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Research Note

Evaluation of elite maize genotypes (Zea mays L.) for nutritional traits

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Abstract
Micronutrients enrichment in the staple food crops is an utmost breeding goal to alleviate 'hidden hunger'. A set of forty eight maize (*Zea mays* L.) genotypes were evaluated for kernel iron (Fe), Zinc (Zn) and carotenoid concentration (β-carotene). The results revealed significant genetic variation among maize genotypes for Fe with values ranging from 22.59-41.03 mg/kg and a mean of 31.92 mg/kg. Whereas CM145 recorded highest kernel iron concentration (41.03 mg/kg) followed by CML193 (40.70 mg/kg) and KI-29 (39.75 mg/kg). For kernel Zn, range varied from 19.38-32.59 mg/kg with a mean of 24.80 mg/kg. The inbred CML169 recorded highest kernel Zn concentration (32.59 mg/kg) followed by CML193 (32.13 mg/kg) and CM152 (32.12 mg/kg). For kernel carotenoid range observed from 10.72-27.61 µg/g with a mean of 19.51 µg/g. The inbred CML162 recorded highest carotenoid concentration (27.09 µg/g) followed by Jaisinghpur Local (27.61 µg/g), BAJIM-06-01 (27.01 µg/g), Rato Makki2 (26.85 µg/g), LM-19-07 (26.57 µg/g) and BAJIM-08-26 (26.28 µg/g). These genotypes were identified as promising genotypes.

Keywords:
Maize, Kernel iron, Zinc, Carotenoid concentration

Maize (*Zea mays* L.) is a major component of the daily diet of most of the needy people of the world (Nestel, 2006) and plays a significant role in human and animal nutrition. In India more than 25 per cent of maize produced is consumed as human food (Kaul *et al.* 2009). Being one of the staple intakes next to rice and wheat, it contributes substantially to the daily caloric requirement of developing countries. However, there are inherently low iron, zinc and carotenoid concentrations in cereal grains 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 (Cakmak, 2008).

Iron (Fe), one of the important micronutrients, is required for various metabolic functions of organisms and, its deficiency leads to severe anemia, growth, reproductive performance and work productivity (WHO, 2009), while deficiency in Zinc (Zn) leads to depression and psychosis, impaired growth and development besides affecting immune system (Cakmak, 2008). Carotenoids are the major sources of dietary precursor of Vitamin A and act as potential antioxidant besides preventing diseases such as night blindness in humans (Wurtzel, 2004). The presence of carotenoid in plant endosperm adds nutritional value and is responsible for proper growth and development of children (Mayer *et al.* 2008).

Hence, breeding maize for nutritional quality will serve as a potential tool in addressing micronutrient malnutrition, often called 'hidden hunger' which is one of the alarming problems in the developing world, afflicting an estimated three billion people (UNSCN, 2004). In India, about 230 million people were estimated to be undernourished, accounting for more than 27% of the world's undernourished population (Lodha *et al.* 2005). 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, Pfeiffer and McClafferty 2007, Bouis and Welch 2010, Gilligan 2012). Research efforts with respect to understanding the variability of kernel micronutrients in maize have been undertaken so far only in few countries under an International Collaborative Programme (Harvest Plus) for biofortification of selected staple food crops (Banziger *et al.* 2000, Oikeh *et al.* 2004). Therefore, studying the genetic variability for kernel Fe, Zn and carotenoid concentrations in the available maize germplasm and their potential to be utilized by adopting various breeding approaches is thus a priority (Agrawal *et al.* 2012).

The experimental material consisted of 48 maize genotypes, comprised of 31 inbred lines and 13 diverse local germplasm. These maize genotypes were selected based on their kernel colour ranging from dark red to white. Forty eight maize genotypes along with checks were evaluated for different morphological and quality traits in α-RBD design during *kharif* 2013 with plot size of 3.0 × 1.2 m^2(2R) with row to row and plant to plant distance of 60 cm and 20 cm, respectively with 2 replications, 12 blocks/replication and 4 entries/block. The present investigation was carried out at the Experimental Farm of the Department of Crop Improvement, CSKHPKV, Palampur situated at 32°8´ N latitude and 76°3´ E longitude and at 1290.8 m above mean sea level (amsl), representing mid-hill zone of Himachal Pradesh, characterized by humid sub-temperate climate with rainfall (2500mm per annum) having acidic soil with pH ranging between 5.0 to 5.6. Standard agronomic practices were followed for raising and maintenance of plants.

Biochemical analysis for kernel Fe and Zn concentration were carried out by digestion with 9:4 diacid mixture (HNO_3: $HClO_4$) followed by observation by the atomic absorption spectrometry (AAS) method as described by Zarcinas *et al.* (1987).After kernel maturation and plant dry down, ear with husk were manually harvested and dried under the shade to lower the post-harvest grain moisture to 14 per cent. Representative grain samples were drawn in duplicate and the individual samples were ground into fine powder. Nitric-perchloric acid digestion was performed, following the procedure recommended by the AOAC (1990). One gram of sample was placed in a 250 ml digestion tube and 15ml of diacid mixture of Nitric acid and perchloric acid was added. The mixture was boiled gently to oxidize all oxidisable matter until dense white fumes appeared. After cooling, 20 ml of distilled water was added and the mixture was boiled further to release fumes. The solution was cooled, further filter through Whatman No. 42 filter paper and transferred quantitatively to a 100 ml volumetric flask by adding water. The concentration of Fe and Zn in the final solution was determined by an Atomic Absorption Spectrometer (AAS).

After kernel maturation and plant dry down, ear with husk were manually harvested and were dried under the shade to lower the post-harvest grain moisture to 14 per cent. Representative grain samples were drawn in duplicate and the individual samples were ground into fine powder. Estimation of carotenoid content was done as per method (AOAC, 1962). Extraction steps were carried out under dark

conditions as carotenoids undergo photo-oxidation in the presence of light (Weber, 1987). One gm of sample was weighed and put in to test tubes. Then n-Butanol solution was prepared and out of this 5ml of solution was added in to test tubes and mixed vigoursly to give a homogenous suspension. Then allowed it to stand for overnight (16hrs) at room temperature under the dark. Shake and filtered completely through the filter paper (Whatman No. 1) and collected in test tubes. For the preparation of standard solution 5mg of β-carotene was dissolved in 20ml of Diethyl ether in 50 ml volumetric flask. Four ml of this solution was pipetted in to the 100ml volumetric flask and volume was made to 50ml by adding water saturated n-butanol. Then 5ml of this solution was placed in to a 100ml volumetric flask and make-up the volume 50ml with water saturated n-butanol (standard solution), and out of this six dilutions of β-carotene standard (SIGMA chemicals) were used to make the standard curve. Absorbance of the clear filtrate was measured at 440 nm. Unfiltered water satured n-butanol was used as blank. Evaluation of the content was done based on a β-carotene calibration curve.

Analysis of variance revealed significant variation for kernel Fe, Zn and carotenoid concentrations (Table 1), 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),Oikeh *et al.*(2003, 2004)and Vignesh *et al.* (2013)also reported the presence of significant variations among the maize genotypes for the kernel Fe, Zn and carotenoid 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 22.59-41.03 mg/kg, kernel zinc from 19.38-32.59 mg/kg and kernel carotenoid ranged from 10.72-27.61 µg/g (Table 2). Inbred line CM145 showed the highest kernel Fe (41.03 mg/kg) and CML169 showed the highest kernel Zn (32.59mg/kg) and Jaisinghpur showed the highest kernel carotenoid (27.61µg/g) content. Banziger and Long (2000) also reported the variation from Kernel Fe ranged from 9.60 to 63.20 mg/kg, while Zn ranged from 12.90 to 57.60 mg/kg in a set of 1814 maize genotypes from different regions of Mexico and Zimbawe. 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 from13.60 to159.43 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. Chen *et al.* (2007) reported carotenoids ranged from 12.20-30.10 µg/g, these findings were in congruence with present study. For kernel Fe, values ranging from 22.59-41.03 mg/kg with a mean value of 31.92 mg/kg. The genotypes CM145 recorded the highest kernel Fe (41.03 mg/kg) content, followed by CML193 (40.70 mg/kg) and KI-29 (39.75 mg/kg). For kernel Zn, values ranging from 19.38 to 32.59 mg/kg, and a mean of 24.80 mg/kg. The genotype CML169 recorded the highest (32.59 mg/kg) followed by CML193 (32.13 mg/kg) and CM152 (32.12 mg/kg). For kernel carotenoid values ranging from 10.72-27.61 µg/g, with a mean of 19.51 µg/g. One of the maize inbred CML162 recorded the highest kernel carotenoid (27.09 µg/g) followed by local germplasm line Jaisinghpur (27.61 µg/g), BAJIM-06-01 (27.01 µg/g), Rato Makki 2 (26.85 µg/g), LM-19-07 (26.57 µg/g) and BAJIM-08-26 (26.28 µg/g).

*Analysis of correlation among micronutrients***:** Positive and significant correlations ($p \leq 0.005$) of kernel Fe with Zn and carotenoid concentrations were observed but carotenoid did not significantly correlated to grains Zn (Table 3), suggesting that the genes responsible for accumulation of kernel Fe and Zn and carotenoid concentrations could be quite different and genetic improvement for these traits could be undertaken independent of each other. 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. No significant correlation was observed among kernel Fe and Zn by Agrawal *et al.* (2012) and Chakraborti *et al.* (2011). 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. Among greater proportion of kernel Fe, Zn and carotenoid concentrations could be due to the sensitivity of these traits to the soil and microclimatic conditions and more variable in different environmental conditions in a more predictable manner. 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). Even minor changes in one factor in combination with other factors may also lead to significant variation in micronutrients traits. Besides, the spatial and temporal variation, system variation caused by the differential management practices can have the significant effects (Pfeiffer and McClafferty, 2007).

The promising genotypes identified in the present study could be potentially utilized for developing kernel micronutrient-enriched maize cultivars. Specific genotypes with high levels of Fe, Zn and total carotenoids need to be further profiled for components of carotenoids; especially β-carotene can be further utilized as potential donors in breeding for nutritionally enriched maize adapted to the Indian context.

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Table 1.Analysis of variance of maize genotypes for Iron, Zinc and Carotenoid contents

Traits/Source	Mean sum of square			
	Replication	Blocks within replication	Lines	Error
d. f.	1	22	47	25
Iron (mg/kg)	11.890*	2.555	31.292*	2.725
Zinc (mg/kg)	1.122	0.927	23.137*	0.599
Carotenoid (µg/g)	0.101	0.629	30.818*	0.650
Iron (mg/kg)	11.890*	2.555	31.292*	2.725

*Significant at P≤0.05, p=parameter.

Table 2. Kernel Fe, Zn (mg/kg) and kernel carotenoid concentration (µg/g) of the 48 maize genotypes

Sr. No.	Genotypes	Iron content (mg/kg)	Zinc content (mg/kg)	Carotenoid content (µg/g)	Sr. No.	Genotypes	Iron content (mg/kg)	Zinc content (mg/kg)	Carotenoid content (µg/g)
1	CML 162	35.05	30.95	27.09	25	KI-29	39.75	25.05	22.37
2	CML 163	36.25	31.47	17.50	26	KI-30	34.03	22.15	15.45
3	CML 170	34.00	30.39	21.55	27	BAJIM-08-26	30.60	20.76	26.28
4	CML 171	33.66	28.99	16.23	28	BAJIM-08-27	35.38	23.63	18.82
5	CML 173	33.55	29.08	12.83	29	BAJIM-06-03	22.59	23.70	18.69
6	CML 180	31.75	27.33	14.92	30	BAJIM-06-15	26.71	26.10	19.79
7	CML 193	40.70	32.13	24.50	31	BAJIM-06-20	28.08	25.60	20.13
8	CML 169	38.10	32.59	23.37	32	LM-18-08	34.90	25.87	26.57
9	VQL2	32.60	26.01	19.63	33	LM-19-07	28.92	23.32	19.80
10	HKI-1348	33.15	25.84	19.69	34	LM-14-11	27.78	22.12	13.75
11	CML472	35.22	28.45	19.87	35	LM-10-11	31.05	20.33	18.21
12	CML 496	30.50	25.77	22.02	36	LM-15-11	29.27	19.65	18.58
13	CM145	41.03	24.09	22.97	37	LM-17-08	28.09	20.81	26.85
14	CM152	28.95	32.12	22.15	38	Rato Makki 2	28.46	19.78	19.11
15	CM212	36.00	25.85	21.36	39	LM-37-07	30.24	23.11	18.61
16	CM128	31.51	23.71	24.05	40	LM-40-07	27.50	21.14	27.61
17	CM129	33.13	26.27	15.64	41	Jaisinghpur	27.17	22.00	15.58
18	332A	29.76	21.95	11.58	42	LM-02-08	26.87	20.80	10.72
19	912A	29.57	23.44	16.31	43	LM-03-11	26.49	22.11	11.52
20	918A	34.86	21.27	19.85	44	PAHNELO MAKKI2	30.32	25.22	24.33
21	KDM 381A	35.21	23.25	18.95	45	VQL1*	36.46	19.38	27.01
22	KDM 905B	32.19	24.29	12.60	46	BAJIM-06-01*	26.03	29.74	20.32
23	KI-16	36.51	23.17	21.51	47	BAJIM-06-10*	27.86	21.18	18.32
24	KI-18	34.93	22.10	20.21	48	BAJAURA MAKKA*	34.90	25.87	26.57

*-Checks

Table 3. Correlation analysis among grains micronutrient concentration

	Zn	Carotenoid
Fe	0.322**	0.294**
Zn		0.107

Level of significance: **p≤0.05