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Iron accumulation in seed of common bean

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Abstract

The effect of soil and genotype on iron concentration [Fe] in common bean (*Phaseolus vulgaris* L.) seed was studied in the greenhouse. Liming an acid soil increased soil pH from 6.0 to 7.3 but had no effect on seed [Fe] of three bean genotypes (Voyager, T39, UI911) from the Middle American gene pool in North Dakota. However, liming decreased seed-manganese concentration [Mn]. The influence of FeEDDHA on Fe accumulation in seed of the three bean genotypes, grown on acid (pH=6.0) and naturally calcareous (pH=8.2) soils, was also studied in North Dakota. Seed from the acid soil contained 25% higher [Fe] than seed from the calcareous soil. FeEDDHA increased seed [Fe] only on the calcareous soil, but reduced seed [Mn] on both soils. Voyager seed, characterized by a relatively low [Fe] in the seed coat, had a higher seed [Fe] than the other two genotypes. The hypothesis that high seed [Fe] is characterized by a low seed-coat [Fe] was next investigated. Voyager, T39 and 10 diverse Latin American genotypes from the Middle American gene pool were grown on a soil (pH=7.0) with Andic properties in Mexico in the presence and absence of FeEDTA. FeEDTA increased seed [Fe]. Seed of Voyager and a Mexican genotype (Bayo 400) had the highest seed [Fe]. However, Bayo 400, unlike Voyager, contained a high percentage of its seed Fe in the seed coat. Consequently, a high seed [Fe] genotype does not necessarily have a low seed-coat [Fe]. Both soil and genotype affect Fe accumulation in bean seed.

Abbreviations: EDDHA – ethylenediamine di(*o*-hydroxyphenylacetic acid); EDTA – ethylenediaminetetraacetic acid

Introduction

Common bean is an important source of protein and minerals, especially Fe, for some vegetarians and for many inhabitants of Latin America and Africa. Both the environment and genotype affect the Fe concentration [Fe] of bean seed (Beebe et al., 2000; Frossard et al., 2000). However, little is known about how liming, Fe chelates and differences in soil-Fe availability affect seed [Fe]. Iron chelates can drastically reduce Mn concentration [Mn] in vegetative tissue (Moraghan, 1979).

Seed consists of maternal seed-coat tissue and filial embryonic tissue. Minerals from the mother plant reach the embryo after passage through the apoplast separating the two tissues during seed development (Wolswinkel, 1992). The distribution of Fe between the seed coat and embryo in *P. vulgaris* is a genotypic trait (Moraghan and Grafton, 2002). Genetic variations in within-seed distribution of Fe could influence the value of common bean as a food-Fe source. For instance, bioavailability of Fe, as determined by a dialysability technique, was greater in whole seed than in cotyledons, due to greater solubility of Fe in the seed coat (Lombardi-Boccia et al., 1995). Therefore, seed of a genotype with a relatively large proportion of its seed Fe in the seed coat may be a better food-Fe

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source than seed of a genotype with a relatively low proportion of Fe in the seed coat.

Iron deficiency in plants is accentuated by high soil pH values, free $CaCO_3$ and high levels of P, Mn and HCO_3^- (Kabata-Pendias and Pendias, 1992). Leaf [Fe] is sometimes poorly related to leaf chlorophyll content (Marschner, 1995). Unlike leaf Fe, the majority of seed Fe is associated with phytic acid and phytoferritin (Lott et al., 1995; Marenthes and Grusak, 1998).

The objective of this investigation was to determine the influence of lime, Fe chelates and type of soil on accumulation of Fe and on its distribution within seed of diverse common bean genotypes from the Middle American gene pool. Data pertaining to within-seed distribution of Ca, Mg, Mn, P and Zn were also obtained during the investigation.

Materials and methods

General

Greenhouse trials were conducted at Fargo, North Dakota and at Montecillo, Mexico to determine the influence of lime, Fe chelates and soil type on seed [Fe] in selected bean genotypes from the Middle American gene pool. Three genotypes known to differ in seed-Fe characteristics, and relatively photoperiod insensitive, were included in the North Dakota studies. Mexico is a rich source of germplasm from the Middle American gene pool. Because of photoperiod sensitivity, however, most Mexican genotypes do not flower in North Dakota. Consequently, in a follow-up Mexican study the seed-Fe characteristics of two of the North Dakota genotypes and 10 Latin American bean genotypes were studied in the presence and absence of FeEDTA.

The experimental unit consisted of 4.5 and 5.0 kg of air-dried soil pot^{-1} at the Fargo and Montecillo locations, respectively. Eight seeds, subsequently thinned to 3 plants pot^{-1}, were planted in each pot. Pots were watered periodically with distilled, deionized H_2O to the approximate field capacity to avoid plant wilting. The watering weights were adjusted periodically to compensate for plant growth. Greenhouse temperatures were generally maintained between 18 and 25 °C.

Basal dressings of NH_4NO_3 (70 μg N g^{-1}), K_2SO_4 (90 μg K g^{-1}), Ca $(H_2PO_4)_2$ (60 μg P g^{-1}), and $ZnSO_4$ (4 μg Zn g^{-1}) were mixed with the air-dried soil in a two-shell blender. A basal dressing of $Na_2B_4O_7$ (1 μg B g^{-1}) and the preplant Fe-chelate treatment, each dissolved in 20 ml of deionized water, was added to the surface of the soil in relevant pots prior to the initial watering with distilled, deionized water to the approximate field capacity. Post-planting applications of N fertilizer, increments of 10 μg NH_4NO_3–N g^{-1}, were added periodically to all pots after the V4 (Brick and Shanahan, 1996) growth stage. Pods in Experiment 1 were harvested at the R8 growth stage, and whole seed was used for chemical analysis. Pods in Experiments 2 and 3 were harvested daily at physiological maturity, the R7 growth stage, until all pods within a given pot were harvested. Pod walls were rinsed with distilled-deionized water within 2 h of harvest, after which seed was removed and counted. Seed coats and embryos were then manually separated, placed in glass jars and dried at 62 °C. When all seed from a given pot was harvested and oven-dried for at least 72 h, the fractions were weighed and then ground in an agate grinder to pass a 60-mesh sieve. Care was taken during seed processing to avoid Fe contamination.

Experiment 1

This greenhouse experiment was designed to determine if addition of lime to an acid Eckman soil reduced [Fe] and [Mn] of common bean seed. The six treatments, arranged in a randomized complete block design with four replications, consisted of two lime treatments (0 and 3.6 g analytical reagent grade $CaCO_3$ kg^{-1} of air-dried soil) applied to each of three bean genotypes (Voyager, T39 and UI911) grown on an Eckman loam soil. The untreated Eckman soil had a pH of 6.0 and contained 62 μg DTPA-Fe g^{-1}. The three bean genotypes belong to race Mesoamerican of the Middle American gene pool of *P. vulgaris*. The harvested seed was analyzed for Fe and Mn.

Duplicate soils without plants were watered to the approximate field capacity, and incubated for 14 d under greenhouse conditions at 16 to 21 °C. The soils were then air-dried, ground and analyzed for pH, DTPA-Fe and DTPA-Mn.

Experiment 2

The purpose of this experiment was to determine the influence of FeEDDHA on seed [Fe] of common bean grown on acid and naturally calcareous soils. The treatments were 12 in number and con-

Table 1. Selected characteristics of 12 common bean genotypes from the Middle American gene pool grown in Mexico in a seed-Fe experiment

Genotype	Race	Growth habit[a]	Seed color	Origin
Voyager	Mesoamerican	II	White	USA
T39	Mesoamerican	III	Black	USA
Bayo 400	Mesoamerican	II	Brown	Mexico
Negro 8025	Mesoamerican	III	Black	Mexico
Tacana	Mesoamerican	II	Black	Mexico
Rio Tibagi	Mesoamerican	III	Black	Brazil
NG 94060	Jalisco	III	Black	Mexico
Mexico 332	Jalisco	III	Black	Mexico
Puebla 152	Jalisco	III	Black	Mexico
A800	Durango	III	Black	Mexico
Negro Durango	Durango	III	Black	Mexico
Pinto Villa	Durango	III	Light brown with spots	Mexico

[a]Type II = indeterminate upright, short vine; Type III = indeterminate, prostrate vine; the genotypes tended to vine more in the greenhouse than is commonly observed in the field.

Table 2. Influence of lime and genotype on dry matter yield and Fe and Mn concentrations of common bean seed

Genotype	Lime ($g\ kg^{-1}$)	Seed ($g\ pot^{-1}$)	Concentration ($\mu g\ g^{-1}$) Fe	Mn
Voyager	0	20.6	100	32
T39	0	25.6	70	25
UI911	0	22.5	78	25
Voyager	3.6	20.1	99	25
T39	3.6	25.1	73	19
UI911	3.6	24.2	75	21
SE		1.0	0.8	0.7
CV		8.9	6.9	4.4
F test probabilities				
Genotype (G)		<0.01	<0.01	<0.01
Lime (L)		0.33	<0.01	<0.01
G × L		0.45	0.46	<0.01

sisted of a factorial combination of two soils (acid and calcareous), two soil Fe treatments (0 and 4 μg FeEDDHA-Fe g^{-1} – half applied preplant and half at flowering) and three bean genotypes (Voyager, T39 and UI911). The flowering application of Fe was added to the soil surface in 20 ml of H_2O prior to a regularly scheduled watering. All treatments were replicated four times and arranged in randomized complete blocks. The acid soil was an Eckman loam (pH=6.0, 62 μg DTPA-Fe g^{-1}, no inorganic C). The calcareous soil was a Wheatville loam (pH=8.2, 4 μg DTPA-Fe g^{-1}, 14.4g of inorganic C kg^{-1}). The harvested seed components were analyzed for Fe, Mn, Zn, Ca, Mg and P.

Experiment 3

Results from Experiment 2 lead to the hypothesis that there is an inverse relationship between seed-coat [Fe] and embryo [Fe] in *P. vulgaris*. This hypothesis was tested by studying in Mexico the influence of FeEDTA on seed-Fe distribution in a more diverse population of 12 bean genotypes, including Voyager and T39 from the North Dakota experiments and 10 Latin American genotypes. Pertinent information concerning the genotypes is given in Table 1.

There were 24 treatments comprising each of the 12 genotypes without and with FeEDTA (4 μg FeEDTA-Fe g^{-1} at planting). All treatments were replicated four times and arranged in a completely randomized design. The greenhouse soil was a Mexican soil with Andic properties (pH=7.0; 28 μg DTPA-Fe g^{-1}; no inorganic C). The seed components were analyzed for Fe at the Mexican location.

Chemical analyses

The seed coat and embryo samples were digested on an aluminum block with 4 ml HNO_3, 2 ml $HClO_4$ and a drop of kerosene. The acid digests were analyzed for Fe, Mn, Zn, Ca and Mg by atomic absorption spectroscopy and for P by a molybdenum-blue procedure. From a knowledge of seed coat and embryo weights and the above analytical data, the concentration of an element in the whole seed was calculated. The percentage of the total seed element in the seed coat was also determined by calculation (μg element seed coat^{-1} $\div$ μg element seed^{-1} $\times$ 100).

Results

Experiment 1

Lime addition and incubation increased the pH of the Eckman soil from 6.0 to 7.3, decreased DTPA-Fe from 62 to 42 $\mu g\ g^{-1}$, and decreased DTPA-Mn from 74 to 13 $\mu g\ g^{-1}$. Liming had no effect on seed yield nor seed [Fe], but decreased seed [Mn] in each of the three bean genotypes (Table 2). Voyager, the lowest-yielding genotype, had higher seed [Mn] and [Fe] than T39 and UI911.

Table 3. Influence of FeEDDHA, soil and bean genotype on seed yield and dry matter distribution in the seed

Treatment			Seed	Dry matter (mg seed^{-1})		
Soil	FeEDDHA-Fe (μg g^{-1})	Genotype	yield (g pot^{-1})	Seed coat	Embryo	Seed
Eckman	0	Voyager	16.4	17.2	153	170
(acid)	0	T39	19.2	19.7	193	212
	0	UI911	17.5	16.3	170	195
	4	Voyager	16.3	16.4	147	164
	4	T39	18.4	18.1	190	208
	4	UI911	16.8	16.2	177	193
Wheatville	0	Voyager	16.4	17.2	156	173
(calcareous)	0	T39	19.7	17.0	174	191
	0	UI911	16.7	15.4	161	176
	4	Voyager	18.2	18.1	159	177
	4	T39	19.4	17.5	187	205
	4	UI911	18.3	16.2	174	190
SE			0.9	0.5	6	7
CV			10.5	5.8	7	7

Experiment 2

Individual seed weight and seed yield were little affected by application of FeEDDHA or by type of soil (Table 3). Analyses of variance for all dry matter and chemical data sets are given in Table 6. As in Experiment 1, T39 had the highest seed yield of the three genotypes. The bean genotypes differed in individual seed weights. Voyager and T39 had the smallest and largest individual seed weights, respectively.

Application of FeEDDHA, type of soil and genotype all influenced the seed-coat, embryo and entire seed [Fe] (Table 4). FeEDDHA increased [Fe] in the three seed components only in plants grown on the calcareous Wheatville soil. Consequently, the soil $\times$ FeEDDHA interactions for [Fe] were significant. Seed [Fe] in the absence of the chelate was higher on plants from the acid soil than from the calcareous soil. Seed [Fe] was substantially higher in Voyager than in T39 and UI911, irrespective of soil or Fe-chelate treatment. However, an inverse relationship existed between seed-coat [Fe] and embryo [Fe]. For instance, seed-coat [Fe] was highest in UI911, intermediate in T39, and least in Voyager. In contrast, embryo [Fe] was highest in Voyager, intermediate in T39 and least in UI911. The total Fe content in the harvested seed (mg 3 plants^{-1}), like [Fe], was higher in Voyager than in T39 and UI911. Seed-Fe content was increased by FeEDDHA applied to the Wheatville soil but not to the Eckman soil.

Seed [Mn] was affected by genotype, FeEDDHA and type of soil (Table 4). Voyager, for given soil-FeEDDHA treatments, contained higher seed [Mn] than did either T39 or UI911. FeEDDHA caused a reduction in seed [Mn], particularly in seed coats, in all three genotypes on both soils. Seed from the acid soil, for given FeEDDHA-genotype treatments, contained higher [Mn] than seed from the calcareous soil. The total Mn content in the harvested seed (mg 3 plants), like [Mn], was greater with the Eckman soil than with the Wheatville soil, and was reduced by FeEDDHA application.

The contrasting patterns of Fe distribution within seed of the three genotypes are best illustrated by comparing percentages of total seed Fe present in the seed coat (Table 5). Neither FeEDDHA nor type of soil greatly affected this distribution. Averaged over FeEDDHA and soil treatments, Voyager, T39 and UI911 had mean percentages of 8, 19 and 37, respectively, of their seed Fe in the seed coat. The corresponding percentages for seed dry matter in the seed-coat fraction were 10, 9 and 9, respectively.

Genotype was the chief factor influencing the relative distribution of dry matter and elements between the seed coat and the embryo (Table 5). However, in contrast to the seed-Fe situation, there was no tendency for the seed-coat fraction of Voyager to contain smaller percentages of Mn, Zn, Ca, Mg and P than the seed-coat fractions of T39 and UI911 (Table 5). In fact, larger percentages of the seed contents of Mn, Zn and P were found in the seed-coat fraction of Voyager than in the seed-coat fractions of T39 and UI911. Seed-coats of all three genotypes were enriched in Ca and depleted in P.

Experiment 3

Genotype and FeEDTA influenced seed [Fe] in the Mexican study (Table 7). The mean seed [Fe] was increased from 73 to 87 μg g^{-1} by application of FeEDTA. The genotype $\times$ FeEDTA interaction was not significant. Voyager, the high seed-Fe accumulator in Experiments 1 and 2, was again a relatively high seed-Fe accumulator in Experiment 3. Bayo 400 was also a high seed-Fe accumulator in this experiment.

FeEDTA had little effect on the distribution of Fe between the seed coat and the embryo. In contrast, genotype had a marked effect on this parameter. The mean percentage of total seed Fe in the seed coat,

Table 4. Influence of FeEDDHA, soil and genotype on the concentration of Fe and Mn in bean seed

Treatment			Fe (μg g^{-1})			Mn (μg g^{-1})			Content (mg 3 plants^{-1})	
Soil	FeEDDHA-Fe (μg g^{-1})	Genotype	Seed coat	Embryo	Seed	Seed coat	Embryo	Seed	Seed Fe	Seed Mn
Eckman (acid)	0	Voyager	120	107	108	33	29	29	1.78	0.48
	0	T39	173	66	76	20	25	24	1.48	0.47
	0	UI911	367	58	84	15	27	26	1.47	0.45
	4	Voyager	90	106	104	28	26	26	1.69	0.42
	4	T39	147	68	75	19	23	23	1.38	0.42
	4	UI911	334	56	79	13	22	21	1.33	0.36
Wheatville (calcareous)	0	Voyager	49	91	87	21	25	25	1.43	0.41
	0	T39	140	55	62	14	21	20	1.23	0.40
	0	UI911	314	44	68	10	22	21	1.13	0.34
	4	Voyager	72	111	107	15	20	20	1.95	0.36
	4	T39	183	75	84	9	17	16	1.64	0.31
	4	UI911	347	58	83	8	17	17	1.51	0.30
SE			12	2	3	1	1	1	0.09	0.02
CV			12	6	6	13	5	5	11	12

Table 5. Influence of FeEDDHA, soil and bean genotype on the percentage of selected seed parameters present in the seed coat

Treatment			Percentage of total seed quantity present in seed coat						
Soil	FeEDDHA-Fe (μg g^{-1})	Genotype	Dry matter	Fe	Mn	Zn	Ca	Mg	P
Eckman (acid)	0	Voyager	10.1	11	11	12	83	16	3.6
	0	T39	9.4	21	8	7	82	16	2.6
	0	UI911	8.3	36	5	8	84	15	1.9
	4	Voyager	10.0	9	11	12	84	17	3.6
	4	T39	8.7	17	7	7	80	15	2.1
	4	UI911	8.4	35	5	8	83	17	1.9
Wheatville (calcareous)	0	Voyager	9.9	6	8	6	83	15	3.5
	0	T39	9.0	20	6	4	80	15	2.6
	0	UI911	8.8	40	4	4	85	16	2.0
	4	Voyager	10.2	7	8	6	83	15	3.9
	4	T39	8.6	19	5	4	81	14	1.9
	4	UI911	8.5	36	4	4	84	14	2.1
SE			0.2	1	1	1	1	1	0.2
CV			5	13	17	15	2	11	17

Table 6. F tests of significance for the effects of FeEDDHA, soil and bean genotype on selected seed characteristics

Parameter	*F* test probabilities						
	FeEDDHA (Fe)	Soil (S)	Genotype (G)	Fe × S	Fe × G	S × G	Fe × S × G
				Table 3			
Seed yield, g pot^{-1}	0.69	0.23	<0.01	0.17	0.59	0.83	0.80
Seed coat, mg seed^{-1}	0.90	0.17	<0.01	<0.01	0.42	<0.01	0.57
Embryo, mg seed^{-1}	0.43	0.23	<0.01	0.08	0.68	0.08	0.92
Seed, mg seed^{-1}	0.48	0.19	<0.01	0.06	0.69	0.06	0.92
				Table 4			
Seed coat-Fe, μg g^{-1}	0.37	<0.01	<0.01	<0.01	0.59	0.02	0.68
Embryo-Fe, μg g^{-1}	<0.01	<0.01	<0.01	<0.01	0.25	0.43	0.72
Seed-Fe, μg g^{-1}	<0.01	<0.01	<0.01	<0.01	0.51	0.13	0.57
Seed coat-Mn, μg g^{-1}	<0.01	<0.01	<0.01	0.31	0.13	<0.01	0.45
Embryo-Mn, μg g^{-1}	<0.01	<0.01	<0.01	0.02	0.10	0.85	0.13
Seed-Mn, μg g^{-1}	<0.01	<0.01	<0.01	0.10	0.10	0.86	0.10
Seed Fe, mg 3 plants^{-1}	<0.01	0.42	<0.01	<0.01	0.73	0.81	0.87
Seed Mn, mg 3 plants^{-1}	<0.01	<0.01	<0.01	0.81	0.33	0.01	0.68
				Table 5			
Dry matter,%	0.23	0.90	<0.01	0.59	0.18	0.29	0.58
Fe,%	0.04	0.41	<0.01	0.81	0.55	0.03	0.05
Mn,%	0.18	<0.01	<0.01	0.69	0.42	0.04	0.89
Zn,%	0.92	<0.01	<0.01	0.46	0.37	<0.01	0.91
Ca,%	0.59	0.92	<0.01	0.33	0.61	0.17	0.24
Mg,%	0.28	0.17	0.11	0.03	0.59	0.68	0.27
P,%	0.48	0.71	<0.01	0.87	0.03	0.76	0.71

averaged over Fe treatments, ranged from 5.5% for Voyager to 32.3% for Rio Tibagi. Of particular interest was the finding that Bayo 400, unlike the other high seed-Fe accumulator Voyager, accumulated a relatively large amount, approximately 22%, of its seed Fe in the seed coat. Voyager was the only genotype that accumulated, relative to dry matter distribution, a smaller percentage of its seed Fe in the seed coat.

Discussion

Seed yield and individual seed weight of Voyager, T39 and UI911 were little affected by liming, type of soil or by application of FeEDDHA. The three genotypes differed in seed weight with mean values of 171, 204 and 189 mg seed^{-1} for Voyager, T39 and UI911, respectively, in Experiment 2. All three genotypes are small-seeded types of common bean. The seed weights of the Mexican genotypes were more variable, with mean weights ranging from 176 to 451 mg seed^{-1}. Seed weight of common bean cultivars range from approximately 200 to 1000 mg seed^{-1} (Gepts and Debouck, 1991).

Plant Fe uptake from well-aerated, moist soil is dependent on the solubility of soil $Fe(OH)_3$, which in turn is affected by soil pH (Lindsay and Schwab, 1982) and plant factors (Brown and Jolley, 1989; Römheld, 1987). The solubility of $Fe\ (OH)_3$ decreases 1000-fold for each unit increase in pH. Due presumably to the greater solubility of soil Fe, seed of Voyager, T39 and UI911 from the acid Eckman soil in the absence of FeEDDHA had 24, 23 and 24% greater [Fe], respectively, than seed from their calcareous Wheatville soil counterparts. The advantage of the acid soil over the calcareous Wheatville soil in regards to seed [Fe] was eliminated by application of FeEDDHA. The EDDHA ligand is effective at maintaining Fe in solution at pH 8.2, the pH value of the calcareous Wheatville soil (Lindsay and Schwab, 1982). Native soil NO_3^- and added NH_4NO_3 were the sources of N for the bean plants in our research.

Table 7. Influence of FeEDTA on dry matter and Fe accumulation in seed of 12 bean genotypes grown in Mexico

Genotype	Seed weight (mg seed^{-1})[a]		Percentage seed dry matter in seed coat[a]		Seed Fe (μg g^{-1})[a]		Percentage total seed Fe in seed coat[a]	
	−	+	−	+	−	+	−	+
Voyager	164	164	10.0	9.5	90	96	5.0	6.0
T39	185	187	9.4	9.4	62	72	17.2	17.4
Bayo 400	389	381	10.4	10.8	86	101	20.5	23.6
Negro 8025	203	193	9.5	9.7	74	91	18.5	16.5
Tacana	184	176	11.0	11.0	78	87	31.5	33.0
Rio Tibagi	182	180	10.4	9.8	78	91	33.8	34.1
NG 94060	359	329	9.1	9.3	65	79	11.6	14.7
Mexico 332	276	280	10.6	10.9	64	76	15.1	16.1
Puebla 152	316	311	8.8	9.7	77	100	24.5	27.5
A800	324	304	8.9	9.3	71	95	20.7	19.3
Negro Durango	444	451	8.8	9.7	65	84	23.3	27.8
Pinto Villa	*407*	*380*	*9.5*	*9.7*	*67*	*71*	*17.9*	*20.4*
$\bar{x}$	286	278	9.7	9.9	73	87	20.0	21.4
SE	10.7		0.3		3.9		1.6	
CV	7.6		5.6		9.8		15.3	
F test probabilities								
Genotype (G)	<0.01		<0.01		<0.01		<0.01	
FeEDTA (Fe)	0.07		0.07		<0.01		0.03	
GxFe	0.80		0.26		0.31		0.67	

[a] − and + indicate 0 and 4 μg FeEDTA-Fe g^{-1} treatments, respectively.

The relative advantage of acid soils for increasing seed [Fe] compared to neutral or alkaline soils would possibly be reduced if dinitrogen fixation, with associated rhizosphere acidification (Marschner, 1995), was an important N source. Considerable variation in dinitrogen fixation by common bean has been reported (Graham, 1981).

Liming the Eckman soil did not cause a reduction in seed [Fe]. This was unexpected but may have resulted from pH and available Fe heterogeneity within the lime-soil matrix. Addition of lime to acid soils with low cation exchange capacities may increase the likelihood of a reduction in seed [Fe].

There is currently a great deal of interest in increasing the nutritional content, including [Fe], of food grains (Beebe et al., 2000; Frossard et al., 2000; Graham et al., 1999). Although soil type and varying soil Fe availability influenced [Fe] of bean seed, genetic differences in seed [Fe] in *P. vulgaris* were expressed over a wide range of environments in the current study. For instance, Voyager was a high seed-Fe accumulator on acid and calcareous soils in North Dakota, on a soil with Andic properties in Mexico, and in the presence and absence of added Fe chelates at both locations.

Voyager, the highest seed-Fe accumulator, had the lowest seed-coat [Fe] and the highest embryo [Fe] in Experiment 2. This lead to the hypothesis that ease of movement of Fe from the seed coat to the embryo during seed development is a characteristic of high seed-Fe accumulators in *P. vulgaris*. This hypothesis was rejected, since in the Mexican study Bayo 400 was a high seed-Fe accumulator but had over 20% of its seed Fe in the seed coat. This relatively high percentage was in marked contrast to the accumulation of only approximately 5% of the seed Fe in the seed coat of Voyager.

The seed coat contained from 5 to 11% of the total seed-Fe content in Voyager, as compared to 17 to 20% for T39, and 35 to 40% for UI911. Voyager has a white seed coat while T39 and UI911 have black seed coats. 'White' beans have very low tannin contents in contrast to higher, but variable, tannin contents in 'colored' beans (Beebe et al., 2000). Tannins can complex Fe. The accumulation of Fe in the seed coats of T39, UI911, and the Mexican lines, all of which had colored seed coats, may have been related to the presence of tannins. Lombardi-Boccia et al. (1995) postulated that higher Fe dialysability in 'white' bean seed as compared to 'mottled' bean seed was due to Fe-tannin interactions in the latter genotype.

Iron in both the seed coats of common bean (Lombardi-Boccia, 1995) and soybean (*Glycine max* L.) (Laszlo, 1988) has higher bioavailability than Fe in the embryo. Therefore, use of only seed [Fe] to compare the value of different common bean genotypes for improved Fe nutrition may not be appropriate; any variability in within-seed Fe distribution also needs to be known. Common beans are consumed after cooking either whole, strained with resultant seed-coat removal or fried in Latin America (Bressani and Elias, 1974). Seed-coat removal from genotypes with high seed-coat [Fe], such as UI911, would proportionately reduce their value as food-Fe sources more than removal from low seed-coat [Fe] genotypes, such as Voyager. Nutrition studies comparing the Fe bioavailability of common bean varieties with inherent differences in within-seed Fe distribution are needed.

Variability in [Fe] between the seed coat and embryo could influence availability of Fe for seedling growth. Iron in the embryo is directly available for plant growth, while Fe in the seed coat is isolated from the newly emerging plant and should be less available for early growth. Thus, Voyager, with up to 95% of its seed Fe in the embryo, may sometimes have an early-growth advantage when grown on calcareous soils. Common bean genotypes differ greatly in susceptibility to Fe chlorosis due apparently to differences in root-Fe reduction (Ellsworth et al., 1997). Whether bean genotypes resistant and susceptible to Fe chlorosis differ in within-seed distribution of Fe between the seed coat and embryo is not known. In fact, little is known about the apoplastic movement of Fe between the seed coat and embryo during seed development.

Seed [Mn] was affected by genotype and reduced by liming and by application of FeEDDHA, especially to the calcareous Wheatville soil. Little can be said on the causes for the liming and FeEDDHA effects in the absence of whole-plant [Mn] data. However, decreases in solubility of soil Mn and resultant decreased Mn uptake is a common occurrence with increasing soil pH (Marschner, 1988). Consequently, the relatively low seed [Mn] with the calcareous Wheatville soil, and the reduction in seed [Mn] with liming the acid Eckman soil, are likely due to such effects. Common bean is a Strategy-I plant that is able to reduce Fe^{3+} in the root environment under conditions of low soil Fe availability (Ellsworth et al., 1997). Soil Mn is also reduced under such conditions (Marschner, 1988), but the increased solubility is suppressed by FeEDDHA application (Moraghan 1979). FeEDDHA possibly influenced root characteristics and reduced seed [Mn] by decreasing Mn solubility in the rhizosphere.

Over 80% of the total seed Ca was located in the seed coat of Voyager, T39 and UI911, despite only approximately 10% of the dry matter being in this fraction. The localization of Ca in the seed coat of common bean is in agreement with other reports (Lombardi-Boccia et al., 1998; Singh et al., 1968; Snyder, 1936), but is in marked contrast to the situation with soybean where less than 25% of the total seed Ca is located in the seed coat (Laszlo, 1990). The hypodermal sclereid layer of the seed coat of common bean is rich in Ca oxalate crystals (Barnabas and Arnott, 1990).

Voyager, the genotype with the smallest percentage of seed Fe in the seed coat, had higher percentages of dry matter and seed Mn, Zn and P in the seed coat than T39 and UI911. The percentages of seed P in the seed coat of the three genotypes, from 1.9 to 3.6%, were much lower than the percentages of dry matter, from 8.3 to 10%. The seed coat of common bean contains less than 0.1% of the seed phytate, the chief form of P in seeds (Lombardi-Boccia et al., 1995). Within-seed distribution of Mg in Voyager, T39 and UI911 was relatively similar, and little affected by FeEDDHA and soil type.

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