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Assessment of Concentrations of Iron and Zinc and Bioavailable Iron in Grains of Early-Maturing Tropical Maize Varieties

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Twenty elite early-maturing (75−90 days) tropical maize varieties grown in three diverse agroecologies in West Africa were evaluated to identify varieties with high kernel-Fe and -Zn and bioavailable Fe levels. Bioavailable iron was assessed using an in vitro digestion/Caco-2 cell model. Significant ($P < 0.001$) varietal differences were observed in mean kernel-Fe and -Zn levels. The ranges were 15.5−19.1 mg kg^{-1} for Fe and 16.5−20.5 mg kg^{-1} for Zn. Genetic component accounted for 34% of the total variation in kernel-Zn and for 11% of the variation in kernel-Fe levels. Mean bioavailable Fe in varieties ranged between 4% below and 49% above the reference control variety. A significant negative relationship was detected between kernel-P concentration and bioavailable Fe ($R = -0.36$; $P < 0.004$; $n = 60$). Two varieties, ACR90POOL16-DT and ACR86TZESR-W, were identified as the most promising for further evaluation to determine their efficacy as improved sources of iron in target populations.

KEYWORDS: Iron biofortification; Caco-2; iron bioavailability; iron and zinc concentrations; tropical maize; West Africa

INTRODUCTION

Maize is a major staple food crop in West Africa. Per capita maize consumption in most coastal countries in West Africa ranges from 30 to 90 kg year^{-1}. Maize kernels are processed into different traditional food products such as porridges, gruels, and pastes. Green maize serves as an important crop in bridging the "hunger gap" after the long dry season, when it is eaten boiled or roasted on the cob. Because of the high consumption of maize, it provides >50% of the total intake of iron and zinc in the diets of the rural people in sub-Saharan Africa (*1*) and 47% of the per capita protein intake (*2*). Because 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, especially resource-poor women, infants, and children.

In sub-Saharan Africa, the prevalence of iron deficiency has been on the increase in the past 50 years, due primarily to the low content of dietary iron and inadequate intake of bioavailable iron (*3*, *4*). In West Africa, the prevalence of iron deficiency is high among women and children. For example, in Burkina Faso, 70% of children under 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 (*5*). Iron deficiencies during childhood and adolescence can retard mental development and learning capacity and impair physical growth, whereas in adults it reduces the capacity to do physical labor (*6*). It has been estimated that in the West African subregion, 20−30% of mortality of children under the age five can be prevented if these children are fed diets adequate in protein, vitamin A, and other micronutrients.

Plant breeding can significantly improve the diet of the rural poor population. This can be done through identification and distribution of cultivars of major staple crops containing enhanced levels of micronutrients, such as iron and zinc, in their grains. Until recently, however, crop improvement programs in Africa placed little emphasis on quality improvement of grains in staple food crops (*7*). Hence, little is known about the genetic variation of micronutrients, such as kernel-iron and -zinc levels, of maize and the potential for enhancement.

Large variations in iron and zinc concentrations in the kernels of maize have been observed. Bānziger and Long (*8*) reported a range of grain-Fe concentration between 9.6 and 63.2 mg kg^{-1} and grain-Zn concentrations between 12.9 and 57.6 mg kg^{-1} from a screening of >1800 maize germplasm in Mexico and Zimbabwe. These variations in kernel-iron and -zinc levels were attributed to both genetic differences and the environments in which the germplasm were grown. Kernel-iron and -zinc

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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 (*8*). Therefore, the evaluation of elite high-yielding varieties for high kernel-iron and -zinc concentrations can circumvent the problem of undesirable association between grain-yield and iron and zinc concentrations, thus allowing the development of high-yielding varieties 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 variety (G) by environment (E) interactions for iron and zinc in grains of maize (*8*) and rice (*9*, *10*). This can ultimately affect the concentrations of these nutrients in extreme environments (*11*). Thus, selecting varieties with stable expressions of micronutrient concentrations across diverse environments is an important aspect of increasing the micronutrient concentration in maize grain through plant breeding.

The nutritional value of selected maize varieties will depend not only on the micronutrient concentration in the kernel but to a large extent on the bioavailability of the micronutrients to humans after consumption. Until recently, the cost of animal and human feeding trials has prevented large-scale screening of samples. An in vitro iron bioavailability model system that mimics the gastric and intestinal digestion of humans, coupled with the culture of human intestinal epithelial cells (Caco-2), has shown great promise for being rapid and inexpensive in addressing bioavailability issues (*12*−*15*). Recently, this model was successfully used to screen and rank rice varieties with increased grain-iron concentration for differences in iron bioavailability in order to advance these genotypes for further testing (*16*). The present study uses this model for the first time to assess the extent of iron bioavailability in a large number of maize varieties grown in diverse environments.

The early-maturing trait of maize is an important adaptation factor for environments with <5 months of annual rainfall in West Africa. The early-maturing varieties (75−90 days) are important in bridging the "hunger gap" after the long dry season and as a source of income generation through early access to the market. It is desirable, therefore, to improve the nutritional quality of these varieties because they serve as a premium crop to the farmer.

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

MATERIALS AND METHODS

Maize Samples. Twenty elite early-maturing maize varieties were grown in a randomized complete block design (RCBD) 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, and southern and northern guinea savannas, respectively. The maize varieties were developed at the International Institute of Tropical Agriculture (IITA), Nigeria. Characteristics of the varieties are described in **Table 1**. Among the 20 varieties, ACR86TZESR-W that has been adopted by some farmers was used for comparing the other varieties that are yet to be released. A widely grown late-maturing variety, open-pollinated (TZB-SR) developed at IITA and released to the farmers of Nigeria in 1986, was included as a "reference control" variety.

Dry grain samples were ground to a uniform fine powder using a stainless steel Waring blender (Waring Products, New Hartford, CT) and stored in a cold room (4 °C) before analysis.

Table 1. Characteristics of the 20 Early-Maturing Maize Varieties Selected for This Study

variety[a]	entry	grain color
AK96DMR-ESR-W	1	white
ACR97TZECOMP3x4	3	white
ACR86TZESR-W[b]	6	white
ACR98TZEMSR-W	7	white
HP97TZECOMP3x4	9	white
ACR94TZECOMP5-Y	13	yellow
TZEEW-SRBC5	15	white
TZEEY-SRBC5	16	yellow
AK9331DMRSR	18	yellow
AK94-DMR-ESR-Y	20	yellow
ACR95TZECOMP4	2	white
ACR90POOL16-DT	4	white
ACR94TZECOMP5-W	5	white
BG97TZECOMP3x4	8	white
TZECOMP3C2	10	white
TZECOMP5C6	11	white
TZEMSR-W	12	white
POOL18SEQ.C4F2	14	yellow
TZESR-YC3	17	yellow
MAKA-SRBC5	19	yellow
TZB-SR[c]	22	white

[a] The first 10 varieties have higher grain yield and more resistance/tolerance to pests and diseases than the others. [b] Adopted early-maturing variety included for comparison. [c] Reference control, widely grown late-maturing variety released to farmers in the 1980s.

Analysis of Mineral Concentrations in Grains. Triplicate ground 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 Corp., Franklin, MA).

Analysis of Iron Bioavailability Using an in Vitro Digestion/Caco-2 Cell Model. Experimental Design. Because of the large number of samples and lack of significant effect of field blocking, the two field replications for each variety were bulked into a composite sample to give a total of 60 samples (20 varieties × 3 locations) that were assessed for iron bioavailability. The experiments were conducted on separate days in 12 trials. Each trial had five samples plus a reference control using six-well plates. There were five independent replicates for each sample, and the same reference control (TZB-SR) was used in all of the trials. Samples were randomized in each six-well plate. TZB-SR had concentrations of 16−21 mg of Fe kg^{-1} and 18−19 mg of Zn kg^{-1}.

Due to the low bioavailable Fe in staple food crops, Glahn et al. (*16*) reported that it was necessary to add ascorbic acid (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 that it was necessary to add 100 μmol of ascorbic acid L^{-1} (approximately 8:1 AA/Fe, molar ratio) to each in vitro digest to increase Fe bioavailability from these early-maturing maize varieties to levels where differences could be exposed (data not shown). Pilot studies also showed no difference in Fe bioavailability between cooking and noncooking 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 passage 17 and used in experiments at passage 25−33. Cells were seeded at a density of 50000 cells cm^{-2} in collagen-treated six-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^{-1} HEPES, and 1% antibiotic antimycotic solution (GIBCO). The cells were maintained in an incubator at 37 °C with 5% CO_2/95% atmospheric air at constant humidity. The medium was changed every 2 days. The cells were used in the iron uptake experiments at 13 days postseeding.

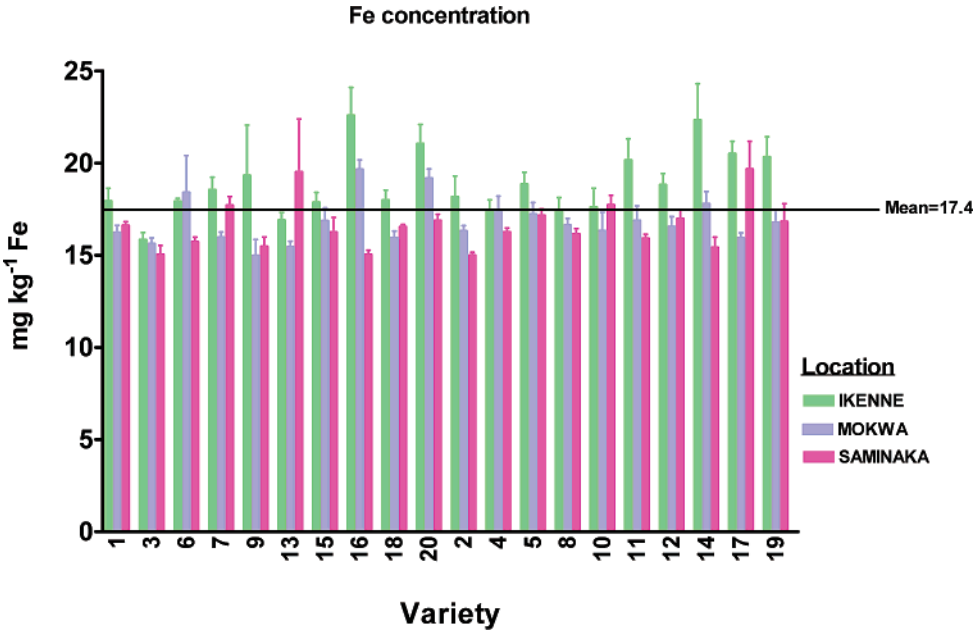

Figure 1. Influence of varieties and locations on kernel-Fe concentration (mg kg^{-1}) of 20 early-maturing tropical maize varieties grown in three field locations in Nigeria. Error bars are standard error of means, $n = 6$. The first 10 varieties on the left have higher grain yield and more resistance/tolerance to pests and diseases than the others.

Table 2. Analysis of Variance of Location, Variety, and Location-by-Variety (G × E) Interactions on Grain Iron and Zinc Concentrations and Fe Bioavailability from 20 Elite Early-Maturing Varieties Grown at Three Field Locations in West Africa

	Pr > *F*						
			Fe bioavailability		contribution to total variance (%)		
source of variation	grain Fe	grain Zn	%	LOG (%)	Fe	Zn	Fe bioavailability
location (Loc)	0.03	0.004	0.444	0.523	15	13	<1
variety (Var)	<0.0001	<0.0001	0.006	0.029	11	34	12
Loc × Var	<0.0001	<0.0001	0.586	0.353	17	19	10
CV (%)	13	7	35	7			

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 (*13*). Preparation of the six-well culture plates with cell monolayers, harvesting of cell monolayers, and ferritin and protein analyses have been previously described (*16*).

Statistics. Statistical analyses of data were carried out using the PROC GLM routine of SAS (*17*). For the analysis of grain-mineral concentrations, field blocks (replications) were treated as random effects. Pearson correlation analyses were run between traits using the PROC CORR routine of SAS (*17*).

RESULTS AND DISCUSSION

Iron and Zinc Concentrations in Kernels. The analysis of variance (ANOVA) showed that locations, varieties, and variety × location (G × E) interaction effects were highly significant for kernel-iron and -zinc concentrations (**Table 2**). Breeders need the information on the magnitude of genetic variation that exists for a given trait in a set of germplasm to justify selection for that trait. This can be estimated by partitioning of the total variation (total SS) into its various components from the ANOVA. Higher magnitude implies a greater genetic potential for improvement for the trait. In the present study, even though significant G × E interactions were detected for grain-Fe and -Zn concentrations, there was still a significant genetic component for grain concentration of Fe (11% of total SS) and Zn (34%) to justify selection for these traits. Considering that these varieties have undergone several cycles of selection in a breeding program, 11% genetic contribution to the total variation, although small, was considered to be high enough genetic potential to warrant further efforts for improvement for this trait.

A large part of the variation in kernel-Fe levels was due to residual or noise that is not interpretable and was thus discarded. The highly significant effects of environment and G × E interaction on kernel-Fe and -Zn levels were similarly observed among maize germplasm evaluated in Zimbabwe and Mexico (*8*). Our results illustrate the importance of testing varieties under representative environmental conditions to identify the most stable varieties with high micronutrient concentration.

Means for grain-Fe levels for varieties averaged over locations varied from 15.5 to 19.1 mg kg^{-1} and for grain-Zn levels from 16.5 to 20.5 mg kg^{-1} (**Figures 1** and **2**). The differences in Fe and Zn concentrations between the best and the worst varieties were only 20% in this trial, unlike the ones reported for wheat and rice showing a 2−4-fold difference (*18*). The narrow range in grain-Fe and -Zn concentrations may be attributed to the fact that these varieties are advanced genetic materials that had undergone several cycles of selection for high yield potential and tolerance/resistance to pests and diseases but not for increased micronutrient content.

Kernel-Fe concentration was highest (18.9 mg kg^{-1}) at Ikenne, whereas the highest kernel-Zn concentration of 19.3 mg kg^{-1} was obtained at Mokwa (**Figures 1** and **2**). Ikenne is known to have the lowest grain-yield potential compared to the other locations (*19*). Greater grain-starch content resulting from higher

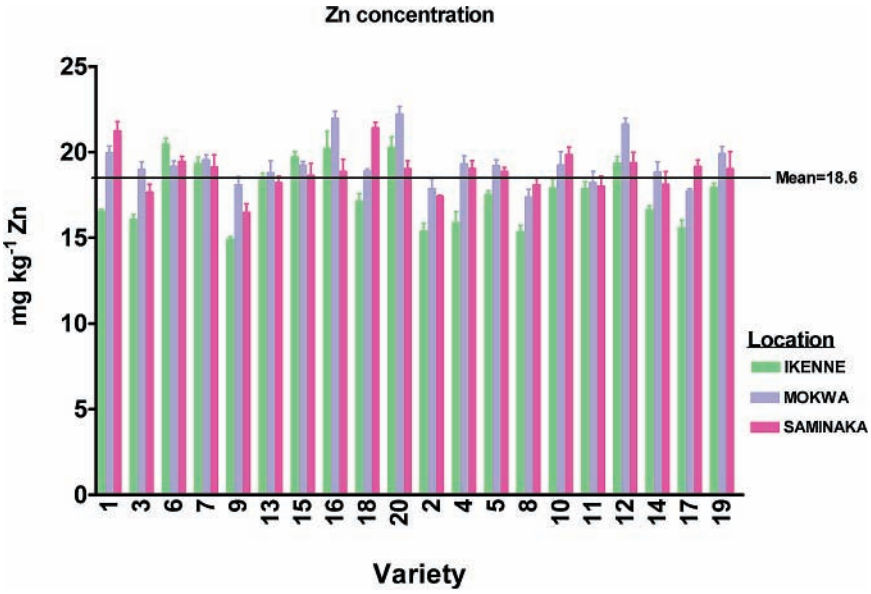

Figure 2. Influence of varieties and locations on kernel-Zn concentration (mg kg^{-1}) of 20 early-maturing tropical maize varieties grown in three field locations in Nigeria. Error bars are standard error of means, $n = 6$. The first 10 varieties on the left have higher grain yield and more resistance/tolerance to pests and diseases than the others.

yields at some locations may have caused biomass dilution effects on grain-Fe concentration. An inverse correlation has been observed between grain-yield and grain-Fe concentration in maize (*8*), possibly because of reduced biomass yield.

No single variety had consistently higher kernel-Fe and -Zn densities above the experimental mean at all three field locations (**Figures 1** and **2**). Selection of the best variety for Fe and Zn concentrations would, therefore, depend on the location. Promising varieties with 25% higher kernel-Fe levels than the adopted variety [ACR86TZESR-W (entry 6)] were TZEEY-SRBC5 (entry 16) and POOL18SEQ.C4F2 (entry 14) for Ikenne, TZEEY-SRBC5 (entry 15) and AK94-DMR-ESR-Y (entry 20) for Mokwa, and TZESR-YC3 (entry 17) and ACR-94TZE-COMP5-Y (entry 13) for Saminaka. In addition to the possible role these micronutrient-dense, early-maturing varieties could play in improving human nutrition, they may also have some significant agronomic advantages, including more viable and vigorous seedlings, higher resistance to diseases, and better use of soil moisture (*20*).

Iron Bioavailability. In the present study, significant differences in bioavailable Fe were observed among varieties, but environment and G × E interaction effects were not significantly different (**Table 2**). Mean bioavailable Fe ranged between 4% below and 49% above the reference control variety TZB-SR (**Figure 3**). Ferritin values for the reference control variety from the 12 trials averaged at 30.5 ± 1.4 ng of ferritin mg^{-1} of cell protein. Four varieties, namely, ACR95TZECOMP4 (entry 2), ACR97TZECOMP3x4 (entry 3), ACR90POOL16-DT (entry 4), and ACR86TZESR-W (entry 6) with similar bioavailable Fe levels, were significantly higher than the reference control variety [TZB-SR (entry 22)] (**Figure 4**). To assess the repeatability of these results, the four varieties plus the reference control were reanalyzed across locations in three trials. Results presented in **Table 3** showed that the four varieties averaged across locations had higher bioavailable iron than the reference control.

A bi-plot of mean bioavailable Fe and mean kernel-Fe concentrations is presented in **Figure 4**. Three yellow varieties [TZEEY-SRBC5 (entry 16), TZESR-YC3 (entry 17), and AK94-DMR-ESR-Y (entry 20)] located in the first quadrant

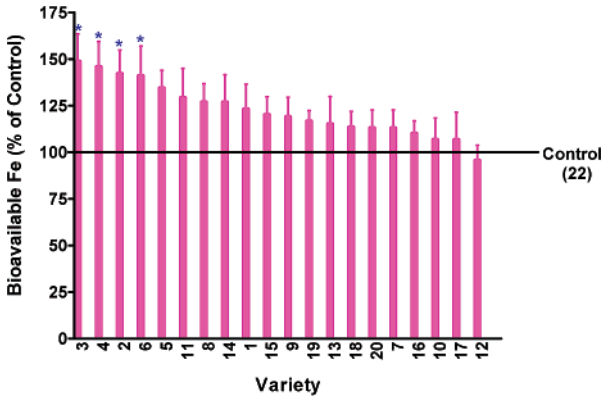

Figure 3. Mean iron bioavailability as measured by Caco-2 cell ferritin formation (as percent of reference control variety, TZB-SR) in 20 early-maturing tropical maize varieties grown in Nigeria. An asterisk (*) above an error bar indicates that the variety was significantly ($P < 0.05$) different from the reference control variety. Error bars are standard error of means, $n = 15$.

(**Figure 4**, quadrant I) with kernel-Fe concentrations 8−10% higher than the experimental mean, but with low bioavailable Fe, are good candidate varieties for further breeding efforts to improve their iron bioavailabilities. Two varieties, ACR90POOL16-DT (entry 4) and ACR86TZESR-W (entry 6) with bioavailable Fe 25−46% higher than the reference control (values from two runs of bioavailability, $n = 15$ each) and with mean kernel-Fe of 17−17.3 mg kg^{-1} close to the experimental mean (17.4 mg kg^{-1}) were identified as the most promising varieties for further evaluation using human subjects. Considering that the two varieties had Fe bioavailability of only 25−46% higher than the reference control, we cannot say with certainty that they will be of great impact in human nutrition. This remains to be determined.

Breeding staple food crops to increase the amount of iron that can be accessible for absorption is known as "biofortification" and is considered to be an additional tool in the fight against micronutrient malnutrition (*21*). The intrinsic iron of maize and other staple food crops represents a significant pool from which more iron can be made available and would result

Table 3. Bioavailable Fe (Second Run) in Four Early-Maturing Varieties That Showed Significantly Higher Fe Bioavailability than the Reference Control from the First Analysis and a Reference Control

		Caco-2 cell ferritin (as % of control)			
variety	entry	Ikenne	Mokwa	Saminaka	mean[a] ($n = 15$)
ACR86TZESR-W[b]	6	175	99	102	**125a** ± 21
ACR95TZECOMP4	2	101	85	133	**107a** ± 12
ACR97TZECOMP3x4	3	139	103	131	**125a** ± 13
ACR90POOL16-DT	4	133	139	96	**123a** ± 10
TZB-SR (ref control)	22	100	100	100	**100a** ± 0
mean ($n = 25$)		**130a** ± 12	**105a** ± 9	**113a** ± 8	**116**

[a] Means followed by the same letter are not significantly different at $P < 0.05$ as determined by Duncan's new multiple-range test. [b] Adopted early-maturing variety.

Table 4. Relationships among Grain-P, -Fe, -Ca, and -Zn and Iron Bioavailability from 20 Elite Early-Maturing Maize Varieties by Location and across Three Locations

location	Fe bioavailability[a]	Fe	Zn
Ikenne ($n = 20$)			
P	$R = -0.43$; $P < 0.06$[b]		$R = 0.84$; $P < 0.0001$
Mokwa ($n = 20$)			
Zn		$R = 0.61$; $P = 0.01$	
P		$R = 0.75$; $P < 0.001$	$R = 0.86$; $P < 0.0001$
Saminaka ($n = 20$)			
P	$R = -0.56$; $P = 0.01$	$R = 0.62$; $P < 0.01$	$R = 0.48$; $P = 0.03$
across locations ($n = 60$)			
P	$R = -0.36$; $P = 0.004$	$R = 0.47$; $P < 0.0001$	$R = 0.54$; $P < 0.0001$
Ca		$R = 0.54$; $P < 0.0001$	

[a] Bioavailable Fe expressed as percent of reference control. [b] R means Pearson correlation coefficient; P is probability of significance.

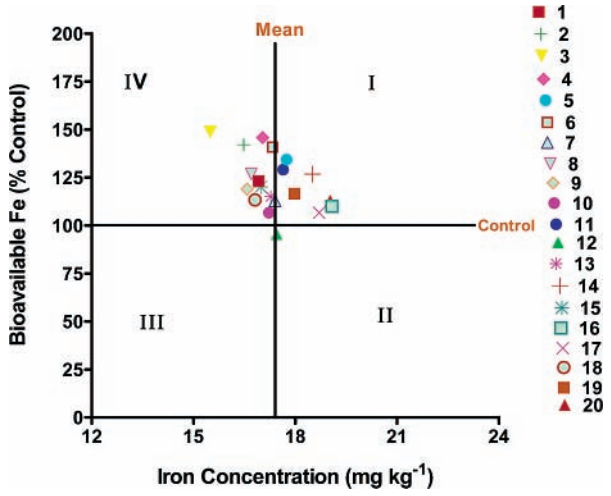

Figure 4. Bi-plot of mean iron bioavailability ($n = 15$) as measured by Caco-2 cell ferritin formation (as percent of reference control variety, TZB-SR) versus mean Fe concentration in mg kg^{-1} ($n = 18$) in 20 early-maturing tropical maize varieties grown in Nigeria.

in an inexpensive and sustainable contribution of bioavailable iron. However, as evident in many human studies, iron bioavailability from staple food crops is relatively low (i.e., 1−6%) if promoters such as meat or ascorbic acid are not consumed in the same meal (*22*, *23*). Pilot studies with this in vitro model also confirmed that the availability of the intrinsic Fe of the maize was very low, thus making it difficult to determine if there are differences among the varieties. For example, baseline ferritin levels in our Caco-2 cell cultures range from 5 to 10 ng of ferritin mg^{-1} of cell protein. Upon exposure of the cells to a maize digest and no other ingredients, cell ferritin levels would only increase by ∼5 ng of ferritin mg^{-1} of cell protein. This increase was deemed to be inadequate to separate differences in varieties as the inherent variation of the model could account for most of the apparent increase in ferritin. The Caco-2 cells of this model have demonstrated a much greater capacity to increase ferritin in response to Fe uptake, as ferritin values of ≥200 have been observed with lesser amounts of Fe from sources such as $FeSO_4$, beef, or fish (*13*). Therefore, as in a similar study with rice (*16*), we found it necessary to add some ascorbic acid to the samples to counteract the inhibitors and allow for cell ferritin increases to levels in the range of 23−45 ng of ferritin mg^{-1} of cell protein. Obviously, the addition of ascorbic acid alters the basis for comparison of the varieties, but we found it to be essential to increase cell iron uptake to levels at which varietal differences could be exposed.

The comparison of the maize in the presence of ascorbic acid should be representative of many common meals in West Africa. On the basis of food composition tables, the amount of ascorbic acid added to the digest would be approximately equal to a small glass of orange juice or one to two pieces of fresh fruit. In this region, maize is often consumed with other foods or with fruits/vegetables that contain some levels of ascorbic acid. For example, in southern Nigeria, roasted or boiled maize is often eaten with fresh mature cocoanut kernel that has some amount of ascorbic acid (AA) (2 mg of AA 100 g^{-1}) (*24*). In some other parts of West Africa, particularly in the urban areas, fruits and/or fruit juices such as *Citrus* spp. (45 mg of AA 100 g^{-1}) (*24*) are taken after meals that can promote Fe bioavailability. We are thus confident that the addition of a small amount of ascorbic acid to the samples represents a reasonable condition for comparison of these varieties.

When a large number of samples are ranked, such as in the present study, it is necessary to choose a reference control most relevant to the experimental objectives. In the design of these experiments, a standard variety of maize currently in use in these regions was deemed to be the most appropriate reference control, as our primary objective was to assess the iron bioavailability of these experimental lines relative to one already in use. Using another reference control such as $FeSO_4$ would not be appropri-

ate as the background matrix of the maize would be absent and Fe uptake would be relatively high (*13*).

In general, use of $FeSO_4$ as a reference is useful in human efficacy studies to provide a reference measure of iron absorption in the subjects (*25*). When $FeSO_4$ is added to a corn meal, it is likely to be of low availability (~4−5%) as it is significantly inhibited by the matrix of the corn (*26*). The intrinsic Fe of the maize is likely to be of similar availability (*23*) and, therefore, represents a significant source of Fe in the diet. However, most human trials comparing Fe availability from staple food matrices were conducted as single-meal, extrinsically radiolabeled trials, often in subjects from the United States or other developed countries. Single-meal feeding trials and extrinsic radiolabeling are both thought to overestimate Fe absorption under certain conditions (*27*, *28*). Testing of efficacy in staple food crops is best done via intrinsic radiolabeling (in single-meal trials) or via long-term assessment of Fe status in subjects native to the targeted region of intervention (*29*).

It is not yet known if determination of the amount of Fe likely to be absorbed in vivo from a given amount of maize can be predicted accurately from this in vitro method. Initial tests on this possibility appear to be promising because almost perfect correlations with select, well-designed human trials have been observed (Glahn et al., unpublished observations). However, much additional research needs to be done. Research in this area needs to focus on generating appropriate conversion factors for Caco-2 cell ferritin formation and human iron absorption. Direct comparison of Caco-2 cell and human uptake from the same food matrices will be necessary to generate this information.

Correlation Studies with Kernel-Mineral Concentrations and Iron Bioavailability. Correlation analyses for each location and across the three locations are presented in **Table 4**. Kernel-Fe and kernel-Zn levels were significantly and positively correlated with kernel-P concentration in at least two locations. A significant positive relationship was detected between kernel-Fe and kernel-Zn levels at the Mokwa location. Across locations, kernel-Fe and kernel-Zn levels were significantly ($P < 0.0001$) and positively correlated with kernel-P and kernel-Ca levels (**Table 4**), but kernel-P concentration was inversely correlated with iron bioavailability ($P < 0.004$; $R = -0.36$, $n = 60$). Surprisingly, there was no clear relationship between iron bioavailability and kernel-Fe concentration.

As observed in the present study, a number of other studies have shown a high correlation between Fe, Zn, and Ca content in cereal grains, indicating that efforts aimed at increasing one may also increase the content of the other (*18*, *30*, *31*). Phytic acid accumulation in cereal grains represents 50−80% total grain-P (*32*). Because phytic acid accumulates as mixed phytate salts of several cations, including Fe, Zn, Ca, and Mg, high phytic acid or total P will result in high kernel-Zn and -Fe concentrations as we found in the present study.

Phytate is a known inhibitor of Fe and Zn bioavailability in humans. Using an in vitro Caco-2 cell model, Glahn et al. (*16*) did not detect a significant relationship between phytate as measured by inositol phosphates (IP5 + IP6) and iron bioavailability from rice grain. In an earlier study, however, Glahn et al. (*33*) showed that an Fe-to-phytic acid molar ratio of 1:10 maximally inhibited Fe bioavailability in Caco-2 cells and that further increases in phytate produced no additional inhibition. In the present study, even though we did not measure phytic acid, if we assume that the phytic acid content of the varieties is 50% of total grain-P (*32*), the variety (entry 2) with the lowest total grain-P of 2.51 g kg^{-1} was estimated to have an Fe-to-phytic acid molar ratio of 1:25. The high molar ratio of Fe-to-phytic acid may explain the inverse relationship observed between Fe bioavailability and total grain-P. Considering the importance of phytic acid to the crop, the health benefit of phytate [inositol hexaphosphate (IP6)] in inhibiting human cancer (*34*, *35*) and the link of phytate with several minerals as found in this study (**Table 4**), caution should be exercised when low-phytate maize is bred as a means of enhancing iron bioavailability. The differences in Fe bioavailability observed among the varieties in this study may be attributed to differences in levels of inhibitory or enhancing compounds among the varieties evaluated, which merit further investigation.

The present paper is part of a larger study with the goal to enhance micronutrients in maize as a sustainable approach to mitigating "hidden" hunger in West Africa. Other components include food consumption and nutrition survey, identification of food processing and cooking techniques to enhance nutrient bioavailability, and nutrition education. The promising varieties identified from the present study will be further evaluated under different food processing and cooking methods. The traditional maize processing method of soaking milled flour or whole grain before milling and food preparation is known to reduce phytate content in maize (*36*) and can further enhance Fe bioavailability from these improved varieties. Furthermore, efficacy studies will be conducted in areas where the food consumption and nutrition survey show acute Fe deficiency to test how the identified Fe-rich varieties in combination with the best food processing method can contribute to improving Fe nutrition in the target populations.

In summary, we found significant differences in Fe and Zn concentrations in the grains of elite early-maturing tropical maize varieties. The varieties also differed in Fe bioavailability in an in vitro human Caco-2 cell model. Positive correlations were found between grain-Fe, -Zn, -Ca, and -P concentrations. Grain-P was inversely correlated with Fe bioavailability. Two varieties (ACR90POOL16-DT and ACR86TZESR-W) were identified as the most promising varieties for further evaluation using human subjects to determine their efficacy to improve the iron nutrition of humans. We anticipate, however, that further breeding effort for increased grain-Fe concentration and Fe bioavailability is needed.

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LITERATURE CITED

(1) Ruel, M. T.; Bouis, H. E. Plant breeding: A long-term strategy for the control of zinc deficiency in vulnerable populations. *Am. J. Clin. Nutr*. **1998**, *68*, 488−494.

(2) Bauernfeind, C. J.; DeRitter, E. Foods considered for nutrient addition: cereal grain products. In *Nutrient Additions to Food: Nutritional, Technological and Regulatory Aspects*; Bauernfeind, C. J., Lachance, P. A., Eds.; Food and Nutrition Press: Westport, CT, 1991.

(3) Mason, J. B.; Garcia, M. Micronutrient deficiency—the global situation. *SCN News* **1993**, *9*, 11−16.

(4) United Nations Administrative Committee on Coordination—Subcommittee on Nutrition. *Second Report on the World Nutrition Situation. United Nations Administrative Committee on Coordination, Subcommittee on Nutrition. Vol. I. Global and Regional Results*; Geneva, Switzerland, 1992; pp 1−80.

(5) FGN/UNICEF. *The Nutritional Status of Women and Children in Nigeria*; National Planning Commission and UNICEF Country Office: Lagos, Nigeria, 1994.

(6) Bouis, E. H. Plant breeding: A new tool for fighting micronutrient malnutrition. *J. Nutr.* **2002**, *132*, 491S−494S.
(7) Oikeh, S. O.; Kling, J. G.; Okoruwa, A. E. Nitrogen fertilizer management effects on maize grain quality in the West African moist savanna. *Crop Sci.* **1998**, *38*, 1056−1061.
(8) Bänzinger, M.; Long, J. The potential for increasing the iron and zinc density of maize through plant breeding. *Food Nutr. Bull.* **2000**, *21*, 397−400.
(9) Senadhira, D.; Graham, R. G. Genetic variation in iron and zinc concentrations in brown rice. *Micronutrients Agric.* **1999**, *No. 3*, 4.
(10) Gregorio, G. B. Progress in breeding for trace minerals in staple crops. *J. Nutr.* **2002**, *132*, 500s−502s.
(11) Graham, R. D.; Senadhira, D.; Beebe, S.; Iglesias, C.; Monasterio, I. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Res.* **1999**, *60*, 57−80.
(12) Glahn, R. P.; Wien, E. M.; Van Campen, D. R.; Miller, D. D. Caco-2 cell iron uptake from meat and casein digests parallels in vivo studies: Use of a novel in vitro method for rapid estimation of iron bioavailability. *J. Nutr.* **1996**, *126*, 332−339.
(13) Glahn, R. P.; Lee, O. A.; Yeung, A.; Goldman, M. I.; Miller, D. D. Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. *J. Nutr.* **1998**, *128*, 1555−1561.
(14) Glahn, R. P.; Lee, O. A.; Miller, D. D. *In vitro* digestion/Caco-2 cell culture model to determine optimal ascorbic acid-to-Fe ratio in rice cereal. *J. Food Sci.* **1999**, *64*, 925−928.
(15) Glahn, R. P.; Rassier, M.; Goldman, M. I.; Lee, O. A.; Cha, J. A. Comparison of iron availability from commercial iron preparations using an *in vitro* digestion/Caco-2 cell culture model. *J. Nutr. Biochem.* **2000**, *11*, 62−68.
(16) Glahn, R. P.; Cheng, Z.; Welch, R. M.; Gregorio, G. B. A. Comparison of iron bioavailability from 15 rice genotypes: Studies using an *in vitro* digestion/Caco-2 cell culture model. *J. Agric. Food Chem.* **2002**, *50*, 3586−3591.
(17) SAS Institute. *SAS Technical Report. SAS/STAT Software: Changes and Enhancements*, release 6.07; SAS Institute: Cary, NC, 1992.
(18) Graham, R. D.; Welch, R. M.; Bouis, H. E. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principle, perspective and knowledge gaps. *Adv. Agron.* **2001**, *70*, 77−142.
(19) Kling, J. G.; Oikeh, S. O.; Akintoye, H. A.; Heuberger, H. T.; Horst, W. J. Potential for developing nitrogen use efficient maize for low input agricultural systems in the moist savannas of Africa. In *Developing Drought and Low Nitrogen-Tolerant Maize*; Edmeades, G. O., Bänziger, M., Mickelson, H. R., Peña-Valdivia, C. B., Eds.; Proc. Symp. 25−29; CIMMYT: El Batán, Texcoco, Mexico, March 1996; CIMMYT: Mexico, D.F., 1997; pp 490−501.
(20) Graham, R. D.; Welch, R. M. Breeding for staple food crops with high micronutrient density. *Working Papers on Agricultural Strategies for Micronutrients 3*; International Food Policy Research Institute: Washington, DC, 1996.
(21) Welch, R. M. Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. *J. Nutr.* **2002**, *132*, 495S−499S.
(22) Bjorn-Rasmussen, E.; Hallberg, L.; Walker, R. B. Food iron absorption in man. I. Isotopic exchange between food iron and inorganic iron salt added to food: studies on maize, wheat and eggs. *Am. J. Clin. Nutr.* **1972**, *25*, 317−323.
(23) Mendoza, C.; Viteri, F. E.; Lonnerdal, B.; Young, K. A.; Raboy, V.; Brown, K. H. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *Am. J. Clin. Nutr.* **1998**, *68*, 1123−1127.
(24) Food and Agriculture Organization. *Agriculture, Food and Nutrition for Africa: Resource Book for Teachers of Agriculture*; Food and Agriculture Organization of the United Nations: Rome, Italy, 1997; 410 pp.
(25) Murray-Kolb, L. E.; Welch, R. M.; Theil, E. C.; Beard, J. L. Women with low iron stores absorb iron from soybeans. *Am. J. Clin. Nutr.* **2003**, *77*, 180−184.
(26) Layrisse, M.; Garcia-Cassals, M. N.; Solano, L.; Barón, M. A.; Arguello, F.; Llovera, D.; Ramírez, J.; Leets, I.; Tropper, E. Bioavailabiliy in humans from breakfasts enrich with iron glycine chelate, phytates and polyphenols. *J. Nutr.* **2000**, *130*, 2195−2199.
(27) Benito, P.; Miller, D. D. Iron absorption and bioavailability: An updated review. *Nutr. Res.* **1998**, *8*, 581−603.
(28) Van Campen, D. R.; Glahn, R. P. Micronutrient bioavailability techniques: Accuracy, problems and limitations. *Field Crops Res.* **1999**, *60*, 93−113.
(29) Haas, J. D.; del-Mundo, A. M.; Beard, J. L. A human feeding trial of iron-enhanced rice. *Food Nutr. Bull.* **2000**, *21*, 440−444.
(30) Cakmak, I.; Ozkan, H.; Braun, J.; Welch, R. M.; Romheld, V. Zinc and iron concentrations in seeds of wild, primitive and modern wheat. *Food Nutr. Bull.* **2000**, *21*, 400−4003.
(31) Maziya-Dixon, B.; Kling, J. G.; Menkir, A.; Dixon, A. Genetic variation in total carotene, iron, and zinc contents of maize and cassava genotypes. *Food Nutr. Bull.* **2000**, *21*, 419−422.
(32) Frossard, E.; Bucher, M.; Mächler, F.; Mozafar, A.; Hurrell, R. Review: Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J. Sci. Food Agric.* **2000**, *80*, 861−879.
(33) Glahn, R. P.; Wortley, G. M.; South, P. K.; Miller, D. D. Inhibition of iron uptake by phytic acid, tannic acid, and $ZnCl_2$: Studies using an in vitro digestion/Caco-2 cell model. *J. Agric. Food Chem.* **2002**, *50*, 390−395.
(34) Vucenik, I.; Kalebic, T.; Tantivejkul, K.; Abulkalam, A. M. Novel anticancer function of inositol hexaphosphate: Inhibition of human rhabdomyosarcoma *in vitro* and *in vito*. *Anticancer Res.* **1998**, *18*, 1377−1384.
(35) Shamsuddin, A. M. Metabolism and cellular functions of IP6: A review. *Anticancer Res.* **1999**, *19*, 3733−3736.
(36) Hotz, C.; Gibson, R. S. Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white maize (*Zea mays*). *J. Agric. Food Chem.* **2001**, *49*, 692−698.

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