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Analysis of genetic variability and genotype × year interactions on kernel zinc concentration in selected Indian and exotic maize (*Zea mays*) genotypes

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ABSTRACT

Analysis of genetic variability for kernel zinc (Zn) concentration was undertaken on 81 maize (*Zea mays* L.) genotypes, including 13 inbreds from CIMMYT-HarvestPlus, in this study. Kernel Zn concentration varied significantly with a range of 3.81 to 35.83 mg/kg across three years of evaluation (2006-2008). Among the genotypes evaluated during 2006, IML 289 showed highest kernel Zn concentration (35.83 mg/kg), followed by HP 12 (31.69 mg/kg), HP 35-6 (29.02 mg/kg), HP 1 (27.24 mg/kg) and IML 119 (25.77 mg/kg). During *kharif* 2007, nine genotypes recorded kernel Zn more than 25 mg/kg. Among the landrace accessions, IML 312 (30.30 mg/kg), IML 185 (28.56 mg/kg), IML 288 (26.34 mg/kg), IML 119 (25.88 mg/kg) and IML 390 (25.75 mg/kg) were found promising, while the inbreds BAJIM 06-5 (28.72 mg/kg), CM 145 (25.89 mg/kg), CM 127 (25.45 mg/kg) and BAJIM 06-12 (25.44 mg/kg) recorded kernel Zn concentration. Among the inbreds evaluated during 2008, BAJIM 06-1 (24.74 mg/kg) was identified as the best genotype, followed by V 336 (23.36 mg/kg), CM 145 (22.18 mg/kg), CM 140 (21.09 mg/kg), V 340 (20.56 mg/kg), V 348 (20.17 mg/kg) and BAJIM-06-6 (20.02 mg/kg). The study revealed significant genotype × year interaction for kernel Zn concentration. Stability analysis revealed that variances due to genotype, year + genotype × year, year (linear) and pooled deviation were significant. Taking into consideration of regression coefficient and deviation from linearity, V 336, VQL 1, V 334 and CM 139 could be identified as stable genotypes for kernel Zn concentration across the years.

Key words: Genotype × year, Maize, Stability, Variability, Zinc

'Hidden hunger' a popular phrase to denote 'micronutrient malnutrition' has become an alarming problem, and affects nearly two billions of people particularly in the resource poor families of the developing world (Kennedy *et al.* 2003, Bouis and Welch 2010). More than five million deaths during the childhood are primarily due to the micronutrient deficiency (Anonymous 2007). 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). Malnutrition in general causes increased morbidity, disability, stunted mental and physical growth and reduced socioeconomic development (WHO and FAO, 2003). Considering the importance of the widespread menace of micronutrient deficiency, Millennium Development Goals (MDGs) were adopted by the General Assembly of the UN, and 'child mortality' and 'maternal health' were included among the problems affecting the world population the most (UN 2000, UNSCN 2004). Among the various mineral elements, iron (Fe), zinc (Zn) and vitamin-A are the most common that have been found deficient predominantly in cereal-based human diet (Pfeiffer and McClafferty 2007, Bouis *et al.* 2011). Deficiency of Zn in the diet leads to anorexia, depression and psychosis, impaired growth and development, altered reproductive biology, gastro-intestinal problems and impaired immunity (Solomons 2003). While mild to moderate Zn deficiency is common throughout the world, approximately half of the world's population is affected by low Zn intake (Sandstead 1995, Cichy *et al.* 2005).

Methods such as food fortification, supplementation and diet diversification have been recommended to ameliorate problems of micronutrient malnutrition (Maberly *et al*. 1994, Underwood 2000). However, these measures in general have

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not been largely successful for an array of reasons including undesirable chemical properties of the substances, faulty food distribution system, and economic background of the resource-poor people (Garcia-Casal 2003, Shrestha *et al*. 2003). Thus, development of micronutrient enriched or 'biofortified' crops through breeding hold significant promise for cost-effective and sustainable food based solutions (Banziger and Long 2000, Graham *et al.* 2001, Pfeiffer and McClafferty 2007, Bouis and Welch 2010, Gilligan 2012). Further, through this approach, the micronutrients would reach the target group in their natural form and can potentially fulfill the daily body requirements (Long *et al*. 2004, Bouis 2002, Pfeiffer and McClafferty 2007). Besides, micronutrient-rich cultivars, in general, have better and deeper root system in micronutrient-deficient soils and are better able to tap the subsoil water and minerals. Micronutrient-dense seeds are also associated with greater seedling vigour.

Maize is an important food and feed crop in the developing world; together with rice and wheat, maize provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw *et al.* 2011). In India, approximately 25% of maize produce is used for human consumption (Kaul *et al.* 2009). Analysis of genetic variability for kernel micronutrients in the available maize germplasm and their potential to utilize them in breeding programmes assume significance. However, such studies were undertaken mostly in the US and in Africa, especially under the HarvestPlus Program, involving mainly the locally adapted germplasm (Banziger and Long 2000, Brkic *et al.* 2003, Oikeh *et al.* 2003, 2004, Menkir 2008). Very few studies have been carried out so far on kernel micronutrients in the Indian maize germplasm (Prasanna *et al.* 2011, Chakraborti *et al.* 2011, Agrawal *et al.* 2012). In view of the importance of maize crop, as both food and feed, and the need for strengthening the maize biofortification programme in the Himachal Pradesh in India, the present study was undertaken. The objectives of the study are to (i) evaluate a set of maize genotypes, including Indian and exotic, for kernel Zn concentration; (ii) assess the stability of performance of the maize genotypes across different years of evaluation, and (iii) identify promising inbreds that can be utilized in breeding for kernel Zn-enriched maize.

MATERIALS AND METHODS

A set of 81 maize genotypes, including 20 inbreds developed by the Chaudhary Shravn Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), 14 elite lines developed under the All-India Coordinated Maize Improvement Programme, 11 inbreds from Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, 13 inbreds from the HarvestPlus-Maize Programme, 3 inbreds (CIMMYT Maize Lines or CMLs) developed by CIMMYT, Mexico, and 20 selected maize landrace accessions from India (Indian Maize Landraces; IMLs), were analyzed for kernel Zn concentration at the CSKHPKV, HAREC, Bajaura Experimental Station across three years during the *kharif* (monsoon) season of 2006, 2007 and 2008. Among these, 80 and 54 diverse genotypes including inbreds and landraces were evaluated during 2006 and 2007, respectively, and 21 inbred lines were analyzed during 2008.

The experiment was conducted in a randomized complete block design (RCBD) with two replications per entry and one row of 3 m length each per replication; 20 cm plant-to-plant spacing and 60 cm row-to-row spacing were maintained in all the three years (2006-2008). Standard agronomic practices, including N:P:K @ 120:60:40 kg/ha, were followed for raising and maintenance of the crop. Soil Zn concentration of the experimental blocks was analyzed, besides recording of the important weather parameters. Ears (including the husk) were hand-harvested and dried under clean shade to lower the grain moisture content to 14%. Representative kernel samples for each entry were drawn in triplicate by the quartering method, and the individual samples were ground into fine powder using a mill. Biochemical estimation of kernel Zn concentration was carried out by digestion with 9:4 diacid mixture (HNO_3: $HClO_4$), followed by recording of observations by the atomic absorption spectrophotometer (AAS) method, as per the protocol described by Zarcinas *et al.* (1987) and Singh *et al.* (2005). The individual datasets were analyzed for analyses of variance (ANOVA) and comparison of means was done using PROC GLM of SAS Version 9.1 (SAS Institute 2005). The dataset of 21 common genotypes, evaluated during 2006-2008, was analyzed for stability parameters using Windostat Version 8.0.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) revealed significant variation for kernel Zn concentration among the genotypes analyzed in all the three years (Table 1). Earlier reports (Banziger and Long 2000, Dixon *et al.* 2000, Menkir 2008, Prasanna *et al.* 2011) indicated wide variation for kernel Zn concentration in maize genotypes.

The kernel Zn concentration during 2006 varied from 3.81 to 35.83 mg/kg, while it ranged from 10.31 to 30.30 mg/kg and 14.65 to 24.74 mg/kg, during 2007 and 2008, respectively. Banziger and Long (2000) reported a range of 12.9-57.6 mg/kg of kernel Zn, among a large set of maize genotypes from Mexico and Zimbabwe. Prasanna *et al.* (2011) reported a similar range (15.14-51.35 mg/kg). In contrast, much narrower ranges of 16.5-24.6 mg/kg (Oikeh *et al*. 2003), 18.4-24.6 mg/kg (Oikeh *et al*. 2004), 16.0-23.6 mg/kg (Brkic *et al.* 2003), 19.1-29.8 mg/kg (Long *et al.* 2004) and 14-41 mg/kg (Menkir 2008) and 7.0-29.9 mg/kg (Agrawal *et al.* 2012) were reported in different studies, including inbreds, open-pollinated varieties and hybrids.

The mean kernel Zn concentration across all genotypes in the present study was found to be low (13.45 mg/kg) during *kharif* 2006, while it was almost similar during the

Table 1 ANOVA for kernel Zn concentration for different sets of inbreds evaluated during 2006-2008

Sources of variation	df	2006		df	2007		df	2008	
		SS	MSS		SS	MSS		SS	MSS
Replication	1	0.0001	0.0001	1	4.09	4.09	1	2.40	2.40
Genotypes	79	5483.88	69.42**	53	2421.96	45.69**	20	309.33	15.47**
Error	79	97.28	1.23	53	69.62	1.31	20	24.27	1.21

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

next two years (19.77 mg/kg and 18.56 mg/kg during 2007 and 2008, respectively). Among the landraces evaluated during 2006, IML 289 (IC 199115 from Nalanda, Bihar) showed highest kernel Zn concentration (35.83 mg/kg), followed by IML 119 (25.77 mg/kg; IC 77433 from Jai Singhpura, Haryana) and IML 205 (19.41 mg/kg; IC 130666 from Kohima, Nagaland). Among the inbred lines, HP 12 recorded the highest kernel Zn (31.69 mg/kg) during *kharif* 2006, followed by HP 35-6 (29.02 mg/kg), HP 1 (27.24 mg/kg), HP 4 (21.07 mg/kg), HP 3 (20.41 mg/kg) and CM 122 (20.30 mg/kg). During *kharif* 2007, nine genotypes recorded kernel Zn more than 25 mg/kg. Among the landraces, IML 312 (30.30 mg/kg; IC 251347; Paheli Makai from Sikkim) was the most promising, followed by IML 185 (28.56 mg/kg; IC 108151 from Mandi, Himachal Pradesh), IML 288 (26.34 mg/kg; IC 199114 from Bhagalpur, Bihar), IML 119 (25.88 mg/kg) and IML 390 (25.75 mg/kg; a landrace from Sikkim). Among the inbreds, BAJIM 06-5 recorded the highest Zn concentration with 28.72 mg/kg, followed by CM 145 (25.89 mg/kg), CM 127 (25.45 mg/kg) and BAJIM 06-12 (25.44 mg/kg). Among the 21 inbreds lines evaluated during 2008, BAJIM 06-1 (24.74 mg/kg) was identified as the most promising, followed by V 336 (23.36 mg/kg), CM 145 (22.18 mg/kg), CM 140 (21.09 mg/kg), V 340 (20.56 mg/kg), V 348 (20.17 mg/kg) and BAJIM 06-6 (20.02 mg/kg). Prasanna *et al.* (2011) also reported similar performance of IML 119, IML 288, CM 145, V 336 and CM 140, while analyzing kernel micronutrients in 30 diverse inbred lines.

Performance of a genotype at a particular location across years is an important factor to identify the promising and stable genotypes for a site specific breeding programme. Keeping this in view, stability parameters were estimated among the 21 common maize genotypes evaluated across 2006-2008 in the present study. The analysis revealed significant effects of genotype × environment interaction (G × Y) on kernel Zn concentration, indicating in general differential behaviour of genotypes under the three different years (Table 2). The sum of squares for G × Y for kernel Zn concentration was 27.48% of the total sum of squares, indicating the influence of year in determining kernel Zn. Agrawal *et al.* (2012) also reported similar degree of G × Y interaction for kernel Zn in maize inbred lines evaluated at VPKAS, Almora. Significant effects of genotype × location × year interaction for kernel Zn in maize were earlier reported by Oikeh *et al.* (2004). Effect of genotype × year interactions for kernel Zn concentration in maize was also reported (Prasanna *et al.* 2011). In contrast, no significant genotype × location interaction for kernel Zn concentration was reported by Menkir (2008).

Since the G × Y interaction was found to have significant effects on Zn concentration, AMMI (Additive Main effect and Multiplicative Interaction) stability model was applied for further partitioning of various variance components (Zobel *et al.*1988). However the AMMI analyses revealed that the dataset from the present study was not of multiplicative type. Therefore, the dataset was further analyzed using the model suggested by Eberhart and Russell (1966). The analyses revealed that variance due to genotypes, year + genotype × year, year (linear) and pooled deviation were significant (Table 3). The sum of squares for pooled deviation for kernel Zn was nearly 5.93% to the total sum of squares, suggesting that kernel Zn concentration could be influenced by the environmental conditions, albeit to a low degree. The environmental indices for kernel Zn concentrations were

Table 2 Analysis of variance and interaction components for kernel Zn concentration in maize

Source of variation	df	SS	MSS
Genotypes	20	750.62	37.53**
Years	2	1598.78	799.39**
Genotype × Year	40	915.77	22.89**
Error	63	66.62	1.05

df: Degrees of freedom; SS: Sum of squares; MSS: Mean sum of squares; *Significant at P = 0.05; *Significant at P = 0.01

Table 3 ANOVA and variance components (based on Eberhart and Russell model)

Source of variation	df	SS	MSS
Replications within year	3	2.36	0.79
Genotypes	20	375.31	18.77*
Year + Gen. × Year	42	1257.28	29.94**
Year (linear)	1	799.39	799.39**
Gen. × Year (linear)	20	284.36	14.21
Pooled deviation	21	173.53	8.26*
Pooled Error	60	30.95	0.51

d.f.: Degrees of freedom; SS: Sum of squares; MSS: Mean sum of squares; *Significance at P = 0.05; *Significance at P = 0.01

–5.03, 2.69 and 2.35 during 2006, 2007 and 2008, respectively, indicating that *kharif* 2006 was the most unfavourable environment for the accumulation of kernel Zn concentration, while *kharif* 2007 was more optimal environment, followed by *kharif* 2008.

The genotype × environment interaction for kernel micronutrients is generally attributed to soil nutrient profile, which influences the accumulation of micronutrients in grain (Oikeh *et al.* 2003). This holds true when experiments are conducted under different locations with different soil profiles. However, in the present study, the trials were undertaken at the same station (CSK-HPKV, HAREC, Bajuara) under similar soil profiles during 2006-2008. The Zn concentration of the soil was 0.50 ppm during 2006, while it was 0.58 ppm and 0.54 ppm, during 2007 and 2008, respectively. Thus, the G × Y interactions could not be attributed to the status of soil Zn alone. As regards to weather parameters, the maximum and minimum temperatures remained almost similar in the *kharif* season (June-September) of 2006, 2007 and 2008. However, *kharif* 2006 and 2007 experienced 380.30 mm and 369.80 mm of total rainfall during the crop growth period, while the total rainfall was substantially higher (627.70 mm) during 2008. It is important to note that kernel Zn reported highest mean during 2007 that experienced least rainfall among three years (Table 4). Similar observation regarding accumulation of high Zn in maize kernel with respect to less rainfall has also been reported by Prasanna *et al.* (2011) and Agrawal *et al.* (2012). Ferreira *et al.* (2012), while experimenting with 10 maize cultivars grown at Rolandia County, Parana State, Brazil also observed similar trend and emphasized significant effects of soil water availability on grain micronutrient concentration. The per se performance of maize genotypes especially for micronutrients is dependent on complex network of diverse factors related to genotypic constitution, its interaction with environment, soil dynamics and micro-climates (Feila *et al.* 2005, Gorsline *et al.* 1964, House 1999). Even changes of minor degree in one factor, in combination with other factors, could lead to significant variation in micronutrient traits. Factors such micro-environmental variations, spatial and temporal variation, system variations caused by the differential management practices are also reported to cause significant effects on the accumulation of micronutrients (Pfeiffer and McClafferty 2007).

In spite of significant G × Y effects on kernel Zn concentration, it is possible to identify genotypes that show considerable stability in mineral concentrations across years (Gregorio 2002). Taking into consideration the regression coefficient and deviation from linearity V336, VQL 1, V 334 and CM 139 were found to be stable across years (Table 4). Among these stable inbreds, V 336 had higher kernel Zn concentration (17.25 mg/kg, across three years) as compared to grand mean (16.21 mg/kg) of the population. The mean kernel Zn concentration of other three inbreds was although lower than the grand mean but was quite comparable to the 'grand mean' of the population. Considering this, V 336, VQL 1, V 334 and CM 139 were thus identified as promising genotypes.

Table 4 Mean kernel Zn concentration (mg/kg) of inbreds in different years and mean estimates of stability parameters

Inbreds	Kernel Zn (mg/kg)				S^2_{di}	b_i
	2006	2007	2008	Grand mean		
BAJIM 06-1	12.64	15.23	17.57	15.15	2.61*	0.49
BAJIM 06-6	6.05	20.53	20.02	15.53	–0.52	1.88*
BAJIM 06-8	8.92	18.40	15.43	14.25	2.88*	1.07
BAJIM 06-10	15.17	22.47	24.74	20.79	2.97*	1.11
BAJIM 06-15	15.09	23.20	16.17	18.15	22.72**	0.64
BAJIM 06-19	6.87	24.66	16.51	16.01	27.82**	1.85
BAJIM 06-20	7.26	13.50	16.77	12.51	6.03**	1.03
CM 128	14.42	23.07	17.25	18.25	14.92**	0.79
CM 129	8.26	20.39	19.63	16.09	–0.50	1.56*
CM 139	12.36	14.64	15.24	14.08	–0.27	0.34
CM 140	8.45	21.58	21.09	17.04	–0.53	1.71*
CM 145	16.03	25.89	22.18	21.37	5.08**	1.08
CM 153	17.13	17.41	14.65	16.40	3.41**	–0.13
CM 212	6.06	11.09	17.71	11.62	23.89**	1.07
HP 2	16.18	24.81	19.69	20.23	11.20**	0.83
V 334	9.91	17.23	15.61	14.25	0.35	0.87
V 336	6.15	22.25	23.36	17.25	1.19	2.20
V 340	10.37	15.55	20.56	15.49	13.79**	0.99
V 348	9.33	15.70	20.17	15.07	11.24**	1.12
VOL 1	12.14	17.23	17.87	15.75	–0.14	0.71
VQL 5	16.00	12.08	17.58	15.22	14.29**	–0.18
Mean	11.18	18.90	18.56	16.21	7.73	1.00
± SE	0.96	0.97	1.10	2.03	2.00	0.47

BAJIM: Bajaura Inbred Maize; CM: Coordinated Maize (from All-India Coordinated Maize Improvement Project); V: Vivek Inbred; VQL: Vivek QPM Line; HP: HarvestPlus-CIMMYT line; b_i: Regression coefficient; S^2_{di}: Deviation from regression; *Significant at P = 0.05; ** Significant at P = 0.01.

The study thus revealed significant genetic variation for kernel Zn concentration in the Indian and exotic maize genotypes, suggesting adequate scope for genetic improvement. Although kernel Zn was affected by years and G × Y, it is possible to identify genotypes that show relatively stable performance. Promising and stable genotypes, identified in the present study could be potentially utilized in development of kernel Zn- enriched maize cultivars adapted to the hill regions of India.

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