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Genetic variation for grain mineral content in tropical-adapted maize inbred lines

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Abstract

Increasing the concentrations of Fe and Zn in staple food crops through breeding has been proposed as one strategy to minimize the adverse effects of widespread mineral deficiencies in humans. This approach requires the presence of adequate genetic differences in concentrations of grain minerals for improvement. Eight trials involving different sets of tropical maize inbred lines adapted to the lowlands and mid-altitudes were, therefore, evaluated for concentrations of grain Fe, Zn and other minerals in two locations. The combined analyses of variance showed significant variation in concentrations of grain minerals among inbred lines in each trial, which was always greater than the variation caused by locations and line × location interactions. The line × location interaction had no significant effect on concentrations of Fe, Zn, Cu, Mg and P in at least three trials of lowland inbred lines. The line × location interaction also did not significantly affect the concentrations of any minerals, except S, in at least three trials of mid-altitude inbred lines. The best-inbred lines identified from each trial had 32–78% more Fe and 14–180% more Zn than their trial average. The first two principal component axes, which accounted for 55–64% of the total variation in kernel mineral concentrations, stratified the inbred lines in each trial into four groups based on differences in their grain mineral compositions. None of the correlations of Fe and Zn with Mn, Cu, Ca, Mg, K, P and S was significant and negative in the various trials, while the correlations of Fe with Zn were positive and significant ($r = 0.55$ to $r = 0.68$, $p < 0.0001$) in almost all the trials. These results suggest that a genetic potential exists for concurrent improvement of Fe and Zn without lowering the concentrations of other grain minerals in maize.

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Keywords: Minerals; Tropical-adapted; Maize inbred lines

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1. Introduction

Micronutrient malnutrition is a widespread problem in many developing countries. The average prevalence of iron deficiency among children in 37 African countries has been estimated at 67% (UNICEF., 2004). Also zinc intake has been considered to be inadequate for an estimated 30% of the populations in 46 African countries (Hotz & Brown, 2004). Deficiencies in these nutrients can hamper early brain development, suppress the immune system, increase both mortality and morbidity, and reduce the capacity to do physical work (Combs, Welch, Duxbury, Uphoff, & Nesheim, 1996; Graham & Welch, 2002; Welch & Graham, 1999). Such deficiencies can perpetuate the cycle of poverty in developing countries (Graham & Welch, 2002; Welch & Graham, 1999). Also, other mineral elements, including calcium, copper, magnesium, manganese, phosphorus, and potassium, are known to be essential for human health (MacDowel, 2003; O'Dell & Sunde, 1997). These minerals are critical for the growth and formation of strong bones, teeth, hair, blood, nerves and skin, synthesis of vitamins, enzymes and hormones, as well as for healthy functioning of the nervous system, blood circulation, fluid regulation, cellular integrity, energy production and muscle contraction (MacDowel, 2003; O'Dell & Sunde, 1997).

In sub-Saharan Africa, a common cause of micronutrient malnutrition is the sub-optimal intake of nutrients. Sustainable solutions should thus emphasize improvement

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of nutrient intake and supply and control of preventable factors that increase requirements. Some believe that crop-based approaches that enhance the nutrient supply in foods are essential to a sustainable reduction and elimination of micronutrient deficiencies in developing countries (Ali & Tsou, 1997; Amoaful, 2001; Combs et al., 1996; Welch & Graham, 1999). This has sparked interest in addressing micronutrient malnutrition through breeding of the major staple food crops for enhanced nutrients. Maize accounts for an average of 50% of the total area devoted to cereal production in 26 countries in Africa and most of the grain produced is used to feed people (Aguino, Carrion, & Calvo, 1999). This staple food crop provides at least 50% of the total intake of iron and zinc in the diets of the poor in sub-Saharan Africa (Ruel & Bouis, 1998). Maize cultivars with increased micronutrient content can thus provide at least part of the nutritional requirements of humans (Mason & D'Croz-Mason, 2002). This approach may promote the self-reliance of rural communities in producing nutritious maize cultivars to meet their nutritional needs on a sustainable basis.

The development of an effective breeding programme to improve mineral content in maize depends on the presence of genetic variation. Significant genetic variation in kernel iron, zinc and other mineral concentrations has been reported among maize inbred lines and hybrids adapted to temperate environments (Ahmadi, Wiebold, & Beuerlein, 1993; Arnold & Bauman, 1976; Arnold, Bauman, & Aycock, 1977; Brkić et al., 2004; Gorsline, Thomas, & Baker, 1964). Marked differences in kernel mineral concentrations were also found among tropical maize varieties (Feil, Thiraporn, & Stamp, 1992; Feila, Mosera, Jampatongb, & Stampa, 2005; Oikeh, Menkir, Maziya-Dixon, Welch, & Glahn, 2003; Oikeh et al., 2004). Significant differences in concentrations of grain Fe and Zn were reported among a large number of landraces, open-pollinated varieties and inbred lines of maize evaluated in different trials in Mexico and Zimbabwe (Bänziger & Long, 2000). However, limited results have been published concerning the range of genetic variation in concentrations of grain mineral elements other than Fe and Zn among diverse inbred lines adapted to the tropics. Furthermore, the successful incorporation of genes for enhanced mineral content from exotic sources into adapted maize germplasm and overcoming the potential negative effect of exotic germplasm on agronomic performance will take several years. Testing elite inbred lines adapted to West and Central Africa can therefore help to identify suitable parental materials useful for development of maize cultivars with enhanced concentrations of minerals in agronomically desirable genetic backgrounds without severely disrupting the broad adaptation already achieved for immediate use by farmers in the region.

As minerals work in combination with each other and with other nutrients in humans (O'Dell & Sunde, 1997), monitoring changes that may occur in other mineral elements when selection is done for increased kernel Fe and Zn content is essential to maximize the potential health benefits of the new maize cultivars to humans. Some studies have found either significant positive or non-significant correlations of kernel iron and zinc concentration with other mineral elements in temperate maize (Arnold & Bauman, 1976; Arnold et al., 1977; Brkić et al., 2004). The potential for enriching Fe and Zn in maize grain without reducing the concentrations of other essential mineral elements would increase in line with our understanding of the relationships of the target minerals with other essential elements in diverse maize inbred lines adapted to the tropics. The objectives of these studies were (i) to explore the extent of genetic variation in kernel mineral concentrations present among tropical-adapted maize inbred lines, (ii) to assess the potential for improvement of Fe and Zn without significant reductions in other minerals and (iii) to determine the effects of line, location and line × location interaction on variability of grain mineral concentrations.

2. Materials and methods

2.1. Genetic materials and field trial

A total of 149 lowland and 129 mid-altitude maize inbred lines were divided into six trials (Tables 1 and 2). The lines were derived from diverse adapted × adapted and adapted × exotic crosses and backcrosses as well as broad-based populations and open-pollinated varieties (DT-SR-W, EV 8749-SR, Obantanpa, P43SRC9, POP 32, TZLCOMP3C1, UCA-SR BC4 ACR97TZLCOMP1-Y, ACR97SYN-Y, TZE-COMP5-Y-C7, Z.diplo.BC4, Taraba-local, Early-W-SR, POP42-SR and TZMSR) with selection for adaptations to the tropical lowlands or mid-altitudes and for resistance to the major diseases at the various stages of inbreeding. The first three trials (Table 1) consisted of sets of 81, 36, and 32 lowland inbred lines and were grown at Saminaka (10°34′N, 8°39′E, altitude 760 m) and Zaria 11°7′N, (7°21′E, altitude 640 m) in Nigeria in 2002, 2005 and 2006, respectively. A fourth trial, involving 24 lowland inbred lines with 14–27 mg/kg of kernel iron selected from the first trial, was evaluated at Ibadan (7°22′N, 3°58′E, altitude 150 m) and Ikenne (3°42′E, 6°54′N, altitude 30 m) in Nigeria in 2004 to further assess the consistency of kernel Fe and Zn concentrations. Trial-5, Trial-6 and Trial-7 (Table 2) were composed of sets of 45, 32, and 42 mid-altitude inbred lines and were grown at Saminaka and Vom (9° 40′N, 8° 50′E, altitude 1300 m) in 2002, 2002 and 2005, respectively. Trial-8 was composed of 18 mid-altitude inbred lines with 16–26 mg/kg of kernel iron selected from Trial-5 and was evaluated at Ibadan and Ikenne in 2004 for assessing the consistency of kernel Fe and Zn concentrations.

The inbred lines included in Trial-1 were arranged in a simple lattice design, whereas the remaining seven trials were planted in a randomized complete block design with two replications. The soil types were Litosols at Ibadan, Distric Nitosols at Ikenne, Plinthic Luvisols at Saminaka,

Table 1
Pedigrees of list of inbred lines included in three trials evaluated for grain mineral concentration at Saminaka and Zaria in 2002, 2005 and 2006, respectively

Pedigree	Number of lines	Pedigree	Number of lines
Trial-1 (2002)		*Trial-2 (2005)*	
1368	1	1368	1
1393	1	((MO17/Tex6)/1368	2
9071	1	1368/GT-MAS-Gk	4
(1368/501)/5012	1	1368/Mi82	20
(1368/9032)/1368	2	(GT-MAS:gk/9450)/GT-MAS:gk	3
(1368/9071)/9071	10	GT-MAS:gk/BABANGOYO	2
(1393/9006)/9006	4	T115/9071	4
(TZMI102/KU1414SR)/TZMI102	1	*Trial-3 (2006)*	
(1368/SAPub Lines 36)/1368	1	(ATPSR/KU1414 SR)/ATP SR	1
(TZMI407/KU1414SR)/TZMI407	1	(KU1414/9450)/9450	1
(9071/9450 STR)	1	(TZMI102/KU1414SR)/TZMI102	1
(9071/Babamgoyo)	7	(CIM116/TZMI302)/CIM 116	2
(KU1403/1368)BC2	4	(MP420/4001)/MP420	1
(M118 /TZMi 302)/CIM 118	2	(1368/B73LPA)/1368	2
(MMB90/TZMi 302)/MMB90	4	(1368/GT-MAS-Gk	2
(TZMSR-W/KU1414SR)/TZMSR-W	1	1368/Mi82	1
(1368/H632F(HI)-1)/1368	1	(1822/73LPA)/1822	1
(1368xHI/4269-1)/1368	4	(4001/B73LPA)/4001	1
(1368/ICAL 224-1)/1368	4	4001	1
(1368/INV 534-1)/1368	5	9450/KI 21	2
(1368/PAC 90038-1)/1368	4	ACR.97 TZL COMP1-Y	2
AGSEEDSCAM	4	(KU1409/MO17LPA)/KU1409	10
CIM116/TZMI302/TZMI302	1	(KU1414/CM 117-1)/KU1414	1
CML 373	1	TZE COMP5	1
DT-SR-W	1	Z. diplo. BC4	1
EV 8749-SR	1		
Mokwa Pioneer-W	1		
Obatanpa	7		
P43SRC9	2		
POP 32	1		
TZLCOMP3C1	1		
UCA-SR BC4	1		

Table 2
Pedigrees of list of inbred lines included in three trials evaluated for grain mineral concentration at Saminaka and Vom in 2002 and 2005

Line	Number of lines	Nsme	Number of lines
Trial-5 (2002)		*Trial-7 (2005)*	
(TZMI101/TZMI501)	9	TZMI407/MO17LPA	23
(TZMI407/H632F(HI)/TZMI407	3	TZMI407′619LPA	1
TZMI501/ ZSR 923 S4BULK	4	TZMI214/619LPA/TZMI214	15
87014/Z28	2	TZMI407	1
87036/87923	1	TZMI102	1
88069/87366	1	TZMI501	1
89302/Z28	4		
TZMI501/90323	2		
C70/88030	1		
CAMINBTCSEL	3		
Early-W-SR	1		
NEW S4/S3	1		
POP43 SR	5		
TZMI102	1		
TZMI407	2		
TZMI407/(8232/TZMSRW-SR/ZM607	4		
TZMSR-W	1		
Trial-6 (2002)			
5057	1		
89207	1		
89365	1		
90251	1		
90301	1		
90313	1		
(87036/89274)	1		
(90301/ 87036)	3		
(TZMI102/TZMI501)	8		
(TZMI407/89183)	1		
TZMI501/MSR123/1137TN-6	1		
1368STR	1		
9030STR	1		
9315-10	1		
TZMI407/TZMI604	1		
TZMI407-Short	1		
TZMI502	1		
TZMI603	1		
TZMSR	3		
UCA SR BC2	2		

Ferric Acrisols at Vom and Ferric Luvisols at Zaria (FAO/UNESCO., 1990). Each inbred line was grown in a single row plot, 5 m long with 0.75 m spacing between rows and 0.25 m spacing between plants within a row. Within a row, three seeds were planted in each hill and thinned to two plants after emergence. A compound fertilizer was applied at the rates of 60 kg N, 60 kg P and 60 kg K per ha at the time of sowing at each location. An additional 60 kg N per ha was applied as top dressing four weeks later. In all inbred trials, Atrazine and Gramoxone were applied as pre- and post-emergence herbicides at 5 litres per ha each of Primextra and Paraquat, respectively. Subsequent manual weeding was done to keep the trials weed-free. At least 10 plants were self-pollinated in each row and the harvested ears were threshed to make a composite sample. A 10 g sample was drawn from each plot for mineral analysis.

2.2. Kernel mineral analysis

Seed samples drawn from all the trials were sent to Waite Analytical Services, University of Adelaide, in Australia for mineral analysis. A protocol, described by Zarcinas, Cartwright, and Spouncer (1987), was used for grinding the grain, pre-digestion and digestion of samples. Grain samples from each plot were ground with a Tecator Cyclotec grinder to pass through a 1 mm stainless steel sieve and stored in screw-top polycarbonate vials. Out of each sample, 0.8 g of flour was digested with 10 ml nitric and 1 ml of perchloric acid overnight in a tube. The tubes were subsequently heated at temperatures varying from 120 °C to 220 °C over a period of 4–5 h. The tubes were left to cool and each sample was diluted to 25 ml final volume. An aliquot of the solution was analyzed with an inductively coupled plasma optical emission spectrophotometer (ICPOES) for mineral element concentrations.

2.3. Statistical analysis

The minerals for Trial-1 obtained at each location were analyzed separately to generate entry means adjusted for block effects according to the lattice design, as described by Cochran and Cox (1957). The adjusted means and effective error mean squares were used for running combined analyses of variance over locations (Cochran & Cox, 1957). In Trial-1, blocks, replications, and locations were considered as random effects whereas lines were considered as fixed effects. In the analyses of variance combined across locations for the remaining seven trials, inbred lines were considered as fixed effects, whereas replications and locations were considered as random effects for each mineral element. All analyses were performed with PROC GLM in SAS (SAS Institute, 2000) using a RANDOM statement with the TEST option. Simple correlations of kernel iron and zinc concentrations with other mineral elements were computed from the line means averaged over locations (SAS Institute, 2000). Principal component analysis was performed using the correlation matrix of the different mineral elements (SAS Institute, 2000). The principal component scores for the first two axes (PC1 and PC2) were plotted to visualize the separation of the lines into groups in Trials 1, 2, 3, 5, 6, and 7.

3. Results

The trials involving sets of maize inbred lines adapted to the lowlands (Trial-1 Trial-2 and Trial-3) and mid-altitudes (Trial-5 Trial-6 and Trial-7) were evaluated at two locations with different soil types. In the combined analyses of variance of the first three trails (Table 3), location was a significant source of variation for Mn and S in two trials, for Zn and Ca in one trial but not for other mineral elements. In addition, a significant line × location interaction was detected for Mn and S in two trials and for Fe, Ca, Mg and K in one trial, but not for P and Zn. The difference in concentration of each kernel mineral element among maize inbred lines was significant in all the trials, except for Fe and P in Trial 2 (Table 3). In the three trials involving mid-altitude inbred lines (Table 4), location significantly affected the concentrations of grain Zn in three trials, grain Mg and K in two trials and grain Mn and S in one trial. The line × location interaction was significant for grain S concentration in two trials and for grain Mn, Cu and Ca concentrations in one trial, but not for other elements. The difference among the mid-altitude adapted inbred lines was significant for the concentrations of all grain mineral elements (Table 4). Inbred lines selected for contrasting Fe concentrations from previous trials were included in

Table 3
Sum of squares of selected sources of variation, expressed as percentages of the corrected total sum of square, from the combined analyses of variance for grain mineral concentration in lowland tropical maize inbred lines tested in four trials at two locations in 2002, 2004, 2005 and 2006

Source	Df	Iron	Zinc	Manganese	Copper	Calcium	Magnesium	Potassium	Phosphorus	Sulphur
Trial-1 (2002)										
Location	1	2.0	6.2	29.5*	0.0	6.8	4.1	1.3	5.5	7.8*
Lines	80	46.4***	55.5***	47.7***	69.4***	75.7***	54.0***	52.6***	39.6**	70.3***
Line × location	80	20.7*	12.4	9.5***	10.4	7.1**	17.3*	18.8*	20.4	9.3*
Trial-2 (2005)										
Location	1	13.9	26.9*	46.3***	0.7	4.6*	2.8	5.9	3.4	22.3***
Lines	35	31.2	39.4***	34.3***	70.7***	70.1***	41.6***	28.3	36.0*	51.1***
Line × location	35	20.0	11.5	8.0	12.2	10.5	17.0	21.3	20.0	12.8*
Trial-3 (2006)										
Location	1	0.2	0.4	5.3	0.0	0.3	4.9	2.7	5.2	0.1
Lines	31	83.0***	59.4***	70.9***	76.5***	79.6***	56.3***	54.1***	51.8**	82.3***
Line × location	31	6.4	13.0	13.9***	6.4	3.9	14.0	16.0	18.8	6.5
Trial-4 (2004)										
Location	1	2.9*	4.2	15.6	0.4	0.2	0.2	0.6	2.1	0.0
Lines	23	84.7***	75.5***	52.7***	90.6***	75.4***	79.7***	68.3***	66.2***	84.6***
Line × location	23	5.7	8.3	8.2	3.7	11.6*	6.4	15.1*	11.5	5.1

*, **, ***Corresponding mean squares significantly different from zero at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively.

Table 4
Sum of squares of selected sources of variation, expressed as percentages of the corrected total sum of square, from the combined analyses of variance for grain mineral concentration in maize inbred lines adapted to the mid-altitudes tested in four trials at two locations in 2002, 2004 and 2006

Source	Df	Iron	Zinc	Manganese	Copper	Calcium	Magnesium	Potassium	Phosphorus	Sulphur
Trial-5 (2002)										
Location	1	3.5	38.0**	2.1*	5.6	1.4	7.2*	17.1*	9.5	0.6
Lines	44	48.2***	31.2**	60.7***	39.0***	42.9**	43.7**	43.9***	40.8***	53.9***
Line × location	44	15.6	12.5	17.6**	13.9	18.5	18.8	13.8	15.3	20.8**
Trial-6 (2002)										
Location	1	17.0	13.3*	0.6	4.0	0.5	0.9	2.7	4.1	2.2
Lines	31	38.6***	44.9***	69.5***	63.2***	83.7***	47.9**	54.4***	39.0*	74.9***
Line × location	31	12.0	13.3	8.7	16.5**	4.1	15.3	16.9	18.6	10.0*
Trial-7 (2005)										
Location	1	7.6	21.4**	0.9	2.7	2.1	18.2*	42.6*	32.1**	4.5*
Lines	41	32.1*	34.9*	54.6***	77.7***	51.3**	44.8***	24.8**	29.4*	67.8***
Line × location	41	18.1	17.1	19.6	6.0	21.3*	12.3	9.9	14.2	11.2
Trial-8 (2004)										
Location	1	6.2	16.8*	3.7	0.2	5.8	1.1	0.0	1.2	0.8
Lines	17	56.6*	48.3*	47.2*	51.0**	52.2**	59.6*	57.8***	57.2**	67.2***
Line × location	17	17.7	15.4	15.5	11.5	12.5	18.8	9.0	15.1	9.2

*,**,***Corresponding mean squares significantly different from zero at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively.

two trials (Trail-4 and Trail-8) and were tested in entirely new sets of locations to further assess the consistency of the concentrations of grain minerals over environments. The combined analyses of variance of these trials showed significant location effect only on Zn in one trial but found no significant line × location interactions for concentrations of the grain mineral elements in the two trials (Tables 3 and 4). The variation in kernel mineral concentrations among lines was always greater than the variation caused by locations and line × location interactions in each of the eight trials.

The extent of variation in the concentrations of minerals observed in the different trials is presented in Table 5. A broad range of variation in concentrations of all grain mineral elements was found among lines included in each of the six trials. The dominant elements in kernels of all inbred lines were Mg, K, P and S. The best-inbred line in each trial had a kernel Fe concentration that exceeded the average of all the inbred lines by 37% in Trial-1, 32% in Trial-2, 52% in Trial-3, 39% in Trial-5, 42% in Trial-6 and 78% in Trial-7 (Table 5). Similarly, the best-inbred line in each trial had 14–180% greater concentrations of Zn and other mineral elements than the average of all inbred lines (Table 5). The number of lines that exceeded the trial average in kernel Fe concentration by at least 25% was 4 in Trial-1, 2 in Trial-2, 5 in Trial-3, and 3 each in Trial-5 Trial-6 and Trial-7 (data not shown). At least one of these inbred lines also had a minimum of 25% more kernel Zn than the respective trial average. Among the lines included in Trial-4 and Trial-8 (Table 6), lines selected for high kernel Fe had higher Fe and Zn, whereas those selected for low kernel Fe had low Fe and Zn across the new sets of locations. These lines had varying concentrations of other minerals that did not fallow any consistent trend in Trail-4 and Trial-8 (Table 6).

Simple correlation analysis was computed to assess the association of Fe and Zn with other mineral elements (Table 7). The correlation of Fe with Zn was positive and significant ($r = 0.55$ to $r = 0.68$, $p < 0.0001$) in five of the six trials. Fe was also significantly correlated with Cu, Mg, K, P and S in at least three trials. The association of Fe with Mn and Ca was not significant in most of the trials. Zn had positive and significant correlations with Cu, Mg, K and P in at least three trials, but its association with Mn, Ca and S was not significant in most trials (Table 7). Principal component analysis was computed to differentiae the lines into distinct groups based on similarity of kernel mineral composition. The first two principal component (PC1 and PC2) axes accounted for 58–64% of the total variation in kernel mineral concentration among the different sets of lowland maize inbred lines (Table 8). The mineral elements that contributed significantly to PC1 axis were Fe, Zn, Mn, Mg, K, P and S in Trial-1, Trial-2 and Trial-3. The contributions of Cu and Ca to the PC1 axis were not significant in two trials. In Trial-1, the high PC2 axis score was associated with increased Fe, Zn and Cu but with reduced Mn and Ca. In Trial-2 and Trial-3, high PC2 scores were associated with increased Cu and Ca but with reduced Fe concentration. Furthermore, the PC1 and PC2 axes accounted for 55–63% of the total variation in kernel mineral concentration among the three sets of mid-altitude maize inbred lines (Table 8). All mineral

Table 5
Mean concentrations of grain mineral elements (mg/kg) and the corresponding ranges for lines adapted to the lowlands (Trials 1–3) and mid-altitudes (Trials 5–7) tested in two locations from 2002 to 2006

Variable	Fe	Zn	Mn	Cu	Ca	Mg	K	P	S
Trial-1									
Minimum	14	18	4	1	17	793	3050	2400	1065
Maximum	27	33	16	4	129	1565	5450	4100	1845
Mean	20	24	10	2	53	1149	3890	3214	1342
Std. error	2	2	1	0	6	83	270	228	50
CV (%)	16	13	16	22	17	12	12	13	6
Trial-2									
Minimum	18	21	8	1	41	955	3250	3064	985
Maximum	29	45	20	4	124	1508	4475	4675	1535
Mean	22	28	13	2	69	1216	3864	3734	1270
Std. error	2	2	2	1	3	17	30	29	18
CV (%)	17	17	19	22	17	16	14	16	8
Trial-3									
Minimum	11	19	5	1	5	913	2825	2478	950
Maximum	31	34	17	3	76	1433	4425	4075	1725
Mean	20	25	10	1	27	1148	3499	3328	1327
Std. error	1	2	2	0	3	71	202	227	55
CV (%)	12	15	17	26	29	12	11	11	8
Trial-5									
Minimum	16	16	6	1	20	988	2950	2650	1073
Maximum	31	27	13	3	118	1580	4702	4275	1423
Mean	22	21	9	2	43	1298	3751	3366	1252
Std. error	1.9	1.6	0.9	0.3	10.0	89.2	232.3	218.4	60.8
CV (%)	17	13	15	30	45	12	11	13	7
Trial-6									
Minimum	16	14	6	1	17	1055	2410	2625	1005
Maximum	28	25	14	3	83	1518	4600	3975	1525
Mean	20	19	8	2	41	1230	3711	3278	1289
Std. error	1.6	1.4	0.6	0.2	3.5	81.0	246.8	227.0	47.5
CV (%)	18	15	16	17	19	14	11	14	6
Trial-7									
Minimum	14	18	6	1	26	950	3050	2600	975
Maximum	34	29	11	3	60	1420	4225	3875	1393
Mean	19	22	8	2	40	1137	3708	3204	1162
Std. error	2.7	1.9	0.7	0.1	4.8	69.3	178.1	201.8	43.2
CV (%)	30	15	14	15	18	12	10	12	6

elements, except Ca, contributed significantly to the PC1 axis in at least two of the three trials (Table 8). The two minerals that contributed positively to PC2 scores in all the trials were Mn and Ca (Table 8). The contributions of K, P and S to the PC2 axis were either negative or positive in one or two trials.

Scatter plots of the PC1 and PC2 axes scores for each set of lowland inbred lines are presented in Fig. 1. These sets of inbred lines exhibited a wider range in PC1 scores. The two axes separated the lines included in each trial into four groups with similar mineral composition. The first group of inbred lines (quadrant I) had negative PC1 and PC2 scores and thus had low kernel concentrations of almost all mineral elements (Fig. 1). The second group of inbred lines (quadrant II) had high concentrations of Fe, Zn, Mn, Mg, K, P and S in two or three trials. The third group (quadrant III) consisted of inbred lines with higher levels of Ca and Cu in two trials. The last group of inbred lines (quadrant IV) had higher levels of Zn, Mn, Cu, Ca, Mg, K, P and S in at least two trials (Fig. 1). The scatter plots for mid-altitude adapted maize inbred lines also showed broad ranges in PC1 scores (Fig. 2). Again the two axes placed each set of these inbred lines into four quadrants. The first group of inbred lines (quadrant I) with negative PC1 and PC2 axes scores represented those with low kernel concentrations of Fe, Zn, Mn, Cu, Ca, Mg, K, P and S. The second group (quadrant II) was composed of inbred lines with high concentrations of Fe, Zn, Mg, K, P and S in at least two trials. Inbred lines included in the third quadrant (quadrant III) had higher levels of Ca and Cu in two trials. The last group of inbred lines (quadrant IV) with positive PC1 and PC2 scores represented those with high concentrations of all the mineral elements recorded from each trial (Fig. 2).

Table 6
Mean concentrations of grain minerals (mg/kg) for selected maize inbred lines adapted to the lowlands (Trial-4) and mid-altitudes (Trial-8) tested for the second time at two new locations in 2004

Lines	Fe	Zn	Mn	Cu	Ca	Mg	K	P	S
Trial-4									
Mokwa Pioneer-Y	28	36	11	4	109	1630	4000	4075	2073
(KU1414*2/CM 116-1)-2-1-B*4	26	41	11	3	62	1315	3850	3850	1453
(KU1414*2/CM 117-1)-7-2-B*4	25	38	17	3	55	1278	3925	3525	1330
(KU1414*2/MIT2-56-1)-4-2-B*4	25	37	24	2	66	1365	4700	4050	1470
(CIM 116*2/TZMi 302)-2-2-B*4	24	33	17	2	62	1440	3500	3700	1508
(4001*2/ 9848)-22-1-1-B*4	16	27	12	3	58	1043	3600	3250	1303
(1393*2/2097)-17-1-1-B*4	17	27	14	3	82	1023	4225	3650	1445
(KU1414/9450*2)-17-3-1-B*4	17	26	9	2	41	1090	3600	3275	1020
UCA-SR BC4-1-5-1-1-B*4	15	26	11	2	61	1070	4275	3375	1318
(1368*2/HIx4269-1)-10-3-B*4	15	23	18	2	85	1170	4500	4100	1230
Mean	20	30	13	3	61	1224	3903	3596	1393
S.E.	1.0	1.5	1.5	0.2	6.3	45.2	176.0	144.6	59.8
Trial-8									
(87014/Z28)-11-27-1-1-1-B*4	26	39	15	4	43	1493	4625	4400	1580
(TZMI101/TZMI501)-31-1-3-1-1-1-B*4	25	35	16	3	59	1455	4025	3950	1385
(TZMI407/ 89183)-8-2-B	24	35	18	3	80	1505	4025	3950	1523
(TZMI101/TZMI501)-69-1-1-4-X*3-B*4	23	33	18	2	69	1383	3550	3225	1123
(TZMI501/ZSR923S4Bulk)-2-2-X*5-B)	23	30	15	3	42	1223	3500	3425	1215
(90301/87036)-25-1-1-1-1-B	18	25	24	3	89	1255	4150	3700	1115
(TZMI101/TZMI501)-1-2-1-1-1-1-B*4	18	26	17	3	61	1360	4200	3650	1310
TZMI407	17	24	13	2	47	1393	3725	3525	1433
(TZMI407*2/H632F(HI))-2-4-2-B*4	17	29	16	3	48	1428	4225	4050	1458
POP43 SR S5-86-2-1-1-1-1-B*5	16	28	20	4	50	1115	4125	3275	1213
Mean	20	30	17	3	61	1367	4036	3769	1353
S.E.	1.6	2.3	2.1	0.3	6.9	66.1	170.0	192.5	73.4

Table 7
Correlation of grain Fe and Zn concentrations with other minerals in different trials evaluated at two locations

Minerals	Trial-1 (2002)		Trial-2 (2005)		Trial-3 (2006)	
	Fe	Zn	Fe	Zn	Fe	Zn
Fe	1.00	0.68^{***}	1.00	0.55^{***}	1.00	0.72^{***}
Zn	0.68^{***}	1.00	0.55^{***}	1.00	0.72^{***}	1.00
Mn	0.15	0.16	0.03	0.10	0.34	0.52^{**}
Cu	0.25	0.38^{***}	0.32	0.26	−0.12	0.10
Ca	−0.10	−0.10	−0.03	−0.04	−0.02	0.23
Mg	0.36^{**}	0.39^{***}	0.62^{***}	0.61^{***}	0.55^{**}	0.70^{***}
K	0.41^{***}	0.23^{*}	0.30	0.39^{*}	0.47^{**}	0.51^{**}
P	0.45^{***}	0.50^{***}	0.47^{**}	0.64^{***}	0.53^{**}	0.69^{***}
S	0.24^{*}	0.21	0.35^{*}	0.29	0.52^{**}	0.65^{***}
	Trial-5 (2002)		Trial-6 (2002)		Trial-7 (2005)	
Fe	1.00	0.56^{***}	1.00	0.23	1.00	0.64^{***}
Zn	0.56^{***}	1.00	0.23	1.00	0.64^{***}	1.00
Mn	0.13	0.20	0.15	0.30	−0.13	−0.09
Cu	0.33^{*}	0.37^{*}	0.37^{*}	0.25	0.48^{**}	0.58^{***}
Ca	0.24	0.01	0.27	0.15	−0.02	0.06
Mg	0.50^{***}	0.38^{*}	0.40^{*}	0.54^{**}	0.12	−0.04
K	0.24	0.25	0.23	0.17	0.43^{**}	0.67^{***}
P	0.53^{***}	0.44^{**}	0.42^{*}	0.58^{***}	0.57^{***}	0.54^{***}
S	0.28	0.32^{*}	0.42^{*}	0.25	−0.08	−0.08

*, **, *** Significantly different from zero at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively.

Table 8
Eigenvectors of the first and second principal component axes (PC1 and PC2) for concentration of minerals in different sets of maize inbred lines evaluated at two locations in 2002, 2005 and 2006

Minerals	Trial-1		Trial-2		Trial-3	
	PC1	PC2	PC1	PC2	PC1	PC2
Fe	0.34^{***}	0.43^{***}	0.36^{***}	-0.23^{**}	0.36^{***}	-0.35^{*}
Zn	0.33^{***}	0.48^{***}	0.39^{***}	−0.21	0.43^{***}	−0.08
Mn	0.34^{***}	-0.36^{***}	0.23^{**}	0.65^{***}	0.32^{***}	0.25
Cu	0.10	0.40^{***}	0.24^{**}	0.30^{*}	0.09	0.60^{***}
Ca	0.19^{***}	-0.50^{***}	0.10	0.58^{***}	0.14	0.62^{***}
Mg	0.45^{***}	−0.16	0.47^{***}	−0.09	0.41^{***}	−0.08
K	0.37^{***}	−0.09	0.30^{***}	−0.10	0.26^{**}	−0.20
P	0.45^{***}	0.01	0.47^{***}	0.02	0.41^{***}	−0.04
S	0.27^{***}	−0.07	0.26^{**}	−0.21	0.39^{***}	0.10
Variance	0.39	0.19	0.42	0.19	0.48	0.16
	Trial-5		Trial-6		Trial-7	
Fe	0.40^{**}	−0.04	0.37^{***}	−0.11	0.44^{***}	−0.08
Zn	0.37^{***}	−0.16	0.40^{***}	−0.06	0.49^{***}	−0.13
Mn	0.20^{*}	0.60^{***}	0.24^{*}	0.43^{**}	0.02	0.52^{***}
Cu	0.23^{**}	0.54^{***}	0.31^{**}	0.50^{***}	0.40^{***}	−0.15
Ca	0.39^{***}	0.27	0.15	0.32^{*}	0.05	0.19^{*}
Mg	0.31^{***}	-0.29^{*}	0.44^{***}	−0.16	0.14	0.52^{***}
K	0.48^{***}	−0.09	0.24^{*}	0.09	0.43^{***}	−0.14
P	0.30^{***}	-0.34^{**}	0.48^{***}	−0.10	0.44^{***}	0.26^{***}
S	0.21^{**}	−0.18	0.23^{*}	-0.64^{***}	0.03	0.54^{***}
Variance	0.38	0.17	0.36	0.15	0.35	0.28

*, **, *** Significantly different from zero at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively.

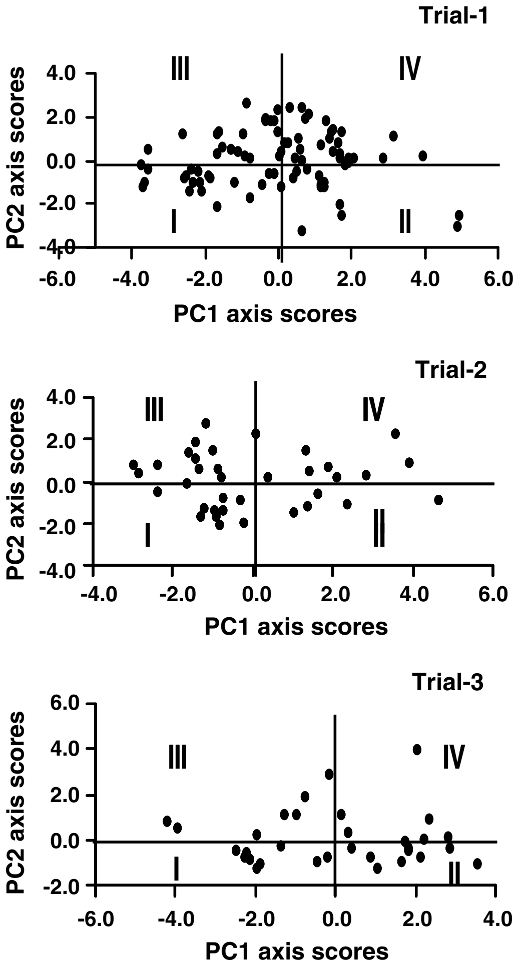

Fig. 1. Scatter plot of PC1 and PC2 axes scores for different sets of maize inbred lines adapted to the lowlands tested at two locations.

4. Discussion

The lines selected for these studies were genetically diverse and represented a broad range of variability in adapted maize germplasm available in the maize breeding programme at IITA. The concentrations of minerals in cereal grains can be affected by soil type and fertility, soil moisture, environmental factors, crop genotype and interactions among nutrients (Arnold & Bauman, 1976; Arnold et al., 1977; Feila et al., 2005; Gorsline et al., 1964; House, 1999). In the current studies, location did not exert significant influence on concentration of any kernel minerals, except Zn, in most of the trials, notwithstanding the differences in soil type under which testing was done. Even in trials where the effect of location was significant, it represented a very small fraction of the total variation in concentration of all minerals. Contrary to these findings, the location–year combination had significant and large effect on variability in Fe and Zn concentrations of both early- and late-maturing maize varieties in other studies (Oikeh et al., 2003, 2004). It thus appeared that soil properties that affect the availability of minerals did not play a prominent role in concentrations of kernel minerals in these sets of homozygous maize inbred lines.

The line × location interaction had no significant effect on concentrations of Fe, Zn, Cu, Mg and P in at least three trials, indicating that the inbred lines had consistent levels of these nutrients in their grains across locations. In agreement with these results, other studies reported the presence of small but significant genotype × environment interactions for concentration of grain minerals in maize (Feila et al., 2005), beans, rice and wheat (Welch & Graham, 2002). However, some trials involving open-pollinated varieties (Oikeh et al., 2003, 2004) and inbred lines (Bänziger & Long, 2000) found significant and high levels of genotype × environment interaction for kernel Fe and Zn concentrations. These contradictory results highlight the importance of evaluating inbred lines in multiple locations for kernel mineral concentrations to obtain reliable results for selecting suitable parental lines.

The genetic variation detected in the different sets of maize inbred lines adapted to the lowlands and mid-altitudes was considerable, accounting for a large proportion of the total variation observed in each trial. This is in agreement with the results of others, who reported a strong

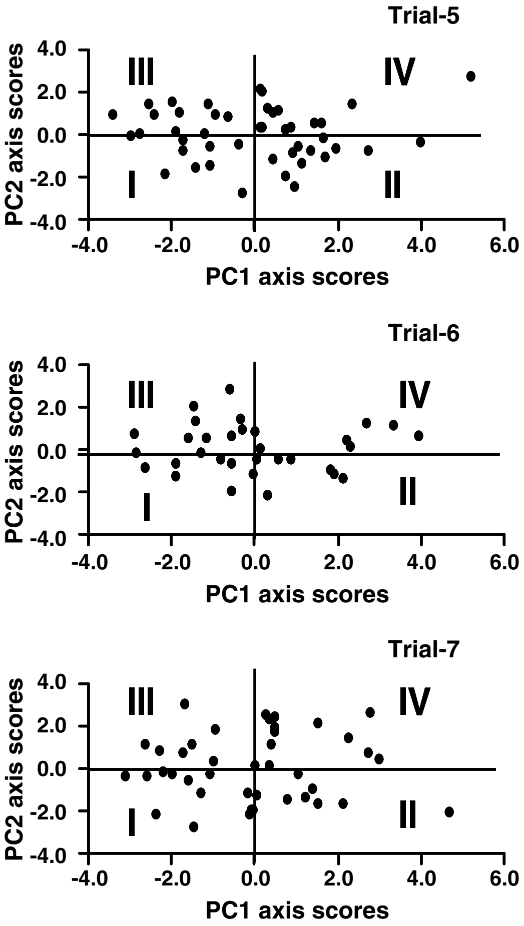

Fig. 2. Scatter plot of PC1 and PC2 axes scores for different sets of maize inbred lines adapted to the mid-altitudes tested at two locations.

genetic component to Fe and Zn concentrations in the grain of rice and wheat (Graham, Senadhira, Beebe, Iglesias, & Monasterio, 1999; Welch & Graham, 2004). Although the physiological basis for differential accumulation of minerals in the grains of different genotypes is yet to be clearly elucidated (Welch & Graham, 2002), the presence of large genotype effects and moderate genotype × location interactions for Fe and Zn and other minerals in the different trials indicate the possibility of attaining a significant increase in concentrations of these minerals in maize grain through hybridization and selection. The inbred lines included in each trial were classified into four groups based on differences in their mineral compositions. One of the four groups constituted inbred lines having high concentrations of almost all mineral elements in their grains. These inbred lines can be used as suitable parental materials for inter-crossing to significantly increase the concentrations of Fe and Zn along with Mn, Cu, Ca, Mg, K, P and S in tropical maize. On the other hand, two of the remaining three groups of lines had high concentrations of specific mineral elements in their grain in two or three trials. A second breeding strategy can thus be pursued by crossing inbred lines selected for high Fe, Zn, Mg, K, P and S from Group II to the inbred lines selected for high Ca and Cu from Group III to increase the concentrations of these mineral elements simultaneously in tropical maize. As none of the correlations of Fe and Zn with other minerals was significant and negative in these and other trials (Arnold & Bauman, 1976; Arnold et al., 1977), breeding for high Fe and Zn concentrations is not likely to have any negative effect on concentrations of other mineral elements in maize grain. The significant and positive correlations between grain Fe and Zn concentrations also indicate the possibility of increasing the two minerals simultaneously.

A few studies have reported a negative correlation between grain yield and concentrations of grain minerals in maize (Bänziger & Long, 2000), triticale (Feil & Fossati, 1995) and wheat (Monasterio & Graham, 2000; Oury et al., 2006; Peterson, Johnson, & Mattern, 1983). On the other hand, yield improvement in maize hybrids over decades in Ontario was not associated with clearly defined changes in concentrations of grain minerals (Vyn & Tollenaar, 1998). Feil and Fossati (1995) and Oury et al. (2006) also found genotypes with comparable yield potential having considerable differences in grain mineral concentrations. Thus, there are indications to breed maize for high concentrations of grain minerals without lowering grain yield potential. Since general combining ability accounts for much of the variation in concentration of grain minerals among maize hybrids (Arnold & Bauman, 1976; Long, Banziger, & Smith, 2004; Simic et al., 2003), the best maize inbred lines selected from the different trials can be used as parents to exploit both additive and non-additive gene action to increase the concentration of grain minerals. In order to minimize the potential dilution effect of yield on mineral concentration, the selected best-inbred lines can be evaluated in hybrid combinations in multiple locations and selection for high mineral concentration can be done among hybrids with high yield potential.

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