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Physiological and biochemical changes in Pearl Millet (*Pennisetum glaucum* L.) Seedlings in response to sulphate based salinity

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Abstract
The seeds of thirteen varieties of Pearl millet (*Pennisetum glaucum* (L.)) were evaluated for biochemical changes in response to sulphate base salinity. Sterilized seeds were kept in filter paper lined Petri dishes and irrigated with water or salt solution (00, 40, 80, 120 m.eq/L). These Petri dishes were kept for germination at 28 ± 2 ^{O}C in seed germinator. Care was taken to see that the filter paper remained moist by periodic additions of water or salt solution as per treatments requirement. The present study was carried out on sulphate dominant salinity. Embryonic axis (including cotyledons) of one and four day old seedlings were used for the estimations of physiological, biochemical, enzyme study by using the standard methodology or standard biochemical techniques. Sulphate dominant salinity levels reduced the per cent of germination of pearl millet, vigour index, root length, shoot length and increase osmolytes or solutes like total soluble sugars, true protein and glycine betaine. Differences in sensitivity to salt in pearl millet varieties are reflected by thirteen varieties of pearl millet. It was observed that cv. GHB-744, GHB-577 and GHB-1126 gave higher response in sense of physiological constituents, osmolytes or solutes, enzyme activity against salt stress. It suggested those that varieties have higher tolerance capacity against salt stress while other pearl millet varieties GHB-905 GHB-935, GHB-1120, GHB-1132, GHB-1138, GHB-757, GHB-538, GHB-719, GHB-732 and GHB-557 were found moderately tolerant or slightly susceptible against salt stress.

Keywords: salt stress, pearl millet, vigour index, true protein, glycine betaine and total sugar.

Introduction
Pearl millet (*Pennisetum glaucum* L.) is a monocot with cross pollinating crop belonging to the family poaceae and sub family penicedae, having relatively small diploid genome (2n = 2x = 14) with DNA content of 1C of 2.36 pg (Budak *et al*., 2003) [4], with a genome size of 2350 Mb. Pearl millet are C4 species with high photosynthetic efficiency and locally known as bajra, is also known as *bulrush millet, cat tail, spiked millet*. It is the sixth most important crop of the world next to rice, wheat and sorghum, maize and barley. In India, pearl millet is ranks third in terms of area, fourth in terms of production and sixth in terms of yield among cereals. In India, principle states under pearl millet cultivation are Rajasthan, Maharashtra, Gujarat, Haryana and Punjab. In the eastern side of the Western Ghats and in Tamilnadu, it is sown as winter crop (George *et al*., 2005) [7]. The primary centre of origin of pearl millet is Africa from where it spread to India. Pearl millet is known by various names in different languages: *pearl, bulrush, cattail*, or *spiked mille*t in English; *bajra* in Hindi; *dukhn* in Arabic and *mil chandelles* in French and cultivated as a cereal or as forage crop. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields in majority of the crop plants by more than 50 % (Boyer, 1982 and Bray *et al*., 2000) [2, 3]. Salinity is a widespread problem around the world. Seed germination and seedling growth of Pearl millet (*Pennisetum glaucum* (L.)), are negatively affected by salinity stress (Hampson and Simpson, 1990) [9]. Poor germination and decreased seedling growth result in poor crop establishment. Poor crop establishment in turn causes decreased crop competitiveness with weeds; lower shading of the soil surface and subsequently higher loss of soil water through evaporation and hence, lower crop water availability (Condon *et al*., 1993) [5].

Materials and Methods

A) Physiological analysis

Determination of germination percentage

Seeds were kept in germination paper folds kept moistened in Petri plates in seed germinator at 27 ^{0}C and allowed to germinate as prescribed in ISTA (1976) [10].

Determination of vigour index

Shoot length and root length of seedlings were recorded and calculate the vigour index as given formula by ISTA (1976) [10].

Vigour Index = Germination percentage x Shoot length

Root and shoot length

Root and shoot length measure by using 30 cm scale from treated and control seedling with three replication and compared with each other (Parida and Das, 2005) [11]

B) Biochemical analysis

Extraction and estimation of total soluble sugars

Total soluble sugars were estimated by following the method suggested by Dubois *et al.*, (1956) [6] with some modifications. Suitable aliquot (0.5 ml) was taken and volume made to 3 ml with the distilled water followed by 0.5 ml distilled phenol and mixed thoroughly. To this, 5.0 ml concentrated sulphuric acid was carefully added at the side of the tube. After the mixing thoroughly the tubes were kept for 30 minutes at room temperature for colour development. The absorbance was measured at 490 nm. The content was calculated with the help of a reference curve prepared from D-glucose as standard and expressed as mg g.$^{-1}$

Estimation of Glycine betaine

Glycine betaine was estimated by the method, described by Grieve and Grattan, (1983) [8]. About 0.5 g leaf powder was taken and shaken in 20 ml distilled water for 24 h at 25 °C. The samples were filtered and supernatant used for analysis. To this 0.5 ml of supernatant and equal volume of 2N H_2SO_4 were kept in ice water for 1h. 0.2 ml of cold KI-I_2 was added and reactants were gently vortex. The tubes were kept 0-4 °C for 16h and centrifuge at 10,000 for 15 minute at 0 °C. Supernatant were discarded. A periodical crystal were dissolved in 9 ml of 1, 2- dichromoethane. After 2 h, the absorbance measured at 365 nm using glycine betaine as standard and expressed in mgg^{-1} dry mass.

Extraction and estimation of protein

Protein content was determined by the method developed by sadasivam and Manickam (1992) [13]. One-gram sample was weighed and homogenized in five ml 0.1 N NaOH and filtered through whatman No.1 filter. The sample extracts (2.5 ml) was taken and made to 3.0 ml volume with distilled water. Five ml of alkaline copper reagent C was added (A) 2 % sodium carbonate in 0.1N sodium hydroxide. (B) 0.5 % copper sulphate in 10 % sodium potassium tartrate. (C) prepared by mixing 50 ml of reagent A with 1.0 ml reagent B. This reagent was prepared fresh at the time of use and mixed well. The content was allowed to stand for 10 min at room temperature followed by addition of 0.5 ml Folin-Ciocalteau reagent (diluted with water 1:1 v/v). The content was kept for 30 min at room temperature and the absorbance was measured at 650 nm. The protein content was calculated using bovine serum albumin as standard.

Results and Discussions

Germination Percentage (%)

The germination per cent of seeds sown in different treatments like Sulphate dominant salinity which imposed to pearl millet varieties was tested and data are presented in Table 1. Among the varieties, mean significantly highest germination per cent was recorded in cv. GHB-1132 (93.58 %) followed by cv. GHB-744(89.99).The germination of the pearl millet seeds obtained from various treatments resulted in to significant differences. Control treatments like T_1 i.e 00 m eq /L had significantly higher germination followed by T_2 i.e 40 m eq /L, T_3 i.e 80 m eq /L and T_4 i.e 120 m eq /L. The result in lower germination per cent (Table. 1) in different pearl millet varieties were due to increasing the respective salt stress treatments.

The result of present study revealed that considerable higher germination per cent in control as compare to salt treatments and increasing levels resulted in decrease per cent germination. The similar results were also in different pearl millet varieties against increasing salinity reported by Turhan *et al.*, (2009) [15].

Table 1: Effect of salt stress on germination percentage (%) of pearl millet seeds.

S. No.	Varieties	Salt treatment				Mean (Vx)
		T1	T2	T3	T4	
1	GHB-1132	98.00	93.66	91.33	91.33	93.58
2	GHB-538	93.66	87. 33	86.66	84.33	88.00
3	GHB-719	70.66	62. 33	53.33	48.66	58.75
4	GHB-577	99.33	93.66	93.00	88.00	93.50
5	GHB-1126	95.33	90.66	86.66	80.66	88.33
6	GHB-744	95.33	91.33	87.33	84.66	89.66
7	GHB-1138	93.33	91.33	86.67	80.67	88.00
8	GHB-905	93.00	86.00	81.33	77.67	84.50
9	GHB-935	72.00	64.66	51.33	47.66	58.91
10	GHB-757	95.33	90.00	86.33	82.66	88.58
11	GHB-557	69.33	62.66	54.66	46.00	58.16
12	GHB-1120	93.33	83.66	78.33	76.33	82.91
13	GHB-732	98.00	95.00	92.66	86.66	93.03
	Mean(Tx)	89.74	84.02	79.20	75.02	

	S.Em.±	C.D. at 5%	CV %
V	0.537	1.517	2.27
T	0.297	0.841	
V X T	1.074	3.035	

Vigour Index

The data on vigour index 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 4.2. In 1st day after germination the highest vigour index was recorded in cv. GHB-577 which followed by cv. GHB-1138. However cv. GHB-557 was at par with cv. GHB-1126 and it's followed by GHB-538, GHB-1132, GHB-1120, GHB-719, GHB-905, GHB-935. In 4th days the highest vigour index was recorded in cv. GHB-577 and it's followed by cv. GHB-1138, GHB-757, GHB-744, GHB-732, GHB-1132, GHB-1126, GHB-538, GHB-1120, GHB-905, GHB-557, GHB-719 and GHB-935 (Table 2. Among the treatment, at 1st day the highest vigour index (481.53) observed in T_1 i.e. 00 m eq /L (control). It was decreased by 394.06, 232.33 and 108.46 under saline conditions at 40, 80, 120 m eq / L in sulphate based salinity respectively, (Table 4.2). In 4th days, it was observed that highest vigour index (728.46) also in T_1 i.e. 00 m eq / L which was decreased with increasing salinity level up to 310.86 at T_4 i.e.120 m eq / L.

The interaction effect between varieties and treatments was found to be significant in both 1st and 4th days (Table 4.2). At 1st and 4th days after germination the vigour index was higher in cv. GHB-577 which was followed by GHB-1126 under control conditions or 00 m eq / L. Cultivar GHB-577 was found superior then the other pearl millet varieties under salinity conditions like 40, 80 and 120 m eq / L sulphate based salinity for their vigour index. Similar pattern of fall in vigour index due to sulphate dominant salinity were observed i.e. increasing in salinity treatments correspondence to decline vigour index. The cv. GHB-577 exhibited superior in vigor index on all days after germination.

Table 2: Effect on vigor index 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	133.36	100.50	73.40	42.66	87.48	372.56	321.53	289.20	258.76	310.51
2	GHB-538	148.20	110.96	62.53	30.93	88.15	365.33	311.46	262.93	224.90	291.15
3	GHB-719	98.86	83.20	46.26	17.80	61.53	261.40	226.56	168.93	129.73	196.65
4	GHB-577	481.53	394.06	232.33	108.46	304.10	728.46	615.16	446.23	310.86	525.18
5	GHB-1126	203.40	127.06	40.46	13.46	96.10	422.66	335.60	239.08	199.00	299.26
6	GHB-744	209.80	158.30	96.03	36.66	125.20	429.06	368.36	296.90	231.40	331.43
7	GHB-1138	360.80	261.90	124.16	67.16	203.51	575.50	471.96	323.50	252.70	405.91
8	GHB-905	108.46	74.46	29.83	5.16	54.43	322.36	272.26	216.90	183.80	248.83
9	GHB-935	72.73	38.83	18.43	5.46	33.87	249.63	198.26	140.30	117.56	176.47
10	GHB-757	270.10	131.97	74.83	35.83	128.18	489.36	338.96	273.40	225.96	331.92
11	GHB-557	185.10	112.60	60.40	33.43	97.88	355.93	271.46	191.13	141.10	239.90
12	GHB-1120	140.13	94.80	44.30	10.16	72.35	354.76	287.23	224.46	185.73	263.05
13	GHB-732	238.43	120.36	61.76	20.30	110.21	463.83	338.86	274.90	219.63	324.30
	Mean(Tx)	203.92	139.15	74.21	32.88		414.68	335.21	257.58	206.24	
		S.Em.±		C.D. at 5%		CV %	S.Em.±		C.D. at 5%		CV %
	V	1.966		5.556		6.05	2.577		7.284		2.94
	T	1.090		3.082			1.429		4.040		
	V X T	3.932		11.113			5.155		14.569		

Root and shoot Length

The data on root length of seedlings of different pearl millet varieties treated with salts treatments Sulphate dominant salinity which recorded at 1st day and 4th days after sowing is presented in Table 3. The mean value for varieties at 1st days (Table 3) showed significantly highest Root length in cv. GHB-577 (3.46 cm) which followed by the cv. GHB-744, GHB-1126, GHB-757, GHB-1138, GHB-732, GHB-1120, GHB-719, GHB-538, GHB-1132, GHB-557, GHB-935, and GHB-905. In 4th days, highest root length was observed in cv. GHB-577 (7.51cm) followed by the cv. GHB-757 and GHB-744. Among the treatments, at 1st days T_1 (i.e. 00 m eq / L) had statistically significant value of root length of the seedlings as compared to the salt stress treatment like T_2, T_3, and T_4. The treatments T_4 having a higher salt constitution which has found lowest root length value. At the time of 4th days (T_1 i.e 00 m eq / L) or control had significantly higher. Those are followed by T_2 and other salt stress treatments like T_3 and T_4 Among the all treatments T_4 having a lowest root length value due to higher salt content 120 m eq /L on sulphate based salinity.

The interaction effect between varieties and treatments was found to be significant in both 1st and 4th days (Table 3). At 1st and 4th days after germination the Root length was higher in cv. GHB-577 which was followed by GHB-757 and GHB-744 under control conditions or 00 m eq / L. The same was also found superior then the other variety like GHB-1126, GHB-1138, GHB-732, GHB-1120, GHB-719, GHB-538, GHB-1132, GHB-557, GHB-935, and GHB-905 in under salinity conditions like 40, 80 and 120 m eq / L sulphate based salinity.

The data on shoot length of seedlings of different pearl millet varieties treated with various salts concentrations Sulphate dominant salinity which recorded at 1st day and 4th days after germination is presented in Table 4. The mean value for varieties at 1st days (Table 4) showed significantly highest Shoot length in cv. GHB-577 (3.25 cm) which followed by the cv. GHB-1138, GHB-557, GHB-757 However varieties like GHB-732, GHB-1126, GHB-1132, GHB-538, GHB-719, GHB-1120, GHB-935 and GHB-905 at par with GHB-744 observed lowest shoot length value. In 4th days it was increased significantly in cv. GHB-577 (5.55 cm) this followed by the cv. GHB-1138 and GHB-557 (Table 4). Among the all treatments at 1st days T_1 i.e. 00 m eq / L had statistically at par with T_2 i.e which was gave higher Shoot length of the seedlings. The salt stress treatments like T_4 i.e 120 m eq / L was found the lowest value of Shoot length. At the time of 4th days after germination T_2 i.e 40 m eq / L which have significantly higher. Shoot length followed by T_1, T_3 and T_4. Among the all treatments T_4 having a lowest Shoot length value due to higher salt content 120 m eq /L sulphate based salinity.

The interaction effect between varieties and treatments was found to be significant in both 1st and 4th days (Table 4). At 1st and 4th days after germination the Shoot length was higher in cv. GHB-577 which was followed by GHB-1138 and GHB-557 under control conditions or 00 m eq / L Sulphate based salinity. In salt stress treatments like 40 and 80 m eq / L, shoot length of the seedlings was declined of as compare to other treatments of the salt stress.

Table 3: Effect on root length (cm) of pearl millet seedlings in response to sulphate dominant salt stress

S. No.	Varieties	1st day				Mean (Vx)	4th day				Mean (Vx)
		T1	T2	T3	T4		T1	T2	T3	T4	
1	GHB-1132	1.46	1.13	0.83	0.50	0.98	4.50	3.46	2.80	1.50	3.06
2	GHB-538	1.46	1.23	0.86	0.56	1.03	5.33	4.33	3.23	2.36	3.81
3	GHB-719	1.46	1.26	1.07	0.93	1.18	2.46	1.76	1.46	1.10	1.70
4	GHB-577	4.56	3.43	3.13	2.73	3.46	9.30	8.13	6.93	5.70	7.51
5	GHB-1126	3.73	3.20	2.93	2.46	3.08	6.36	5.80	4.26	3.23	4.91
6	GHB-744	4.03	3.50	3.06	2.36	3.24	7.53	6.20	5.83	3.20	5.69
7	GHB-1138	2.86	2.13	1.53	0.90	1.85	5.36	3.83	2.46	1.26	3.23
8	GHB-905	0.30	0.16	0.13	0.00	0.15	2.66	1.90	1.06	0.80	1.60
9	GHB-935	1.06	0.87	0.73	0.46	0.78	2.36	1.86	1.16	0.77	1.54
10	GHB-757	4.20	3.50	2.43	1.77	2.97	8.46	7.33	6.57	5.13	6.87
11	GHB-557	1.20	1.13	0.86	0.50	0.92	3.03	2.46	2.06	1.26	2.20
12	GHB-1120	1.86	1.50	1.20	0.80	1.34	1.86	1.36	0.86	0.46	1.14
13	GHB-732	2.80	2.03	1.56	0.80	1.80	4.86	3.26	2.46	1.73	3.08
	Mean(Tx)	2.38	1.93	1.56	1.13		4.93	3.97	3.16	2.19	
		S.Em.±	C.D. at 5%		CV %		S.Em.±	C.D. at 5%		CV %	
	V	0.019	0.054		3.82		0.027	0.076		2.63	
	T	0.010	0.030				0.015	0.042			
	V X T	0.038	0.109				0.054	0.152			

Table 4: Effect on shoot length (cm) 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	1.43	1.13	0.86	0.53	0.99	3.73	3.43	3.16	2.83	3.29
2	GHB-538	1.60	1.26	0.73	0.36	0.99	3.90	3.56	3.03	2.66	3.29
3	GHB-719	1.40	1.33	0.86	0.36	0.99	3.70	3.63	3.16	2.66	3.29
4	GHB-577	5.03	4.26	2.50	1.23	3.25	7.33	6.57	4.80	3.53	5.55
5	GHB-1126	2.13	1.40	0.46	0.16	1.04	4.43	3.70	2.76	2.46	3.34
6	GHB-744	2.20	1.73	1.10	0.43	1.36	4.5	4.03	3.40	2.73	3.66
7	GHB-1138	3.86	2.86	1.43	0.83	2.25	6.16	5.16	3.73	3.13	4.55
8	GHB-905	1.16	0.86	0.36	0.06	0.60	3.46	3.17	2.66	2.37	2.91
9	GHB-935	1.16	0.77	0.43	0.16	0.63	3.46	3.06	2.73	2.46	2.93
10	GHB-757	2.83	1.47	0.86	0.43	1.40	5.13	3.76	3.16	2.73	3.70
11	GHB-557	2.83	2.03	1.20	0.76	1.70	5.13	4.33	3.50	3.06	4.03
12	GHB-1120	1.50	1.13	0.56	0.13	0.83	3.80	3.43	2.86	2.43	3.13
13	GHB-732	2.43	1.26	0.66	0.23	1.15	4.73	3.56	2.96	2.53	3.45
	Mean(Tx)	2.27	1.65	0.92	0.44		4.57	3.95	3.22	2.74	
		S.Em.±	C.D. at 5%		CV %		S.Em.±	C.D. at 5%		CV %	
	V	0.019	0.056		5.20		0.017	0.054		1.90	
	T	0.011	0.031				0.014	0.032			
	V X T	0.039	0.112				0.032	0.110			

Total Soluble Sugars

The data on total soluble sugar of seedlings of different pearl millet varieties treated with salts treatments viz. Sulphate dominant salinity which recorded at 1st day and 4th days after germination is presented in Table 5 The total soluble sugars content in seedlings of pearl millet varieties at 1st days after germination is presented in Table 5. The mean total soluble sugars were varied from 1.40 % to 2.39 % among pearl millet varieties. The maximum content was observed in cv. GHB-719 (2.39 % fr. Wt.) which followed by the GHB-732 and GHB-905 and it was at par with GHB-757. The lowest total soluble sugars content was found in GHB-1126 (1.40 % fr. wt.). At 4th days the mean of total soluble sugars was varied from 3.77 % to 4.79 %. The maximum total soluble sugars content was observed in cv. GHB-719 (4.79 % fr. wt.). However, cv. GHB-538 at par with GHB-719. The lowest total soluble sugars content was found in GHB-744 (3.77 % fr. wt.).

The data on total soluble sugars showed significant variation among the treatments at 1st days and 4th days after germination. At 1st days the content of total soluble sugars varied from 1.46 % to 2.08 %. The treatment T_4 i.e 120 m eq/L had higher total soluble sugars as compared to control treatments T_1 i.e 00 m eq/L. This indicated that as increased in salt stress total soluble sugars content of seedlings was also increased. Now, in 4th days after germination the maximum value of total soluble sugars content was found in treatments like T_4 i.e 120 m eq/L. The lowest total soluble sugars percentage observed in treatment T_1 i.e 00 m eq/L. Increase in salt stress resulted to elevate total soluble sugars content in seedlings.

Table 5: Effect on total soluble sugars (%) of pearl millet seedlings in response to sulphate dominant salt stress.

S. No.	Varieties	1st day				Mean	4th day				Mean
		T1	T2	T3	T4	(Vx)	T1	T2	T3	T4	(Vx)
1	GHB-1132	1.30	1.70	2.20	2.37	1.89	3.70	4.10	4.60	4.77	4.29
2	GHB-538	1.35	1.28	1.55	1.91	1.53	4.45	4.38	4.65	5.01	4.63
3	GHB-719	2.11	2.20	2.56	2.67	2.39	4.51	4.60	4.96	5.07	4.79
4	GHB-577	1.45	1.74	1.93	2.09	1.80	3.75	4.04	4.23	4.39	4.10
5	GHB-1126	1.05	1.26	1.55	1.75	1.40	3.65	3.86	4.15	4.35	4.00
6	GHB-744	1.56	1.79	1.95	2.20	1.87	3.46	3.69	3.85	4.10	3.77
7	GHB-1138	1.26	1.56	1.76	1.89	1.62	3.45	3.75	3.95	4.08	3.81
8	GHB-905	1.55	1.82	1.94	2.15	1.86	3.74	4.01	4.13	4.34	4.05
9	GHB-935	1.71	1.84	2.13	2.26	1.98	3.90	4.03	4.32	4.45	4.17
10	GHB-757	1.54	1.73	1.83	2.12	1.81	4.16	4.35	4.45	4.74	4.43
11	GHB-557	1.14	1.30	1.52	1.71	1.42	3.76	3.92	4.14	4.33	4.04
12	GHB-1120	1.26	1.42	1.47	1.62	1.44	3.71	3.87	3.92	4.07	3.89
13	GHB-732	1.78	1.90	2.15	2.30	2.03	4.23	4.35	4.60	4.75	4.48
	Mean (Tx)	1.46	1.66	1.89	2.08		3.88	4.07	4.30	4.50	

	S.Em.±	C.D. at 5%	CV %	S.Em.±	C.D. at 5%	CV %
V	0.021	0.060	4.21	0.022	0.061	1.79
T	0.011	0.033		0.012	0.033	
V X T	0.043	0.121		0.044	0.122	

The interaction effect of varieties and treatments were reported to be significant at both 1st and 4th days after germination (Table 5). At 1st days the total soluble sugars content was maximum in cv. GHB-719 with 120 m eq/L (T_4) as compared to rest of varieties at this treatment. The significantly minimum total soluble sugars were found in cv. GHB-1126 with treatments T_1 i.e 00 m eq/L Sulphate based salinity. In case of the 4th days after germination, the maximum total soluble sugars content found in cv. GHB-719 with T_4 i.e 120 m eq/L.

The present study supported by Thakur and Sharma (2005) [14] as they found the total soluble sugar content was increased under saline stress condition in seedlings of *Sorghum bicolor* (L.).

True protein content

The data on true protein 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 6.

True protein content in pearl millet varieties, Salt stress treatments and their combinations are depicted in Table 6. At 1st days, among the varieties, the true protein was significantly higher in GHB-1126 (14.03 mg g^{-1} fr. wt.). The GHB-1126 at par with cv. GHB-557 (13.74 mg g-1 fr. wt.) and it was followed by GHB-577. The lowest true protein value found in cv. GHB-538 (8.43 mg g-1 fr. wt). While in case of 4th days after germination, there was declined in the true protein content. The highest true protein value found in cv. GHB-1126 (10.23 mg g^{-1} fr. wt.) which was at par with the cv. GHB-557 (9.94 mg g^{-1} fr. wt.) and same was lowest value foun d in cv. GHB-538 (4.63 mg g^{-1} fr. wt.).

Table 6: Effect on true protein (mg g^{-1} fresh weight) of pearl millet seedlings in response to sulphate dominant salt stress.

S. No.	Varieties	1st day				Mean	4th day				Mean
		T1	T2	T3	T4	(Vx)	T1	T2	T3	T4	(Vx)
1	GHB-1132	9.37	11.07	12.53	14.53	11.88	5.57	7.27	8.73	10.73	8.08
2	GHB-538	6.33	7.70	9.43	10.23	8.43	2.53	3.90	5.63	6.43	4.63
3	GHB-719	6.33	8.37	10.23	11.53	9.12	2.53	4.57	6.43	7.73	5.32
4	GHB-577	11.20	12.07	14.10	15.10	13.12	7.40	8.27	10.30	11.30	9.32
5	GHB-1126	11.57	13.40	15.10	16.07	14.03	7.77	9.60	11.30	12.27	10.23
6	GHB-744	8.70	9.83	11.07	12.40	10.50	4.90	6.03	7.27	8.60	6.70
7	GHB-1138	10.10	11.93	13.50	14.67	12.55	6.30	8.13	9.70	10.87	8.75
8	GHB-905	9.80	10.50	12.10	13.07	11.37	6.00	6.70	7.30	9.27	7.32
9	GHB-935	9.60	10.50	12.20	13.37	11.42	5.80	6.70	8.40	9.57	7.62
10	GHB-757	8.30	9.87	12.37	13.73	11.07	4.50	6.07	8.57	9.93	7.27
11	GHB-557	11.87	13.43	14.27	15.40	13.74	8.07	9.63	10.47	11.60	9.94
12	GHB-1120	9.33	10.40	11.50	12.53	10.94	5.53	6.60	7.70	8.73	7.14
13	GHB-732	10.53	11.73	12.63	13.70	12.15	6.73	7.93	8.83	9.90	8.35
	Mean (Tx)	9.46	10.83	12.39	13.56		5.66	7.03	8.51	9.76	

	S.Em.±	C.D. at 5%	CV %	S.Em.±	C.D. at 5%	CV %
V	0.067	0.189	2.01	0.101	0.286	4.53
T	0.037	0.105		0.056	0.158	
V X T	0.134	0.379		0.202	0.572	

The lowest true protein content found with control treatment like T_1 i.e 00 m eq/L (9.46 mg.g^{-1} fr. wt.) and it was increased with progress of treatments like T_2, T_3 and T_4 (Table 6).

The combined effect of varieties and treatments at 1st days resulted significantly highest true protein content with 120 m eq/L in cv. GHB-1126 (16.07 mg.g^{-1} fr. wt.) and the same was minimum in cv. GHB-538 and GHB-719 (6.33 mg.g^{-1} fr. wt.) with treatment T_1 00 m eq/L. At 4th days, significantly increase in true protein content was observed in cv. GHB-1126 from T_1 to T_4.

The present study was supported by Ali, (1998) [1] who reported that maize seed were germinated on filter paper moistened with solutions 0, 50, and 150 ml molar NaCl salt, with resulted significantly increased in protein content in the seedling.

Glycine betaine

The data on glycine betaine content 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 7.

The glycine betaine content in pearl millet varieties, salt stress treatments and their combinations are presented in Table 7. At 1st days among the varieties the glycine betaine was significantly higher in cv. GHB-719 (320.09 µg g^{-1} fr. wt) which was at par with GHB-1120 (317.92 µg g^{-1} fr. wt). However, varieties GHB-1132 it was followed by GHB-935, GHB-757, GHB-744, GHB-905, GHB-732, GHB-538, GHB-577, GHB-557 and GHB-1138. The lowest glycine betaines content were found in cv. GHB-1126 (161.78 µg g^{-1} fr. wt). While in case of 4th days after germination, there was increase in the glycine betaine content of the seedlings of pearl millet varieties. Among the highest glycine betaines value were found in cv. GHB-719 (417.89 µg g^{-1} fr. wt) which at par with the GHB-1120. It was followed by cv. GHB-1132, GHB-935, GHB-757, GHB-744, GHB-905, GHB-732, GHB-538, GHB-577, GHB-557 and GHB-1138. The lowest glycine betaine content was found in cv. GHB-1126 (259.58 µg g^{-1} fr. wt).

The mean of glycine betaine at 1st days was highest with treatment T_4 i.e 120 m eq/L (411.97 µg g^{-1} fr. wt) which followed with T_3 i.e 80 m eq/L (355.53 µg g^{-1} fr. wt), T_2 i.e 40 m eq/L (298.90 µg g^{-1} fr. wt) and T_1 i.e 00 m eq/L (213.97 µg g^{-1} fr. wt). The lowest glycine betaine content found with control treatment like T_1 i.e 00 m eq/L (213.97 µg g^{-1} fr. wt). While in case of 4th days after germination highest glycine betaine content was found in T_4 i.e 120 m eq/L (509.77 µg g^{-1} fr. wt.) and its was declined in control treatment like T_1 i.e 00 m eq/L (312.43 µg g^{-1} fr. wt) as compared with other treatments, as seen in table 7.

The interaction effect of varieties and treatments was found significant during the experimental studied at 1st and 4th day after germination, Among all varieties GHB-719 gave 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.

This pronouncement conform with finding of Rao *et al*., (2013) [12] who reported that, increase in salt stress resulted in increase the amount of glycine betaine content in tolerance genotype as compare to susceptible genotype of wheat.

Table 7: Effect on glycine betaine content (µg g^{-1} fresh weight) 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	186.00	256.10	310.87	386.60	284.89	283.80	353.90	408.67	484.40	382.69
2	GHB-538	157.07	190.87	226.03	300.23	218.55	254.87	288.67	323.83	398.03	316.35
3	GHB-719	213.97	298.90	355.53	411.97	320.09	311.77	396.70	453.33	509.77	417.89
4	GHB-577	145.07	197.47	233.30	277.90	213.43	242.87	295.27	331.10	375.70	311.23
5	GHB-1126	121.77	146.03	178.47	200.83	161.78	219.57	243.83	276.27	298.63	259.58
6	GHB-744	165.93	198.53	245.77	302.27	228.13	263.73	296.33	343.57	400.07	325.93
7	GHB-1138	133.13	166.93	212.17	255.07	191.83	230.93	264.73	309.97	352.87	289.63
8	GHB-905	148.10	194.70	244.60	306.90	223.58	245.90	292.50	342.40	404.70	321.38
9	GHB-935	195.93	246.03	277.73	317.60	259.33	293.73	343.83	375.53	415.40	357.13
10	GHB-757	159.30	205.17	246.33	317.03	231.96	257.10	302.97	344.13	414.83	329.76
11	GHB-557	142.50	177.63	213.00	263.30	199.11	240.30	275.43	310.80	361.10	296.91
12	GHB-1120	214.63	298.67	343.97	414.40	317.92	312.43	396.47	441.77	512.20	415.72
13	GHB-732	166.97	195.97	226.53	299.60	222.27	264.77	293.77	324.33	397.40	320.07
	Mean (Tx)	165.41	213.31	254.95	311.82		263.21	311.11	352.75	409.62	
		S.Em.±	C.D. at 5%		CV %		S.Em.±	C.D. at 5%		CV %	
	V	0.510	1.441		1.75		0.512	1.443		1.53	
	T	0.282	0.799				0.289	0.795			
	V X T	1.020	2.882				1.021	2.888			

Conclusions

Over all, physiological parameter clearly distinguished tolerant and susceptible varieties of pearl millet: GHB-744, GHB-577 and GHB-1126 give batter germination percentage and vigour index for treatment compared with others. The osmolytes were found higher in tolerant GHB-1132 and GHB-577 under salt condition. However total sugar, protein, glycine betaine are varies in moderate and susceptible varieties under salt stress condition.

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