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# Genotypic Differences in Concentration and Bioavailability of Kernel-Iron in Tropical Maize Varieties Grown Under Field Conditions[#]

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## ABSTRACT

Iron deficiency is estimated to affect over one-half the world population. Improving the nutritional quality of staple food crops through breeding for high bioavailable iron represents a sustainable and cost effective approach to alleviating iron malnutrition. Forty-nine late maturing tropical elite maize varieties were grown in a lattice design with two replications in three locations representing three agroecologies in West and Central

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Africa to identify varieties with high levels of kernel-Fe. Bioavailable iron was assessed for some varieties selected for high Fe concentration in kernel and improved agronomic traits using an in vitro digestion/Caco-2 cell model. Significant differences in kernel-Fe and -zinc concentration were observed among varieties ($P < 0.001$). Kernel-Fe levels ranged from 16.8 to 24.4 mg kg$^{-1}$, while kernel-Zn levels ranged from 16.5 to 24.6 mg kg$^{-1}$. Environment did not have a significant effect on kernel-iron and -zinc levels, but genotype by environment (G × E) interaction was highly significant. The genetic component accounted for 12% of the total variation in kernel-Fe and 29% for kernel-Zn levels. Kernel-Fe was positively correlated with kernel-Zn ($R^2 = 0.51$, $P < 0.0001$). Significant differences in iron bioavailability were detected among selected Fe-rich varieties grown at one location. Mean bioavailable Fe ranged between 30% below to 88% above the reference control variety. The results indicate that genetic differences exist in kernel-Fe and -Zn concentrations and Fe bioavailability. These differences may be useful in biofortification intervention programs, but additional research is needed to determine the efficacy of iron-rich maize varieties in alleviating iron deficiency in humans.

*Key Words:* Caco-2; Grain-iron and -zinc concentrations; Iron bioavailability; Tropical maize; West and Central Africa.

## INTRODUCTION

Iron deficiency is the most common nutritional deficiency affecting over one-half of the world population. In West Africa, the prevalence of iron deficiency is high especially among women and children. For example, in Burkina Faso, 70% of children under the age five and 40% of pregnant women suffer from iron deficiency anemia. In southeastern Nigeria, over 50% of children and 61% of women of childbearing age are anemic.[1] Iron deficiencies can retard mental development and learning capacity and impair physical growth during childhood and adolescence while in adults it reduces the capacity to do physical labor.[2]

In West Africa maize is a major staple food crop with a per capita consumption in most coastal countries ranging from 30 to 90 kg year$^{-1}$. Maize kernels are processed into different traditional food products such as pastes, gruels and porridges. Green maize serves as an important vegetable crop in bridging the "hunger gap" after the long dry season, when it is eaten boiled or roasted on the cob. Most rural people rely on cereal- and legume-based diets for their major sources of essential micronutrients. Improving the nutritional quality of cereals such as maize can have a significant impact on the nutritional

status of the rural populace dependent on these staple foods, especially resource-poor women, infants, and children.

Plant breeding can significantly contribute to dietary improvement to alleviate iron deficiency anemia among the rural poor through the identification and distribution of cultivars of major staple crops with enhanced levels of iron in their seeds. Recent studies showed that large differences exist in iron concentration in maize kernels. Grain-Fe concentrations have been reported to range between 9.6 and 63.2 mg $kg^{-1}$.[3] These variations in kernel-iron levels were attributed to both genetic differences and the environments in which the germplasm were grown. Grain-iron and -zinc concentrations have been reported to correlate inversely with grain-yield as a result of dilution effect caused by enhanced grain-starch content, posing difficulties in breeding efforts.[3] Therefore, the evaluation of elite genotypes, which are inherently high yielding, for high kernel-iron and -zinc levels can circumvent the problem of the undesirable association between grain-yield and iron and zinc concentrations, thus allowing the development of high yielding genotypes with enhanced levels of iron and zinc in the kernels.

It is important to have desirable levels of micronutrients expressed consistently in all growing environments. There is some evidence of significant genotype by environment (G×E) interactions for iron levels in grains of maize[3] and rice.[4,5] This can ultimately affect the iron concentrations in extreme environments.[6] Breeders, however, can consider G × E interactions as heritable and exploitable component of variation through selection for broad or specific adaptation. Thus, selecting genotypes with stable expression of high kernel-iron and -zinc levels across diverse environments (broad adaptation) may be as important as increasing the concentration of these nutrients in maize kernels.

The nutritional value of selected maize genotypes to humans will depend to a large extent on the bioavailability of the micronutrients to humans after consumption. An in vitro iron bioavailability model system that mimics the gastric and intestinal digestion of humans, coupled with culture of human intestinal epithelial cells (Caco-2) has been shown to have great promise in addressing bioavailability issues because it is rapid and inexpensive.[7,8] Recently, this model was used to screen and rank promising rice genotypes with increased grain-iron level for differences in iron bioavailability in order to advance these lines in breeding programs.[9] This model was selected to screen a large number of maize genotypes for differences in iron bioavailability.

The objectives of this study were: (i) to evaluate iron and zinc concentrations in the grains of elite late-maturing maize varieties grown in diverse environments; and (ii) to rank the bioavailability of iron in selected iron-dense varieties using an in vitro digestion/Caco-2 cell model.

## MATERIALS AND METHODS

### Maize Samples

Forty-nine elite late-maturing maize varieties were grown in a Lattice Design with two replications at Ikenne (6°54′N, 3°42′E, 60 m asl; rainfall, 1421 mm), Mokwa (9°18′N, 5°40′E, 210 m asl; rainfall, 1235 mm), and Saminaka (11°11′N, 7°38′E, 686 m asl; rainfall, 900–1200 mm), representing the forest agroecology, southern and northern guinea savannas, respectively. The maize varieties were developed at the International Institute of Tropical Agriculture (IITA), Nigeria. The control variety, Entry 22 is a widely grown late-maturing variety released to the farmers in the 1980s. Dry grain samples were ground to uniform fine powder using a stainless steel Waring blender (Waring Products, New Hartford, CT)[a] and stored in a cold room (4°C) before analysis.

### Analysis of Mineral Concentrations in Grains

Triplicate samples (150 mg) were digested using concentrated nitric acid and perchloric acid. The concentrations of trace minerals including iron and zinc in the samples were analyzed using inductively coupled argon plasma emission spectrometry (ICP-ES) (ICAP Model 61E Trace Analyzer, Thermo Jarrell Ash Corporation, Franklin, MA).

### Analysis of Iron Bioavailability Using an In Vitro Digestion/Caco-2 Cell Model Experimental Design

Twenty genotypes with high grain-iron concentrations and with improved agronomic traits were selected from genotypes grown at Ikenne to assess bioavailable iron using the Caco-2 cell model. Because of the large number of samples and lack of significant effect of field blocking, the two replications for each genotype were bulked into a composite sample for bioavailability study. The experiments were conducted on separate days in four trials. Each trial had five samples plus a reference control (Entry 22) using 6-well plates. There were five replicates for each sample. The same reference control was used in all the four trials. Samples were randomized in each 6-well plate.

[a]Mention of trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the United State Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Due to the low available Fe in staple food crops, Glahn et al.[9] reported that it was necessary to add ascorbic (10 : 1 AA : Fe, molar ratio) to the digest of rice in order to express differences in bioavailable iron between genotypes. With maize, pilot studies demonstrated it was necessary to add 100 μmol/L ascorbic acid (approximately 8 : 1 AA : Fe, molar ratio) to each in vitro digest to increase Fe bioavailability from these maize varieties to measurable levels (data not shown). Pilot studies also showed no difference in Fe bioavailability between cooking and non-cooking of samples, hence samples were not cooked before in vitro digestion.

## Cell Culture

Unless otherwise stated, all chemicals, enzymes and hormones were purchased from Sigma Chemical Co. (St. Louis, MO). Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD) at cell passage 17, and used in experiments at cell passage 25–33. Cells were seeded at a density of 50,000 cells/cm$^2$ in collagen-treated 6-well plates (Costar Corp., Cambridge, MA). The cells were grown in Dulbecco's Modified Eagle Medium (GIBCO, Grand Island, NY) with 10% (v/v) fetal calf serum (GIBCO), 25 mmol/L HEPES, and 1% antibiotic antimycotic solution (GIBCO). The cells were maintained in an incubator at 37°C with a 5% $CO_2$ and 95% atmospheric air at constant humidity. The medium was changed every two days. The cells were used in the iron bioavailability experiments at 13 days post seeding.

## In Vitro Digestion

Ground maize (0.5 g) was used for each sample digestion. The preparation of digestion solutions including pepsin, pancreatin and bile extract and in vitro digestion procedures were performed as previously reported.[7] Preparation of the 6-well culture plates with cell monolayers, harvesting of cell monolayers, and ferritin and protein analyses have been previously described.[9]

## Statistical Analysis

Data were subjected to analysis of variance using the general linear model procedure of SAS[10] and means were separated by LSD test at $P < 0.05$.

## RESULTS AND DISCUSSION

### Iron Concentrations in Kernels

The statistical analysis (ANOVA) showed that there was no significant effect of environment (location), but varietal and variety × environment (G × E) interaction effects were highly significant for kernel-iron and -zinc concentrations (Table 1). Even though significant variety × environment (G × E) interactions were detected for grain-Fe and -Zn concentrations in the present study, there was still a significant and large genetic component for kernel-Fe concentration (12% of total sum of squares) and kernel-Zn (29%) to justify further efforts for improvement for these traits. It was quite encouraging to find such significant variation among these elite varieties that were selected for high yield and not increased in kernel-mineral concentration. A similar observation of significant G × E interaction effects on kernel-Fe concentration was reported among maize germplasm evaluated in Zimbabwe.[3] Our results illustrate the importance of testing genotypes under representative environmental conditions to identify genotypes with high and stable kernel-Fe concentration across a broad range of growing environments.

Means of varieties averaged over environments varied from 16.8 to 24.4 $mg\,kg^{-1}$ for kernel-Fe (Table 2) and from 16.5 to 24.6 $mg\,kg^{-1}$ for kernel-Zn (Table 3). The difference in kernel-Fe and -Zn concentrations between the best variety and the poorest variety in this trial was 45–49%,

***Table 1.*** Statistical significance of environment, genotype, and environment by genotype (G × E) interactions on kernel-Fe and -Zn concentrations from 49 late-maturing maize varieties, grown at three field locations, and bioavailable Fe from 20 selected iron-rich varieties at one location in Nigeria.

| | *Pr > F* | | | | Contribution to total sum of squares (%) | |
|---|---|---|---|---|---|---|
| | Kernel | | Bioavailable Fe | | Kernel | |
| Source of variation | Fe | Zn | % | LOG (%) | Fe | Zn |
| Environment (E) | 0.48 | 0.26 | | | 17 | 4 |
| Variety (G) | <0.0001 | <0.001 | 0.006 | 0.001 | 12 | 29 |
| G × E | <0.0001 | <0.0001 | | | 8 | 28 |
| CV (%) | 11 | 7 | 46 | 8 | | |

***Table 2.*** Kernel-iron concentration of 49 late-maturing maize varieties grown at three locations in Nigeria.

| | Kernel-Fe concentration ($mg\,kg^{-1}$) | | | |
|---|---|---|---|---|
| Variety/entry no. | Ikenne | Mokwa | Saminaka | Mean |
| 1 | 18.75 | 22.81 | 18.49 | 20.01 |
| 2 | 17.85 | 21.01 | 18.29 | 19.05 |
| 3 | 16.25 | 18.38 | 15.76 | 16.79 |
| 4 | 18.39 | 24.67 | 17.26 | 20.11 |
| 5 | 16.62 | 20.86 | 17.37 | 18.29 |
| 6 | 21.11 | 20.38 | 20.70 | 20.73 |
| 7 | 19.95 | 19.08 | 18.50 | 19.17 |
| 8 | 17.01 | 23.13 | 20.56 | 20.23 |
| 9 | 16.57 | 20.64 | 19.11 | 18.77 |
| 10 | 17.16 | 21.52 | 16.07 | 18.25 |
| 11 | 18.57 | 22.08 | 17.39 | 19.35 |
| 12 | 18.91 | 20.62 | 18.37 | 19.30 |
| 13 | 18.00 | 23.50 | 18.05 | 19.85 |
| 14 | 26.84 | 25.92 | 18.94 | 23.90 |
| 15 | 19.76 | 22.46 | 17.82 | 20.01 |
| 16 | 17.71 | 21.95 | 18.20 | 19.29 |
| 17 | 18.84 | 20.30 | 16.75 | 18.63 |
| 18 | 18.92 | 24.23 | 17.65 | 20.27 |
| 19 | 15.51 | 22.01 | 17.12 | 18.21 |
| 20 | 21.78 | 25.69 | 17.47 | 21.65 |
| 21 | 19.32 | 19.16 | 16.96 | 18.48 |
| 22 (control) | 16.04 | 21.39 | 16.23 | 17.89 |
| 23 | 17.69 | 23.58 | 18.15 | 19.80 |
| 24 | 15.70 | 21.50 | 17.95 | 18.38 |
| 25 | 16.17 | 17.35 | 18.17 | 17.23 |
| 26 | 19.87 | 21.39 | 18.03 | 19.76 |
| 27 | 20.81 | 21.85 | 19.24 | 20.63 |
| 28 | 19.14 | 26.50 | 17.91 | 21.18 |
| 29 | 17.48 | 18.30 | 17.85 | 17.88 |
| 30 | 19.70 | 21.91 | 19.28 | 20.30 |
| 31 | 17.59 | 20.80 | 16.38 | 18.26 |
| 32 | 16.92 | 21.07 | 16.47 | 18.16 |
| 33 | 16.94 | 20.32 | 22.17 | 19.81 |
| 34 | 22.80 | 23.08 | 17.11 | 20.99 |
| 35 | 24.37 | 26.56 | 22.20 | 24.38 |
| 36 | 18.50 | 20.91 | 19.64 | 19.69 |
| 37 | 17.47 | 20.32 | 18.56 | 18.78 |
| 38 | 17.07 | 23.54 | 18.23 | 19.61 |

(*continued*)

*Table 2.* Continued.

| Variety/entry no. | Kernel-Fe concentration (mg kg$^{-1}$) | | | |
|---|---|---|---|---|
| | Ikenne | Mokwa | Saminaka | Mean |
| 39 | 20.04 | 22.88 | 18.20 | 20.37 |
| 40 | 19.78 | 22.81 | 18.72 | 20.44 |
| 41 | 17.69 | 22.10 | 20.05 | 19.95 |
| 42 | 19.26 | 23.71 | 22.89 | 21.96 |
| 43 | 17.35 | 18.80 | 17.80 | 17.98 |
| 44 | 16.94 | 22.70 | 18.96 | 19.53 |
| 45 | 18.50 | 22.64 | 20.22 | 20.45 |
| 46 | 19.83 | 25.14 | 18.96 | 21.31 |
| 47 | 21.57 | 23.48 | 19.92 | 21.66 |
| 48 | 20.34 | 24.87 | 18.60 | 21.27 |
| 49 | 15.29 | 20.14 | 16.34 | 17.26 |
| Mean | 18.67 | 22.04 | 18.39 | 19.70 |

LSD ($P < 0.05$) for environment (E) = not significant
LSD ($P < 0.05$) for variety (G) = 1.77
LSD ($P < 0.05$) for G × E = 3.06

suggesting that a genetic potential exists to increase concentration of these trace minerals in maize kernels. Since these varieties had undergone selection for high yield potential and tolerance/resistance to pests and diseases, the presence of significant varietal differences for iron and zinc contents would indicate a possibility that the undesirable negative association between grain-Fe and -Zn concentrations and grain-yield as a result of dilution effect previously reported[3] could have been circumvented.

Three varieties, namely Entries 35, 47, and 6, had grain-Fe concentration consistently higher than the experimental mean (19.7 mg Fe kg$^{-1}$) in all environments, indicating higher stability for this trait than the other varieties. Entry 35 was the most Fe- and Zn-rich variety with a mean of 24.4 mg Fe and 24.6 mg per kg kernels across environments, and the most stable variety with 13–35% higher kernel-Fe concentration compared to that of the experimental mean in all environments. This variety may have much broader adaptation to adverse environments than others. These Fe-rich varieties could contribute to the improvement of human iron nutrition and could have some significant agronomic advantages, such as more viable and vigorous seedlings at the early growth stage, higher resistance to diseases, and better use of soil moisture.[11]

Significant and positive correlations were observed between kernel-Fe and kernel-Zn concentrations in each of the locations (Ikenne, $R^2 = 0.76$,

***Table 3.*** Kernel-Zn concentration of 49 late-maturing maize varieties grown at three locations in Nigeria.

| | Kernel-Zn concentration ($mg\,kg^{-1}$) | | | |
|---|---|---|---|---|
| Variety/entry no. | Ikenne | Mokwa | Saminaka | Mean |
| 1 | 17.62 | 21.11 | 19.79 | 19.51 |
| 2 | 14.25 | 20.26 | 119.54 | 18.01 |
| 3 | 15.80 | 16.63 | 16.90 | 16.45 |
| 4 | 17.16 | 22.15 | 18.84 | 19.39 |
| 5 | 17.42 | 18.32 | 17.93 | 17.89 |
| 6 | 25.51 | 21.62 | 19.81 | 22.31 |
| 7 | 25.46 | 20.86 | 19.07 | 21.79 |
| 8 | 17.13 | 20.60 | 21.36 | 19.70 |
| 9 | 14.37 | 19.53 | 21.69 | 18.53 |
| 10 | 17.09 | 18.99 | 20.57 | 18.88 |
| 11 | 19.16 | 17.73 | 17.18 | 18.02 |
| 12 | 20.67 | 20.47 | 20.11 | 20.41 |
| 13 | 19.79 | 21.65 | 20.10 | 20.52 |
| 14 | 27.71 | 19.92 | 21.20 | 22.94 |
| 15 | 20.79 | 20.51 | 19.62 | 20.31 |
| 16 | 15.40 | 21.42 | 18.66 | 18.49 |
| 17 | 20.21 | 20.60 | 20.40 | 20.40 |
| 18 | 21.95 | 19.73 | 22.43 | 21.37 |
| 19 | 18.89 | 19.46 | 20.98 | 19.78 |
| 20 | 21.32 | 21.51 | 19.37 | 20.73 |
| 21 | 19.54 | 19.45 | 19.35 | 19.45 |
| 22 (control) | 18.56 | 18.72 | 17.95 | 18.41 |
| 23 | 17.31 | 18.78 | 20.53 | 18.88 |
| 24 | 18.14 | 19.39 | 23.67 | 20.40 |
| 25 | 16.95 | 18.28 | 18.21 | 17.81 |
| 26 | 19.22 | 20.23 | 19.70 | 19.72 |
| 27 | 17.85 | 19.53 | 19.33 | 18.91 |
| 28 | 20.41 | 20.07 | 20.74 | 20.41 |
| 29 | 20.52 | 21.38 | 18.52 | 20.14 |
| 30 | 21.51 | 18.62 | 23.45 | 21.19 |
| 31 | 17.52 | 20.59 | 23.53 | 20.54 |
| 32 | 18.19 | 19.64 | 20.14 | 19.32 |
| 33 | 19.17 | 21.74 | 21.92 | 20.94 |
| 34 | 23.22 | 18.49 | 19.46 | 20.39 |
| 35 | 26.11 | 23.81 | 23.88 | 24.60 |
| 36 | 21.88 | 21.75 | 23.06 | 22.23 |
| 37 | 17.79 | 21.52 | 19.33 | 19.55 |
| 38 | 14.43 | 22.88 | 22.34 | 19.88 |

(*continued*)

*Table 3.* Continued.

| Variety/entry no. | Kernel-Zn concentration ($mg\,kg^{-1}$) | | | |
|---|---|---|---|---|
| | Ikenne | Mokwa | Saminaka | Mean |
| 39 | 20.36 | 20.92 | 20.66 | 20.65 |
| 40 | 23.62 | 20.61 | 19.95 | 21.39 |
| 41 | 17.08 | 21.83 | 19.73 | 19.54 |
| 42 | 22.33 | 23.77 | 21.02 | 22.37 |
| 43 | 17.45 | 20.90 | 20.36 | 19.57 |
| 44 | 20.51 | 22.51 | 22.10 | 21.71 |
| 45 | 18.65 | 21.39 | 19.83 | 19.96 |
| 46 | 18.30 | 21.77 | 20.61 | 20.23 |
| 47 | 22.38 | 24.61 | 23.22 | 23.40 |
| 48 | 19.14 | 25.54 | 23.96 | 22.88 |
| 49 | 16.70 | 19.38 | 21.57 | 19.21 |
| Mean | 19.44 | 20.64 | 20.48 | 20.19 |

LSD ($P < 0.05$) for environment (E) = not significant
LSD ($P < 0.05$) for variety (G) = 1.17
LSD ($P < 0.05$) for G × E = 2.01

$P < 0.0001$; Mokwa, $R^2 = 0.42$, $P < 0.003$; Saminaka, $R^2 = 0.37$, $P < 0.01$) and across locations ($R^2 = 0.51$, $P < 0.0001$). Other studies have also shown a high positive relationship between Fe and Zn content in cereal grains, indicating that efforts aimed at increasing one may also increase the content of the other.[12,13]

## Iron Bioavailability

Significant differences in bioavailable Fe were observed among selected Fe-rich varieties grown at Ikenne (Table 1). Mean bioavailable Fe ranged between 30% below to 88% above the reference control variety, Entry 22 (Fig. 1). Ferritin values for the reference control variety from all four trials averaged $36.9 \pm 3.9$ ng ferritin per mg of cell protein. Three varieties (Entries 14, 4, and 28) with similar bioavailable Fe were significantly higher than the reference control. Entry 14 showed specific adaptation to Ikenne, because it had the highest kernel-Fe concentration of $26.8\,mg\,kg^{-1}$ and ranked the highest in bioavailable Fe compared with other varieties at this location.

There was no significant correlation between kernel-Fe and -Zn concentration and Fe bioavailability. A similar observation of lack of significant

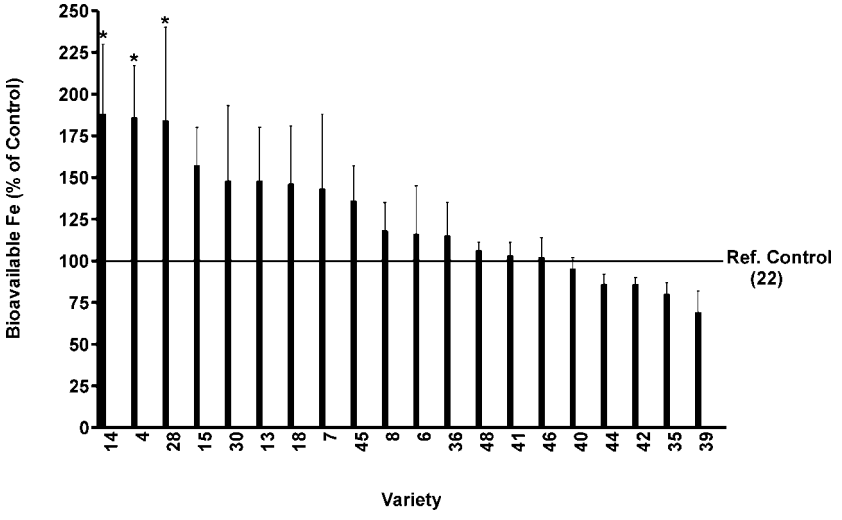


***Figure 1.*** Bioavailable Fe as measured by Caco-2 cell ferritin formation (as % of reference control, Entry 22) in 20-selected iron-rich late-maturing maize varieties grown at Ikenne, Nigeria. Bars are standard error of mean. Asterisks indicate varieties that are significantly higher than the reference control variety at $P < 0.05$.

relationship between kernel-Fe levels and Fe bioavailability from rice had been reported.[9] The presence of polyphenolic compounds and high levels of phytic acid (inositol phosphates) in some of the rice genotypes was reported to have profound inhibitory effects on Fe bioavailability.[9] In the present study, these compounds were not measured, but since most cereal crops contain high levels of these compounds, the differences in Fe bioavailability observed may be attributed to variable levels of these organic compounds in the varieties screened. This merits further investigation. Furthermore, the presence of multiple-aleurone layers (MAL) can increase kernel-Fe and -Zn concentrations in maize. There may be varietal differences in the existence of MAL, but this was not evaluated in the present study.

## CONCLUSIONS

We found significant differences in Fe and Zn concentrations in the kernels of elite late-maturing tropical maize varieties. Genetic component accounted for 12% of the total variation in kernel-Fe concentration and 29% in kernel-Zn concentration. Bioavailable Fe of selected Fe-rich varieties from one

location was significant. However, no significant relationship was observed between kernel-Fe concentration and Fe bioavailability. Further studies are needed to establish if the observed differences in kernel-Fe concentration and Fe bioavailability would contribute to alleviate iron deficiency in humans.

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