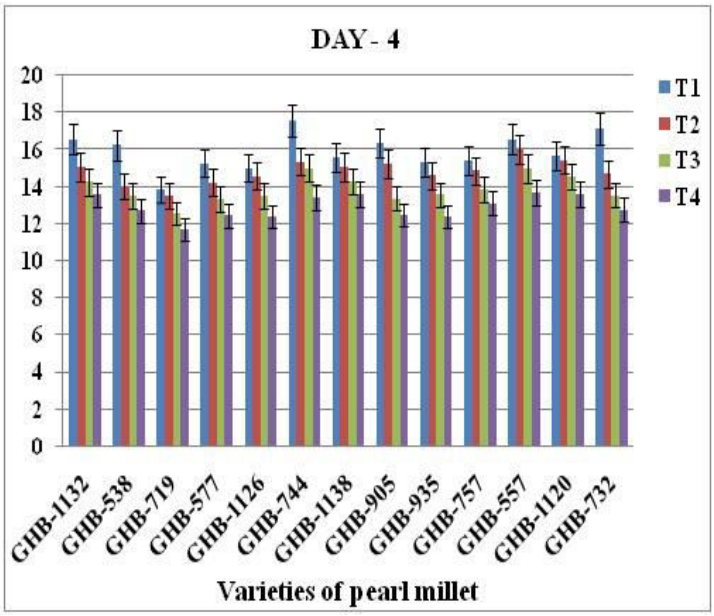

Fig. 1(b)

Interaction effect on α--amylase activity (maltose released, mg g^{-1} fresh weight per 20 min.) of pearl millet seedlings in response to sulphate dominant salt stress.

The maximum α--amylase activity was observed in cv. GHB-744 (7.10 mg maltose released g^{-1} fr. wt. 20 min^{-1}) which followed by the cv. GHB-577, GHB-1132, GHB-1120, GHB-1138, GHB-732, GHB-905, GHB-757, GHB-538, GHB-935, GHB-1126 and GHB-577. The lowest α--amylase activity was found in GHB-719 (4.65 mg maltose released g^{-1} 20 min^{-1}). While in case of at 4th days after germination the mean of α--amylase activity was increased from 12.89 to 15.34 (mg maltose released g^{-1} 20 min^{-1}). In this day the maximum α--amylase activity was observed in cv. GHB-744 (15.34 maltose released mg g^{-1} fr. wt. 20 min^{-1}) which followed by cv. GHB-577, GHB-1132, GHB-1120, GHB-1138, GHB-732, GHB-905, GHB-757, GHB-538, GHB-935, GHB-1126 and GHB-577. The lowest α--amylase activity was found in cv. GHB-719 (12.89 mg maltose released g^{-1} fr. wt. 20 min^{-1}).

The data on α--amylase activity showed significant variation among the treatments at 1st days and 4th days after germination (Table 1 and Fig.1(a)). At 1st day the content of α--amylase activity varied from 4.67 to 7.65 (mg maltose released g^{-1} fr. wt. 20 min^{-1}). The treatments of T_1 i.e 00 m eq/L observed higher α--amylase activity as compared to salt treatments like T_4 i.e 120 m eq/L. In these treatments showed that increased in salt stress resulted α--amylase activity of seedlings was decreased. Now, in 4th days after germination the maximum α--amylase activity found in treatments like T_1 i.e 00 m eq/L and The lowest α--amylase activity observed in treatment T_4 i.e 120 m eq/L Sulphate based salinity. Here, in this case also found that increase in salt stress resulted to decrease in α--amylase activity of seedlings.

The interaction effect of varieties and treatments were reported to be significant at both 1st and 4th days after germination (Table 1 and Fig.1(a), 1(b)). At 1st day the α--amylase activity was maximum in cv. GHB-744 with 00 m eq/L (T_1) as compared to rest of varieties at these treatments. The significantly lowest α--amylase activity was found in cv. GHB-719 with treatments T_4 i.e 120 m eq/L. In case of the 4th days after germination the maximum α--amylase activity found in T_1 i.e 00 m eq/L, were as significantly lower activity found in T_4 i.e 120 m eq/L sulphate based salinity.

The overall result found that, it was increase in the salt concentration in salt stress treatments observed that decreasing α--amylase activity of seedlings. The greatest fall in α--amylase activity in response to salinity was seen in pearl millet varieties on 1st and 4th days. The maximum α--amylase enzyme activity was found in cv. GHB-744 and GHB-557 in response to salt stress. Maximum effect on α--amylase activity was seen in sulphate dominant salinity. The rate of α--amylase activity of the seedlings was higher at 4th days compare to 1st days after germination. With progress of treatments, the α--amylase activity in the seedlings decreased in all salt stress treatments.

The finding was in line and its result also supported with Sharma and Gupta (1986) [6] and Datta *et al.,* (2009)

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[7] who reported that low salinity of NaCl and Na_2SO_4 level α--amylase activity was higher and decline at higher salt concentration in germinating wheat seedling.

3.2 Effect on Protease activity

The data on protease activity of seedlings of different pearl millet varieties treated with salts treatments Sulphate dominant salinity which recorded at 1st day and 4th days after germination is presented in Table 2 and Fig. 2(a), 2(b).

The protease activity was in seedlings of pearl millet varieties at 1st day after germination is presented in Table 2 and Fig. 2(a). The mean protease activity was varied from 5.84 to 7.69 (mg peptides released g^{-1} fr. wt. 20 min^{-1}) among pearl millet varieties. The maximum protease activity was observed in cv. GHB-905 (7.69 mg peptides released g^{-1} fr. wt. 20 min^{-1}) which at par with GHB-757. It was followed by the cv.GHB-1126, GHB-719, GHB-1120, GHB-935, GHB-744, GHB-732, GHB-557, GHB-1138, GHB-538 and GHB-1132. The lowest protease activity was found in GHB-577 (5.84 mg peptides released g^{-1} 20 min^{-1}). While in case of at 4th days the mean of protease activity was increased from 13.27 to 15.12 (mg peptides released g^{-1} 20 min^{-1}) see at the Table 2 and Fig. 2(b). In this day the maximum protease activity was observed in cv. GHB-905 (15.12 mg peptides released g^{-1} fr. wt. 20 min^{-1}) which at par with cv. GHB-757 which were followed by GHB-1126, GHB-719, GHB-1120, GHB-935, GHB-744, GHB-732, GHB-557, GHB-1138, GHB-538 and GHB-1132. The lowest protease activity was found in cv. GHB-577 (13.27 mg peptides released g^{-1} fr. wt. 20 min^{-}.

The data on protease activity showed significant variation among the treatments at 1st days and 4th days after germination (Table 2 and Fig.2(a), 2(b)). At 1st day the content of protease activity varied from 5.30 to 8.11 (mg peptides released g^{-1} fr. wt. 20 min^{-1}). The treatments of T_4 i.e 120 m eq/L observed higher protease activity as compared to control treatment like T_1 i.e 00 m eq/L. In these treatments showed that increasing salt stress resulted protease activity of seedlings was increased. Now, in 4th days after germination the maximum protease activity found in treatments like T_4 i.e 120 m eq/L and the lowest protease activity observed in treatment T_1 i.e 00 m eq/L Sulphate based salinity. Here, in this case also found that increased in salt stress resulted protease activity of seedlings was increased.

Table 2: Effect on protease activity (peptides released, mg g^{-1} fresh weight per 20 min.) of pearl millet seedlings in response to sulphate dominant salt stress

Sr. No.	Varieties	1st day				Mean (Vx)	4th day				Mean (Vx)
		T1	T2	T3	T4		T1	T2	T3	T4	
1	GHB-1132	4.66	5.35	6.47	7.18	**5.92**	12.09	12.78	13.90	14.61	**13.35**
2	GHB-538	5.15	5.42	6.28	6.87	**5.93**	12.58	12.85	13.71	14.30	**13.36**
3	GHB-719	5.20	6.81	7.30	9.06	**7.09**	12.63	14.24	14.73	16.49	**14.52**
4	GHB-577	4.22	5.40	6.35	7.37	**5.84**	11.65	12.83	13.78	14.80	**13.27**
5	GHB-1126	6.23	6.54	7.83	8.54	**7.28**	13.66	13.97	15.26	15.97	**14.71**
6	GHB-744	5.23	6.40	7.25	8.70	**6.90**	12.66	13.83	14.68	16.13	**14.33**
7	GHB-1138	4.55	5.91	6.68	7.28	**6.10**	11.98	13.34	14.11	14.71	**13.53**
8	GHB-905	6.03	6.52	8.37	9.83	**7.69**	13.46	13.95	15.80	17.26	**15.12**
9	GHB-935	5.20	6.64	7.45	8.47	**6.94**	12.63	14.07	14.88	15.90	**14.37**
10	GHB-757	6.23	6.64	7.83	8.54	**7.31**	13.66	14.07	15.26	15.97	**14.74**
11	GHB-557	5.55	5.92	6.71	7.28	**6.36**	12.98	13.35	14.14	14.71	**13.79**
12	GHB-1120	5.60	6.64	7.48	8.47	**7.05**	13.03	14.07	14.91	15.90	**14.48**
13	GHB-732	5.03	6.52	7.37	7.83	**6.69**	12.46	13.95	14.80	15.26	**14.12**
	Mean (Tx)	**5.30**	**6.21**	**7.18**	**8.11**		**12.73**	**13.64**	**14.61**	**15.54**	

	S.Em.±	C.D. at 5%	CV %	S.Em.±	C.D. at 5%	CV %
V	0.028	0.079	1.45	0.026	0.077	0.69
T	0.015	0.044		0.014	0.041	
V X T	0.056	0.158		0.055	0.155	

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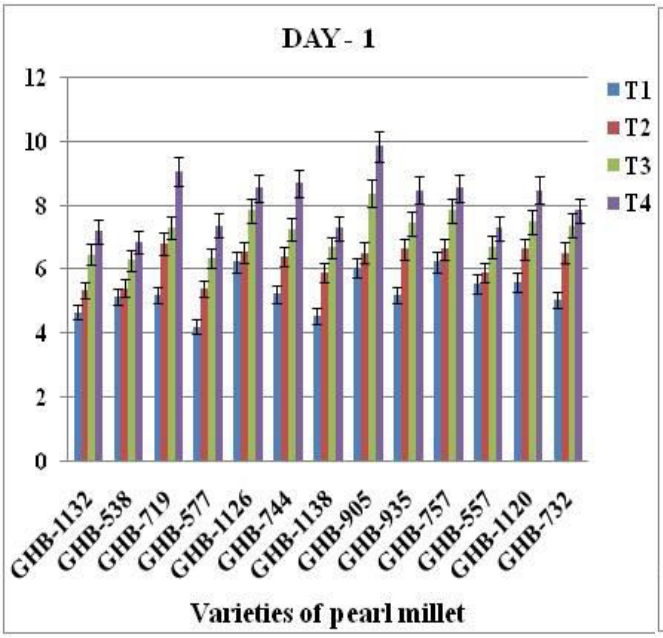

Fig. 2(a)

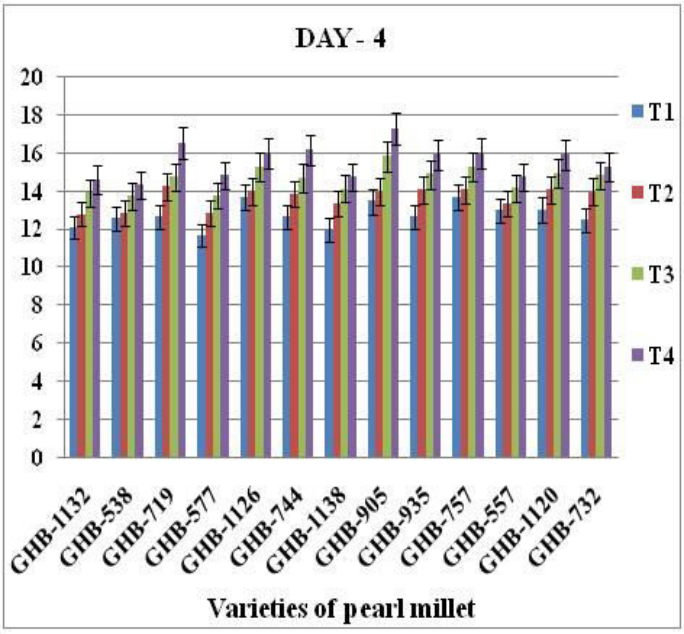

Fig. 2(b)

Interaction effect on protease activity (peptides released, mg g^{-1} fresh weight per 20 min.) of pearl millet seedlings in response to sulphate dominant salt stress.

The interaction effect of varieties and treatments were reported to be significant at both 1st and 4th days after germination (Table 2 and Fig.2(a), 2(b)). At 1st day the protease activity was to maximum in cv. GHB-905 followed by cv. GHB-757 and GHB-1126 with 120 m eq/L (T_4) as compared to rest of varieties at these treatments. The minimum protease activity was found in cv. GHB-577 with respect to treatments T_1 i.e 00 m eq/L. In case of the 4th days after germination the maximum protease found in cv. GHB-905 with T_4 i.e 120 m eq/L, however, significantly lower enzyme activity was found in the cv. GHB-577 with 00 m eq/L.

The overall result found that, there was increase in the salt concentration in treatments resulted to increase protease activity of seedlings. The greatest fall in protease activity in response to salinity was seen in pearl millet varieties on 1st and 4th days. The maximum protease enzyme activity was found in cv. GHB-905 in response to salt treatments. The protease activity of the seedlings was higher at 4th days compare to 1st day after germination. With progress of treatments, the protease activity in the seedlings increased in all salt stress treatments except at 40 m eq/L in some cases.

This finding is in conforming to the findings of earlier work done by Shigong *et al.* (1999)[8] and Gautam *et al.*, (2011)[9] reported that the seeds of salt resistance wheat varieties showed that 1.2 % NaCl stress increased the protease activity. The tolerance varieties were significantly increased protease activity as compare to susceptible varieties.

3.3. Peroxidase Activity

The data on peroxidase activity of seedlings of different pearl millet varieties treated with salts treatments sulphate dominant salinity which recorded at 1st day and 4th days after germination is presented in Table 3 and Fig. 3(a), 3(b).

The peroxidase activity in seedlings of pearl millet varieties at 1st day after germination is presented in Table 3 and Fig. 3(a), 3(b). The mean peroxidase activity was varied from 14.17 to 19.1(Δ OD min^{-1} g^{-1} fr. wt.) among pearl millet varieties.

The maximum peroxidase activity was observed in cv. GHB-905 (19.1 Δ OD min^{-1} g^{-1} fr. wt.) which followed by the cv. GHB-935, GHB-538, GHB-1120, GHB-757, GHB-744, GHB-557, GHB-1132, GHB-577, GHB-1138, GHB-1126 and GHB-732. The lowest peroxidase activity was found in GHB-719 (14.17 Δ OD min^{-1} g^{-1} fr. wt.). While in case of at 4th days, among the varieties, the mean of peroxidase activity was increased from 20.47 to 24.98 (Δ OD min^{-1} g^{-1} fr. wt.) see at the table 3 and fig. 3a. In this day the maximum peroxidase activity was observed in cv. GHB-905 (24.98 Δ OD min^{-1} g^{-1} fr. wt.) which followed by cv. GHB-935, GHB-538, GHB-1120, GHB-757, GHB-744, GHB-557, GHB-1132, GHB-577, GHB-1138, GHB-1126

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and GHB-732. The lowest peroxidase activity was found in cv. GHB-719 (20.47 Δ OD min^{-1} g^{-1} fr. wt.).

Table 3: Effect on peroxidase activity (Δ O.D. min^{-1} g^{-1} fresh weight) of pearl millet seedlings in response to sulphate dominanat salt stress

Sr. No.	Varieties	1st day				Mean (Vx)	4th day				Mean (Vx)
		T1	T2	T3	T4		T1	T2	T3	T4	
1	GHB-1132	12.26	14.36	18.06	21.86	**16.64**	18.06	20.66	24.36	28.16	**22.81**
2	GHB-538	13.13	14.36	19.50	22.76	**17.44**	19.76	20.66	25.80	29.06	**23.82**
3	GHB-719	11.06	12.36	15.73	17.53	**14.17**	17.36	18.66	22.03	23.83	**20.47**
4	GHB-577	11.93	15.56	18.30	19.83	**16.40**	18.26	21.86	24.83	26.16	**22.78**
5	GHB-1126	11.33	14.16	17.36	20.60	**15.86**	17.63	20.46	23.66	26.90	**22.16**
6	GHB-744	12.20	15.70	18.96	20.83	**16.92**	18.33	22.00	25.27	27.13	**23.18**
7	GHB-1138	12.56	13.93	17.73	20.43	**16.16**	18.86	20.23	24.03	26.73	**22.46**
8	GHB-905	14.83	16.70	19.90	24.96	**19.10**	21.13	23.00	26.20	29.60	**24.98**
9	GHB-935	13.10	15.86	19.53	23.10	**17.90**	19.10	22.13	25.83	29.40	**24.11**
10	GHB-757	12.60	15.20	19.30	20.83	**16.98**	19.10	21.50	25.60	27.13	**23.33**
11	GHB-557	12.26	15.40	18.43	21.56	**16.91**	18.56	21.70	24.73	27.83	**23.20**
12	GHB-1120	12.86	15.70	19.30	21.50	**17.34**	19.16	22.00	25.60	28.13	**23.72**
13	GHB-732	11.20	13.36	16.06	18.53	**14.79**	17.40	19.66	22.36	24.83	**21.06**
	Mean (Tx)	**12.41**	**14.82**	**18.32**	**21.10**		**18.67**	**21.12**	**24.64**	**27.30**	

	S.Em.±	C.D. at 5%	CV %	S.Em.±	C.D. at 5%	CV %
V	0.163	0.462	3.40	0.149	0.422	2.26
T	0.090	0.256		0.082	0.234	
V X T	0.327	0.925		0.299	0.845	

The data on peroxidase activity showed significant variation among the treatments at 1st days and 4th days after germination (Table 3 and Fig. 3(a), 3(b)). At 1st day the content of peroxidase activity varied from 12.41 to 21.10 (Δ OD min^{-1} g^{-1} fr. wt.). The control treatment have lower peroxidase activity which increased with progress of treatment and maximum activity were recorded at T_4 (120 m eq/L). However, in 4th days similar pattern of increase in peroxidase activity with salt treatment also recorded.

The interaction effect of varieties and treatments were reported to be significant at both 1st and 4th days after germination (Table 3 and Fig. 3(a), 3(b)). At 1st day the peroxidase activity was maximum in cv. GHB-905 with 120 m eq/L (T_4) as compared to rest of varieties at these treatments. The significantly minimum peroxidase activity was found in cv. GHB-719 with treatments T_1 i.e 00 m eq/L. In case of the 4th days after germination the maximum peroxidase activity found in cv GHB-905 with T_4 i.e 120 m eq/L, Which was significantly lower in the cv. GHB-719 with 00 m eq/L. indeed, in both the day 1st and 4th, lowest activity in control were recorded in GHB-719. However, maximum peroxidase activity was found in GHB-905 variety at T_4 treatment.

The overall result found that, increase in the salt concentration in treatments resulted to increasing peroxidase activity of seedlings. The greatest fall in peroxidase activity in response to salinity was seen in pearl millet varieties on 1st and 4th days. The maximum peroxidase enzyme activity was found from cv. GHB-905 and GHB-935 as compare to other pearl millet varieties in

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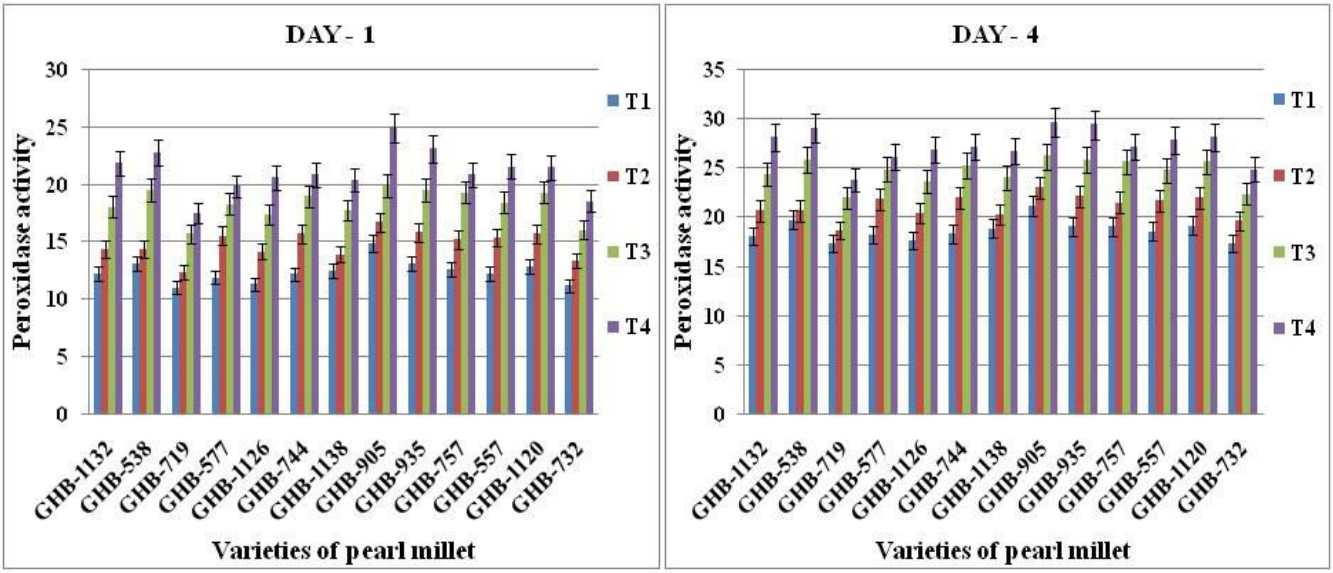

Fig. 3(a) **Fig. 3(b)**

Interaction effect on peroxidase activity (Δ O.D. min^{-1} g^{-1} fresh weight) of pearl millet seedlings in response to sulphate dominanat salt stress.

response to salt treatments. The peroxidase activity of the seedlings was higher at 4th days compare to 1st days after germination. With progressive days of germination, the peroxidase activity in the seedlings increased in all salt stress treatments.

The finding of present study is an agreement with earlier work done by Sreenivasulu *et al.,* (1999)[10] and Mahatma *et al*., (2009)[11] who reported that the effect on total peroxidase activity was increased under NaCl salinity and the degree of elevation in the activity was dependent on salt concentration.

4. Summary and Conclusions

4.1. α-amylase

The greater fall in **α** -Amylase activity in response to salinity have been seen in pearl millet varieties on 1st and 4th days. The maximum α-Amylase enzyme activity was found in cv. GHB-744 and GHB-557 in response to salt stress. Maximum effect on **α** Amylase activity was seen in sulphate dominant salinity with progressive days of germination. The **α** -Amylase activity in the seedlings decreased in all salt stress treatments.

4.2. Protease

Increasing the salt concentration of treatments, accordingly increase in protease activity of seedlings. The greatest fall in protease activity in response to salinity was seen in pearl millet varieties on 1st and 4th days. The maximum protease enzyme activity was found in cv. GHB-905 in response to salt treatments. The protease activity of the seedlings was increasingly higher at 4th days compare to 1st days after germination. With progressive days of germination, the protease activities in the seedlings were increased in all salt stress treatments.

4.3. Peroxidase

Peroxidase activities of seedlings were increased in pearl millet seedlings with increasing salt concentrations in treatments. The greater fall in peroxidase activity in response to salinity was seen in pearl millet varieties on 1st and 4th days. The maximum peroxidase enzyme activity was found from cv. GHB-905 and GBH-935 as compare to other pearl millet varieties in response to salt treatments. The peroxidase activity of the seedlings was increasingly higher at 4th days compare to 1st day after germination. With progressive days of germination, the peroxidase activity in the seedlings was increased in all salt stress treatments.

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