Genetic Selection for Enhanced Bioavailable Levels of Iron in Bean (*Phaseolus vulgaris* L.) Seeds†

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The bioavailability of Fe from 24 select genotypes of bean (*Phaseolus vulgaris* L.) seeds containing a range of concentrations of Fe, *myo*-inositol pentaphosphate plus phytic acid (IP5+IP6), and tannins was studied using a rat model. Bean accessions, selected from field trials for their variations in Fe, phytate, and tannin seed concentrations, were grown in a greenhouse in nutrient solutions radiolabeled with ^{59}Fe. Mature seeds were autoclaved and lyophilized. Test meals (containing 1 g of dried bean, 0.5 g of sucrose, and 1 g of basal Fe-deficient diet) were fed to marginally Fe-depleted weanling rats over a 3-h period; rats were radioassayed in a γ-spectrometer immediately after feeding and daily thereafter for the next 10 d. Radioiron retention data were used to calculate percent Fe absorption (i.e., Fe bioavailability) from the meals. Seed Fe concentrations ranged from 52 to 157 μg g^{-1} dry weight. There was a tendency to also select for higher Zn concentrations in the beans when selecting for high Fe concentrations. The Fe bioavailability to rats from test meals depended on the genotype and varied from 53% to 76% of the total Fe. Bean genotypes with higher seed Fe concentrations resulted in increased amounts of bioavailable Fe to rats. There was no significant correlation between the Fe concentration in different bean genotypes and Fe bioavailability to rats attributable to variations in IP5+IP6 or tannins, even though these antinutrients varied widely (i.e., from 19.6 to 29.2 μmol of IP5+IP6 g^{-1} and from 0.35 to 2.65 mg of tannins g^{-1}) in the test meals. Other unknown seed factors (i.e., antinutrients or promoter substances) may be contributing factors affecting Fe bioavailability from bean seeds.

Keywords: *Iron bioavailability; nutritional quality; rats; plant breeding*

INTRODUCTION

Nearly two billion people are currently iron-deficient, and the incidence is increasing globally (United Nations Administrative Committee on Coordination−Subcommittee on Nutrition, 1992; World Bank, 1994). This is especially true for resource-poor women, infants, and children living in the developing world (Mason and Garcia, 1993). Even in the United States iron deficiency has been considered by the Surgeon General to be a public health issue (Department of Health and Human Services, 1988) seriously affecting the health and well-being of certain population groups including Native American Alaskans, pregnant black women, and children in low-income families (Looker et al., 1997). Iron deficiency results in increased mortality and morbidity rates, decreased labor productivity, and impaired mental development, which reduces the capacity of people to live healthy and productive lives and thereby stagnates national development efforts in some developing nations. Almost all international programs to alleviate iron deficiency have depended on food fortification and iron supplementation interventions. Unfortunately, most of these programs have not proven to be sustainable in many world regions for various reasons (Yip, 1997). Importantly, neither the nutrition community nor the agriculture community has viewed agricultural production systems as an important tool to use in intervention programs to eliminated iron deficiency. Most policymakers, however, now agree that food-based sustainable solutions to iron deficiency must be developed; these types of solutions may best be found in holistic food system models that view agriculture as the primary source of all nutrients (Combs et al., 1997). Significantly increasing the bioavailable concentrations of iron in staple plant foods through genetic selection for this trait via plant breeding is such an agricultural tool that can be used. Doing so could contribute greatly to reducing the number of people afflicted with iron deficiency and would employ a sustainable food-based approach to the problem (Graham et al., 1999; Graham and Welch, 1996; Welch and Graham, 1999).

The bioavailability of Fe to individuals eating plant foods depends on a complex set of interacting factors including (but not limited to) meal composition, Fe status of the individual, the presence of dietary antinutrients (such as phytic acid and tannins) or promoter substances (e.g., “meat factors”), metabolic demand for Fe, and genetic propensity for absorbing Fe (House, 1999; Van Dokkum, 1992). However, all mature legume seeds contain high levels of phytic acid, and many contain significant levels of tannins (Burk and Solomons, 1985; Deshpande and Deshpande, 1991), antinutrients that reportedly depress Fe bioavailability (Fairweather-Tait, 1992; Viteri, 1999; World Health

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Organization, 1996). Thus, both of these substances are important factors to consider when selecting genotypes of legume seeds that accumulate higher levels of Fe as compared to current commercial varieties. Additionally, certain substances (i.e., promoter substances such as "meat factors" found in animal meats and ascorbic acid) are known to promote the bioavailability of non-heme-iron from plant foods high in antinutrients such as phytic acid and tannins. Some evidence has been presented that suggests that the S-containing amino acids, methionine and cysteine, can promote both non-heme-Fe and -Zn bioavailability to rats and that these amino acids may be associated with the "meat factor" (House et al., 1996, 1997; Mulvihill and Morrissey, 1998a,b; Welch and House, 1995).

An important grain legume directly consumed by humans is the common bean (*Phaseolus vulgaris* L.) (Deshpande and Deshpande, 1991). This staple plant food is an important source of nutrients for resource-poor people worldwide. It provides important quantities of protein and calories and is an excellent sources of some minerals and vitamins (Pennington and Young, 1990; Holland et al., 1991). There are over 25 000 accessions of the domesticated common bean held in the gene bank at CIAT (Centro Internacional de Agricultural Tropical) in Cali, Colombia. From these accessions, a core collection containing 1441 accessions was selected to represent the full range of agroecological environments and morphological variability of common beans (Tohme et al., 1998). This collection offers a good approximation of the potential of the species for a given trait.

Using the CIAT core collection as a genetically variable source of bean seeds, we selected lines that differed in their ability to accumulate Fe, phytic acid, tannins, and total S (a measure of S-containing amino acids) (Welch, 1993) in their mature seed to study the effect of these seed factors on the amount of bioavailable Fe in bean seeds using a rat model. Zinc concentrations were also determined because Zn is another limiting nutrient in many diets of people dependent on beans as a staple food. These data will be used in a breeding program to develop high-yielding bean lines that are significantly enriched in bioavailable Fe levels (Graham and Welch, 1996).

MATERIALS AND METHODS

Bean Accessions Selection and Propagation. Seeds from the CIAT core bean collection (grown at the same location during the same season) were initially screened in Cali, Columbia, for seed iron concentrations using inductively coupled, argon-plasma emission spectrometry (ICPES), and 24 contrasting accessions were selected. These accessions represented a range in seed types differing not only in their iron concentrations but also in their seed colors, total S, and total P concentrations (total S and P concentrations include both inorganic and organic forms; Table 1).

White-seeded beans are known to contain low tannin levels; therefore, two accessions of white-colored bean were included to ensure variability in seed tannin content among accessions. Total S concentration is an indicator of S-containing amino acids (i.e., methionine and cysteine−cystine) in seeds. Total P is an indicator of seed phytate concentrations in seeds. Variability was likewise sought in seed S and seed P concentrations among accessions.

Seeds were germinated between layers of water-saturated filter paper in glass-covered Petri dishes at 25 °C in the dark. After hypocotyl and radical emergence, the plantlets were transferred to nutrient solutions of 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 Fe(III)−EDDHA chelate]. At flowering, Fe was supplied as radiolabeled Fe(III)−EDDHA [740 MBq ^{59}Fe (mmol of Fe)$^{-1}$]. Seeds were harvested at maturity, and subsamples were autoclaved for 15 min to cook the beans. The autoclaved beans were homogenized in a Polytron homogenizer, and the homogenate was lyophilized to dryness.

Table 1. Characteristics of 24 Bean (*Phaseolus vulgaris* L.) Accessions Used in This Study (Received from CIAT)

CIAT i.d.	common name	origin[a]	seed color	seed size (g/100 seeds)
G87	De Seda	ELS	cream	20
G734	Otz k'al tsaik	GTA	cream	32
G1678	Baetao manteiga	BZL	purple mottled	51
G1844		CRA	cream	22
G2572	Rosado	ECD	cream	40
G2774		MEX	cream striped	34
G3096	Blanco Mono Ligero	GTA	black mottled	26
G4825	Carioca	BZL	cream striped	23
G5706	Jalpatagua 72	GTA	black	20
G8465	Guatemala 0370	GTA	red	17
G11350	Chiapas 36-3	MEX	red	19
G11419	Puebla 44-2	MEX	cream striped	40
G12610	Bayo mediano	PER	cream	48
G13220	Tolima	CLB	yellow	44
G14519	Hickman pole bean	USA	brown	38
G15137		CMR	red	58
G16267	Chimbolo	CRA	pink	16
G18372	Coscorron No. 4	CLB	white	55
G18811	Alubia de Asturia	SPN	white	27
G19022	Chiapas 115	MEX	cream & black	32
G21078		ARG	cream	47
G21242		CLB	cream mottled	43
G21725	IPA 6	BZL	cream	19
G23063	Line 227	MWI	purple	31

[a] ELS, El Salvador; GTA, Guatemala; BZL, Brazil; CRA, Costa Rica; ECD, Ecuador; MEX, Mexico; PER, Peru; CLB, Colombia; USA, United States; CMR, Cameroon; SPN, Spain; ARG, Argentina; MWI, Malawi.

Bean Seed Analyses. Subsamples (0.2 −0.5 g) of the dried bean seed homogenates were wet digested (using nitric−perchloric acids), and the acid digestates were analyzed via ICPES for Fe, S, P, and Zn concentrations. Phytate was determined in extracts (Lehrfeld, 1994) of subsamples of dried bean meal via a Dionex liquid ion chromatography method (Dionex, 1990). Tannins and polyphenolics were determined in subsamples of a methanol extraction of dried bean meal as catechin equivalents (Deshpande and Cheryan, 1987).

Bioavailability Determinations. The dried homogenates of intrinsically ^{59}Fe-labeled mature beans were used to prepare single meals. The meals were fed to male Sprague−Dawley weanling rats (fasted overnight) weighing between 103 and 120 g (mean, 111 g) that were maintained on a marginally Fe-deficient diet (modified AIN93-G containing 20 μg of Fe g^{-1} dry wt diet) for 7 d (blood-hemoglobin averaged 10.6 ± 0.2 g dL^{-1}). The meal contained 1.0 g of dried bean homogenate, 1.0 g of basal diet, and 0.5 g of sucrose, and the container holding the meal was radioassayed before the meal was offered to the rats. The meal was fed ad libitum for 3 h, and any meal spillage was collected. Immediately after being fed the meals, the rats were radioassayed for radioactivity and daily thereafter for the next 10 days (Welch et al., 1978). Meal containers plus bean contents and spillage were also radioassayed postprandial to determine the amount of the bean meal consumed. All radioassays were conducted using a "whole body" γ-spectrometer. Rats were then provided, ad libitum, the basal diet for the remainder of the experiment. Retention data plotted as a function of time postprandial were described by exponential functions, and the functions were used to calculate Fe absorption (Welch et al., 1974). The percent of ^{59}Fe absorbed represented the percent of Fe in the beans that was bioavailable to the rats. Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC).

Table 2. Concentrations (Dry Weight Basis) of Fe, Zn, Total P, Total S, IP5+IP6, and Tannins in Mature Bean Seeds from 24 Genotypes Grown in ^{59}Fe-Radiolabeled Nutrient Solutions

bean	Fe (μg g^{-1})	Zn (μg g^{-1})	total P (mg g^{-1})	total S (mg g^{-1})	IP5+IP6[a] (μmol g^{-1})	tannins[b] (mg g^{-1})
G12610	51.63	30.42	4.62	1.47	19.57	2.65
G8465	55.67	35.88	6.84	1.89	25.00	1.64
G21725	60.25	37.64	6.58	1.47	26.45	1.18
G21242	69.16	38.15	7.04	1.90	27.77	1.56
G21078	74.53	42.80	6.24	1.33	23.68	1.88
G2774	75.27	35.30	5.26	1.37	20.66	0.96
G19022	81.45	39.47	5.36	1.97	22.99	1.28
G15137	82.07	52.59	7.17	1.41	27.48	1.55
G4825	82.26	40.41	5.91	1.47	23.23	1.85
G14519	85.53	49.17	8.46	2.14	33.53	0.90
G18372	88.01	43.45	5.55	1.95	19.60	0.35
G23063	88.88	36.24	5.19	1.73	21.94	2.60
G1678	89.74	41.62	7.05	1.72	25.68	1.21
G11419	91.96	41.80	6.05	1.46	23.58	0.91
G1844	92.53	62.51	6.42	2.06	29.16	1.15
G11350	98.71	44.04	6.40	2.13	25.91	1.32
G5706	100.51	47.26	6.16	2.03	24.27	0.65
G2572	103.55	49.28	6.74	2.30	23.06	2.22
G16267	110.59	53.69	6.30	2.09	25.41	1.32
G13220	122.70	44.02	5.51	1.38	23.74	2.49
G18811	130.70	56.64	5.45	1.50	22.94	0.54
G3096	139.91	57.76	6.90	1.56	25.27	0.89
G87	149.86	60.87	6.45	2.04	26.15	1.97
G734	156.91	62.06	6.16	2.13	24.09	1.41
SEM[c]	3.58	0.68	0.13	0.16	0.94	0.10
LSD[d]	± 10.18	± 1.93	± 0.36	± 0.45	± 2.75	± 0.40

[a] IP5+IP6 = concentration of *myo*-inositol pentaphosphoric acid plus phytic acid. [b] Determined as catechin equivalences in bean homogenates. [c] SEM, pooled standard error of the mean calculated form error mean square of ANOVA (n = 72 for Fe, Zn, total P, and total S and 48 for all others). [d] LSD, least significant difference ($P \leq 0.05$).

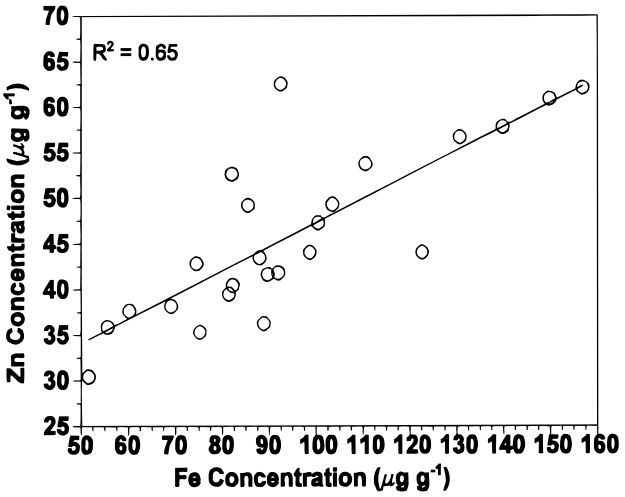

Figure 1. Relationship between the concentrations (dry weight basis) of Fe and Zn in 24 genotypes of bean seeds from plants grown in ^{59}Fe-labeled nutrient solutions. R^2 is the correlation coefficient squared.

RESULTS AND DISCUSSION

The intrinsically ^{59}Fe-labeled bean seed genotypes contained a wide range of Fe, Zn, total P, total S, *myo*-inositol pentaphosphoric acid + phytic acid (IP5+IP6), and tannin concentrations (Table 2), with concentrations (dry weight basis) ranging from 51.63 to 156.91 μg g^{-1} for Fe, from 4.62 to 8.46 mg g^{-1} for total P, from 1.33 to 2.30 mg g^{-1} for total S, from 19.57 to 29.16 μmol g^{-1} for IP5+IP6, and from 0.35 to 2.65 μg g^{-1} for tannins. However, there was a significant positive correlation (Figure 1) between bean genotypes and seed Fe and seed Zn levels. Apparently, selecting for high bean seed Fe concentrations also tended to increase Zn concentrations in bean seeds simultaneously. Both Fe and Zn may accumulate in the same seed mineral storage pool and may be bound to phytate that accumulates as globoid crystals within protein bodies of seeds (Welch, 1993).

Table 3. Bioavailability of Fe to Marginally Fe-Depleted Rats Fed Bean Meals Prepared from 24 Bean Genotypes Intrinsically Labeled with ^{59}Fe

genotype	Fe consumed (μg)	Fe absorption (%)	bioavailable Fe (μg)
G12610	50.65	64.09	32.40
G21242	63.72	53.81	34.29
G21725	58.26	60.55	35.28
G8465	55.03	68.24	37.69
G14519	77.44	53.56	41.48
G19022	79.74	53.08	42.33
G1678	80.09	56.13	44.96
G21078	71.63	62.88	45.04
G15137	80.59	56.29	45.36
G2774	71.79	67.94	48.77
G23063[a]	83.58	61.84	51.66
G4825	78.08	67.08	52.38
G1844	91.47	58.67	53.67
G11419	89.86	60.72	55.50
G11350	96.13	56.87	56.14
G5706	97.86	60.13	58.54
G18372	90.75	72.34	62.39
G16267	117.01	60.05	65.31
G2572	106.85	65.99	66.94
G13220	120.00	59.37	71.24
G3096	134.20	58.81	78.92
G734	141.06	59.23	89.35
G18811[a]	122.40	76.24	93.27
G87[a]	146.24	72.09	105.50
SEM[b]	3.60	4.13	4.23
LSD[c]	± 10.11	± 8.98	± 11.88

[a] Meal contained 0.5 g of dried beans, but values were normalized to 1.0 g for comparison with all other genotypes fed in 1.0-g amounts. [b] SEM, pooled standard error of mean calculated form error mean square of ANOVA (n = 120). [c] LSD, least significant difference ($P \leq 0.05$).

There were no significant relationships between seed Fe concentrations in seeds of the different bean genotypes and total P, total S, IP5+IP6, or tannins in the seeds. Therefore, the genetic basis for increased Fe concentrations in bean seeds does not appear to be related to any of these factors.

On the basis of a food composition table (Holland et al., 1991), conventional beans contain about 60 μg of Fe g^{-1} dry weight (4.56 mg of Fe per half-cup serving). The data in Table 2 shows that it is possible to increase the concentration of Fe in common beans to as high as 157 μg g^{-1} (11.55 mg of Fe per half-cup serving). This is an Fe enrichment factor of over 2.5 as compared to conventional beans. Therefore, based solely on bean Fe concentrations, it would be worthwhile to breed for Fe-enrichment traits in common beans.

Table 3 shows the amount of bioavailable Fe present in the 24 bean accessions as determined using the rat model. Iron bioavailability varied between 53.1 and 76.2% depending on the genotype. The amount of bioavailable Fe varied from 32.4 (genotype G19022) to 105.5 μg of Fe (genotype G87). There were not enough beans harvested from certain bean genotypes (i.e., genotypes G23063, G18811, and G87) to supply 1.0 g of dried bean to rats receiving meals prepared from these genotypes. Rats fed these genotypes only received 0.5 g of dried bean in their meals. The data presented in Table 3 was normalized to 1 g to account for these differences in bean-meal size for these genotypes.

There were no significant correlations between IP5+IP6 or tannin concentrations and bioavailable Fe

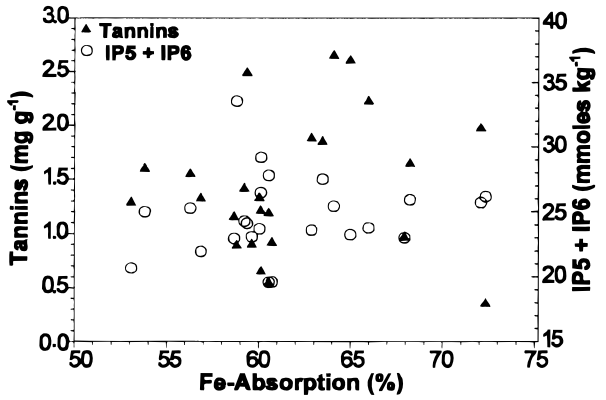

Figure 2. Percent bioavailable Fe to rats in 24 bean seed accession meals as affected by total tannins and IP5+IP6 concentrations in the mature seeds. No significant correlations were found between either total tannins or IP5+IP6 and bioavailable Fe in the seed meals.

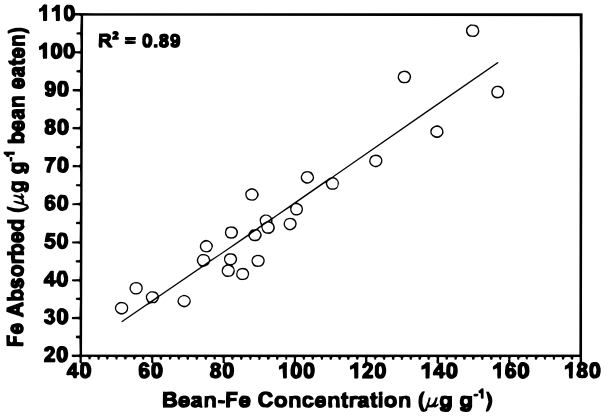

Figure 3. Relationship between bean−Fe concentrations in 24 bean accessions and Fe absorbed form bean meals fed in single meals to marginally Fe-depleted rats. R^2 is the correlation coefficient squared.

in the intrinsically radiolabeled bean seeds (Figure 2). Thus, wide differences in seed IP5+IP6 and seed tannin concentrations did not appear to effect bean Fe bioavailability. Apparently other unknown seed substances or combinations of substances were responsible for the differences in bioavailable Fe found between the different bean accessions.

This was unexpected in that numerous researchers have reported that phytic acid and tannins affect Fe bioavailability to rats from edible plant seeds (House, 1999; Michaelsen and Friis, 1998). Why these findings were not observed in the present study is not known. Possibly, unknown non-heme-Fe promoter substances (Welch, 1993) within the bean seeds could have affected the results reported. Further research is needed to determine what specific substances in bean seeds, besides those studied here, affect Fe bioavailability (either positively or negatively) before improvements in bioavailable Fe in bean seeds can be accomplished efficiently through plant breeding.

The genotypes G87 and G18811 (Table 3) possessed both high Fe concentrations [i.e., 149.9 and 130.7 μg (g of Fe)$^{-1}$, respectively] and high bioavailable Fe levels (i.e., 72.1% and 76.2%, respectively). Therefore, these genotypes may be appropriate for using in plant breeding programs directed at increasing the nutritional quality of common beans with respect to Fe. However, other characteristics of these bean genotypes would have to be addressed in a breeding program before releasing progeny from these accessions to growers. Additional factors to consider include yield potential, disease resistance, stability of the high iron concentration and high bioavailability traits when grown under various environmental conditions and soil types, and consumer acceptance. Additionally, human bioavailability studies should to be conducted to substantiate the bioavailability findings reported here using a rat model.

Interestingly, the white-colored bean genotypes, G18372 and G18811 (selected by color to contain very low total tannin concentrations), demonstrated high percent bioavailable Fe levels, suggesting that the white seed-coat color might be used to select for bean seeds containing highly bioavailable Fe. However, as mentioned previously, total tannin concentrations as determined in the bean seeds were not shown to be directly related to bioavailable Fe concentrations in the bean seeds. Therefore, seed color may not be related to tannin content alone, and the white color may reflect either higher levels of other unknown components in the seeds that promote Fe bioavailability, or lower levels of undetermined specific antinutrients that inhibit Fe bioavailability (Tables 2 and 3).

Tannin is a chemical term used to describe a very broad class of compounds that include all plant polyphenolic substance having a molecular weight greater than about 500 (Singleton and Kratzer, 1973). Possibly only certain types of tannins contribute to reduced bioavailable Fe levels in beans. Thus, determining total tannins may not be an appropriate assay to use for determining the effects of certain types of tannins on Fe bioavailability. Perhaps, specific types of tannin compounds should be determined to ascertain their effects on Fe bioavailability in the future if it is found that different types of tannins have different effects on Fe bioavailability.

The relationship between bioavailable Fe in the bean meals and the concentration of Fe in the bean seeds is shown in Figure 3. Clearly, increasing the Fe concentrations in beans via selecting for high Fe genotype seeds resulted in increased bioavailable amounts of Fe when compared to low Fe genotypes irrespective of antinutrient levels (i.e., IP5+IP6 and tannins) or Fe concentrations in the seeds of the different bean genotypes studied.

While a rat model is not ideal for determining Fe bioavailability to humans (because rats are much more efficient at absorbing Fe from plant foods as compared to humans and have an intestinal phytase that is not known to be expressed in humans; Reddy and Cook, 1991; Wienk et al., 1999), rats can be used to give qualitative data on the bioavailable Fe from staple plant foods (House, 1999). These qualitative estimates can be used to rank promising genotypes of staple foods for use in later feeding trials with humans, therby greatly reducing the numbers of genotypes that would have to be tested in humans without use of the rat.

Besides the rat model used here, in vitro screening models have also been used to qualitatively rank foods as sources of bioavailable Fe. One such in vitro model is the caco-2 cell method (Van Campen and Glahn, 1999). However, because of the complexities of determining the bioavailability of Fe in plant foods to humans, ultimately, human feeding trials performed under free-living conditions should be conducted with the most promising genotypes before these genotypes are released for distribution to breeding programs worldwide.

Our results demonstrate that selecting beans for increased Fe concentrations in plant breeding programs can result in significant increases in bioavailable Fe to monogastric animals fed bean meals. This is true even though some of the bean genotypes contained very high levels of the "antinutrient" substances, phytic acid and tannins. Thus, our results support the contention that breeding for higher Fe density in common beans could contribute significantly to improving the Fe status of individuals dependent on beans as staple foods.

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LITERATURE CITED

Burk, R. F.; Solomons, N. W. Trace elements and vitamins and bioavailability as related to wheat and wheat foods. *Am. J. Clin. Nutr.* **1985**, *41*, 1091−1102.

Combs, G. F., Jr.; Duxbury, J. M.; Welch, R. M. Food systems for improved health: linking agricultural production and human nutrition. *Eur. J. Clin. Nutr.* **1997**, *51*, *S16*−*S33*.

Department of Health and Human Services. *The Surgeon General's Report on Nutrition and Health, 1988*; DHHS (PHS) Pub. 88-50210; U.S. Government Printing Office: Washington, DC, 1988.

Deshpande, S. S.; Cheryan, M. Determination of phenolic compounds of dry beans using vanillin, redox and precipitation assays. *J. Food Sci.* **1987**, *52*, 332−334.

Deshpande, U. S.; Deshpande, S. S. Legumes. In *Foods of Plant Origin*; Salunkhe, D. K., Deshpande, S. S., Eds.; AVI Books, Van Nostrand Reinhold: New York, 1991.

Dionex. *Application Note 65*−*analysis of inositol phosphates*; Dionex Corporation: Sunnyvale, CA, 1990.

Fairweather-Tait, S. J. Bioavailability of trace elements. *Food Chem.* **1992**, *43*, 213−217.

Graham, R. D.; Welch, R. M. *Breeding for staple-food crops with high micronutrient density*; Agricultural Strategies for Micronutrients, Working Paper 3; International Food Policy Research Institute: Washington, DC, 1996.

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.

Holland, B.; Welch, A. A.; Unwin, I. D.; Buss, D. H.; Paul, A. A.; Southgate, D. A. T. *McCance and Widdowson's The Composition of Foods*, 5th ed.; The Royal Society of Chemistry: Letchworth, U.K., 1991.

House, W. A. Trace element bioavailability as exemplified by iron and zinc. *Field Crops Res.* **1999**, *60*, 115−141.

House, W. A.; Van Campen, D. R.; Welch, R. M. Influence of dietary sulfur-containing amino acids on the bioavailability to rats of zinc in corn kernels. *Nutr. Res.* **1996**, *16*, 225−235.

House, W. A.; Van Campen, D. R.; Welch, R. M. Dietary methionine status and its relation to the bioavailability to rats of zinc in corn kernels with varying methionine content. *Nutr. Res.* **1997**, *17*, 65−76.

Lehrfeld, J. HPLC separation and quantification of phytic acid and some inositol phosphates in foods: Problems and solutions. *J. Agric. Food Chem.* **1994**, *42*, 2726−2731.

Looker, A. C.; Dallman, P. R.; Carroll, M. D.; Gunter, E. W. Prevalence of iron deficiency in the United States. *J. Am. Med. Assoc.* **1997**, *277*, 973−976.

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

Michaelsen K. F.; Friis, H. Complementary feeding: A global perspective. *Nutrition* **1998**, *14*, 763−766.

Mulvihill, B.; Morrissey, P. A. Influence of the sulphhydryl content of animal proteins on *in vitro* bioavailability of non-haem iron. *Food Chem.* **1998a**, *61*, 1−7.

Mulvihill, B.; Morrissey, P. A. An investigation of factors influencing the bioavailability of non-haem iron from meat systems. *Irish J. Agric. Food Res.* **1998b**, *37*, 219−226.

Pennington, J.A. T.; Young, B. Iron, zinc, copper, manganese, selenium and iodine in foods from the United States total diet study. *J. Food Comp. Anal.* **1990**, *3*, 166−184.

Reddy, M. B.; Cook, J. D. Assessment of dietary determinants of nonheme-iron absorption in humans and rats. *Am. J. Clin. Nutr.* **1991**, *54*, 723−728.

Singleton, V. L.; Kratzer, F. H. Plant phenolics. In *Toxicants Occurring Naturally in Foods*; National Academy of Sciences: Washington, DC, 1973; Chapter 15, pp 309−345.

Tohme, J.; Jones, P.; Beebe, S.; Iwanaga, M. The combined use of agroecological and characterization data to establish the CIAT *Phaseolus vulgaris* core collection. In *Core Collections of Plant Genetic Resources*; Hodgkin, T., Brown, A. H. C., van Hintum, Th. J. L., Morales, E. A. V., Eds.; John Wiley and Sons: Chichester, U.K., 1998; pp 95−107.

United Nations Administrative Committee on Coordination−Subcommittee on Nutrition. *Second Report on the World Nutrition Situation. Vol. I. Global and Regional Results*; World Health Organization: Geneva, 1992.

Van Campen, D. R.; Glahn, R. P.. Micronutrient bioavailability techniques: Accuracy, problems and limitations. *Field Crops Res.* **1999**, *60*, 93−113.

Van Dokkum, W. Significance of iron bioavailability for iron recommendations. *Biol. Trace Element Res.* **1992**, *35*, 1−11.

Viteri, F. E. Prevention of iron deficiency. In *Prevention of Micronutrient Deficiencies. Tools for Policymakers and Public Health Workers*; Howson, C. P., Kennedy, E. T., Horwitz, A., Eds.; National Academy Press: Washington, DC, 1999; Chapter 3, pp 45−102.

Welch, R. M. Zinc concentrations and forms in plants for humans and animals. In *Zinc in Soils and Plants*; Robson, A. D., Ed.; Kluwer Academic Publishers: Dordrecht, Boston, London, 1993; Chapter 13, pp 183−195.

Welch, R. M.; Graham, R. D. A new paradigm for world agriculture: meeting human needs. Productive, sustainable, nutritious. *Food Crops Res.* **1999**, *60*, 1−10.

Welch, R. M.; House, W. A. Meat factors in animal products that enhance iron and zinc bioavailability: Implications for improving the nutritional quality of seeds and grains. In *1995 Cornell Nutrition Conference for Feed Manufacturers*; Department of Animal Science and Division of Nutrition, Cornell University Agricultural Experiment Station: Ithaca, NY, 1995; pp 58−66.

Welch, R. M.; House, W. A.; Allaway, W. H. Availability of zinc from pea seeds to rats. *J. Nutr.* **1974**, *104*, 733−740.

Welch, R. M.; House, W. A.; Van Campen, D. R. Availability of cadmium from lettuce leaves and cadmium sulfate to rats. *Nutr. Rep. Int.* **1978**, *17*, 35−42.

Wienk, K. J. H.; Marx, J. J. M.; Beynen, A. C. The concept of iron bioavailability and its assessment. *Eur. J. Nutr.* **1999**, *38*, 51−75.

World Bank. The Challenge of Dietary Deficiencies of Vitamins and Minerals. In *Enriching Lives: Overcoming Vitamin and Mineral Malnutrition in Developing Countries*; World Bank: Washington, DC, 1994; Chapter 1, pp 6−13.

World Health Organization. *Trace elements in human nutrition and health*; World Health Organization: Geneva, 1996.

Yip, R. The challenge of improving iron nutrition: limitations and potentials of major intervention approaches. *Eur. J. Clin. Nutr.* **1997**, *51*, S16−S24.

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