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FULL-LENGTH RESEARCH ARTICLE

Genetic Variability, Diversity and Interrelationship for Twelve Grain Minerals in 122 Commercial Pearl Millet Cultivars in India

M. Govindaraj[1] · O. P. Yadav[2,3] · B. S. Rajpurohit[2] · A. Kanatti[1] · K. N. Rai[1] · S. L. Dwivedi[1]

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Abstract Pearl millet contributes to the major source of dietary calories and essential micronutrients intake among rural populations in certain regions of India as its grains are more nutritious than other cereals. The aims of this investigation were to profile cultivar nutrition, diversity and interrelationship for grain minerals (Ca, K, Mg, Na, P, S, Cu, Fe, Mn, Zn, Mo and Ni) among 122 pearl millet hybrids and open-pollinated varieties in India. Trials were evaluated in randomized complete block design with three replications at two locations (Patancheru and Mandor) representing two major cultivation zones. The grain minerals in cultivars exhibited two- to- four-fold variation. Positive and significant correlations were noted among different minerals. A higher magnitude of positive and significant association between Fe and Zn ($r = 0.71$, $P < 0.01$) and with other minerals suggested the existence of greater genetic potential for the concurrent improvement of Fe and Zn without lowering the other grain minerals in pearl millet. The first two principal components accounted for 49% of variation. Euclidian distance-based cluster analysis grouped the 122 cultivars into seven clusters. Cluster I had higher mean for Fe (56 mg kg^{-1}) and Zn (49 mg kg^{-1}), in which ICTP 8203, Ajeet 38, Sanjivani 222, PAC 903 and 86 M 86 were identified as rich sources of iron, zinc and calcium with considerable levels of other nutrients. About 65% of cultivars for iron and 100% of cultivars for zinc have met the minimum standards set forth by the Indian Council of Agricultural Research. This indicates the feasibility of breeding nutrient-rich hybrids with competitive yields through mainstreaming in future.

Keywords Biofortification · Correlation · Hybrid · Micro-nutrient · Malnutrition

Introduction

Pearl millet [(*Pennisetum glaucum* (L.) R. Br.)] is a staple food crop grown in marginal arid and semi-arid tropical regions of Sub-Saharan Africa and Asia. The crop is of prime importance among the poor and low-income groups since it contributes substantially to food and nutritional security in dry regions worldwide [3]. The pearl millet grains, when compared to maize, rice or wheat, are more nutritious and contain balanced amino acid profile [4, 23]. India is the largest producer of pearl millet, with about 9.1 million metric tons of grains produced from an area of 7.4 million ha [1]. Pearl millet in major crop-growing states contributes to 19–63% and 16–56% of the total dietary intake of Fe and Zn [30].

A healthy human requires more than 40 nutrients that are essential to meet the metabolic needs of the body and

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✉ M. Govindaraj
m.govindaraj@cgiar.org

[1] International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana 502 324, India

[2] ICAR - All India Coordinated Pearl Millet Improvement Project, Mandor, Jodhpur, Rajasthan 342 304, India

[3] ICAR - Central Arid Zone Research Institute, Jodhpur, Rajasthan 342 003, India

which include proteins, lipids, macro-nutrients, micro-nutrients and vitamins. Inadequate consumption of any of these will result in adverse metabolic disturbances leading to sickness, poor health, impaired development in children and large economic cost to the society [44]. A rapid survey on children in India revealed that about 18.6% of newborns, 34.6% of children up to age of 3 years and 62.5% of adolescent girls suffer from malnutrition and the deficiencies arising due to micro-nutrients in India which causes annual losses amounting to 2.5 billion US dollars [27]. Low dietary diversity and monotonous cereal-based diets have been associated with micro-nutrient malnutrition.

FAO/WHO [11] recommended dietary allowance (RDA) among men and women of 25 to 50 years of age daily intake of 800 mg of Ca and P, 280–350 mg of Mg, 500 mg of Na, 2000 mg of K, 750 mg of Cl, 10–15 mg of Fe and Zn, 1.5–3.0 mg of Cu, 55–70 µg of Se, 150 µg of I, 2–5 µg of Mn, 75–250 µg of Mo, 50–200 µg of Cr and 1.5–4.0 mg of F [2, 11]. Although no RDA has been established for Ni, it is suggested that the estimated daily intake of Ni from food and water worldwide should be in the range of 80–130 µg per day [34]. In the past, the genetic uniformity of cultivars has led to several devastating pest and disease attacks on major crops. However, care was taken by pearl millet breeding programs to considerably increase its productivity in India and to minimize the chances of losses due to major outbreaks of diseases [29]. Therefore, major breeding efforts in the past decades are being focused on the yield potential of the cultivars with built-in genetic diversity. The research on the variations in the concentrations of multiple elements in grains of pearl millet hybrids has not been performed anywhere except for variations in Fe and Zn [13, 19, 33]. The present study is the first attempt in the direction of documenting the existing diversity in commercial cultivars and of assessing the associations among minerals (Ca, K, Mg, Na, P, S, Cu, Fe, Mn, Zn, Mo and Ni) in them.

Materials and Methods

Experimental Details

The trial consisted of 122 entries, including twenty-one (21) public-sector-bred hybrids and a hundred and one (101) released/commercialized as truthfully labeled seed/pipeline hybrids from thirty private-sectors and a high Fe control (ICTP 8203), which were evaluated at Mandor and Patancheru in the rainy season of 2011. The trial was conducted in a randomized complete block design with three replications. The field consisted of plots with two rows of 4 m length. The space in between the rows of the plots was 75 cm and 50 cm at Patancheru and Mandor, respectively. The space within the rows was 15 cm at both the locations. All the recommended agronomic practices were followed for raising good crop.

At maturity, the main panicles of ten random plants from each plot were harvested, sundried for 10–12 days and bulk threshed to collect 30 g of grain samples from each plot for the analyses of micro- and macro-nutrients following ICP-OES (Thermo Fisher Scientific, Waltham, MA) [42, 45]. In this method, grain samples were oven-dried overnight at 85 °C prior to digestion, grounded enough to pass through 1 mm stainless steel sieve using Christie and Norris hammer mill and stored in screw-top polycarbonate vials. The samples were digested with diacid (nitric/perchloric acid) mixture. After digestion, the volume of the digest was made to 25 mL using distilled water; and the content was agitated for 1 min by vortex mixer. The digests were filtered, the concentrations of micro- and macro-minerals were read at the respective wavelengths using ICP-OES, and the nutrients were expressed as mg kg^{-1}. All possible care was taken at each step to avoid any contamination of the grains with dust particles and any other extraneous matter by analyzing the index element ‘aluminum’ [40].

Statistical Analysis

The analysis of variance (ANOVA) was done following Gomez and Gomez [12]. The ANOVA for both individual environments and pooled data was carried out using the PROC GLM procedure in SAS 14.1 software [37]. The components of variance attributable to the genotypes (σ^2g) in the individual location and over the two locations (pooled) were estimated for all the traits. In pooled analysis, the variance components for genotype × environment (σ^2ge) were estimated for all traits. Principal component analysis (PCA) and diversity cluster analysis were carried out using R v3.5.1 [32]. PCA was performed using the PRCOM program [17]. Ideally, PCA components with cumulative variability must explain at least 75% of total variability to have a meaningful dispersion of genotypes in the graphical presentation. For calculating the pair-wise genetic dissimilarity index, the Euclidean distance (using Ward’s minimum variance method) was employed [43]. Significance of differences between cluster means was compared using Newman–Keuls procedure [22, 28]. Phenotypic correlations were estimated among all the traits and tested for their significance [39]. Promising entries were identified for Fe, Zn and other nutrients based on per se performance and grouping pattern.

Results and Discussion

Per se Performance and Genetic Variance

The pooled analysis revealed that environment (E) had significant effect on grain densities of minerals in cultivars. Greater genetic variation for Fe, Zn, Mn, Cu, Mo, Ni, Ca, Mg and Na was noted at Mandor (1.34 folds for P to 4.67 folds for Na) than at Patancheru, while genetic variation for K, P and S were almost similar at both locations (1.3 to 1.5 folds at Patancheru and 1.4 to 1.6 folds at Mandor). Both cultivar (C) and cultivar × environment interaction ($C \times E$) were significant ($P < 0.01$) for most mineral, except for Na in case of $C \times E$ (Table 1). The contribution of cultivars (cultivar sum of square) to the total variability (total sum of square) was higher compared to $C \times E$ and E components for all macro-nutrients. The pooled analysis revealed very large differences for each element: fivefold for Na; threefold for Ni; 2.5 fold for Ca; twofold for Fe, Mo and Mn; nearly, twofold for Cu and Zn; and between 1.3 to 1.5 fold for Mg, K, P and S (Table 2). Greater $C \times E$ interaction for Zn content than Fe content has been reported in pearl millet [19], and $C \times E$ interactions for Fe and Zn were equal or lesser than $C \times E$ interaction to grain yield [20].

Hybrid and open-pollinated varieties (OPVs) among the category of top twenty Fe- and Zn-rich cultivars (Table 3) and few of these hybrids have also produced higher grain yield in a previous study [50]. ICTP 8203 (2120 kg ha^{-1}) among the varieties and Ajeet 38 (3912 kg ha^{-1}), Sanjivani 222 (3775 kg ha^{-1}), PAC 903 (3812 kg ha^{-1}) and 86 M 86 (4863 kg ha^{-1}) among hybrids are the ones that combine both high yield (3324 to 5386 kg ha^{-1} in other cultivars) and multi-essential elements (Fe, Zn and Ca). ICTP 8203 is an OPV with the highest Fe and Zn concentrations which makes it incomparable to any of its counterparts. A few cultivars in addition to being rich in Fe and Zn, were also rich in P (N 68, PAC 931), Ca (HTBH 4204, ICMH 356) and K (N 68, PAC 931) which may be utilized as resource to derive populations/inbreds high in these minerals. Both Fe and Zn are very important minerals for human health. Iron is an important component of various enzyme systems, such as the cytochromes, which are involved in oxidative metabolism. However, zinc is essential for catalytic, structural and regulatory functions in the body. Over 300 different enzymes depend on zinc for their ability to catalyze vital chemical reactions [24].

Malnutrition is a serious health issue in India, and to realize India free from malnutrition ("Kuposhan Mukt Bharat" by 2022), the Indian Council of Agricultural Research (ICAR) declared that any new pearl millet cultivar released in India must contain a minimum 42 mg kg^{-1} of iron and 32 mg kg^{-1} of zinc. In this context, 78 cultivars for iron and all 122 cultivars for zinc have met the minimum standards of Fe and Zn set forth by ICAR. This indicates that the baseline is well accomplished and being maintained for Zn and to an acceptable extent for Fe. Interestingly, this progress happened in Fe and Zn even though they were not cnsidered as target traits in the past. The challenge is that most of these cultivars were

Table 1 Mean square from analysis of variance for grain macro- and micro-nutrients in pearl millet across two locations

Trait	Environment (E) 1[a]	REP 1	Cultivars (C) 121	$C \times E$ 121	Error 121
Macro-nutrients					
P	9,733,008**	680,691**	160,512**	51,738**	29,544
K	26,460,996**	672,459**	523,856**	173,578**	52,623
Mg	66,268**	28,627**	58,331**	7384**	2707
Ca	87,304**	3939**	2739**	1049**	290
Na	1782**	1128**	168**	115$^{\text{NS}}$	101
S	1,257,148**	80,535**	21,574**	5433*	4043
Micronutrients					
Fe	8314**	123.00**	189.30**	51.80**	19.60
Zn	122**	326.90**	81.70**	32.00**	9.80
Cu	10**	0.120$^{\text{NS}}$	1.76**	0.34**	0.20
Mn	284**	10.70**	18.90**	7.30**	1.90
Mo	40**	0.030$^{\text{NS}}$	0.10**	0.04**	0.02
Ni	38**	0.002$^{\text{NS}}$	0.25**	0.05**	0.02

[a] Indicate degrees of freedom

*, **Significant at probability levels of 0.05 and 0.01, respectively

Table 2 Mean and range for grain macro- and micro-nutrients in pearl millet across two locations

Trait	Mean	Range	SE±	CV (%)	LSD (5%)	No. of cultivars > trial mean
Macro-nutrients (mg kg^{-1} seed)						
P	3442	2925–3900	85.9	0.3	239.4	62
K	4394	3675–5375	114.7	0.2	319.5	56
Mg	1257	1060–1650	26.0	0.4	72.5	58
Ca	135	82–210	8.5	2.2	23.7	56
Na	20	9–42	5.0	11.1	14.0	54
S	1245	1045–1405	31.8	0.5	88.6	64
Micronutrients (mg kg^{-1} seed)						
Fe	45.00	31–73	2.2	3.3	6.2	59
Zn	41.00	32–55	1.6	3.0	4.4	58
Cu	5.03	3.48–6.91	0.2	9.4	0.6	59
Mn	15.00	11–22	0.7	5.5	1.9	50
Mo	1.00	0.67–1.42	0.1	25.4	0.2	53
Ni	1.30	0.62–1.93	0.1	20.9	0.2	66
Grain yield (Kg ha^{-1} Patancheru)						
GY	3903	984–6089	188	15.7	373	65

SE—standard error, *LSD*—least significant difference, *CV*—coefficient of variation

Table 3 Top-twenty high-grain Fe- and Zn-dense pearl millet cultivars and their performance for other grain minerals

Genotype	Sector	Fe	Zn	Mn	Cu	Mo	Ni	Ca	Mg	Na	K	Na:K ratio	P	S	GY[a]
ICTP 8203	Public	73	55	16	5.28	1.33	1.51	153	1255	12	4275	0.005	3575	1300	2132
Ajeet 38	Private	61	46	19	6.45	0.92	1.56	195	1365	15	4575	0.006	3675	1200	3887
Sanjivani 222	Private	61	52	13	5.07	0.94	1.32	131	1373	14	4275	0.005	3400	1305	4004
PAC 903	Private	60	46	16	5.48	1.06	1.07	99	1363	10	4450	0.004	3550	1365	3685
86 M 86	Private	59	50	17	5.61	0.81	1.91	126	1278	17	3975	0.005	3550	1285	4796
HTBH 4204	Private	57	43	19	4.58	0.67	1.21	210	1538	34	4750	0.012	3700	1205	3889
XL 51	Private	57	46	19	6.20	1.11	1.55	187	1320	15	4575	0.005	3550	1268	3541
Ajeet 39	Private	57	49	16	6.91	1.32	1.60	98	1198	29	4950	0.010	3625	1300	3963
Shradha	Public	56	51	13	5.33	0.79	1.24	116	1273	16	3675	0.007	3500	1310	2051
PAC 982	Private	56	46	11	6.11	1.18	1.20	102	1285	13	3825	0.006	3350	1285	3753
RBH 9	Public	55	47	15	6.20	1.04	1.12	145	1260	29	4525	0.011	3575	1260	4399
Sanjivani 2312	Private	55	48	13	5.10	0.90	1.42	135	1335	14	4000	0.006	3375	1223	3469
ICMH 356	Public	54	42	20	5.03	0.99	1.54	207	1528	30	4625	0.011	3775	1300	3297
NBH 1134	Private	54	44	16	5.33	1.00	1.93	181	1220	22	4200	0.009	3675	1245	4343
KBH 7201	Private	53	44	15	4.92	1.03	1.63	151	1130	18	4050	0.008	3600	1280	4613
HTBH 4203	Private	53	37	16	4.22	0.95	1.22	131	1258	23	4250	0.009	2925	1178	4023
PAC 931	Private	52	44	16	6.46	1.10	1.49	154	1295	29	5150	0.009	3800	1240	4711
Navbharat Banas Express	Private	51	44	18	5.18	1.28	1.69	133	1153	21	4750	0.007	3575	1310	3993
Saburi	Public	51	48	14	4.17	1.15	1.46	91	1105	17	3975	0.007	3300	1268	3775
N 68	Private	50	40	20	4.50	1.00	1.37	167	1410	22	5000	0.007	3875	1350	3312

[a] Grain yield from Patancheru location

withdrawn from cultivation due to product replacement strategy by the private and public sectors involved in seed business. This also indicates that there is a wider breeding gap (differences of baseline and target) for achieving targeted nutritional levels in future hybrids. Considering the higher prevalence of iron and zinc deficiency in India, there is a need to set breeding targets (instead of following baseline recommendation) in national program in order to meet at least 50% of the daily Fe and Zn requirements and gradually reaching the Fe target (72 mg kg^{-1}) of the global breeding programs [14].

A lower level of Na and Na/K ratio in the plant is an indicator of tolerance to salinity [10, 34]. Hence, in the present study, ratio of Na/K in all the hybrids was estimated first by converting the values of Na/K from mg kg^{-1} to m mol (23 mg Na = 1 m mol Na; 39 mg $K = 1$ m mol K), and secondly, the Na/K ratio for all the hybrids is obtained by dividing the value of Na by that of K. For instance, salinity tolerance mechanism in rice is related to its capacity to maintain a low Na/K ratio in the shoot tissue [38, 41, 42], and otherwise, maintaining a higher shoot K content could tolerate salt stress [35]. In a previous study, variation for leaf Na/K ratio was 0.48 to 2.58, and the Na/K ratio in the stem was 0.50 to 3.50 among the pearl millet hybrids [34]. It appears that this ratio is lower in grains compared to the samples from other plant parts. This merits further investigation on this direction in pearl millet. With respect to human health, Na maintains the electrolyte balance in the body besides plays important role in transport of sugars and amino acids into the cells. K is essential to both cellular and electrical function. K and Na together regulate the water balance and the acid-base balance in the blood and tissues [9].

The level of Na, K and Na/K ratio is of much importance to maintaining the optimal blood pressure in human. The daily recommended dose of K as prescribed by WHO is 3510 mg kg^{-1} while that of Na is 184–230 mg/day [6, 48]. The World Health Organization recommends the ratio of Na to K to be lesser than 1 [47, 49]. The uptake of potassium needs to be higher than sodium to keep a balanced blood pressure and to avoid cardiovascular diseases or stroke. The normal ratio of Na:K in humans is recorded at 1 to 1.36–1.47, though the dietary ratio for a very healthy living could be as good as 1:14–16 [16].

The Na/K values in hybrids of our study ranged from 0.004 to 0.015 (Table 3). Pearl millet grains have much lower level of Na/K ratio in comparison with rice (0.05 to 0.3), wheat (0.08–1.22) or maize (0.03–1.34) [5, 8, 25]. This is due to the higher levels of potassium present in the pearl millet grains. PAC 903, 86 M 01, NBH 1717 and ICPT 8203 showed lower grain Na/K ratio. This reduced Na/K ratio in pearl millet grains will significantly contribute to the improved nutritional status of the people consuming pearl millet to preventing the early onset of cardiovascular diseases and in maintaining a balanced blood pressure.

Principal Component Analysis (PCA) and Clustering

PCA was used to assess the patterns of variations and relationships among the twelve elements (Table 4). The first five PCs accounted for 74.63% of the variability. PC1 accounted for 31.53% of the total variation with P and Mn together, with the highest negative coefficients. PC2 explained 17.55% of the total variation with Fe and Zn, having the highest positive loadings, while PC3 accounted to 10.54% variation with Ca having the highest positive and K and Mo with the highest negative coefficients. PC4 and PC5 have contributed to variations in the range of 6.5% to 8.08%, with S and Cu of PC4 having the highest positive coefficients. In the case of PC5, Mg had the highest positive and Ni had the negative loadings. The first two PCs explained 49.1% of the total variation in grain mineral among the 122 cultivars (Fig. 1).

Fe, Zn, P and Mn had higher vector length indicating the presence of large variability, while Mo and Na had smaller vector length indicating low variability for these traits. This was in accordance with the estimates of variance. When Fe and Zn are grouped together, this is a strong confirmation to the high positive association between these two traits. The acute coordinate angle ($< 90°$) between Fe and Zn with Cu, Ni, S and P further implies the positive association between these traits. A slightly more than 90° angle of Fe and Zn with Ca, Mn, K and Na indicate non-significant or low negative association with these traits. P had $< 90°$ angle with other traits indicating the presence of positive association. These relationships in the PCA graph were in accordance with the Pearson correlation values observed among the traits (Table 5).

The 122 entries were grouped into seven different clusters using Euclidian distance (Fig. 2). Cluster III had the highest number of genotypes with 34 entries followed by cluster II with 19 and I with 15 entries, cluster IV and V, each with 17 entries, cluster VI with 14 entries and cluster VII with only seven entries. The entries in clusters I, II and VII were more promising with respect to higher nutrient densities for multiple elements (S Table 1). A few entries in cluster I showed greater Fe (56–73 mg kg^{-1}; ICTP 8203, Ajeet 38, Sanjivani 222, PAC 903, 86M86, XL 51, Shradha) and Zn (50–55 mg kg^{-1}; ICTP 8203, NBH 4903, Dhamal, Sanjivani 222, 86M86, Shradha).

Cluster II had the highest mean value among all the seven clusters for Mn, Ca, Mg, Na and P, whereas cluster VII had the highest mean value for Cu, Mo, Ni, K and S. Cluster III represented the lowest mean values for Mn, Cu, Ni and Ca, while cluster IV had the lowest values for P and S. In contrast, cluster V had the lowest value for Fe and Zn, and cluster VI had the lowest mean value for Mo, Mg, Na and K. The cluster patterns of the present study are mostly

Table 4 Eigen value and proportion of variance contributed by the five principal components (PCA) for grain macro- and micro-nutrients in pearl millet over two locations

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.78	2.11	1.26	0.97	0.83
Variance (%)	31.53	17.55	10.54	8.08	6.94
Cumulative variance (%)	31.53	49.08	59.61	67.70	74.63
P	– **0.41**	0.07	0.02	– 0.04	0.34
K	– 0.31	– 0.27	– **0.38**	0.08	– 0.12
Mg	– 0.34	– 0.22	0.21	– 0.10	**0.37**
Ca	– 0.34	– 0.11	**0.32**	– 0.22	– 0.34
Na	– 0.21	– 0.29	– 0.30	– 0.41	0.21
S	– 0.32	0.07	– 0.12	**0.53**	0.31
Fe	– 0.18	**0.56**	– 0.02	– 0.24	0.05
Zn	– 0.11	**0.56**	– 0.03	– 0.40	0.11
Cu	– 0.23	0.28	– 0.18	**0.41**	0.02
Mn	– **0.41**	– 0.20	0.16	– 0.14	– 0.24
Mo	– 0.04	0.02	– **0.72**	– 0.16	– 0.28
Ni	– 0.30	0.19	0.14	0.23	– **0.57**

Bold values indicate higher loadings in respective PC

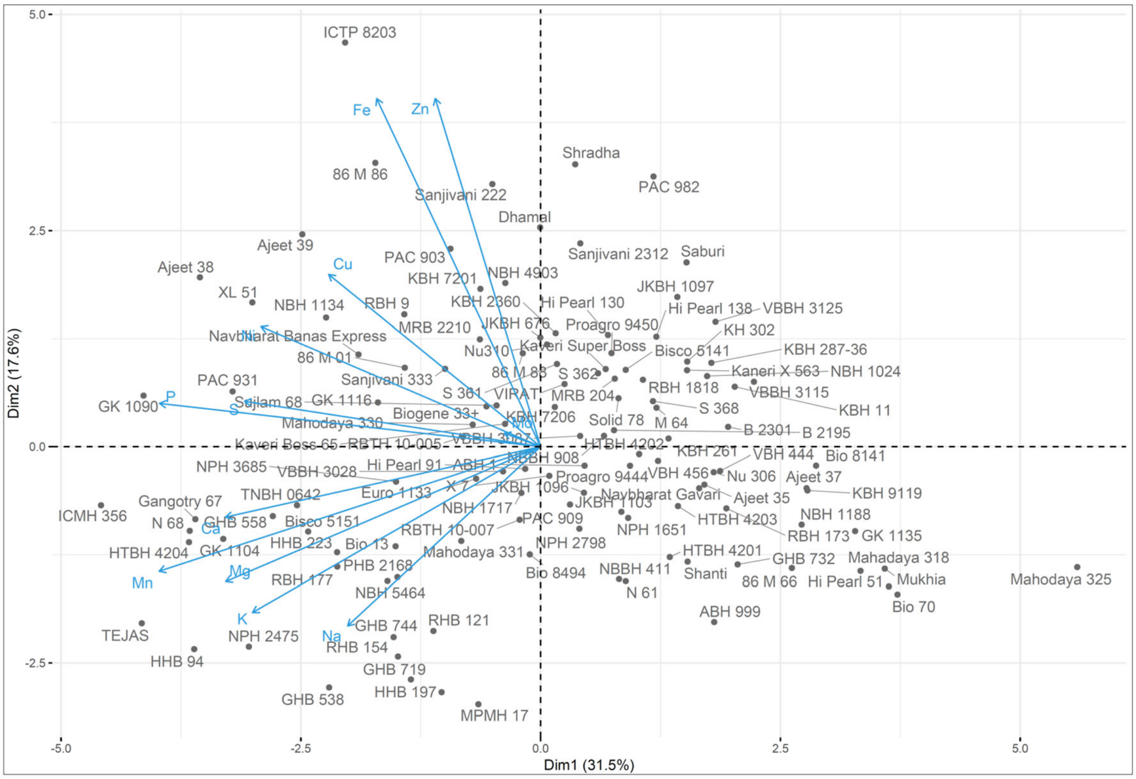

Fig. 1 Principal component analysis (PCA) for grain macro- and micro-nutrients among 122 commercial cultivars in pearl millet

in confirmation with the cluster patterns of a previous research on the yield traits of pearl millet [51]. For instance, nine (9) cultivars of clusters I and II, eleven (11) in cluster II and fourteen (14) in cluster III were grouped together in both the studies. This implies similarity within the clusters and diversity between clusters which is equally represented for grain yield and mineral traits.

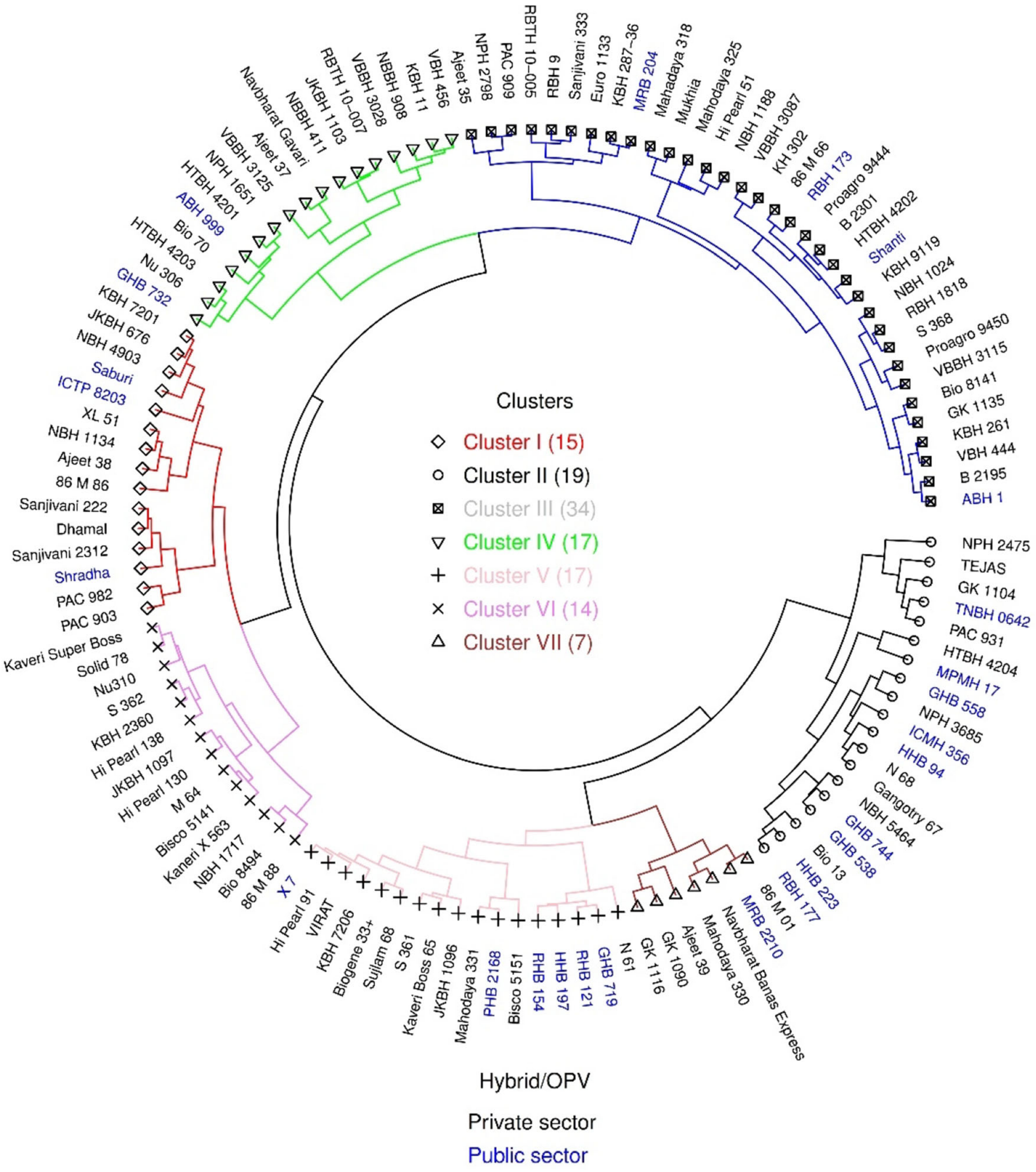

Fig. 2 Cluster analysis based on Euclidian distance for grain macro- and micro-nutrients among 122 commercial cultivars in pearl millet

Grain Mineral Correlation

Pearson correlation coefficients (r) were determined for pooled data across locations (Table 5). Most of the correlations in the present study, except for Fe and Zn (r = 0. 73, $P \leq 0.01$), were of moderate degree, indicating that the breeding for high Fe and Zn is not likely to reduce the level of other mineral elements in pearl millet. The significant and positive correlations between Fe and Zn concentrations indicate the effectiveness of concurrent selection for Fe and Zn in pearl millet. P was positively associated with all elements; however, the coefficients varied: moderately high (r = 0.50–0.56; $P < 0.01$) with Mn and Mg but low to moderate (r = 0.25–0.42; $P < 0.01$) with Fe, Zn, Cu, Ni, Ca, Na and K. Seven elements were positively associated with K: moderate (r = 0.45–0.49; $P < 0.01$) with Mn and Na but of low magnitude (r = 0.19–0.33; $P < 0.01$) with Cu, Mo, Ni, Ca and Mg. Mn was positively and moderately (r = 0.61–0.65; $P < 0.01$) associated with Ca and Mg.

S was positively and moderately associated (r = 0.52; $P < 0.01$) with P, while the coefficients of S with Mn, Cu, Ni, Mg and K were of low magnitude (0.21–0.35;

Table 5 Phenotypic correlation coefficient among grain macro- and micro-nutrients in pearl millet over two locations

	P	K	Mg	Ca	Na	S	Fe	Zn	Cu	Mn	Mo	Ni
Macro-nutrients												
K	0.38**											
Mg	0.56**	0.30**										
Ca	0.42**	0.33**	0.41**									
Na	0.25**	0.45**	0.28**	0.22*								
S	0.52**	0.35**	0.32**	0.17	0.11							
Micro-nutrients												
Fe	0.31**	− 0.07	− 0.01	0.13	− 0.07	0.21*						
Zn	0.28**	− 0.15	− 0.05	0.04	− 0.09	0.08	0.73**					
Cu	0.29**	0.23*	0.11	0.17	0.04	0.34**	0.37**	0.24**				
Mn	0.50**	0.49**	0.61**	0.65**	0.34**	0.35**	0.09	− 0.03	0.11			
Mo	0.04	0.24**	− 0.06	− 0.12	0.12	0.05	0.05	0.08	0.05	0.01		
Ni	0.37**	0.19*	0.18	0.34**	0.01	0.35**	0.31**	0.22*	0.3**	0.46**	0.01	

*, **Significant at probability levels of 0.05 and 0.01, respectively

$P < 0.01$). Correlations involving Ni with Fe, Zn and Cu were of low magnitude ($r = 0.22$–0.31; $P < 0.01$); however, it was positive but moderate ($r = 0.46$; $P < 0.01$) with Mn. Cu was positively associated with Fe and Zn but of low magnitude ($r = 0.24$–0.37; $P < 0.01$). This multi-elemental study has found out that the genetic linkage of Fe and Zn with P. Fe and Zn have also demonstrated positive linkages with most of the other elements. This is very interesting to discover this sort of positive association of Fe and Zn with other grain nutrients in the course of biofortifying Fe and Zn in the parental lines of pearl millet hybrids.

The present study supports the previous reports of positive correlations among macro- and micro-nutrients, Mg with Ca, Na with P and K, P with S and Fe with Zn and Cu or between macro- and micro-nutrients, P and S linked with Cu and Fe in wheat [29]. As documented in our study, the seed P was positively correlated with Ca, Mg, Cu, Fe and Zn, K with Cu or Cu with Fe and Zn in similar research in barley [15]. Similar results to that of ours were published in lentil, where P was positively associated with Mg and Zn [21].

Significant positive associations of P with Ca, Mg and Na, S with K, Mg and P, K with Ca, Mg and Na, and Mg with Ca among macro-nutrients; Fe with Zn and Cu and Zn with Cu among micro-nutrients; P with Cu, Fe, Mn and Zn, K with Cu and Mn, Mn with Ca, Mg and Na, and S with Cu, Fe and Mn between the macro- and micro-nutrients suggest that there might be a common physiological pathway (genes network) involved in the uptake of these nutrients from soils and/or the translocation and storage of minerals in plant organs [46]. Sulfur-containing amino acids act as promoters to enhance the bioavailability of elements. The positive association between S and Mn, Cu and Mg may lead to greater bioavailability of these elements [18, 47]. Sulfur also promotes phytosiderophore production and organic substances such as nicotinamine, mugeniec acid and avenic acid, which are produced by plants under Fe or Zn deficient soils. They enhance the mobilization of Fe and Zn in the soil, their uptake into plants and their translocation into grains [7, 31].

Conclusions

Sufficient genetic variation for grain minerals (macro- and micro-nutrients) density has been noted among 122 pearl millet hybrids and OPVs in India as evidenced by range variation, principal component analysis and cluster analysis based on Euclidian distance matrix. High magnitude of correlation coefficients between Fe and Zn, and positive correlation with other grain minerals, suggests that selecting for high Fe will also lead to a proportional increase of Zn density without compromising other grain minerals in pearl millet hybrid breeding programs. A few cultivars such as an open-pollinated variety ICTP 8203 or hybrids such as Ajeet 38, Sanjivani 222, PAC 903 and 86 M 86 were identified as good source for Fe. However, most of them are discontinued. Hence, we need target breeding for high Fe and Zn cultivars in addition to the regular monitoring of the newly developed hybrids for other essential mineral elements to ensure that the new generation hybrid cultivars are not deprived of essential elements. Pearl millet biofortification research at ICRISAT and NARS has demonstrated that it is indeed feasible to combine high Fe and Zn contents into improved genetic background with specific adaptation.

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