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Potential for Improving Bioavailable Zinc in Wheat Grain (*Triticum* Species) through Plant Breeding

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A "whole-body" radioassay procedure was used to assess retention and absorption by rats of Zn in mature kernels of whole grain wheat harvested from 28 genotypes (*Triticum* spp.) grown in nutrient solution supplied with 2 μM $ZnSO_4$ radiolabeled with ^{65}Zn. Grain-Zn concentration differed among genotypes and ranged from 33 to 149 μg g^{-1} of dry weight (DW); similarly, grain-Fe concentration varied ~4-fold, from 80 to 368 μg g^{-1} of DW. Concentrations of Zn and Fe in the grain were positively correlated. Therefore, selecting genotypes high in grain-Zn also tends to increase grain-Fe concentration. Concentrations of *myo*-inositolhexaphosphate (phytate) in the wheat grain varied from 8.6 to 26.1 μmol g^{-1} of DW. Grain intrinsically labeled with ^{65}Zn was incorporated into test meals fed to Zn-depleted rats. All rats readily ate the test meals, so that Zn intake varied directly with grain-Zn concentration. As determined by the percentage of ^{65}Zn absorbed from the test meal, the bioavailability to rats of Zn in the wheat genotypes ranged from about 60 to 82%. The amount of bioavailable Zn (micrograms) in the grain was positively correlated to the amount of Zn accumulated in the grain. There was a significant negative correlation between grain-phytate levels and percentage of Zn absorbed from the wheat grain, but the effect was not large. These results demonstrate that concentrations of Zn in whole-wheat grain, as well as amounts of bioavailable Zn in the grain, can be increased significantly by using traditional plant-breeding programs to select genotypes with high grain-Zn levels. Increasing the amount of Zn in wheat grain through plant-breeding contrivances may contribute significantly to improving the Zn status of individuals dependent on whole grain wheat as a staple food.

KEYWORDS: Zinc; bioavailability; biofortification; grain; plant breeding; rats; phytate, micronutrient malnutrition

INTRODUCTION

Wheat grain (*Triticum* species) is a significant source of food containing protein, calories, and certain vitamins and minerals, particularly for resource-poor people globally (*1*). Wheat grain can also contain significant amounts of antinutrients, such as phytate and certain fibers. These generally accepted antinutritives affect the nutritional quality of wheat grain by depressing Zn bioavailability to monogastric animals and humans (*2*, *3*).

Failure to consume adequate amounts of bioavailable Zn or the consumption of factors that reduce Zn absorption from the diet may contribute to the development of Zn deficiency in people (*4*). The full extent of Zn deficiency worldwide is not known because specific and sensitive clinical procedures to assess marginal Zn status are lacking (*5*). Zinc deficiency has been identified among some low-income pregnant women, infants, and children in many developing countries, as well as in some children in Canada and the United States (*6*, *7*). In countries where plant foods are the major source of essential minerals, increasing the amount of bioavailable Zn, increasing the amount of factors that promote Zn absorption, and decreasing the amount of putative antinutritive factors in wheat or in other important staple plant foods represent significant steps toward developing food-based approaches to reducing Zn deficiency in at-risk populations (*8*, *9*). However, relatively little research has been conducted to improve the nutritional quality of plant foods as sources of Zn (*9*).

From the wheat collection at the Centro International de Mejoramiento de Maiz y Trigo (CIMMYT), El Batan, Mexico, we selected 27 wheat genotypes that accumulate various amounts of Zn in their mature grain. Subsequently, these genotypes were used to assess the effect of increasing grain-Zn concentrations on the bioavailability of Zn to rats from whole-wheat grain labeled intrinsically with ^{65}Zn.

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MATERIALS AND METHODS

Mature wheat grains, from plants grown by scientists at CIMMYT at various field locations, were initially screened for Zn concentrations. On the basis of initial Zn analyses, 27 lines were selected for the study reported here. These genotypes encompass several *Triticum* species (including *T. aestivum* L., *T. dicoccoides* Aarons., *T. dicoccon* Schrank, *T. durum* Desf., and *T. boeticum* Boiss.) as well as landraces (i.e., endemic wheat lines) and released varieties that differed in grain-Zn [from 13 to 143 μg g^{-1}, dry weight (DW)] and grain-Fe (from 16 to 93 μg g^{-1}, DW) concentrations when grown at various CIMMYT field locations in Mexico.

Grains of each genotype were germinated in the dark and then transferred to pots and grown in hydroponic nutrient solutions with the following composition (in mM): N, 16; K, 6; P, 2; Mg, 1; S, 1; Ca, 4; (in μM) Cl, 50; B, 12.5; Mn, 2; Zn, 2; Cu, 0.5; Mo, 0.1; Ni, 0.1; Fe, 50 [as the iron(III) *N*-(2-hydroxyethyl)-ethylenediamine-*N*,*N*,*N*′-triacetate chelate]. At flowering, Zn was supplied as ^{65}Zn-labeled $ZnSO_4$ (7.4 MBq of ^{65}Zn mmol^{-1} of Zn). Mature grains were harvested and hulled, and subsamples of whole grain were autoclaved at 250 °C for 15 min, homogenized, and lyophilized to dryness. Samples of dried grain and dried grain homogenates were wet-digested in a mixture of HNO_3 and $HClO_4$ (9:1, v/v) and analyzed for mineral elements using inductively coupled argon plasma atomic emission spectrometry with appropriate standards and reference materials as described (*10*). The concentration of *myo*-inositolhexaphosphate (i.e., phytate; IP6), in acid extracts of subsamples of dried grain meal was determined using a liquid ion chromatography method (*11*, *12*).

The 27 genotypes of wheat grain were used to assess the effect of increasing grain-Zn concentration on the amount of bioavailable Zn in the grain as determined using a rat model (*13*). Because of the relatively large number of genotypes to be tested (i.e., 27), the wheat genotypes were assigned to three groups and the amount of bioavailable Zn in the whole grain was determined in three feeding trials (8, 13, and 6 genotypes used in trials 1, 2 and 3, respectively). Additionally, a commercial wheat genotype, Grandin (*Triticum aestivum* L.), commonly grown in the United States, was also intrinsically labeled with ^{65}Zn and included in each trial as a control. Results for Grandin did not differ ($P > 0.05$) between trials so these data were used to normalize the bioavailability results between trials and to rank all genotypes with respect to bioavailable Zn content. All trials were performed using the protocol described below.

Male Sprague−Dawley rats (Charles River Laboratories, Wilmington, MA) were housed individually as described previously (*10*). All rats had free access to deionized water and, except as noted, the rats had free access to a basal, egg-white-based diet (Dyets Inc., Bethlehem, PA) modified from the AIN-93G formulation to contain 8 mg of Zn/kg of diet (*14*). After a 7-day adjustment period, the rats were denied food for 12 h, weighed, and assigned by body weight (BW) to 9, 14, or 7 groups of five rats each depending on the trial; the BW of rats did not differ among trials and averaged ~83 g at the start of all trials before the rates were fed the radiolabeled meal. The rats were then fed test meals that contained either wheat grain intrinsically labeled with ^{65}Zn or the basal diet labeled extrinsically with ^{65}Zn-labeled $ZnSO_4$.

Meals with wheat grain contained 1.0 g of dried grain homogenate, 1.0 g of a low-Zn diet (6.9 μg of Zn g^{-1} of diet) and 0.5 g of sucrose. The extrinsically labeled meals contained 2.0 g of basal diet, 0.5 g of sucrose, and 18 kBq of ^{65}Zn as $ZnSO_4$ added to the entire meal. The test meals were offered to the rats for 3 h and then were replaced with the basal diet. As described previously (*15*), rats were assayed for radioactivity in a "whole body" γ-spectrophotometer immediately after they were fed the test meals and then at about daily intervals for 10 days. Briefly, the γ-spectrophotometer was especially constructed for radioassaying small rodents and consisted of a multichannel analyzer connected to a special scintillation crystal assembly. In part, the crystal detector assembly consisted of a thallium-activated NaI well crystal (23 cm × 18 cm with a 9.5 cm × 10 cm well) that was optically coupled to four 7.6 cm photomutiplier tubes with external magnetic shield and low background tube base assemblies. The NaI−crystal−photomutiplier assembly was housed in a steel safe (61 cm × 61 cm × 122 cm) lined with 10-cm-wide Pb bricks (top, sides, and bottom) to reduce background radiation.

Table 1. Concentrations (Dry Weight Basis) of Zn, Fe, and *myo*-Inositolhexaphosphoric Acid (IP6) in Whole-Wheat Grain from 28 Genotypes Grown in ^{65}Zn-Labeled Nutrient Solutions

genotype	Zn (μg g^{-1})	Fe (μg g^{-1})	IP6 (μmol g^{-1})
CWI44509	32.75	112.68	17.91
Nainari 60	35.62	124.54	12.50
Anza	40.70	125.01	19.99
Tepoca M89	41.96	139.01	23.48
CWI44264	45.55	146.86	16.69
CWI66057	46.01	137.13	15.57
Maringa	46.05	111.42	8.64
Pato Blanco	47.40	146.39	24.55
CBME1YC 78	52.94	207.41	23.45
Sonora 64	54.05	254.18	24.75
Bichena	55.09	80.42	18.53
CWI44510	56.10	215.15	18.06
Papago	67.74	109.40	22.91
CWI65289	69.12	133.48	17.51
CWI44321	75.13	131.36	19.09
Baviacora	76.83	120.76	18.30
CWI65243	85.89	149.10	16.23
CWI44134	89.94	238.34	15.90
CWI65313	100.20	216.88	13.93
CWI44507	102.45	208.52	17.44
CWI44258	114.11	204.55	20.12
CWI4601	122.33	283.94	20.50
CWI66058	123.60	208.71	15.19
CWI4202	128.20	334.97	26.06
C-306	130.54	219.51	14.03
CWI65278	142.06	111.58	15.10
CBME1YC 19	148.99	368.13	15.40
Grandin control	56.7	137.5	14.8
SEM[a]	3.47	22.01	0.08
LSD[b]	10.58	26.67	1.62

[a] Pooled standard error of the mean calculated from ANOVA ($n = 60$). [b] Least significant difference ($P \leq 0.05$).

Whole-body retention of ^{65}Zn data were used to calculate Zn absorption using a second-order exponential equation as follows: fraction of dose retained $= A\ e^{-at} + B\ e^{-bt}$. The first equation component ($A\ e^{-at}$) reflects clearance of ^{65}Zn from the body, whereas the second component ($B\ e^{-bt}$) represents losses of endogenous ^{65}Zn. The coefficient *B*, obtained by extrapolating the terminal portion of the curve to time zero, is an indicator of the percentage of the dose absorbed from the meal (see ref *13* for details). The calculated percentage of ^{65}Zn absorbed represented the percentage of bioavailable Zn in the test meal. All animal care and use procedures were approved by an Institutional Animal Care and Use Committee.

Data were evaluated statistically using a one-way analysis of variance; means were compared using a least significant difference (LSD) procedure. Both linear and multiple regression techniques were utilized to evaluate associations among variables.

RESULTS AND DISCUSSION

Wheat Grain Composition. Table 1 shows concentrations of Zn, Fe, and IP6 (i.e., phytate) in mature grain harvested from 28 genotypes of wheat grown in nutrient solutions radiolabeled with ^{65}Zn. The amount of Zn in the grain varied among genotypes and ranged from about 33 to 149 μg g^{-1}, DW. The average concentration of Zn in all 28 genotypes tested (77 μg g^{-1}, DW) was higher than the average (31 μg g^{-1}, DW) reported by Wolnik et al. (*16*) for whole dry wheat grain collected from the major wheat-producing regions of the United States. Similarly, a food composition table reported that whole-wheat grain contained ~37 μg of Zn g^{-1}, DW (*17*). The difference in the overall concentration of Zn that we observed compared to that reported by others may be attributed partially to the

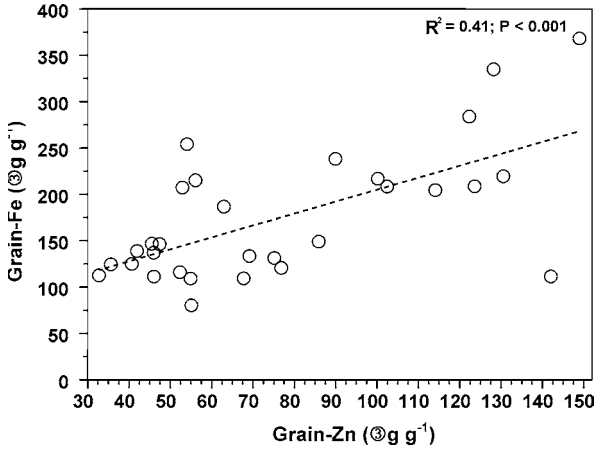

Figure 1. Relationship between Zn concentration and Fe concentration in 27 lines of intrinsically ^{65}Zn-labeled wheat genotypes (points also include three Grandin control samples from each of the three trials). Each point is an average of two replicate analyses. R^2 = correlation coefficient squared ($n = 60$); P = significance probability.

genotypes selected and the manner in which the wheat genotypes were grown. We purposely selected genotypes that had relatively high concentrations of Zn compared to commercially available varieties. Additionally, wheat grain included in previous studies was from plants grown in the field, and soils generally contain less plant-available Zn (*18*) compared to hydroponic nutrient solutions containing highly available $ZnSO_4$ (2 μM Zn) as was used to grow wheat in our study. Nevertheless, our results indicate that genotypes of wheat can be selected that contain >3 times as much Zn as many conventional varieties.

As with Zn, the concentration of Fe in the grain varied ~4-fold, ranging from 80 μg g^{-1}, DW (genotype, Bichena, a durum wheat) to 335 μg g^{-1}, DW (genotype, CWl4202) (see **Table 1**). The concentrations of Zn and Fe in the grain were positively correlated (**Figure 1**; [Fe] = 75.74 + 1.29 × [Zn], $R^2 = 0.41$; $P < 0.001$). This significant positive linear relationship between Fe and Zn concentrations in the grain suggests that selecting wheat genotypes with high concentrations of grain-Zn will also tend to increase the amount of Fe in the grain.

The phytate concentrations in the wheat grain varied from 8.64 to 26.06 μmol g^{-1} (**Table 1**). These values are similar to phytate values reported by others for various wheat lines (i.e., 9.15−15.07 μmol g^{-1}) (*19*, *20*). Because the phytate content of seeds can be affected by various abiotic and biotic factors, differences in the amount of phytate observed in the wheat genotypes that we grew and those surveyed by others could be the result of differences in the genotypes grown and to numerous agronomic factors, especially available soil-P levels (*21*).

Zinc Bioavailability. The rats readily ate the test meals containing the wheat grain radiolabeled intrinsically with ^{65}Zn; the small differences in the percentage of the meal consumed did not differ ($P > 0.05$) among treatments (data not shown). Because all rats ate ~99% of the wheat meals, the amount of Zn consumed was directly related to the amount of Zn in the grain. As indicated by absorption of ^{65}Zn, the percent bioavailability to rats of Zn in the grain varied among genotypes and ranged from 60 to 82% of the Zn in the grain (**Table 2**). In rats fed meals without wheat grain added and with the diet radiolabeled extrinsically with ^{65}Zn-labeled $ZnSO_4$, the bioavailability of Zn from the $ZnSO_4$ meal averaged ~89% (±2.5% SEM) of the dose, indicating a high Zn absorption efficiency by the rats from the diet fed. The amount of bioavailable Zn in the grain varied >4-fold, ranging from 22.4 to 95.5 μg of Zn g^{-1} of dry wt in genotypes CWl44509 and CMBE1YC 19, respectively. **Figure 2** shows the relationship between grain-Zn concentration in the 28 genotypes studied and their bioavailable Zn content (i.e., amount of Zn absorbed from the test meal) to rats. Clearly, genotypes with increased grain-Zn concentrations contained increased amounts of bioavailable Zn to rats supporting the concept that breeding for Zn-enhanced wheat grain may contribute to decreasing Zn deficiency in target populations.

Table 2. Bioavailability of Zn in 28 Genotypes of Wheat Grain Intrinsically Labeled with ^{65}Zn and Fed to Marginally Zn-Deficient Rats in a Single Meal[a]

genotype	trial	Zn absorption[b] (% of dose)	bioavailable Zn[c] (μg g^{-1} of meal)	normalized Zn bioavailability[d] (as % of Grandin control)
CWl44509	2	68.4	22.4	51.3
Nainari 60	2	69.0	24.6	57.6
Anza	1	63.0	25.6	102.6
Tepoca M89	1	66.0	27.7	112.5
CWl44264	2	75.0	34.1	82.1
CWl66057	3	82.1	37.8	85.8
Maringa	2	73.0	33.6	79.2
Pato Blanco	1	63.7	30.2	120.0
CBME1YC 78	1	65.5	34.7	139.6
Sonora 64	1	63.8	34.5	139.3
Bichena	1	66.5	36.6	149.1
CWl44510	2	76.4	42.8	102.1
Papago	1	67.0	45.4	115.1
CWl65289	3	80.7	55.8	127.1
CWl44321	3	76.0	57.1	130.5
Baviacora	1	68.2	52.4	209.5
CWl65243	3	80.9	69.5	158.9
CWl44134	2	76.5	68.8	162.1
CWl65313	2	73.7	73.8	174.3
CWl44507	2	68.9	70.6	165.3
CWl44258	2	72.5	82.7	194.2
CWl4601	3	62.5	76.5	174.1
CWl66058	2	68.5	84.7	199.08
CWl4202	3	66.7	85.5	196.1
C-306	2	65.6	85.7	200.1
CWl65278	2	60.3	85.7	202.7
CBME1YC 19	2	64.1	95.5	223.4
Grandin control	1	67.6	25.4	100
Grandin control	2	70.9	44.6	100
Grandin control	3	79.9	43.9	100
LSD[e]		12.6	10.0	43.6

[a] Meals contained 1.0 g of dried wheat grain homogenate, 1.0 g of low-Zn diet, and 0.5 g of sucrose. [b] Calculated from whole-body ^{65}Zn retention data. [c] Calculated as % radiolabeled Zn absorbed × % Zn consumed in 1 g of meal. [d] Calculated as [(μg of Zn absorbed g^{-1} of meal of genotype)/(μg of Zn absorbed g^{-1} of meal Grandin control)] × 100. [e] Least significant difference ($P \leq 0.05$).

Zinc absorption can be affected by the concentration of Zn in the diet fed prior to the test meal and by the amount of Zn in the test meal (*22*). Moreover, it is well established that Zn status affects the amount of Zn absorbed (*2*). Rats used in our study were fed a marginally Zn-deficient diet and, as indicated by plasma-Zn concentration (i.e., 0.83 ± 0.06 μg mL^{-1}), the rats were marginally Zn-deficient (an adequate Zn range is reported to be 1 μg of Zn mL^{-1} of plasma or greater). Marginal Zn deficiency was induced in the rats to enhance Zn absorption and thereby provide a more sensitive model to assess Zn bioavailability. The Zn in the wheat grain studied here was highly bioavailable to rats and averaged ~70% of the Zn in the grain meals. The total amount of Zn absorbed from the meals averaged 53.3 μg of Zn per gram of meal) (**Table 2**).

Mature wheat grain usually contains relatively high amounts of phytate, and an inhibitory effect of phytate on Zn absorption

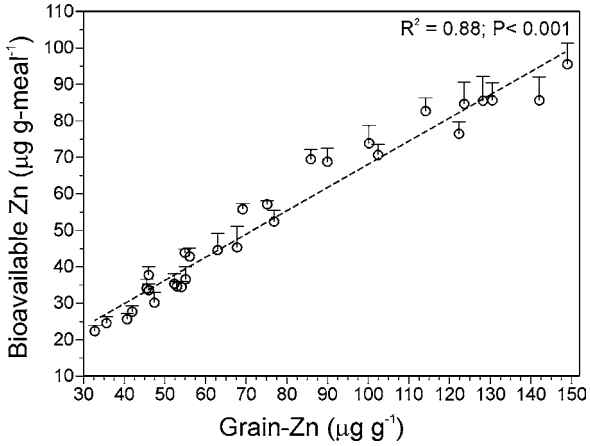

Figure 2. Effects of increasing grain-Zn concentrations in 27 wheat lines and 3 Grandin wheat control samples on Zn bioavailability to Zn-depleted rats fed single meals containing intrinsically ^{65}Zn-labeled wheat grain. Error bars represent standard error of the means (SEM) for bioavailable Zn (n = 5). Line plotted was calculated using a linear regression model; R^2 = correlation coefficient squared (n = 30); P = significance probability.

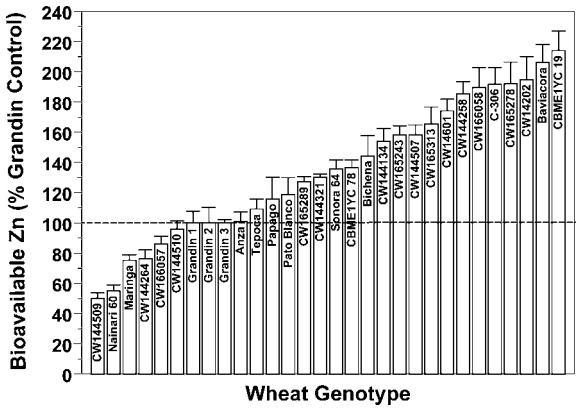

Figure 3. Ranking of 28 wheat genotypes with respect to bioavailable grain-Zn plotted as a percent of the bioavailable Zn in the control genotype, Grandin (i.e., Grandin = 100%) that was included in each of the three rat trials. Error bars represent standard error of the means (SEM; n = 5).

has been demonstrated repeatedly (*23*). On the basis of dietary phytate content, several indices have been proposed to predict Zn bioavailability including the phytate/Zn molar ratio, the (phytate × Ca)/Zn molar ratio, and the total amount of phytate in the plant food (*2*). About half of the variation in Zn bioavailability in several cereals (wheat, corn, oats, barley) and legumes (beans, peas, and lupines) could be explained by the Zn/phytate-P ratio. The current study found a weak but significant negative correlation between the percent of Zn absorbed by the rats and the phytate concentration (micromoles) in the grain-meal (% Zn = 83.5 − 0.707[phytate]; R^2 = 0.10; P = 0.02), but there was no marked effect of grain-phytate concentration on the total amount of Zn absorbed from the meal (i.e., total micrograms of Zn absorbed). Apparently, although phytate reduced the percent of Zn absorbed from the meal, its negative effect on Zn bioavailability was not great enough to negate the positive effects of increasing grain-Zn concentrations from high-Zn genotypes.

Figure 3 depicts the rankings of the 28 wheat genotypes studied with respect to bioavailable Zn content. The normalized values represent the bioavailable Zn plotted as a percent of the control genotype, Grandin (taken as 100%), which was used in each of the three rat trials. The genotype with the lowest bioavailable Zn ranking was CWl44509, which contained only ~50% of the bioavailable Zn found in the Grandin control. The CBME1YC 19 genotype ranked highest with ~220% more bioavailable Zn when compared to the Grandin control. Thus, there was a >4-fold range in bioavailable Zn levels observed within the 28 genotypes of wheat studied. These results show that there is enough genetic variation in bioavailable grain-Zn levels within the wheat genome to allow for significant improvements in the nutritional quality of wheat by selecting for high grain-Zn in wheat-breeding programs.

It is neither practical nor appropriate to extrapolate the Zn bioavailability data presented in this study directly to human nutritional needs. The rat is not an ideal quantitative model for assessing the bioavailability of Zn for people (*24*), but rats can be used to rank genotypes with respect to bioavailable Zn in plant foods (*3*). As shown in this study, this approach can be used to evaluate various wheat genotypes for bioavailable Zn and, thereby, provide an objective basis for selecting genotypes for further testing. Before any particular wheat genotype studied here is promoted in a breeding program, it is recommended that selected wheat genotypes containing highly bioavailable grain-Zn be further tested in a pig model. After the latter studies, the most promising genotypes could be tested in human subjects to ensure an impact on improving the Zn status of target populations.

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