Improving Human Nutrition Through Agriculture: The Role of International Agricultural Research

October 5-7, 1999

Research on Trace Minerals in Common Bean

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A Workshop hosted by the International Rice Research Institute, Los Banos, Philippines organized by the International Food Policy Research Institute

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Introduction

Food legumes in general contain appreciable quantities of iron and other minerals. Although legumes are often cited as a complement to cereals in terms of amino acid content, they also make a particularly important contribution to micronutrient nutrition. Decreasing legume per capita consumption in India is considered to be one possible cause of increasing iron deficiency, illustrating the importance of legumes in the diet. The common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption, being especially important in Eastern Africa and Latin America. CIAT has participated in the DANIDA funded project on micronutrients in an attempt to assess the feasibility of improving common beans for micronutrient content, especially iron and zinc.

Genetic variability in mineral content

The first essential question regarding whether bean can be improved for micronutrient status is to determine the degree of genetic variability of the species for mineral content. For this purpose we evaluated the common bean core collection for mineral content using ICP technology at Adelaide University. A core collection is a systematic sample of the germplasm of a species, taken in such a fashion as to represent the broadest possible genetic diversity in a limited and manageable number of accessions. In the evaluation of more than a thousand accessions in the cultivated core collection a range of 34 to 89 ppm was found, with an average of 55 ppm (Table 1). A clear relationship of iron content and geographical distribution was not evident, although accessions from the Andean gene pool tended to present higher values than those from the Mesoamerican pool. Wild and cultivated bean presented similar standard deviations, and wild bean presented only a narrow advantage in iron content. Initially we thought that use of the wild bean was not warranted in the breeding program, but recent developments in breeding methodology for the use of wild germplasm have induced us to include a wild accession in the populations under study. Given that some of the accessions with high iron content originated in Peru, we subsequently evaluated additional germplasm from this region, finding accessions that averaged as much as 100 ppm over sites and seasons (Table 2).

Zinc content of beans is one of the highest among vegetable sources, and is nearly equal to dairy products but is far inferior to meats. Evaluation of the bean core collection revealed a range of 21 to 54 ppm in Zn content, with an average value of 35 ppm (Table 1). Germplasm from Guatemala presented especially high values of zinc, but it has not been possible to find germplasm that exceeds the levels found in the initial evaluation.

These initial data suggested that sufficient genetic variability exists to improve iron content by about 80%, and zinc by about 50%.

Table 1: Mineral content (ppm) of wild and cultivated common bean.

	Wild (n=119)			Cultivated (n=1031)		
	Ave	SD	High	Ave	SD	High
B	18	5.9	58	10	1.8	18
Ca	3207	1327	6450	1466	412	3152
Cu	6	2.0	12	9	1.8	14
Fe	60	10.2	96	55	8.3	89
K	16,271	1629	20055	14782	2481	21255
Mg	2151	231	2705	1874	207	2510
Mn	23	9.0	74	15	4.4	29
Na	16	7.1	38	12	4.0	50
P	6044	705	7782	3684	696	7095
S	2354	314	3073	2120	259	3078
Zn	29	4.5	43	35	5.0	54

Table 2: Accessions of bean with the highest iron content (ppm) over sites and seasons.

	DARIEN		POPAYAN		
Accession Nº	**Season 1**	**Season 2**	**Season 1**	**Season 2**	**Average**
G23834E	106	104	111	87	102
G23818B	106	97	106	71	95
G23823E	103	88	101	77	92

An essential question for the improvement of any trait is to what degree is the trait stable across environments. In the case of seed mineral content, one might well expect an effect of varying soil type and soil chemistry over sites. Trials were planted to assess the stability of mineral content over tow growing environments, and two seasons in one of these sites. For both iron and zinc, the varietal mean square was highly significant, although location and location x variety effects were also significant. Visual inspection of data confirmed that several accessions were superior independent of site and season, as demonstrated in Table 2. This indicates that the superior mineral content selected at one experimental site should not be lost when the materials are planted at other sites, although degree of expression of the trait will vary.

Table 3: Analysis of variance for iron content of 29 bean varieties grown in three environments.

Source	Df	Mean square	F value	Probability
Location	2	1514.89	66.83	.0001
Rep (Loc)	6	103.92	4.58	.0002
Variety	28	755.60	33.33	.0001
Location * Variety	55	45.08	1.94	.0005
Error	167	22.67		

Table 4: Analysis of variance for zinc content of 29 bean varieties grown in three environments.

Source	Df	Mean square	F value	Probability
Location	2	951.98	86.86	.0001
Rep (Loc)	6	25.66	2.34	.0338
Variety	28	215.87	19.70	.0001
Location * Variety	55	19.59	1.79	.0026
Error	167	10.96		

One criticism of the breeding approach to resolve micronutrient deficiencies is that it addresses deficiencies one element at a time. In fact, this might not be the case. In the core evaluation we noted that positive correlations existed among several important elements. For our purposes, suffice it to say that iron and zinc presented a correlation of 0.52*** across different genotypes. Even more convincing are the results of mineral evaluation of Recombinant Inbred Lines (RIL) developed for genetic studies. Two sets of data are presented in Tables 5 and 6, in the first case, for RIL involving Andean parents, and in the second case, involving Middle American parents. Although these two crosses involve accessions of wide genetic diversity from contrasting gene pools, results are very similar in most cases. Again, highly significant positive correlations are found among several elements, including iron, zinc, sulfur, manganese and phosphorus. The implication of these correlations is that some genetic factors for different minerals are co-segregating, and that selection for one element (for example, iron) will in fact result in an increase in other elements (such as zinc).

Table 5. Correlation among mineral contents in a cross of two accessions of common bean (G21657 x G 21078) of Andean origin.

	Mn	Zn	Ca	Mg	K	P	S
Fe	0.451**	0.663**	0.141^{NS}	0.113^{NS}	0.029^{NS}	0.598**	0.641**
Mn		0.376**	0.211*	0.268**	0.118^{NS}	0.356**	0.233*
Zn			0.253**	0.144^{NS}	0.120^{NS}	0.772**	0.757**
Ca				0.247**	-0.257**	0.211*	0.079^{NS}
Mg					0.543**	0.181^{NS}	0.077^{NS}
K						0.032^{NS}	0.068^{NS}
P							0.724**
S							

Table 6. Correlation among mineral contents in a cross of two accessions of common bean (G 11350 x G 11360) of Mesoamerican origin.

	Mn	Zn	Ca	Mg	K	P	S
Fe	0.375**	0.571**	-0.0197^{NS}	0.336**	0.381**	0.469**	0.602**
Mn		0.527**	0.272*	0.268*	0.521**	0.429**	0.442**
Zn			0.027^{NS}	0.190^{NS}	0.491**	0.756**	0.797**
Ca				0.392**	-0.036^{NS}	0.098^{NS}	-0.017^{NS}
Mg					0.368**	0.221*	0.191^{NS}
K						0.401**	0.464**
P							0.723**
S							

Variability in tannin content

Tannins are widely cited as an important antinutrient that precipitates iron in food preparation or in the gut. One possible avenue for the improvement of iron nutrition is to reduce the tannin content or activity. With this purpose we evaluated the core collection for tannin content. This variability was considered within the context of grain colors since tannins are closely related to the seed coat pigments, and the possibilities for altering the tannin content must be evaluated within a color class. For example, white beans have very low tannins but if this trait is related to lack of pigment, it might not be possible to transfer this trait from white beans into other colors. It was necessary to establish that the low tannin trait can be obtained in other colors of beans as well. The following table indicates the range of tannin content found within each color class. There is apparently ample variability even within color classes to be able to alter the total content of tannin in the seed coat. The darker colored beans (red and black seeded) in fact had more tannins but there was more variation within color classes than between classes. In some degree this was related to gene pool as well, especially in black beans, in which Mesoamerican black beans presented high tannin and Andean black beans presented very low values.

It can be noted in passing that in one trial to measure bioavailabilty, two white beans did in fact present the highest values of per cent bioavailable iron: over 70% versus about 50% or less for other accessions. This suggests that lower tannin could in fact be beneficial, although the analysis of all accessions in the trial did not reveal an effect of tannin, possibly due to inadequate variability for tannin content among the other accessions.

Table 7: Variability in tannin content within color classes of common bean

	g Tannin / g Seed coat	
Seed Color	**Mean**	**Range**
Cream	0.116	0.036-0.168
Yellow	0.116	0.060-0.184
Pink	0.120	0.072-0.164
Red	0.128	0.048-0.196
Purple	0.120	0.048-0.196
Black	0.128	0.060-0.188

One concern with the possibility of lowering tannin content was related to possible collateral effects of this, since tannins may have other functions such as serving as resistance factors or flavor components. We have compared data on tannin content with disease reaction data on the core collection, using simple linear correlations. While the relationship is not consistent, it was found that positive correlations are more common than negative correlations. That is to say, high tannin in the seed coat is often associated with more disease, not less disease. This effect was actually quite strong in the case of Common Bacterial Blight (caused by *Xanthomonas campestris* pathovar phaseoli), and was also observed with reaction to *Rhizoctonia solani*. The most consistent negative correlation (ie, more tannin associated with fewer symptoms) was observed in relation to an insect pest, *Empoasca kraemerii*. Thus, although the relationship between seed coat tannin and pest resistance is not consistent, there appear to be few negative effects on plant resistance associated with reduced tannin.

Variability in sulfur containing amino acids

We considered the possibility of genetically increasing sulfur containing amino acids (SAA) as uptake promoters of iron, looking at the genetic variability of sulfur content in the core, under the assumption that this reflected SAA. Subsequently a subset of the core was analyzed for amino acid content by HPLC, and a Near InfraRed Spectrophotometer in CIAT was calibrated to detect methionine and cystine. Results suggested that some accessions presented levels of SAA about 25% above the mean of the core. Furthermore, the genetic component of SAA content was expressed over localities and seasons (Table 8). However, we have not pursued this. Although legumes are limited in SAA, they have abundant lysine, which has also been reported to improve uptake. The question remains whether lysine can supplant the SAA in the uptake promoter role.

Table 8: Analysis of variance of methionine + cystine content of 29 varieties of bean grown in three environments.

Source	Df	Mean square	F value	Probability
Locality	2	.01695	38.37	.0001
Rep (Loc)	6	.00136	3.07	.0070
Variety	28	.01222	27.66	.0001
Variety * Locality	55	.00071	1.61	.0117
Error	167	.00044		

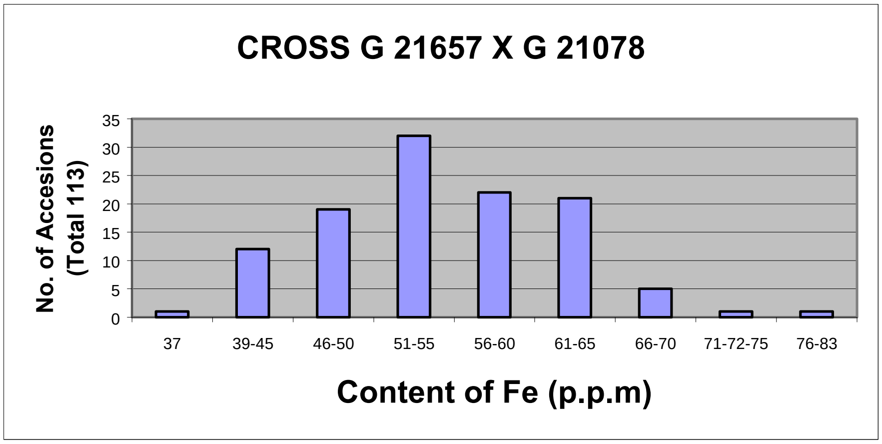
Genetics of mineral content

Four populations had been prepared as RIL for the development of molecular markers, three of which have been analyzed. Two of these populations were derived from crosses of Mesoamerican parents, and one population from a cross of Andean parents. Thus the two major gene pools of common bean were represented. The recombinant lines reveal aspects of the genetics of iron and zinc content in the parental materials. Results of all three populations are similar, and only the results of one population are presented graphically (Figure 1).

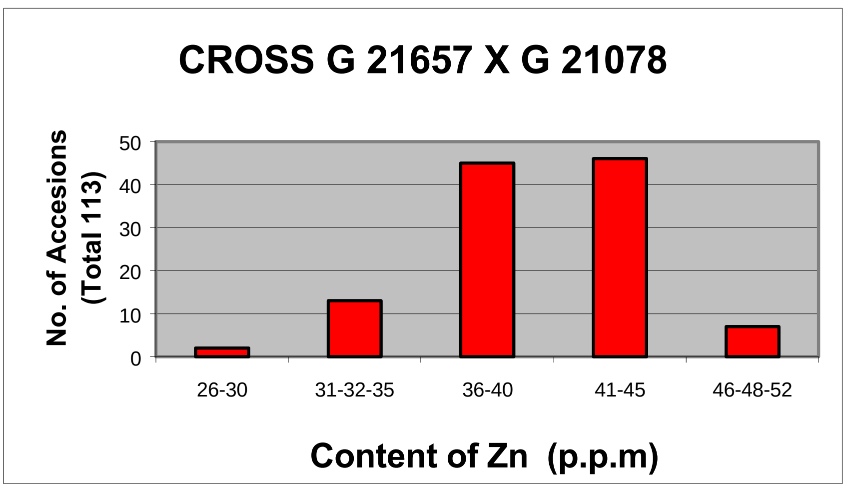
In all three populations both iron and zinc content in the RILs presented a continuous distribution, which is to say, mineral content behaves as a quantitative trait. The parental accessions were in each case very close to the extremes of the populations, and little evidence of transgressive segregation was observed. This suggests that almost all of the favorable alleles came from the high iron parent. The only exception to this rule was the population of G11350 x G11360, a Mesoamerican cross, in which several progeny either inferior or superior to the parents were observed in iron content. The number of segregating lines that presented iron contents similar to the high parent suggest that the number of genes involved could be in the range of 4 to 7.

Figure 3. Distribution of iron (A) and zinc (B) content in Recombinant Inbred Lines of the cross (G21657 x G21078)

A)



B)



QTL analysis of iron and zinc content

Although molecular analysis of the core collection was part of a separate project, the existence of data on mineral content of the core accessions permitted establishing relationships between specific DNA bands and mineral content. Of the two major gene pools, the Andean pool is quite uniform genetically. However, introgression has occurred from Mesoamerican beans that have been introduced into the Andean zone. Given the relatively uniform background of the Andean beans, this introgression can be traced and quantified using DNA markers. Subsequently we could use standard QTL analysis to relate introgressed fragments of Mesoamerican DNA to changes in the mineral contents in the Andean beans.

Eight primers were used to generate 150 RAPD bands on about 600 accessions of Andean germplasm. Of these accessions about 10% displayed evidence of introgression from Mesoamerican beans. A simple t-test was used to compare the mineral contents in accessions that presented a specific Mesoamerican band versus those accessions that lacked the band. Twenty-five bands so studied presented a significant effect on iron content, and forty-eight presented effects on zinc content. Of the 150 bands generated, 47 could be mapped to the CIAT mapping population. Seven regions were identified for iron, in chromosomes 3, 4, 8, 9, 10 and 11. Eleven regions were identified for zinc content, these appearing in all chromosomes except 5 and 7. Bands in four regions expressed an effect for both iron and zinc. Thus, loci for mineral content have already been placed on a reliable genetic map that includes RFLP as well, thus permitting extrapolation to other maps of common bean.

This preliminary mapping exercise confirms observations on the segregation patterns of the RILs to the effect that mineral content is quantitatively inherited. In the case of iron the number of loci identified by QTL analysis corresponded rather well to the estimate based on the segregation pattern, while in the case of zinc, the QTL analysis revealed more loci than would have been expected. The loci that were found to be in common could be the basis of the positive correlation that was found for iron and zinc content in the three populations.

It must be stressed that this is a very preliminary estimate of where QTL are to be found and what their effects might be. Furthermore, all the markers identified are RAPD and therefore are not of adequate quality to use in Marker Assisted Selection. The search for reliable markers represents another phase in the task of increasing mineral content of the grain.

Conclusions

Studies to date suggest that iron content of common bean could be increased by 60-80%, while potential gains in zinc content would be more modest, perhaps around 50%. Genetic differences have been expressed over environments and seasons, offering prospects that genotypes selected in one environment for high iron or zinc will express superior levels of minerals in other environments as well. The genetics of iron and zinc content appears to be complex, with from seven to eleven loci being involved. However, allelic variation remains to be explored, and it might yet be possibly to identify alleles at specific loci with relatively major effects. Correlations among mineral contents suggest that the improvement of one mineral may simultaneously improve content of other minerals, thus multiplying the impact of the effort. The fact that white beans presented higher bioavailability of iron suggests that lower tannin content could be beneficial, but the role of tannin is still not well elucidated.