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Genotype × environment interactions for grain micronutrient contents in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract

Assessment of the stability of grain iron (Fe) and zinc (Zn) content across growing regions is a pre-requisite in breeding for micronutrient enrichment in grains. Grain Fe and Zn contents were estimated in a set of 68 pearl millet genotypes grown at four environments. The Fe and Zn in grain ranged from 37.73-111.35 mg/kg and 26.48-111.35 mg/kg, respectively, across the environments. Significant genotype × environment (G × E) interactions were observed for both grain Fe and Zn, indicating differential nutrient accumulation by the genotypes. Additive main effects and multiplicative interaction (AMMI) analysis indicated that the first two principal components were significant and contributed more than 75% of G × E sum of squares. As the PCA I contributed more in G X E interaction, than other PCA, for both Fe and Zn, were pooled into residual, thus AMMI model with PCA I axis was accepted. Though environment (linear) variance was significant indicating linear sensitivity, significant pooled deviation for both the traits suggested that expression in some of the genotypes fluctuated significantly from their respective linear path of response to environments. None of parents or hybrids was found consistently stable for all the characters in any environment. The four promising cross combinations which had average stability for both micronutrient can be recommended for more rigorous testing in an array of environments to assess their stability or they may be used for generating new inbreds or varieties with wider adaptability for micronutrient, yield and yield contributing characters over location. The stable parents S-12/30088 could be used as sources for further genetic improvement of micronutrients. Environmental specific crosses may also be identified with high heterotic and sca effects, and available gene pool may be strengthened with the inclusion of more charge source of parental genotypes.

Keywords: grain Fe, grain Zn, pearl millet, genotypes, AMMI, G x E interaction

Introduction

The WHO has estimated that over 3 billion people in the world suffer from micronutrient malnutrition and that about 2 billion people of these have a Fe deficiency (Govindaraj *et al*., 2011) [4]. In Asia about 35 % of children between 0-5 years of age suffer from Fe and Zn deficiency. It affects large segment of population mostly women, infants and children in resource poor families in the country (Singh *et al*., 2009) [10]. Prevalence of anemia in pregnant women is highest (87.5%) in India. It is a highly nutritious cereal with high levels of metabolizable energy, protein and more balanced amino acid profile (Andrews and Kumar, 1992) [1]. The levels of Fe and Zn are far higher than those reported in improved wheat and maize varieties (Rai *et al*., 2008) [9]. Large genetic variation has been found for Fe and Zn and other minerals in pearl millet (Jambunathan and Subramanian, 1988) [6]. It is thought that increasing Fe and Zn concentrations in these crops could increase the dietary intake of Fe and Zn. Crop biofortification is a sustainable and cost-effective approach to address micronutrient malnutrition, especially in the developing world (Stein *et al*., 2007; Bouis *et al*., 2011) [11, 2]. It has the potential to help alleviate the suffering, death, disability, and failures to achieve human potential, which result from micronutrient deficiency-related diseases. Keeping these problems in the view, development of genotypes with high micronutrient is, vital to address human health problems.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major warm-season cereal grown on more than 27 million ha in some of the harshest environments in the arid and semi-arid tropical regions of Africa (17 million ha) and Asia (10 million ha), with India being the largest producer, cultivating this crop on about 7.95 million ha and production of 8.79 million tones with national average productivity of 1106 kg/ha. Grain is always the principal object of cultivation. The nutritive value of pearl millet is higher than rice, sorghum, and wheat (Uprety and Austin, 1972) [12].

Among the major cereal producing regions, per capita consumption is highest (92 kg/year) in the rural population in the western region of Rajasthan, followed by dry areas of Gujrat. The other major pearl millet consuming regions are inland central Maharashtra, western Maharashtra, Saurashtra, the northern plains of Gujrat and northeastern Rajasthan. In these regions, pearl millet contributes to about 20-40 % of the total energy and protein intake. Its contribution of micronutrient especially Fe and Zn is higher, varying from 30 to 50 % of the intake of these micronutrients from cereals (Parthasarathy *et al*., 2006) [8]. Pearl millet grain provides a low-cost solution to combating malnutrition due to micronutrient deficiency. Also it has additional health related advantages because of its higher levels of insoluble dietary fiber and more balanced amino acid profile. Thus, pearl millet with enhanced nutritional quality could contribute significantly to improving the nutritional value of the diets of people depend on improving the nutritional value of the diets of people dependent on pearl millet as a major energy source. Apart from this, the knowledge on stability of the trait across growing conditions or seasons is another important pre-requisite, though not given adequate attention in most of the breeding programmes beforehand. Assessment of the stability of expression of grain micronutrients is important for effective utilization of identified genotypes in breeding programmes. Hence, an attempt was made to study the stability of grain Fe and Zn contents in sixty eight pearl millet genotypes across four environments.

Materials and Methods

The experimental material for the present study comprised of 11 inbred lines obtained from Bajra Research Scheme, College of Agriculture, Dhule and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana. These inbreds were crossed in diallel fashion to obtain F_1's excluding reciprocals. Sufficient quantity of seed for 55 cross combinations was obtained.

These fifty five crosses along with eleven inbreds and two checks were used to study stability over four environments *viz.*, E_1: *Kharif*-2013, Dhule, E_2: *Kharif*-2013, Rahuri, E_3: Summer-2014, Dhule and E_4: Summer-2014, Rahuri. The 68 genotypes were raised in a randomized block design with three replications and two rows (of 3.0m length) plots per replication. Standard agronomic practices were followed for raising and maintenance of plants.

Representative soil samples were collected from each plot at 15 cm depth before sowing and at harvest of crop. The collected soil samples were air dried in shade on paper sheet, gently ground in wooden mortar and pestle, mixed and sieved through 2 mm plastic sieve. The processed soil samples were used for determination of Fe and Zn on Automic Absorption Spectrophotometer at Micronutrient laboratory, MPKV, Rahuri by DTPA (Diethylene triamine penta acetic acid) extraction method (Lindsay and Norvell, 1978) [7]. The data on available soil Fe and Zn content (mg/ kg) (DTPA extractable) in the experimental fields were given in Table 2. Representative grain samples from the middle of the ear head of the plants from net plot area were collected at harvest stage. The grains were manually cleaned and care was taken to avoid any contamination of the grains with dust particles and any other extraneous matter. The samples were dried in an oven at 70^0C, ground to fine powder using sample mill consisting of hard plastic and used for micronutrient analysis. A known quantity (0.2 g) of grain samples powder were digested following wet digestion of the dried grain sample material with triacid mixture HNO_3:$HClO_4$:H2SO$_4$ in the ratio of 9:3:1 and this acid extract was used for determination of Fe and Zn on Automic Absorption Spectrophotometer (Lindsay and Norvell, 1978) [7].

Additive Main effects and Multiplicative Interaction (AMMI) and stability analyses based on the Eberhart and Russell, (1966) [3] model were undertaken using INDOSTAT statistical package at Mahatma Phule Krishi Vidyapeeth, Rahuri.

Results and Discussion

The Fe and Zn in grain ranged from 37.73-111.35 mg/kg and 26.48-111.35 mg/kg, respectively, across the environments. Among the environments, the overall mean grain Fe content was the highest in samples from *Kharif*-2013, Rahuri (E_2) (67.82 mg/kg) and the least was from Summer-2014, Dhule (E_3), (62.17 mg/kg). The coefficient of variation (C.V.) for grain Fe was high in samples from Summer-2014, Dhule (E_3) (5.07%) followed by *Kharif*-2013, Rahuri (E_2) and *Kharif*-2013, Dhule (E_1), while it was around 3.48 % in Summer-2014, Rahuri (E_4) (Table 1). The environmental index (deviation of site mean from general mean) was positive for *Kharif*-2013, Dhule (E_1), and *Kharif*-2013, Rahuri (E_2), while for rest of the environments it was negative indicating the unfavourable environment for accumulation of grain Fe. In case of grain Zn, the highest mean value was 45.94 mg/kg (*Kharif*-2013, Rahuri (E_2)) and least was 41.01 mg/kg (Summer-2014, Rahuri (E_4)). The variation within the locations was higher for grain Zn compared to Fe content and the C.V. was high (3.29-7.64%). Environmental index was positive for *Kharif*-2013, Dhule (E_1), and *Kharif*-2013, Rahuri (E_2), indicating better overall grain Zn status or favourable environment in these two environments (Table 1).

Analyses of variance revealed significant variation for grain Fe and Zn at all the environments (Table 2). Presence of significant variation for grain Fe and Zn in pearl millet has been reported (Velu, 2006; Hariprasanna *et al*., 2012) [13, 5] and different ranges of values have been presented for both grain Fe and Zn in these studies. The status of soil Fe and Zn in the test environments is given in Table 2. Among the four environments, *Kharif*-2013, Rahuri (E_2) had high soil Fe while *Kharif*-2013, Dhule (E_1) had high soil Zn content.

The parent S-12/30060 produced maximum grain Fe in E_1 (115.24), E_3 (104.78), E_4 (111.28) and pooled over environments (107.12), while in E_2 parent S-12/30074 produced the maximum grain Fe. Among, the hybrids, the mean Fe values ranged from 31.64 (DHLBI 967 x S-12/30071) to 105.37 (RHRBI 138x S-12/30088) in E_1, 36.47 (DHLBI 731 x S-12/30069) to 116.34 (S-12/30074 x S-12/30088) in E_2, 31.44 (DHLBI 731 x S-12/30069) to 109.30 (S-12/30060 x S-12/30088) in E_3, 33.34 (S-12/30069 x S-12/30071) in E_4 and 37.73 (DHLBI 731 x S-12/30071) to 111.35 (S-12/30060 x S-12/30074) in pooled over environments.

The parent S-12/30069 produced maximum value for grain Zn in E_1 (63.33), E_2 (59.00), E_3 (65.00) and pooled over environments (64.00); while in E_4 parent S-12/30088 produced maximum grain Zn. Among hybrids, the mean values ranged from 45.33 (DHLBI 967 x ICMB 98222) to 61.33 (S-12/30060 x S-12/30074) in E_1, 45.33 (RHRBI 138 x S-12/30088) to 58.67 (ICMB 98222 x S-12/30060) in E_2, 47.67 (RHRBI 138 x RHRBI 458) to 66.00 (DHLBI 731 x S-12/30109) in E_3, 56.67 (RHRBI 458 x ICMB 98222) to 72.00 (S-12/30069 x S-12/30109) in E_4 and 51.17 (RHRBI 138 x RHRBI 458) to 62.17 (DHLBI 731 x S-12/30060) in pooled over environments. The differential response of the genotypes over the environments necessitated the genotype × environment (G × E) interaction study. We did not observe higher mean soil micronutrient status (Table 2).

As the G × E interaction was significant for both the grain Fe and Zn concentrations, AMMI analysis (Zobel *et al*., 1988) [15] was undertaken for estimation of variance components. In case of grain Fe, the first two principal component axes (PCA I and PCA II) were significant at P = 0.01 and 0.05 level, respectively (Table 3), and cumulatively contributed to more than 75% (48.22% and 31.15%) of interaction sum of squares. Similarly, the first two component axes captured nearly 81.9% of interaction sum of squares for grain Zn, with PCA I accounting for 46.81% and PCA II for 35.09% of sum of squares. The mean squares for the PCA 1 and PCA II were significant at P = 0.01 level (Table 3). As the PCA I contributed more in G X E interaction, than other PCA, for both Fe and Zn, were pooled into residual. Thus, AMMI model with PCA I axis was accepted for further study.

The result of the AMMI analysis can also be easily comprehended with the help of AMMI bioplot as represented in Fig. 1 for grain Fe and in Fig. 2 for grain Zn. The mean performance PCA I score for both the genotypes and environment used to construct bioplot are represented in Table 4. The main effects (genotype means and environment mean) are shown along abscissa (X-axis) and the ordinate (Y - axis) represent the first PCA. Both the main effects and interaction component are very clearly depicted in the figures.

The stability analysis following Eberhart and Russell model (Eberhart and Russell, 1966) [3] undertaken to study the behaviour of these traits indicated that the mean sum of squares due to genotype and environments were significant for both characters studied. The values of genotype x environment (G x E) and environment + (genotype x environment) [E+ (G x E)] were also significant for grain Fe and Zn. Similar trend was observed in respect of environment (Linear) and G x E (Linear) which were found to be significant (Table 3).

The significant environment (linear) variance implies that the variation among environments was linear, which signify unit changes in environmental index for each unit change in the environmental conditions. Highly significant values of mean squares due to environments (linear) for all the characters indicated that the linear responses of genotypes to environment differed significantly for the said characters. However, significant pooled deviation for both the traits suggests importance of non-linear component in the manifestation of G × E interaction, or in other words, expression of some of the genotypes fluctuated significantly from their respective linear path of response to environments. This may also be due to the difference in the soil micronutrient status in the test environments, though no significant association between soil Fe and Zn status and overall environment mean was observed. More elaborate experiments may be necessary to understand the differential response of the genotypes for grain Fe and Zn content. This genotype × environment interaction can be better understood by studying the performance of genotypes in multiple environments as well as at different growing seasons. Though G × E interactions for grain Fe and Zn contents appear to play an important role, it is possible to identify the genotypes with stable micronutrient concentrations across environments, and thus it could be feasible to combine high micronutrient traits with high grain yield (Velu *et al*., 2011) [14]. G x E interaction significant and linear component was higher in the present study for grain Zn. However, Velu, 2006 [13] reported non linear component higher than linear component for grain Zn.

The results revealed that, out of 68 genotypes, 31 had higher mean than population mean and 31 genotypes showed non-significant deviation from linear regression. Among parental genotypes 3 had non-significant deviation from liner regression and higher mean than parental mean (64.98 mg/kg), however among hybrids and checks 14 genotypes exhibited non-significant deviation from linear regression with higher mean than hybrid mean (64.57 mg/kg).

The results revealed that among the parental genotypes, inbred RHRBI 458 and S-12/30088 had average stability, as high mean, bi, S^2di non-significant and PCA I near to zero (Mean>parental mean, bi=1.00 ns, S^2di=0 ns and PCA I near to 0), while parent ICMB 98222 was grouped as below average stable and suitable for favourable environment as (Mean>population mean, bi>1.00 and significant and S^2di=0 ns). Among crosses, 10 cross combinations and check Dhanshakti (Mean>population mean, bi=1.00 ns and S^2di=0 ns) exhibited higher mean and non-significant bi, S^2di and PCA I value nearer to zero, which revealed that these ten crosses would have wide adaptation over a range of environment for grain Fe, while cross DHLBI 967 x S-12/30060 (P_3 x P_9) was adapted to poor environment showing above average stability. However, four crosses were adapted to better environment showing below average stability as having high mean, significant and bi higher than unity. Out of ten stable cross combinations, two had one of the parents RHRBI 458 and four have common parent S-12/30088 which is grouped as stable parent for grain Fe (Table 4 and 5).

Three parents ICMB 98222, S-12/30060 and S-12/30088 had average stability for grain Zn. Out of, 68 genotypes, ten, one and one had average (Mean>population mean, bi=1.00 ns, S2di=0 ns and PCA I near to 0), above average (Mean>population mean, bi<1.00 and significant and S^2di=0 ns) and below average stability(Mean>population mean, bi>1.00 and significant and S^2di=0 ns) and check Dhanshakti had similar trend like grain Fe for Zn as it exhibited average stability and suitable for wide range of environment (Hariprasanna *et al*., 2012) [5].

Among the genotypes studied, parent and cross combinations identified as stable for average, favourable and poor environment for grain Fe and grain Zn are summarized in (Table 4 and 5). None of parents or hybrids was found consistently stable for all the characters in any environment. The persual of results for grain micronutrient indicated that parent S-12/30088 as well as cross combinations *viz*., RHRBI 138 x S-12/30060 (P_1 x P_9), ICMB 98222 x S-12/30060 (P_5 x P_9), S-12/30060 x S-12/30074 (P_9 x P_{10}), S-12/30060 x S-12/30088 (P_9 x P_{11}), S-12/30074 x S-12/30088 (P_{10} x P_{11}) and check variety Dhanshakti had average stability and wider adaptability; whereas, DHLBI 731 x ICMB 98222 was grouped as below average stable and suitable for favourable environments for both characters grain Fe and Zn.

These four promising cross combinations can be recommended for more rigorous testing of crosses in an array of environments to assess their stability or they may be used for generating new inbreds or varieties with wider adaptability for micronutrient, yield and yield contributing characters over location. The stable parents S-12/30088 could be used as sources for further genetic improvement of micronutrients. Environmental specific crosses may also be identified with high heterotic and sca effects, and available gene pool may be strengthened with the inclusion of more charge source of parental genotypes.

Table 1: Mean performance of parents and hybrids for grain Fe (mg/kg) and grain Zn (mg/kg) in *Kharif*-2013, Dhule (E_1), *Kharif*-2013, Rahuri (E_2), Summer-2014, Dhule (E_3), Summer-2014, Rahuri (E_4) environments and over seasons (Pooled).

	Cross/Parent	**Grain Fe (mg/kg)**					**Grain Zn (mg/kg)**				
SN	**Parents**	**E_1**	**E_2**	**E_3**	**E_4**	**Pooled**	**E_1**	**E_2**	**E_3**	**E_4**	**Pooled**
1	RHRBI 138	60.21	50.12	45.21	66.19	55.43	41.38	33.57	31.47	36.34	35.69
2	RHRBI 458	69.57	77.37**	61.00	57.11	66.26	47.66*	53.28*	43.61	38.21	45.69
3	DHLBI 967	37.14	49.67	44.28	42.31	43.35	28.81	33.24	28.84	25.35	29.06
4	DHLBI 731	48.17	38.24	32.61	51.67	42.67	31.25	26.34	31.07	34.27	30.73
5	ICMB 98222	91.24**	87.18**	71.44**	76.51**	81.59*	57.46**	65.47**	54.61**	49.91**	56.86**
6	S-12/30069	54.38	44.63	35.92	43.22	44.54	37.78	28.16	26.17	24.02	29.03
7	S-12/30109	41.51	46.51	51.73	37.94	44.42	35.00	30.07	29.05	21.54	28.92
8	S-12/30071	34.62	38.73	49.21	30.64	38.30	31.67	34.00	35.50	31.29	33.12
9	S-12/30060	115.24**	97.18**	104.78**	111.28**	107.12**	62.41**	63.68**	61.74**	56.36**	61.05**
10	S-12/30074	79.34**	119.34**	94.64**	104.37**	99.42**	54.34**	68.18**	63.08**	66.34**	62.99**
11	S-12/30088	97.26**	101.26**	87.55**	80.64**	91.68**	56.60**	70.61**	59.63**	51.38**	59.56**
	Parent Mean	66.24	68.20	61.67	63.81	64.98	44.03	46.05	42.25	39.55	42.97
	F_1s										
1	P_1 x P_2	70.91**	76.24**	68.04*	61.84	69.26	49.07**	47.61	46.51**	42.37	46.39
2	P_1 x P_3	48.32	50.97	46.50	48.40	48.55	39.51	29.13	26.15	27.14	30.48
3	P_1 x P_4	39.98	56.57	35.71	62.53	48.70	31.27	37.52	32.64	41.22	35.66
4	P_1 x P_5	73.51**	75.12**	60.27	68.74**	69.41	51.38**	52.24*	48.33**	47.38**	49.83
5	P_1 x P_6	67.12	47.21	47.82	53.08	53.81	45.72	29.37	35.21	29.17	34.87
6	P_1 x P_7	54.35	47.24	53.21	46.55	50.34	39.61	27.22	32.04	24.35	30.81
7	P_1 x P_8	51.67	43.61	33.76	55.21	46.06	41.37	24.28	25.16	32.00	30.70
8	P_1 x P_9	91.56**	81.60**	82.54**	88.00**	85.93**	49.66**	54.39**	52.17**	53.07**	52.32*
9	P_1 x P_{10}	81.29**	85.24**	73.14**	77.34**	79.25*	54.32**	56.17**	53.19**	56.21**	54.97**
10	P_1 x P_{11}	105.37**	101.54**	76.31**	83.44**	91.67**	59.74**	63.08**	49.00**	52.48**	56.08**
11	P_2 x P_3	67.84	75.59**	50.76	43.61	59.45	47.22	47.37	30.17	26.52	37.82
12	P_2 x P_4	64.53	47.85	54.91	45.72	53.25	42.31	32.32	45.05*	30.14	37.46
13	P_2 x P_5	86.33**	87.28**	72.33**	75.31**	80.31*	53.53**	60.88**	55.33**	57.25**	56.75**
14	P_2 x P_6	50.73	45.49	44.50	45.20	46.48	40.41	33.64	33.21	29.50	34.19
15	P_2 x P_7	42.29	61.34	57.22	54.22	53.77	33.62	43.26	32.50	33.37	35.69
16	P_2 x P_8	60.41	52.94	60.34	39.64	53.33	43.74	38.50	41.64	36.22	40.03
17	P_2 x P_9	76.49**	88.11**	93.47**	84.77**	85.71**	54.68**	61.53**	63.75**	57.86**	59.46**
18	P_2 x P_{10}	82.64**	69.30	65.91	88.31**	76.54	46.37	52.35*	46.51**	55.61**	50.21
19	P_2 x P_{11}	82.31**	94.84**	84.43**	80.13**	85.43**	54.53**	58.88**	55.33**	58.45**	56.80**
20	P_3 x P_4	48.78	48.64	36.84	47.22	45.37	34.88	30.28	30.28	26.56	30.50
21	P_3 x P_5	54.67	76.32**	46.27	50.06	56.83	37.00	47.11	31.44	31.05	36.65
22	P_3 x P_6	38.64	50.71	33.07	38.17	40.15	27.11	29.04	28.15	26.77	27.77
23	P_3 x P_7	42.38	39.62	47.24	43.38	43.16	30.17	28.44	25.87	26.14	27.66
24	P_3 x P_8	31.64	50.81	41.64	35.26	39.84	28.39	36.31	22.08	23.95	27.68
25	P_3 x P_9	76.10**	65.61	82.33**	71.02**	73.77	50.14**	45.07	47.33**	44.72**	46.82
26	P_3 x P_{10}	69.89**	77.35**	69.17**	73.64**	72.51	42.36	53.19*	48.05**	55.19**	49.70
27	P_3 x P_{11}	64.04	64.95	66.20	62.56	64.44	40.45	48.67	34.00	40.21	40.83

Table 1: contd….

		Grain Fe (mg/kg)					**Grain Zn (mg/kg)**				
SN	**F_1s**	**E_1**	**E_2**	**E_3**	**E_4**	**Pooled**	**E_1**	**E_2**	**E_3**	**E_4**	**Pooled**
28	P_4x P_5	72.15**	77.00**	59.61	62.47	67.81	48.53*	54.75**	47.84**	39.35	47.62
29	P_4x P_6	58.58	36.47	31.44	52.34	44.71	39.28	23.30	23.13	31.11	29.21
30	P_4 x P_7	39.85	48.61	47.97	36.89	43.33	25.00	29.33	27.03	24.57	26.48
31	P_4 x P_8	37.29	41.81	32.31	39.51	37.73	32.61	32.67	26.16	28.53	29.99
32	P_4 x P_9	58.74	53.27	78.06**	94.77**	71.21	41.29	41.00	53.22**	56.24**	47.94
33	P_4 x P_{10}	79.56**	75.61**	64.15	69.34**	72.17	52.08**	53.28*	46.24**	48.43**	50.01
34	P_4 x P_{11}	54.39	69.30	68.27*	52.41	61.09	34.22	55.22**	51.47**	37.00	44.48
35	P_5 x P_6	48.33	51.91	47.19	52.95	50.10	44.37	43.06	32.55	33.33	38.33
36	P_5 x P_7	71.49**	70.81	51.21	48.68	60.55	49.07**	47.60	42.04	30.38	42.27
37	P_5 x P_8	67.89	50.34	53.64	49.00	55.22	48.66*	41.28	41.63	40.12	42.92
38	P_5 x P_9	100.21**	96.19**	92.98**	94.51**	95.97**	62.31**	64.32**	63.65**	61.28**	62.89**
39	P_5 x P_{10}	96.84**	79.10**	71.62**	96.06**	85.91**	63.24**	61.27**	56.31**	63.09**	60.98**
40	P_5 x P_{11}	95.39**	104.15**	72.47**	70.17**	85.55**	51.00**	71.45**	55.60**	52.55**	57.65**
41	P_6 x P_7	36.48	41.24	46.00	43.29	41.75	28.63	28.39	26.56	24.24	26.96
42	P_6 x P_8	49.87	43.00	40.72	33.34	41.73	36.02	30.22	27.70	27.00	30.24
43	P_6 x P_9	55.28	76.37**	66.34	80.81**	69.70	43.11	51.00	47.54**	42.47	46.03
44	P_6 x P_{10}	51.29	56.12	49.06	61.54	54.50	42.09	40.63	37.87	41.07	40.42
45	P_6 x P_{11}	59.71	62.41	62.64	62.92	61.92	45.38	48.37	44.35	43.51*	45.40
46	P_7 x P_8	39.20	41.34	53.07	36.52	42.53	30.50	30.28	31.20	23.18	28.79
47	P_7 x P_9	65.94	89.39**	84.79**	85.34**	81.37**	45.25	53.69**	53.17**	49.26**	50.34

48	P_7 x P_{10}	52.34	98.61**	81.72**	61.28	73.49	43.64	51.05	45.00*	37.09	44.20
49	P_7 x P_{11}	68.17	59.27	61.53	66.08	63.76	48.77*	43.51	41.03	41.12	43.61
50	P_8 x P_9	51.64	84.67**	82.04**	80.55**	74.73	41.00	53.18*	53.08**	51.54**	49.70
51	P_8 x P_{10}	74.53**	87.34**	62.64	62.31	71.71	51.82**	57.84**	40.39	45.00**	48.76
52	P_8 x P_{11}	60.18	56.09	64.07	51.34	57.92	40.28	44.55	44.11	37.22	41.54
53	P_9 x P_{10}	102.61**	111.19**	108.54**	123.04**	111.35**	64.69**	68.72**	73.64**	71.05**	69.53**
54	P_9 x P_{11}	96.28**	86.17**	109.30**	95.22**	96.74**	57.05**	65.38**	64.95**	58.59**	61.49**
55	P_{10} x P_{11}	101.30**	116.34**	97.37**	110.43**	106.36**	60.37**	72.17**	66.09**	68.11**	66.69**
Hybrid Mean		64.90	67.75	62.27	63.57	64.62	44.34	45.92	42.45	41.30	43.50
General Mean		65.12	67.82	62.17	63.61	64.68	44.29	45.94	42.42	41.01	43.41
Range Parents		34.62-115.24	38.24-119.34	32.61-104.78	30.64-111.28	38.30-107.12	28.81-62.41	26.34-70.61	26.17-63.03	21.54-66.34	28.92-62.99
Range Hybrids		31.64-105.37	36.47-116.34	31.44-109.30	33.34-123.04	37.73-111.35	25.00-64.69	23.30-72.17	22.08-73.64	23.18-71.05	26.48-69.53
S.E.±		1.33	1.44	1.82	1.28	4.53	1.20	2.03	0.82	0.78	2.46
CD at 5%		3.71	4.04	5.09	3.58	12.63	3.36	5.67	2.29	2.18	6.85
CD at 1%		4.91	5.34	6.72	4.73	16.66	4.44	7.49	3.03	2.88	9.03
CV %		3.53	3.69	5.07	3.48	14.01	4.70	7.64	3.35	3.29	11.31
Environmental index		0.377	2.886	-2.329	-0.934	--	0.8	2.569	-1.028	-2.342	--

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

Table 2: ANOVA for grain Fe and Zn content and soil micronutrient status in the test environments.

Sr. No.	**Characters**	**Mean sum of squares**		**Soil Fe (mg/kg)**	**Soil Zn (mg/kg)**
		Grain Fe	**Grain Zn**		
1.	*Kharif*-2013, Dhule (E_1)	1255.51**	303.24**	5.65	0.74
2.	*Kharif*-2013, Rahuri (E_2)	1450.56**	570.25**	5.98	0.73
3.	Summer-2014, Dhule (E_3)	1192.54**	503.92**	4.83	0.66
4.	Summer-2014, Rahuri (E_4)	1393.03**	520.73**	5.06	0.63

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

Table 3: Analysis of variance and variance components for grain Fe and Zn content in pearl millet (based on AMMI & Eberhart and Russell model).

Source	**DF**	**Grain Fe (mg/kg)**	**Grain Zn (mg/kg)**
Rep. within Env.	8	2.83	3.28*
Genotypes	67	1522.04**	561.53**
Environments	3	357.47**	312.42**
Geno. x Env.	201	80.61**	23.73**
PCA I	69	113.24**	32.35**
PCA II	67	75.33**	24.98**
PCA III	65	40.91**	10.22**
Residual	63	10.85**	3.16**
Env. + (Geno. x Env.)	204	84.68**	27.97**
Environments (Lin.)	1	1072.40**	937.27**
Geno. x Env. (Lin.)	67	130.52**	39.70**
Pooled deviation	136	54.84**	15.51**
Pooled error	536	2.22	1.67
Total	271	440.04	159.89

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

Table 4: Stability parameters for grain Fe (mg/kg) and grain Zn (mg/kg).

			Grain Fe (mg/kg)				**Grain Zn (mg/kg)**			
S. No	**Parents / Crosses**	**Code**	**Mean**	**b_i**	**S^2d_i**	**PCA I**	**Mean**	**b_i**	**S^2d_i**	**PCA I**
1	RHRBI 138	P_1	55.43	0.02	113.34**	-1.27	35.69	0.14	25.72**	-0.88
2	RHRBI 458	P_2	66.26	1.21	0.96	0.19	45.69	2.75*	-1.00	-0.06
3	DHLBI 967	P_3	43.35	1.00	10.61*	0.91	29.06	1.41	0.25	0.2
4	DHLBI 731	P_4	42.67	0.29	93.11**	-1.26	30.73	-1.42**	0.60	-0.13
5	ICMB 98222	P_5	81.59	3.26**	10.77	-0.91	56.86	2.99	0.68	0.19
6	S-12/30069	P_6	44.54	1.71	42.49**	-1.21	29.03	1.21	39.90**	-1.34
7	S-12/30109	P_7	44.42	-0.41	30.60**	0.69	28.92	1.51	22.07**	-0.88
8	S-12/30071	P_8	38.30	-1.25	61.95**	0.82	33.12	0.21	3.94*	0.3
9	S-12/30060	P_9	107.12	-0.80*	7.19*	-1.43	61.05	1.32	2.01	-0.09
10	S-12/30074	P_{10}	99.42	3.82**	293.16**	2.58	62.99	-0.11	54.72**	1.56
11	S-12/30088	P_{11}	91.68	3.36	11.05	-0.03	59.56	2.27	2.38	0.81
	Parental Mean		64.98				42.97			
12	RHRBI 138 x RHRBI 458	P1 x P2	69.26	2.07	2.11	0.24	46.39	1.06	7.03*	-0.34
13	RHRBI 138 x DHLBI 967	P1 x P3	48.55	0.80	-0.94	0.04	30.48	1.16	45.62**	-1.39

14	RHRBI 138 x DHLBI 731	P1 x P4	48.70	2.49	160.94**	0.5	35.66	-0.63	26.97**	0.81
15	RHRBI 138 x ICMB 98222	P1 x P5	69.41	2.70	5.64*	-0.43	49.83	1.07	-5.31*	-0.12
16	RHRBI 138 x S-12/30069	P1 x P6	53.81	0.04	106.25**	-1.79	34.87	0.55	86.53**	-1.8
17	RHRBI 138 x S-12/30109	P1 x P7	50.34	-0.72	2.06	-0.4	30.81	0.85	60.14**	-1.43
18	RHRBI 138 x S-12/30071	P1 x P8	46.56	0.90	125.77**	-1.25	30.70	-0.39	91.01**	-1.78
19	RHRBI 138 x S-12/30060	P1 x P9	85.93	-0.30	3.17	-0.92	52.32	0.12	2.90	0.53
20	RHRBI 138 x S-12/30074	P1 x P10	79.25	2.31**	-1.09	-0.1	54.97	0.11	1.51	0.2
21	RHRBI 138 x S-12/30088	P1 x P11	91.67	5.22	70.72**	-1.2	56.08	2.66	12.40**	-0.32
22	RHRBI 458 x DHLBI 967	P2 x P3	59.45	5.74	65.01**	-0.04	37.82	4.82**	21.33**	-1.17
23	RHRBI 458 x DHLBI 731	P2 x P4	53.25	-0.55	86.38**	-1.17	37.46	0.00	78.87**	-0.87
24	RHRBI 458 x ICMB 98222	P2 x P5	80.31	3.09*	1.39	-0.43	56.75	0.60	2.64	0.68
25	RHRBI 458 x S-12/30069	P2 x P6	46.48	0.31	1.23	-0.53	34.19	1.13	20.50**	-0.94
26	RHRBI 458 x S-12/30109	P2 x P7	53.77	0.61	75.52**	1.52	35.69	1.92	11.39**	0.59
27	RHRBI 458 x S-12/30071	P2 x P8	53.33	-0.14	121.01**	-0.25	40.03	0.46	3.47	-0.57
28	RHRBI 458 x S-12/30060	P2 x P9	85.71	-0.93	47.24**	1.22	59.46	0.06	22.33**	0.86
29	RHRBI 458 x S-12/30074	P2 x P10	76.54	-0.33	147.49**	-1.52	50.21	-0.47	27.90**	0.74
30	RHRBI 458 x S-12/30088	P2 x P11	85.90	2.13	1.91	0.78	56.80	0.09	7.44*	0.48
31	DHLBI 967 x DHLBI 731	P3 x P4	45.37	1.96	1.87	-0.51	30.50	0.90	10.14**	-0.64
32	DHLBI 967 x ICMB 98222	P3 x P5	56.83	5.85**	1.62	0.9	36.65	3.31**	6.81**	0.28
33	DHLBI 967 x S-12/30069	P3 x P6	40.15	3.27**	0.91	0.43	27.77	0.34**	-0.89	0.19
34	DHLBI 967 x S-12/30109	P3 x P7	43.16	-1.37**	-1.22	-0.01	27.66	0.68	1.30	-0.31
35	DHLBI 967 x S-12/30071	P3 x P8	39.84	1.94	55.81**	1.41	27.68	2.70*	8.14**	0.18
36	DHLBI 967 x S-12/30060	P3 x P9	73.77	-2.71**	1.98	-0.33	46.82	0.19	2.45	-0.51

Table 4: contd….

			Grain Fe (mg/kg)				**Grain Zn (mg/kg)**			
SN	**Parents / Crosses**	**Code**	**Mean**	**b_i**	**S^2d_i**	**PCA I**	**Mean**	**b_i**	**S^2d_i**	**PCA I**
37	DHLBI 967 **x** S-12/30074	P_3 x P_{10}	72.51	1.33	0.92	0.32	49.70	-0.59	45.41**	1.32
38	DHLBI 967 **x** S-12/30088	P_3 x P_{11}	64.44	-0.07*	1.18	0.12	40.83	2.05	23.55**	0.34
39	DHLBI 731 **x** ICMB 98222	P_4x P_5	67.81	3.54**	1.81	-0.17	47.62	2.77*	2.45	0.09
40	DHLBI 731 **x** S-12/30069	P_4x P_6	44.98	0.29	222.98**	-2.31	29.21	-0.39	85.73**	-1.67
41	DHLBI 731 **x** S-12/30109	P_4 x P_7	43.33	0.62	26.37*	0.81	26.48	0.73	1.78	0.36
42	DHLBI 731 **x** S-12/30071	P_4 x P_8	37.73	1.53	1.15	0	29.99	1.19	4.05*	-0.28
43	DHLBI 731 **x** S-12/30060	P_4 x P_9	71.71	-6.52	252.00**	0.07	47.94	-3.48**	9.25**	0.92
44	DHLBI 731 **x** S-12/30074	P_4 x P_{10}	72.17	2.31	8.78*	-0.77	50.01	1.28	2.87	-0.18
45	DHLBI 731 **x** S-12/30088	P_4 x P_{11}	61.09	0.85	101.97**	1.38	44.48	2.16	128.97**	1.93
46	ICMB 98222 **x** S-12/30069	P_5 x P_6	50.10	0.59	0.64	0.07	38.33	2.51	13.42**	-0.76
47	ICMB 98222 **x** S-12/30109	P_5 x P_7	60.55	4.51	54.16**	-0.66	42.27	3.43	25.46**	-0.89
48	ICMB 98222 **x** S-12/30071	P_5 x P_8	55.22	0.01	90.46**	-1.41	42.92	0.60	18.40**	-0.89
49	ICMB 98222 **x** S-12/30060	P_5 x P_9	95.97	0.72	1.47	-0.49	62.89	0.45	-0.31	0.18
50	ICMB 98222 **x** S-12/30074	P_5 x P_{10}	85.91	0.56	211.91**	-2.04	60.98	0.18	13.82**	-0.28
51	ICMB 98222 **x** S-12/30088	P_5 x P_{11}	85.55	3.92	7.13	-0.21	57.65	3.21	59.91**	1.47
52	S-12/30069 **x** S-12/30109	P_6 x P_7	41.75	-1.00	1.52	0.55	26.96	0.86	-0.54	-0.17
53	S-12/30069 **x** S-12/30071	P_6 x P_8	41.73	1.15	37.64**	-0.62	30.24	1.07	15.57**	-0.85
54	S-12/30069 **x** S-12/30060	P_6 x P_9	69.70	0.76	166.98**	1.34	46.03	1.25	11.64**	0.64
55	S-12/30069 **x** S-12/30074	P_6 x P_{10}	54.50	0.65	22.65*	0.04	40.42	0.22	2.86	-0.19
56	S-12/30069 **x** S-12/30088	P_6 x P_{11}	61.92	-0.14*	0.89	0.2	45.40	0.95	-1.19	0.14
57	S-12/30109 **x** S-12/30071	P_7 x P_8	42.53	-1.60	38.66**	0.67	28.79	1.15	10.40**	-0.18
58	S-12/30109 **x** S-12/30060	P_7 x P_9	81.87	0.26	151.60**	1.85	50.34	0.30	20.83**	0.94
59	S-12/30109 **x** S-12/30074	P_7 x P_{10}	73.49	3.84	506.36**	3.66	44.20	2.39	8.06**	0.32
60	S-12/30109 **x** S-12/30088	P_7 x P_{11}	63.76	-0.56	2.37	-0.78	43.61	0.90	12.52**	-0.75
61	S-12/30071 **x** S-12/30060	P_8 x P_9	74.73	-0.17	337.13**	2.76	49.70	-0.42	48.39**	1.52
62	S-12/30071 **x** S-12/30074	P_8 x P_{10}	71.71	5.15**	1.31	0.33	48.76	3.13	18.65**	-0.17
63	S-12/30071 **x** S-12/30088	P_8 x P_{11}	57.92	-0.85	15.11*	-0.02	41.54	1.03	8.95**	0.33
64	S-12/30060 **x** S-12/30074	P_9 x P_{10}	111.35	-0.55	6.00	0.48	69.53	-1.00	2.1	0.79
65	S-12/30060 **x** S-12/30088	P_9 x P_{11}	96.74	-3.96**	1.38	-0.07	61.49	0.70	2.52	0.85
66	S-12/30074 **x** S-12/30088	P_{10} x P_{11}	106.36	2.98	3.35	0.56	66.69	0.46	3.03	1.2
67	Shanti (Check)		39.80	-0.98	23.64*	-0.92	29.87	1.65	14.13**	0.29
68	Dhanshakti (Check)		84.04	-0.75**	-1.06	0	56.66	0.71	3.80	0.61
	Hybrid with check Mean		64.57				43.49			

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

Table 5: Classification of pearl millet parents and hybrids based on their well adaptation in average, poor and better environments.

Characters	**Average stability and wide/ general adaptability**	**Above average stability and adapted to poor environment**	**Below average stability and adapted to better environment**
	Parents		
Grain Fe (mg/kg)	RHRBI 458(P_2), S-12/30088 (P_{11})	---	ICMB 98222(P_5)
Grain Zn (mg/kg)	ICMB 98222(P_5), S-12/30060(P_9), S-	---	RHRBI 458(P_2)

	12/30088(P_{11})		
	Hybrids		
Grain Fe (mg/kg)	RHRBI 138 x RHRBI 458(P_1xP_2), RHRBI 138 x S-12/30060(P_1xP_9), RHRBI 458 x S-12/30088(P_2xP_{11}), DHLBI 967 x S-12/30074(P_3xP_{10}), ICMB 98222 x S-12/30060(P_5xP_9), ICMB 98222 x S-12/30088(P_5xP_{11}), S-12/30060 x S-12/30074(P_9xP_{10}), S-12/30060 x S-12/30088(P_9xP_{11}), S-12/30074 x S-12/30088(P_{10}xP_{11}), Dhanshakti (Check)	DHLBI 967 x S-12/30060 (P_3xP_9)	RHRBI 138 x S-12/30074(P_1xP_{10}), RHRBI 458 x ICMB 98222(P_2xP_5), DHLBI 731 x ICMB 98222(P_4xP_5), S-12/30071 x S-12/30074(P_8xP_{10})
Grain Zn (mg/kg)	RHRBI 138 x S-12/30060(P_1xP_9), RHRBI 138 x S-12/30074(P_1xP_{10}), RHRBI 458 x ICMB 98222(P_2xP_5), DHLBI 967 x S-12/30060(P_3xP_9), DHLBI 731 x S-12/30074(P_4xP_{10}), ICMB 98222 x S-12/30060(P_5 xP_9), S-12/30060 x S-12/30074(P_9xP_{10}), S-12/30060 x S-12/30088(P_9xP_{11}), S-12/30074 x S-12/30088(P_{10} x P_{11}), Dhanshakti (Check)	S-12/30069 x S-12/30088(P_6xP_{11})	DHLBI 731 x ICMB 98222(P_4xP_5)

E_1: *Kharif*-2013, Dhule, E_2: *Kharif*-2013, Rahuri, E_3: Summer-2014, Dhule and E_4: Summer-2014, Rahuri.

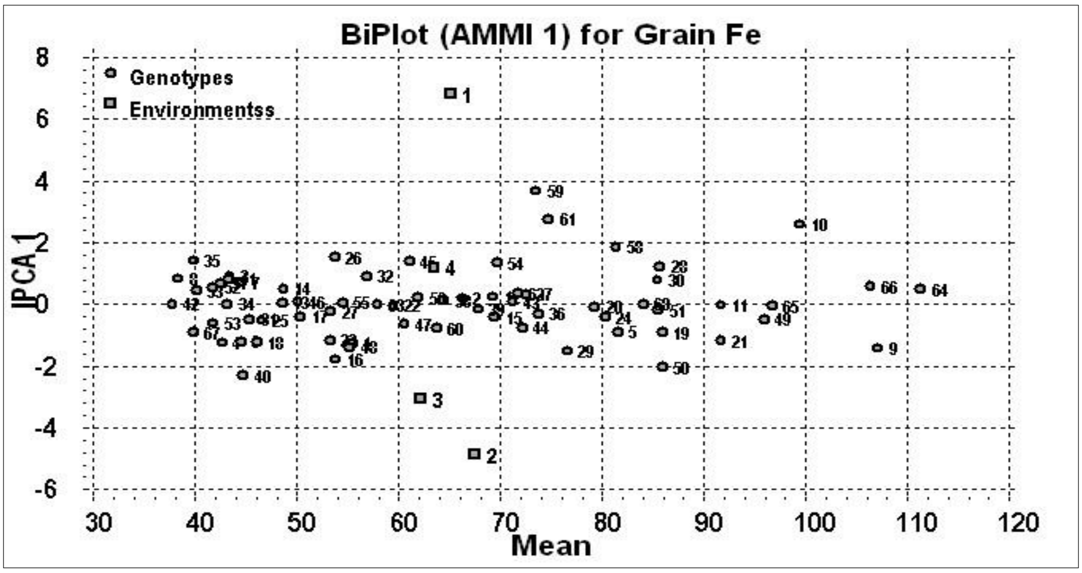

Fig 1: Bioplot of AMMI-1 model for the pearl millet for grain Fe (mg/kg) with sixty eight genotypes(•) and four environments (■). The vertical line represents the grand mean of the experiment and horizontal lines PCA-I.

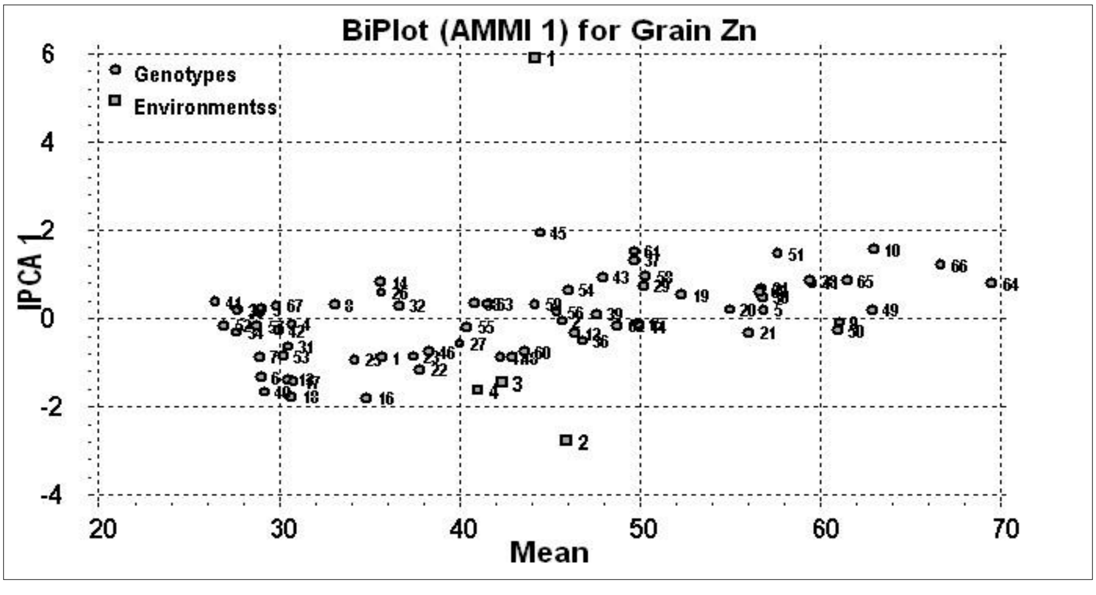

Fig 2: Bioplot of AMMI-1 model for the pearl millet for grain Zn (mg/kg) with sixty eight genotypes(•) and four environments (■). The vertical line represents the grand mean of the experiment and horizontal lines PCA-I.

Conclusion

To conclude, the G × E interactions play a significant role in the expression of grain Fe and Zn content in pearl millet. As grain pearl millet is grown in different soil types with varying levels of soil fertility and management in India, it is necessary to examine the stability of grain micronutrient content across different soil types and soil fertility. The environmental conditions, especially soil micronutrient status may affect the grain Fe and Zn content, but no significant association was observed, thus reiterating the differential response of genotypes with regards to Fe and Zn accumulation in the grains. Though linear response to environmental conditions was observed in some of the genotypes, nonlinear response was also equally evident in other genotypes, necessitating multi-location as well as multi-season evaluation of genotypes before identifying stable genotypes that can be used as donor parents in breeding for micronutrient enrichment in pearl millet. Proper understanding of micronutrient accumulation in the grains, genetic control and identification of genotypes that accumulate high Fe and Zn contents irrespective of growing conditions will pave the way for development of micronutrient rich hybrid and varieties to address the problem of micronutrient malnourishment among the target populations.

Authors’ contribution

Conceptualization of research (VYP, NSK, HTP, GPD); Designing of experiments(VYP, NSK, HTP, GPD); Contribution of experimental material (VYP, HTP, RKG); Execution of field/lab experiments and data collection (VYP, NMM, HTP, VRA, RKG, DGK); Analysis of data and interpretation (VYP, NMM); Preparation of manuscript (VYP, NSK).

Declaration

The authors declare no conflict of interest.

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