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Effect of salinity on bio-chemical parameters of pearl millet (*Pennisetum glaucum* L.) varieties

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Abstract
A pot experiment was conducted at Department of Agricultural Chemistry and Soil Science, College of Agriculture, Junagadh Agricultural University, Junagadh to assess the "Effect of salinity on bio-chemical parameters, nutrient composition and yield of pearl millet (*Pennisetum glaucum* L.) varieties" during the summer season of 2017.The result revealed that the yield and yield attributes, quality parameters, bio-chemical parameters and nutrient uptake were significantly influenced by the different varieties of pearl millet and salinity levels. The significant differences were observed in proline accumulation among varieties. The highest (1.17 µmole gf.wt^{-1}) proline accumulation was found with variety GHB-538 (V_1) at 45 DAS. The proline content in varieties was observed in decreasing order of GHB-538> GHB-732> GHB-744> GHB-905> GHB-558. Among the different tested varieties, variety V_1 (GHB-538) produced significantly higher RWC, chlorophyll a, chlorophyll b and total chlorophyll content than other varieties. In case of chlorophyll a, chlorophyll b and total chlorophyll content, it was also remained at par with variety V_3 (GHB-732) at 45 DAS. The proline accumulation significantly increased with increasing salt concentration. The maximum (1.22 µmole gf.wt^{-1}) proline accumulation observed at salinity level S_4 (EC 8 dS m^{-1}). The different salinity levels produce significant effect on biochemical parameters like RWC, chlorophyll a, chlorophyll b and total chlorophyll content. These parameters are highest at salinity level S_1 (EC 2 dS m^{-1}). In case of RWC and chlorophyll a content which remains statistically at par with salinity level S_2 (EC 4 dS m^{-1}). The combined effect of variety and salinity was significantly affected on proline accumulation with variety GHB-538 (V_1) and salinity level S_4 (8 dS m^{-1}) at 45 DAS. The combine effect of variety and saline irrigation water on RWC and chlorophyll a content was found with variety V_1 (GHB-538) and salinity level S_1 (< 2 dS m^{-1}). In case of cholophyll and total chlorophyll content, the combine effect was found with variety V_3 (GHB-732) and variety V_5 (GHB-558) at salinity level S_1 (< 2 dS m^{-1}), respectively at 45 DAS.

Keywords: Pearl millet**,** salinity levels, varieties, bio-chemical parameters, proline, chlorophyll

Introduction
Pearl millet (*Pennisetum glaucum* (L) R. Br.) is one of the major coarse grain cereal crop and is considered to be poor people's food. It is widely grown in Africa and Asia since pre-historic times. In Asia, important pearl millet growing countries are India, China, Nigeria, Pakistan, Sudan, Egypt, Arabia and Russia. In India major bajara production state is Rajasthan followed by Maharashtra, Haryana, Gujarat, and Uttar Pradesh. In India, pearl millet is one of the important millet crops. Now a day, water scarcity is one of the most limiting factors in agricultural production. This limitation particularly in arid and semi-arid region because of salt accumulation in this area. Salinization of agricultural areas due to intense practices and irrigation is an important feature for limiting crop yield and production. Soil salinity adversely effects on plant growth and development. Worldwide, about one-third of irrigated arable land is already affected and that level is still rising (Lazof and Bernstein, 1999) [4]. An excess of soluble salts in the soil leads to osmotic stress, which results in specific ion toxicity and ionic imbalances and the consequences of these can be plant demise (Rout and Shaw, 2001) [9]. Proline accumulation is an important mechanism for osmotic regulation under salt stress. Proline is multifunctional amino acids and also acting as a plant growth regulator. Proline preferred as a common osmolyte in plants and get up-regulated against different stresses. Its accumulation in plants provides protection against salinity and drought stress. Exogenous application of proline improves the crop tolerance against various abiotic stresses particularly salinity by protecting them from the severe effects of Reactive Oxygen Species (ROS). Plants tend to enhance its endogenous level with continuously increasing levels of salinity.

Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell, which is reported in salt tolerant and salt sensitive cultivars of many crops (Misra and Gupta, 2005) [5]. Increasing crop salt tolerance is a highly attractive approach to overcoming the salinity threat. The need of the hour is to explore and select salt-tolerant genotypes within a species in comparison to relatively salt-sensitive ones through conventional selection and breeding techniques. Keeping this view an attempt has been made to the performance of different pearl millet genotype.

Materials and Methods

A pot experiment was conducted during *summer*- 2017 at the Department of Agricultural Chemistry and Soil Science, College of Agriculture, Junagadh Agricultural University, Junagadh. The experimental soil was clayey in texture and alkaline in reaction with pH 8.0, EC 0.58 dS m^{-1}, $CaCO_3$ 31.05% and CEC 36.2 cmol (p^+) kg^{-1}.The soil was low in available nitrogen (242 kg ha^{-1}), medium in available phosphorus (39.20 kg ha^{-1}), high in available potassium (298 kg ha^{-1}), high in available sulphur (29.50 kg ha^{-1}), low in available iron (3.25 mg kg^{-1}), high in available zinc (0.55 mg kg^{-1}), medium in available manganese (5.20 mg kg^{-1}) and high in available copper (1.25 mg kg^{-1}). The experiment consists of 20 treatments combinations comprising four levels of salinity water *viz.* < 2, 4, 6 and 8 dS m^{-1} and five levels of varieties *viz.* GHB-538, GHB-744, GHB-732, GHB-905 and GHB-558. Complete Randomized Design with total twenty treatments replicated thrice was employed in this study. Standard agronomic practices were adopted for raising healthy crop. Observations were recorded on five tagged plants in each treatment for growth and yield attributes. The collected data for various parameters were statistically analysed using F' test (Panse and Sukhatme, 1985) [7].

Result and Discussion

Effect of Variety

Bio-chemical parameters

The results showed that the bio-chemical parameters were significantly affected by different varieties of pearl millet at 45 DAS (Table 1). Significantly the highest proline content (1.17 µmole g^{-1} of fresh weight) was observed in variety V_1 (GHB-538) as compared to other varieties of pearl millet at 45 DAS. This might be due to tolerance of the variety to salinity. The proline accumulation in various variety was found in order of $V_1 > V_3 > V_2 > V_4 > V_5$. Different varieties of pearl millet significantly influenced on the relative water content (RWC) of pearl millet leaves at 45 DAS which was recorded higher with variety V_1 (GHB-538) in value of 80.10%. RWC directly reflects the water status of plants and its reduction indicated that salinity resulted in water deficit in plants. The negative effect on plant water relations was induced by an increase in soluble salts which decrease the uptake of water and nutrients causing osmotic effects and toxicity (Yang *et al.* 2009) [13]. Chlorophyll-a content of pearl millet leaves at 45 DAS among the varieties was significant the highest (13.88 mg g^{-1} of fresh weight) recorded with variety V_1 (GHB-538), which was found statistically at par with V_3 (GHB-732). Chlorophyll-b content of pearl millet leaves at 45 DAS was significantly highest (10.34 mg g^{-1} of F.W) recorded in variety V_1 (GHB-538), which remain statistically at par with V_3 (GHB-732) and V_4 (GHB-905). The total chlorophyll content was significantly influenced by the varieties of pearl millet (Table 1). The total chlorophyll was recorded higher with variety V_1 (GHB-538) with values of 23.30 mg g^{-1} of fresh weight. It was also remained at par with V_3 (GHB-732). The total chlorophyll content of pearl millets was observed in decreasing order of $V_1 > V_3 > V_4 > V_2 > V_5$.

Effect of Salinity

The calculation of proline content increasing with increasing levels of salinity (Table-1). This is due to pyrroline s-carboxylic acid is a key intermediate in proline biosynthesis pathway. The key enzyme responsible for diverting this precursor towards pathway other than proline biosynthesis is pyrroline s-carboxylic acid dehydrogenase. Significantly higher proline (1.22 µmole g^{-1} of fresh weight) content was recorded under application of 8.0 dS m^{-1} (S_4) saline irrigation water. Application of 2.0 dS m^{-1} (S_1) saline irrigation water gave significantly higher value of RWC (77.10%) which remain statistically at par with 4.0 dS m^{-1} (S_2). The RWC decreased with increased salinity levels. This may be due to the salt stress caused negative effect on water uptake in plant (Sheldon *et al.*, 2004) [11]. The valuation of chlorophyll-a content of pearl millet leaves at 45 DAS decreased with increasing levels of salinity. Significantly higher chlorophyll-a content (14.21 mg g^{-1} of fresh weight) was recorded under 2.0 dS m^{-1} (S_1) saline irrigation water, which remains statistically at par with 4.0 dS m^{-1} (S_2) and 6.0 dS m^{-1} (S_3). The chlorophyll content decreased with increasing salinity levels. This may be due to the reduced level of chlorophyll content under high salt stress condition in the leaves which may be due to membrane deterioration of the cell membrane of the chloroplastid leading towards lesser accumulation of chlorophyll and lesser photosynthetic efficiency as reported by several workers (Seeman *et al.*, 1985) [10]. The chlorophyll-b content was noticeably affected by increasing salinity levels. Result revealed that chlorophyll-b content decreased with an increasing salinity levels (Table 1). In general, decrease of these pigments under salt stress is considered to be a result of slow synthesis or fast breakdown of the pigments in cells (Ashraf and Neilly, 1987) [2]. The highest chlorophyll-b (12.00 mg g^{-1} of fresh weight) observed in salinity level (S_1) 2.0 dS m^{-1}. The total chlorophyll (24.08 mg g^{-1} of fresh weight) which was recorded higher under application 2.0 dS m^{-1} (S_1) saline irrigation water. Result revealed that total chlorophyll content decreased with an increasing salinity levels. This may be due to salt stress causes various effects on plant physiology such as increased respiration rate, ion toxicity, changes in plant growth, mineral distribution, and membrane instability resulting from calcium displacement by sodium, membrane permeability and decreased photosynthetic rate (Gupta *et al.*, 2002) [3].

Salinity decreases the amount of chlorophyll by suppression of the specific enzyme which is responsible for the synthesis of green pigments i.e., chlorophyll. Later on Reddy and Vora (1983) [8] reported that NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: cholorophyllase and inducing the destruction of the chloroplast structure and the instability of pigment protein complexes.

Table 1: Effect of varieties and salinity on biochemical parameters in leaves of pearl millet at 45 DAS

Treatments	Proline (µmole gf.wt^{-1})	RWC (%)	Chlorophyll-a (mg gf.wt^{-1})	Chlorophyll-b (mg gf.wt^{-1})	Total chlorophyll (mg gf.wt^{-1})
Variety (V)					
V_1-GHB-538	1.17	80.10	13.88	10.34	23.30
V_2-GHB-744	0.82	73.95	12.96	9.39	20.87
V_3-GHB-732	1.01	75.35	13.66	10.14	22.32
V_4-GHB-905	0.68	72.41	13.30	9.85	21.99
V_5-GHB-558	0.66	70.57	13.24	8.79	20.55
S.Em.+	0.02	1.27	0.20	0.19	0.36
C.D. (P=0.05)	0.05	3.63	0.57	0.55	1.04
Salinity level (S) dS m^{-1}					
S_1 - < 2.0 (tap water)	0.52	77.10	14.21	12.00	24.08
S_2 - 4.0	0.78	76.37	13.72	9.97	22.47
S_3 - 6.0	0.95	72.33	13.16	8.80	20.80
S_4 - 8.0	1.22	72.09	12.54	8.03	19.87
S.Em.+	0.02	1.14	0.18	0.17	0.32
C.D. (P=0.05)	0.04	3.25	0.51	0.49	0.93
Vx S Interaction					
S.Em.+	0.03	2.54	0.40	0.38	0.73
C.D. (P=0.05)	0.10	7.26	1.14	1.10	2.08
C.V.%	6.86	5.91	5.17	6.86	5.77

Interaction effect

The interaction effect between varieties and salinity levels on the proline content is given in Table 2. The result indicated that the highest proline content (1.68 µmole g^{-1} of fresh weight) was observed in variety V_1 (GHB-538) at S_4 (8.0 dS m^{-1}). Which indicated that out of five varieties, variety V_1 (GHB-538) found better against salt stress condition. These may be due to salt concentration increased biochemical component which act as compatible solute in plant to maintain the osmotic balance and also scavenge the free radicle under salt stress condition in pearl millet (Sneha *et al*., 2013) [12]. The lowest proline content was observed in V_4-GHB-905 under S_1 (2.0 dS m^{-1}). These results are supported by those by Moghaieb *et al*. (2009) [6] in wheat and Agarwal *et al*. (2016) [1] in finger millet. The combine effect of variety and saline irrigation water on RWC (Table 3) and chlorophyll a (Table 4) content was found with variety V_1 (GHB-538) and salinity level S_2 (< 2 dS m^{-1}). In case of cholophyll b (Table 5) and total chlorophyll (Table 6) content, the combine effect was found with variety V_3 (GHB-732) and variety V_5 (GHB-558) at salinity level S_2 (< 2 dS m^{-1}), respectively.

Table 2: Interaction effect of varieties and salinity on proline (µmole gf.wt^{-1}) content in leaves of pearl millet at 45 DAS

	S_1 - < 2.0 dS m^{-1} (tap water)	S_2 - 4.0 dS m^{-1}	S_3 - 6.0 dS m^{-1}	S_4 - 8.0 dS m^{-1}	Mean
V_1-GHB-538	0.75	0.94	1.30	1.68	1.17
V_2-GHB-744	0.53	0.79	0.82	1.16	0.82
V_3-GHB-732	0.47	0.83	1.22	1.51	1.01
V_4-GHB-905	0.30	0.74	0.76	0.91	0.68
V_5-GHB-558	0.55	0.62	0.66	0.82	0.66
Mean	0.52	0.78	0.95	1.22	
S.Em.±	0.03		C.D. (P=0.05)	0.10	

Table 3: Interaction effect of varieties and salinity on relative water content (%) in leaves of pearl millet at 45 DAS

	S_1 - < 2.0 (tap water) dS m^{-1}	S_2 - 4.0 dS m^{-1}	S_3 - 6.0 dS m^{-1}	S_4 - 8.0 dS m^{-1}	Mean
V_1-GHB-538	84.96	80.63	78.90	75.91	80.10
V_2-GHB-744	73.41	75.55	72.40	74.44	73.95
V_3-GHB-732	80.93	76.16	76.54	67.75	75.35
V_4-GHB-905	79.55	77.96	66.98	65.15	72.41
V_5-GHB-558	66.68	71.56	66.83	77.19	70.57
Mean	77.10	76.37	72.33	72.09	
S.Em.±	2.54		C.D. (P=0.05)	7.26	

Table 4: Interaction effect of varieties and salinity on chlorophyll-a (mg gf.wt^{-1}) content in leaves of pearl millet at 45 DAS

	S_1 - < 2.0 (tap water) dS m^{-1}	S_2 - 4.0 dS m^{-1}	S_3 - 6.0 dS m^{-1}	S_4 - 8.0 dS m^{-1}	Mean
V_1-GHB-538	15.40	14.61	12.88	12.65	13.88
V_2-GHB-744	14.36	13.77	12.16	11.53	12.96
V_3-GHB-732	14.80	13.58	13.85	12.40	13.66
V_4-GHB-905	13.31	13.24	12.86	13.77	13.30
V_5-GHB-558	13.17	13.39	14.05	12.34	13.24
Mean	14.21	13.72	13.16	12.54	
S.Em.±	0.40		C.D. (P=0.05)	1.14	

Table 5: Interaction effect of varieties and salinity on chlorophyll-b (mg gf.wt^{-1}) content in leaves of pearl millet at 45 DAS

	S_1 - < 2.0 dS m^{-1} (tap water)	**S_2 - 4.0 dS m^{-1}**	**S_3 - 6.0 dS m^{-1}**	**S_4 - 8.0 dS m^{-1}**	**Mean**
V_1-GHB-538	12.19	11.16	9.18	8.83	10.34
V_2-GHB-744	11.30	9.53	9.15	7.57	9.39
V_3-GHB-732	12.97	10.17	8.53	8.90	10.14
V_4-GHB-905	12.03	10.45	9.39	7.50	9.85
V_5-GHB-558	11.53	8.54	7.74	7.33	8.79
Mean	12.00	9.97	8.80	8.03	
S.Em.$\pm$	0.38		C.D. (P=0.05)	1.10	

Table 6: Interaction effect of varieties and salinity on total chlorophyll (mg gf.wt^{-1}) content in leaves of pearl millet at 45 DAS

	S_1 - < 2.0 (tap water) dS m^{-1}	**S_2 - 4.0 dS m^{-1}**	**S_3 - 6.0 dS m^{-1}**	**S_4 - 8.0 dS m^{-1}**	**Mean**
V_1-GHB-538	25.10	24.47	22.43	21.20	23.30
V_2-GHB-744	21.89	20.97	20.76	19.88	20.87
V_3-GHB-732	24.40	23.04	21.63	20.20	22.32
V_4-GHB-905	22.87	21.77	20.66	22.67	21.99
V_5-GHB-558	26.17	22.10	18.53	15.40	20.55
Mean	24.08	22.47	20.80	19.87	
S.Em.$\pm$	0.73		C.D. (P=0.05)	2.08	

Conclusion

On the basis of results, the pearl millet variety GHB-538 showed significantly higher values of bio-chemical parameters (proline, RWC, chlorophyll a, cholophyll b and total chlorophyll) over the salinity level. The pearl millet hybrid variety GHB-538 is found better up to EC 4 dS m^{-1} irrigation water. The tolerance order of pearl millet varieties to salinity in decreasing order of GHB-538 > GHB-732 > GHB-744 > GHB-558 > GHB-905 against salinity in silty clay soil.

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