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Genetic variability and genotype × environment interactions for kernel iron and zinc concentrations in maize (*Zea mays*) genotypes

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ABSTRACT

Micronutrient enrichment in the major staple food crops is an important breeding goal in view of the extensive problem of ‘hidden hunger’ caused by micronutrient malnutrition. Kernel iron (Fe) and zinc (Zn) concentrations were evaluated in a set of 30 diverse maize genotypes during rainy (*kharif*) season of 2006, 2007 and 2008. The ranges of kernel Fe and Zn concentrations were 11.28–60.11 mg/kg and 15.14–52.95 mg/kg, respectively, across the three years. Based on the performance of the entries across the years, four highly promising inbreds and three landrace accessions were identified as highly promising for kernel Fe concentration, including a HarvestPlus line, HP2 (42.21 mg/kg). Similarly, for kernel Zn concentration, three inbreds and one landrace were identified as highly promising, including V340 (43.33 mg/kg). No significant association was found between kernel Fe and Zn concentrations indicating the need for independent selection for enhancing the concentration for these traits. Stability analysis revealed significant role of environment and genotype × environment (G × E) interaction in determining the levels of kernel Fe and Zn. The study also identified HP2 and BAJIM 06-17 for kernel Fe concentration and IML467 for kernel Zn concentration as the most stable genotypes across the environments.

Key words: Environment, Kernel iron, Maize, Stability, Variability, Zinc

Nearly one billion people worldwide are undernourished due to insufficient food, and more than double this population, mostly the resource-poor in the developing world, suffer from ‘hidden hunger’, a term more often used to describe malnutrition due to micronutrient deficiencies in staple food diet (Bouis and Welch 2010, FAO 2010). Two-thirds of all deaths among the children below five years were reported due to micronutrient deficiencies (Welch and Graham, 2004). The micronutrient deficiencies are particularly concentrated in the semi-arid tropics, especially in South and South East Asia and Sub-Saharan Africa (Reddy *et al.* 2005). In India, 230 million people were reported to be undernourished, accounting for more than 27% of the world’s undernourished population (Lodha *et al.* 2005).

Among various micronutrient deficiencies, Fe deficiency is the most common nutritional disorder, affecting 4–5 billion of people worldwide, with more than two billion people in the developing countries (Ghandilyan *et al.* 2006). In India, nearly 70 % of the children (under five years) suffer from anemia (Lodha *et al.* 2005). Fe-related deficiencies also affect cognitive development, growth, reproductive performance and work productivity (Lynch 2003, Bouis 2002). Zinc deficiency leads to anorexia, depression and psychosis, impaired growth and development, altered reproductive biology, gastro-intestinal problems and impaired immunity (Solomons 2003). Cichy *et al.* (2005) reported that 49% of the world’s population is affected by low zinc intake. Medical supplements and fortification of food products have been attempted in several countries for decades to ameliorate problems of ‘hidden hunger’ (Underwood 2000), but these measures have their inherent limitations in terms of their reach, besides lack of purchasing power of the poor (Pfeiffer and McClafferty 2007).

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Maize is the staple food for more than 1.2 billion people worldwide, particularly in Latin America, Sub-Saharan Africa, and many of the South East Asian countries, including India. Development of micronutrient-enriched staple plant foods through breeding holds significant promise for sustainable food-based solutions (Banziger and Long 2000, Graham *et al.* 2001, Pfeiffer and McClafferty 2007). Development of 'golden rice' is one of the classical examples of plant biofortification to fight the menace of vitamin A deficiency (Ye *et al.* 2000). Micronutrient-enriched or biofortified maize would not only serve as the logical vehicle for providing Fe and Zn in the diets of the people (Long *et al.* 2004) but also shall be a cost-effective and sustainable approach to alleviate micronutrient deficiencies (Bouis 2002). Development of micronutrient-rich cultivars would produce more yield in a micronutrient-deficient soil than the micronutrient inefficient variety. Ascertaining the genetic variability for kernel micronutrients (like Fe and Zn) in the available maize germplasm and their potential to utilize in the breeding programme assumes significance. In addition, assessment of the stability of genotypes with respect to target traits is important for effective utilization of such genotypes in breeding strategies.

Research efforts with respect to kernel micronutrient traits in maize were undertaken so far only in the South American and African countries (Banziger and Long 2000, Oikeh *et al.* 2003, 2004, Dixon *et al.* 2000, Menkir 2008), particularly under HarvestPlus, an international collaborative programme for biofortification of selected staple food crops. The present research effort was the first of its kind in India, involving selected inbred lines and landraces, for ascertaining the genetic variability and stability for kernel micronutrient traits.

MATERIALS AND METHODS

A set of 30 maize entries (Table 1), including 24 inbred lines developed at important maize breeding centres in India

Table 1 Details of maize entries analyzed in the study

Entries[1]	Pedigree/collection site	Seed source[2]
BAJIM 06-6	Derivative of HEYPool-204	CSKHPKV, Bajaura
BAJIM 06-7	Derivative of HAREC 95 Pool-98	CSKHPKV, Bajaura
BAJIM 06-10	Derivative of HAREC 95 Pool-99	CSKHPKV, Bajaura
BAJIM 06-12	Derivative of Pool 98- (6618)	CSKHPKV, Bajaura
BAJIM 06-13	Derivative of Pool 98- (6615)	CSKHPKV, Bajaura
BAJIM 06-14	Derivative of HAREC K Pool-703	CSKHPKV, Bajaura
BAJIM 06-15	Derivative of HAREC K Pool-719	CSKHPKV, Bajaura
BAJIM 06-17	Derivative of HAREC 95 Pool-98-4	CSKHPKV, Bajaura
BAJIM 06-19	Derivative of HAREC K Pool-729	CSKHPKV, Bajaura
CM139	Derivative of (Tarun × MS 1) -Y63	PAU, Ludhiana
CM140	Derivative of J 617-61	PAU, Ludhiana
CM128	Derivative of (Anantnag local × WF9 × M14)	VPKAS, Almora
CM145	Derivative of Pop 31 C4 HS bulk (Alm.) -70	VPKAS, Almora
CM152	Derivative Pop 31 C4 HS bulk (Alm.)	VPKAS, Almora
CM153	Derivative of intercrosses of three inbreds from Pop 31 C4 HS bulk (V198, V270 and V273)	VPKAS, Almora
CM212	Derivative of USA/Acc No. 2132 (Alm.)	VPKAS, Almora
V334	Derivative of TZI-9	VPKAS, Almora
V336	Derivative of CML145, P63.COH-C-181 (CIMMYT)	VPKAS, Almora
V340	Derivative of (CM128 × CM129)	VPKAS, Almora
V341	Derivative of Mexico Acc. No. 3136	VPKAS, Almora
V348	Derivative of Pop 31	VPKAS, Almora
VQL-1	QPM version of CM212 (CML180 as donor)	VPKAS, Almora
IML119 (IC77433)	Jai Singhpura, Ambala Dt., Haryana	NBPGR, New Delhi
IML273 (IC131229)	Majilar, E. Sikkim Dt., Sikkim	NBPGR, New Delhi
IML288 (IC199114)	Ramchandrapur, Bhagalpur Dt., Bihar	NBPGR, New Delhi
IML312 (IC251347)	Jatraham, Kamalpur Dt., Tripura	NBPGR, New Delhi
IML434 (IC298558)	Sompur, Sabarkanta Dt., Gujarat	NBPGR, New Delhi
IML467 (IC353812)	Oorgoundannur Pudur, Thiruvannamalai Dt., Tamil Nadu	NBPGR, New Delhi
HP1	B.I.Z.T.V.C. 1-3-1-1-1-1-B-B-B-B	CIMMYT, Mexico
HP2	B.I.Z.T.V.C. 4-2-1-3-1-2-B-B-B-B	CIMMYT, Mexico

[1]BAJIM, Bajaura inbred maize; CM, coordinated maize (from All-India Coordinated Maize Improvement Project); V, Vivek inbred; VQL: Vivek QPM Line; IML, Indian maize landrace/local; IC, indigenous collection; HP, harvestplus-CIMMYT line; [2]CSKHPKV, Chaudhary Sravan Kumar Himachal Pradesh Krishi Viswavidyalay; VPKAS; Vivekananda Parvatiya Krishi Anusandhan Sansthan; PAU, Punjab Agricultural University; NBPGR, National Bureau of Plant Genetic Resources

(nine lines developed by the CSKHPKV, Bajaura, seven elite lines developed under the All-India Coordinated Maize Improvement Programme (AICMIP); six inbred lines from VPKAS, Almora, besides two of the highly promising lines identified under the Maize-HarvestPlus Programme (CIMMYT, Mexico), and six selected maize landrace accessions from India (Indian Maize Landraces/Locals; IMLs), were analyzed for kernel Fe and Zn concentrations at the IARI Experimental Farm, New Delhi during rainy (*kharif*) season for three consecutive years (2006 to 2008).

The genotypes were planted in a randomized complete block design (RCBD) with two replications per entry and one row (5 m length) per replication with a plant-to-plant spacing of 20 cm and row-to-row spacing of 75 cm. Standard agronomic practices were followed for raising and maintenance of plants. The soil at Delhi is basically deep, well drained, light alluvium and classified as Typic Haplustept belonging to Mehrauli series. The soil status of the experimental block and the major meteorological parameters recorded during the experimental seasons (2006–08) are presented in Table 2.

After kernel maturation and plant dry down, ears with the husk were hand harvested and was dried under the clean shade to lower post-harvest grain moisture content to 14%, as per the protocol suggested by HarvestPlus. Representative grain samples were drawn in triplicate by quartering method and the individual samples were ground into fine powder using a Cyclotec Sample Mill (Model 1093; Hooganas, Sweden). The flour sample (1 g) was digested using standard di-acid protocol (Singh *et al.* 2005). The concentrations of trace minerals (Fe and Zn) in the samples were analyzed using an Atomic Absorption Spectrophotometer (AAS-Perkin Elmer). The individual datasets were analyzed for analyses of variance (ANOVA) and comparison of means using PROC GLM of SAS Version 9.1 (SAS Institute, 2005). Stability analysis was undertaken based on the Eberhart and Russell model (1966) using Windostat Version 8.0.

RESULTS AND DISCUSSION

ANOVA revealed significant variation for both kernel Fe and Zn concentrations in all the three years (table not presented), suggesting the presence of wider genetic variability to be utilized for the genetic improvement of kernel micronutrient traits in maize. Banziger and Long (2000), Dixon *et al.* (2000) and Oikeh *et al.* (2003, 2004) also reported the presence of significant variations among the maize genotypes for the kernel Fe and Zn concentrations. Abundant genetic variations for kernel-Fe and Zn concentrations were also reported in all the major cereal crops including maize (Ghandilyan *et al.* 2006 and Menkir 2008).

In the present study, kernel Fe ranged from 11.28 to 60.11 mg/kg, while kernel Zn was found to vary from 15.14 to 52.95 mg/kg across all the three years. Banziger and Long (2000) reported the ranges of kernel Fe and Zn as 9.6–63.2 mg/kg and 12.9–57.6 mg/kg, respectively, while undertaking a set of experiments involving a set of 1 814 maize genotypes in different places of Mexico and Zimbabwe. Oikeh *et al.* (2003) reported a range of 16.8–24.4 mg/kg for kernel-Fe and 16.5–24.6 mg/kg for kernel Zn concentration. Dixon *et al.* (2000) found that the Fe concentration varied from as low as 13.60 mg/kg to as high as 159.43 mg/kg, while it was 11.65–95.62 mg/kg for kernel Zn concentration. Chen *et al.* (2007) reported kernel Fe concentration as high as 68.1 mg/kg among the maize lines.

The mean value for kernel Fe concentration was found to be 35.01 mg/kg in 2006, while the mean values for 2007 and 2008, were 30.37 and 24.04 mg/kg, respectively (Table 3). In 2006, V336 recorded the highest kernel Fe concentrations (49.66 mg/kg), while HP1 revealed the least (20.88 mg/kg) (Table 3). BAJIM 06–14 (45.67 mg/kg), IML273 (44.07 mg/kg), BAJIM 06–19 (42.98 mg/kg), HP2 (42.78 mg/kg), IML288 (42.75 mg/kg) and BAJIM 06–1 (41.73 mg/kg) with kernel Fe more than 40 mg/kg were found highly promising. In 2007, BAJIM 06-19 was identified as the most promising with kernel Fe as high as 60.11 mg/kg. Other promising

Table 2 Soil profile and meteorological parameters during the experimental period

Experiment	*p*H	Zn (ppm)	Fe (ppm)	Temperature (°C)			Rainfall (mm)	RH (%)	EVP (mm)	SH (hr)	AWS (km/hr)
				Avg. max.	Avg. min.	Mean					
2006 (27 June-7 October) #	7.9	1.038* (1.330)	4.630* (4.697)	33.8	25.0	29.4	482.00	69.1	7.5	6.7	4.8
2007 (26 June-8 October) #	7.9	0.811* (0.751)	4.422* (4.152)	34.0	24.9	29.4	401.60	75.6	7.8	5.8	3.4
2008 (26 June-18 October) #	7.8	0.852* (0.780)	4.934* (4.850)	33.2	24.1	28.7	583.70	74.1	5.3	5.3	2.2

#Dates of sowing and harvesting

*Data from 0–15 cm soil depth; data in parentheses from 15–30 cm depth

RH, Relative humidity; EVP, evaporation; SH, sunshine hours, AWS, average wind speed

Table 3 Mean kernel Fe and Zn concentrations (mg/kg) of the entries in different years and mean estimates of stability parameters

Entries	Kernel Fe						Kernel Zn					
	2006	2007	2008	Mean	b_i	S^2_{di}	2006	2007	2008	Mean	b_i	S^2_{di}
BAJIM 06-6	41.73	14.49	21.37	25.86	1.68	227.02**	21.17	37.95	24.15	27.76	2.08	83.43**
BAJIM 06-7	35.78	36.61	25.78	32.72	0.96	14.40*	24.46	36.85	31.07	30.79	1.92	11.16 **
BAJIM 06-10	32.99	15.41	22.44	23.61	0.84	111.35**	18.17	51.35	31.78	33.76	4.78	157.20**
BAJIM 06-12	31.89	18.13	31.96	27.33	–0.13	123.22**	32.27	37.95	32.64	34.29	0.65	11.18**
BAJIM 06-13	28.39	35.32	20.54	28.08	0.81	66.99**	21.82	35.80	22.95	26.86	1.61	73.58**
BAJIM 06-14	45.67	35.88	13.57	31.71	2.96	6.93	35.62	34.50	26.11	32.07	–0.95	36.68**
BAJIM 06-15	26.35	21.31	23.86	23.84	0.19	7.98*	24.18	30.90	18.98	24.69	0.28	68.36**
BAJIM 06-17	35.74	34.34	34.56	34.88	0.10	–1.98	33.52	20.98	27.25	27.25	–1.91	13.78**
BAJIM 06-19	42.98	60.11	22.31	41.80	2.13	440.11**	18.52	22.70	34.33	25.18	1.83	74.35**
CM128	32.67	25.19	24.78	27.55	0.68	8.88*	22.34	30.75	24.34	25.81	1.09	16.36**
CM139	26.08	23.83	27.19	25.70	–0.13	2.36	31.18	30.00	24.45	28.54	–0.71	15.26**
CM140	26.97	23.71	30.17	26.95	–0.33	11.55*	24.51	17.25	30.86	24.21	–0.23	89.98**
CM145	30.58	25.45	14.95	23.66	1.44	–1.07	20.80	24.75	31.48	25.68	1.36	24.54**
CM152	30.10	23.80	33.52	29.14	–0.38	37.19**	27.06	26.35	36.23	29.88	0.72	49.91**
CM153	29.69	12.99	19.50	20.72	0.81	99.12**	21.80	18.79	31.86	24.15	0.55	86.72**
CM212	30.43	16.12	15.00	20.51	1.33	37.61**	20.34	36.70	36.76	31.26	3.20	–0.96
V334	32.77	36.45	28.63	32.61	0.43	16.99**	35.01	37.85	30.27	34.37	–0.10	27.43**
V336	49.66	43.26	29.19	40.70	1.89	0.86	23.22	32.45	30.16	28.61	1.60	–0.29
V340	36.41	37.23	12.78	28.80	2.26	74.75**	36.65	43.68	49.67	43.33	1.90	20.73**
V341	22.88	21.69	11.28	18.62	1.09	6.61	20.95	22.01	23.84	22.26	0.37	0.21
V348	47.10	42.99	27.13	39.07	1.86	9.91*	31.37	32.45	24.47	29.43	–0.48	31.68**
VQL-1	30.74	30.03	16.48	25.75	1.35	16.18**	19.04	52.95	34.54	35.51	5.03	135.64**
IML119	38.91	35.97	24.61	33.16	1.33	3.86	34.13	24.65	23.80	27.53	–1.93	–0.51
IML273	44.07	35.86	33.16	37.69	0.96	6.07	24.73	16.05	50.06	30.28	1.26	94.91**
IML288	42.73	43.33	24.75	36.94	1.72	42.00**	27.78	34.70	30.38	30.95	0.98	6.13*
IML312	34.59	24.67	23.44	27.56	0.97	15.44**	29.22	15.14	40.14	28.17	–0.58	306.65**
IML434	37.37	38.19	17.63	31.06	1.89	53.035**	22.19	43.95	30.23	32.12	3.06	77.78**
IML467	41.28	36.85	29.33	35.82	1.10	–2.25	30.94	39.90	41.35	37.40	1.88	0.58
HP1	20.88	18.75	20.73	20.12	–0.01	0.32	22.20	28.03	25.44	25.22	0.91	0.80
HP2	42.78	43.20	40.65	42.21	0.21	–1.35	30.10	26.25	32.94	29.76	–0.17	20.30**
Mean	35.01	30.37	24.04	29.81	1.00		26.18	31.45	31.08	29.57	1.00	
SE	1.44	3.04	1.94	5.01	0.91		1.90	1.98	1.80	5.89	2.00	

b_i regression coefficient; S^2_{di} deviation from regression; **P* = 0.05; ***P* = 0.01

genotypes include IML288 (43.33 mg/kg), V336 (43.26 mg/kg), HP-2 (43.20 mg/kg) and V348 (42.99 mg/kg), while some genotypes like CM153, BAJIM 06–6, BAJIM 06-10, CM212, HP1 and BAJIM 06-12 recorded low kernel Fe content. In 2008, the mean kernel Fe concentration across all the genotypes was found to be much lesser compared to the previous two years. HP2 (40.66 mg/kg) was found to be the only genotype to surpass 40 mg/kg kernel Fe, while some genotypes like BAJIM 06-17 (34.56 mg/kg), CM152 (33.52 mg/kg), IML273 (33.16 mg/kg), BAJIM 06-12 (31.96 mg/kg) and CM140 (30.17 mg/kg) recorded more than 30 mg/kg kernel Fe. Taking into account the performance of the test entries in all the three years, HP2 (42.21 mg/kg) could be identified as the most promising, followed by BAJIM 06-19 (41.80 mg/kg) and V336 (40.70 mg/kg). V341 consistently performed poorly in all the three years (18.62 mg/kg) (Table 3).

For kernel Zn concentration, the ranges varied from 18.17 to 36.65 mg/kg, 15.14 to 52.95 mg/kg and 18.98 to 50.06 mg/kg, respectively, in the three years (Table 3). V340 recorded the highest kernel Zn concentration (36.65 mg/kg) in 2006, followed by BAJIM 06-14 (35.62 mg/kg), and V334 (35.01 mg/kg) with more than 35 mg/kg kernel Zn (Table 3). In 2007, VQL1 (52.95 mg/kg) was found to be the most promising, followed by BAJIM 06-10 (51.35 mg/kg), IML434 (43.95 mg/kg), V340 (43.68 mg/kg), IML467 (39.90 mg/kg), BAJIM 06-6 (37.95 mg/kg), BAJIM 06-12 (37.95 mg/kg), V334 (37.85 mg/kg) BAJIM 06-7 (36.85 mg/kg), and BAJIM 06-13 (35.80 mg/kg). IML312 (15.14 mg/kg) recorded the least kernel Zn concentration, followed by IML273, CM140 and CM153.

While the mean of kernel Fe concentration across genotypes in 2008 was found to be much lower as compared to those in the previous two years, the mean kernel Zn concentration across all genotypes was at par with the previous two years. IML273 (50.06 mg/kg) and V340 (49.67

mg/kg) were identified as the best performing genotype in 2008. Other promising entries included IML467 (41.35 mg/kg), IML312 (40.14 mg/kg), CM212 (36.75 mg/kg) and CM153 (36.23 mg/kg). Considering the three years data, V340 (43.30 mg/kg) was identified as the most promising genotype, followed by IML467 (37.40 mg/kg) and VQL1 (35.51 mg/kg) (Table 3). V341, interestingly was identified to have the least kernel Fe and Zn concentrations across the three years.

Taking into account both the kernel Fe and Zn concentrations, HP2 (Fe: 42.21 mg/kg; Zn: 29.76 mg/kg), V336 (Fe: 40.70 mg/kg; Zn: 28.61 mg/kg) and V348 (Fe: 39.07 mg/kg; Zn: 29.43 mg/kg) could be identified as the most promising genotypes with kernel Fe and Zn concentrations near or crossing the threshold levels of 40 mg/kg and 30 mg/kg, respectively (Table 3).

The study also revealed no significant correlation between kernel Fe and Zn concentrations in all the individual datasets as well as in pooled analysis, suggesting that the genes and pathways responsible for accumulation of kernel Fe and Zn concentrations could be quite different, and genetic improvement for these two traits could be undertaken independent of each other. In contrast, Dixon *et al.* (2000), Oikeh *et al.* (2003) and Menkir (2008) found significant and positive association between the kernel Fe and Zn concentrations.

The analyses further revealed significant variation for genotype × environment (G × E) interaction for both kernel Fe and Zn concentrations (Table 4). The sum of squares for G × E for kernel Fe was 31.77% of the total sum of squares, while for the kernel Zn it was as high as 58.37%. Greater proportion of G × E variance in case of kernel Zn than the kernel Fe is perhaps indicative of the sensitivity of kernel Zn to the soil and microclimatic conditions. This is also evident from the greater proportion of pooled deviation in case of kernel Zn than the kernel Fe, which suggests that in general, the kernel Zn could be much more variable in different environmental conditions in a more unpredictable manner than kernel Fe (Table 5).

Micronutrient concentration is affected by a range of factors, including soil type and fertility, soil moisture, environmental factors, crop genotype, and interactions among the nutrients (Feila *et al.* 2005). The G × E has been attributed to various micro-environmental conditions, besides soil profile (Oikeh *et al.* 2003). Although, the soil micronutrient status is one of the major factors for kernel micronutrient variations, micro-environmental variations could have profound effects on kernel micronutrients, particularly zinc concentration (Pfeiffer and McClafferty 2007). Planting seasons in rice and pearl millet and planting dates within a specific season in case of wheat have been found to have the significant effects on the kernel mineral concentrations. Besides, the spatial and temporal variation, system variations caused by the differential management practices can have the significant effects (Pfeiffer and McClafferty 2007). Significant effects of genotype × year interactions for kernel Fe, and genotype × location × year interaction for kernel Zn in maize were earlier reported by Oikeh *et al.* (2004). Significant genotype × location interactions for both kernel Fe and Zn concentrations in maize was also reported by Chakraborti *et al.* (2009) while experimenting with a set of inbred lines at two locations (Delhi and Bajaura). On the contrary, Menkir (2008) reported no significant genotype × location interaction for kernel Fe (except one out of eight trials) and Zn concentrations, although significant G × E were reported in some trials for kernel Mn, Ca, Mg, K and S concentrations in maize.

Table 4 Analysis of variance and interaction components for kernel Fe and Zn concentrations in maize

Sources of variation	d f	Kernel Fe		Kernel Zn	
		SS	MSS	SS	MSS
Genotypes	29	7724.45	266.36**	3572.75	123.19
Years	2	3634.91	1817.45**	1042.32	521.16**
Genotype × year	58	5496.07	94.76**	6907.39	119.09**
Error	90	451.45	5.02	313.43	3.48

df, Degrees of freedom; SS, sum of squares; MSS, mean sum of squares; *$P = 0.05$; **$P = 0.01$

Table 5 ANOVA and variance components (based on Eberhart and Russell model)

Sources of variation	df	Kernel Fe		Kernel Zn	
		SS	MSS	SS	MSS
Replications within year	3	4.14	1.37	0.68	0.23
Genotypes	29	3862.22	133.18**	1786.37	61.59
Year + gen. × year	60	4565.49	76.09	3974.85	66.24
Year (linear)	1	1817.44	1817.44**	521.15	521.15*
Gen. × year (linear)	29	1238.69	42.71	1367.84	47.16
Pooled deviation	30	1509.34	50.31**	2085.85	69.52**
Pooled error	87	221.59	2.55	156.03	1.79

df, Degrees of freedom; SS, sum of squares; MSS, mean sum of squares *$P = 0.05$; **$P = 0.01$

Since the initial G × E interaction was found to be significant for both the kernel Fe and Zn concentration, AMMI (additive main effect and multiplicative interaction) was used for further estimation of various variance components. However, for both the target traits, the models were found to be non-multiplicative type. In contrast, Oikeh *et al.* (2004) reported the presence of multiplicative type of G × E interaction for kernel Fe and Zn concentrations in maize. The present analyses using Eberhart and Russell model revealed that variance due to environment (linear) and pooled deviation were significant for both the kernel micronutrient traits, while G × E (linear) was found to be non-significant (Table 5). The sum of squares for pooled deviation for kernel-Fe was found to be 11.42% of the total sum of squares, while it was almost double (21.08%) for kernel Zn. The environmental indices for kernel Fe were 5.20, 0.56 and 5.74 during *kharif* 2006, 2007 and 2008, respectively. While the environmental index for kernel Zn during *kharif* 2006 was –3.40, the indices for *kharif* 2007 and 2008 were found to be 1.88 and 1.51, respectively. This suggests that *kharif* 2006 was the most favourable environment for expression of kernel Fe, while it was the most unfavourable environment for kernel Zn. *kharif* 2007 was identified as the most favourable environment for the kernel Zn concentration, followed closely by *kharif* 2008 as the next best environment.

The unpredictable effects of the year in a same location can be attributed to various micro-environmental factors, and even meteorological parameters. In the present study, the soil profile and the meteorological parameters of the three seasons were of similar nature with the exception of receiving more rainfall during *kharif* 2008 (583.70 mm) as compared to *kharif* 2006 (482.00 mm) and *kharif* 2007 (401.60 mm). Interestingly, the mean kernel-Fe across 30 genotypes was much less (24.04 mg/kg) during *kharif* 2008, while it was 35.01 mg/kg and 30.37 mg/kg during *kharif* 2006 and 2007, respectively. Therefore, higher rainfall alone or in interaction with other factors could be one of the factors contributing towards lesser kernel-Fe concentration. Interestingly, higher rainfall did not affect the mean kernel-Zn concentration during *kharif* 2008. More elaborate experiments are therefore, required to be undertaken to establish the role of excess rainfall and its interaction with other soil and meteorological factors, on the accumulation of kernel micronutrients, particularly kernel Fe.

The performance of a genotype with regard to traits like kernel micronutrient concentrations could be a function of its genetic constitution, its interaction with environment, and the influence of a complex network of diverse factors related to soil dynamics and micro-climates. Even minor changes in one factor, in combination with other factors, could lead to significant variation in kernel micronutrient traits. This genotype × year interaction can be better understood by analyzing the performance of a set of genotypes not only in multiple locations but also at different growing seasons each of the locations (Oikeh *et al.* 2004). Although G × E interactions for kernel Fe and Zn concentrations appear to play an important role, it is possible to identify the genotypes with stable mineral concentrations across environments, and thus, it could be feasible to combine high micronutrient traits with high yield (Gregorio 2002).

Taking into consideration the mean performance, regression coefficient and deviation from linearity, HP2, IML273, IML467, BAJIM 06 17 and IML119 were found to be the stable genotypes in terms of kernel Fe concentration (Table 3). Among these five genotypes, HP 2 and BAJIM-06 17 were identified as the most stable genotypes during *kharif* 2006, 2007 and 2008. Genotypes having higher mean value with high value of regression coefficients reflect the higher sensitivity of specific genotypes for micro- and macro-environmental changes; such genotypes could be performing better in more favourable environments. Considering this, V336, V348 and BAJIM 06-14 were found to accumulate

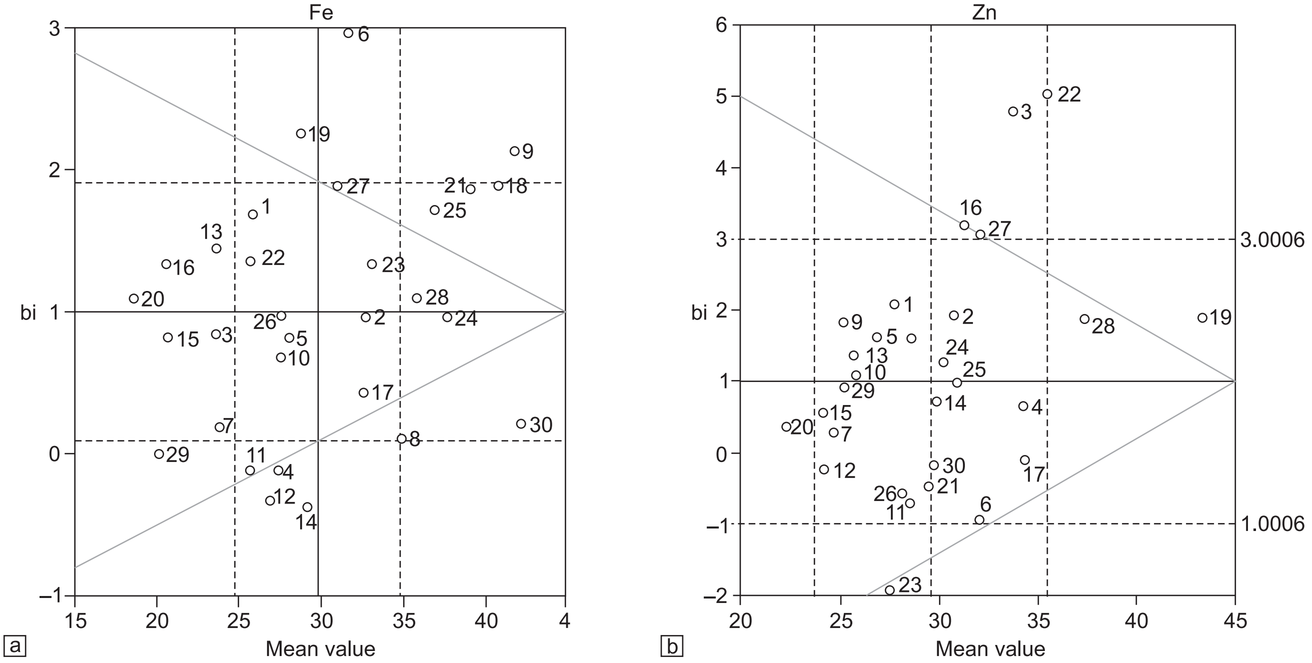

Fig 1 The relationship between the regression coefficients and (*A*) mean kernel Fe and (*B*) Zn concentrations (mg/kg) for 30 maize entries

more kernel-Fe during *kharif* 2006 than the other years, while BAJIM 06-19, IML288 and IML434 performed better during *kharif* 2007. In contrast, HP1 and CM139 were identified with poor performance for the target traits in all the three environments, as they failed to perform even under the more favourable environments. For kernel Zn, almost all the genotypes were found to be highly variable with regard to their performance, with an exception of IML467, which showed stable performance for kernel Zn in all the three years. The relationship between regression coefficients and the mean kernel Fe and Zn concentrations have been depicted in Fig 1. However, BAJIM 06–10, VQL1, CM212 and IML434 were identified as the promising genotypes for kernel Zn in the favourable environment (*kharif* 2007). Genotypes such as V341 and IML119 revealed poor performance in all the three years. Taking into consideration both the target traits, IML467 was found to be most stable (Table 3).

The promising genotypes identified in the present study could be potentially utilized for developing kernel micronutrient-enriched maize cultivars. Besides offering nutritional advantages, such cultivars could also offer other possible benefits, including a root system with better capacity to tap subsoil water and minerals, and greater seedling vigour. Besides identifying genotypes with high mean values for the target traits, the present study also revealed some genotypes with consistently low mean values for the target traits (e.g. HP1 and CM139 for kernel Fe, and V341 for kernel Zn). Such genotypes with contrasting and stable performance for the kernel micronutrient traits could be potentially useful as parental lines for developing mapping populations for QTL analysis of the target traits.

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