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Genetic variability and correlation of kernel micronutrients among exotic quality protein maize inbreds and their utility in breeding programme

M. G. Mallikarjuna, T. Nepolean, Firoz Hossain, K. M. Manjaiah[1], A. M. Singh and H. S. Gupta*

Division of Genetics, [1]Division of Soil science and Agricultural Chemistry, Indian Agricultural Research Institute (IARI), New Delhi 110 012

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Abstract

Micronutrient malnutrition is a widespread problem known to affect millions of children and women. However, the adverse effects of micronutrient deficiency can be overcome through self-targeting, cost-effective and sustainable genetic biofortification approach, which is mainly based on staple food crops. Since maize has emerged as a prominent future crop especially for India, developing maize hybrids that are rich in kernel micronutrients will help in reducing the problems of micronutrient malnutrition. Here, we report variability of kernel Fe and Zn in 120 exotic Quality Protein Maize (QPM) inbreds and kernel Mn and Cu in a representative subset of 68 lines. A wide range of genetic variation was found among genotypes for all the kernel micronutrients: Fe (16.6-83.4 ppm), Zn (16.4-53.2 ppm), Mn (1.7-34.8 ppm) and Cu (0.5-9.5 ppm). Higher mean for kernel Fe and Zn in QPM inbreds suggests possible influence of *opaque2* gene and/or modifiers present in them. Significant and positive correlation was observed among kernel Fe, Zn, Mn and Cu. Genetic diversity based on all four micronutrients grouped 68 lines into three major clusters with a mean coefficient of genetic dissimilarity of 2.46. Parental combinations were selected from different heterotic pools and maturity groups to develop high-yielding hybrids enriched with micronutrients.

Key words: Correlation, diversity, kernel micronutrients, maize, *opaque2,* variability

Introduction

Deficiency of micronutrients including mineral elements causes micronutrient malnutrition, and leads to poor health in human beings and finally perpetuates in the vicious circle of poverty in developing countries by lowering the working efficiency of the population [1]. Iron (Fe), one of the important micronutrients, is required for various metabolic functions of organisms and, its deficiency leads to severe anemia. Almost 2 billion people worldwide, especially in the underdeveloped and developing countries are known to suffer from Fe deficiency [2]. Zinc (Zn) is critical for cellular growth and differentiation of the organisms [3]. Its deficiency causes impaired growth, increased morbidity and mortality of children, along with abnormal neurobehavioral development [1]. Manganese (Mn) and Copper (Cu) are known to play important role in synthesis of vitamins, hormones and enzymes, in addition to healthy functioning of nervous system, blood circulation, fluid regeneration, cellular integrity and energy production [4].

Maize (*Zea mays* L., 2n=20) is an important cereal contributing nearly 34.5 percent of total cereal production in the world [5]. Asia produces almost half of the developing world's maize and three-quarters of the maize in South Asia is consumed directly as food; however, in East Asia a major part of maize is used as animal feed (http://www.idrc.ca). Being one of the staple intakes next to rice and wheat, it contributes substantially to the daily caloric requirement of developing countries. Hence, breeding maize for nutritional quality will serve as a potential tool in addressing hidden hunger.

Presence of sufficient variation is a prerequisite for the improvement of any target trait. Significant differences in concentration of kernel Fe, Zn and other

*Corresponding author's e-mail: hsgupta.53@gmail.com
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mineral elements were reported among large number of landraces, open-pollinated varieties and inbred lines of maize [6, 7]. Quality Protein Maize (QPM) genotypes posses enhanced level of lysine and tryptophan content in the maize kernel as a result of mutation in *opaque2* (*o2*) locus, which helps in reducing protein malnutrition [8]. Therefore, identification of Fe, Zn and other micronutrients rich QPM lines will help in combining improved protein quality with higher level of micronutrients, which may serve as a potential base for the future biofortification programmes. Furthermore, successful incorporation of genes for enhanced mineral concentration from exotic sources into adapted maize germplasm will take prolonged time. Therefore, screening of exotic QPM inbreds adapted in Indian condition for kernel micronutrients will help in identification of genotypes that can be directly used for developing potential maize hybrids with enhanced kernel Fe and Zn. The present investigation was undertaken to (i) study the extent of variability for kernel Fe, Zn, Mn and Cu in exotic QPM inbreds and find out the role of *o2* gene in kernel micronutrient variation, (ii) examine the extent of correlation among kernel Fe, Zn, Mn and Cu and (iii) assess the genetic diversity for kernel micronutrients among exotic QPM lines and to identify the possible heterotic combination for kernel micronutrient concentration.

Materials and methods

Experimental material

A set of 1600 new QPM maize genotypes developed by the CIMMYT breeding programme (S. K. Vasal, Personal Communication) and eight CIMMYT maize lines (CMLs) possessing *o2* genes, which are the part of many national and international breeding programme were selected for the experiment. The new QPM genotypes (here after SKV lines) and the CMLs were grown in 3m rows following standard agronomic practices at the Indian Agricultural Research Institute (IARI) farm, New Delhi during *kharif* 2012. Seeds of 120 QPM lines (Full set) with yellow seed colour were selected based on their adaptability, which includes 114 SKV lines and six CMLs to study the variation for the kernel Fe and Zn concentration. Among these 120 QPM inbreds, a representative subset of 68 inbreds was further selected to understand kernel Mn and Cu variation, and to study the correlation and genetic diversity among all four kernel micronutrients.

Determination of kernel micronutrient concentration

After the crop reached physiological maturity, 3-5 selfed cobs per entry were harvested with husk and were dried under shade. The husk was removed from each ear and seeds were manually shelled by clean hands wearing contaminant-free gloves on a dust free and clean surface. Seeds were placed in a clean plastic tray and representative grain samples were sampled by quartering method. The seed samples were further dried at 40ºC for 5 days in a clean, contaminant-free and uncorroded oven. Utmost care was taken at every step to exclude dust and metal contamination. From each representative sample, 15-20 g of seeds were ground into fine powder using a Retsch Mill (Retsch, Mixer Mills: MM 400) with zirconium oxide grinding jar (http://www.harvestplus.org/content/crop-sampling-protocols-micronutrient-analysis). Three technical replications were drawn from each ground sample. The flour sample (0.5 g) was digested as per modified di-acid protocol [9] using microwave digestion system (Anton Parr: Multiwave ECO) available at Division of Genetics, IARI, New Delhi. The concentration (in ppm) of Fe, Zn, Mn and Cu were analysed using an inductively coupled plasma-mass spectrometry (ICP-MS) with auto-sampling protocol (Perkin Elmer, Model: NexION 300 ICP-MS) at Division of Soil Science and Agricultural Chemistry, IARI, New Delhi.

Statistical analysis

Correlation, variability, and standard error were analysed using SAS v 9.2 for Windows [10]. Dissimilarity matrix was calculated using kernel Fe, Zn, Mn and Cu concentrations for assessing the genetic diversity and a dendrogram was generated by weighted-neighbour joining method based on usual Euclidean distance using Darwin 5.01 [11] to visualise the grouping pattern.

Results and discussion

Variability for kernel Fe and Zn in the full set

A significant variation for kernel Fe and Zn concentration was observed among 120 QPM lines. The kernel Fe concentration ranged from 16.6-83.4 ppm with a mean of 36.6 ppm whereas, it was 16.4-53.2 ppm with a mean of 29.8 ppm for kernel Zn (Table 1). Such variations suggested that the genes necessary for micronutrient enrichment were available within the maize germplasm and therefore could be used for improving kernel Fe and Zn concentration through

Table 1. Mean and range of kernel Fe and Zn in the full set (120) and kernel Fe, Zn, Mn and Cu in the representative subset (68) of QPM inbreds

Micronutrients	Full set			Subset		
	Range (ppm)	Mean (ppm)	S.E (±)	Range (ppm)	Mean (ppm)	S.E (±)
Fe	16.6-83.4	36.6	1.1	16.6-83.4	36.5	1.9
Zn	16.4-53.2	29.8	0.7	16.4-53.2	29.5	1.1
Mn	–	–	–	1.7-34.8	11.9	0.8
Cu	–	–	–	0.5-9.5	3.4	0.2

appropriate breeding strategies. Significant variation among maize genotypes for kernel Fe and Zn concentration were also reported by Agrawal *et al*. [12] Banziger and Long [6], Chakraborti *et al*. [13], Guleria *et al.* [14], Menkir [15], Oikeh *et al*. [16] and Pixley *et al.* [17]. Among inbreds, SKV616 recorded the highest kernel Fe concentration (83.4 ppm) and SKV3 recorded the lowest (16.6 ppm). Whereas, for kernel Zn, SKV343 recorded the highest (53.2 ppm) and CML163 (16.4 ppm) recorded the lowest concentration. However, the inbreds, SKV343, SKV616, SKV612, SKV672, SKV617, SKV613, SKV675, SKV263 recorded both kernel Fe and Zn concentration above the target level (Fe: >60 ppm, Zn: >38 ppm) [18]; whereas, 13 inbreds recorded higher concentration of kernel Zn only. The present investigation revealed the presence of higher kernel Fe (Mean: 36.6 ppm) and Zn (Mean: 29.8 ppm) as compared to earlier reports which were predominantly based on non–QPM lines: Agrawal *et al*. [12] (Fe: 33.17 ppm, Zn: 20.29 ppm), Chakraborti *et al*. [13] (Fe: 23.6 ppm, Zn: 29.7 ppm) and Menkir [15] (Fe: 20.0 ppm, Zn: 30.0 ppm). Furthermore, Agrawal *et al*. [12] reported slightly higher mean kernel Fe and Zn concentration in QPM inbreds (Fe: 34.77 ppm; Zn: 20.34 ppm) as compared to the mean of non-QPM inbreds (Fe: 32.95 ppm; Zn: 20.28 ppm). In the present investigation, we recorded higher concentration of kernel Fe as compared to kernel Zn which is in accordance with that of the results reported by Agrawal *et al*. [12], Banziger and Long [6] and Simic *et al*. [7] but in contrast to Chakraborti *et al*. [13] who reported higher kernel Zn concentration.

Despite high mean values, the variation for kernel Fe and Zn observed among the QPM lines was very broad, indicating possible influence of *o*2 gene and/or modifiers present in the genetic background of QPM inbred lines. Arnold *et al*. [19] reported higher concentration of Zn in QPM kernels as compared to non-QPM lines. Further, increase in concentration of kernel minerals including kernel Fe and Zn was observed in *o*2 version of several non-QPM maize lines, though it was not found to be universal [20]. It is assumed that in addition to *o*2, the endosperm modifiers, which are known to alter the endosperm structure and protein composition [21], may play a major role in kernel micronutrient concentration. The number of endosperm modifiers and their interactions vary from genotype to genotype; thus a huge variation for kernel micronutrients was found among the *o*2 genotypes. Another possible reason for the presence of wide variation for kernel micronutrients in QPM lines could be due to dilution effect which arises as a result of negative association between kernel mineral concentrations and kernel size [6].

Variability for kernel Mn and Cu in the representative subset

A representative subset of 68 lines capturing the full variation for Fe and Zn was selected from 120 lines for estimating kernel Mn and Cu concentrations. The mean of selected subset for kernel Fe and Zn was 36.5 ppm and 29.5 ppm, respectively reflecting the true representation of the full set (Fe: 36.6 ppm and Zn: 29.8 ppm). The result of kernel Mn and Cu estimation in the subset showed that the QPM genotypes possessed good variation for these two micronutrients as well. Kernel Mn ranged from 1.7-34.8 ppm with a mean of 11.9 ppm, while it was 0.5-9.5 ppm with a mean of 3.4 ppm for kernel Cu (Table 1). CML193 and CML194 recorded the lowest kernel Cu concentration (0.5 ppm), whereas SKV390 showed the highest kernel Cu concentration (9.5 ppm). For kernel Mn, CML163 recorded the lowest (1.74 ppm) and SKV672 recorded the highest (34.8 ppm) value.

The variation observed for kernel Mn was in accordance with Menkir [15], but for the other kernel micronutrients *viz.*, kernel Fe, Zn and Cu, it was slightly wider. SKV343, SKV616, SKV617 and SKV672

recorded higher concentration of all four kernel micronutrients (Fe: >60 ppm, Zn: >38 ppm, Mn: >20 ppm and Cu: >4 ppm). The good variation for all four micronutrients in QPM lines suggested that simultaneous genetic improvement of kernel micronutrient traits in maize is possible through appropriate breeding strategies.

In our study, we observed, higher mean kernel micronutrients concentration in late maturity inbreds (Fe: 42.8 ppm; Zn: 32.8 ppm; Mn: 15.1 ppm and Cu: 4.1 ppm) as compared to early maturity inbreds (Fe: 29.4 ppm; Zn: 27.2 ppm; Mn: 14.2 ppm and Cu: 3.3 ppm). It could be due to prolonged vegetative stage in late maturing genotypes led to uptake and accumulation of more kernel micronutrients. On the contrary the early maturing inbreds SKV49 and SKV343 recorded higher kernel micronutrients, which suggested that these lines are efficient in uptake and accumulation of the micronutrients.

Correlation among the kernel micronutrients

A significant positive correlation (0.79) was observed between kernel Fe and Zn concentrations in the subset followed by Fe and Mn (0.61) (Table 2). Positive correlation between kernel Fe and Zn were also reported earlier in maize [15, 16] and in other cereals too [22-24]. On the contrary, no significant correlation was observed among kernel Fe and Zn by Agrawal *et al*. [12] and Chakraborti *et al*. [13]. This difference could be due to inherent nature of the specific type of genetic material used and thus it may not be a general phenomenon. Strong correlation among kernel micronutrients in QPM lines was recorded and highlighted by Arnold *et al*. [19]; however, compared to other micronutrient combinations, the correlation between kernel Fe and Cu was very low (0.34) though it was significant in the present investigation.

The presence of strong positive correlation between kernel Fe and Zn is probably due to pleiotropic effect of genes which control the network of Fe and Zn uptake, transportation and accumulation [25, 26]. The strong correlation between kernel micronutrients emphasizes the role of common transporters for several micronutrients or accumulation of several genes coding transporter for various elements. Plants of Gramineae family including maize predominantly adopted strategy II for mineral uptake, which involves secretion of chelating compounds *viz*., phytosiderophores and mugineic acid which binds to Fe (III) and facilitate their absorption [27]. The *Zea mays* Zinc-regulated transporters and Iron-regulated transporter-like Proteins (*ZmZIP*) genes encode functional transporters that may be responsible for the uptake, translocation and storage of divalent metal ion including Zn and Fe in plant cells [28-30]. Previous reports highlighted the existence of cross-talk between Fe and Zn transport pathways.

Table 2. Correlation among micronutrients in a representative subset of (68) QPM inbreds

Micronutrients	Zn	Mn	Cu
Fe	0.79**	0.61**	0.34**
Cu	0.46**	0.41**	
Mn	0.48**		

**Significant at p< 0.01

Inbreds having high kernel Fe, Zn and appreciable level of Mn and Cu concentration (Table 3) can be directly used as parents in hybrid breeding and line development through transgressive segregants to further enrich the micronutrient concentration. Moreover, the presence of positive correlation among all four micronutrients offers opportunity of increasing the minerals simultaneously in the QPM genotypes. The above-mentioned approach can lead to the development of biofortified maize hybrids that are rich in essential amino acids and micronutrients and thus could help in alleviating micronutrient malnutrition.

Diversity analysis among the genotypes of subset

Presence of genetic diversity for target trait(s) is of pivotal importance for any crop improvement

Table 3. List of selected inbreds having high kernel Fe and Zn concentrations and appreciable kernel Mn and Cu concentrations

S.No.	Inbreds	Micronutrients concentration (ppm)			
		Fe	Zn	Mn	Cu
1	SKV263	60.2	45.1	13.4	3.0
2	SKV343	64.6	53.2	32.8	8.5
3	SKV612	75.7	39.1	16.1	2.2
4	SKV613	65.0	38.3	19.0	3.0
5	SKV616	83.4	44.3	25.1	4.2
6	SKV617	66.1	44.7	23.6	5.5
7	SKV672	73.1	40.0	34.8	4.9
8	SKV675	61.3	39.7	27.8	3.7

programme. The genetic diversity of 68 lines was assessed using all four kernel micronutrients (Fig. 1). The coefficient of genetic dissimilarity varied from 0.26-7.97 with a mean of 2.46. The highest genetic dissimilarity value was observed between QPM inbreds SKV343 and CML163 indicating that these lines highly differed from each other with respect to kernel micronutrient status and can be effectively used in developing a mapping population to map QTLs for micronutrients. The dendrogram revealed that the maize genotypes used in present investigations were grouped into three major clusters namely, A, B and C. Cluster A had 23 genotypes which all were SKV lines and it was further divided into two sub-cluster A1 (22 genotypes) and A2 (one genotype). Cluster B comprised of maximum number of genotypes (44) and all the CMLs were clustered in this group. Sub-cluster B1 emerged as largest sub-cluster by comprising 33 genotypes. Sub-cluster B2 had 11 genotypes of which five were CMLs. Cluster C emerged as the smallest cluster with single genotype. The QPM lines analysed in our experiment belonged to flint and dent categories and formed different yield heterotic pools (S. K. Vasal, Personal Communication). Attempting high × high or high × low crosses (for kernel micronutrient concentration) between the lines that belong to genetically diverse pools can generate high yielding hybrids enriched with high kernel micronutrients.

In flint × flint inbreds, the highest coefficient of genetic dissimilarity was observed for SKV672 × SKV40 (5.57) and the lowest between SKV10 × SKV1 (0.26) with a mean of 2.36. Among high/high combinations in flint heterotic group, SKV672/SKV244, SKV675/SKV244, SKV693/SKV244 are possible as they all belong to late maturity. In the same flint group, SKV244/SKV49, SKV675/SKV49, SKV672/SKV49, SKV693/SKV49 are belong to late/early maturity, whereas in high/low combinations, all crosses belongs to late/early maturity groups (Table 4).

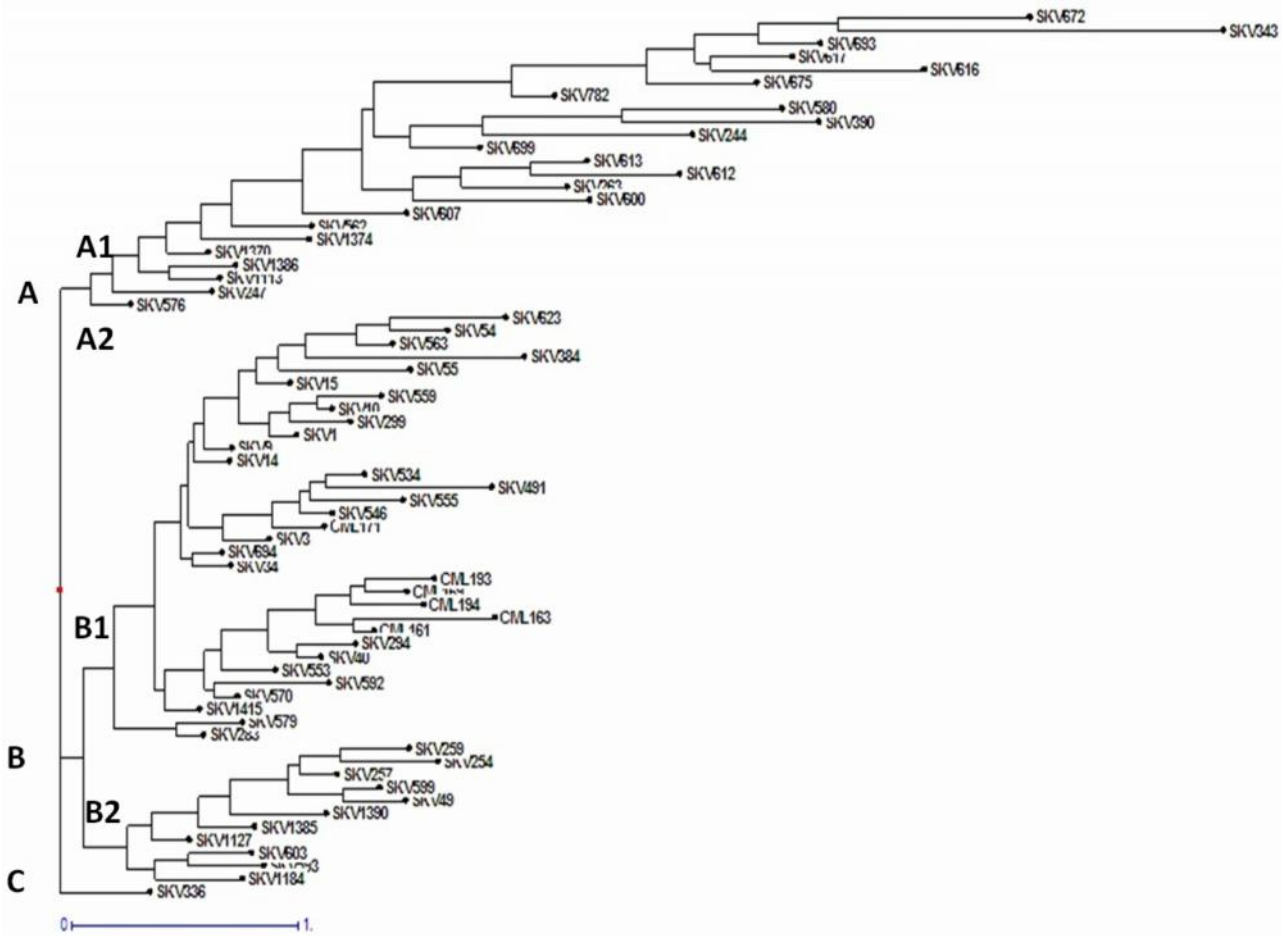

Fig. 1. Dendrogram showing genetic relationship of 68 selected QPM inbreds

Table 4. Parental combinations selected based on kernel micronutrients, maturity and genetic diversity in different heterotic pools

Parental combinations (at coefficient of genetic dissimilarity >3)			
A. High/High (for kernel micronutrient)			
Heterotic pool	Early/Early	Late/Late	Early/Late
Flint/Flint	–	SKV672/SKV244, SKV675/SKV244, SKV693/SKV244	SKV244/SKV49, SKV675/SKV49, SKV672/SKV49, SKV693/SKV49
Dent/Dent	–	SKV390/SKV259, SKV390/SKV263	SKV343/SKV259, SKV343/SKV263, SKV390/SKV343
Dent/Flint	SKV343/SKV49	SKV259/SKV672, SKV259/SKV693, SKV263/SKV672, SKV390/SKV244, SKV390/SKV672, SKV390/SKV675, SKV390/SKV693	SKV343/SKV675, SKV390/SKV49
B. High/Low (for kernel micronutrient)			
Flint/Flint	–	–	SKV244/SKV3, SKV244/SKV9, SKV244/SKV10, SKV244/SKV14, SKV244/SKV15, SKV244/SKV34, SKV244/SKV40, SKV244/SKV55, SKV672/SKV1, SKV672/SKV3, SKV672/SKV9, SKV672/SKV10, SKV672/SKV14, SKV672/SKV15, SKV672/SKV34, SKV672/SKV40, SKV672/SKV55, SKV675/SKV1, SKV675/SKV3, SKV675/SKV9, SKV675/SKV10, SKV675/SKV14, SKV675/SKV15, SKV675/SKV34, SKV675/SKV40, SKV675/SKV55
Dent/Dent	–	SKV390/SKV257, SKV390/SKV283, SKV390/SKV294, SKV390/SKV299, SKV390/SKV336, SKV390/SKV384, SKV782/SKV294, SKV782/SKV336	SKV343/SKV257, SKV343/SKV283, SKV343/SKV294, SKV343/SKV299, SKV343/SKV336
Dent/Flint	SKV343/SKV1, SKV343/SKV3, SKV343/SKV9, SKV343/SKV10, SKV343/SKV14, SKV343/SKV15, SKV343/SKV34, SKV343/SKV40, SKV343/SKV54, SKV343/SKV55	SKV263/SKV694, SKV390/SKV694, SKV782/SKV694	#SKV263/SKV1, SKV263/SKV3, SKV263/SKV9, SKV263/SKV10, SKV263/SKV14, SKV263/SKV15, SKV263/SKV34, SKV263/SKV40, SKV390/SKV1, SKV390/SKV3, SKV390/SKV9, SKV390/SKV10, SKV390/SKV14, SKV390/SKV15, SKV390/SKV34, SKV390/SKV40, SKV390/SKV54, SKV390/SKV55, SKV343/SKV247, SKV343/SKV694, SKV782/SKV3, SKV782/SKV40
	–	*SKV254/SKV672, SKV254/SKV675, SKV254/SKV693, SKV257/SKV244, SKV257/SKV672, SKV257/SKV675, SKV257/SKV693, SKV283/SKV244, SKV283/SKV672, SKV283/SKV675, SKV283/SKV693	–

High dent/Low flint; * High flint/Low dent

In the dent heterotic pool, SKV343/SKV294 had the highest coefficient of genetic dissimilarity (6.76) whereas the lowest was observed in SKV259/SKV257 (0.68) pair with a mean of 2.96. In high/high late maturity combination, SKV390/SKV259, SKV390/SKV263 and in early/late maturity combination, SKV343/SKV259, SKV343/SKV263, SKV390/SKV343 are assumed to produce high yield coupled with kernel micronutrients. Several combination are available in high/low late maturity combination (SKV390/SKV257, SKV390/SKV283, SKV390/SKV294, SKV390/SKV299, SKV390/SKV336, SKV390/SKV384, SKV782/SKV294, SKV782/SKV336) as well as in early/late combination (SKV343/SKV257, SKV343/SKV283, SKV343/SKV294, SKV343/SKV299, SKV343/SKV336) (Table 4).

It is also possible to generate heterotic combination between flint × dent heterotic groups. In high × high kernel micronutrient concentration, SKV343/SKV49 combination is possible in early maturing group, SKV259/SKV672, SKV259/SKV693, SKV263/SKV672, SKV390/SKV244, SKV390/SKV672, SKV390/SKV675, SKV390/SKV693 combinations in late/late and SKV343/SKV675, SKV390/SKV49 combinations in early/late maturity groups. In high dent/low flint groups, early maturing dent inbred SKV343 can be crossed with a set of early maturing flint inbreds such as SKV1, SKV3, SKV9, SKV10, SKV14, SKV15, SKV34, SKV40, SKV54, SKV55 and SKV263/SKV694, SKV390/SKV694, SKV782/SKV694 pairs in late/late inbred groups to generate heterotic combinations (Table 4). The selected parental pairs can be exploited to develop kernel micronutrients-rich high-yielding QPM hybrids of different maturity groups.

The present study reveals that *o*2 gene plays positive role in increasing kernel Fe and Zn concentration in maize, although broad variability for these micronutrients were noticed among the QPM genotypes. The variation for kernel micronutrients could be due to the presence of endosperm modifiers in the QPM genotypes. In addition, dilution effect associated with kernel size and genetic background of the genotypes could also cause variation. Significant and positive correlation among the micronutrients concentration in maize kernel presents an opportunity to improve various micronutrients simultaneously. The genotypes identified with contrasting value for kernel micronutrients can be used for developing a mapping population to map QTLs for respective traits. The parental pairs identified with high genetic dissimilarity in different heterotic pools can be useful in development of high-yielding, QPM maize cultivars enriched with kernel micronutrients.

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