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Minerals (Zn, Fe, Ca and Mg) and Antinutrient (Phytic Acid) Constituents in Common Bean

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ABSTRACT

In this study, the variation of zinc (Zn), iron (Fe), calcium (Ca) and magnesium (Mg) and the interference of phytic acid (PA) on their availability was investigated in 29 US grown and CIAT breeding genotypes of common bean. Fe levels showed the highest variation (8.9-112.9 mg kg⁻¹) followed by Ca (58.67-122.98 mg kg⁻¹) and Zn (30.90-64.60 mg kg⁻¹) while variability of Mg concentration (6.47- 11.05 mg kg⁻¹) is the least among the mineral components. PA showed a wide range of variability (12.52- 316.42 mg kg⁻¹) and inversely correlated with Fe, Ca and Mg concentrations. The results of the minerals and PA concentration can be interpreted in terms of expected bio-availability of minerals and the correlation study indicated that the presence of high concentration of PA inhibit the availability of most minerals under study in common beans. We suggest that the genotypes, MIB466, MIB465, MIB152 and JaloEEP 558 could be considered as sources of high Zn and Vista and NUA56-1770 for high seed Fe. We also identified G122 for high Ca and JaloEEP558 genotype for high Mg. We conclude that there is scope for the enhancement of mineral contents of common bean by selecting suitable genotype and bean products require processing for dephytinization for the improvement of mineral availability.

Key words: *Phaseolus vulgaris* L., minerals, phytic acid, interference, availability

INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) are the most important grain legumes for direct human consumption in the world (Broughton *et al.*, 2003). It has been considered as an important and inexpensive source of protein, dietary fiber, vitamins, minerals and bioactive compounds (Nyombaire *et al.*, 2007). Compared with meat-based diets, plant-based diets are often limited in the content and bioavailability of essential minerals such as zinc (Zn), iron (Fe), calcium (Ca) and magnesium (Mg). A major constraint to the availability of minerals is the presence of toxic and antinutrient constituents like Phytic Acid (PA). Phytic acids chelate several mineral elements, especially Zn, Fe, Ca, Mg and Mo and interfere with their absorption and utilization (Ologhobo, 1980). The world population, particularly in Latin America, Sub-Saharan Africa, the Caribbean and Southeast Asia, is at risk for micronutrient intake (Brown and Pearson, 2001). Recent reports indicate that Fe deficiency is the most prevalent micronutrient problem in the world, affecting over 2 billion people globally, many of whom depend on beans as their staple food (Welch, 1999). An estimated 49% of the world

population is at risk for low Zn intake (Brown and Peerson, 2001). Children diets high in cereals suffer from Zn deficiencies (Ranum, 1999) and nutritional Zn deficiency is common throughout the world, including the USA (Ganapathy and Volpe, 1999). Calcium content in rural diets in developing countries is not adequate (Rosado *et al.*, 1992; Wyatt and Triana-Tejas, 1994) and dietary Ca deficiency has been epidemiologically linked to several chronic diseases, including osteoporosis. Without adequate Mg, energy production falters and protein production is insufficient for normal growth and development of infants, children, adolescents and pregnant women (Mangels and Havala, 1994). The American diet is rich in proteins, carbohydrates and fats but it is commonly poor in magnesium (Mg). The minerals in beans are readily available, which is important in reducing the risks of osteoporosis (Dawson-Hughes *et al.*, 1990) and hypertension (Appel *et al.*, 1997).

Phytic acid represents from 65 to 85% of the total seed P (Reddy *et al.*, 1989). As a polyanion, PA is an effective chelator of positively charged molecules and has the potential to form stable insoluble complexes with minerals and proteins. These complexes confer PA with antinutritional properties, particularly in the humans and nonruminants lacking the hydrolytic enzyme phytase in their digestive tract (Cheryan, 1980). In some aspects, such as PA is considered beneficial source of nutrients during bean seed germination (Raboy, 2001), an antioxidant and anticarcinogen and may have beneficial role on human health, particularly for aged people (Zhou and Erdman, 1995). In order to improve availability of minerals such as Fe and Zn availability in foods, studies have been conducted to reduce the levels of PA in the seeds of various plants (Lucca *et al.*, 2001). For instance, development of low PA grains of maize (*Zea mays*), barley (*Hordeum vulgare*) and rice (*Oryza sativa*) to improve availability of micronutrients (Larson *et al.*, 1998, 2000; Raboy *et al.*, 2001).

Common bean is considered as one of the most beneficial crop species and the knowledge of micronutrients and their availability in this species is essential for human health management.

The level, bioavailability of different micronutrients and their relationships with antinutrients, PA are crucial for pedigree selection and breeding common beans aiming to enhance micronutrients content (Welch and Graham, 2004). We conducted this study using 29 genotypes consist of US grown and CIAT (International Center for Tropical Agriculture) developed genotypes to understand the level of different minerals and the interference of PA on their availability.

MATERIALS AND METHODS

Plant material: The 29 common bean genotypes in this study consisted of 14 genotypes from CIAT, 13 from the USA and one each from Brazil and India. All common bean genotypes and their origin, are listed in Table 1. Common bean genotypes were grown in the greenhouse in 18×19 cm pots filled with Sunshine mix 1 (Sun Gro Horticulture Canada Ltd., formulated with Canadian Sphagnum peat moss, coarse grade perlite, gypsum and Dolomitic lime) as substrate. The seeds were planted on March 27, 2008 following a Randomized Complete Block Design (RCBD) with three replications. Two seeds for each genotype were placed in each pot for germination but one plant was allowed to grow until harvest of the seeds. Pots were watered periodically with tap water to the approximate field capacity to facilitate normal plant growth. No additional fertilizer or pesticide was applied during the period of experimentation.

Table 1: Common bean genotypes, gene pool group, origin, seed color

No.	Genotype	Gene pool	Origin	Market class	Seed color
1	Jalo EEP558	Andean	Brazil	-	Cream
2	NUA45	Andean	CIAT breeding line	-	Purple mottled
3	NUA35	Andean	CIAT breeding line	-	Purple mottled
4	NUA56-1770	Andean	CIAT breeding line	-	Purple mottled
5	NUA59	Andean	CIAT breeding line	-	Purple mottled
6	G122	Andean	Indian landrace	Cranberry	Red mottled
7	NY6020-4	Andean	USA breeding line	Snap	White
8	Benton	Andean	USA	Snap	White
9	Dorado	Mesoamerican	CIAT	Small red	Dark red
10	BAT93	Mesoamerican	CIAT breeding line	-	Cream
11	A55	Mesoamerican	CIAT breeding line	Black	Black
12	XAN176	Mesoamerican	CIAT breeding line	Black	Black
13	MIB151	Mesoamerican	CIAT breeding line	-	Light Cream
14	MIB152	Mesoamerican	CIAT breeding line	-	Black
15	MIB154	Mesoamerican	CIAT breeding line	-	Brown mottled
16	MIB217	Mesoamerican	CIAT breeding line	-	Black
17	MIB465	Mesoamerican	CIAT breeding line	-	Black
18	MIB466	Mesoamerican	CIAT breeding line	-	Pink with black specks
19	ND88-106-04	Mesoamerican	USA	Navy	White
20	Aztec	Mesoamerican	USA	Pinto	Cream mottled
21	Voyager	Mesoamerican	USA	Navy	White
22	Albion	Mesoamerican	USA	Navy	White
23	Mayflower	Mesoamerican	USA	Navy	White
24	T39	Mesoamerican	USA	Black	Black
25	Jaguar	Mesoamerican	USA	Black	Black
26	Othello	Mesoamerican	USA	Pinto	Cream mottled
27	Ryder	Mesoamerican	USA	Small red	Red
28	Vista	Mesoamerican	USA	Navy	White
29	BelNeb-RR-1	Mesoamerican	USA breeding line	Great Northern	White

Chemical analysis: After harvesting, seeds from each pod of individual plant were mixed thoroughly and randomly selected 100 seed were weighed. For chemical analysis, 10 randomly selected seeds were washed with deionized water containing Joy detergent (Proctor and Gamble, Cincinnati, OH) and later rinsed with deionized water only. Samples were oven-dried at 70°C for 48 h, weighed and ground in an agate mortar with an agate pestle (Brinkmann Instruments Co., Westbury, NY). A 300 mg aliquot of the ground material was processed for concentrated nitric acid digestion, followed by 30% hydrogen peroxide. Fe and Zn concentrations were determined following the method described by Moraghan and Grafton (2001). Magnesium and Ca were determined from the samples by dry ashing method that described by Chapman and Pratt (1961). Concentrations of Zn, Fe, Mg and Ca were converted and expressed in mg kg⁻¹ from the absorbance using Atomic Absorption Spectroscopy.

Phytic acid was determined using the ferric precipitation method as described by Raboy *et al.* (1984). Briefly, samples were extracted in 0.4 M HCl: 10% (w/v) sodium sulfate. Following centrifugation, supernatant PA was precipitated as a ferric salt. Ferric phytates were washed, wet ashed and digest phytic acid phosphorus content determined colorimetrically using the method as described (Chen *et al.*, 1956). Spectrophotometric reading was converted to PA by multiplying with the conversion factor 3.5484 and expressed as mg kg⁻¹.

Statistical analysis: Each determination was carried out on three separate replications and analyzed in triplicate and values were then averaged. Data was assessed by the Analysis Of Variance (ANOVA) following Tukey's multiple range tests and significance was accepted at $p < 0.05$ (Tukey, 1953). The PC software Excel Statistics (Version 5.0, Esumi Co. Ltd., Japan) was used for the calculations.

RESULTS AND DISCUSSION

Seed mineral concentrations: The four mineral constituents (Zn, Fe, Ca and Mg) and the antinutrient component PA of US and CIAT common bean genotypes were determined (Table 2). Seed weight (100 seeds) ranged from 15.88 to 78.85 g with a mean of 29.97 g. The highest seed weight was recorded for Andean genotype NUA45 (78.85 g) and lowest for Mesoamerican genotype MIB152 (19.27 g), both of these genotypes were collected from the CIAT breeding program. Analysis of variance reveals that the seeds of 29 genotypes are significantly ($p < 0.05$) differed in Zn, Fe, Ca and PA concentration except Mg. Seed Zn concentration ranged from 30.90 to 64.60 mg kg^{-1} and seven bean genotypes namely, MIB466 (64.6 mg kg^{-1}), MIB465 (64.4 mg kg^{-1}), JaloEEP558 (59.5 mg kg^{-1}), MIB151 (58.9 mg kg^{-1}), MIB152 (58.3 mg kg^{-1}), Ryder (54.9 mg kg^{-1}) and BAT93 (54.7 mg kg^{-1}) had consistently higher seed Zn than others. Most of the MIB genotypes from CIAT have considerably high seed Zn than the US genotypes. The MIB genotypes are from CIAT breeding program and are selected for nutritional quality (Matthew Blair, personal communication), NUA59 also from CIAT but found lowest in Zn concentration. The Brazil originated Andean genotype JaloEEP558 and the US genotype Ryder also possess considerably high seed Zn concentration.

Wide range of variability of seed Fe concentration was found among the genotypes (8.9 to 112.9 mg kg^{-1}). The higher level of seed Fe concentration was observed in the US navy bean genotype Vista (112.9 mg kg^{-1}) and CIAT genotype NUA56-1770 (106.2 mg kg^{-1}). Among all genotypes Brazil originated Andean genotype JaloEEP558 (8.9 mg kg^{-1}) had the lowest seed Fe concentration. Seven bean genotypes, Vista (112.9 mg kg^{-1}), NUA56-1770 (110.6 mg kg^{-1}), MIB154 (92.8 mg kg^{-1}), MIB465 (84.7 mg kg^{-1}), Dorado (84.3 mg kg^{-1}), Voyager (80.9 mg kg^{-1}) and NUA45 (74.6 mg kg^{-1}) had higher seed Fe concentration than those of others. NUA genotypes identified as high iron content developed by CIAT however, in our study, we did not find similar iron accumulation efficiency for all genotypes. The CIAT genotypes NUA35 and NUA59 have relatively lower level of Fe concentrations, while the genotype NUA45 has considerable high Fe concentration.

In an analysis of 2000 accessions at CIAT, a range of 34 to 89 mg kg^{-1} (average = 55 mg kg^{-1}) for Fe and 21 to 54 mg kg^{-1} (average = 35 mg kg^{-1}) for Zn were reported (Beebe *et al.*, 2000). Some bean accessions from Peru were also found to contain high levels of Fe, averaging $>100 \text{ mg kg}^{-1}$ (Beebe *et al.*, 2000; Islam *et al.*, 2002). In this study we found some genotypes with higher level of Fe and Zn than previously reported by Beebe *et al.* (2000) and Islam *et al.* (2002). Beebe *et al.* (2000) also suggested that the seed Fe content in the Andean gene pool tended to present higher values than those from the Mesoamerican pool. In our study we found the Mesoamerican US bean genotype, Vista has the higher iron concentration than all of Andean genotypes. Cichy *et al.* (2005) reported high Zn concentration in Albion than Voyager, which is reverse in our findings. Recently, common bean genotypes, NUA35 and NUA56 have been registered for high seed mineral content at the CIAT (Blair *et al.*, 2010). To our knowledge, these are the first registered genotypes specifically for nutritional quality in common beans. In our study we found high Fe concentration

Table 2: Zinc (Zn), iron (Fe), calcium (Ca), magnesium (Mg) and phytic acid (PA) concentrations in the seeds of 29 common bean genotypes

No.	Cultivar/line	100 seeds Weight (g) ^a	Concentration (mg kg ⁻¹) ^a				
			Seed Zn	Seed Fe	Seed Ca	Seed Mg	Seed PA
1	Jalo EEP558	27.62f-i	59.5a-c	8.9k	122.98b	11.05a	12.52p
2	NUA45	78.85a	40.8f-h	74.6cd	80.53g-j	7.50i-l	44.18j-o
3	NUA35	61.67b	47.4c-g	22.9i-k	81.53f-j	7.74g-l	65.21g-j
4	NUA56-1770	56.27b	34.5gh	110.6ab	79.10i-l	7.24k-m	63.81g-k
5	NUA59	59.74b	30.9h	34.2f-k	78.66j-l	6.47m	62.12l-o
6	G122	29.46e-h	41.1f-h	42.0e-j	129.84a	8.71c-f	35.33no
7	NY6020-4	31.05d-g	47.0c-g	20.6i-k	74.17l-n	8.27	59.73h-m
8	Benton	25.48f-j	53.6a-f	74.5cd	91.03cd	7.28j-l	54.38i-n
9	Dorado	22.05g-h	42.4e-h	84.3a-d	78.40j-l	8.68c-f	68.46g-i
10	BAT 93	19.13h-j	54.7a-f	42.1e-j	78.44j-l	8.56c-f	107.92f
11	A55	22.60g-j	52.6a-f	22.2i-k	58.67o	7.13lm	65.15g-j
12	XAN176	26.66f-j	44.0c-h	17.2i-k	69.79n	8.06f-j	31.26op
13	MIB151	15.88j	58.9a-d	64.4c-f	78.77j-l	9.14b-d	172.74c
14	MIB152	19.27h-j	58.3a-e	66.7c-e	81.87f-j	7.94f-k	64.17g-k
15	MIB154	22.04g-j	48.6b-g	92.8a-c	80.10h-k	8.59c-f	72.62g-i
16	MIB217	21.50g-j	42.8e-h	34.2f-k	70.13n	7.02lm	43.11k-o
17	MIB465	17.63ij	64.4ab	84.7a-d	74.53k-n	9.33bc	80.91gh
18	MIB466	23.59g-j	64.6a	57.6d-h	86.66d-f	8.29e-h	131.95h-l
19	ND88-106-04	16.43j	49.8a-g	27.4h-k	84.46f-i	9.04b-e	40.48m-o
20	Aztec	39.16c-e	53.9a-f	24.3i-k	72.06mn	7.25kl	113.11n-p
21	Voyager	18.50h-j	43.4d-h	80.9b-d	90.19c-e	9.50b	33.75g
22	Albion	19.70h-j	53.9a-f	28.8g-k	87.12d-f	7.56h-l	316.42a
23	Mayflower	19.48h-j	53.6a-f	12.2jk	77.53j-m	7.38j-l	121.96l-o
24	T39	19.05h-j	45.0c-g	29.0g-k	78.79j-l	8.17f-i	94.68op
25	Jaguar	22.04g-j	51.8a-f	55.2d-h	79.76h-l	9.56b	225.79b
26	Othello	41.28cd	45.1c-h	58.9d-g	80.97g-j	7.37j-l	93.06cd
27	Ryder	42.34c	54.9a-f	33.3g-k	93.33c	8.36d-g	87.25g-i
28	Vista	19.05h-j	49.2a-g	112.9a	85.08e-h	9.51b	30.95e
29	BelNeb-RR-1	34.68c-f	49.6a-g	43.3e-i	86.18d-g	8.43d-g	151.10de

^aMeans having common letter do not differ significantly at p<0.05

in NUA56 but the level of Zn concentration lower than some of the genotypes. The discrepancy of our finding in mineral concentration might be due to difference in growing environment. We conducted our study in greenhouse condition which is reflected mostly the efficiency of accumulation of mineral by common bean genotypes. Our findings also indicate that seed Fe and Zn concentration was simple inherited and highly heritable traits across environments and soil types. Calcium concentrations were significantly (p<0.05) higher in Brazilian Andean genotype JaloEEP558 (122.98 mg kg⁻¹) and Indian Andean genotype G122 (129.84 mg kg⁻¹), where as the Mesoamerican CIAT genotypes XAN176 (69.79 mg kg⁻¹) and MIB217 (70.13 mg kg⁻¹) has lower concentration of Ca. On average, US bean genotypes namely Ryder, Voyager, BelNeb-RR-1 and Vista have high level of Ca concentration. Magnesium concentration ranged from 6.47 to 11.05 mg kg⁻¹. The Brazilian Andean genotype JaloEEP558 (11.05 mg kg⁻¹) has the highest seed Mg concentration, followed by US bean genotypes Vista (9.51mg kg⁻¹) and Voyager (9.50 mg kg⁻¹). The CIAT breeding lines NUA59 (6.47 mg kg⁻¹), MIB217 (7.02 mg kg⁻¹) and A55 (7.13 mg kg⁻¹) has the lower level of seed Mg concentration.

The PA concentration of dry beans differed significantly ($p < 0.05$) among genotypes and market classes (Table 2). PA concentration ranged from 12.52 to 316.42 mg kg⁻¹ for the Brazilian Andean genotype JaloEEP558 and the US bean genotype Albion, respectively. Those two genotypes representing 25 folds difference in PA concentration between these two genotypes. Colored beans, particularly from the black and red market classes (Canadian navy bean cultivars), had significantly lower PA content than those of white bean (Oomah *et al.*, 2008) but we did not find this trend in our study. Cichy *et al.* (2005) did not find any difference for PA concentration of seed between genotypes Albion and Voyager but we found significant differences between the two genotypes. Results (Table 2) showed that genotypes with low PA concentration have high contents Fe (e.g., Vista) and Ca (e.g., JaloEEP558), moderate level of Zn concentration (e.g., JaloEEP558). Again, in some cases genotypes with higher concentration showed low concentration of minerals. Research has shown that PA significantly inhibits the absorption minerals in the PA-laden whole grains and fresh legumes. A study on Fe absorption in cereal porridges reported, in some cases, a twelve-fold increase in the absorption of Fe when the PA was removed from the food (Hurrell *et al.*, 2003). In several studies, it has been reported that human body can absorb about 30% of Mg and Zn without PA, however with PA, human body absorbed only 13 and 23%, respectively (Egli *et al.*, 2004; Bohn *et al.*, 2004). These results clearly indicate a low PA food product can provide a greater availability of minerals. In our study we also find similar trends. We identified few low PA contained genotypes namely, JaloEEP558, Vista, Xan176, Albion, Voyager and G122 with cumulatively high level of minerals concentration.

Correlations among phytic acid and other minerals: Non significant or weak correlations were found between 100 seeds weight and the concentration of seed Zn ($r = 0.11$; $p < 0.05$), Fe ($r = 0.02$), Ca ($r = 0.46$) and Mg ($r = 0.27$) concentration, where as PA inversely correlated with 100 seeds weight ($r = -0.26$; (Table 3). Marschner (1997) reported a significant correlation between seed weight and seed mineral concentration. However, Hacisalihoglu *et al.* (2004) indicated that largest seeded genotypes have high amount of total seed mineral content but not concentration. In our study, we found seed minerals, Zn, Fe, Mg and Ca were non significantly correlated with each other except Fe and Zn, which was inversely correlated ($r = -0.11$; $p < 0.05$). It has been reported that the accumulation of seed Fe and Zn is significantly correlated and the genetic factors for increasing Fe are cosegregating with genetic factors for increasing Zn (Gregorio, 2002; Hacisalihoglu *et al.*, 2005). Inverse correlation between Zn and Fe in our result did not support their findings which may be attributed with the wider variation in genomic constituents of the genotypes under study. Our results suggest the accumulation and enhancement of one mineral do not influence on the concentration of others and they are independently inherited in bean genome which is in agreement with Welch and Graham (2004).

Table 3: Correlation coefficients (r ; $p < 0.05$) for 100 seeds weight (SW), PA, Zn, Fe, Ca and Mg concentrations of beans

	SW	PA	Zn	Fe	Ca	Mg
SW		-0.26	0.11	0.02	0.46	0.27
PA	-0.26		0.25	-0.11	-0.14	-0.07
Zn	0.11	0.25		-0.11	0.06	0.40
Fe	0.02	-0.11	-0.11		0.03	0.03
Ca	0.46	-0.14	0.05	0.03		0.49
Mg	0.27	-0.07	0.40	0.03	0.49	

Phytic acid concentration was inversely related to seed Fe ($r = -0.11$; $p < 0.05$), Ca ($r = -0.14$) and Mg ($r = -0.07$) concentration. Among 29 genotypes, many genotypes contain higher concentration of phytic acid and low concentration of Fe, Ca and Mg (Table 2). Lower concentrations of Fe, Ca and Mg may be due to presence high concentration of PA. Because PA or phytate is a chelating agent, which is involved in binding minerals (such as K^+ , Ca^{2+} , Zn^{2+} , Fe^{2+} , etc.) and making them unavailable for dietary absorption (Hirschi, 2009). It has been reported that cereals and legumes are rich in minerals but the bioavailability of these minerals is usually low due to the presence of antinutrient factor such as PA (Ann-Sofie 2002). Some genotypes, BelNeb-RR-1, Aztect or MIB151, we identified with high content of PA and correspondingly low content of most of the minerals (Table 2). Therefore, in order to evaluate mineral availability, PA should be considered as a major factor for common bean. Weak but non significant correlation ($r = 0.25$) between phytic acid and Zn concentration was observed in the genotypes tested, which suggest that breeding for increasing seed Zn may increase seed PA. We recommend screening PA levels in bean with high seed Zn concentration to ensure that increased levels of PA do not negate the value of gains in Zn in the diet.

CONCLUSION

We have identified a set of common bean genotypes contained high concentrations of seed minerals. Our data represent a comprehensive report on the genetic variation for several human health related nutrient concentrations in US grown and CIAT breeding genotypes representing both the Andean and Mesoamerican gene pools of common beans. We identified MIB466, MIB465, MIB152 and JaloEEP558 could be considered as sources of high seed Zn; Vista and NUA56-1770 for high seed Fe; G122 for high Ca and JaloEEP558 for high seed Mg genotypes. Our study also suggested that high concentration of PA inhibit the availability of most minerals in common beans. Thus, selection of low PA common bean may be important particularly for nutritional point of view.

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