Indian J. Genet., 79(3) 552-562 (2019)
DOI: 10.31742/IJGPB.79.3.4

Identification of new stable and high iron rich fertility restorers in pearl millet

Tripti Singhal[3], C. Tara Satyavathi[1*], S. P. Singh, S. Mukesh Sankar, N. Anuradha[2], C. Bharadwaj, Aruna Kumar[3], M. Mallik and Nirupma Singh

Division of Genetics, ICAR-Indian Agriculture Research Institute, New Delhi 110 012; [1]ICAR-All India Coordinated Research Project on Pearl Millet, Jodhpur 342 304, Rajasthan; [2]Acharya NG. Ranga Agricultural University, Vizianagaram, Andhra Pradesh; [3]Amity Institute of Biotechnology, Amity University Campus, Sector 125, Noida 201 303, Uttar Pradesh

(Received: May 2019; Revised: July 2019; Accepted: August 2019)

Abstract

Biofortification of pearl millet (*Pennisetum glaucum* (L.) R. Br.) with improved iron (Fe) and zinc (Zn) will have great impact as it is an indispensable component of nutritional security of inhabitants of arid and semi-arid regions. Ten genotypes along with checks were evaluated in RBD in six locations during *kharif,* 2016 under rainfed conditions. Significant differences were observed in genotype, environment and genotype × environment interaction mean squares for grain Fe and Zn contents, indicating differential nutrient accumulation by the genotypes. The first two principal components obtained in AMMI analysis were significant and cumulatively explained the total variation were 81.47 % for Fe and 73.97 % for Zn. A positive and moderately high correlation (r=0.6) between Fe and Zn contents suggests good prospects of simultaneous improvement for both micronutrients. Among the ten genotypes, PPMI 953 was found to be more stable with high mean Fe (90 ppm) and Zn (59 ppm) contents. On crossing with designated A lines of pearl millet, the line PPMI 953 found to be restorer for A_1 system with complete fertility restoration of F_1 panicle of the cross, $ICMA_{(1)}$ 863 x PPMI 953 under bagged condition and resulting F_1 with 78-84% fertility measured by seed setting % under bag. The F_2 individuals showed 9:7 fertility-sterility ratio (χ^2 value=0.002, P value=0.964). The promising line, PPMI 953 may be used as source for further genetic improvement with respect to grain micronutrient content or can be directly used as male parent in development of high iron pearl millet hybrids.

Keywords: Pearl millet, iron, zinc, AMMI, fertility, restorer

Introduction

Hidden hunger or Micronutrient deficiency affects more than 2 billion people worldwide (FAO 2015). The most striking of these are Fe and Zn deficiencies that rank 9th and 11th, respectively, among the top 20 risk factors contributing to global burden of disease (Stein 2010). Deficiency of Fe in the diet leads to anaemia (Gregory et al. 2017), and also causes stunted growth, low birth weight and delayed mental development etc. (Singhal et al. 2018). Similarly, Deficiency of Zn in the diet can have symptoms of hypogonadism, dwarfism and geophagia, as well as mortality during childhood. Prolonged Zn deficiency leads to increased susceptibility to infectious diseases such as pneumonia and diarrhoea reduced physical performance and work productivity, and poor birth outcomes in pregnant women (Cakmak and Kutman 2018). Deficiencies of Fe and Zn have been estimated to reduce the Gross Domestic Product of developing countries by 2-5% (Kumssa et al. 2015). Biofortified pearl millet serves as the logical vehicle for providing Fe and Zn in the diets of the people to alleviate micronutrient deficiencies (Govindaraj et al. 2019a). Breeding of pearl millet varieties/hybrids with high yield and improved nutritional value is one of the priority areas for providing nutritional security in the developing world. Breeders have identifyied the available variability for the trait and utilising them in development of a handful of varieties and hybrids rich in micronutrients, which now playing a major role in nutritional security in dry lands (Govindaraj et al. 2019b). However, hybrid released so far in India is having as mean Fe content at par or less than HarvestPlus target level of 77 ppm

*Corresponding author's e-mail: csatyavathi@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

and hence there is a great need to make constant effort to breed more and more parental lines which can combine both for yield and micronutrient content along with resistance/tolerance to drought, downy mildew and blast diseases, good keeping quality etc. and showing a stable performance under different geographical locations.

Genotypes by environment interactions (G × E) are a major obstacle for developing and popularising micronutrient rich pearl millet hybrids over zone. Micronutrient status vary greatly in dry lands where pearl millet cultivation is concentrated. Under such conditions, G × E interaction for agronomic and grain nutrient traits is expected to be large and may not permit differentiation of performance of genotypes across environments (Satyavathi et al. 2015; Anuradha et al. 2017). Multivariate models such as additive main effects and multiplicative interaction (AMMI) model are the most widely used statistical models for the analysis of data from multi-environment testing of genotypes to understand G x E interactions (Gauch 2006). In addition, AMMI stability value (ASV), developed by Purchase et al. 2000 help us to quantify and rank the genotypes based on their yield stability, is established upon the first and the second IPC scores for each genotype. The AMMI model fits the sum of several multiplicative terms rather than only one multiplicative term in assessing the performance of genotypes in different environments (Bose et al. 2014). AMMI analysis can be used to determine stability of the genotypes across locations using the PCA (principal component axis) scores and AMMI stability value (ASV). Genotypes having the least ASV are considered as widely adapted genotypes. Similarly, IPCA2 score near zero indicates more stable genotypes whilst large values represent more responsive and less stable genotypes (Singhal et al. 2018). YSI and the GSI are based on the sum of the ranking due to ASV scores and yield or performance ranking. Lower Yield Stability Index (YSI) and Genotype Stability Index (GSI) values indicate genotypes that combine high performance with stability (Farshadfar et al. 2011).

In pearl millet, a variable number of fertility restorers categories are reported among which A_1, A_4 and A_5 systems are commonly employed (Amiribehzadi et al. 2012). In CGMS systems, restorer categories are routinely identified by test crossing prospective lines with available CMS lines and evaluating F_1s for pollen and spikelet fertility (Prasad et al. 1993). Pollen fertility or spikelet fertility or both have been used as an index to fix the restoration ability of the lines. Precise knowledge of fertility restorer category is an essential prerequisite for efficient heterosis breeding and a successful seed production programme. Biofortified pearl millet hybrid released so far in India is having as mean Fe content is at par or less than HarvestPlus target level of 77 ppm. In order to have a high Fe pearl millet hybrid, both the parent involved in a specific cross must possess higher level of Fe and Zn, as both traits are governed by additive gene effect (Kannati et al. 2014). So in order to utilise the promising line identified having high Fe (>90 ppm) and greater stability to a specific restorer category, the line were crossed with designated A lines in pearl millet and recorded the set % under baggage. Hence, the present study was focussed on to identify a stable genotype over the locations for grain micronutrient in a pearl millet in $F_{6:7}$ mapping population and to find out the restoration category and fertility restoration percentage of promising lines identified by AMMI analysis for using them in development pearl millet hybrids.

Materials and methods

Experimental material and field trials

The experimental material comprised of ten genotypes namely, (PPMI 951, PPMI 952, PPMI 953, PPMI 954, PPMI 955, PPMI 956, PPMI 957, PPMI 958, PPMI 959 and PPMI 960) which were selected from RIL mapping population derived from the cross, PPMI 683 × PPMI 627. The RILs were selected considering the superior agronomic performance with grain Fe and Zn contents. PPMI 683 possessed 90.8 ppm of Fe and 45.2 ppm Zn whereas PPMI 627 had lower concentration of Fe and Zn contents (60.7 and 35.21 ppm, respectively) under multi-environment trials conducted during 2014-2015 (Anuradha et al. 2017). These genotypes along with two checks, ICMB 98222 and Dhanshakti were evaluated in Randomised Block Design (RBD) for grain Fe and Zn contents over the six diverse environments at. Mandor (MDR), Jaipur (JPR), Hisar (HSR), Jamnagar (JMR), New Delhi (NDL) and Coimbatore (CBE) during *kharif,* 2016 under rainfed conditions.

The test environments were chosen to represent environments typically rainfed regions, where pearl millet is grown as primary staple food crop during *kharif* season (Table 1). Soil Fe and Zn content at the experimental sites was estimated using standard

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

Falpos	Locations					
	Mandor	Jaipur	Jamnagar	New Delhi	Hisar	Coimbatore
Geographical identity						
Latitude	26.3415° N	26.9124° N	22.4707° N	28.6139° N	29.1492° N	11.0168° N
Longitude	73.0463° E	75.7873° E	70.0577° E	77.2090° E	75.7217° E	76.9558° E
Altitude	383m	431m	27.6m	219m	215m	411m
Climatic factors						
Temp^{o}C (Max.)	35±0.6	32.4±2.7	30.6±1.8	33±8.8	34.3±2.3	32.1±0.7
Temp^{o}C (Min.)	24±7.6	24.4±1.4	25.2±1.1	22±.3.8	25.2±1.4	22.2±0.7
RH (%)	63.26	58.19	73.77	75.2	69.99	70.43
Rainfall(mm)	433	484	438	1146.7	452	153
Soil factors						
Soil pH	8.2	8.2	7.9	7.9	7.8	8.4
Soil texture	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam
Electrical conductivity (dSm^{-1})	0.9	1.45	0.43	0.42	0.10	0.14
Organic Matter (%)	0.27	1.84	0.32	0.52	0.61	0.33
Available Fe (mg kg^{-1})	2.27	4.64	7.00	4.52	5.5	4.26
Available Zn (mg kg^{-1})	0.35	0.40	0.65	0.94	0.6	0.82

procedures. The entries were planted in single row plots of 4 m length with two replications. Plant to plant spacing of 15 cm and row to row spacing of 75 cm was followed. Five random plants from each plot excluding the border plants were handled following standard procedure of HarvestPlus (Stangoulis and Sison 2008).

Grain micronutrient analysis

The grain samples collected from open pollinated panicles individually from each of the six sites, harvested at physiological maturity, threshed manually were analysed for grain Fe and Zn content at ICRISAT, Patancheru, using an Energy-Dispersive X-Ray Fluorescence (EDXRF) spectrometry method standardized at the flinders university, Australia (Paltridge et al. 2012). Procedure to estimate the contents was strictly followed to minimise the contamination from soil or other means by adopting Stangoulis et al. (2010).

AMMI stability analysis

After testing of error variance for homogeneity using Bartlett's homogeneity test, combined analysis of variance (ANOVA) was performed over locations. Stability analysis was performed using AMMI with the Genstat 17th Edition software (VSN International, Hemel Hempstead, UK). AMMI1 biplot using main effect means with first Interaction Principal Component Analysis (IPCA) score and AMMI2 biplot using IPCA1 & IPCA2 were drawn as described by Zobel et al. 1988. Quantitative estimation of stability, AMMI stability value (ASV) for each genotype according to the relative contributions of the principal component axis scores (IPCA1 and IPCA2) to the interaction sum of squares as described by Purchase et al. (2000) was calculated as follows:

$$ASV = \text{“} [((IPCA1_{\text{sum of squares}}/IPCA2_{\text{sum of squares}})(IPCA1_{\text{score}}))^2 + (IPCA2_{\text{score}})^2]$$

Where $IPCA1_{\text{sum of squares}}/IPCA2_{\text{sum of squares}}$ of squares is the weight given to IPCA 1. The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller ASV scores indicate a more stable genotype across environments.

In addition, the genotype selection index (GSI) for both Fe and Zn was calculated using the following formula:

GSI = RASV + R

where RASV is the ranking of the AMMI stability value and R is the ranking of Fe or Zn in all environments.

Fertility restoration studies

The promising lines having high mean Fe content (>90 ppm) and having stable Fe and Zn contents (ASV~±1.5) were chosen to identify the restorer class and per cent restoration in F_1 hybrid and F_2 population under baggage. The promising line was crossed as male parent with designated A-lines for three different sterile cytoplasmic systems in pearl millet (A_1, A_4 and A_5). Then the resulting F_1 and F_2 were evaluated for pollen fertility and per cent seed setting under baggage at three different locations (Jodhpur, Delhi and Dharwad) during *kharif,* 2018. At flowering, each plant was assessed for its pollen fertility with 2% aceto-carmine solution. Completely round and well stained pollen grains were counted as fertile while, the shrivelled, unstained or partially stained ones were considered as sterile. Counts were taken in each cross and fertility/ sterility was expressed in percentage. Similarly, same panicle was bagged to understand the seed set under baggage. Number of seeds/cm^2 were counted randomly in each panicle in three locations in selfed panicle and expressed as percentage. F_2 Plants with <5% SSS (Selfed Seed Set) were considered as male sterile irrespective of whether plants are tagged as fertile or sterile on the basis of pollen shed data. Chi-square (χ^2) test was used to verify if fertility segregation agreed with the expected ratios at 5% significance level (Yadav et al. 2010).

Results and discussion

During the pearl millet growing period, average daily air temperature ranged between 22.2ºC to 35ºC at all the six experimental locations (Table 1), which was optimal temperature for pearl millet growth according to Garcia-Huidobro et al. (1982). During 2016, the annual rainfall in CBE was less than 200 mm, while in the remaining five places it was more than 500 mm. NDL location received superfluous rainfall of around 1146.7 mm. The observed relative humidity was between 58.2% to 75.2%. The available Fe and Zn were estimated as DTPA extractable Fe and Zn are presented in Table 1. The availability was higher in HSR followed by NDL and JPR and least in MDR. Three important soil properties that determine fertilizer response of a crop are soil pH (to regulate nutrient availability), texture (to regulate water transmission properties and fixation and release of nutrients) and organic matter (to realise the cascading effect on whole range of soil physical as well chemical properties, including the biological properties). The range of soil pH over the locations was 7.6 to 8.2, which indicate the soil was neutral to slightly alkaline. A slightly alkaline (pH 7.4-7.8) or higher soil pH can cause a problem with the availability of Fe and Zn (Kumar et al. 2017a). Pearl millet will yield best on fertile, well-drained loamy soils and table 1 indicates, the soil texture of all the locations was sandy loam. Organic content in soil ranges from 0.27 to 0.61 % and studies shown that higher the soil OC % or incorporation of organic material in soil can reduce the soil pH, increase the Cation Exchange Capacity (CEC) and increase the clay content. Hence, increasing organic carbon content in soils indirectly improves the availability of iron and zinc as indicated by significant positive correlations probably due to higher degree of adsorption and chelation as reported by Kumar et al. (2006).

The mean trait performance of pearl millet genotypes at six locations during 2016 are summarised in Table 2. Significant phenotypic variation was observed for days to 50% flowering, grain Fe and Zn contents. Mean performance for grain Fe and Zn content in all genotypes was higher than HarvestPlus target level of 77 ppm except one genotype, PPMI 960 (Fe: 70 ppm & Zn: 55 ppm) (Fig. 1). The range for grain Fe content was 34 to 151 ppm when considering

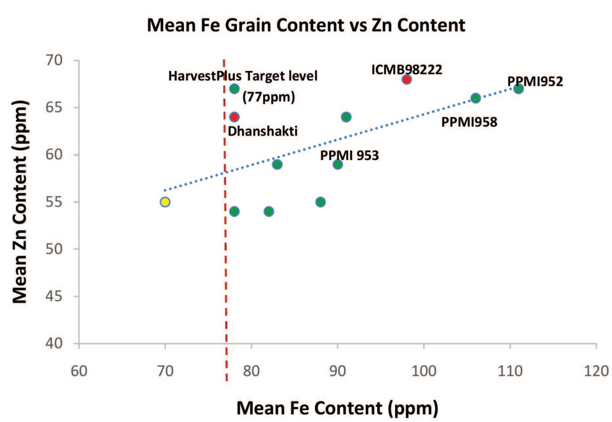

Fig. 1. Variability and relationship between grain Fe and Zn density in newly developed inbred and hybrid of pearl millet (mean of six locations) (A) AMMI1 Biplot for 10 high Fe entries (B) AMMI1 Biplot for 10 high Zn entries (C) AMMI2 Biplot for 10 high Fe entries (D) AMMI2 Biplot for 10 high Zn entries

individual locations and genotypes with overall mean of 87.7 ppm. The genotype, PPMI 952 topped 3 times over six locations in having highest mean Fe content

Table 2. Mean performance of pearl millet genotypes along with checks in multi-location test conducted during *kharif*, 2016 in high iron joint biofortification trials of All India Coordinated Pearl millet improvement programme

Genotype	MDR			JPR			HSR			JMR			NDL			CBE			GM		
	DF	Fe	Zn	DF	Fe	Zn	DF	Fe	Zn	DF	Fe	Zn	DF	Fe	Zn	DF	Fe	Zn	DF	Fe	Zn
PPMI 683	54	99	56	55	83	40	58	103	62	57	101	60	54	85	52	54	103	69	55	96	57
PPMI 627	53	36	26	52	40	23	54	37	24	54	36	26	51	33	24	51	41	35	53	37	26
PPMI 951	56	56	43	50	88	71	56	103	72	48	96	56	54	98	57	44	56	52	51	83	59
PPMI 952	65	78	44	57	130	88	69	130	74	55	129	65	54	107	67	49	89	65	58	111	67
PPMI 953	57	61	53	56	95	71	61	121	65	51	115	63	53	95	52	44	54	47	53	90	59
PPMI 954	55	48	36	54	90	70	56	134	81	48	104	48	54	78	40	48	75	53	52	88	55
PPMI 955	61	45	46	47	91	75	59	89	72	53	88	61	52	86	82	48	70	67	53	78	67
PPMI 956	54	45	42	49	86	71	62	113	62	51	71	43	52	79	50	50	74	58	53	78	54
PPMI 957	55	58	40	50	87	45	56	100	66	51	82	56	48	92	55	42	71	59	50	82	54
PPMI 958	62	88	50	52	96	63	67	120	70	53	111	64	51	151	89	43	70	62	54	106	66
PPMI 959	57	53	44	49	100	71	65	122	82	50	111	60	54	80	57	44	80	69	53	91	64
PPMI 960	48	34	32	46	91	74	52	81	56	48	77	52	49	60	43	45	77	71	48	70	55
ICMB 98222 (Check)	67	81	50	50	86	69	60	109	69	54	104	64	50	120	88	50	87	66	55	98	68
Dhanshakti (Check)	54	79	51	45	57	65	54	95	84	45	78	57	48	102	67	45	54	62	48	78	64

Source: Annual Report of All India Coordinated Pearl millet Improvement Project 2016-17

DF = Degrees of freedom, Fe = Iron, Zn = Zinc, MDR = Mandor, JPR = Jaipur, HSR = Hisar, JMR = Jamnagar, NDL = New Delhi and CBE = Coimbatore locations; GM = Grand mean

of 111 ppm followed by PPMI 958 (106 ppm). Grain Zn content was 32 to 89 ppm (mean 60 ppm). The genotype, PPMI 952 topped 2 times over six locations with mean grain Zn content of 67 ppm. Highest overall mean grain Zn content was in recorded ICMB 98222 (68 ppm). Among the environments, the mean grain Fe content was observed highest in samples from Hisar (HSR) with 110 ppm of Fe and least from Mandor (MDR) with 60.5 ppm of Fe. In grain Zn content, showed a similar trend. Highest was from HSR (71.1 ppm) and lowest from MDR (44.3 ppm). It was evident from soil test analysis that available Fe and Zn were higher in HSR and least in MDR. As availability of micronutrient from soil increases with decline in soil pH and increase in soil organic content (Kumar et al. 2006). A strong positive correlation among grain Fe and Zn content over each location with overall correlation, $r=0.6$ ($p<0.01$) was found, suggesting that the genes and pathways responsible for accumulation of grain Fe and Zn concentrations could be same. Genetic improvement for these two traits could be under taken simultaneously. Significant and positive association between the grain Fe and Zn content in pearl millet has been reported earlier (Kanatti et al. 2014; Anuradha et al. 2017; Pawar et al. 2018; Singhal et al. 2018).

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 contents indicating experiments were homogeneous. Combined ANOVA indicate significant differences ($p<0.01$) among the genotypes, environments and G x E interactions (GEi) (Table 4). Significant G x E interactions explained 20.65 % and 26.16 % of total sum of squares for grain Fe and Zn content respectively, which shows that although both the micronutrients are influenced by environments, grain Zn is relatively more sensitive to environmental fluctuations. As Zn availability from soil is more influenced by physical and chemical properties of soil than that for Fe. Several factor such as soil pH, organic matter, soil texture and soil type mutually interacts to govern its solubility in soil solution (Kumar et al. 2017b). Genotypic contribution towards total sum of squares was 19.4% and 13.29% for Fe and Zn content. Significant genotypic differences suggested that genes

Table 3. Morpho-agronomic characters of promising pearl millet genotypes identified through AMMI analysis evaluated at three locations during *kharif,* 2017

Genotype	Codes given	Days to 50% flowering	Days to maturity	Plant height (cm)	Panicle length (cm)	Panicle diameter (cm)	Panicle/ plant	1000-SWt (g)	Fe (ppm)	Zn (ppm)	OP seed set%
PPMI 683	-	64	93	120	19.0	2.99	3.0	12.75	98	91	95
PPMI 627	-	61	91	123	22.0	2.77	3.0	8.28	47	27	90
PPMI 952	G2	58	88	154	24.9	2.63	1.5	11.1	111	67	95
PPMI 953	G3	53	84	153	25.6	2.48	1.8	8.65	90	59	90
PPMI 958	G8	54	84	150	25.2	2.31	1.6	10.6	106	66	95

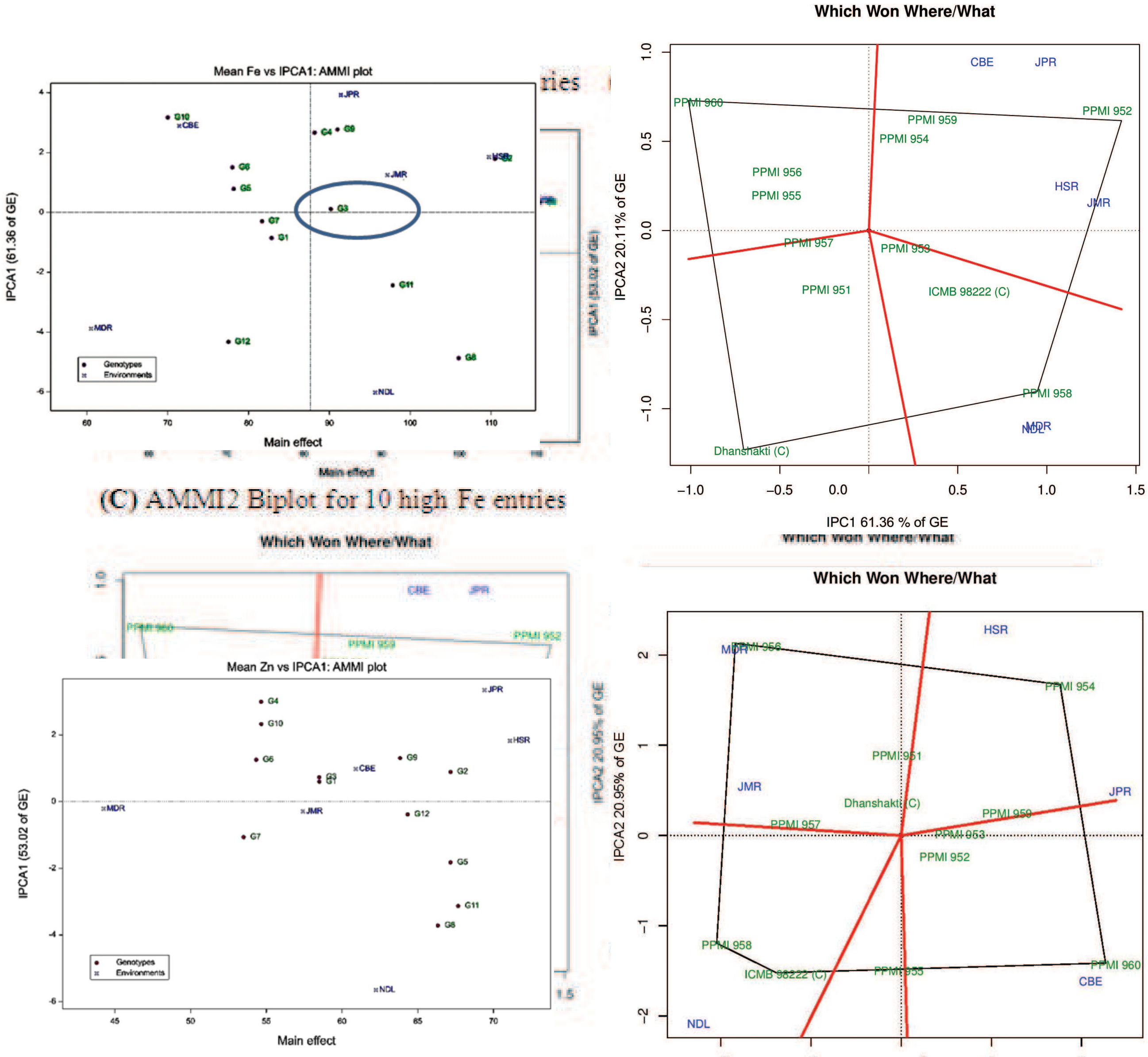

Fig. 2. AMMI and GGE biplot based on symmetrical scaling fo[r] of interaction (PC1) and mean over six locations at year 2016: (A) AMMI1 Biplot for 10 high Fe entries, (B) AMMI1 Biplot for 10 high Zn entries, (C) AMMI2 Biplot for 10 high Fe entries, (D) AMMI2 Biplot for 10 high Zn entries

necessary for micronutrient enrichment 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 x E interactions. The application of AMMI model for partitioning the GE interaction effect showed that only first two interaction principal components (IPCAs) of AMMI were significant based on Gollob's F-test (Gollob 1968), leaving 27 residuals which means that the first two interaction components (Table 3) could elucidate the interaction variation sufficiently and model holds good (Gauch 2013). For Fe content, IPC 1 and IPC 2 explained 61.36 % and 20.11 % of total G × E interactions and for Zn content, the first two PC explained 53.02 % and 20.95 %, respectively.

In case of AMMI 1 biplot the genotypes with high mean yield positioned near the line, showing IPCA=0 have negligible or no G × E interaction and have general adaptability over all locations (Rad et al. 2013). The genotypes away from the line with IPCA1=0 would be adapted to a specific environment. AMMI1 biplot of grain Fe content indicates the genotype PPMI 953 (G3) scattered at the right-hand side of the grand mean level and close to IPCA-1=0 line as having general adaptability in all the six locations. But this genotype was found more suited in JMR location. Even though, slightly unstable for grain Fe concentration, the genotype PPMI 952 (G2), PPMI 958 (G8) and ICMB 98222 (G11) were high in mean Fe concentration but slightly deviated from the origin of biplot were identified as specifically adapted to favorable environments. Favorable locations for a particular genotype means are those with high mean and high IPCA1 score with same sign for both the genotype and location indicating positive interaction. For instance, HSR was found favorable environment for PPMI 952 and NDL being the favorable location for PPMI 958 and ICMB 98222 (Fig. 2A). Similarly, for grain zinc content, G12 followed by G2 found to be closer towards IPCA1=0 and can be regarded as genotypes with general adaptability to all six locations. The genotype ICMB 98222 (G11) having high grain Zn of 68 ppm found more favorable towards NDL. PPMI 953 (G3) is one among the stable lines for grain Fe content was also found to be stable for grain zinc content, its level is approximately near as that of grand mean (60 ppm) (Fig. 2B).

A "which won where" polygon view (Figs. 2C, 2D) of the relationship between genotypes and environments was calculated. Genotypes appeared on the vertices of the polygon suggest the best or the poorest in one or other environment (Yan and Hunt 2002). The genotypes PPMI 952, PPMI 958, Dhanshakti, and PPMI 960 appeared on the corners of "which won where" polygon revealing that they were the best genotypes in specific environments. In case of grain iron content, genotype PPMI 958 appeared on the corner of the polygon where NDL and MDR environments fell. This suggests that PPMI 958 was the best cultivar for NDL and MDR. Likewise, the PPMI 952 genotype remained on the vertex of the polygon where the HSR, JMR, JPR and CBE environments fell; indicating PPMI 952 was the best cultivar for these environments. The genotypes PPMI 960, PPMI 955, PPMI 956, Dhanshakti and PPMI 951 located on the vertices did not fall in any environment, suggesting that these genotypes were not the best in any of the environments (Fig. 3C). The genotypes, *viz*., PPMI 953 and PPMI 957 remained in the centre of origin showed stable performance across the environments. Similarly, for grain Zn content, PPMI 952, Dhanshakti and PPMI 953 remained in the centre of origin showed stable performance across the environments. Also, genotypes suited for specific locations were given by AMMI2 biplot as PPMI 954 for HSR, PPMI 959 for JPR, PPMI 960 for CBE, PPMI 958, ICMB 98222 and PPMI 955 for NDL (Fig. 3D). The genotypes, PPMI 956, PPMI 951 and PPMI 957 were found to be the best genotype for location MDR and JMR. Hence, evaluation of test environment could help in identifying environments that could be utilized for selecting superior genotypes for mega environment (Singhal et al. 2018 and Jha et al. 2019).

According to ASV ranking (Table 5), PPMI 953

Table 4. Mean sum of squares of grain Fe-Zn content obtained by combined analysis over six locations

Source of variation	d.f.	Grain Fe content		Grain Zn content	
		MSS	% TSS	MSS	% TSS
Genotypes	11	892.9**	19.4	197.1**	13.29
Environments	5	3979.4**	39.3	1121.8**	34.38
Interactions	55	190.1**	20.65	77.6**	26.16
IPCA 1	15	427.6**	61.36	150.9**	53.02
IPCA 2	13	161.7*	20.11	68.8	20.95
Residuals	27	71.8		41.2	

Where, *=p-value < 0.05; **=p-value<0.01

Table 5. Mean grain Fe and Zn contents, ranking, AMMI stability values (ASV), ranking orders (RASV), and genotypic selection index (GSI) of the 12 genotypes tested across six environments

S.No.	Genotype	Code	Mean Fe	Fe-rank	IPCA [1]	IPCA [2]	ASV_{Fe}	Rank ASV_{Fe}	Fe_{GSI}	Rank Fe_{GSI}	Mean Zn	Zn-rank	IPCA [1]	IPCA [2]	ASV_{Zn}	Rank ASV_{Zn}	Zn_{GSI}	Rank Zn_{GSI}
1	Dhanshakti (Check)	G1	77.5	11	-4.33	0.63	9.29	11	22	8	64.3	4	-0.38	2.21	2.29	3	7	2
2	ICMB 98222 (Check)	G2	97.8	3	-2.44	1.71	5.35	7	10	3	67.7	1	-3.13	-0.95	5.07	10	11	5
3	PPMI 951	G3	82.8	7	-0.85	-1.15	2.01	3	10	3	58.5	6	1.90	1.58	3.41	8	14	6
4	PPMI 952	G4	110.5	1	1.79	-1.02	3.70	6	7	2	67.2	2	0.89	-1.80	2.30	4	6	1
5	PPMI 953	G5	90.2	5	0.11	-1.46	1.54	1	6	1	58.5	6	0.73	0.80	1.41	1	7	2
6	PPMI 954	G6	88.2	6	2.66	-2.26	6.09	9	15	7	54.7	7	3.00	1.81	5.10	11	18	9
7	PPMI 955	G7	78.2	9	0.78	1.61	2.28	4	13	5	67.2	2	-1.82	-1.30	3.18	7	9	3
8	PPMI 956	G8	78.0	10	1.50	1.43	3.33	5	15	7	54.3	8	1.26	-1.02	2.25	2	10	4
9	PPMI 957	G9	81.7	8	-0.30	1.62	1.80	2	10	3	53.5	9	-1.06	1.88	2.53	6	15	7
10	PPMI 958	G10	106.0	2	-4.87	-0.84	10.60	12	14	6	66.3	3	-3.71	-0.02	5.91	12	15	7
11	PPMI 959	G11	91.0	4	2.77	-1.16	5.84	8	12	4	63.8	5	1.31	1.20	2.40	5	10	4
12	PPMI 960	G12	70.0	12	3.17	3.28	7.76	10	22	8	54.7	7	2.33	-3.09	4.82	9	16	8

Table 6. Segregation for fertility restoration in F_2 populations involving PPMI 953 with three classes of designated seed parent (A-Lines)

Designated A-lines	F_1 plant tested	F_2 plants tested																		Over all	
		Jodhpur						Delhi						Dharwad							
		Total	Fertile	Sterile	Ratio	χ^2	P value	Total	Fertile	Sterile	Ratio	χ^2	P value	Total	Fertile	Sterile	Ratio	χ^2	P value	χ^2	P value
ICMA 863(A_1)	62	223	130	93	9:7	0.379	0.537	205	120	85	9:7	0.435	0.509	213	110	103	9:7	1.836	0.175	0.002	0.964
ICMA 04111 (A_4)	65	F_1 Plant got sterile; no sterile seed developed																			
ICMA 02555 (A_5)	61	F_1 Plant got sterile; no sterile seed developed																			

had the lowest value for Fe, followed by RIL PPMI 957, and PPMI 951 was the most stable genotype for Fe, whereas PPMI 953, PPMI 956 and Dhanshakti were found stable for Zn. PPMI 953 remained only genotype having lower ASV value for both Fe and Zn. Stability per se is not a desirable selection criterion, because the most stable genotypes would not necessarily give the best performance, hence, the simultaneous consideration of mean performance and ASV in a single non-parametric index (Mohammadi et al. 2007). Therefore, the ranking of ASV and mean performance (as ranking of Fe and Zn) are incorporated in a single selection index, GSI. The lowest GSI indicates the most stable genotype with high mean performance. GSI discriminated PPMI 953 for Fe and Zn, with general adaptability and high mean performance for rainfed condition. This was in agreement with the results of biplot analysis. Hence PPMI 953 were found to be promising for the most stable with higher mean micronutrient content among newly developed RILs as per AMMI biplots, ASV and GSI analysis under rainfed conditions.

Fertility restoration studies

Promising line, PPMI 953 which has mean iron content above 90 ppm and ASV value (~±1.5) along with parental lines and other high iron lines, PPMI 952 and PPMI 958 were evaluated for morpho-agronomic characters during *kharif* 2017 at three location *viz*., New Delhi, Dharwad and Jodhpur (Table 3). At the same time, the F_1 hybrids derived from the crosses with PPMI 953 as male parent with designated A-lines for three different sterile cytoplasmic systems in pearl millet (A_1, A_4 and A_5) showed 78-84% seed setting under baggage in the cross, $ICMA_{(1)}$ 863 x PPMI 953 whereas $ICMA_{(4)}$ 04111 x PPMI 953 and $ICMA_{(5)}$ 02555 x PPMI 953 set no seed indicating that fertility restoration in F_1 plants under bag did not take place at all the locations. The result indicated that fertility restoration in A-line with A_1 cytoplasm is due to the presence of A_1 restorer genes in PPMI 953 and is non functional in restoring the sterility due to A_4 and A_5 cytoplasmic system. Then F_2 population of the cross $ICMA_{(1)}$ 863 x PPMI 953 were evaluated for pollen fertility and % seed setting under baggage at three different zones (Jodhpur, Delhi, Dharwad) during *kharif,* 2018.

In F_2 generation, population derived from cross between ICMA $_{(1)}$ 863× PPMI 953 showed a good fit to 9 fertile: 7 sterile plants (χ^2 value=0.002, p-value=0.964), suggesting fertility restoration by PPMI 953 is governed by two complimentary genes (Table 6). Similar results suggesting involvement of two genes functioning in complementary epistatic manner. Thus the promising line may be readily available to use as pollen parent for high iron rich A-lines with A_1 cytoplasm to derive high yielding high micronutrient rich hybrid through heterotic breeding or alternatively serve as donor parents for transferring the high micronutrient traits along with fertility restoring genes to non-restoring genotypes having high combining ability.

Authors' contribution

Conceptualization of research (CTS, SPS, AK); Designing of the experiments (TS, MSS); Contribution of experimental materials (SPS, CTS, NA); Execution of field/ lab experiments and data collection (TS, MM, NS); Analysis of data and interpretation (MSS, CB); Preparation of manuscript (TS, MSS).

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This research work has been funded by CRP on biofortification (ICAR funding), all of the facilities availed upon from the Division of Genetics, IARI to carry out phenotyping and Fe and Zn estimation was done at AICPMIP, Jodhpur is gratefully acknowledged. First author also thank Amity University, Noida to pursue doctoral degree program at IARI, New Delhi.

Reference

Amiribehzadi A. and Satyavathi C. T. 2012. Fertility restoration studies in different cytoplasms of pearl millet [*Pennisetum glaucum* (L.) R. BR.]. AOAS, **33**(3): 136-142.

Anuradha N., Satyavathi C. T., Meena M. C., Sankar S. M., Bharadwaj C., Bhat J., Singh O. and Singh S. P. 2017. Evaluation of pearl millet [*Pennisetum glaucum* (L.) R. Br.] for grain iron and zinc content in different agro climatic zones of India. Indian J. Genet., **77**: 65-73. doi.org/10.5958/0975-6906.2017.00009.8.

Bose L. K., Jambhulkar N. N., Pande K. and Singh O. N. 2014. Use of AMMI and other stability statistics in the simultaneous selection of rice genotypes for yield and stability under direct-seeded conditions. Chil. J. Agr. Res., **74**(1): 3-9. doi.org/ 10.4067/S07 18-58392014000100001.

Cakmak I. and Kutman U. B. 2018. Agronomic biofortification of cereals with zinc: a review. Eur. J.

Soil Sci., **69**: 172-180. doi.org/10.1111/ejss.12437.

FAO. 2015. The Food and Agriculture Organization of United Nations report. Webpage http://www.fao.org/3/a-i3300e.

Farshadfar E., Mahmodi N. and Yaghotipoor A. 2011. AMMI stability value and simultaneous estimation of yield and yield stability in bread wheat (*Triticum aestivum* L.). Aust. J. Crop Sci., **5**(13): 1837.

Garcia-Huidobro J., Monteith J. L. and Squire G. R. 1982. Time, temperature and germination of Pearl millet (*Pennisetum typhoides* S. & H.) I. Constant temperature. J. Exp. Bot., **33**: 288-96. doi.org/10.1093/jxb/33.2.297.

Gauch HG. 2006. Statistical analysis of yield trials by AMMI and GGE. Crop Sci., **46**: 1488-500. doi.org/10.2135/cropsci2005.07-0193.

Gauch H. G. 2013. A simple protocol for AMMI analysis of yield trials. Crop Sci., **53**: 1860-9. doi.org/10.2135/cropsci2013.04.0241.

Gollob H. F. 1968. A statistical model which combines features of factor analytic and analysis of variance techniques. Psychometrika., **33**: 73-115. https://link.springer.com /article/10.1007/BF02289676.

Govindaraj M., Rai K. N., Kanatti A., Rao A. S. and Shivade H. 2019b. Nutritional security in drylands: Fast-track intra-population genetic improvement for grain iron and zinc densities in pearl millet. Front. Nutr., **6**: 74. doi.org/10 .3389/fnut.201 9.00074.

Govindaraj M., Rai K. N., Cherian B., Pfeiffer W. H., Kanatti A. and Shivade H. 2019a. Breeding Biofortified Pearl Millet Varieties and Hybrids to Enhance Millet Markets for Human Nutrition. Agriculture., **9**(5): 106. doi.org/10.3390/agricu lture9050 106.

Gregory M. A., Silva B. S., Narlon C., Gill D. P., McGowan C. L., Liu-Ambrose T., Shoemaker J. K., Hachinski V., Holmes J. and Petrella R. J. 2017. Combined Dual-Task Gait Training and Aerobic Exercise to Improve Cognition, Mobility, and Vascular Health in Community-Dwelling Older Adults at Risk for Future Cognitive Decline 1. J. Alzheimers Dis., **57**: 747-763. doi.org/10.3233/JAD-161240.

HarvestPlus, Available at: www.harvestplus.org [Accessed February 27, 2019].

Jha Uday Chand, Paresh Chandra Kole, Narendra Pratap Singh, Sandip Shil and Hemant Kumar 2019. GGE bi-plot analysis for grain yield in chickpea (*Cicer arietinum*) under normal and heat stress conditions. Indian J. agric. Sci., **89**: 721-5. epubs.icar.org.in/ejournal/index.php/IJAgS/article/view/88873/36383.

Kanatti A., Rai K. N., Radhika K., Govindaraj M., Sahrawat K. L. and Rao A. S. 2014. Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. Springer Plus., **3**: 763. doi.org/10. 1186/2193-18 01-3-763.

Kumar G., Singh A. and Kumar R. 2017a. Status of Available Zinc and their Relationship with Soil Properties in Soils of Rae Bareli District of Uttar Pradesh, India. Int. J. Curr. Microbiol. App. Sci., **6**(12): 1968-1971. doi.org/10.2054 6/ijcmas.2017.612.225.

Kumar M. B., Subbarayappa C. T. and Ramamurthy V. 2017b. Distribution of Available (DTPA-extractable) Zinc and Iron and their Relationship with Some Soil Properties in Rice Soils of Chamarajanagar District, Karnataka, India. Int. J. Curr. Microbiol. App. Sci., **6**(5): 1423-1428. doi.org/10.20546/ijcmas.2017.605.155.

Kumar R., Pal S. and Singh N. B. 2006. Status of available micronutrient in alluvial soils and their relationship with soil properties. Ann. plant soil res., **8**(2): 181-182.

Kumssa D. B., Joy E. J., Ander E. L., Watts M. J., Young S. D., Walker S. and Broadley M. R. 2015. Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. Scientific Rep., **5**: 10974. doi.org/10.1038/srep10974.

Mohammadi R., Abdulahi A., Haghparast R. and Armion M. 2007. Interpreting genotype× environment interactions for durum wheat grain yields using nonparametric methods. Euphytica, **157**: 239-251. doi.org/10.1007/s10681-007-9417-3.

Paltridge N. G., Palmer L. J., Milham P. J., Guild G. E. and Stangoulis J. C. 2012. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. Plant Soil, **361**: 251-260. doi.org/10.1007/s11104-011-1104-4.

Pawar R. R., Kim M., Kim J. G., Hong S. M., Sawant S. Y. and Lee S. M. 2018. Efficient removal of hazardous lead, cadmium, and arsenic from aqueous environment by iron oxide modified clay-activated carbon composite beads. Appl. Clay Sci., **162**: 339-350. doi.org/10.1016/j.clay.2018.06.014.

Prasad M. N., Thiyagarajan K., Jeyamani P. and Rangaswamy M. 1993. Isolation of maintainers and restorers for cytopalsmic male sterile (CMS) lines. Int. Rice Res. Notes, **18:** 10.

Purchase J. L., Hatting H. and Van Deventer C. S. 2000. Genotype × environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa: II. Stability analysis of yield performance. S. Afr. J. Plant Soil., **17**(3): 101-7. doi.org/ 10.1080/02571862.2000.10634878.

Rad M. N., Kadir M. A., Rafii M. Y., Jaafar H. Z., Naghavi M. R. and Ahmadi F. 2013. Genotype environment interaction by AMMI and GGE biplot analysis in three consecutive generations of wheat (*Triticum aestivum*) under normal and drought stress conditions. Aust. J. Crop Sci., **7**: 956.

Satyavathi C. T., Sankar S. M., Singh S. P., Bhowmick P., Bhat J., Singh O. and Anuradha N. 2015. Stability analysis of grain iron and zinc content in pearl millet [*Pennisetum glaucum* (L.) R. Br]. Int. J. Trop. Agric., **33** (2 (Part III)): 1387-94.

Singhal T., Satyavathi C. T., Kumar A., Sankar S. M., Singh S. P., Bharadwaj C., Aravind J., Anuradha N., Meena M. C. and Singh N. 2018. Genotype × environment interaction and genetic association of grain iron and zinc content with other agronomic traits in RIL population of pearl millet. Crop and Pasture Sci., **69**: 1092-1102. doi.org/10.1071/ CP18306.

Stangoulis J. 2010. Technical aspects of zinc and iron analysis in biofortification of the staple food crops, wheat and rice. In: 19th World Congress of Soil Science, Soil Solutions for a Changing World, **42**. https://www.iuss.org/19th%20WCSS /Symposi um/pdf/ 2499. pdf.

Stangoulis J. and Sison C. 2008. Crop sampling protocols for micronutrient analysis. HarvestPlus Tech Monogr Ser., 7. https://www.researchgate.net/p rofile/James Stang oulis/publication265181444 CropSampling Protocolsfor MicronutrientAnalysis/links/546f396 b0cf216f8cfa9cbb6.pdf.

Stein A. J. 2010. Global impacts of human mineral malnutrition. Plant Soil, **335**: 133-54. doi: org/ 10.1007/s11104-009-0228-2.

Yadav D., Gupta S., Kulkarni V., Rai K. and Behl R. 2010. Inheritance of A 1 system of cytoplasmic-nuclear male sterility in pearl millet [*Pennisetum glaucum* (L). R. Br.]. Cereal Res. Commun., **38**(2): 285-93. doi.org/10.1556/CRC.38.2010.2.15.

Yan W. and Hunt L. A. 2002. Biplot analysis of diallel data. Crop Sci., **42**: 21-30. doi.org/10.2135/cropsci2002. 2100.

Zobel R. W., Wright M. J. and Gauch H. G. 1988. Statistical analysis of a yield trial. Agron J., **80**: 388-393. doi.org/ 10.2134/agronj1988.0002196200 8000030002x.

Erratum

The article titled "Whole genome SNP identification and validation in *Cucumis melo* L. cultivars using genome resequencing approach" published in Vol. **78**(4): 478-486 (2018) contained an inadvertent error in the corresponding author's affiliation. The correct affiliation of the corresponding author may be read as given below:

Sanjay Kumar*[1], Sharad Vats[1], Paramananda Barman, Neetu Tyagi, Ratna Kumari, Pooja Bangar, Bhavana Tiwari, Supriya Sachdeva, Ambika B. Gaikwad and K. V. Bhat

Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110 012; [1]Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan 304 022.
*corresponding author.