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DISTRIBUTION OF SEED MINERAL NUTRIENTS AND THEIR CORRELATION IN *PHASEOLUS VULGARIS*

GOKHAN HACISALIHOGLU,[1]* LEON V. KOCHIAN[2]
AND C. EDUARDO VALLEJOS[3]
[1]*Florida A&M University*
Biology Department
207 Jones Hall
1530 S. M. L. King, Jr. Blvd.
Tallahassee, FL 32307

[2]*USDA-ARS Plant, Soil and Nutrition Laboratory*
Ithaca, NY 14853

[3]*Department of Horticultural Sciences,*
and Plant Molecular and Cellular Biology Program
University of Florida
Gainesville, FL 32611

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Abstract. **Mineral nutrients are essential for plant growth and reproduction and nutrient deficiencies can limit yield. In addition, plant products represent an important source of minerals in the human diet. A genetic approach has been taken to advance our understanding of nutrient use efficiency in plants. Towards this purpose, we have selected the common bean (*Phaseolus vulgaris*, L.) as a model genetic system. An Andean (Calima), and a Mesoamerican (Jamapa) genotype, as well as a family of 76 recombinant inbreds (F10) lines from the (Jamapa × Calima) cross were used in the analysis. Mineral nutrient concentration and Zn efficiency were analyzed. Eleven macro- and micronutrients tested were detected in bean seeds. Seed Zn concentration was segregating in the RI family, and ranged from a low of 32.5 μg·g^{-1} in RIL148 to 67.1 μg·g^{-1} in RIL135. RIL5, RIL6, RIL15, RIL47, RIL137 and RIL150. The latter had consistently the highest mineral concentrations among all lines. Significant correlation was found between seed Zn concentration and the concentrations of Fe, Cu, Mg, Mn and P, but not the concentrations of Se, B, Ca, and S. These correlations are discussed together with Zn accumulation and Zn efficiency trait in bean.**

It is believed that beans were domesticated in South America and Mexico by 5000 BC (Sauer, 1993). At present, common bean is one of the world's most important food legumes, especially in Latin America and Africa. In those regions, beans offer a low cost alternative to beef and milk because beans are a good source of protein, Fe, dietary fiber and complex carbohydrates. Deficiency of mineral nutrients such as iron Fe, Zn, and P restrict the yield in beans (Polson, 1968).

Macro- and micro-nutrient composition can vary both within and between species. Common bean has been collected from a wide range of geographic areas. The natural variation in seed mineral content among different accession can be exploited for the genetic analysis of different traits related to mineral metabolism including Zn and Fe use efficiencies (Hacisalihoglu and Kochian, 2003). To begin dissecting the genetic mechanisms of nutrient accumulation in beans, a series of Zn-efficient and Zn-inefficient bean genotypes have been identified and characterized (Hacisalihoglu et al., 2004). Moreover, a series of recombinant inbred lines (RILs) have been generated by Vallejos et al. (2001) and are readily available for mapping and identifying genes controlling characters of interest.

Limited information is known about macro- and micronutrient concentration of seeds and their interactions. Khera et al. (2004) reported that large and medium seeds of *D. sissoo* showed better germination in laboratory and nursery, respectively. Furthermore, the authors stated that seed size in *A. nilotica* and *A. lebbek* had positive correlation with plant growth and development.

The first objective of this research was to survey the variability in multiple macro- micro-nutrient accumulation among currently available bean recombinant inbred lines. The second objective was to identify a core of bean lines with the highest mineral nutrient concentration. The results of this research will assist bean breeding programs in increasing the bio-availability of key nutrients.

Materials and Methods

Two parental common bean genotypes of Andean (Calima) and Mesoamerican (Jamapa) origin, and a family of 76 recombinant inbreds from the (Jamapa × Calima) cross were analyzed (Vallejos et al., 2001). All seeds were harvested from greenhouse plants grown in soil [Metromix 300] supplemented with 30 g of Osmocote [N:P:K, 14-14-14] in Gainesville, Florida. Four independent aliquots of 5 harvested seeds were bulked, ground with a coffee grinder, and 0.5 g samples from each pool were prepared for elemental analysis. Seed samples were digested in concentrated HNO3 overnight at 120 °C. Samples were then dissolved in HNO3:HClO4 (1:1, v/v) at 220 °C, re-suspended in 5% HNO3 and analyzed for elemental composition via simultaneous inductively coupled argon-plasma emission spectrometry (ICAP 61E trace analyzer, Thermo-Jarrel Ashe, Franklin, Mass.).

The experiments were set up in a complete randomized design with four replicates, and variation within means is presented as the standard error (S.E.). Statistical analyses were performed using Sigma Plot (SPSS, Inc., Chicago, Ill.) as described previously (Hacisalihoglu et al., 2004).

Results and Discussion

Seed Mineral Concentration. Seed mass ranged from 0.108 g to 0.539 g with a mean of 0.277 g (SE = 0.01) (Fig. 1C). RIL66 (0.539 g), Calima (0.489 g), and RIL11 (0.471 g) were recorded as largest seeds with 70-95% greater than average seed mass of 0.277 g (Fig. 1C). Lines with the lowest seed mass were RIL138 (0.108 g) and RIL150 (0.115 g). Generally, largest seed lines have high seed mineral content but not concentration, such as RIL11, RIL 46, and RIL66 (Fig. 1A, 1B).

*Corresponding author; e-mail: gokhan.h@famu.edu

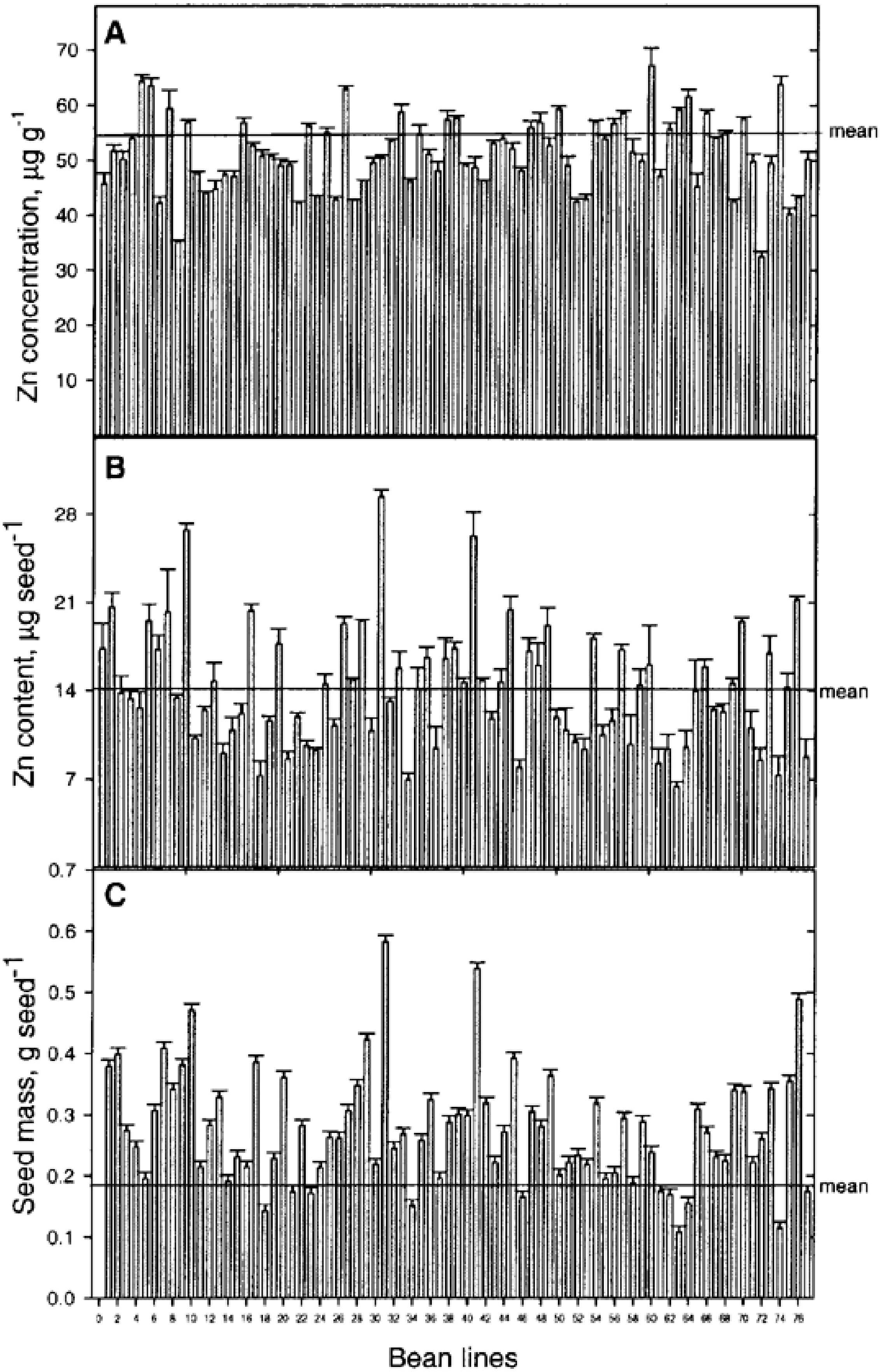

Fig. 1. Zn concentration (A), Zn content (B), and seed mass (C) in 77 bean recombinant inbred lines. Bars are standard errors (S.E.) of mean which is marked as straight line.

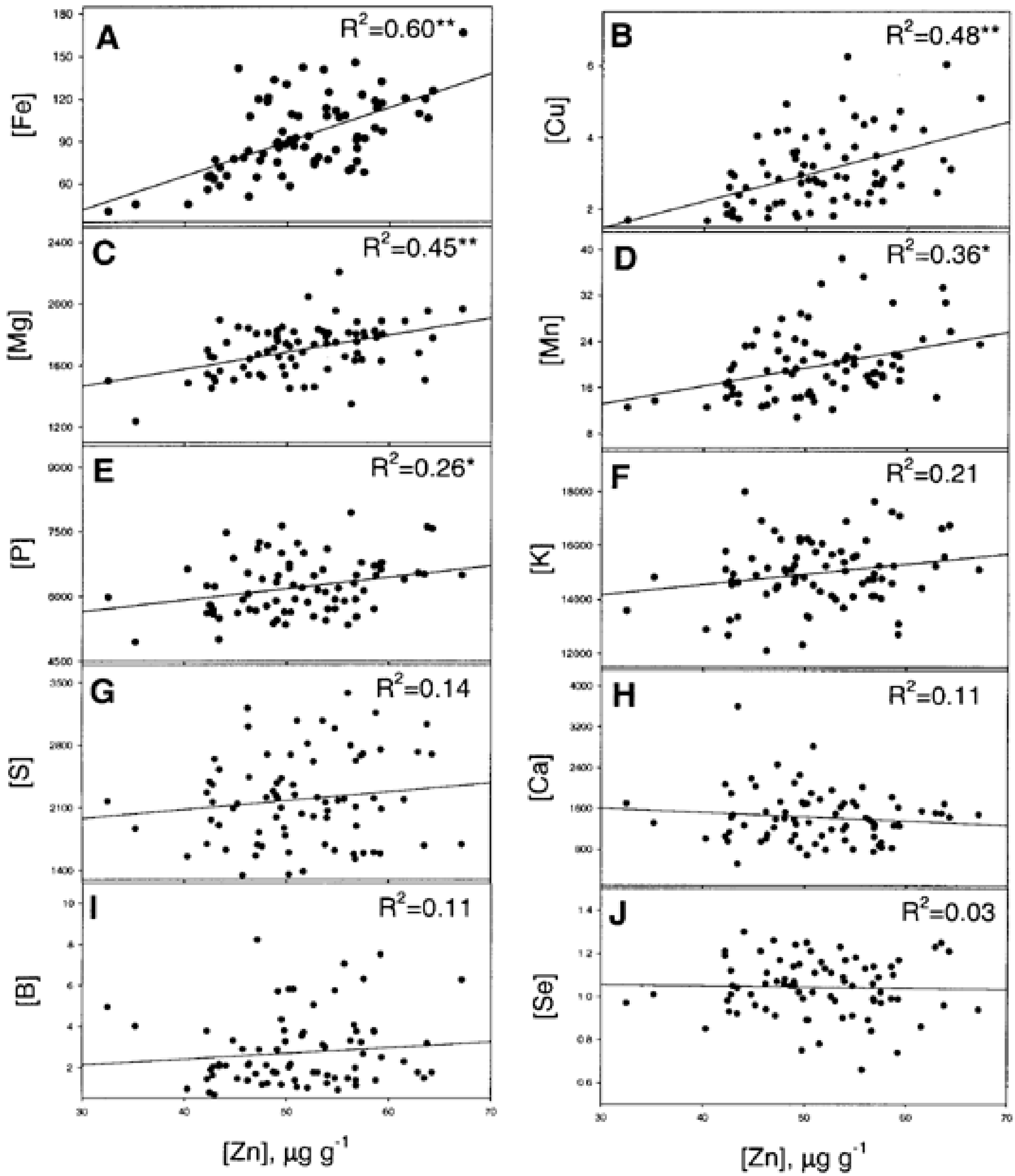

Fig. 2. Correlation between Zn concentration and the concentrations of seed Fe (A), Cu (B), Mg (C), Mn (D), P (E), K (F), S (G), Ca (H), B (I), and Se (J) of 79 bean lines. * and ** statistically significant at $P < 0.05$ and $P < 0.01$ levels, respectively; as determined using simple linear regression; R^2 = linear regression coefficient squared.

The mineral nutrient concentrations were determined for all lines (Fig. 1). Large differences were observed among the parental lines and the 76 RI lines in all nutrients tested. However, there were variations among differences and less dramatic variation found for Mg, S, and Se concentrations.

Based on the results of the large scale screening of seeds of the 77 lines, six bean lines were chosen namely RIL5, RIL6, RIL19, RIL47, RIL137, and RIL150 had consistently highest seed mineral concentration as superior to others (Table 1). There was a significant correlation between Zn efficiency (ZE) and efficiency of other nutrients. Six superior bean lines chosen for high nutrient concentration showed high Zn efficiency (Table 1).

Correlation between Zinc and other Nutrients. Significant and positive correlation was observed between seed mineral concentration in each of bean lines (Fig. 2). Seed Zn concentration varied from 32.5 to 67.2 $\mu g \cdot g^{-1}$ with a mean of 51.3 $\mu g \cdot g^{-1}$ (SE = 0.97) (Fig. 1A). Bean lines of RIL135, RIL5, and RIL150 had the highest Zn concentration, while RIL9, RIL148, and RIL151 had the lowest Zn concentration (Fig. 1 and Table 2).

Figure 2 shows the relationship between Zn concentration and other ten nutrients. Concentration of Fe, Cu, and Mg showed strong positive relationship with zinc (Zn), while concentration of Phosphorus showed moderate positive relationship. Moreover, Potassium (K), Sulfur (S), Calcium (Ca), Boron (B), and Selenium (Se) showed weak relationship with Zn concentration.

Significant variation for seed weight, content and concentration of mineral nutrients has been found in the recombinant inbred line population of common bean. However, there was limited genetic variation in seed selenium Se, S, and Mg concentrations, therefore Se, S, and Mg accumulation may respond little to selection in breeding programs. The results of this study showed that there was greater genetic variation in bean lines for Zn, Fe, Mn, Cu, P, and K concentrations.

There was a positive correlation between seed weight and seed mineral content and this is consistent with Marschner (1997) that a cultivar with relatively larger seed may show higher nutrient accumulation. Moreover, seed Zn concentration values were positively correlated with the concentrations of Fe, Cu, Mg, Mn, and P in all bean lines. These results suggest that seed nutrient concentration values may be used as an additional selection tool for nutrient efficiency in common bean.

In conclusion, we have identified a core set of lines that had superior nutrient concentration. Our data represent the first reported extensive survey of genetic variation for multiple nutrient accumulations in bean seeds. Future genetic and physiological analysis can be carried out with these lines to understand the accumulation of key nutrients in seeds together with the genes involving in these processes.

Table 1. Superior bean lines with high elemental concentrations.

Line	Seed mass, *g*	Zn efficiency rating*	[Zn] $\mu g\ g^{-1}$
RIL5	0.196	1.6	64.3
RIL6	0.307	1.5	63.5
RIL19	0.228	2.2	50.8
RIL47	0.305	2.4	56.0
RIL137	0.169	2.3	55.7
RIL150	0.115	2.0	63.8

*Zn efficiency rating was visual scoring of whole plant based on a scale of 1 to 5: 1 = healthy plants, and 5 = severe Zn deficiency symptoms.

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