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Generation mean analysis of kernel iron and zinc concentrations in maize (*Zea mays*)

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ABSTRACT

Alleviating micronutrient malnutrition through biofortification of food crops is an important objective of breeding programmes. Of particular importance is breeding for micronutrient enrichment, especially iron and zinc in crops, like maize (*Zea mays* L.). In the present study, generation mean analysis was undertaken for the first time to estimate the nature and magnitude of gene effects for kernel iron and zinc concentrations in 2 specific crosses of maize, with contrasting parental lines. Scaling test indicated the influence of epistasis on the expression of both micronutrients and inadequacy of additive-dominance model to explain the variation in different generations. Similar types of components of gene action for a particular micronutrient were observed, albeit some differences in the direction and magnitude of effects. Additive gene action was of higher magnitude as compared to dominance for kernel iron, while dominance was relatively higher in case of kernel zinc. Additive×dominance component was significant in both the crosses for kernel Fe, whereas additive×additive component was predominant for kernel zinc. Different non-additive components acted in opposite directions in both the crosses for kernel Fe, which could be a bottleneck to exploit heterosis. Recurrent selection and population improvement programmes, coupled with development of superior heterotic pools of inbred lines with enhanced kernel iron and zinc concentrations, could be effective in improving concentrations.

Key words: Epistasis, Generation means, Genetic variance, Iron, Kernel micronutrients, Maize, Zinc

More than half of the world's population, especially women and children of the developing countries suffer from micronutrient malnutrition or 'hidden hunger' resulting from the consumption of diets with meager bioavailable vitamins and minerals (UNSCN 2004, Lodha *et al.* 2005). Even mild levels of micronutrient deficiency may damage cognitive development and lower the resistance to diseases in children. Plant breeding can aid in producing staple foods enriched with micronutrients, a process referred to as 'biofortification' (Bouis 2002). Available data indicate that genes necessary for micronutrient enrichment traits are available in genomes of major staple crops like rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L. cmend. Fiori & Paol.) and maize (*Zea mays* L.) which could allow for substantial increases in kernel iron and zinc content without any negative impact on grain yield (Banziger and Long 2000). Biofortified maize holds considerable significance both as a food and feed worldwide, particularly in countries like India, where a significant proportion (~25%) of maize produced is used for human consumption. 'HarvestPlus', an International Collaborative Programme is presently focusing on biofortification for 3 micronutrients, kernel Fe, Zn and Provitamin A in target crops like maize (Pfeiffer and McClafferty 2007).

The estimates of gene action in crop improvement programmes have direct bearing upon the choice of breeding procedures to be followed. Biofortification programmes should focus, first, on exploring the available genetic diversity for the targeted micronutrients to identify potential genotypes that can be used in crosses, genetic studies, gene pool development, mapping population development, etc. Studies aimed at exploring the nature and magnitude of gene action (additive, dominance and epistasis), mapping of quantitative trait loci (QTL) and quantifying their interactions may prove to be useful. There is no published report till date on detailed genetic dissection on kinds and magnitude of epistatic gene action controlling the kernel iron and zinc concentrations in maize. The present study, therefore, was aimed at utilizing generation mean analysis for understanding the genetic components influencing kernel micronutrient traits in maize.

MATERIALS AND METHODS

Four genetically diverse maize inbred lines ('CM145', 'V334', 'CM128' and 'V340') with significant phenotypic

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contrast for kernel Fe and Zn concentrations were selected for this study. The mean kernel Fe and Zn concentrations (mg/kg) in each of these lines, based on experiments undertaken at the IARI experimental farm, New Delhi during 2006–07 are (*i*) 'CM145': Fe 13.92; Zn 25.14; (*ii*) 'V334': Fe 28.12; Zn 31.27; (*iii*) 'CM128': Fe 27.61; Zn 24.71; (*iv*) 'V340': Fe 16.56; Zn 35.64. Six generations [P_1, P_2, F_1, F_2, $BC(P_1)$ and $BC(P_2)$] were derived during rainy (*kharif*) season of 2007 and winter (*rabi* 2007–08) at IARI, New Delhi, and Maize Winter Nursery, Hyderabad from two experimental crosses, namely 'CM145'×'V334' (Cross 1) and 'CM128'×'V340' (cross 2).

The 6 generations of each cross combination were evaluated in experimental trials during rainy (*kharif*) season 2008 at IARI experimental farm, New Delhi. Soil analysis indicated Fe concentration of 4.93 ppm at 0–15 cm depth, and 4.85 ppm at 15–30 cm depth, while the soil Zn concentrations were 0.85 ppm and 0.78 ppm at 0–15 cm and 15–30 cm respectively. The average maximum temperature during the season was 33.2°C, while the average minimum temperature was found to be 24.1°C. High rainfall (583.70 mm) was recorded during the experimental season. The genotypes were control-pollinated (by selfing) and samples of 10 ears each from the parental lines, 20 from F_1, 60 from F_2, and 40 each from $BC(P_1)$ and $BC(P_2)$ were selected randomly from each of the populations and were analyzed for the kernel Fe and Zn concentrations, following the protocol suggested by 'Harvest Plus'. After kernel maturation and plant dry down, ears with the husk were hand-harvested and dried under clean shade to lower the grain moisture content to 14%. The individual ears were hand-shelled and triplicate grain samples were collected by quartering method. The grain samples were dried in an oven for 48 hr at 60°C.

The grain samples were ground into a fine powder using a mill (Cyclotech Sample Mill 1093, Hooganas, Sweden). Kernel iron and zinc concentrations were determined by open air digestion with 9 : 4 di-Acid (HNO_3 : $HClO_4$), followed by atomic absorption spectrometry (AAS) method using ECIL AAS (Perkin Elmer). The basic protocol described by Zarcinas *et al.* (1987) with some modifications suggested by Singh *et al.* (2005) was followed for estimation of kernel micronutrients.

The generation means were estimated by considering means of kernel micronutrient concentration of the grain samples of ears analyzed for that generation. The adequacy of the additive-dominance model was tested by scaling test (Mather 1949) as well as joint scaling test (Cavalli 1952). The adequacy of the additive-dominance model, consisting of mean, additive, and dominance genetic effects was tested via Chi-square test. When this model was not found to be sufficient to explain variation among generation means, non-allelic interaction parameters, ie additive × additive, additive × dominance, and dominance × dominance were included in the model and tested to identify the best fit model, based on the procedure suggested by Mather and Jinks (1982). The number of major gene blocks affecting kernel Fe and Zn concentrations was calculated by Castle-Wright's equation as described by Sharma (1998). Statistical analysis was carried out using Windostat Version 8.0 software.

RESULTS AND DISCUSSION

Joint scaling test for the three parameter model indicated the inadequacy of additive-dominance model and the influence of non-allelic interactions on the expression of kernel Fe concentration (Table 1). The results obtained from the best fit model for kernel Fe showed that in case of 'CM145'×'V334', additive×dominance type of epistatic interaction played an important role, besides additive and dominance effects. While the estimates of additive and dominance components were in the negative direction, the additive×dominance was found to act in the positive direction. Interestingly, additive×dominance component detected in this cross combination was found to contribute maximum for kernel Fe concentration as compared to the individual main effects (Table 1). The non-additive gene effect (dominance and additive×dominance) was observed to be predominant over the additive gene effect. In case of 'CM128'×'V340', all the three types of epistatic interactions (additive×additive, additive×dominance and dominance×dominance) were found to be significant. Again the total contribution of epistatic effects was observed to be higher than the additive main effects (Table 1). Among the different epistatic components, dominance×dominance effects had the maximum contribution and was in the positive direction along with the additive×additive effects, while the additive×dominance epistasis acted in the negative direction. The non-additive gene action (additive×dominance and dominance× dominance) were predominant over the additive gene action (additive and additive×additive).

The study also revealed the presence of significant non-allelic interactions for kernel Zn concentration in both the cross combinations (Table 1). In case of 'CM145'×'V334', the best fit model detected the importance of additive×additive type of epistasis, besides additive and dominance main effects. The overall contribution of non-additive component (particularly dominance) was relatively more as compared to the additive gene action. In various generations of 'CM128'×'V340', we found that additive×dominance played a relatively more important role than additive×additive, unlike the case of 'CM145'×'V334', where additive×dominance did not show significant effect. Except for the additive component, all the other components acted in the positive direction in case of 'CM128'×'V340'. The study also revealed the pre-dominance of non-additive gene actions over the additive type. Among all the relevant components, additive×dominance, followed by dominance, revealed maximum contributions towards the kernel Zn concentration.

Table 1 Estimates of joint scaling test, genetic components in the best fit model, heterosis and heritability for kernel Fe and Zn concentrations

Parameters	'CM145'×'V334'		'CM128'×'V340'	
	Kernel Fe	Kernel Zn	Kernel Fe	Kernel Zn
Joint scaling test				
m (Mean)	21.23±0.49**	29.14±0.73**	21.52±0.49**	28.60±0.59**
d (Additive)	–6.69±0.49 **	–2.58±0.76 **	5.35±0.48**	–3.09±0.58**
h (Dominance)	–5.91±0.92**	–5.83±1.10**	1.21±1.20 ns	5.72±1.19**
χ^2	9.95**	14.77**	30.75**	53.91**
Best fit model				
m (Mean)	20.94±0.50**	34.42±1.82**	16.79±1.18**	23.96±2.10**
d (Additive)	–7.15±0.52**	-2.65±0.76**	5.53±0.50**	–5.46±0.66**
h (Dominance)	–5.54±0.92**	-12.01±2.24**		12.74±2.87**
i (Add.×Add.)		-6.49±2.05**	5.26±1.31**	6.27±2.24**
j (Add.×Dom.)	9.40±3.31**		–7.62±3.65**	19.36±2.90**
l (Dom.×Dom.)			10.88±2.19**	
χ^2	1.85ns	4.76ns	0.23ns	0.34ns
Genetic parameter				
Average heterosis (%)	–25.87	–19.73	25.62	21.09
Heterobeltiosis (%)	–44.58	–27.60	0.48	2.60
Broad sense heritability (%)	78.4	70.8	73.1	76.3

**$P \leq 0.01$; ns, non-significant

The present study thus revealed the significant contribution of non-allelic interactions or epistasis, besides the main effects, for determining the gene action for both kernel Fe and Zn concentration. The study perhaps was the first to analyze the genetics of kernel Fe and Zn concentrations in maize using generation mean analysis thereby dissecting the gene action in depth. In contrast, studies carried out till date to dissect the genetics of kernel Fe and Zn concentration in maize were based on diallel approach (Long *et al.* 2004 and Chen *et al.* 2007) with an assumption of absence of non-allelic interactions and main effects such as additive and dominance effects playing major role. In general, among the three epistatic components, additive × dominance component was common in both the cross combinations for kernel Fe. Similarly, additive×additive type of epistasis was found to be prominent in both the sets of crosses for kernel Zn, indicating the suitability of recurrent selection for general combining ability (GCA) for accumulating desirable gene combinations for kernel Zn concentration. However, different non-additive components were acting in opposite directions in case of kernel Fe for both the crosses, which could act as a bottleneck in exploiting heterosis for kernel Fe concentration. On the basis of diallel analysis, Chen *et al.* (2007) reported predominance of additive gene action along with low level of heterosis for kernel Fe and suggested that hybrid breeding may not be efficient for improving this trait. In the present study, in case of 'CM128'×'V340', all the three types of epistatic interactions (additive × additive, additive × dominance and dominance × dominance) were found to be significant for kernel Fe concentration, among which, dominance× dominance effects had the maximum contribution and was in the positive direction along with the additive × additive effects, while the additive×dominance epistasis acted in negative direction. Despite of predominance of non-additive gene action, significant positive heterobeltiosis was not detected (Table 1), which could be attributed to the 'dilution effect' in the hybrids due to higher endosperm volume (Pfeiffer and McClafferty 2007). The study also demonstrated that for a specific kernel micronutrient, the nature of gene effects could be similar in different crosses, although the magnitude and the directions could vary. Overall, the study revealed the importance of both additive and non-additive gene action in the expression of the kernel Fe and Zn concentrations of maize. The relative importance of additive gene action in expression of kernel Fe and Zn was reported in several studies using diallel analysis (Long *et al.* 2004, Chen *et al.* 2007).

The broad sense heritability ($H= V_G/V_P$) values for kernel Fe concentrations were 78.4% and 73.1% in case of 'CM145'×'V334' and 'CM128'×'V340', respectively, and 70.8% and 76.3% for kernel Zn concentration, in the respective crosses, suggesting the possibility of genetic improvement through breeding (Table 1). Analysis with Castle-Wright's equation for number of effective genes/ blocks (represented as n) revealed that at least one major gene block (n = 0.90 in case of 'CM128'×'V340' and n = 1.03 in case of 'CM145'×'V334') could be involved for the kernel Fe concentration in both the cross combinations besides several minor genes. In case of kernel Zn, the Castle-

Wright's equation showed no such major gene block (n = 0.43 in case of 'CM128'×'V340' and n = 0.12 in case of 'CM145'×'V334'), thereby indicating that several genes with minor effects could be contributing towards accumulation of kernel Zn. These observations suggest that although kernel micronutrient concentrations are polygenically inherited, one or a few major gene blocks might have a major influence on micronutrient concentration in crop plants like maize. Interestingly, kernel Fe and Zn content in crop plants was earlier considered to a be qualitative trait rather than quantitative until results from multi-environment experiments revealed significant G×E interactions, possible influence of several gene blocks and heritability of intermediate magnitude (Pfeiffer and McClafferty 2007).

Based on the results obtained in the present study, recurrent selection and population improvement programmes, coupled with development of superior heterotic pools with enhanced kernel Fe and Zn concentrations could be effective in improving the kernel Fe and Zn concentrations in maize. However, the need for intensive phenotyping (including reliable biochemical analyses for kernel micronutrients) at every stage could be a major challenge in implementing such a breeding programme.

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