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Evaluation of pearl millet [*Pennisetum glaucum* (L.) R. Br.] for grain iron and zinc content in different agro climatic zones of India

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Abstract

Micronutrient malnutrition, especially the paucity of iron (Fe) and zinc (Zn) is posing a big threat to the world affecting nearly 25% of worldwide population. Pearl millet is endowed with huge amount of variability for micronutrients especially for grain Fe and Zn content. Micronutrient enrichment in pearl millet is possible by identifying stable genotypes for high levels of micronutrients and utilising them in breeding programme. In this context, a set of 40 pearl millet genotypes along with one check, Dhanshakti (G30), were evaluated at three different agro climatic zones during the year 2014 for grain iron (Fe) and zinc (Zn) contents using Atomic Absorption Spectrometry. The genotypes contributed 58.3% and 52.8% of the total variation for grain Fe and Zn content, respectively. The magnitude of variation contributed by interaction component was also relatively high (39.7% and 32.5% for grain Fe and Zn). Both AMMI and GGE biplot analysis identified desirable genotypes; PPMI 708 (G40), PPMI 1102 (G25) and PPMI 683 (G39) for grain Fe content, whereas PPMI 708 (G40), PPMI 1116 (G24) and PPMI 683 (G39) for grain Zn content. The Pearson correlation coefficient for grain Fe and Zn content showed that both traits are highly associated (r = 0.8, p <0.01) and these traits did not associate significantly with grain yield. Hence, there is possibility for simultaneous improvement of both grain Fe and Zn content without compromising for grain yield.

Key words: Pearl millet, grain Fe and Zn content, GEI, AMMI analysis, GGE biplot

Introduction

Micronutrient malnutrition or 'Hidden hunger' is caused due to inadequate accessibility of minerals and vitamins in diet. Dearth of micronutrients especially iron and zinc in diet is affecting more than two billion people globally and the most susceptible being pregnant women and children below the age of five (WHO 2012). Anaemia, caused by iron (Fe) deficiency is the most common disorder mainly observed in low income countries and the inhabitants in such countries who consume low quality diet are prone to possible risk of child mortality and other physiological disorders (Tako et al. 2015). Zinc is also an important micronutrient which is required for proper growth, the deficiency of which may lead to stunting, increased susceptibility to many infectious diseases, morbidity and low mental ability (Deshpande et al. 2013). Among all the possible ways to combat this micronutrient deficiency, crop biofortification is better and viable approach (Bouis et al. 2011; Stein 2010).

Among the cultivated crops in arid and semi-arid regions of Africa and Asia, pearl millet is one of the staple crop grown over an extent of 29 million ha (Kannan et al. 2014). India is the largest producer of this crop in Asia. In terms of quality, it is nutritious compared to various other cereals as it contains premier content of macro as well as micro nutrients (Anonymous, 2013). In areas growing pearl millet, 35% of total consumption of energy, protein, iron and zinc is from this millet. It is found to be the economical source for rural residents to get micronutrients compared to other cereals such as rice and wheat (Rao et al. 2006). However the stable expression of these nutrients is required for the benefit of mankind in terms of nutritional security.

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To reveal the GEI (Genotype Environment Interaction), Additive Main Effects and Multiplicative Interactions (AMMI) developed by Gauch (1988) and Genotype plus Genotype by Environment Interactions (GGE) developed by Yan et al. (2000) are most frequently used by plant breeders (Gauch 2013). Both of them are useful to understand complex GEI, mega-environment delineation and selecting genotypes specific to certain environments. Both of them employ biplots for visualisation, which are powerful tools to summarize the data. The only difference existing between these two models is that while GGE biplot analysis is based on environment-centered principal component analysis (PCA), double-centered PCA is used in AMMI analysis (Gauch 2006).

Pearl millet enhancement for grain Fe and Zn content can be achieved by utilizing stable genotypes with increased levels of grain Fe and Zn in hybridization or population development programme. Hence, the present study was attempted to identify stable genotypes for micronutrient content in pearl millet grains across different locations.

Materials and methods

Plant materials and field trials

The experimental material for the present study comprised of 40 pearl millet genotypes and one check variety (Dhanshakti, G30) (Table 1). All the genotypes included in the study were inbred lines maintained through selfing except check variety Dhanshakti, which is open pollinated variety.

The experiment was conducted at three diverse geographical locations, representing all the three pearl millet growing agro-climatic zones of India, (i) ICAR-Indian Agricultural Research Institute Research farm, New Delhi (28^{o} 382' N, 77^{o} 802' E) representing Zone A receiving more than 400 mm annual rainfall (ii) ICAR-National Bureau of Plant Genetic Resources, Regional Station farm, Jodhpur (26^{o} 252' N, 72^{o} 992'E) falling in zone A_1 with annual rainfall less than 400 mm and (iii) ICAR-IARI Regional Centre farm, Dharwad (15^{o} 212' N, 75^{o} 052' E) from zone B (covering the southern peninsular India). In all locations, the trial was taken for two consecutive years (*kharif*, 2014 and 2015). The planting of genotypes was taken up in randomized complete block design (RCBD) in two lines each of 4m length with a spacing of 50 cm between rows and 15 cm from plant to plant, replicated thrice per entry. At the time of land preparation, diammonium phosphate was applied at 100 kg ha^{-1} as a basal dose, followed by application of 100 kg ha^{-1} of urea at 3-4 days after thinning. Field was irrigated as and when required to protect from moisture stress. Standard agronomic practices were followed for a normal healthy crop across all locations. Soil Fe and Zn content at the test locations were estimated using Diethylene triamine penta acetic acid (DTPA) extraction as per Singh et al. (2005) (Table 2).

Grain sampling and micronutrient analysis

Open-pollinated panicles following Rai et al. (2015a) from ten representative plants were harvested for each accession at physiological maturity, sun dried and then threshed with a wooden hammer, cleaned, while taking utmost care to avoid dust or metal contamination of the samples in every step. Threshed samples were transferred to paper covers and oven dried at 60^{o}C for 48 h before samples were utilized for estimation of grain Fe and Zn content on triplicate samples as per Singh et al. (2005) followed by reading on Atomic Absorption Spectrometer (AAS).

Statistical analysis

Data was subjected to analysis using Cropstat (v.7.2) and Genstat (v.18.1). After testing the error variance for homogeneity, combined analysis was performed over locations. Stability analysis was performed using AMMI model and GGE model. AMMI1 biplot using main effect means *vs* first Interaction Principal Component Analysis (IPCA) score as described by Zobel *et al*. (1988). GGE biplots were drawn as described by Yan and Kang (2003). Genotype-focused scaling was used in visualizing for genotypic comparison. Association analysis was performed using SPSS (v16).

Results and discussion

Pooled ANOVA was carried out after Bartlett’s homogeneity test, where the test result was found to be non-significant for both grain Fe and Zn content indicating experiments were homogeneous. While conducting combined ANOVA, locations were taken as random and genotypes were considered as fixed effects. It was observed that, Genotypes (G), Environmental effects (E) and GEI effects were highly significant ($P<0.01$) for grain Fe and Zn content (Table 3). The genotypes explained highest proportion of variation, for grain Fe and Zn content, accounting for 58.3% and 52.8% respectively, followed by GEI effects (39.7% and 32.5%). High observed genotypic variations indicate that there is much scope for

Table 1. Codes and details of genotypes, grand mean (GM) and range of grain Fe and Zn content in pearl millet over locations

S. No.	Genotype	Fe (mg/kg)		Zn (mg/kg)	
		Mean	Range	Mean	Range
G1	PPMI 1220	62.7	38.7-103.1	30.7	28.9-32.9
G2	PPMI 1224	64.7	41.9-85.1	49.2	40.6-62.1
G3	PPMI 1225	85.0	76.2-102.7	46.5	32.9-53.9
G4	PPMI 1233	55.4	32.0-70.2	42.4	24.9-60.6
G5	PPMFeZMP 37	77.0	64.0-97.2	46.3	43.1-48.8
G6	PPMFeZMP 47	61.2	37.9-89.3	32.2	29.0-35.7
G7	PPMFeZMP 87	61.2	39.1-90.4	34.9	24.6-40.3
G8	PPMI 1275	58.5	41.9-88.3	40.5	32.6-50.6
G9	PPMI 1276	65.9	63.9-68.4	39.7	27.6-49.0
G10	PPMDMgMP 99	93.8	70.6-125.9	47.0	44.3-52.0
G11	PPMDMgMP 148	59.7	49.9-76.1	35.1	33.8-36.3
G12	PPMI 1218	52.3	50.9-54.6	43.3	32.1-54.7
G13	PPMI 1267	71.9	63.9-81.6	50.6	43.4-55.3
G14	PPMI 1268	36.3	31.8-40.2	32.4	28.0-37.5
G15	PPMFeZMP 126	77.8	48.7-101.7	54.2	39.6-74.5
G16	PPMI 1285	74.4	46.4-119.6	53.5	38.6-72.7
G17	PPMI 1287	51.2	48.6-55.9	42.2	33.7-49.4
G18	5540 B	29.0	26.1-33.5	26.0	24.8-26.7
G19	6030 B	50.6	44.8-60.4	36.1	31.5-40.4
G20	PPMI 1084	87.6	65.4-99.5	44.6	32.6-62.5
G21	PPMI 1089	57.2	35.8-71.1	41.1	26.8-56.5
G22	PPMI 1092	49.5	34.8-61.1	38.2	27.3-50.8
G23	H77/833-2	54.0	40.0-68.9	37.7	32.6-42.0
G24	PPMI 1116	73.3	71.4-75.5	66.4	64.3-68.8
G25	PPMI 1102	111.6	105.3-115.5	64.6	56.8-75.2
G26	PPMI 1107	56.6	45.7-74.8	44.2	29.4-58.6
G27	ICMB 04222	61.9	50.5-71.0	50.1	41.8-56.3
G28	ICMR 06222	56.0	49.0-59.6	43.7	23.7-60.9
G29	ICMR 07111	60.9	42.5-76.6	34.8	25.8-39.8
G30	Dhanshakti	78.0	71.1-87.4	49.7	39.0-59.1
G31	IPC 1657	42.7	34.3-51.6	29.1	27.1-32.2
G32	J 108	42.5	37.5-51.5	30.7	21.7-36.5
G33	PIB 228	80.4	45.5-88.5	51.7	27.7-81.1
G34	PIB 686	42.2	33.0-50.4	35.0	28.9-45.9
G35	PPMI 59	52.3	29.2-77.0	38.6	36.2-42.5
G36	PPMI 214	77.6	58.5-99.6	44.8	26.5-63.1
G37	PPMI 265	68.9	56.4-76.9	51.4	34.3-83.9
G38	PPMI 275	51.2	38.1-59.7	47.3	41.7-52.2
G39	PPMI 683	92.4	84.8-106.6	58.5	55.9-61.1
G40	PPMI 708	114.6	108.4-121.6	76.4	68.2-81.5
G41	PPMWGI 152	59.1	44.4-78.5	39.4	36.8-41.2
GM		64.9		43.9	
LSD		30.3		16.4	

Mean is average over three locations. Range is given for three locations

enhancing pearl millet with these micronutrients through appropriate breeding approach. Also, substantial part of interaction variance obtained for grain Fe and Zn content, emphasize the importance of the GEI. These results are akin to genotype environment interaction studies conducted on micronutrient content in pearl millet and other related species (Bashir et al. 2014; Tara Satyavathi et al. 2015; Mallikarjuna et al. 2015; Gopalareddy et al. 2015), suggesting that both grain Fe and Zn are sensitive to environmental fluctuations and there is necessity to breed varieties for specific regions.

Further, stability analysis was conducted by both AMMI and GGE model to identify specific and widely adapted genotypes for grain Fe and Zn content.

AMMI analysis

In order to establish the suitability of the dataset to AMMI analysis Genotype Environment signal (GE_S) was calculated as per Gauch (2013). The sums of squares (SS) for genotype is 122514.0 and 39609.0 for grain Fe and Zn content respectively (Table 3). GE noise (GE_N) was calculated by multiplying the error mean sum of square by the degrees of freedom (df) for (GE) (15.6 × 80 = 1248.0 and 9.8 x 80 = 784.0 for grain Fe and Zn) and then GE_S was calculated by subtracting GE_N from GEI (83503.0–1248.0 = 82255.0 for grain Fe; 24345.0–784.0 = 23561 for grain Zn). As per Gauch et al. (2013), when SS due to GE_N is almost equal to the SS due to GEI obtained in ANOVA, then the GEI is said to be buried in noise. In this case, SS due to GE_N for Fe and Zn were far less than SS for GEI. So, the interaction was not buried in noise and was almost signal rich. Therefore, AMMI analysis is appropriate in this context (Similar results were obtained by Ndhlela et al. (2014).

The ANOVA for AMMI2 model showed that the first two interaction components explained 100% of the interaction variation leaving no residual

Table 2. Climatic and edaphic factors of different locations

Locations	New Delhi	Jodhpur	Dharwad
Climatic factors			
Temp (Avg. max)	38.8 °C	34.4 °C	30.7°C
Temp (Avg. min)	25.0 °C	25.0 °C	18.7 °C
RH%	69.1	77.7	68.6
RF in mm	482	389	761
Soil factors			
Texture	Sandy loam	Loamy sand	Silty clay
p^H	8.1	8.4	7.1
EC	0.23	0.09	0.18
Fe (mg/kg)	11.0	6.4	27.6
Zn (mg/kg)	4.6	3.2	1.7
Cu (mg/kg)	3.7	2.7	4.5
Mn (mg/kg)	27.0	15.0	23.3

All the climatic parameters except Rainfall are mean over crop growing period, June to October. Rainfall is total rain received in the crop growing period. RH% (Relative humidity) was mean of RH taken during morning and afternoon during a day.

Table 3. Pooled ANOVA for grain Fe and Zn content across the locations

Source	d.f.	Fe		Zn	
		MSS	Variance (%)	MSS	Variance (%)
Genotypes (G)	40	3062.8**	58.3	990.2**	52.8
Environ-ments (E)	2	106.8**	0.1	4215.3**	11.2
Rep within Env	6	18.7		29.1**	
G X E	80	1043.8**	39.7	304.3**	32.5
Error	240	15.6		9.8	
Total	368	570.9		203.5	

**Significance at p<0.01

(Table 4). This is in confirmation with Amare et al. (2014), which means that the first two interaction components could elucidate the interaction variation sufficiently and AMMI2 model holds good (Gauch, 2013). AMMI1 biplot which was plotted between the mean and the first IPC of GEI (Fig. 1a and 1b) showed that 17 genotypes had above average performance while G40 and G25 (with IPC1 scores of 0.62 and –0.28 respectively) (IPC1 data not shown) were having highest mean grain Fe content (114.6 and 111.6 mg/kg, respectively) and were also nearly stable in expression. G24 being the most stable genotype with an IPC1 score nearing to zero (–0.02) and mean grain Fe content of 73.3 mg/kg, whereas G10 being the most unstable and specifically adapted genotype (IPC1 score of –5.17) having more mean Fe content (93.8 mg/kg) and at Dharwad it recorded grain Fe content of 125.9 mg/kg (range is from 70.6 to 125.9).

Regarding Zn content, G24 is the most stable (IPC1 score was 0.007) and having more Zn content in grains (66.4mg/kg). Among above average grain Zn content genotypes, G39, G13, G3 and G5 are more stable. G40 with highest grain Zn content (76.4 mg/

Table 4. ANOVA for AMMI2 model for grain Fe and Zn content

Source	d.f.	Fe			Zn		
		MSS	% GE explained	% cumulative	MSS	% GE explained	% cumulative
Total	368	570.9			203.5		
Treatments	122	1690.4**			593.3**		
Genotypes	40	3062.8**			990.2**		
Environments	2	106.8**			4215.3**		
G X E	80	1043.8**			304.3**		
IPCA 1	41	1055.5**	51.13	51.13	340.8**	57.39	57.39
IPCA 2	39	1031.5**	48.17	100.0	266.0**	42.61	100.0
Residual	0	0			0		
Error	240	15.6			9.8		

**Significance at p<0.01

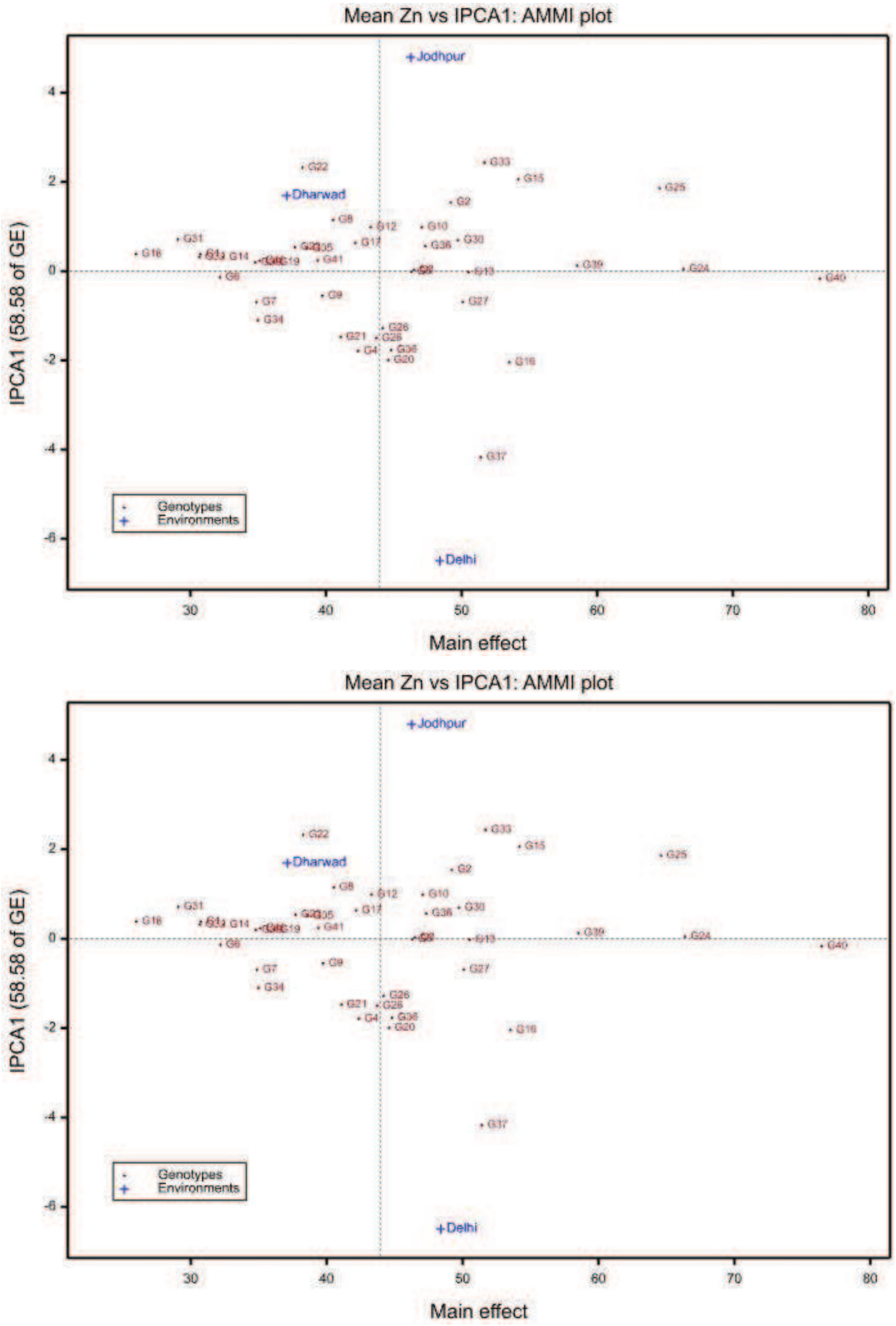

Fig. 1. AMMI1 biplot for (a) grain Fe content, (b) grain Zn content

kg) is relatively stable (with IPC1 score of –0.62) (IPC1 data not shown). G25 and G37 being unstable in grain Zn content across locations. Genotypes which have IPC1 scores near zero display little interaction across environments and are considered to be more stable. Genotypes which fall on right side of the ordinate are above average performers for the trait under consideration (Crossa et al. 1990). Rai et al. (2015b) reported that many high iron and zinc containing lines, including the check, Dhanshakti were derived from iniadi germplasm which is of African origin. In this study, G40 (PPMI 708) is best, both for Fe and Zn, considering the levels of micronutrient content in grain and stability of these micronutrient. One of the parents from which this inbred line is derived is from west African germplasm.

Generally, mega-environment delineation is studied for adaptive traits, but there are reports on mega-environment delineation based on grain micronutrients in pearl millet by Bashir et al. (2014) in three locations, among them, one location is repeated in two years, hence totally conducted in four environments, likewise Mallikarjuna et al. (2015) in maize studied at six locations. It is observed in most of the mega-environment delineation studies, more number of locations are included. Here, the number of locations taken are only three, but, they represent all the three pearl millet growing agro climatic zones of India. Even though the main objective of this study is identifying stable genotypes, mega-environment delineation was also studied to know how the three agro climatic zones were related in terms of grain micronutrient expression through GGE biplot analysis.

Which won where identification using GGE biplots

The most attractive feature of GGE biplot is to make out which genotypes ranked first at which environment. In this study 'which-won-where' biplots for grain Fe and Zn content showed 7 and 6 sectors giving rise to a heptagon (with seven genotypes at the vertices) and hexagon (with six genotypes at the vertices) (Fig. 2a & b) respectively.

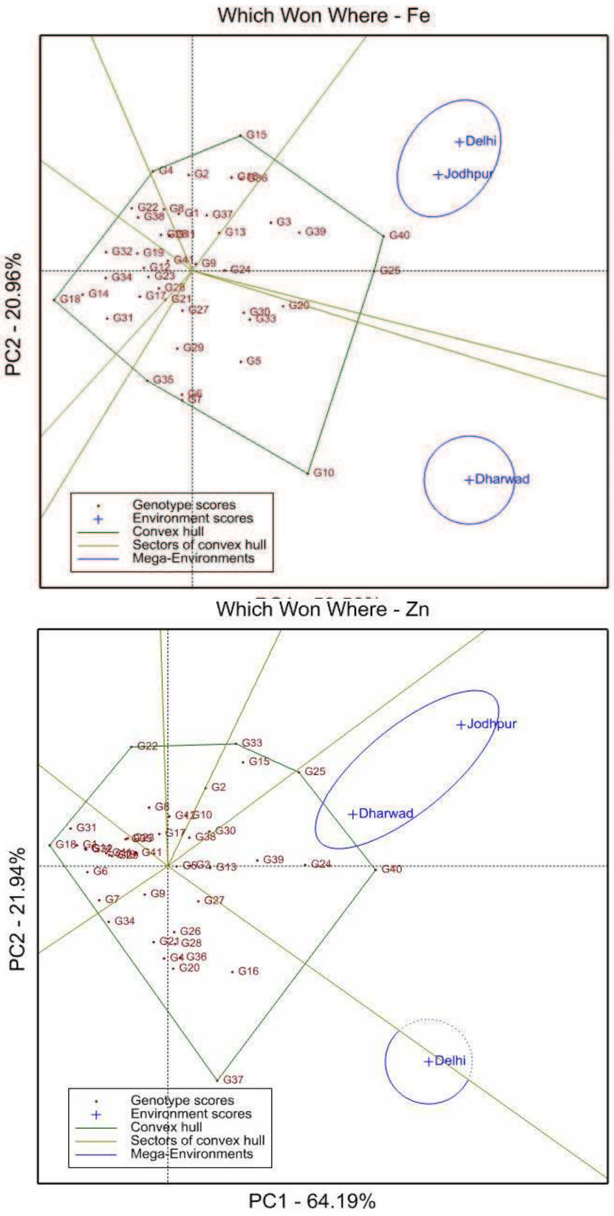

Fig. 2. **GGE Biplots for Mega environment delineation and Which-won-where pattern for (a) grain Fe content, (b) grain Zn content**

For both grain Fe and Zn contents, the three locations belonging to three different agro-climatic zones were now reduced to two mega-environments. Jodhpur and Delhi fell in one mega-environment for grain Fe content where G40 is winning genotype. In another mega-environment which included Dharwad only, G10 is the highest Fe containing genotype. For grain Zn content, Jodhpur and Dharwad fell under one mega-environment, G40 being the winning genotype and since Delhi fell in another sector, it alone forms another mega-environment where G37 ranked first for grain Zn content. As different genotypes won in different environments, cross over GEI exists between different genotypes across the locations for grain Fe and Zn content (similar results were reported in maize by Mallikarjuna et al. (2015) for kernel Fe and Zn content. As said above, locations taken here for evaluation were chosen such that they fell in different agro-climatic zones. This demarcation of geographical region is based on the rainfall they receive and other climatic conditions, but here those three different zones were grouped into two mega-environments. This seems to be based on their soil mineral content (Table 2). For grain Fe content, Dharwad which is having more soil Fe content compared to remaining two locations fell in a separate mega-environment and similar is the pattern for grain Zn content, Delhi having more Zn content fell in another mega-environment.

Association analysis

Association of mean grain micronutrient with soil micronutrient content of three locations (Table 5) revealed that, there is a non significant positive correlation between grain Zn and soil Zn content (0.945) and in case of Fe also, association is non-significant (–0.102). This implies that there is no significant role of soil micronutrient status to that of sequestration of micronutrients in grain. This can be due to different genotypes responding differently in the three locations and thus giving similar grand mean values of grain Fe content at all the three locations, though there is more difference in soil micronutrient content (Table 5). For instance, Dharwad soil Fe is more than double than

Table 5. Correlation between Fe and content of soil and grain Fe and Zn content

Location	Mean Fe	Content mg/kg)	Mean Zn	Content (mg/kg)
	Soil	Grain	Soil	Grain
Delhi	11	65.90	4.6	48.41
Jodphur	6.4	64.13	3.2	46.34
Dharwad	27.6	64.52	1.7	37.02
Overall	15	64.85	3.2	43.92
Correlation between soil and grain micronutrient content	-0.1022NS		0.9453NS	

NS: Non Significant

Table 6. Correlation between grain Fe and Zn content with agronomic traits

Trait	Zn	PH	PL	PD	NPT	GY	TSW	DFF
Fe	0.803**	–0.056	0.037	0.277	–0.235	–0.168	0.448**	0.055
Zn		0.003	–0.1	0.211	–0.19	–0.142	0.350*	0.082

Grain iron content in mg/kg (Fe); Grain zinc content in mg/kg (Zn); Plant height in cm (PH); Panicle length in cm (PL); Panicle Diameter in cm (PD); Number of productive tillers per plant (NPT); Grain yield per plant in g (GY); Thousand seed weight in g (TSW); Days to 50% flowering (DFF); **significance at $p < 0.01$ and *$p < 0.05$

Delhi and more than four times that of Jodhpur, but the mean values of grain Fe content were similar. Similarly, for Zn content, Dharwad is lesser by 1.88 times than Jodhpur, and by 2.7 times to Delhi, but the fall in grain Zn content did not follow that trend. Another reason for non significance is because, all the locations included in the study have micronutrient content above the lower critical limit (4.5 mg/kg for Fe and for 0.6 mg/kg for Zn as given by Alloway 2008). Similar results were obtained by Zhao et al. (2013), Vragolovic et al. (2007), where they did not observe any association between grain Fe and Zn content with soil Fe and Zn content in wheat and maize crop respectively.

The Pearson correlation coefficient among the trait means showed that, association was very high ($r = 0.8$, $p < 0.01$) between grain Fe and Zn content (Table 6). Earlier reports on pearl millet also showed similar relationship (Govindaraj et al. 2016; Kanatti et al. 2014, 2016), as they may share some common physiological process, right from uptake of micronutrients from soil to finally sequestration in to the grains. Previous study in pearl millet by Kumar et al. (2010), reported two co-mapped QTLs for both of these traits. Similarly in other crops like Rice (Anuradha et al. 2012) and in chickpea (Upadhyaya et al. 2016) co-located QTLs were reported. Increased accumulation of Fe and Zn in roots, shoots and mature seeds were observed in transgenic rice, by over expressing *OsIRT1*gene (Lee et al. 2009). Hence, simultaneous selection for both high Fe and Zn is possible. Both of the micronutrients exhibited significant positive association with thousand seed weight (0.448, $p < 0.01$ and 0.35, $p < 0.05$ for Fe and Zn respectively) while association with other traits, including grain yield was non-significant. Similar results were obtained on pearl millet studies (Kanatti et al. 2014; Velu et al. 2008a&b). This indicated that high grain Fe and Zn containing genotypes can be easily combined with higher grain weight without compromising for grain yield and other important agronomic traits. The study demonstrated the usefulness of both AMMI and GGE biplot analysis in identifying stable genotypes with higher levels of micronutrient. It also revealed that there is much potential for pearl millet breeding towards developing more micronutrient content in grains. There is scope to increase both grain Fe and Zn content along with grain weight without compromising for grain yield in pearl millet.

Authors' contribution

Conceptualization of research (CTS, CB, NA); Designing of the experiments (NA, CTS, SMS); Contribution of experimental materials (CTS, SPS, SMS); Execution of field/lab experiments and data collection (NA, JB, SPS, MCM); Analysis of data and interpretation (NA, CTS, SPS, OS); Preparation of manuscript (NA, CTS, SPS).

Declaration

The authors declare no conflict of interest.

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