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Stability analysis of Grain Iron and Zinc content in Pearl millet (Pennisetum glaucum (L.) R. Br.)

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ABSTRACT: *Pearl millet (Pennisetum glaucum (L.) R. Br.) is a quick growing summer cereal crop which forms the staple food in arid and semi arid regions of Indian subcontinent and Africa. It is the fourth important staple food in India after rice, wheat and maize, and is nutritionally superior to the major cereals. Micronutrient malnutrition (Hidden hunger) especially for iron and zinc is a big threat to the world. Since, pearl millet possesses huge amount of variability for nutritional traits like grain iron and zinc than other cereals, efforts were initiated to biofortify this crop for increased levels of grain iron and zinc. Breeding programme aiming at developing pearl millet lines with high iron and zinc content was taken up at IARI, New Delhi. In the present study, promising pearl millet lines with high iron and zinc content were investigated for the stability over location. Twelve promising lines with high iron and zinc content were tested for stability in iron and zinc content over 8 locations representing different pearl millet growing zones of the country. It was observed that the pearl millet genotypes PPMI 903, PPMI 904 and PPMI 906 showed better mean performance for iron and zinc content with moderate stability across locations in the AMMI analysis.*

Keywords: *AMMI analysis, Fe and Zn, GE interaction, Pearl millet*

INTRODUCTION

'Hidden hunger', is a term more often used to describe malnutrition due to micronutrient deficiencies in staple food diet caused due to non/poor availability of minerals in diet. Malnutrition hinders the development of human potential and the nation's social and economic development especially women and pre-school children [1]. Globally about 11% of all deaths under the age of five are attributable to micronutrient deficiencies [2]. Fe and Zn deficiency are the two most widespread nutritional disorders. It is estimated that two billion of the world's population are Fe deficient, with consequent diminished work performance, impaired body temperature regulation, impaired psychomotor development and intellectual performance, detrimental behavioral changes (e.g. significantly decreased responsiveness and activity, and increased body tension and fearfulness), decreased resistance to infection and increased susceptibility to Pb poisoning [3]. Women and children are particularly at risk of Fe deficiency because of their elevated requirements for child-bearing and growth respectively. An estimated 58% of the pregnant women in developing countries are anaemic, and their infants are more likely to be born with a low birth weight [4]. Zn deficiency, thought to be widespread, can expresses it's symptom as hypogonadism, dwarfism, heptosplenomegaly, anaemia and geophagia and mortality during childhood if it is prolonged [5]. Furthermore, Zn deficiency in man has been linked to Vitamin A underutilization. Even in the developed countries, micronutrient deficiencies affect a significant number of the population. Taken together, micronutrient deficiencies affect a far greater number of the world' population than protein-energy malnutrition [6].

Pearl millet (*Pennisetum glaucum*) is one of the staple cereal food and fodder crop for 90 million resource poor people, grown especially in arid and semi-arid regions of Asia and Sub-Saharan Africa

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covering an area of more than 29 m.ha where micronutrient deficiencies are particularly concentrated [7]. Prevalence of iron deficiency anaemia (IDA) is still wide spread among 34% of adolescent girls of Bikaner, Rajasthan and Gujarat where millet as their major food crop [8]. Human nutritionists have focused on supplementation, fortification and dietary diversification to address micronutrient deficiencies [9]. Fortified food and food supplements do not reach all those affected in the developing countries because of weak market infrastructure and also because these products have high recurring costs. Biofortification approach involves enchancing the levels of specific, limiting micronutrients in edible tissues of crops by combining crop management, breeding, and genetic approaches [10]. Micronutrient-enriched or biofortified pearl millet would not only serve as the logical vehicle for providing Fe and Zn in the diets of the people but also shall be a cost-effective and sustainable approach to alleviate micronutrient deficiencies.

Development of micronutrient-enriched genotypes would produce more micronutrient yield in a micronutrient-deficient soil than by a micronutrient inefficient variety. Hence assessment of the stability of genotypes under different environment with respect to target traits is important for effective utilization of such genotypes in breeding strategies. In India, pearl millet is grown under varied edaphic and environmental conditions and they are known to exhibit high degree of genotype and environmental interactions. Generally, Indian soils are reported to have deficiency of about 1 to 35% and 12 to 87% Fe and Zn respectively [11]. An understanding of the causes of genotype x environment interaction can help in identifying traits and environments for better genotype evaluation and those lines suitable for cultivation or further improvement through breeding. Genotypes that show low G x E interaction have high stable grain micronutrient yields and are desirable for plant breeders and farmers, as it shows the lesser effect of environment on the performance of genotypes and their yields are largely due to their genetic composition. Therefore, evaluation of newly developed pearl millet genotypes for high yield and stability in grain micronutrient content over a wide range of environments will remain an important criterion in pearl millet breeding [12].

There are several models like ANOVA (analysis of variance), PCA (principal component analysis) and linear regression (LR) which can be used to estimate the stability of genotypes, each having its own advantage and disadvantages. The main lacuna of above methods was it ignores the interaction component as noise and it will consider with the residuals. AMMI analysis provides a graphical representation (biplot) to summarise information on the main effects and interaction of the both genotypes and environment simultaneously. AMMI model is a hybrid model which involves both additive and multiplicative component of the two way data structure. The AMMI model separates the additive variance from the multiplicative variance and then applies principal component analysis (PCA) to the interaction portion to extract a new set of coordinate axes which explain more the interaction component through least square principle [13]. The utility of AMMI in stability analysis was well established by Zobel [14] and Crossa [15] in maize using multilocation trial data.

The present study attempts to analyse the stability of newly developed inbred lines, derived from a RIL population for mapping high grain iron and zinc content across pearl millet growing regions of India using AMMI model.

MATERIALS AND METHODS

Experimental Material

The experimental material for the present study comprises of 12 elite genotypes, among which 9 genotypes (PPMI 901, PPMI 902, PPMI 903, PPMI 904, PPMI 905, PPMI 906, PPMI 907, PPMI 908and PPMI 909) were selections from a RIL mapping population (PPMI 683 X PPMI 627) for mapping grain iron and zinc content; selected based on their superior agronomic performances and two genotypes (841B and D 23) developed at IARI, New Delhi and a check variety (ICTP 8302 Fe) developed at ICRISAT, India centre. These genotypes were analysed for grain Fe and Zn concentrations over 8 diverse environments during kharif, 2014 (rainy) season.

Field Trials

The test environments were chosen to represent environments typically rainfed regions, were pearl millet is grown as primary staple food crop during kharif season. The location details, climatic patterns and soil types were presented in Table 1. Soil Fe and Zn concentration at the experimental sites was estimated using standard procedures. The entries were planted in Randomized Complete Block Design (RCBD) with three replications per entry (4 rows per replication) with plant to plant spacing of 15cm and

Table 1
Geographical location, climatic and edaphic factors present for each location is given

Locations	*Dharwad*	*New Delhi*	*Tabiji*	*Karnal*	*Shikopur*	*Ludhiana*	*Jodhpur*	*Pune*
Geographical Identity								
Latitude	15°212 N	28°382 N	26°222 N	29°752 N	28°372 N	30°562 N	26°252 N	18°552 N
Longitude	75°052 E	77°802 E	74°352 E	76°982 E	76°982 E	75°482 E	72°992 E	73°802 E
Altitude	750 m	219 m	444 m	254 m	255 m	247 m	233 m	571 m
Climatic Factors								
Temp (Max.)	30.7°C	33.8°C	32.6°C	35.1°C	33.4°C	34.7°C	34.4°C	29.8°C
Temp (Min.)	18.7°C	25.0°C	24.0°C	24.8°C	25.0°C	25.6°C	25.0°C	21.2°C
RH (%)	68.6	69.1	64.7	68.5	71.3	73.6	77.7	74.6
Rainfall (mm)	761	482	536	491	400	440	389	623.6
Soil Factors								
Soil PH	7.3	7.8	8.03	7.5	8.4	7.8	8.2	7.9
Texture	clay loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy loam

row to row spacing of 75 cm. Five random plants from each plot were handled following the procedure suggested by Harvest Plus [16].

Grain Micronutrient Analysis

The grain samples collected from the open pollinated panicles harvested at physiological maturity were threshed manually using wooden mallot were analyzed at ICRISAT using an energy-dispersive X-ray fluorescence Spectrometry (EDXRF) method that had been standardized at the Flinders University, Australia.[17]

Statistical Analysis

In multi-environmental trials, two most commonly used statistical methods are additive main effect and multiplicative (AMMI) interaction model proposed and used by Gauch [18] and Zobel [14] and the AMMI model is:

$$Y_{ij}=\mu+g_i+e_j+\Sigma h_k\alpha_{ik}+\tau_{jk}+R_{ij},$$

Where Yij is the grain yield of the i-th genotype in the j-th environment, μ is the grand mean, g_i and e_j are the genotype and environment deviation from the grand mean, respectively, h_k is the eigenvalue of the PCA axis k, α_{ik} and τ_{jk} are the genotype and environment principal component scores for axis k, N is the number of principal components retained in the model, and R_{ij} is the residual term. The interaction, GEI sum of squares was sub divided into PCA axes, where axes k is regarded as having t+s - 1-2k degrees of freedom and t and s are the no: of genotypes and environment respectively. The data were analysed using Indostat v.9.2 statistical package, a software developed by Indostat Services, Hyderabad, India.

RESULTS AND DISCUSSIONS

The mean data of grain Fe and Zn and grain yield for 12 pearl millet genotypes evaluated in 8 locations were given in Table 2. The means of the genotypes and the environments along with the first principal components scores corresponding the genotypes and the environments are also presented. The ranges for grain Fe content were 35.33- 98.17 ppm (mean 55.42 ppm). The genotype PPMI 904 topped 5 times over eight locations in having higher mean Fe content. The range for grain Zn content was 39.62-80.67 ppm (mean 54.33 ppm). The genotype PPMI 904 topped 6 times over eight locations. Similarly for grain yield, the range was 1158- 2066 Kg/ha with mean yield of 1332 Kg/ha. The genotype ICTP 8203Fe topped 6 times over eight locations for mean grain yield. The environmental index for Fe and Zn in grain and corresponding yield varied from location to location (data not presented). But there exists a strong correlation between grain Fe and Zn content over each location with overall correlation, r=0.74 ($p<0.01$), suggesting that the genes and pathways responsible for accumulation of grain Fe and Zn concentrations could be quite same, and genetic improvement for these two traits could be undertaken simultaneously. Thinh [19], Velu *et al.* [20] and Kanatti *et al.* [7] found significant and positive association between the grain Fe and Zn concentrations in pearl millet.

AMMI Analysis

Pooled ANOVA was carried out after analysing the homogeneity of error variance using Bartlett's test and the grain iron and zinc content and grain yield is presented in Table 3. There were significant differences ($p<0.01$) among the genotypes,

Table 2
Mean data for grain Fe & Zn(ppm) and grain yield (Kg/ha) of 8 pearl millet genotypes grown in 8 locations and the first PCA scores for the GEI effect as derived from AMMI analysis

Genotype	*Grain Iron content (ppm)*										*Grain Zinc content (ppm)*										*Grain Yield (Kg/ha)*									
	L1	*L2*	*L3*	*L4*	*L5*	*L6*	*L7*	*L8*	*Geno. Mean*	*PCA* **I**	*L1*	*L2*	*L3*	*L4*	*L5*	*L6*	*L7*	*L8*	*Geno. Mean*	*PCA* **I**	*L1*	*L2*	*L3*	*L4*	*L5*	*L6*	*L7*	*L8*	*Geno. Mean*	*PCA I*
PPMI 901	38.00	52.00	44.33	51.33	53.33	47.00	48.00	32.67	**45.83**	-0.148	42.67	50.00	64.33	36.00	40.00	45.33	35.67	38.33	**44.04**	-1.492	1751	1220	973	1238	1083	1233	967	1646	**1264**	-3.470
PPMI 902	37.67	46.33	42.00	52.67	46.00	31.33	40.00	31.67	**40.96**	-0.188	48.33	45.33	40.33	30.67	38.33	34.00	35.00	45.00	**39.62**	0.935	1778	1258	902	1274	1052	1260	906	1457	**1236**	-1.719
PPMI 903	67.67	98.00	65.00	92.00	91.00	67.00	85.33	65.33	**78.92**	-2.576	63.67	99.00	69.00	52.00	75.67	83.00	72.00	60.00	**71.79**	-0.771	1671	1187	910	1193	839	1188	856	1621	**1183**	-1.858
PPMI 904	93.00	126.7	78.00	111.0	111.0	73.67	105.00	87.00	**98.17**	-4.034	107.00	114.33	48.00	80.00	82.00	33.00	78.00	103.00	**80.67**	7.961	1635	1183	898	1228	838	1177	828	1474	**1158**	-2.217
PPMI 905	29.33	50.00	44.00	34.67	43.00	41.00	38.00	34.33	**39.29**	0.498	46.33	61.00	48.00	36.33	39.67	74.67	37.00	43.67	**48.33**	-2.049	1626	1423	1341	1341	825	1397	1169	1744	**1358**	-9.913
PPMI 906	74.33	94.00	60.33	97.00	66.00	65.00	66.00	87.67	**76.29**	-2.790	80.67	97.00	70.00	59.00	55.00	69.00	52.00	77.67	**70.04**	1.131	1629	1179	1303	1158	821	1182	800	1535	**1201**	-5.751
PPMI 907	29.67	57.00	46.00	55.00	39.67	39.67	34.00	24.00	**40.62**	0.416	46.00	64.00	56.00	46.00	36.33	57.00	33.33	43.00	**47.71**	-1.181	1741	1429	925	1212	924	1365	885	1793	**1284**	-0.524
PPMI 908	41.00	59.00	60.00	62.00	46.33	26.67	42.67	37.33	**46.88**	0.767	54.00	67.00	66.00	54.67	45.33	46.00	40.67	49.33	**52.88**	-0.117	1648	1201	1276	1344	844	1158	928	1486	**1236**	-5.754
PPMI 909	36.00	49.00	85.00	58.00	46.33	43.00	39.00	28.67	**48.13**	4.645	48.00	62.00	78.00	52.67	42.33	65.67	39.00	44.67	**54.04**	-2.787	1603	1405	907	1235	883	1486	989	1732	**1280**	-5.331
841B	32.00	45.00	42.00	38.67	38.67	27.00	33.00	26.33	**35.33**	0.476	42.00	46.00	50.67	38.00	39.67	42.00	37.00	39.33	**41.83**	-0.576	1999	1342	1178	1229	1073	1230	976	1633	**1332**	-0.498
D 23	33.00	48.00	69.00	46.00	39.67	28.67	34.33	27.67	**40.79**	3.121	46.00	44.33	50.00	39.00	41.00	37.33	36.00	41.00	**41.83**	-0.022	2133	1783	1030	1083	819	1236	986	2033	**1388**	6.788
ICTP 8203	47.00	97.00	76.00	95.67	83.33	74.33	77.00	40.67	**73.87**	-0.188	54.00	74.00	69.00	52.00	57.33	62.67	54.00	50.67	**59.21**	-1.033	3185	2246	1600	2534	1528	1843	1030	2563	**2066**	30.250
Loc. Mean	**46.56**	**68.50**	**59.31**	**66.17**	**58.69**	**47.03**	**53.53**	**43.61**	**55.42**	*	**56.56**	**68.67**	**59.11**	**48.03**	**49.39**	**54.14**	**45.81**	**52.97**	**54.33**	*	**1867**	**1405**	**1104**	**1339**	**961**	**1313**	**943**	**1726**	**1332**	*
PCA I	-0.531	-2.644	6.924	-1.129	-1.068	1.290	-1.369	-1.473			-0.676	-0.777	1.146	1.280	1.307	-1.786	-1.842	1.348			20.828	6.030	-10.401	10.244	-4.826	-7.374	-19.736	5.237		

Where,

*Overall mean, L1: IARI, RS, Dharwad; L2: IARI, New Delhi; L3: Tabiji; L4: IARI, RS, Karnal; L5 : KVK, Shikopur; L6 : Ludhiana; L7: NBPGR, RS, Jodhpur and L8: IARI, RS, Pune.

Table 3
Pooled AMMI analysis of variance for grain Fe and Zn and grain yield of 12 pearl millet genotypes grown across eight locations in India

Source of Variations	*df*	*Grain Iron Content (ppm)*			*Grain Zinc Content (ppm)*			*Grain Yield (Kg/ha)*		
		Sum of Squares	*Mean Squares*	*% of TSS*	*Sum of Squares*	*Mean Squares*	*% of TSS*	*Sum of Squares*	*Mean Squares*	*% of TSS*
Genotypes	11	37434.70	3403.15**	70.75	15884.90	1444.08**	52.17	5125344.00	465940.38**	30.02
Environments	7	7253.20	1036.17**	13.71	4464.39	637.77**	14.66	9457210.00	1351030.00**	55.40
G*E Interaction	77	7689.30	99.86**	14.53	9898.95	128.56**	32.51	2417560.25	31396.88**	14.16
PCA I	17	4011.05	235.94**	52.16	6909.33	406.43**	69.80	1387464.50	81615.55**	57.39
PCA II	15	1920.94	128.06**	24.98	1535.43	102.36**	15.51	497000.09	33133.34**	20.56
PCA III	13	844.97	65.00**	10.99	847.80	65.22**	8.57	286380.19	22029.25**	11.85
PCA IV	11	684.34	62.21**	8.90	452.79	41.16**	4.57	185942.38	16903.85**	7.70
PCA V	9	183.56	20.40**	2.39	150.04	16.67**	1.52	34409.41	3823.27**	1.42
PCA VI	7	42.93	6.13*	0.56	3.58	0.51	0.04	22374.77	3196.40**	0.93
Residual	5	1.52	0.30	0.02	-0.03	-0.01	0.000	3988.90	797.78	0.17
Pooled residual	60	3678.25	61.30**		9898.95	128.56**		1030095.75	17168.26**	
Error	192	533.56	2.78		198.44	1.03		71220.66	370.94	
Total	287	52910.75	184.36		30446.68	106.09		17071334.00	59482.00	

environments and G × E interactions (GEI). Significant G × E interactions explained 14.53%, 32.51% and 14.16% of total sum of squares for grain Fe, Zn content and grain yield respectively, which shows that although both the micronutrients are influenced by environments in which grain Zn is relatively more sensitive to environmental fluctuations than grain Fe. Genotypic contribution towards total sum of squares was 70.75 and 52.17% for Fe and Zn content and 30.02% for grain yield. Significant genotypic differences suggested that genes necessary for micronutrient enrichment along with yield traits are available within the pearl millet genome that could allow for substantial increases in grain Fe and Zn content by recombination and directional selection. However, the ranges and means of seed Fe and Zn concentration varied widely at different locations due to the differences attributable to genotypes, environments as well as G × E interactions.

The GEI which was highly significant was further partitioned into six PCA axes. In the AMMI analysis employing Gollob's test, first two PC explained 77.14%, 85.31% and 77.95% of the total G × E variation for grain Fe, Zn contents and grain yield respectively. The graphical method was employed by using two PCs to investigate environmental variation and interpret the G × E interaction for Fe (Fig. 1a), Zn (Fig. 2a) and grain yield (Fig.3a). Also, the AMMI biplot analysis between the mean and the first PCA of G x E interactions (Figs. 1b, 2b & 3b) indicated the distinct behaviour of the environment. The graphical representation of AMMI I analysis reveals the main effect (character means) on the abscissa and IPCA-1 scores of genotypes as well as the environments simultaneously on the ordinate. The genotypes fall close to the X-axis (zero value of the first PCA of G x E interaction) were the most stable genotypes across environments for that particular trait and when the genotype and environment have same sign on the PCA axis, then their interaction is positive, if different then interaction is negative.

The pearl millet genotypes closer to the origin of biplot were stable across the eight test locations. For grain Fe content, the genotypes PPMI 901, PPMI 902 and PPMI 907 were high and widely adapted for eight locations. Even though slightly unstable for grain Fe concentration, the genotype PPMI 904, PPMI 903 and PPMI 906 were high in mean Fe concentration but slightly deviated from the origin of biplot were identified as specifically adapted to favourable environments. Favourable locations for a particular genotypes means are those with high mean and high PCA 1 score with same sign for both the genotype and location indicating positive interaction. For instance Delhi, Karnal, Jodhpur and Shikopur were found favourable environment for PPMI 904, PPMI 903 and PPMI 906 (Fig. 1b). Among which, genotype PPMI 909 found to be more unstable among genotypes investigated, displayed more deviation from the origin of biplot.

Genotypes D 23, PPMI 908 and 841B were highly stable for grain Zn content and are generally adapted for eight locations. From Fig. 2a, it is clear that the genotypes PPMI 904, PPMI 903 and PPMI 906 are significantly superior to others in mean value and simultaneously had moderate G x E interaction due to being closer to X axis. The genotypes PPMI 903 and PPMI 906 responded well in locations IARI, New Delhi and KVK Shikopur (Fig.2b).

Genotypes 841B, PPMI 903 and PPMI 902 are found to be more stable genotypes in terms of grain yield across eight test locations. From Fig. 3a ICTP 8203 Fe found to have higher mean yield, but deviated significantly from the origin of biplot, which was found responding well in Dharwad, Delhi and Pune (Fig. 3b).

CONCLUSION

The study was aimed at selection of superior, stable genotype for grain micronutrient and yield as donor for trait introgression. PPMI 904, PPMI 903 and PPMI 906 were found to have high mean grain iron and zinc content with moderate stability. Hence these genotypes can be employed for further biofortification programme in pearl millet.

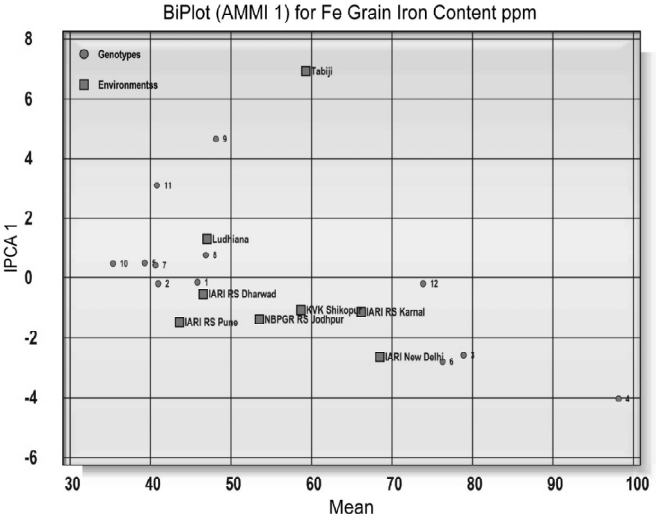

Figure 1a: AMMI (additive main effects and multiplicative interactions model) plots for grain Fe content between mean and first PC

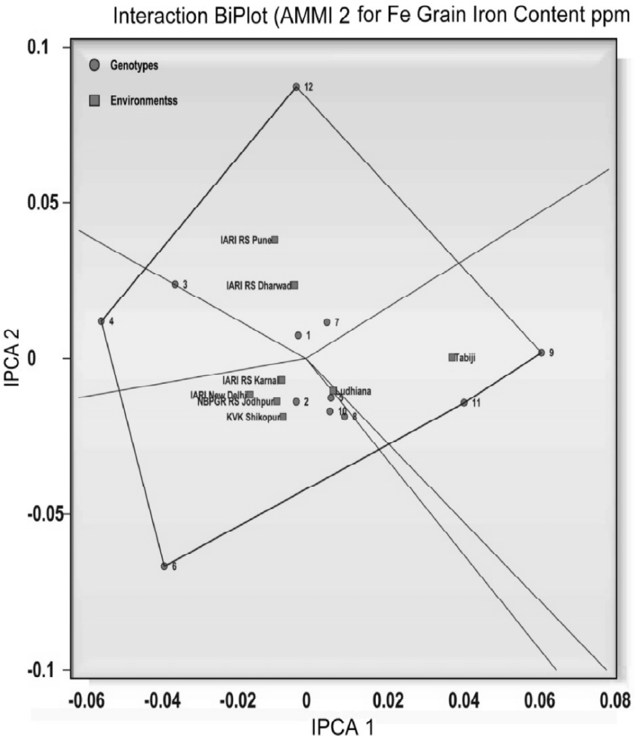

Figure 1b: AMMI (additive main effects and multiplicative interactions model) plots for grain Fe content between first two PCs

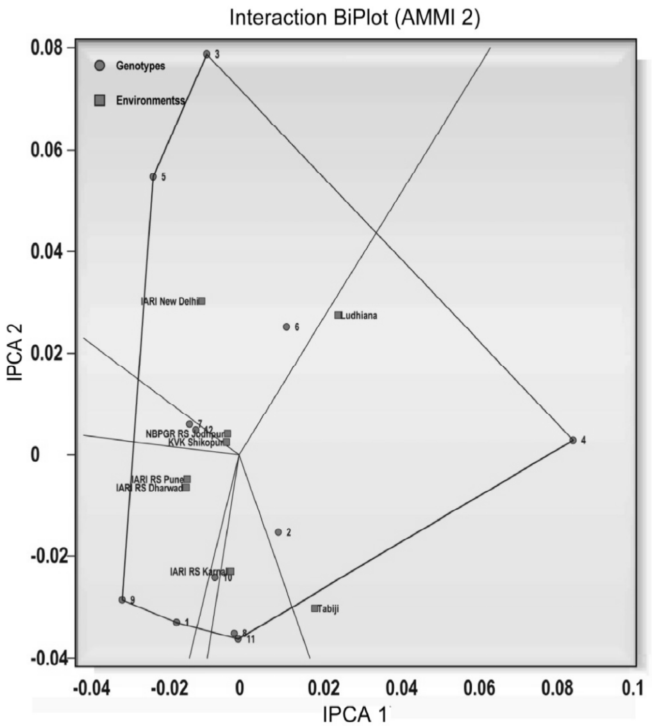

Figure 2b: AMMI (additive main effects and multiplicative interactions model) plots for grain Zn content between first two PCs

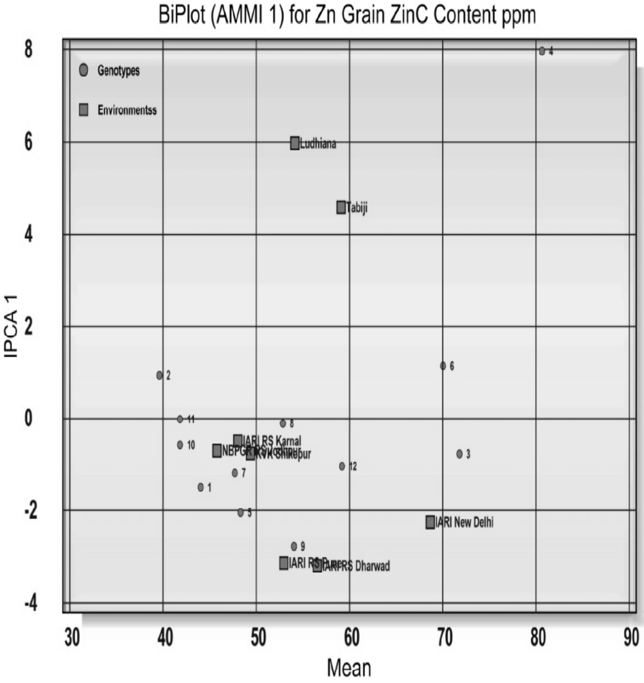

Figure 2a: AMMI (additive main effects and multiplicative interactions model) plots for grain Zn content between mean and first PC

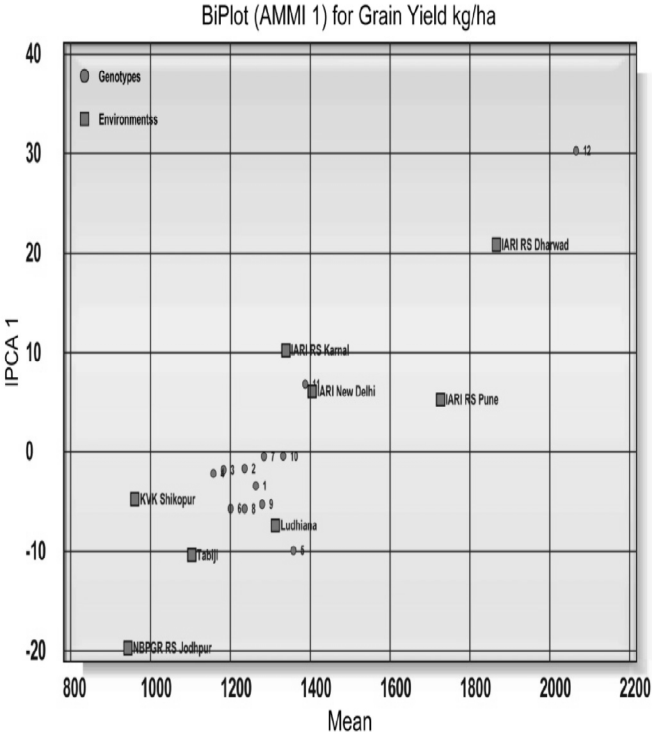

Figure 3a: AMMI (additive main effects and multiplicative interactions model) plots for grain yield /ha between mean and first PC

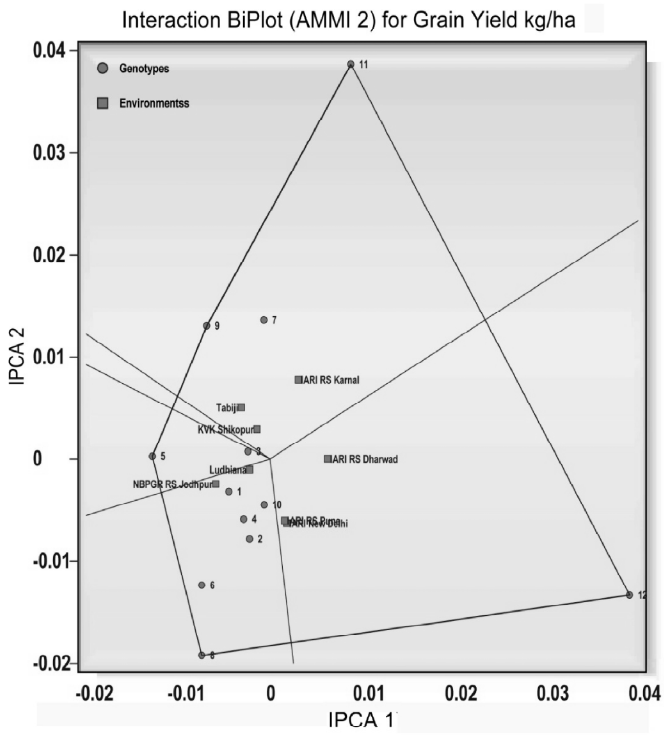

Figure 3b: AMMI (additive main effects and multiplicative interactions model) plots for grain yield/ha content between first two PCs

REFERENCE

Stein, A.J. (2010), Global impacts of human mineral malnutrition, *Plant Soil.* 335: 133–154.

Black, R.E., Allen, L.H., Bhutta, Z.A., Caulfield, L.E., de Onis, M. & Ezzati, M. (2008), Maternal and child undernutrition: global and regionalexposures and health consequences. *Lancet* 371: 243–260.

Khush, G.S. (2008), Biofortification of crops for reducing malnutrition. biofortification of crops for reducing malnutrition. *Proc. Indian Natn. Sci. Acad.* 74(1): 21-25.

Ma, G, Jin, Y., Piao, J., Kok, F., Guusje, B & Jacobsen, E. (2005), Phytate, calcium, iron, and zinc contents and their molar ratios in foods commonly consumed in China. *J. Agric. Food. Chem.* 53(26):10285-10290.

Ackland, M.L. & Michalczyk, A. (2006), Zinc deficiency and its inherited disorders – A review. *Genes & Nutrition.* 1(1): 41-50.

Robin, D., Graham., Ross, M. & Welch. (1996), Breeding for staple food crops with "high micro nutrient density. IFPRI, Washington, D.C. April.

Kanatti, A., Rai, K. N., Radhika, K., Govindaraj, M., Sahrawat, K. L., & Rao, A. S. (2014), Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. *SpringerPlus*, *3*(1), 763.

Shukla, A.K., Tiwari, P.K. & Prakash, C. (2014), Micronutrients Deficiencies vis-a-vis Food and Nutritional Security of India. *Indian J. Fert.* 10 (12): 94-112.

Prasanna, B.M., Mazumdar, S., Chakraborti, M., Hossain, F., Manjaiah, K.M., Agrawal, P.K., Guleria, S.K. & Gupta, H.S. (2011), Genetic variability and genotype × environment interactions for kernel iron and zinc concentrations in maize (*Zea mays*) genotypes. *Ind. J. Agric. Sci.* 81 (8): 704–711.

Hirschi, K.D. (2009), Nutrient Biofortification of Food Crops. *Annu. Rev. Nutr.* 29:401-421.

Singh, M. V. (2009), Micronutrient nutritional problems in soils of India and improvement for human and animal health. *Ind. J. Fertilisers*, *5*(4): 11-16.

Rai, K.N., Govindaraj,M. & Rao, A.S. (2012), Genetic enhancement of grain iron and zinc content in pearl millet. *Quality Assurance & Safety of Crops & Foods.* 4(3): 119-125.

Roostaei, M., Mohammadi, R. & Amri, A. (2014), Rank correlation among different statistical models in ranking of winter wheat genotypes. *The Crop Journal.* 2(2–3):154–163.

Zobel, R.W., Wright, M.J. & Gauch, H.G. (1988), Statistical analysis of a yield trial. *Agron. J.* 80: 388-393.

Crossa, J., Gauch, H.G. & Zobel, R.W. (1990), Additive main effect and multiplicative interaction analysis of two international maize cultivar trials. *Crop Sci.* 30: 493-500.

http://www.harvestplus.org/concentration/cropsampling-protocals-micronutrient-analysis.

Paltridge, N.G., Palmer, L.J., Milham, P.J., Guild, G.E. & Stangoulis, J.C.R. (2012), Energy dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grains. *Plant Soil. 361*(1-2): 251-260.

Gauch, Jr, H.G. (1992), *Statistical analysis of regional yield trials: AMMI analysis of factorial designs*. Elsevier Science Publishers.

Thinh, N.T. (2011), Assessment of diversity for morphological and nutritional quality traits in pearl millet [*Pennisetum glaucum* (L).R.Br.]. M.Sc. Dissertation. IARI, Division of Genetics, New Delhi.

Velu, G., Rai, K. N., Muralidharan, V., Kulkarni, V. N., Longvah, T., & Raveendran, T. S. (2007), Prospects of breeding biofortified pearl millet with high grain iron and zinc content. *Plant Breeding*, *126*(2): 182-185.