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DISTRIBUTION OF SELECTED ELEMENTS BETWEEN THE SEED COAT AND EMBRYO OF TWO BLACK BEAN CULTIVARS

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ABSTRACT

Differences in distribution of minerals between the seed coat and embryo of plant seed can affect their bioavailability. Concentrations of iron [Fe], manganese [Mn], zinc [Zn], calcium [Ca], magnesium [Mg], potassium [K], and phosphorus [P] in these seed fractions of two black bean (*Phaseolus vulgaris* L.) cultivars (T39 and UI-911) were studied in field experiments conducted on acid and calcareous soils. Seed of T39 and UI-911 at the acid soil site had 34% and 45% higher [Fe], respectively, than at the calcareous soil site. Seed-coat [Fe] in UI-911 was substantially higher than that in T39. In contrast, embryo [Fe] was higher in T39 than UI-911. Mean percentages of the total seed Fe located in the seed coat were 33% and 32% for UI-911 at the acid and calcareous soil sites, respectively. The corresponding percentages for T39 were 14% and 18%, respectively.

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Although UI-911 accumulated higher percentages of Ca, Mg, and Zn in the seed coat than T39, the cultivar differences were relatively small. Mean percentages of dry matter and elements in the seed, averaged over cultivars and sites, found in the seed-coat fraction were: Dry matter = 10%, Ca = 84%, Fe = 24%, Mg = 18%, K = 8%, Zn = 7%, Mn = 3%, and P = 2%. There is apparently a genetically-controlled barrier to the movement of Fe between the seed coat and embryo during seed development in *P. vulgaris*.

INTRODUCTION

Relatively little is known about genotypic variability in within-seed distribution of minerals in common bean (*Phaseolus vulgaris* L.). Such information is of potential importance since there is evidence that the seed coat and embryo of *P. vulgaris* differ in bioavailability of Fe (1) and Ca (2) in human nutrition.

Abrasive dehulling and presoaking techniques are commonly used to separate seed coats from embryos of mature, dry seed. However, incomplete separation of the two tissues during abrasive dehulling (3) and loss of minerals during soaking of the seed (4,5) affect the efficacy of these procedures for plant nutrition purposes. Seeds reach physiological maturity when pod color of common bean changes from green to yellow, tan, or purple, and vascular connections between the pod and stem cease to function, i.e. the R7 growth stage (6). Unlike the situation with mature, dry seed, seed coats and embryos can be easily separated without presoaking at the R7 growth stage.

Preliminary research indicated that [Fe] in seed coats varies appreciably among common bean genotypes. For example, the cultivar UI-911 had a higher [Fe] in the seed coat than the cultivar T39. Both T39 and UI-911 are black beans derived from the Middle American gene pool, race Mesoamerican of *P. vulgaris*. The objective of our research was to confirm the differential accumulation of Fe, and to determine if the two genotypes differed in seed coat-embryo relationships for Ca, Mg, K, Zn, Mn, and P.

MATERIALS AND METHODS

Two field experiments were conducted in 2000 to determine differences in the accumulation and distribution of Fe, Mn, Zn, Ca, Mg, K, and P between the seed coat and embryo fractions of the black bean cultivars T39 and UI-911 grown on acid and calcareous soils. The soil at Site 1 (Erie, ND) was an Eckman (coarse-silty, mixed, superactive, frigid Calcic Hapludolls). The soil at Site 2 (Hatton, ND) was a Glyndon (coarse-silty, mixed, superactive, frigid Aeric Calciaquolls). The 0 to 15-cm soil depths at Sites 1 and 2 had pH values of 6.4 and 8.0, respectively.

The two cultivars were grown in randomized complete block experiments with twelve replications. Each plot consisted of four rows, 6.1 m in length, with an interrow spacing of 0.76 m. When at least 50% of the pods in a plot had reached the R7 growth stage (6), thirty-five such pods were harvested from each plot. The harvested pods were rinsed in deionized water to remove soil and dried with cellulose tissue. Seed was removed from pods and mixed. Thirty-five seeds were randomly selected; seed coats and embryos were manually separated, care being taken to avoid contamination. The two fractions were dried at 62°C for 36 h and weighed. Each seed-coat sample was immediately added to a digestion tube for chemical analysis. The embryo fraction was ground in an agate mill with an agate pestle, and redried at 62°C prior to chemical analysis.

The plant materials were digested on an aluminum block with 4 ml HNO_3, 2 ml $HClO_4$, and a drop of kerosene (7). Particular care was taken to shake the seed-coat digests, to avoid forming problems, as the block temperature was initially raised from room temperature to 100°C. The acid digests were analyzed for Fe, Mn, Zn, Ca, K, and Mg by atomic absorption spectroscopy and for P by a molybdenum-blue procedure. Standard Reference Material 1515 from the National Institute of Standards and Technology (Gaithersburg, MD) was digested and analyzed concurrently with the bean material to provide an indication of the accuracy of the analytical procedures.

Differences between T39 and UI-911 for the various parameters studied were analyzed statistically by testing the null hypothesis that the difference was zero (8).

RESULTS

T39 and UI-911 had similar embryo, seed coat, and seed weights (Table 1). The seed coat comprised approximately 10% of the dry weight of the seed. T39 had substantially lower [Fe] in the seed coat than UI-911 at both sites. In contrast, T39 had higher [Fe] in the embryo than UI-911. The [Fe] in the entire seed was higher at Site 1, the acid soil site, than at Site 2, the calcareous soil site. Seed of T39 and UI-911 at Site 1 had 34% and 45% higher [Fe], respectively, than at Site 2.

In agreement with the [Fe] data, UI-911 had higher [Ca], [Mg], and [Zn] in seed coats than T39 (Table 2). However, these latter cultivar differences were markedly less than those for [Fe]. There was no tendency for UI-911 to have higher seed-coat [Mn], [K], or [P].

Table 1. Differences in Accumulation of Dry Matter and Fe in Seeds of Two Black Bean Cultivars at Two Sites

		Site 1			Site 2		
Parameter	Cultivar	Seed Coat	Embryo	Seed	Seed Coat	Embryo	Seed
Dry matter, mg	T39 (a)	18.8	180	198	17.7	165	182
	UI-911 (b)	*19.4*	*187*	*207*	*17.9*	*162*	*180*
	a-b	0.6^{NS}	7^{NS}	9^{NS}	0.2^{NS}	3^{NS}	2^{NS}
Fe, $mg\,kg^{-1}$	T39 (a)	82	53	55	74	37	41
	UI-911 (b)	*226*	*48*	*64*	*143*	*33*	*44*
	a-b	−144***	5*	−9**	−69**	4**	−3***
Fe, μg	T39 (a)	1.5	9.5	11.0	1.3	6.1	7.4
	UI-911 (b)	*4.4*	*8.9*	*13.3*	*2.6*	*5.4*	*7.9*
	a-b	−2.9***	0.6*	−2.3**	−1.3***	0.7*	-0.5^{NS}

*, **, ***, ^{NS}Significant at 0.05, 0.01 and 0.001 probability levels and not significant, respectively.

Relative distribution of an element between seed coats and embryos can be given by (a) a comparison of seed-coat concentration/embryo concentration ratios, or (b) a comparison of percentages of the total quantity of the elements present in the seed coat. The concentration-ratio data (Table 3) show that Fe, Mg, and especially Ca are concentrated in the seed coat. Mean seed-coat concentration/embryo concentration ratios for T39, averaged over sites, were: Ca = 45, Fe = 1.9, Mg = 1.9, K = 0.85, Zn = 0.65, Mn = 0.36, and P = 0.22. The corresponding ratios for UI-911 were: Ca = 55, Fe = 4.6, Mg = 2.2, K = 0.85, Zn = 0.8, Mn = 0.32, and P = 0.19. Proportionately, the greatest difference between T39 and UI-911 was in Fe accumulation in the seed coat.

From a bioavailability point-of-view a comparison of percentages of the total quantity of elements present in the seed coat is more important than a ratio comparison. Mean percentages, averaged over cultivars and sites, of the total quantity of dry matter and elements found in the seed coat fraction were: Dry matter = 10%, Ca = 84%, Fe = 24%, Mg = 18%, K = 8%, Zn = 7%, Mn = 3%, and P = 2% (Table 4). The cultivar differences for Fe distribution were particularly pronounced. Mean percentages of total seed Fe located in the seed coat of UI-911 were 33% and 32% at Sites 1 and 2, respectively (Table 4). The corresponding percentages for T39 were 14% and 18%, respectively. Although UI-911 accumulated higher percentages of Ca, Mg, and Zn in the seed coat than T39, the cultivar differences were relatively small.

Table 2. Concentration of Selected Elements Within Seed of Two Black Bean Cultivars at Two Sites

		Element					
		Mn	Zn	Ca	K	Mg	P
Plant Part	Cultivar	($mg\,kg^{-1}$)		($g\,kg^{-1}$)			
				Site 1			
Seed coat	T39 (a)	6.4	16	16.4	12.3	3.9	0.80
	UI-911 (b)	*6.2*	*21*	*19.9*	*12.7*	*4.2*	*0.73*
	a-b	0.2^{NS}	−5**	−3.5***	-0.4^{NS}	−0.3**	0.07*
Embryo	T39 (a)	19.0	25	0.4	14.6	2.1	4.03
	UI-911 (b)	*19.5*	*27*	*0.4*	*14.8*	*1.9*	*4.14*
	a-b	-0.5^{NS}	−2**	0	-0.2^{NS}	0.2***	-0.11^{NS}
				Site 2			
Seed coat	T39 (a)	7.6	19	17.5	12.8	3.6	0.76
	UI-911 (b)	*6.7*	*24*	*21.8*	*11.6*	*3.9*	*0.70*
	a-b	0.9*	−5**	−4.3***	1.2*	−0.3**	0.06*
Embryo	T39 (a)	20.0	29	0.4	14.7	1.8	3.32
	UI-911 (b)	*20.5*	*30*	*0.4*	*14.9*	*1.8*	*3.70*
	a-b	-0.5^{NS}	-1^{NS}	0	-0.2^{NS}	0	−0.38***

*, **, ***, ^{NS}Significant at 0.05, 0.01 and 0.001 probability levels and not significant, respectively.

Table 3. Ratios of Elemental Concentration in Seed Coat to Elemental Concentration in Embryo for Two Black Bean Cultivars

	Concentration (Seed Coat)/Concentration (Embryo)						
Cultivar	Fe	Mn	Zn	Ca	K	Mg	P
				Site 1			
T39 (a)	1.6	0.34	0.6	42	0.8	1.8	0.20
UI-911 (b)	*4.8*	*0.32*	*0.8*	*54*	*0.9*	*2.2*	*0.18*
a-b	−3.2***	0.02*	−0.2*	−12***	-0.1^{NS}	−0.4***	0.02^{NS}
				Site 2			
T39 (a)	2.1	0.38	0.7	47	0.9	2.0	0.23
UI-911 (b)	*4.3*	*0.33*	*0.8*	*56*	*0.8*	*2.2*	*0.19*
a-b	−2.2***	0.05**	−0.1**	−9**	0.1^{NS}	−0.2*	0.04**

*, **, ***, ^{NS}Significant at 0.05, 0.01 and 0.001 probability levels and not significant, respectively.

Table 4. Percentage of Dry Matter and Selected Elements in Seed Located in Seed Coats of Two Black Bean Cultivars

Cultivar	Total Seed Quantity in Seed Coat (%)							
	Dry Matter	Fe	Mn	Zn	Ca	K	Mg	P
				Site 1				
T39 (a)	9.4	14.1	3.4	6.2	81.4	8.1	16.1	2.0
UI-911 (b)	*9.4*	*33.1*	*3.2*	*7.6*	*85.0*	*8.2*	*18.5*	*1.8*
a-b	0	−19.0***	0.2^{NS}	−1.4*	−3.6***	0.1^{NS}	−2.4***	0.2^{NS}
				Site 2				
T39 (a)	9.6	17.9	3.8	6.4	83.0	8.3	17.7	2.3
UI-911 (b)	*10.0*	*32.0*	*3.3*	*7.6*	*86.0*	*7.5*	*19.3*	*1.9*
a-b	−0.4**	−14.1***	0.5***	−1.2**	−3.0***	0.8*	−1.6**	0.4**

*, **, ***,^{NS}Significant at 0.05, 0.01 and 0.001 probability levels and not significant, respectively.

DISCUSSION

There is general agreement that movement of minerals from plants to the seed coat, with the possible exception of Ca, occurs mainly in the phloem (9). Since there are no vascular connections between maternal tissue of the seed coat and filial embryonic tissue, phloem unloading of minerals other than Ca in the seed coat must occur during seed development (10). The higher and lower [Fe] in the seed coat and embryo, respectively, of UI-911, compared to those concentrations in T39, indicate that there is a barrier to the movement of Fe from the seed coat to the embryo of UI-911. Differences between T39 and UI-911 for both embryonic and seed-coat [Ca], [Mg], [K], [Zn], [Mn], and [P] are relatively small or non-existent. Consequently, the hypothesized barrier has little effect on the migration of these latter elements to the embryo. Movement of Fe from the seed coat to the embryo is not well understood. The Fe exiting the phloem in pea (*Pisum sativum* L.) seed appears to move symplastically in seed-coat cells to the apoplast separating the filial and maternal tissues before uptake into the embryo occurs (11). The seed-coat accumulation of Fe in UI-911 could result from unloading difficulties from seed-coat cells into the apoplast separating the seed coat and embryo. Alternatively, movement of Fe within the seed coat of UI-911 could be impeded.

The [Fe] of seed of both cultivars was highest at Site 1, the acid-soil site. Soil-Fe availability is known to increase under acid-soil conditions (9). However, there are surprisingly few literature citations concerning the influence of soil pH on seed [Fe]. Liming an acid soil decreased [Fe] of soybean (*Glycine max* L.) seed (12).

Although only 10% of the dry weight of seed was located in the seed coat, over 80% of the seed Ca was located in this fraction. Similarly high amounts of Ca in the seed coat, 70% for "mottled" bean seed and 73% for "white" bean seed, were also found in *P. vulgaris* (2). Formation of calcium oxalate in the seed coat of *P. vulgaris* is a possible explanation for this segregation of Ca (13). Such localization of Ca in the seed coat apparently does not occur to the same extent in soybean seed (14).

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