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Genetic evaluation of kernel Fe and Zn concentrations and yield performance of selected Maize (*Zea mays* L.) genotypes

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Abstract

Twenty-five maize inbred lines, including 10 QPM and 15 non-QPM lines, were analyzed for kernel micronutrient concentrations (iron and zinc) at two different locations (Delhi and Bajaura) in India. The study revealed considerable genetic variability and significant genotype x environment (GxE) interaction for the target traits under study. Genotypes with high kernel Fe and Zn concentrations at both locations were identified. The study also revealed significant and positive correlation between kernel Fe and Zn concentrations, indicating the possibility of simultaneous improvement of both the traits. The genotypes also showed significant variation for various yield related traits. Most of the yield components displayed significant positive correlation with yield, except days to 50% anthesis and silking, which also displayed negative correlation with kernel Zn concentration. Among all the traits, kernel number per ear row was found to have significant direct effect on grain yield in both genotypic and phenotypic path analysis, whereas no significant direct or indirect effects of kernel iron and zinc concentration on grain yield was observed. The study, thus, provides a foundation for breeding kernel micronutrient-rich and high yielding maize genotypes in the Indian context.

Key words : Biofortification, Iron, Maize, Zinc

Introduction

Maize (*Zea mays* L.) provides a large proportion of the daily intake of energy and other nutrients, including micronutrients for poor populations in many areas of South East Asia and sub-Saharan Africa. Besides, it is also used as green fodder for livestock and feed for poultry and piggery. However, most of the staple food crops, including maize, display very low concentration of micronutrients in the kernels, especially Fe and Zn. More than half of the world's population, especially women and children from the developing countries, suffer from micronutrient malnutrition or 'hidden hunger', resulting from the consumption of staple foods with very low levels of bioavailable vitamins and minerals (UNSCN, 2004). Micronutrient deficiency in the grains also reduces its nutritional value as feed. To combat this problem, the strategy of 'biofortification' is now being employed to develop genotypes of staple foods whose edible portions are denser in bioavailable minerals and vitamins (Lodha *et al.*, 2005). Biofortified maize holds considerable significance both as a food and feed worldwide, particularly in countries like India where a major portion of maize produced is used as feed for poultry and piggery sector and significant proportion (~25%) is used for human consumption (Kaul *et al.*, 2009).

Improving the nutritional quality, especially for micronutrients, is now an important breeding goal in maize, particularly for kernel Fe and Zn concentrations and Provitamin A (Pfeiffer and McClafferty, 2007). To breed high yielding crops with improved quality, evaluation of genetic variability for the target traits, besides grain yield and its components, is one of the important steps towards successful implementation of the biofortification strategy. It is also useful to study the interrelationships among various component characters to develop selection criteria for improvement of the target traits. Therefore, the present study was undertaken to analyze the variability for kernel Fe and Zn concentration, besides assessing the performance of the genotypes for yield-related traits and analyzing the inter-relationships among the kernel micronutrient and yield-related traits.

Materials and Methods

Twenty-five inbred lines, including 10 QPM and 15 non-QPM genotypes, were evaluated at two locations: (i) IARI, New Delhi (28^{0}40'N, 77^{0}12'E, 218 amsl) and (ii) CSK-HPKV Regional Research Station, Bajaura (Himachal Pradesh) (28^{0}40'N, 77^{0}12'E, 218 amsl) during *kharif* 2008.

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The materials consisted of a set of 25 inbred lines, including 14 inbred lines developed at CSK-HPKV Regional Research Station, Bajaura [designated as 'BAJIM' lines], one inbred line (V334) from VPKAS, Almora; eight QPM lines developed at different research institutes [designated as 'DQPM', DMRQPM, 'VQL' lines] and two QPM inbred lines from CIMMYT, Mexico [CML162 and CML189].

The materials were planted in randomized complete block design (RCBD) with two replications per entry (one row 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 the plants. Five random plants from each row were selfed and the rest was allowed to open-pollinate. For kernel micronutrient analysis, only the selfed ears were hand-harvested, following the procedure suggested by HarvestPlus (http://www.harvestplus.org). Data for yield components were recorded on the ears of five randomly sampled open-pollinated ears for each entry, while data for kernel micronutrient was taken from the control-pollinated ears. Data were recorded on ten relevant yield components - grain yield (kg/plot), ear length (cm), ear diameter (cm), number of kernel rows, kernel number per row, hundred kernel weight (g), plant height (cm), ear height (cm), days to 50% anthesis and silking, besides kernel Fe and Zn concentrations.

Biochemical analysis for kernel Fe and Zn concentrations was carried out using the protocol described by Zarcinas *et al.* (1987) with some modifications suggested by Singh *et al*. (2005). The analysis was done on triplicate samples by open air digestion with 9:4 diacid mixture, followed by atomic absorption spectrometry (AAS) method using ECIL AAS (Perkin Elmer) at the Grain Quality Laboratory, Division of Genetics, IARI, New Delhi.

ANOVA was carried out using SAS-6.12 (SAS Institute; 1989) while D^2 analysis, estimation of correlation coefficients and path analysis were undertaken using the Windostat (Version 8.0, Indostat Services, Hyderabad).

Results and Discussion

ANOVA of individual datasets revealed significant variation for both kernel Fe and Zn concentrations at both Delhi and Bajaura (Table not shown), indicating the presence of considerable genetic variability that can be utilized for genetic improvement of kernel micronutrient traits in maize. Several workers (Banziger and Long, 2000; Dixon *et al.,* 2000; Oikeh *et al*., 2003a & b, 2004) also reported the presence of significant variation among maize genotypes for the kernel Fe and Zn concentrations. Thus, the study confirmed that genes necessary for micronutrient enrichment traits are available within the maize genome that could allow for substantial increases in kernel Fe and Zn content.

Mean kernel micronutrient concentration of the genotypes is presented in Table 1. The range for kernel Fe concentration was found to be 13.60-31.83 mg/kg (mean 23.23 mg/kg) at Delhi, 13.23-40.09 mg/kg (mean 22.18 mg/kg) at Bajaura and 13.41-31.12 mg/kg (mean 22.58 mg/kg) based on the mean across two locations. In a similar study at CIMMYT, Mexico and Zimbabwe, Banziger and Long (2000) reported a range of 9.6-63.2 mg/kg for Fe, while, Dixon *et al.* (2000) found that the Fe content varied from 13.60 to 159.43 mg/kg. Oikeh *et al.* (2003a) reported a very narrow range of 16.8-24.4 mg/kg Fe in a different set of maize genotypes. In the present study, mean kernel Fe concentration for all the genotypes at different locations showed similar pattern. DQPM-8 was the most promising genotype with mean Fe concentration of 31.12 mg/kg, closely followed by CML162 (29.89 mg/kg).

The range for kernel Zn concentration was found to vary from 21.92 to 46.39 mg/kg (mean 32.96 mg/kg) at Delhi, while the range for Bajaura and across locations were 13.44-24.14 mg/kg (mean 18.93 mg/kg) and 19.47-32.85 mg/kg (mean 25.94 mg/kg), respectively. Mean kernel Zn concentration for all the genotypes at two different locations was found to be significantly different. The Zn concentration of all the genotypes grown at Bajaura was much lower as compared to Delhi. CML162 was identified as the most promising genotype with an overall mean of 32.85 mg/kg kernel Zn concentration (Table 1), followed by DQPM-4 (32.26 mg/kg). Interestingly, four among the top five inbred lines for kernel Zn concentration across locations were the QPM lines. The highest kernel Zn concentration among the non-QPM lines was 28.26 mg/kg (BAJIM-08-06). Banziger and Long (2000) reported a range of 12.9-57.6 mg/kg for kernel Zn in a multi-location trial of 1800 maize genotypes including inbreds and landraces. Interestingly, narrow ranges of 14.7-24.0 mg/kg and 18.5-28.6 mg/kg were recorded in their study for the same trait on a smaller set (20 genotypes) at two different locations. Dixon *et al*. (2000) observed a range of 12 to 96 mg/kg for mid-altitude inbred lines and 24 to 96 mg/kg for lowland inbred lines. Oikeh *et al.* (2003a) reported a narrow range of 16.5-20.5 mg/kg kernel Zn. In a separate experiment on 49 inbred lines, Oikeh *et al.* (2003b) found comparatively wider range for kernel zinc concentration (16.5 to 24.6 mg/kg).

Table 1: Mean micronutrient content of inbred lines grown at two environments.

Genotype	Fe (mg/kg)			Zn (mg/kg)		
	Delhi 08	Bajaura 08	Mean	Delhi 08	Bajaura 08	Mean
DQPM-1	25.70	24.35	25.03	36.41	15.67	26.04
DQPM-2	23.24	16.93	20.09	37.59	13.47	25.53
DQPM-3	22.80	13.83	18.32	33.90	20.59	27.24
DQPM-8	22.15	40.09	31.12	33.06	23.97	28.52
DQPM-4	26.34	18.34	22.34	43.60	20.92	32.26
DMRQPM 58	26.33	13.41	16.73	32.85	18.25	25.55
VQL1	26.46	27.65	27.05	37.13	19.29	28.21
VQL5	22.29	21.31	21.80	35.06	18.23	26.65
CML162	31.83	27.96	29.89	46.39	19.30	32.85
CML189	22.06	21.17	21.62	40.02	23.87	31.95
BAJIM-06-20	22.70	19.83	21.30	26.98	14.12	20.55
BAJIM-08-01	22.30	23.97	23.13	30.08	21.71	25.90
BAJIM-08-02	23.70	23.56	23.63	21.92	17.02	19.47
BAJIM-08-03	16.39	17.46	16.93	36.26	20.26	28.26
BAJIM-08-04	13.60	13.23	13.41	23.84	17.18	20.51
BAJIM-08-05	22.59	35.20	28.89	27.82	24.14	25.98
BAJIM-08-06	17.94	17.16	17.55	29.44	20.37	24.91
BAJIM-08-07	23.99	18.75	21.37	31.32	19.47	25.39
BAJIM-08-08	18.09	20.73	19.41	34.12	13.44	23.78
BAJIM-08-09	26.61	20.42	23.52	24.38	22.16	23.2
BAJIM-08-10	24.06	20.40	22.23	33.62	17.67	25.63
BAJIM-08-11	21.30	25.94	23.62	34.36	20.25	27.30
BAJIM-08-12	26.75	26.14	26.44	23.32	16.02	19.67
BAJIM-08-13	25.03	22.54	23.79	35.48	20.78	28.13
V334	26.53	24.10	25.31	34.94	15.09	25.01
Grand mean	23.23	22.18	22.58	32.96	18.93	25.94
LSD	3.00	4.27	2.63	4.35	4.65	3.21

Fe: Kernel iron concentration; Zn: Kernel zinc concentration

The kernel Fe and Zn concentration of the 25 inbred lines were subjected to pooled ANOVA (Table 2). The role of environment was found to be prominent for kernel Zn concentration, while for kernel Fe the effect was not significant. However, G×E interaction was found to play an important role in the expression of both the micronutrient traits. Mean kernel Fe content for all the genotypes under different environments was found to follow the similar trend, while mean kernel Zn content for all the genotypes under different environment was found to be significantly different indicating greater influence of environment on kernel Zn concentration as compared to kernel Fe. Besides, the relative ranking of the genotypes changed for both the micronutrients in different environments for both the trials, signifying once again the possible influence of G × E interaction on the expression of the target traits, confirming similar observations made in some earlier studies on maize (Oikeh *et al.,* 2003a,b, 2004; Menkir, 2008)

Table 2 : ANOVA (Pooled) of micronutrient trials at two environments

Source of variation	Mean Sum of Squares		
	d.f.	Fe	Zn
Replication	1	0.75	0.02
Genotypes	24	36.17**	25.33**
Locations	1	16.11	2459.74**
Genotype × Location	24	16.12**	21.31**
Error	49	3.43	5.12

*Significant at P = 0.05; ** Significant at P = 0.01;

Fe: Kernel iron concentration; Zn: Kernel zinc concentration

Kernel micronutrient concentration is affected by an array of factors, including soil type and fertility status, soil moisture, microenvironment variation, G × E interaction, genotypic variation, soil profiles, crop management practices, interactions among nutrients, etc. (Gorsline *et al.*, 1964; Arnold and Bauman, 1976; Arnold *et al.*, 1977; House, 1999; Oikeh *et al.*, 2003a, b; Feil *et al.*, 2005; Pfeiffer and McClafferty, 2007). In the present study, it was evident from the fact that the Zn content of all the genotypes grown at Bajaura was much lower (mean 18.93 mg/kg) as compared to Delhi (mean 32.96 mg/kg). Among the factors that contribute to Zn deficiency in plants are low organic matter in soil, low soil water regime, low soil temperature, and high light intensity (Moraghan and

Mascagni, 1991). With respect to micronutrient status, the soil at the IARI Experimental Farm in general is slightly deficient in Fe as well as Zn. In contrast, analysis of the soil samples at CSK-HPKV Research Station, Bajaura revealed it to be micronutrient-deficient, particularly for Zn. Besides lower micronutrient status at Bajaura, relatively lower temperatures during crop growth could also have affected the kernel micronutrient concentrations. Zinc deficiency was reported to be more prevalent under low soil temperature conditions, possibly due to reductions in root growth, VA mycorrhizal infection, Zn uptake by roots and Zn translocation into shoots (Moraghan and Mascagni, 1991). Despite various factors affecting the kernel micronutrient status and the differential behavior of the same set of genotypes in different locations, the study was successful in identifying some promising inbred lines for kernel micronutrients, especially Zn, in the germplasm analysed.

Considering the mean values across the two locations for kernel Fe concentration, DQPM-8 was found to be the most promising genotype with mean Fe content of 31.12 mg/kg, followed by another QPM genotype, CML162, with a mean of 29.89 mg/kg. However, these values were still lower than the threshold levels identified by HarvestPlus, that is, 40 mg/kg or above for Fe (Pfeiffer and McClafferty, 2007). For kernel Zn concentration, CML162 was found to be promising (32.85 mg/kg across locations), followed by DQPM-4 (32.26 mg/kg) (Table 1). With respect to Zn, a threshold level of 30 mg/kg and above has been recommended by HarvestPlus (Pfeiffer and McClafferty, 2007); this criterion is fulfilled by the promising genotypes identified in this study.

The present study demonstrated that kernel micronutrient status of the QPM genotypes is relatively better than the non-QPM genotypes. The QPM genotypes recorded comparatively higher kernel Zn concentrations as compared to those of the non-QPM genotypes. Two tailed-'t' test revealed highly significant difference between the QPM and non-QPM group of genotypes for kernel Zn concentration at Delhi as well as in pooled analysis. However, such a difference was not observed for kernel Fe concentration at any location and for kernel Zn concentration at Bajaura. The micronutrient advantage of QPM genotypes observed in the present study may be considered as a preliminary indication, considering the limited set of QPM and non-QPM lines evaluated in this study, and needs further confirmation. It is interesting to note that some researchers also reported higher micronutrient density in *opaque*2 genotypes as compared to the normal maize (Bauman, 1975; Arnold *et al.*, 1977; Gupta *et al.*, 1980; Welch *et al.*, 1993). Arnold *et al.* (1977) attributed the high Zn concentration in kernels of *o2/o2* genotypes to the pleiotropic effect of *opaque2* allele or its close linkage with Zn enhancement genes. Gupta *et al.* (1980) reported that some maize genotypes having *o2* gene accumulate higher concentrations of Fe, Zn, Mn, Mg and Ca as compared to normal maize.

Besides kernel micronutrient status, it is important to ascertain the *per se* performance of the genotypes. In the present study, the genotypes were also evaluated for various yield related traits (only at Delhi location) to consider their potential use in breeding programmes. D^2 analysis revealed that the genotypes used in present study were highly diverse (Fig. 1). The BAJIM lines (developed at Bajaura, Himachal Pradesh) which are adapted to hill regions, were mostly grouped in cluster 1 and the QPM lines analyzed in the study were found in two clusters (2 and 3), except DQPM-1 which behaved as an outlier. BAJIM-08-3 and BAJIM-08-12 were also found to be distinct among the lines analyzed. Besides some yield components, kernel Fe and Zn concentrations made significant contribution towards the genetic divergence of the lines, again indicating the presence of significant variability for these traits. Presence of such genetic variation for the micronutrient traits, coupled with grain yield and its attributes, is highly encouraging as this provides a possibility to breed micronutrient-enriched maize genotypes with no yield penalty due to the 'dilution effect' (Oikeh *et al.*, 2003a & b).

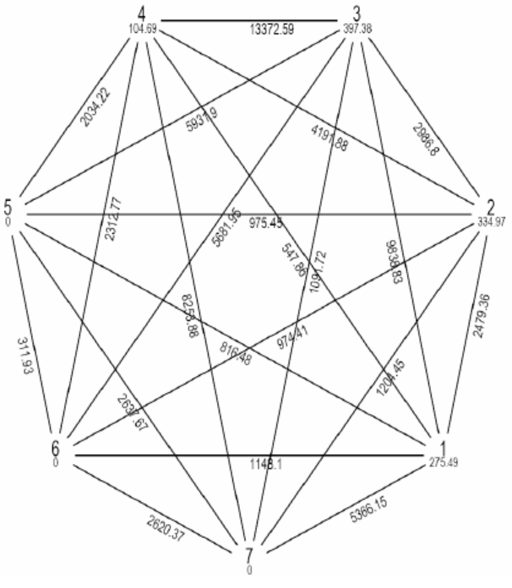

Fig. 1:D^2 clustering pattern among 25 maize inbred lines