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Research Article

Nutrient Analysis Based Grouping of Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract

The present investigation comprising four hybrids viz., Shraddha, Shanti, DHBH 9070 and DHLB 1307 and their female and male par-ents viz., RHRB 1A, RHRBI 138, RHRB 13A, RHRBI 1314, DHLB 8A, DHLBI 967, DHLB 17A and DHLBI 731 respectively developed by Bajara Research Scheme, MPKV, Rahuri. All these genotypes were evaluated in RCBD with three replications under two environments created by adopting two dates of sowing and field locations (*Kharif-2014* and *Summer-2015*). Mineral composition (Fe, Zn, protein, ash and CHO) was determined for grouping of pearl millet hybrids and their parents. The Hy. Shraddha recorded the highest values among all genotypes for Iron (61.08 ppm), Zinc (49.20 ppm), protein (10.11%), ash (2.25 g/100g) and CHO (64.99 g/100g). Both the parents of Hy. Shraddha i.e. RHRB 1A, and RHRBI 138 and Hy. DHLB 1307 with its 'R' line i.e. DHLBI 731 recorded high values (Above 52.55 ppm) for Iron. Hybrid DHBH 9071 grouped under high protein (Above 8.71%) and high CHO (Above 60.08 g/100g) content category. Hy. DHBH 9071 and Hy. DHLB 1307 were grouped as high (Above 1.87 g/100g) ash content genotypes. The lowest values for Iron (35.49 ppm), Zinc (28.44 ppm), protein (5.91%), ash (1.12 g/100g) and CHO (50.27 g/100g) were recorded in RHRB 13A, RHRBI 1314, DHLBI 731, DHLB 17A and RHRB 1A respectively. Results leads to conclude that, all the hybrids viz., Shraddha, Shanti, DHBH 9071 and DHLB 1307 were found distinct from each other. Quantitative traits were differed in expressions among the geno-types due to two different growing seasons.

Keywords: Pearl millet; Nutrients; Grain composition; Fe; Zn; Protein; CHO; Ash

Abbreviations

Hy.: Hybrid; g: grams; ppm: parts per million; CHO: carbohydrates; Fe: Iron; Zn: Zinc; Env.: environment; Gen.: geno-type; S.E.: Error of significance; CD.: critical difference; CV.: coefficient of variation

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br., 2n = 2x = 14, Family: *Poaceae;* Subfamily: *Panicoideae*; Tribe: *Paniceae;* Subtribe: *Panici-nae*; Section: *Penicillaria*; and Genus: *Pennisetum*) is also known as bulrush or cattail millet, is an important grain and forage crop in Africa and South Asia and a forage crop in the Americas. The genus Pennisetum contains about 140 species. The important wild relatives of cultivated pearl millet include the progenitor, *Pennisetum glaucum* subsp. *monodii Maire*; *P. purpureum K. Schumach.*; *P. pedicellatum* Trin.; P. orientale Rich.; P. mezianum Leeke; and P. squamulatum Fresen. Previous names are P. typhoideum L. C. Rich. and P. americanum (L.) Leeke. The four cultivated forms of pearl millet are typhoides (found mainly in India and Africa), nnigritarum (dominant in eastern Sahel), globosum (dominant in the western Sahel) and leonis (dominant on the West African coast) [1,2]. The nutritive value of pearl millet is higher than rice, wheat, and even maize [3,4]. In addition, it is a short-duration (60-90 days) crop that allows double cropping in manyplaces. The germplasm of its land races show a wide range of variation for several characters including plant type, photoperiod sensitivity, asynchronous flowering maturity period and sugar content [5].

Morris., *et al*. [6] assessed the relative contribution of wheat genotype and environment to variation in ash content, protein content, test weight, and kernel hardness, weight, and size. They concluded that, wheat grain ash is more greatly influenced by crop year and location than by genotype. However, sufficient genotype variation is present to plausibly manipulate this grain trait through traditional plant breeding. Hariprasanna., *et al.* [7] assessed the stability of ten sorghum genotypes grown at six locations for grain iron (Fe) and zinc (Zn) content as a prerequisite in breeding for micronutrient enrichment in grains. Significant genotype × environment (G × E) interactions were observed for both grain Fe and Zn, indicating differential nutrient accumulation by the genotypes. Jahan., *et al*. [8] determined Iron (Fe) content of rice grain samples and suggested that there were inherent genetic differences among the genotypes. Thus, local landraces can be a good source for biofortification of popular rice cultivars using different breeding methods. Keeping all these facts in view, present investigation was undertaken with the objective of determination of nutrient content and grouping the pearl millet genotypes accordingly.

Materials and Methods

The present investigation was conducted at Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri during Kharif- 2013 and Summer-2014. Geographically, Rahuri is situated at 19° 34' N latitude and 74° 64' E longitude with an altitude of 536 meters above Mean Sea Level. The experimental material was developed at and provided by the AICPMIP, Bajra Research Scheme, Dhule, Mahatma Phule Agriculture University, Rahuri during Kharif-2012. Randomized Complete Block Design (RBD) was followed with 3 replications each consisting 6 rows of 6m in length; 60 × 15 cm row to row and plant to plant distance was maintained on well drained deep black soil having good water holding capacity. All recommended agronomical practices and plant protection measures were followed as and when required for raising good crop. Mineral (Fe & Zn) content of grain samples was determined by using Atomic Absorption Spectrophotometer (AAS) as stated by Lindsay and Novell [9]. In this process the samples were digested by the application of di-acid mixture [10,11] which includes nitric acid (HNO_3): and perchloric acid ($HClO_4$) in 2:1 ratio. The detailed procedure of this process described below.

From the each genotypes one gram (1g) amount of grain powder was taken in 150 ml conical flask and 10 ml of di-acid mixture (HNO3: HClO4 = 2:1) added to it. It was kept overnight at room temperature (2.00 PM to 11.00 AM). Then the conical flask was placed on sand bath at temperature 180~200°C for 30~40 minutes. After a few minutes brown fume was evolved. This indicated the starting of digestion process. Finally white fume was seen by clearing the solution. At the bottom of the conical flask about 2-3 ml solution was noticed. After that heating was stopped and the digested sample was cooled for 20 minutes. Then about 20~30 ml distilled water was added to each conical flask. Then this solution was filtered into a 50 ml volumetric flask and the volume was made up to the mark (50 ml) by adding distilled water. The 50 ml solution was then transferred into a plastic bottle for each genotype for the further use in future. The plastic bottle was stored at a room temperature.

Fe and Zn content determination by AAS

It is based on the principle that atoms of iron (Fe/Zn) which is normally remain in ground state, under flame condition absorb energy when subjected to radiation is proportional to the specific wavelength. The absorption of radiation is proportional to the concentration of iron or zinc. Iron and zinc content was estimated in the aliquot of seed extract by using Atomic Absorption Spectrophotometer (AAS) at 248.33 nm.

Ash Content (g/100 gm)

1g of the oven-dried sample in powder from was placed in crucible of known weight. This was ignited in a muffle furnace for 4h at 550°C. The crucible was cooled and weighed and the ash content was expressed in terms of the oven-dried weight of the sample [12].

Protein (%) and Carbohydrates content (g/100 gm) estimation

Protein and carbohydrates estimation was done on Zeutec SpectraAlyzer machine, which estimates mineral content of grain sample automatically within few seconds.

Based on composition, genotypes were grouped accordingly for, Iron content Low: Below 44.02 ppm, Medium: below 44.02 to 52.55 ppm, and High: Above 52.55 ppm; Zinc content: Low: Below 35.36 ppm, Medium: 35.36 to 42.28 ppm, and High: Above 42.28 ppm; Ash content: Low: Below 1.50 g/100g, Medium: 1.50 to 1.87 g/100g, and High: Above 1.87 g/100g; Carbohydrates content: Low: Below 55.17 g/100g, Medium: 55.17 to 60.08 g/100g, and High: Above 60.08 g/100g; Protein content Low: Below 7.31%, Medium: 7.31 to 8.71%, and High: Above 8.71%.

Results and Discussion

The seed mineral densities were differed significantly among all the hybrids and their parents as well between seasons. As a pre-requisite in breeding for micronutrient enrichment in grains iron is very valuable micronutrient. The parents studied, representing potential

breeding lines which can be advantageously utilized in breeding programmes designed to improve the nutritive quality of pearl millet. The ANOVA table revealed significant variation present in genotypes, which helped in grouping and ultimately enables the identification of genotypes. The mean seed iron content during *Kharif*-2013 (49.99 ppm) was found more than *Summer*-2014 (45.56 ppm). It was differed among all studied lines and found to little more during *Kharif*-2013. However, it ranged from 38.63 (RHRB 13A) to 62.57 ppm (Shraddha) and 32.35 (RHRB 13A) to 59.58 ppm (Shraddha) during *Kharif*-2013 and *Summer*-2014 respectively. Pooled analysis showed significantly highest mean iron content in Shraddha (61.08 ppm) and lowest in RHRB 13A (35.49 ppm). Based on the means of pooled data, the genotypes viz., Shraddha, RHRB 1A, RHRBI 138, DHLB 1307 and DHLBI 731 were grouped high (> 52.55 ppm), DHLB 17A medium (44.02 to 52.55 ppm) and Shanti, RHRB 13A, RHRBI 1314, DHBH 9071, DHLB 8A and DHLBI 967 as low (< 44.02 ppm). The results suggested that there were inherent genetic differences among the genotypes. Thus, these lines can be a good source for bio-fortification of popular pearl millet cultivars using different breeding methods. The study also showed the necessity of repeated multi-location as well as multi-season evaluation of genotypes for identifying stable donors that can be used in breeding programmes for micronutrient enrichment. The result were consent with the findings of Abdalla., *et al.* [13] and Buerkert., *et al*. [12] in pearl millet, Hariprasanna., *et al*. [7] in sorghum and Jahan., *et al.* [8] in rice.

Source of variation	df	Mean sum of square											
		Iron (ppm)						Zinc (ppm)					
		Kharif-2013		Summer- 2014		PEVs		Kharif-2013		Summer-2014		PEVs	
Replications	2	0.04	NS	0.02	NS	-		0.49	NS	0.06	NS	-	
Environments	1	-		-		352.86	**	-		-		320.90	**
Genotypes	11	247.31	**	274.85		520.17	**	104.11	**	107.58	**	209.42	**
Env.× Gen.	11	-		-	**	1.99	*	-		-		2.27	**
Error	22(44)	1.41		0.16		0.78		0.42		0.06		0.24	
S.E. ±	-	0.69		0.23		0.51		0.37		0.14		0.28	
C.D. 5%	-	2.0		0.68		1.46		1.09		0.41		0.80	
C.V.%	-	2.37		0.88		1.85		1.72		0.72		1.37	

Source of variation	df	Mean sum of square											
		Protein (%)						Ash (gm/100g)					
		Kharif-2013		Summer- 2014		PEVs		Kharif-2013		Summer-2014		PEVs	
Replications	2	0.50	NS	0.10	NS	-		0.001	NS	0.001	NS	-	
Environments	1	-		-		68.12	**	-		-		2.13	**
Genotypes	11	5.89	**	6.82		12.27	**	0.48	**	0.48	**	0.95	**
Env.× Gen.	11	-		-	**	0.43	*	-		-		0.01	**
Error	22(44)	0.36		0.06		0.21		0.01		0.01		0.01	
S.E. ±	-	0.35		0.14		0.26		0.05		0.04		0.04	
C.D. 5%	-	1.01		0.42		0.75		0.14		0.11		0.04	
C.V.%	-	7.20		3.86		6.23		4.74		4.76		0.12	

Table 1: Analysis of variance for individual and pooled over the environments (PEVs) for chemical composition like iron (ppm), zinc (ppm), protein (%) and ash (gm/100g) content of pearl millet genotypes.

*, ** Significant at 0.05 and 0.01 levels of probability, respectively; figure in parenthesis is pooled error df.

Source of variation	df	Mean sum of square					
		CHO (g/100 gm)					
		Kharif-2013		Summer-2014		PEVs	
Replications	2	0.03	NS	0.04	NS	-	
Environments	1	-		-		421.29	**
Genotypes	11	57.09	**	59.68	**	112.21	**
Env.× Gen.	11	-		-		4.57	**
Error	22(44)	0.02		0.07		0.04	
S.E. ±	-	0.07		0.15		0.12	
C.D. 5%	-	0.22		0.44		0.34	
C.V.%	-	0.22		0.49		0.37	

Table 2: Analysis of variance for individual and pooled over the environments (PEVs) for CHO (gm/100g) content of pearl millet genotypes. *, ** Significant at 0.05 and 0.01 levels of probability, respectively; figure in parenthesis is pooled error df.

The mean zinc content of hy. Shraddha was observed highest (51.01 ppm and 47.40 ppm during) Kharif-2013 and Summer-2014 respectively (Table 3), while the lowest Zn content was recorded in RHRBI 1314 during Kharif-2013 (30.78 ppm) and Summer-2014 (26.10 ppm). Based Zinc composition, genotypes viz., RHRB 1A, RHRBI 138, DHLB 8A and DHLB 1307 grouped in medium (35.36 to 42.28 ppm) and Shanti, RHRB 13A, RHRBI 1314, DHBH 9071, DHLBI 967, DHLB 17A and DHLBI 731 in low (< 35.36 ppm) Zn content group (Table 4.3.3). Shraddha was the only genotype grouped under high (> 42.28 ppm) Zn content genotype, which facilitates its easy identification. Likewise, the significant differences noted among the genotypes which could be used as the criteria for grouping and identification of genotypes. The studied genotypes represent breeding lines by wide variability in their zinc concentration which could be advantageously utilized in breeding programmes designed to improve the nutritive quality of pearl millet. The results were confirmed with that of Buerkert., *et al.* [12] (2001) in pearl millet and Hariprasanna., *et al*. [7] in sorghum.

The mean protein content; pooled over environments (PEVs) of pearl millet hybrid Shraddha was significantly highest (10.11%) while lowest was recorded in DHLBI 731 (5.91%). The protein content was ranged from 6.98 (DHLBI 731) to 11.10 (Shraddha) and 4.52 (RHRBI 1314) to 9.11% (Shraddha) during Kharif-2013 and Summer-2014 respectively. hybrids, Shraddha and DHBH 9071 were categorized as high (>8.71%) protein content genotype, while hybrids Shanti and DHLB 1307 along with RHRBI 138 and DHLB 8A categorized as medium (7.31 to 8.71%) protein content genotype. The genotypes viz., RHRB 1A, RHRB 13A, RHRBI 1314, DHLBI 967, DHLB 17A and DHLBI 731 were grouped under low (< 7.31%) protein group. A significant reduction was observed in protein content with change growing season. While the results need to be studied in replicated multi-location trial studies, to underline the potential of parental lines in breeding programs to concurrently improve grain quality. The results were consent with the findings of Buerkert., *et al.* [12] in pearl millet.

Genotypes	Iron (ppm)				Zinc (ppm)				Protein (%)			
	Kharif 2013	Summer 2014	PEVs	Groups	Kharif 2013	Summer 2014	PEVs	Groups	Kharif 2013	Summer 2014	PEVs	Groups
Shraddha	62.57	59.58	61.08	High	51.01	47.40	49.20	High	11.10	9.11	10.11	High
RHRB 1A	57.63	53.07	55.35	High	44.05	37.31	40.68	Medium	7.05	5.05	6.05	Low
RHRBI 138	60.70	55.06	57.88	High	42.31	38.95	40.63	Medium	8.48	6.34	7.41	Medium
Shanti	40.10	34.22	37.16	Low	35.15	29.57	32.36	Low	8.70	7.70	8.20	Medium
RHRB 13A	38.63	32.35	35.49	Low	33.44	28.69	31.06	Low	7.52	5.42	6.47	Low
RHRBI 1314	40.16	36.40	38.28	Low	30.78	26.10	28.44	Low	7.54	4.52	6.03	Low

DHBH 9071	43.12	39.46	41.29	Low	35.36	30.19	32.78	Low	10.46	8.38	9.42	High
DHLB 8A	42.91	37.83	40.37	Low	37.06	34.21	35.63	Medium	8.01	6.93	7.47	Medium
DHLBI 967	45.32	41.13	43.23	Low	32.74	28.95	30.85	Low	7.03	5.42	6.23	Low
DHLB 1307	58.04	55.12	56.58	High	41.02	37.60	39.31	Medium	9.50	7.25	8.38	Medium
DHLB 17A	52.99	49.75	51.37	Medium	32.72	30.29	31.50	Low	7.17	5.25	6.21	Low
DHLBI 731	57.68	52.75	55.22	High	35.63	31.35	33.49	Low	6.98	4.83	5.91	Low
Mean	49.99	45.56	-	-	37.61	33.38	-	-	8.30	6.35	-	-
Min.	38.63	32.35	35.49	-	30.78	26.10	28.44	-	6.98	4.52	5.91	-
Max.	62.57	59.58	61.08	-	51.01	47.40	49.20	-	11.10	9.11	10.11	-
S.E. ±	0.69	0.23	0.51	-	0.37	0.14	0.28	-	0.35	0.14	0.26	-
C.D. 5%	2.01	0.68	1.46	-	1.09	0.41	0.80	-	1.01	0.42	0.75	-
C.V.%	2.37	0.88	1.85	-	1.72	0.72	1.37	-	7.20	3.86	6.23	-

Table 3: Means table for Iron (ppm), Zinc (ppm) and protein (%) composition of pearl millet genotypes.

ANOVA reveals that the ash content differed among genotypes and environments significantly (Table 4.3.1). During Kharif-2013 hy-brid Shraddha recorded highest (2.44g/100g) but in Summer-2014 hybrid DHBH 9071 recorded highest (2.07g/100 gm), while lowest noted in DHLB 17A during both the seasons (Table 4.3.4). based on ash determination, three genotypes viz., Shraddha, DHBH 9071 and DHLB 1307 were categorized under high (> 1.87g/100 gm), two genotypes viz., RHRBI 138 and DHLBI 967 under medium (1.50 to 1.87 g/100 gm) and seven genotypes viz., RHRB 1A, Shanti, RHRB 13A, RHRBI 1314, DHLB 8A, DHLB 17A and DHLBI 731 under low (< 1.4967 g/100 gm) category. The genotypes judged noteworthy because they had the highest ash content based on pooled data of Kharif-2013 and Summer-2014 seasons, i.e. hybrid Shraddha (2.25g/100 gm) followed by hybrid DHBH 9071 (2.22g/100 gm). The grain ash was more greatly influenced by crop year and location as well by genotype. Khalili., *et al*. [14] reported that with water stress in forage crops, ash percent decreased significantly. However, sufficient genotype variation is present to manipulate this grain trait through appropriate plant breeding. These variations of ash contents were conformity with conclusions of Buerkert., *et al.* [12] and Morris., *et al.* [6] in wheat. Carbohydrates (g/100g) content ranged from 52.22 to 68.28g/100g and 48.32 to 61.69 g/100g during *Kharif*-2013 and Summer-2014, respectively. Based on the means of pooled environmental data (Table 4.3.5), significantly highest CHO content was recorded in Shraddha (64.99g/100g) and lowest in RHRB 1A (50.27g/100g). Further, based on CHO content in the genotypes were grouped into three catego-ries as low (< 55.17g/100 gm), medium (55.17 to 60.08 g/100 gm) and high (> 60.08g/100 gm). Out of four hybrids, Shraddha and DHBH 9071 grouped under high and Shanti and DHLB 1307 under medium CHO content group, while low CHO content group includes eight genotypes viz., RHRB 1A, RHRBI 138, RHRB 13A, RHRBI 1314, DHLB 8A, DHLBI 967, DHLB 17A and DHLBI 731.

Genotypes	Ash (g/100g)				CHO (g/100g)			
	Kharif-2013	Summer-2014	PEVs	Groups	Kharif-2013	Summer-2014	PEVs	Groups
Shraddha	2.44	2.06	2.25	High	68.28	61.69	64.99	High
RHRB 1A	1.46	1.05	1.25	Low	52.22	48.32	50.27	Low
RHRBI 138	1.76	1.29	1.53	Medium	55.14	53.31	54.23	Low
Shanti	1.54	1.19	1.36	Low	60.78	57.58	59.18	Medium
RHRB 13A	1.30	1.04	1.17	Low	56.38	50.33	53.35	Low
RHRBI 1314	1.34	1.06	1.20	Low	56.50	51.47	53.99	Low
DHBH 9071	2.37	2.07	2.22	High	63.21	60.19	61.70	High
DHLB 8A	1.61	1.11	1.36	Low	57.33	49.54	53.44	Low

DHLBI 967	1.73	1.35	1.54	Medium	55.15	51.13	53.14	Low
DHLB 1307	2.11	1.83	1.97	High	59.69	55.28	57.48	Medium
DHLB 17A	1.28	0.96	1.12	Low	54.67	48.82	51.75	Low
DHLBI 731	1.51	1.32	1.41	Low	58.02	51.64	54.83	Low
Mean	1.70	1.36	-	-	58.11	53.28	-	-
Min.	1.28	0.96	1.12	-	52.22	48.32	50.27	-
Max.	2.44	2.07	2.25	-	68.28	61.69	64.99	-
S.E. ±	0.05	0.04	0.04	-	0.07	0.15	0.12	-
C.D. 5%	0.14	0.11	0.04	-	0.22	0.44	0.34	-
C.V.%	4.74	4.76	0.12	-	0.22	0.49	0.37	-

Table 4: Means table for ash (g/100g) and CHO (g/100g) composition of pearl millet genotypes.

The results suggested that, CHO content is also one of the important traits which could be effectively used for grouping and identifi-cation of genotypes. An inherent genetic difference among the genotypes and season is also one of the contributing factors for variation in carbohydrate content. The results were confirmative with that of Buerkert., *et al.* [12] in pearl millet, Gopalan., *et al.* [15] in millets, Hariprasanna., *et al.* [7] in sorghum and Jahan., *et al.* [8] in rice.

Conclusion

The concentrations of minerals in grains were might be get influenced by numerous complex, dynamic and interacting factors, including genotype, soil properties, environmental conditions and nutrient interactions. In the present study, differences among the genotypes in absorbing different minerals from the soil and accumulating them in the grains to achieve nutritional benefits were clearly expressed, but since data were obtained from only two sites, and from open pollinated panicles, further investigations regarding the stability of grain nutrient contents are required. Based on nutrient composition; the hybrid Shraddha recorded highest values for Iron (61.08 ppm), Zinc (49.20 ppm), protein (10.11%), ash (2.25 g/100 gm) and CHO (64.99 g/100 gm). In contrast, lowest values for Iron (35.49 ppm), Zinc (28.44 ppm), protein (5.91%), ash (1.12 g/100 gm) and CHO (50.27 g/100 gm) were recorded in RHRB 13A, RHRBI 1314, DHLBI 731, DHLB 17A and RHRB 1A respectively.

Hybrid DHBH 9071 grouped under low Iron (47.29 ppm) and Zinc (32.78 ppm) while, high protein (9.42%), ash (2.22 g/100 gm) and CHO (61.70 g/100 gm) content category. Hybrid Shanti grouped under low Iron (37.16 ppm), Zinc (32.36 ppm) and ash (1.36 g/100 gm) while, medium protein (8.20%), and CHO (59.18 g/100 gm) content category. Hybrid DHLB 1307 grouped under high Iron (56.58 ppm) and ash (1.97 g/100 gm) while, medium Zinc (39.31 ppm), protein (8.38%) and CHO (57.48 g/100 gm) content group. Significant effect growing environments were observed for grain Fe, Zn, protein, ash and CHO which resulted in differential nutrient accumulation by the genotypes.

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Conflict of Interest

The authors hereby declare that there is no any conflict of interest with any financial organization regarding the material discussed in the manuscript and research conducted.

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