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Studies on physio-chemical attributes of barnyard millet [*Echinochloa frumentacea* (Roxb.) Link] under sodicity

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Abstract
Barnyard millet (*E. frumentacea*) is a miracle crop due to its early mature, climate resilient capacity and nutrient potential. It is a hardy crop which can withstand a variety of abiotic stresses and one such limiting stress is sodicity. The crop also possesses immense potential in future as it holds an important stature in the quest for food and nutrition security. A study was conducted using ninety-seven barnyard millet germplasm along with two commercial checks *viz.,* MDU 1 and CO (KV) 2. Among the genotypes evaluated, 25 were selected (best and poor yielder) for physio-chemical analysis. Attributes related to sodicity stress like proline content, chlorophyll index and Na^+/K^+ ratio and iron nutrient content were estimated. These analyses found high yielding barnyard millet genotypes *viz*., BAR-242, BAR-252 and BAR 264 had high proline with low Na^+/K^+ ratio and moderate iron nutrient content. This study revealed that, the genotypes BAR242 followed by BAR252 and BAR264 were observed to produce better yield than checks CO (KV) 2 and MDU 1 with optimum nutrient (8.21mg/100g iron). Hence, BAR242 and BAR252 could be considered as sodicity tolerant genotypes which could be exploited for future breeding programmes under sodicity.

Keywords: Barnyard millet, Proline content, Iron nutrient, Na^+/K^+ ratio, Sodicity.

INTRODUCTION

Millets are a traditional staple food of the dry land regions of the world, grown for both food and fodder. In India, millets are grown on about 17 million ha with annual production of 18 million tonnes and contribute 10 percent to the country's food grain basket (Rao *et al*., 2017). They are nutri-cereals which includes protein, essential fatty acids, dietary fibre, B-Vitamins, minerals such as calcium, iron, zinc, potassium and magnesium (Rao *et al.,* 2017). It needs less water for cultivation that makes these crops suitable for arid and semi-arid farming in the world.

Barnyard millet is one among them and it had unique adapting capacity at both biotic and abiotic stress condition. It is a stable cereal in areas where climatic and edaphic conditions are unsuitable for rice cultivation (Yabuno, 1987). It is a fair source of protein and is an excellent source of dietary fibre with good amount of soluble and insoluble fractions (Veena *et al.,* 2005). It is a very good source of Iron (Sampath *et al.,* 1989) and it ranges from 2.29 to 18.00 mg/100g (Renganathan *et al.,* 2017) and many other phytochemicals. The grain has low carbohydrate content and it is slowly digestible and hence is suitable for people suffering with diabetes mellitus (Ugare, 2008).

Globally, 37 per cent of the arable land is sodic in nature (Szabolcs, 1989 and Leland *et al.,* 1999). Sodicity is one of the major abiotic stresses which affects the barnyard millet crop yield. Sodic soils are characterised by high pH (>8.5) and Exchangeable Sodium Percentage (ESP > 15 %), low EC (< 4.0 dS/m) and imbalanced nutrition with ion toxicity which exhibits poor physical and chemical features (Khan and Duke, 2001) that hinders seed emergence and crop growth. Barnyard millet has also been used for the reclamation of sodicity, arsenic and cadmium affected soils (Abe *et al*., 2011). The high alkaline pH nature of sodic soil results in lesser availability of micronutrients and deteriorates the soil structure and porosity which in turn causes water logging (Rengasamy, 2002). In sodic soil, plants suffer from micronutrient deficiency (e.g., Cu, Fe, Mn and Zn) as the solubility of micronutrients is at an all time low. Under sodic soil condition, biochemical responses on plants *viz*., proline content, chlorophyll content and sodium potassium ratio provided better knowledge for selection best genotypes (Tripathi *et al.,* 2018).

Iron is one of the important mineral nutrients that plays a major role in our body as it is a vital component of haemoglobin. Its deficiency causes anemia and according to the World Health Organization, it is one of the top nutritional disorders in the world and 30% of people are affected by anemia, especially in the developing countries. Hence, the proper growth and development needs iron nutrient from our diets. Among the different millet crops, barnyard millet is endowed with high iron content of upto 16 mg/100g of grain (Vanniarajan *et al.*, 2018). Estimation of physiological attributes related to sodicity stress like proline content, chlorophyll content, and sodium/ potassium ratio provides reliable knowledge for selecting best genotypes under sodic condition. Considering the importance of nutritional security and necessity of nutrient food for health benefits, this study was also estimated the iron content present in grain part of selected barnyard millet germplasm accessions under sodic condition. There is paucity of information about the above facts. Hence, this research was undertaken, and this is the first study to assess the performance of barnyard millet germplasm accessions by physio-chemical analyses in natural sodic soil condition.

MATERIALS AND METHODS

This study was carried out in natural sodic soil (pH: 9.07, EC: 0.95dS/m and ESP: 43.69%) at Anbil Dharmalingam Agricultural College and Research Institute, Trichy, Tamil Nadu during summer 2019. The experimental material consists of 97 germplasm lines of barnyard millet obtained from Indian Institute of Millets Research (IIMR) and two commercial check varieties viz., MDU1 and CO(KV) 2. The experiment was laid out in randomized block design with two replications. Observations were recorded on yield contributing biometrical traits *viz*., days to fifty percent flowering, plant height (cm), inflorescence length (cm), lower raceme length (cm), harvest index, thousand grain weight (g) and grain yield per plant (g) as per the descriptors of Barnyard millet (IPGRI, 1983). Among the genotypes evaluated, 26 were selected based on yield under sodic soil condition (13 best and 13 poor yielder) for the purpose of physio-chemical studies. The indirect estimation of total chlorophyll content was done by using SPAD meter which gives the relative amount of chlorophyll present in the sample by measuring the absorbance of the leaves in two different wavelength regions (red 650nm and infrared 940nm). The amino acid, proline content was estimated in fully expanded leaves at flowering stage following the method of Bates *et al.* (1973) and is expressed in µg g^{-1} on fresh weight basis. Sodium and potassium content were estimated by flame photometer method using the triple acid extract of dry sample at maturity stage as proposed by Jackson (1973). Iron content was estimated in grains of selected high yielding 25 barnyard millet genotypes as suggested by Jackson (1973) and expressed in mg $100g^{-1}$ on seed weight basis.

Data from mean of individual genotypes were subjected to usual method of analysis of variance (ANOVA) as per the (Panse and Sukhatme*,* 1967). The association analysis was done as per Singh and Chaudhary (1977).

RESULTS AND DISCUSSION

In the present study, under the sodic condition, the evaluation of selected barnyard millet accessions for physio-chemical traits exhibited considerable variation which indicating the existence of more variability among the genotypes presenting excellent avenue for selection of desirable genotypes for sodicity. While evaluating the genotypes, high mean value is considered as the primary criterion for a long time among the breeders. Mean performance of physio-chemical attributes is presented in **Table 1.**

Sodium-potassium ratio showed wide variation among the genotypes and ranged from 0.08 (BAR 242) to 0.67 (BAR 198) with a mean value of 6.79. The highest Na^+/K^+ ratio was recorded by BAR 198 followed by BAR 228, BAR 208 and BAR 317, but these genotypes recorded low grain yield per plant. Low Na^+/K^+ ratio was observed in BAR 242 followed by BAR 252, CO (KV) 2 and MDU 1, but these genotypes exhibited higher mean for grain yield per plant. This indicates the negative association between Na^+/K^+ ratio and grain yield. This could be because under sodicity stress, tolerant plants accumulate more potassium as a tolerance mechanism and produce better yield. This result is in conformity with the findings of Joshi *et al.* (1980), Qadar (1995), Qadar and Azam (2007) in rice.

Proline is an excellent osmolyte (Hayat *et al.,* 2012) and its accumulation was correlated with improved plant performance under stress condition by down regulation of the genes involved in proline catabolism. Under sodicity, proline content exhibited wide range of variation and ranged from 140 µg/g (BAR 198) to 790 µg/g (BAR 242) with a

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Table 1. Mean performance of 26 selected barnyard millet genotypes for physio-chemical and biometrical attributes in sodicity

S. No.	Genotypes	Sodium-potassium ratio	Proline content µg/g	Chlorophyll content (SPAD value)	Days to fifty percent flowering	Plant height (cm)	Inflore-scence length (cm)	Lower raceme length (cm)	Harvest index	Thousand grain weight (g)	Grain yield per plant (g)
1	BAR 242	0.08*	790**	31.66	39.5	49.63	9.92	1.55	0.45**	2.46	41.88**
2	BAR 252	0.11*	610**	34.25	45**	73.11**	11.1	2.84**	0.44**	2.31	28.14**
3	CO (KV) 2	0.22	710**	31.55	42	56.37*	11.04	2	0.44**	2.58	27.05**
4	MDU 1	0.27	670**	32.11	43.5*	56.94*	11.39	2.38	0.43**	3.06**	24.5**
5	BAR 264	0.12*	650**	29.2	38	32.26	7.58	0.72	0.42**	2.15	22.96**
6	BAR 353	0.12*	420	31.25	36	39.7	8.05	1.2	0.42**	2.16	22.95**
7	BAR1365	0.13	620**	32.9	39.5	47.17	9.68	1.73	0.42**	2.13	22.05**
8	BAR 351	0.15	620**	34.81	36.5	43.79	9.33	1	0.42**	2.43	21.3**
9	BAR 263	0.17	610**	29.82	35.5	66.42**	12.08	2.32	0.41**	1.9	19.1**
10	BAR 372	0.19	600**	35.7	39	40.73	10	1.97	0.41**	2.09	17.26
11	BAR 119	0.2	580**	33.4	40	56.85*	12.3	1.75	0.41**	2.49	16.97*
12	BAR 131	0.2	530**	28.15	44*	60.99**	11.27	2.27	0.4**	2.62*	16.26
13	BAR 350	0.2	460	34.66	36	48.94	15.2	3.35**	0.4**	2.06	16.11
14	BAR 366	0.21	440	25.97	36	46.02	10.55	1.49	0.4**	2.12	15.75
15	BAR 203	0.23	360	29.91	35	35.33	6.91	1.35	0.31	2.03	6.43
16	BAR 199	0.25	340	34.15	35	45.49	8.73	1.41	0.27	2.22	4.66
17	BAR 254	0.26	350	30.82	39.5	40.93	8.93	1.42	0.25	2.15	4.26
18	BAR 206	0.28	320	31.24	35	49.35	9.22	1.41	0.25	2.07	4.15
19	BAR 160	0.34	320	34.61	36	41.03	10.23	2.03	0.25	1.95	4.1
20	BAR 215	0.35	300	29.12	35.5	54.69	11.22	1.74	0.25	2.1	4.02
21	BAR 178	0.36	310	35.43	35.5	47.56	10.03	1.58	0.25	2.06	3.99
22	BAR 207	0.39	270	28.36	35.5	46.84	8.63	1.57	0.24	1.91	3.97
23	BAR 317	0.41	210	31.73	35	35.57	8.09	1.12	0.24	1.92	3.75
24	BAR 208	0.42	220	29.67	35	42.89	7.87	1.54	0.26	2.11	4.61
25	BAR 228	0.46	170	33.75	46**	58.89**	10.88	2.5*	0.24	1.87	3.67
26	BAR 198	0.67	140	35.57	35	38.2	10.13	1.17	0.22	1.59	3.1
Mean		0.26	446.92	31.92	38.02	48.3	10.01	1.75	0.34	2.17	13.96
S. Ed		0.07	15.2	3.55	2.43	3.58	1.24	0.31	0.02	0.21	1.31
CD (5%)		0.14	31.31	7.31	5.05	7.38	2.55	0.64	0.04	0.44	2.7
CD (1%)		0.19	42.41	9.91	6.85	10	3.45	0.87	0.06	0.59	3.66

mean value of 447 µg/g. Proline content was observed to be high in the genotype BAR 242 followed by CO (KV) 2, MDU 1 and BAR 252. Incidentally these genotypes were also observed to record the highest grain yield per plant. This indicates that under stress condition, accumulation of proline in plants takes place that retain permanence of mitochondrial electron transport which directly enhance photosynthetic yield (Hayat *et al.,* 2012) and this helps the plants to attain stability in performance. This was further confirmed by the fact that minimum proline content was recorded in BAR 198 followed by BAR 228, BAR 317 and BAR 208 which were low yielders. This result corroborates with the findings of Sabir *et al.* 2011 and Dubey *et al.* 2018 who reported that free proline accumulation increased in leaves under stress condition.

The chlorophyll meter provides a simple, rapid, and non-destructive method for estimating leaf chlorophyll content in relation with leaf nitrogen concentration (Zhang *et al.,* 2019). Leaf nitrogen is closely related to photosynthetic rate and grain yield in rice (Peng *et al.,* 1995). Hence, high nitrogen count leads to increase the chlorophyll content that could be increase the grain yield. Wide variation was also observed for chlorophyll content, ranged from 25.97 (BAR 366) to 35.70 (BAR 372) with a mean value of 31.92. The genotypes BAR 372 showed high chlorophyll content followed by BAR198, BAR 178 and BAR 351which indicates no chlorophyll reduction that supported photosynthetic rates for better tolerance of these genotypes under sodicity. Lowest chlorophyll content was noted by BAR

366 followed by BAR 131, BAR 207 and BAR 215 which implies that influence of sodicity effect on these genotypes *i.e.,* chlorophyll degradation. This is accordance with the conclusion derived by Singh *et al.* (1990) in mustard under sodic condition.

Proline content is positively correlated with salt stress whereas chlorophyll content and Na^{+}/K^{+} ratio were negatively correlated with it (Kumar *et al.,* 2017). This could be because of accumulation of the Reactive Oxygen Species (ROS) under stress condition which causes degradation of chlorophyll content (Kumar *et al.,* 2017).

Correlation studies on physio-chemical traits revealed that (**Table 2**), grain yield per plant for 25 selected genotypes expressed significant positive correlation with proline content, days to fifty percent flowering, harvest index and thousand grain weight. This indicated that accumulation of as proline increases in tolerant genotypes in response to stress, better performance of the genotypes with reference to the above yield component traits is achieved. Similar association between proline and grain yield were recorded by Gopikannan and Ganesh (2013) in rice under sodicity. Sodium and potassium ratio showed negative significant correlation with grain yield per plant. This could be because of the fact that accumulation of more potassium takes place in tolerant genotypes (Joshi *et al.,* 1980, Qadar, 1995). Chlorophyll content had non-significant negative correlation with yield.

Sodium and potassium ratio expressed significant and positive correlation with chlorophyll content and also showed significant negative correlation with proline content (Kumar *et al.,* 2017), days to fifty percent flowering, harvest index and thousand grain weight. Proline content was observed to have highly significant positive correlation with days to fifty percent flowering, harvest index and thousand grain weight. This could be because, proline maintains the osmotic balance, integrity of cell membrane and prevents the damages caused by ROS in plants (Peng *et al.,* 1995, Sabir *et al.,* 2011 and Dubey *et al.,* 2018). Chlorophyll content (SPAD value) was found to have highly significant and positive correlation with inflorescence length, days to fifty percent flowering, and lower raceme length. This augurs well for the fact that high chlorophyll content induces more photosynthesis which ultimately leads to better performance of the plant.

Iron content was estimated in 25 high yielding barnyard millet genotypes (**Fig. 1**). It recorded wide range of variation with a minimum of 1.87 mg/100g (BAR 220) to a maximum of 11.88 mg/100g (BAR 370) with a mean value of 5.22 mg/100g. Highest iron content was recorded by the genotype BAR 370 followed by MDU 1, BAR 372, BAR 252, BAR 242, CO (KV) 2, BAR 366, BAR 131 and BAR 329. Low content was exhibited by BAR 220 followed by BAR 269, BAR 389, BAR 193, BAR 358, BAR 263 and BAR 353 which also simultaneously exhibited higher mean value for grain yield per plant. Utilizing high yielding

Table 2. Correlation coefficient among yield and yield components with physio-chemical attributes in barnyard millet genotypes

Characters	Sodium-potassium ratio	Proline content	Chlorophyll content (SPAD value)	Days to fifty percent flowering	Plant height	Inflore-scence l ength	Lower raceme length	Harvest index	Thousand grain weight	Grain yield per plant
Sodium- potassium ratio	1	-0.880**	0.565**	-0.410*	-0.24	-0.14	-0.18	-0.910**	-0.690**	-0.810**
Proline content	-0.780**	1	-0.12	0.482*	0.345	0.317	0.197	0.945**	0.801**	0.921**
Chlorophyll content (SPAD value)	0.077	0.008	1	0.981**	0.236	1.207**	0.537**	-0.16	-0.450**	-0.08
Days to fifty percent flowering	-0.12	0.383*	-0.04	1	0.700**	0.437*	0.624**	0.483*	0.666**	0.512**
Plant height	-0.19	0.325	-0.06	0.548**	1	0.753**	0.784**	0.352	0.473*	0.346
Inflorescence length	-0.07	0.243	0.104	0.274	0.575**	1	0.983**	0.378	0.168	0.276
Lower raceme length	-0.08	0.18	0.192	0.437*	0.653**	0.689**	1	0.287	0.306	0.195
Harvest index	-0.760**	0.909**	-0.05	0.366	0.301	0.273	0.216	1	0.722**	0.936**
Thousand grain weight	-0.410*	0.612**	-0.04	0.472*	0.309	0.235	0.114	0.541**	1	0.695**
Grain yield per plant	-0.700**	0.911**	0.001	0.397*	0.3	0.205	0.169	0.922**	0.544**	1

Values above diagonal indicates genotypic correlation and values below indicates phenotypic correlation coefficients, respectively.
* Significant at 5% level
** Significant at 1% level

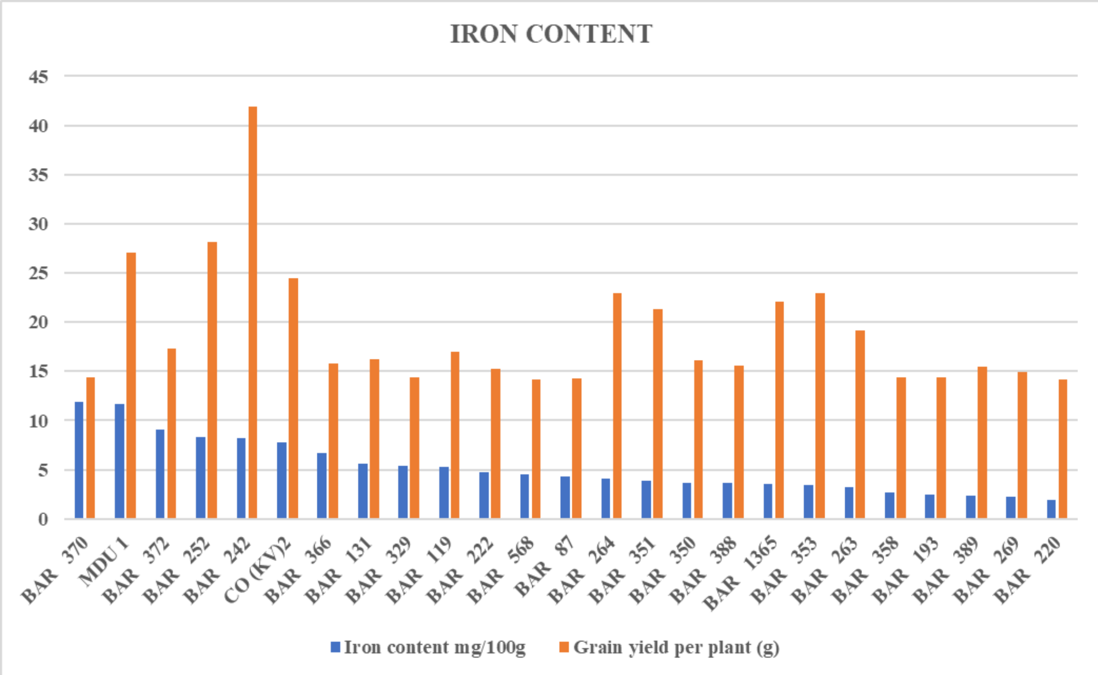

Fig. 1. Iron content in 25 high yielded barnyard millet genotypes

barnyard millet genotypes which coupled with high iron nutrient content for breeding programme could be provide better nutrient benefits for health as well as reduce the risk of chronic diseases and also high yielding nature improves commercial benefits. Chandra *et al.* (2018), Renganathan and Vanniarajan (2018), Vishnuprabha and Vanniarajan (2018), Prakash *et al.* (2016) and Verma *et al.* (2015) estimated iron content in barnyard millet and they concluded that high iron content is present in barnyard millet than the other millets.

This study supports that the iron content could be increased with increasing the grain yield hence, improving high yielding genotypes leads to enrich the iron content. In any crop the yield will be inversely proportional to the quality parameters (Prakash *et al.,* 2016). This is in accordance with the findings of Babu *et al.* (2013), who observed negative correlation between grain yield and grain iron content in rice genotypes. On the contrary in the present study, it was observed that BAR 370 followed by MDU 1, BAR 372, BAR 252, BAR 242, CO (KV) 2, BAR 366, BAR 131 and BAR 329 had high yielding as well as good in iron content. Similar findings were reported by Prakash *et al.* (2016) in barnyard millet.

This is the first study to our realization to provide detailed prospective details on biochemical response among barnyard millet genotypes cultivated under natural sodic soil condition. This study provides insights about sodicity stress responsive biochemicals on barnyard millet and its influence with yield. In general proline content was positively correlated with stress condition whereas chlorophyll content and Na^+/K^+ ratio were negatively correlated with it. It was confirmed by the present study under sodicity for proline, one of stress state for plant growth. Correlation study of physio-chemical attributes with biometrical traits revealed that significant positive correlation on grain yield per plant was showed by proline content, days to fifty percent flowering, harvest index and thousand grain weight. Better performance of the genotypes with reference to the above yield component traits could be due to accumulation of high proline in response to sodicity stress whereas, Na^+/K^+ ratio showed negative significant correlation with grain yield per plant. The reason is accumulation of more potassium takes place in tolerant genotypes. This research found that chlorophyll content had no effect with yield in barnyard millet.

Based on physio-chemical attribute like the Na^+/K^+ ratio, chlorophyll content and proline content, the genotypes BAR 242 followed by BAR 252, BAR 264, BAR 353, BAR 1365, BAR 351, BAR 263, BAR 372, BAR 119 were observed to produce better yield than checks CO (KV) 2 and MDU 1 which indicated that these genotypes were more suitable under sodicity. On the nutritional front, highest iron content was recorded by the genotype BAR 370 followed by MDU 1, BAR 372, BAR 252, BAR 242, CO (KV) 2, BAR 366, BAR 131 and BAR 329. These genotypes with high yield and iron content could be exploited for future breeding programmes in the crop. Nevertheless, some encouraging results regarding iron content in grains by nutrient analysis provide the motivation for conducting further nutrient studies to find iron rich genotypes under sodic condition and it also helps for confirmation the result of iron content in grains of selected genotypes which could be exploited for future breeding programmes.

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