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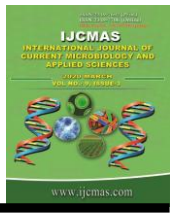

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Multivariate Diversity Analysis for Grain Micronutrients Concentration, Yield and Agro-morphological Traits in Pearl millet (*Pennisetum glaucum* (L) R. Br.)

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Micronutrient malnutrition resulting from the dietary deficiency of important minerals such as Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn) in the staple food crops like pearl millet leads to ubiquitous food-related health problems. In context to this present investigation was undertaken to study the phenotypic diversity among 48 maintainer (B) and restorer lines (R) of pearl millet genotypes for grain micronutrients concentration, yield and agro-morphological traits using multivariate approach. Higher range, large value of Shannon-weaver Diversity Index for both traits and genotypes and large differences in mean values for most of the characters showed that sufficient diversity existed among the genotypes and traits. Cluster analysis using unweighted pair group method of arithmetic averages (UPGMA) grouped the genotypes into five clusters with varied number which suggested the clear differentiation among B and R lines with some exceptions. Clustering of pearl millet genotypes from different geographical locations or source/origin into same cluster has confirmed that they are genetically related, and possibly from the same progenitor, but could have been separated by geographical or ecological barrier. The principal component analysis (PCA) revealed that most of the variation (68.83%) was accounted by first four PCA and genotypes from maintainer were clustered into left side of the biplot graph while the lines from the restorer category were distributed throughout the PCA biplot graph. Average Diversity Index of 1.883 and 3.792 for genotypes and traits respectively, further validated that the genotypes were more diverse among themselves and for all the traits studied. Association studies revealed significant positive correlation of grain Fe content with the grain Zn and Cu content; grain yield per plant with the plant height, panicle weight and dry fodder yield per plant; panicle weight with plant height, panicle length and dry fodder yield; panicle length with plant height and dry fodder yield per plant and dry fodder yield per plant with plant height. It indicated the likely effectiveness of simultaneous improvement of all these characters along with grain micronutrients in pearl millet. Grain yield per plant showed non-significant positive or negative correlation with grain micronutrients concentration thus suggesting improvement in nutrient content without compromising yield. The significant negative association between the grain yield and panicle weight with days to flowering has the great advantages in pearl millet cultivation as crop can fit into multiple cropping system in arid and semi-arid environments.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a prominent tropical C4 small-grained cereal crop having a very high photosynthetic effectiveness and dry matter production capability. It is typically grown under the most unfavourable agro-climatic conditions where other cereals like sorghum and maize fail to produce economic yields. Moreover, pearl millet has a notable capacity to act in response to favourable environments because of its short duration and capacity for fast growth rate, thus making it an outstanding crop for short growing seasons under improved crop management (Yadav and Rai, 2013). Pearl millet is highly resilient to diverse climate conditions and is cultivated in marginal environments of arid and semi-arid tropics of Sub-Saharan Africa and Asia (Radhika Ramya *et al*., 2018). In India, pearl millet is the third most widely cultivated food crop after rice and wheat. It is grown on 9 million ha with an average productivity of 1,000 kg per hectare. It shows a higher degree of tolerance to severe drought, heat stress and high temperature (Anuradha *et al*., 2018 and Govindaraj *et al*., 2018). Pearl millet grains are the rich source of the several macro and micro-nutrients (like iron, zinc, phosphorus, and magnesium), high fiber content, α-amylose, metabolizable energy, proteins, essential amino acids, thus ensuring food and nutritional security (Kumar *et al*., 2018). Pearl millet is primarily grown for food and dry fodder. Dry fodder of pearl millet is a major component of livestock ration during the dry period of year. It is also an excellent forage crop because of its lower hydrocyanic acid content than sorghum. Its green fodder is rich in protein, calcium, phosphorous and other minerals with oxalic acid within safe limits.

Development of high-yielding hybrids is an important breeding objective for pearl millet worldwide. The availability, assessment, and exploitation of genetic diversity and distance among genotypes help to develop heterotic groups which aid in selection of parents for further crossing. Moreover, assessing the extent of diversity for the economically important traits and identification of promising germplasm in untapped genetic resources of pearl millet is essential to generate knowledge useful for germplasm conservation and breeding programs. In addition to it, evaluation, characterization and classification of genotypes based on estimates of genetic divergence will help to identify diverse parental lines which can be used in hybridization program to develop potential hybrids or varieties.

Worldwide serious and widespread human health problems have been recognized due to dietary deficiency of mineral micronutrients such as iron and zinc (WHO, 2002 and Welch & Graham, 2004). Micronutrient malnutrition increases mortality & morbidity rates, health-care costs and reduces labour productivity, thus affect the development of any nation (Darnton-Hill *et al*., 2005 and Stein, 2010). Pearl millet is grown as major staple food crop in developing and under-developed countries of Africa and Asia, so these regions are largely affected by malnutrition. Genetic resources of pearl millet are relatively untapped and need further attempts to improve the grain micronutrients such as iron, zinc, copper and manganese content.

Biofortification of staple crops through genetic means is a sustainable and most cost effective approach to tackle malnutrition problem. It has always been promising to improve the mineral nutritional status and health of poor population in both rural and urban areas of developing world (Kumar *et al*., 2016). Fe (iron), Zn (zinc), Cu (copper) and Mn (manganese) are known to play an important role in many crucial biological processes and are necessary for survival of

human beings. For instance, Fe is an important component of haemoglobin and myoglobin; Zn stimulates the activities of many enzymes in the human body and is closely related to intelligence development in children and adult reproductive function; Cu plays key a role in the formation of red blood cells, energy production and maintaining nerve cells and the immune system and Mn is vital for the human body and helps in the metabolism of amino acids, cholesterol, glucose and carbohydrates (Ansari *et al*., 2004).

Deciphering the genetic diversity of grain micronutrients, yield and agro-morphological traits will be useful to identify contrasting parents, to maximize heterozygosity and achieve yield stability along with nutrient value in variable and changing climates (Haussmann *et al*., 2007 and Hausmann *et al*., 2012). The pattern of genetic relationships between and within accessions can be studied by multivariate analysis methods. Principal component analysis (PCA) and clustering are the two useful multivariate statistical tools for studying the relationship among the related genotypes. Cluster analysis is used to study the association between landraces while relationships between traits are statistically analyzed using PCA. These techniques have been previously applied for genetic diversity study in many crops such as finger millet (Lule *et al*., 2012), rice (Gana *et al*., 2013), and pearl millet (Pucher *et al*., 2015 and Kumari *et al*., 2016). Therefore, the present study was aimed to characterise and evaluate 48 pearl millet genotypes for grain micronutrients, yield and agro-morphological traits by clustering and PCA approach.

Materials and Methods

Experimental materials and location

Forty eight pearl millet genotypes (24 maintainer and 24 restorer lines) were sown during *kharif*, 2016 at Centre of Crop Improvement (CCI), S. D. Agricultural University, S.K.Nagar, Gujarat. The parental lines were of diverse origin which represented the A (PMRS, JAU, Jamnagar, Gujarat India) and B zone (ICRISAT, Patancheru, Hyderabad, Telangana India) of the three agro-ecological zones (A, A_1 and B) of pearl millet in India (table 1). Experiment was conducted at Centre for Crop Improvement (CCI), Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar (SDAU), Gujarat. SK. Nagar is situated at $24^{o}19^{'}26^{''}$ North latitude and $72^{o}18^{'}53^{''}$ East longitude with an altitude of 172.00 meters above the mean sea level (Arabian Sea). The soil of experimental sight was loamy sand in texture with a pH of 7.5 and climatic condition falls under the category of semi-arid, characterized by less than 400 mm of annual average rainfall.

Field experiments and observations recorded

The genotypes were planted in randomized complete block design (RCBD) with 2 replications. Each genotype was represented by 2 rows of 3 m length with distance of 45 cm between rows and 15 cm between plants in a row. Thinning was performed after 15 days of germination to ensure single plant per hill. From sowing till harvesting, all the recommended agronomic package of practices was followed to raise the good crops. Five plants were randomly selected and tagged for taking observations. The observations were recorded for quantitative morphological traits such as days to 50% flower, plant height (cm), panicle length (cm), panicle girth (cm), panicle weight (g) and dry fodder yield per plant (g). During harvest main panicles of five random plants from each entry were harvested and stored separately in a cloth bag for grain micronutrients (Fe, Zn, Cu and Mn content) analysis.

Grain micronutrients analysis

The cleaned grains from each entry were oven dried at 60°C for 48 hours and grinded to fine powder using mortal and pestle. Grinded samples were properly labeled and stored in butter paper cover for further analysis. The grain micronutrients were estimated from the acid extract prepared by wet digestion procedure of Singh *et al*., (2005) using diacid mixture at Centre for Bio Science Research Laboratory, S. D. Agricultural University, S. K. Nagar, Gujarat. One gram of grinded sample was pre-digested by adding 10 ml concentrated nitric acid (HNO_3) and kept for overnight. Further, prepared diacid mixture (HNO_3 and $HClO_4$) of approximately 10 ml was added to predigested sample. Then the samples were kept on hot plate at 200°C temperatures until the fume that comes out becomes colourless. After that heating was stopped,the digested sample was cooled down for 20 minutes andabout 50-60 ml double distilled water was added. The volume of digest was filtered with Whatman filter paper and final volume of 100 ml was made by adding double distilled water in conical flask. Care was taken at each step to avoid any contamination of samples with foreign dust particles. The samples were analyzed for iron and zinc content in Atomic Absorption Spectrophotometer (AAS), ELICO SL 194.

Statistical analysis

Descriptive statistics

The major descriptive statistics such as mean, range and coefficient of variation for each traits were computed using excel sheet program.

Cluster analysis

Hierarchical cluster analysis for grain micronutrients, yield and agro-morphological characters was performed to produce dendrogram based on the average distance between genotypes. The intergenotypic divergence was calculated using the unweighted pair group method of arithmetic averages (UPGMA). Data of cluster analysis were analyzed using PAST software, version 3.25 (Hammer, 2019).

Principal components analysis

Principal components analysis (PCA) is the data reduction technique applicable to quantitative type of data. PCA transforms multi-correlated variables into another set of uncorrelated variables for further study. These new set of variables are linear combinations of original variables.

It is based on the development of eigen-values and mutually independent eigen-vectors (principal components) ranked in descending order of variance size. Such components give scatter plots of observations with optimal properties to study the underlying variability and correlation. Suppose x_1, x_2,…….,x_n be the original data in a study, then principal components may be defined as:

$$\boldsymbol{\alpha}'\mathbf{1}\boldsymbol{x} = \alpha 11x1 + \alpha 12x2 + \cdots + \alpha 1pxp = \sum_{j=1}^{p} \alpha 1jxj$$

Where, $\boldsymbol{\alpha}'_1x$ is the a linear function of the elements of x having maximum variance, and α1 is a vector of p constants α11, α12,...,α1p, and ‘ denotes transpose, so that Next, look for a linear function $\boldsymbol{\alpha}'_2x$, uncorrelated with $\boldsymbol{\alpha}'_1x$ having maximum variance, and so on, so that at the k^{th} stage a linear function $\boldsymbol{\alpha}'_kx$ is found that has maximum variance subject to being uncorrelated with $\boldsymbol{\alpha}'_1x$, $\boldsymbol{\alpha}'_2x$.., $\boldsymbol{\alpha}'_{k-1}x$. The k^{th} derived variable, $\boldsymbol{\alpha}'_kx$ is the k^{th} PC (Jolliffe, 2002). The biplot based on two principal components were also generated to depict the two-dimensional view of accession scores.

Data of principal component analysis were analyzed using PAST software, version 3.25. (Hammer, 2019).

Shannon-weaver Diversity Index

Shannon diversity indices ($H^{'}$) were calculated to study diversity among all genotypes studied as well as among genotypes from each category (B/R lines) with the formula given by Shannon (1948):

$$_{i=1}^{N} H^{'} = -\sum pi.lnpi$$

where p_i is the proportion of accessions in the i^{th} class of an n-class character and n is the number of phenotypic classes for a character and for quantitative traits 'n' equalled 8, based on Sturge's rule, n, the number of frequency classes = 1 + Log_2 (*N*), where, n = the observed number of accessions.

The indices are standardised by dividing each value of $H^{'}$ by $loge_n$ to keep the value in a range of 0 to 1 in order to estimate the importance of phenotypic diversity. The data were analysed to calculate the diversity index using R statistical software, version 3.4.1 (R Development Core Team, 2017).

Association analysis

Pearson r correlation was used to measure the degree of relationship between linearly related variables. The following formula was used to calculate the Pearson r correlation:

$$r_{xy} = \frac{n\sum xiyi - \sum xi\sum yi}{\sqrt{n\sum xi2-(\sum xi)2}\sqrt{n\sum yi2-(\sum yi)2}}$$

where, r_{xy} = Pearson r correlation coefficient between x and y variables; n = number of observation; x_i = value of x for the i^{th} observation and y_i = value of y for i^{th} observation. The data were analysed for the Pearson correlation analysis using R statistical software, version 3.4.1 (R Development Core Team, 2017).

Results and Discussion

Genetic diversity analysis

The major descriptive statistics such as mean, range and coefficients of variation revealed wider ranges of variations among genotypes for most of the traits studied. The range of variation was maximum for grain Fe content (14.00-122.00 ppm) followed by grain Zn content (11.50-56.00 ppm), plant height (61.67-153.80 cm), panicle length (13.00-28.84 cm), panicle weight (0.02-0.44 kg), grain yield per plant (0.01-0.22 kg) and dry fodder yield per plant (0.33-2.93 kg) while it was minimum for grain Cu content (4.5-10.5 ppm) and grain Mn content (4.5-17.5 ppm) (Table 2). Higher range, more diversity Index and large differences in mean values for most of the characters revealed that sufficient diversity existed among the genotypes and traits. The present findings were similar with previous reports in pearl millet (Dapke *et al.*, 2014; Anuradha *et al*., 2018; Sharma *et al*., 2018 and Mahendrakar *et al*., 2019).

Cluster analysis

Grouping of genotypes into few numbers of homogenous clusters facilitates the selection of diverse lines for the crossing purpose. It permits precise comparison among all the possible pair of individuals and provides an opportunity for bringing together gene combinations and yielding desirable segregants from crossing between these lines. The cluster analysis through the UPGMA method grouped the genotypes into five clusters with varied number of genotypes (Fig.1). In the present study cluster analysis on the basis of mean values for grain micronutrients, yield and agro-morphological traits were studied. It was clearly observed

that cluster I possessed genotypes with high grain iron and zinc content, dry fodder yield and panicle length. Similarly, cluster II comprised only B lines with high grain iron and zinc content, panicle girth and late flowering type. These results were in agreement with the previous findings by Sangwan *et al*., (2019) in pearl millet. Cluster III comprised most of the genotypes from B lines with higher panicle girth and Mn content. Similarly, grouping in cluster IV with genotypes both from B and R lines with early flowering habit and high panicle girth showed the findings in close agreement with previous reports of Kumari *et al*., (2016) and Sangwan *et al*., (2019). The genotypes which possessed more plant height, longer panicle length, panicle girth, panicle weight, grain yield, dry fodder yield and Mn content were grouped in cluster V (Fig.1). These results of cluster analysis for various grain micronutrients, yield and agro-morphological characters suggested clear differentiation of B and R lines with some exceptions. The initial assessment of genetic materials to enable identification of potent parents for hybridization programme based on morphological data is easy, simple and can be considered as a universal approach for evaluating genetic diversity among genotypes. Similarly, grouping of genetic materials based on quantitative data in pearl millet was reported by Shanmuganathan *et al*., (2006), Vidhyadhar and Devi (2007), Govindaraj *et al*., (2011), Drabo *et al*., (2013), Sathya *et al*., (2013), Upadhyaya *et al*., (2013), Sankar *et al*., (2014), Chaudhary *et al*., (2015), Kumar *et al.*, (2015), Kumari *et al*., (2016) and Sangwan *et al*., (2019). Genotypes from different source/origin falls under the same cluster, so grouping did not happened on the basis of origin or geographical location. These results are in agreement with the findings of Burson *et al*., (2015) and Animasaun *et al.*, (2017) who observed that accessions of pearl millet did not necessarily assemble into the same cluster based on their geographical origins. Clustering of pearl millet accessions together regardless of their source supports the possibility of a common progenitor but separation by geographical or ecological isolation mechanisms (Jauhar, 1981). Therefore, for any hybridization programs in pearl millet, the choice of suitable diverse parents based on genetic divergence analysis would be more rewarding than the choice based on the geographical distances.

Principle component analysis

The PCA based on correlation was used to study interrelationships among the different traits and genotypes. The first four principal components having eigen value greater than one were extracted from the mean of 11 traits and they explained 68.83% variance in pearl millet genotypes. The first principal component (PC1) was the most important and accounted 31.33% of variation. The major contributors for variation observed in first principle component were plant height, panicle length, panicle weight, grain yield per plant, dry fodder yield per plant, grain Mn content. A variance of 15.40, 12.78 and 9.32 were extracted from second, third and fourth principal components, respectively. The variation in PC2 were mainly due to panicle weight, grain yield per plant, grain Fe, Zn and Cu content. PC3 imparted 12.78% variance mainly through days to 50% flower, plant height, panicle length, grain Fe and grain Cu content. Likewise major contributor to the variation observed in PC4 was panicle girth (Table 3). The results indicated the role of traits (specific to each PC) which contributed more towards genetic divergence in discriminating the genotypes of pearl millet. The present study was in agreement with the PCA traits analysis of Kumari *et al*., (2016), Animasaun *et al*., (2017), Radhika Ramya *et al*., (2017), Sangwan *et al*., (2019) in pearl millet. The Genotype-trait biplot based on two

principal components were also generated to represent the two-dimensional view of eleven traits and different genotypes of pearl millet (Fig. 2). Two dimensional PCA biplot of 48 pearl millet genotypes showed that most of the lines from maintainer category were clustered in left side of the biplot graph. The lines from the restorer category were mainly present on the right side of the PCA biplot graph (Fig. 3). The two dimensional view of genotype-trait biplot based on 2 PCs in pearl millet revealed that genotypes from B lines were clustered in the left side of the biplot graph and the lines from the restorer category or R lines were distributed throughout the PCA biplot graph (Radhika Ramya *et al*., 2017 and Sangwan *et al*., 2019).

Estimation of Shannon-weaver Diversity Index

Shannon-weaver Diversity Index was estimated which revealed sufficient diversity for both genotypes and traits. The pearl millet genotypes were diverse for all the quantitative traits and also diverse among themselves ($H^{'}$>0.5). Diversity index ranged from 1.712 to 2.002 for the genotypes (Table 2). Average Diversity Index of 1.883 and 3.792 for genotypes and traits respectively revealed that pearl millet genotypes were more diverse among themselves and for all the quantitative traits. The highest (2.002) and lowest (1.712) diversity indices were reported in genotypes 225SU14B and ICMB 92777, respectively from the maintainer category. Ten genotypes from B lines and fifteen genotypes from R lines exhibited above average diversity indices.

Genotypes, 225SU14B (2.002), 226SU14B (1.998), 106SB13 (1.976), 123SM14 (1.957), 95SM13 (1.951) and ICMB 08444 (1.950) exhibited greater diversity (Table 2). While genotypes, ICMB 92777 (1.712), 211SU14B (1.772), 131SM14 (1.790), 212SU14B (1.811), 237SM13 (1.815) and 218SU14B (1.819) showed less diversity for all the traits studied (Table 2). However, the Shannon-weaver Diversity Index for all the traits together revealed that days to 50% flower (3.869) has contributed most for the diversity among the genotypes followed by panicle girth (3.862), grain Cu content (3.858), panicle length (3.855), plant height (3.850), grain Mn content (3.835) and grain Zn content (3.801) (Table 2). The similar indices for trait specific Shannon-weaver Diversity Index was calculated by Kumari *et al*., (2016) in pearl millet germplasm accessions for determining morphological diversity.

Association studies

Correlation study of micronutrients concentration revealed that grain Fe content showed significant positive correlation with the grain Zn (0.33) and Cu content (0.40) but significant negative correlation with plant height (-0.31), dry fodder yield per plant (-0.30) and grain Mn content (-0.45). Grain Zn content was significantly negatively correlated with plant height (-0.30) and panicle length (-0.41). Significant positive correlation were observed for the grain yield per plant with the plant height (0.39), panicle weight (0.95), dry fodder yield per plant (0.57) and significantly negative correlation with days to 50% flower (-0.41). Interestingly, in the present study grain yield per plant does not show any significant positive or negative correlation with grain micronutrients concentration. Similarly, panicle weight showed significant positive correlation with plant height (0.45), panicle length (0.32), dry fodder yield (0.60) and negative correlation with days to 50% flower (-0.43). Panicle length was positively correlated with plant height (0.46) and dry fodder yield per plant (0.30) and dry fodder yield per plant exhibited significant positive correlation with plant height (0.64) (Fig. 4).

Table.1 List of genotypes, pedigree, Source / Origin and their category

Sr. No.	Genotype Name	Pedigree	Source / Origin	B/R line
1	ICMB 99222	(BSECBPT/91-40 x SPF3/S91-94)-3-1-1-2	ICRISAT, Patancheru	B line
2	ICMB 05888	(SRC II C3 S1-1-1-2 x HHVBC)-5-1-1-2	ICRISAT, Patancheru	B line
3	ICMB 92777	[843 B x (ICMPES-500-4-4-3 x ICMPES-800-3-1-2(3-4)]-7-1-3	ICRISAT, Patancheru	B line
4	ICMB 06777	(ICMB 96333 x HHVBC-2-D2-HS-259-2)-4-B	ICRISAT, Patancheru	B line
5	ICMB 95222	{[843B x (GNS x SS-48-40-4)-29-7-4-B] x (843B x ICMPES-29)-23-2-3}-16	ICRISAT, Patancheru	B line
6	213-SU-14B	[ICMB 96111 x 4017-2-1-B)-7-2-3 x (SRC II C3 S1-19-3-2 x HHVBC)-17-3}-1-3-19-2-2-2-B-2-4	PMRS, JAU, Jamnagar	B line
7	230-SU-14B	{[78-7088/3/SER3 AD//B282/(3/4)EB x PBLN/S95-359]-7-4-B-B-2-B-B x HHVDBC HS-10-1-2-1-1-1-4-1-1}-10-3-3-B	PMRS, JAU, Jamnagar	B line
8	ICMB 05333	(MC 94 S1-30-2-B x HHVBC)-16-3-1-1	ICRISAT, Patancheru	B line
9	ICMB 89111	[843B x (CNS x SS-48-40-4)-1-9-8]	ICRISAT, Patancheru	B line
10	ICMB 96222	126B x (81B x SLR 50-1)-1-1-2 x 852B)-69-1-1	ICRISAT, Patancheru	B line
11	225-SU-14B	{(MC 94 S1-34-1-B x HHVBC)-16-2-1-1-1-1-B-B-5 x (MC 94 S1-34-1-B x HHVBC)-10-4-1-2-1-B-B-1-30-2-4-3-6-4-3	PMRS, JAU, Jamnagar	B line
12	ICMB 04999	(EBC-Gen-S1-40-2-2-1 x B-line bulk)-25-B-B	ICRISAT, Patancheru	B line
13	218-SU-14B	[(SRC II C3 S1-1-1-2 x HHVBC)-2-2-1-1-1-B-B x (81B x 4017-5-4-B)-12-3-1-3]-6-2-3-3-2	PMRS, JAU, Jamnagar	B line
14	227-SU-14B	[(MC 94 S1-34-1-B x HHVBC)-16-1-3-1-2-2-B-B-2-B-B x ICMB 99222]-13-2-1-2	PMRS, JAU, Jamnagar	B line
15	212-SU-14B	(ICMB 04777 x ICMB 04111)-2-2-2-2-1	PMRS, JAU, Jamnagar	B line
16	211-SU-14B	[ICMB 96111 x 4017-2-1-B)-7-2-3 x ((SRC II C3 S1-19-3-2 x HHVBC)-17-3]-1-3-19-2-2-2-B-2-2	PMRS, JAU, Jamnagar	B line
17	215-SU-14B	{[(843B x ICTP 8202-161-5)-20-3-B-B-3 x B-bulk]-2-B-1-2-2-B-B-B-11-1 x B-bulk (3981-4011/S06 G1)}-3-2-4-	PMRS, JAU, Jamnagar	B line
18	ICMB 05222	(ICMR-312S1-8-3-3-B x HHVBC)-9-4-1-1	ICRISAT, Patancheru	B line
19	224-SU-14B	[EEDBC S1-425-2-1-2-3-B-1-B-7-1 x B-bulk (3981-4011/S06 G1)]-2-2-1-B	PMRS, JAU, Jamnagar	B line
20	ICMB 08444	HHVBC II D2 HS-410-1-2-4-1-3-B-2-2-3-2	ICRISAT, Patancheru	B line
21	226-SU-14B	(ICMB 99555 x ICMB 00555)-11-1-3-B-2-B-2	PMRS, JAU, Jamnagar	B line
22	ICMB 98222	ARD-288-1-10-1-2(RM)-5	ICRISAT, Patancheru	B line
23	ICMB 841	DM.RESI.SELE.FROM SEED LOT NO.8015 OF 5141B	ICRISAT, Patancheru	B line
24	261-SU-14B	09888B x [(HHVDBC HS-246-1-2-1-2 x ICMB 01222)-4-2-1-1]-2-1	PMRS, JAU, Jamnagar	B line

Sr. No.	Genotype Name	Pedigree	Source / Origin	B/R line
25	121-SM-13	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-17-4-3-2	PMRS, JAU, Jamnagar	R line
26	J-2510	JBV 3-S1-231	PMRS, JAU, Jamnagar	R line
27	J-2500	AIB-1-2-B	PMRS, JAU, Jamnagar	R line
28	J-2480	AS-14 (B-Senegal-2-5 x 700651)-2-1-4 (IPC-655)	PMRS, JAU, Jamnagar	R line
29	123-SB-14	(J-2340 x J-2454)-15-10-8-3-1-1-1	PMRS, JAU, Jamnagar	R line
30	106-SB-13	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-34-4-1	PMRS, JAU, Jamnagar	R line
31	131-SM-14	(MRC HS-86-1-1-5-B-B-B-B-B x MRC S1-54-2-3-B-B-1-B-B)-19	PMRS, JAU, Jamnagar	R line
32	J-2523	MRC HS 130-2-2-1-B-B-1-B-B	PMRS, JAU, Jamnagar	R line
33	113-SB-13	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-34-4-1	PMRS, JAU, Jamnagar	R line
34	124-SB-14	(J-2405 x J-2480)-13-10-2-1-1-1-1	PMRS, JAU, Jamnagar	R line
35	J-2549	(IPC 107 × SDMV 90031-S1-84-1-1-1-1)-1-2-1-3-B	PMRS, JAU, Jamnagar	R line
36	237-SM-13	ICTP 8203	PMRS, JAU, Jamnagar	R line
37	94-SB-13	(EERC-HS-8)-20-1-5	PMRS, JAU, Jamnagar	R line
38	128-SM-14	(EERC-HS-32)-B-8-1-1-B	PMRS, JAU, Jamnagar	R line
39	199-SM-13	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-22-2-1	PMRS, JAU, Jamnagar	R line
40	109-SB-13	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-34-4-1	PMRS, JAU, Jamnagar	R line
41	123-SM-14	(J-2340 x J-2454)-15-10-8-3-1-1-1	PMRS, JAU, Jamnagar	R line
42	99-SM-13	(SRC II C3 S1-19-3-2 x HHVBC)-3-5-1P2 x ((ICMV IS 94206 S1-15-2) x {(SRC II C3 S1-19-3-2 x HHVBC)-5-3-1})-B-1-2-1-1)-B-2-2-2-1	PMRS, JAU, Jamnagar	R line
43	93-SM-13	CGP S1-14-1	PMRS, JAU, Jamnagar	R line
44	95-SM-13	(ICMB 04888 x ICMB 02333)-3-1-3-1	PMRS, JAU, Jamnagar	R line
45	110-SB-13	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-17-4-3-2-B-B	PMRS, JAU, Jamnagar	R line
46	108-SB-13	(J 834 x 700516)-1-4-4-2-4-B-2-2-B-B-B-1	PMRS, JAU, Jamnagar	R line
47	73-SB-13	ICMR 312 S1-8-1-1-1-1-B-B-B-1-B	PMRS, JAU, Jamnagar	R line
48	J-2512	(PPMI-362 x IP-5738-1) x RIB-3135-18-5-3-8-B	PMRS, JAU, Jamnagar	R line

Table.2 Mean values, Shannon-weaver Diversity Index ($H^{'}$) of 48 genotypes and descriptive statistics of 11 traits in pearl millet

Sr. No.	Genotype	Days to 50% Flower	Plant Height (cm)	Panicle Length (cm)	Panicle Girth (mm)	Panicle Weight (kg)	Grain Yield (kg)	Dry Fodder Yield (kg)	Grain Fe Content (ppm)	Grain Zn content (ppm)	Grain Cu content (ppm)	Grain Mn content (ppm)	Shannon-weaver Diversity Index ($H^{'}$)
1	ICMB-99222	56.00	105.67	16.17	26.80	0.09	0.05	1.06	76.50	56.50	8.00	9.00	1.823
2	ICMB-05888	60.50	109.49	28.84	21.38	0.15	0.05	1.05	85.50	32.50	8.00	11.50	1.833
3	ICMB-92777	59.50	129.17	18.84	22.48	0.10	0.04	2.93	17.00	15.50	6.00	14.00	1.712
4	ICMB-06777	63.50	105.67	16.17	27.85	0.08	0.05	0.74	56.50	27.00	9.50	11.00	1.852
5	ICMB-95222	59.00	93.33	15.41	19.62	0.09	0.04	0.33	42.00	38.00	8.50	10.50	1.873
6	213SU-14B	60.50	94.50	22.00	23.74	0.05	0.05	0.66	56.00	36.50	9.50	8.00	1.899
7	230SU-14B	62.00	62.50	14.91	20.31	0.15	0.07	0.46	103.00	32.00	9.50	11.00	1.861
8	ICMB-05333	61.00	76.67	20.67	26.28	0.06	0.05	0.42	97.00	42.50	8.00	6.50	1.875
9	ICMB-89111	56.50	87.17	16.50	20.35	0.14	0.06	0.54	77.00	56.00	7.50	10.00	1.893
10	ICMB-96222	56.00	85.00	16.50	19.03	0.02	0.01	0.42	122.00	39.50	8.50	12.00	1.826
11	225SU-14B	55.50	61.67	16.50	28.62	0.16	0.07	0.81	22.50	21.00	7.00	14.00	2.002
12	ICMB-04999	61.00	92.50	16.50	22.51	0.08	0.03	0.37	107.00	44.00	9.00	14.00	1.879
13	218SU-14B	56.00	131.00	22.50	26.58	0.37	0.14	2.07	92.00	24.50	8.00	6.00	1.819
14	227SU-14B	65.00	76.67	15.00	27.88	0.02	0.01	0.61	39.50	24.00	7.00	11.50	1.951
15	212SU-14B	62.00	118.35	23.34	18.55	0.13	0.04	0.90	89.00	20.50	7.00	7.50	1.811
16	211SU-14B	60.50	134.17	28.84	19.60	0.05	0.02	0.49	19.50	16.00	6.50	13.00	1.772
17	215SU-14B	56.50	80.00	14.00	28.11	0.13	0.04	0.42	95.50	37.50	9.50	8.00	1.909
18	ICMB-05222	61.00	87.50	23.67	26.36	0.10	0.04	0.79	94.00	23.00	7.00	8.50	1.898
19	224SU-14B	59.50	120.32	19.50	24.70	0.09	0.03	1.34	69.50	30.00	8.00	15.50	1.910
20	ICMB-08444	53.50	108.00	22.34	22.23	0.44	0.21	1.38	57.00	25.50	7.00	16.00	1.950
21	226SU-14B	60.50	82.67	13.00	28.79	0.11	0.04	0.55	47.00	53.50	8.00	17.50	1.998
22	ICMB-98222	57.00	102.50	14.67	26.91	0.18	0.09	0.85	91.00	45.50	7.50	7.00	1.894
23	ICMB-841	57.50	122.17	24.00	21.01	0.18	0.07	0.73	57.00	15.50	8.00	7.50	1.850
24	261SU-14B	50.00	115.84	17.50	24.79	0.05	0.01	0.32	56.50	18.00	7.00	8.50	1.859
25	121SM-13R	59.00	108.00	20.00	27.76	0.14	0.04	0.84	55.00	34.50	6.00	12.50	1.946
26	J2510R	53.00	117.67	19.83	24.22	0.14	0.08	0.63	53.50	21.00	10.50	10.00	1.908
27	J2500R	61.50	140.17	19.50	25.12	0.10	0.04	1.17	96.00	24.00	8.50	7.00	1.822
28	J2480R	61.00	117.00	16.33	19.88	0.16	0.07	0.61	110.00	23.00	8.50	9.00	1.827

29	123SB14	60.50	122.67	21.34	19.34	0.08	0.03	1.10	24.50	28.00	6.00	11.50	1.866
30	106SB13	55.50	103.50	24.00	23.13	0.34	0.15	1.22	33.50	24.00	8.50	13.50	1.976
31	131SM14	58.50	147.80	19.00	21.23	0.31	0.16	2.00	21.00	21.50	7.50	9.50	1.790
32	J2523	53.50	127.65	20.34	24.16	0.23	0.11	1.06	51.00	25.00	6.50	13.50	1.904
33	113SB13	54.50	124.35	24.50	25.51	0.41	0.15	1.49	54.50	26.50	7.00	11.50	1.932
34	124SB14	60.00	119.17	17.84	21.59	0.10	0.05	0.42	54.50	23.00	5.50	13.50	1.891
35	J2549	61.00	153.80	24.50	22.41	0.33	0.14	2.13	85.50	22.50	8.00	8.00	1.838
36	237SM13	61.00	131.85	20.00	24.26	0.09	0.03	1.00	14.00	11.50	9.00	17.00	1.815
37	94SB13	60.00	140.00	17.67	22.93	0.14	0.04	0.96	43.50	37.50	6.50	11.00	1.872
38	128SM14	61.50	125.00	21.50	19.65	0.06	0.02	1.20	48.00	47.00	8.00	11.00	1.928
39	199SM13	53.50	116.00	21.34	29.88	0.18	0.05	1.18	31.50	25.00	6.00	10.50	1.926
40	109SB13	53.00	120.30	21.50	28.51	0.27	0.13	0.85	66.00	24.50	7.50	9.50	1.924
41	123SM14	64.50	98.00	18.67	29.39	0.08	0.04	1.28	55.50	17.00	8.50	10.50	1.957
42	99SM13	59.00	137.65	21.50	26.47	0.29	0.11	1.62	35.00	13.50	7.00	12.50	1.858
43	93SM13	54.50	130.84	19.34	26.68	0.15	0.05	1.07	52.50	47.50	4.50	8.50	1.902
44	95SM13	52.00	98.35	15.50	20.97	0.11	0.04	0.77	53.00	25.00	6.50	12.50	1.951
45	110SB13	59.00	140.50	21.12	24.22	0.38	0.16	1.60	24.50	30.50	5.50	13.00	1.869
46	108SB13	54.00	139.99	17.17	18.63	0.33	0.21	2.02	48.50	45.00	8.00	11.00	1.901
47	73SB13	63.50	79.17	18.50	23.09	0.06	0.03	0.49	56.50	25.00	6.00	4.50	1.944
48	J2512	55.00	127.67	19.67	24.86	0.35	0.19	1.61	64.00	21.00	8.50	8.50	1.904
	Mean	58.30	111.45	19.54	23.93	0.17	0.071	1.01	60.44	29.54	7.58	10.79	1.883
	Minimum	50	61.67	13	18.55	0.02	0.01	0.33	14	11.5	4.5	4.5	1.712
	Maximum	65	153.8	28.84	29.88	0.44	0.22	2.93	122	56	10.5	17.5	2.002
	CD @ 5%	6.84	31.29	5.53	6.14	0.15	0.07	NS	12.30	9.60	NS	NS	
	CV%	5.83	13.96	14.05	12.75	45.52	48.63	72.66	10.15	16.12	30.65	44.28	
	H'	3.869	3.850	3.855	3.862	3.657	3.622	3.732	3.767	3.801	3.858	3.835	3.792

H' = Shannon-weaver Diversity Index; CD @ 5% = Critical Difference @ 5% level of significance; CV% = Per cent Coefficient of Variation

Table.3 Principal Components of eleven quantitative traits in pearl millet

Principal Component	PC1	PC2	PC3	PC4
Loadings				
Days to 50% Flower	-0.38	-0.37	0.47	0.06
Plant Height(cm)	0.76	-0.14	0.27	-0.10
Panicle Length(cm)	0.54	-0.20	0.54	0.10
Panicle Girth(mm)	-0.07	0.05	-0.48	0.82
Grain Weight(kg)	0.84	0.43	-0.13	0.00
Grain Yield/Plant(kg)	0.78	0.51	-0.13	-0.06
Dry Fodder Yield/Plant(kg)	0.79	0.00	0.05	0.03
Grain Fe Content(ppm)	-0.45	0.67	0.37	0.01
Grain Zn Content(ppm)	-0.42	0.41	-0.30	-0.38
Grain Cu Content(ppm)	-0.32	0.47	0.23	-0.06
Grain Mn Content(ppm)	0.13	-0.46	-0.52	-0.43
Eigen Value	3.45	1.69	1.41	1.02
% Variance	31.33	15.40	12.78	9.32

Significant positive association of various traits gives an insight on simultaneous selection of characters and it means selection for these traits will lead to simultaneous improvement in grain yield and micronutrients level in crop plants.

Conclusively, trait association study of yield and agronomical traits revealed that significant positive correlation existed between grain Fe content with the grain Zn (0.33) and Cu content (0.40); grain yield per plant with the plant height (0.39), panicle weight (0.95), dry fodder yield per plant (0.57); panicle weight with plant height (0.45), panicle length (0.32), dry fodder yield (0.60); panicle length with plant height (0.46) and dry fodder yield per plant (0.30) and dry fodder yield per plant with plant height (0.64).

The similar association was also reported by several workers for yield and related traits in pearl millet namely, Pareek, (2002), Borkhataria *et al*., (2005), Izge *et al*., (2006), Kale *et al*., (2011), Atif *et al*., (2012), Dapke *et al*., (2014), Kumar *et al*., (2016), Singh and Singh (2016), Bhaskar *et al*., (2017), Talawar *et al*., (2017), Anuradha *et al*., (2018), Sharma *et al*., (2018). The positive correlation of grain micronutrients and yield with these characters implies that improving one or more components traits could result in higher nutrient contents and grain yield in pearl millet. For any biofortification programme, an important aspect is to take are that enhancement in nutrient value should not be at the cost of grain yield.

Therefore, a correlation study was conducted between grain yield per plant and grain Fe, Zn, Cu and Mn content. Interestingly, in the present study grain yield per plant showed non-significant positive or negative correlation with grain micronutrients concentration thus suggesting scope of improvement in nutrient value without sacrificing yield. This is supported by the studies of Gupta *et al*., (2009), Rai *et al*., (2012), Kanatti *et al*., (2014) and Sangwan *et al*., (2019). Earlier studies on pearl millet for grain micronutrients concentration by Velu *et al*., (2007, 2008a, 2008b), Gupta *et al*., (2009), Govindaraj *et al*., (2012, 2013), Rai *et al*., (2012, 2013, 2015) and Anuradha *et al*., (2018) also showed a highly significant and positive correlation between the Fe and Zn contents indicating that these two traits can be improved simultaneously.

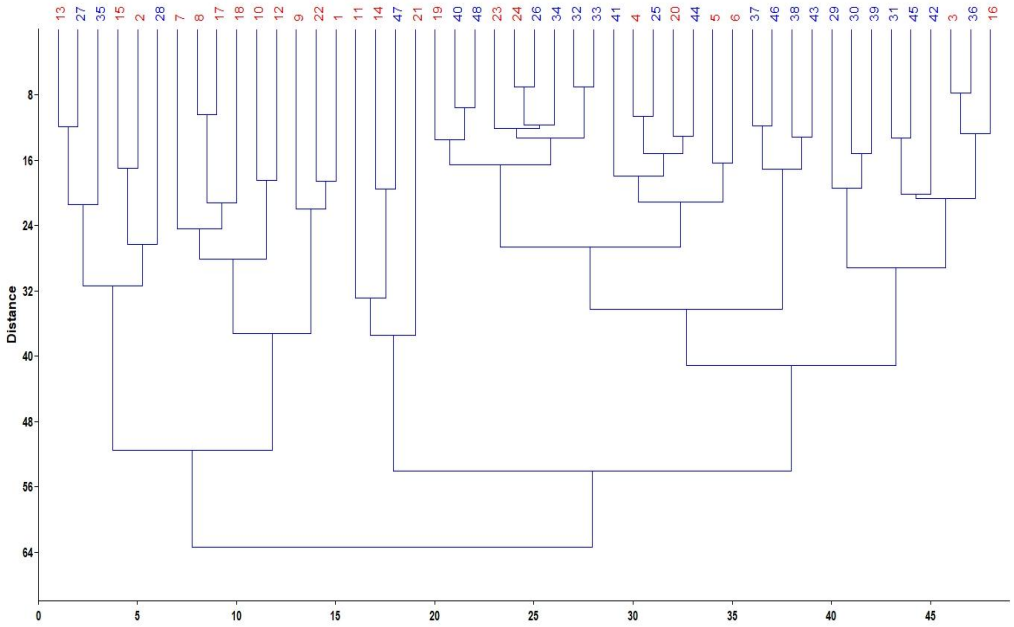

Fig.1 Dendrogram showing 24 maintainer lines (red colour) and 24 restorer lines (blue colour) of pearl millet

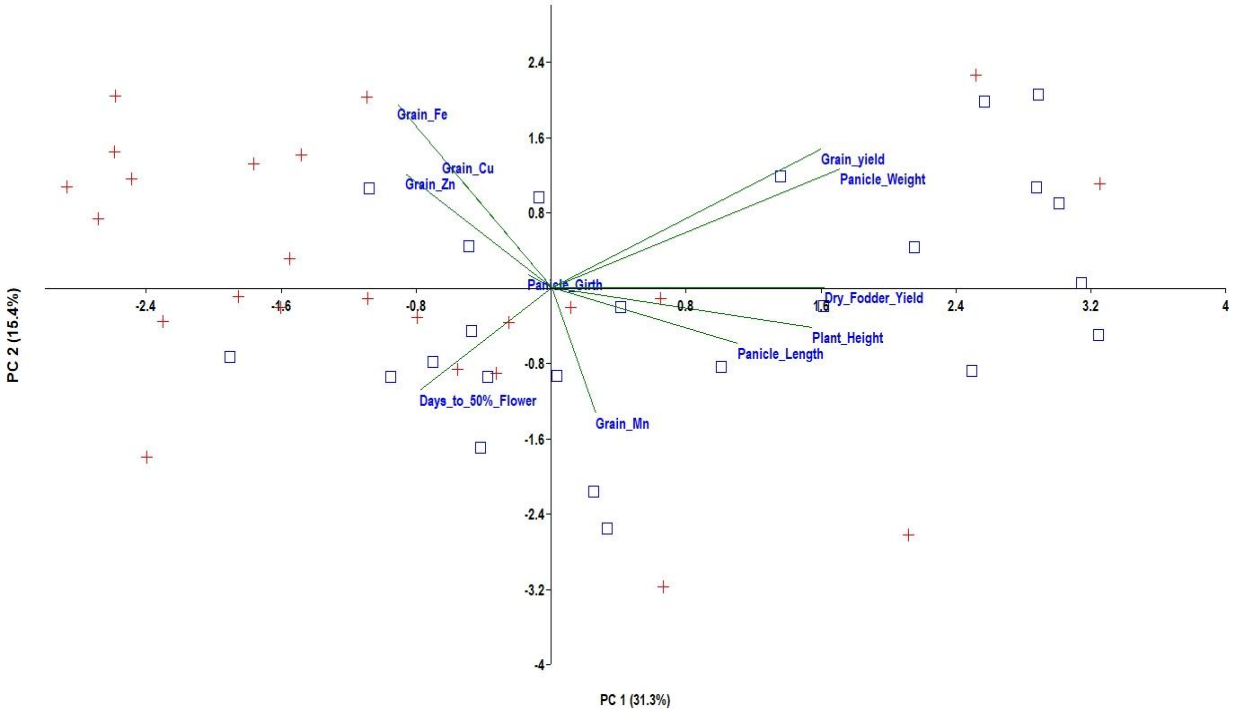

Fig.2 PCA biplot showing 11 quantitative traits along with 24 maintainer lines (red colour) and 24 restorer lines (blue colour) of pearl millet

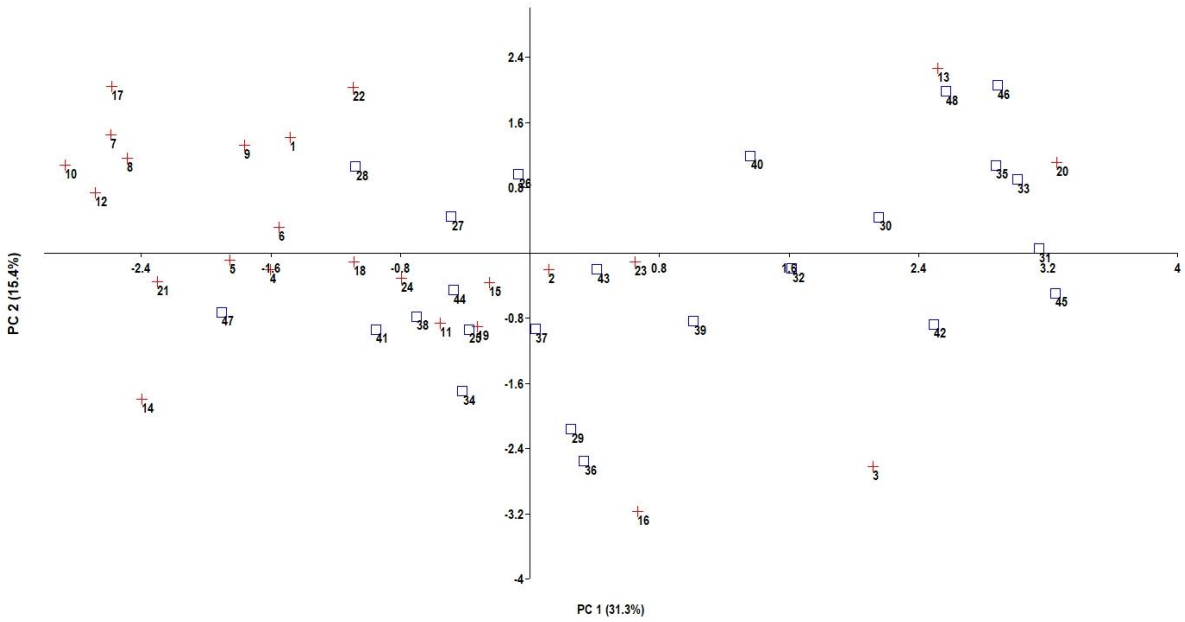

Fig.3 PCA biplot showing 24 maintainer lines (red colour) and 24 restorer lines (blue colour) of pearl millet

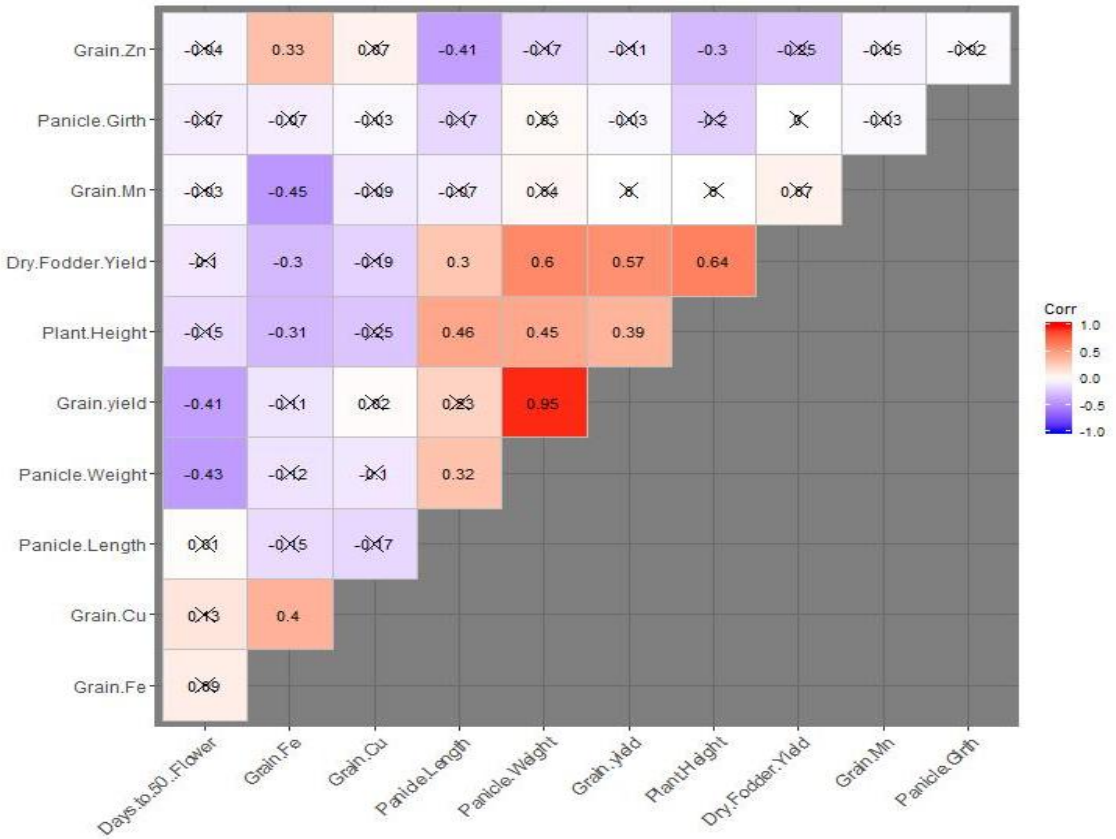

Fig.4 Pearson's correlation among 11 quantitative traits in pearl millet

The present findings showed significant positive association between grain Fe and Cu content in contrast to earlier work of Anuradha *et al*., (2018) who reported non-significant association between them. This finding would further enable simultaneous improvement of grain Fe and Cu content in pearl millet breeding programme The grain Fe content showed significant negative correlation with plant height (-0.31), dry fodder yield per plant (-0.30) and grain Mn content (-0.45).

Likewise, grain Zn content showed negative association with plant height (-0.30) and panicle length (-0.41); grain yield per plant with days to 50% flower (-0.41) and panicle weight with days to 50% flower (-0.43). The significant negative association between the grain yield and panicle weight with days to flowering has great advantages in pearl millet cultivation in arid and semi-arid environments. Early flowering habit is preferred trait for its cultivation as it enables the crop to mature earlier and consequently escape terminal heat and water stress in short season environments and permits multiple cropping system. Likewise, negative association between the grain yield and days to flowering in pearl millet was reported by several workers (Dapke *et al*., 2014 and Sangwan *et al.*, 2019).

An accurate assessment of genetic diversity is important in crop breeding and improvement. It is established that considerable phenotypic diversity existed among the maintainer and restorer lines of pearl millet in the present study. Clustering of the genotypes independent of the geographical location or source/origin suggested that hybridizing the genetically diverse parents belonging to different clusters could provide an opportunity for bringing genes of diverse nature together for its improvement. Clustering of pearl millet genotypes from different source/origin into same cluster has confirmed that they are genetically related, and possibly from the same parent, but could have been separated by geographical or ecological isolation mechanisms. Cluster formation on the basis of grain micronutrients, yield and agro-morphological characters suggested clear differentiation of B and R lines with some deviations. Principal component biplot supported the result obtained by cluster analysis which further validated the diversity pattern in the pearl millet population. Further characterisation and genetic diversity analysis in pearl millet genotypes using molecular techniques should be conducted.

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