AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
Batch 54

1. SUBJECT CLASSIFICATION	A. PRIMARY: Serials	Y-AF30-1410-0000
	B. SECONDARY: Food production and nutrition--Plant breeding--Leguminous grains and vegetables--Beans	

2. TITLE AND SUBTITLE
Improving yield and quality of bean seed protein and the development of superior populations for cooperative utilization in breeding programs; progress report,1976

3. AUTHOR(S)
(100) Bliss,F.A. (101) Wis. Univ. Dept.of Horticulture

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1977	49p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Wis.

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*
(Research summary)

9. Abstract
Project: The three objectives of this project are to identify strains of the common bean, Phaseolus vulgaris, which consistently produce higher levels of seed protein, to combine high-yielding cultivars with protein-modified strains having enhanced nutritive value, and to develop cooperative arrangements with other improvement programs to evaluate selected materials and provide maximum utilization of promising selections.
Dates: February 5, 1975-December 31, 1977
Development: This report discusses the research done in Wisconsin, Colombia, and Puerto Rico, on developing and evaluating high protein genotypes with wide-adaptability and multiple trait superiority. The results of the studies are presented and illustrated with many tables. Since food legumes are the sole protein source for nearly 50% of the population in many LDC's it is desirable to develop high-yielding, protein rich legume cultivars which complement cereals nutritionally and are economically competitive. Large seeded food legumes are able to biologically fix nitrogen and accumulate large amounts of seed protein. There is great variability among bean genotypes to accumulate protein in the seed. There is now a sound basis for developing strains with improved methionine content, and research is well underway in determining the effects of tannin. These studies are relevant for improving protein content in high yielding bean populations and for providing a model that is applicable to improving other traits in bean populations and other self-pollinated crops.

10. CONTROL NUMBER PN-AAD-380	11. PRICE OF DOCUMENT
12. DESCRIPTORS Beans	13. PROJECT NUMBER
	14. CONTRACT NUMBER AID/ta-C-1173 Res.
	15. TYPE OF DOCUMENT

AID 590-1 (4-74)

PN-AAD-380

Annual Report

on

Improving Yield and Quality of Bean Seed Protein and the Development of Superior Populations for Cooperative Utilization in Breeding Programs.

January 1, 1976 thru December 31, 1976

NOT FOR PUBLICATION

Prepared by:

Fredrick A. Bliss, Project Leader
Department of Horticulture
College of Agricultural and Life Sciences
University of Wisconsin
Madison, WI 53706

Contract No. AID /ta-C-1173 PDS

Submitted to:

Agency for International Development
Department of State
Washington, D.C.

REPORT SUMMARY

A. 1. Project Title and Contract No.: Improving the Yield and Quality of Bean Seed Protein and The Development of Superior Populations for Cooperative Utilization in Breeding Programs. AIDta-C-1173.
2. Principal Investigator, Fredrick A. Bliss; Contractor, Board of Regents of the Univeristy of Wisconsin; 750 Univ. Ave., Madison, WI 53706.
3. Contract Period: from Feb. 5, 1975 to Dec. 31. 1977.
4. Period Covered by Report: from Jan. 1, 1976 to Dec. 31, 1976.
5. Total AID funding of contract to Date: $13,120.15.
6. Total Expenditures and obligations through previous contract year: $8,196.25.
7. Total Expend. and Oblig. for current year: $4,923.90.
8. Total Expend. and Oblig. for next contract year: $11,594.85.

B. Narrative Summary of Accomplishments and Utilization.

Separation of bean seed protein into its constituent fractions shows that globulin, particularly G1, is the predominant fraction. However, the alkali-soluble portion (glutelin) often makes up 20-30% of the total protein. Amino acid analyses show that glutelin is more than twice as rich in methionine as is globulin. Development of new genotypes through breeding or identification of major mutants in which the G1 is suppressed and/or the glutelin fraction is enhanced presents a well-defined strategy for alleviating the poor quality of bean protein that results from methionine deficiency. Several additional mutants that affect the polypeptide subunits of the G1 protein have been identified. Their effects on protein quantity and quality are being studied. Genetic analysis of families segregating for tannin content of the testa show a complex pattern of inheritance but indicate that relatively few genes are involved. It appears that lines of nearly any color with simultaneous low tannin can be developed if this is desired.

Additional high protein genotypes have been identified in the protein uniformity trials. Several of these strains are materials received from MITA, Puerto Rico, which because of their potential disease resistance may be of even greater value.

Two cycles of recurrent selection for improved seed yield and percentage protein have produced substantial gains for both traits. The best selections from the 1st cycle performed very well at CIAT, Cali, Colombia, when compared with standard checks. Performance is expected to increase through further selection and addition of genes for pest resistance.

Exchange of plant materials with MITA and CIAT provides an effective means of evaluating promising selections for wide-adaptability as well as multiple trait superiority. A procedure for developing segregating breeding lines from good local stocks crossed to the improved composite population is proposed. It should be particularly useful for national or regional programs where personnel and facilities are limited but there is a need to improve local varieties.

A. General Background

Food legumes provide about 20% of the protein available to humans on a world-wide basis, and in many less developed countries they are the sole protein source for nearly 50% of the population. The large-seeded food legumes possess the unique combination of ability to biologically-fix nitrogen and to accumulate large amounts of seed protein. These characteristics are particularly attractive at a time of high fertilizer costs and a need for low-priced protein. Research aimed at developing high-yielding, protein-rich legume cultivars which complement cereals nutritionally and are economically competitive, must be successful to reduce the shift from production of much-needed legumes to larger acreages of cereals. Genetic improvement of protein quantity and quality has the special advantage of being integral to a traditional food plant. People requiring better sources of protein need not be induced to adopt new foods, and no new economic infrastructure for processing, fortification and distribution is required.

There is evidence that quantity and quality of bean seed protein can be modified genetically and that such changes will have dietary significance. Maximum improvement will be realized only if these traits can be incorporated into high-yielding, well-adapted bean strains that are being developed at various locations for optimum response to different environments and for different uses. We feel that the desired improvement of yield and protein can be accomplished through the development of genetically-diverse populations, within which recurrent selection and intercrossing is practiced. Promising superior selections are used for further improvement or sent to cooperative programs throughout the world for testing, selection and utilization within local programs.

B. Project Objectives as Stated in the Contract Are:

1. To identify strains of common bean, Phaseolus vulgaris, that consistently produce enhanced levels of seed protein and limiting essential amino acids, and determine the nature of heritable differences regarding these traits.
2. To construct breeding populations that combine protein-modified strains having enhanced nutritive value with well-adapted high-yielding cultivars and other accessions possessing desirable traits; and to improve these populations for seed yield and protein.
3. To develop cooperative arrangements for the exchange of plant materials with other improvement programs in such a way as to promote extensive evaluation of selected materials for important traits and to provide maximum utilization of promising selections that are superior for one or several characters.

C. Continued Relevance of Objectives.

The stated objectives are perhaps more relevant now than at the initiation of the project. As more plant materials are assessed it is evident that there is great variability among bean genotypes as to their capacity to accumulate protein in the seed. With a better knowledge of the constituent fractions, we now have a sound basis for developing strains with improved methionine content. Since legume protein quality is limited by other factors as well, additional work must be done in other areas. We are well underway in determining the effects of tannins.

The concept of forming improved populations from which cultivars can be selected for local needs is being followed by various groups involved in bean improvement. This becomes a more difficult task as we attempt to integrate many superior traits into a population and to serve more diverse needs with the end products. These present studies are relevant not only for improving protein content within high yielding bean populations, but also for providing a model that is applicable to improving other traits in bean populations and other self-pollinated crops.

D. Accomplishments to Date.

1. Findings:

The results of our studies show that globulin-1 (G1) is the predominant seed protein fraction, followed by glutelin, albumin, globulin-2 (G2), free amino acids, prolamine and a small undefined residue. This knowledge in addition to amino acid profiles of each fraction and the total seed protein suggests that substantial improvement in methionine content should result by materially reducing the low-methionine G1 fraction and commitantly raising the proportion of methionine-rich glutelin. There is some indication of genotypic variability in methionine content of each fraction, so this additional possibility should not be discounted. Our early studies of a single population suggested that improvement of total protein through selection was largely the result of selecting for more G1 protein. Since G1 is low in methionine, the results could lead to the undesirable situation of lowering the percentage of methionine while raising the total protein. Further analyses suggest that there is genetic variability for glutelin content as well as G1 and that selection for high protein based on high glutelin and reduced G1 should be explored.

The Grain Quality Analyzer total seed protein provides a rapid, accurate and economical means of estimating total seed protein. While biochemical separation of the fractions is accurate, it is slow and costly. We are now using rocket immunoelectrophoresis to quantitatively estimate the amount of G1 protein. This is particularly useful for studying genetic control of G1 synthesis, and we feel this technique together with estimation of total protein

will allow us to develop a procedure to indirectly screen for high glutelin beans.

The polypeptide subunit composition of the G1 protein of P. vulgaris has been studied using acrylamide gel electrophoresis. This is a highly-reproducible means of describing genetic differences between genera, species and strains. We have identified several additional sources of genetic variability which affect the G1 polypeptides. These polypeptides constitute the G1 protein which is of major importance, but we do not know whether these variants have substantial effects on total protein quantity or quality.

Considerable variation in seed tannin content has been found between different strains. This does not appear to be strongly related to intensity of testa color, except that white seeds are free from tannins. Genetic analyses of several segregating populations show complex inheritance patterns, but suggest that relatively few genes are involved. While in some families color and tannin level are somewhat related, it is possible to identify a range of tannin levels within each testa color group.

Protein uniformity trials included many lines from MITA, Puerto Rico. Several entries were found to have high percentage protein and may be of even greater value if they contain multiple disease resistance.

Two cycles of recurrent selection for improved yield and percentage protein have shown substantial gain for both traits. An assessment of the genetic variability present showed that 13 different sources of germplasm were still represented in the improved Composite Open Population. Additional genetic variability has been intercrossed

prior to the 3rd cycle. Performance of the best selections from cycle 1 which were grown at CIAT, Cali, Colombia was quite promising. Materials sent to MITA for evaluation included several sources of multiple disease resistance.

We have initiated a procedure designed to cross locally-adapted strains to the improved Composite Open Population as a means of utilizing improved germplasm, for breeding programs where facilities and highly trained personnel may be limited.

2. Interpretation of data and supporting evidence.

   a. Constituent fractions of bean seed protein (studies by Yu Ma, Research Assistant)

      Bean seed protein was separated into constituent fractions based on properties of solubility (Fig. 1). Optimum conditions for time of extraction were established using the cv. 'Sanilac', and the reproducibility varified using the strains 'BBL 240', PI 207227, 302542 and 229815. Extensive analyses of 10 F_5 lines derived from the cross PI 207227 x BBL 240 and selected for both high and low percentage protein show that globulin-1 (G-1) and glutelin (alkali-soluble) fractions are of major importance (Table 1). G-1 accounted for from 34 to 51% of the total and glutelin from 20 to 29% of the total seed protein. The remaining seed protein fractions in order of percentage of total were albumin, free amino acids, G-2, prolamine and an undefined residue.

      This information provides a clear, comprehensive picture of the fractional composition of the seed protein. Prior studies suggested that globulins, were of major importance, but they did not account for nearly all the protein.

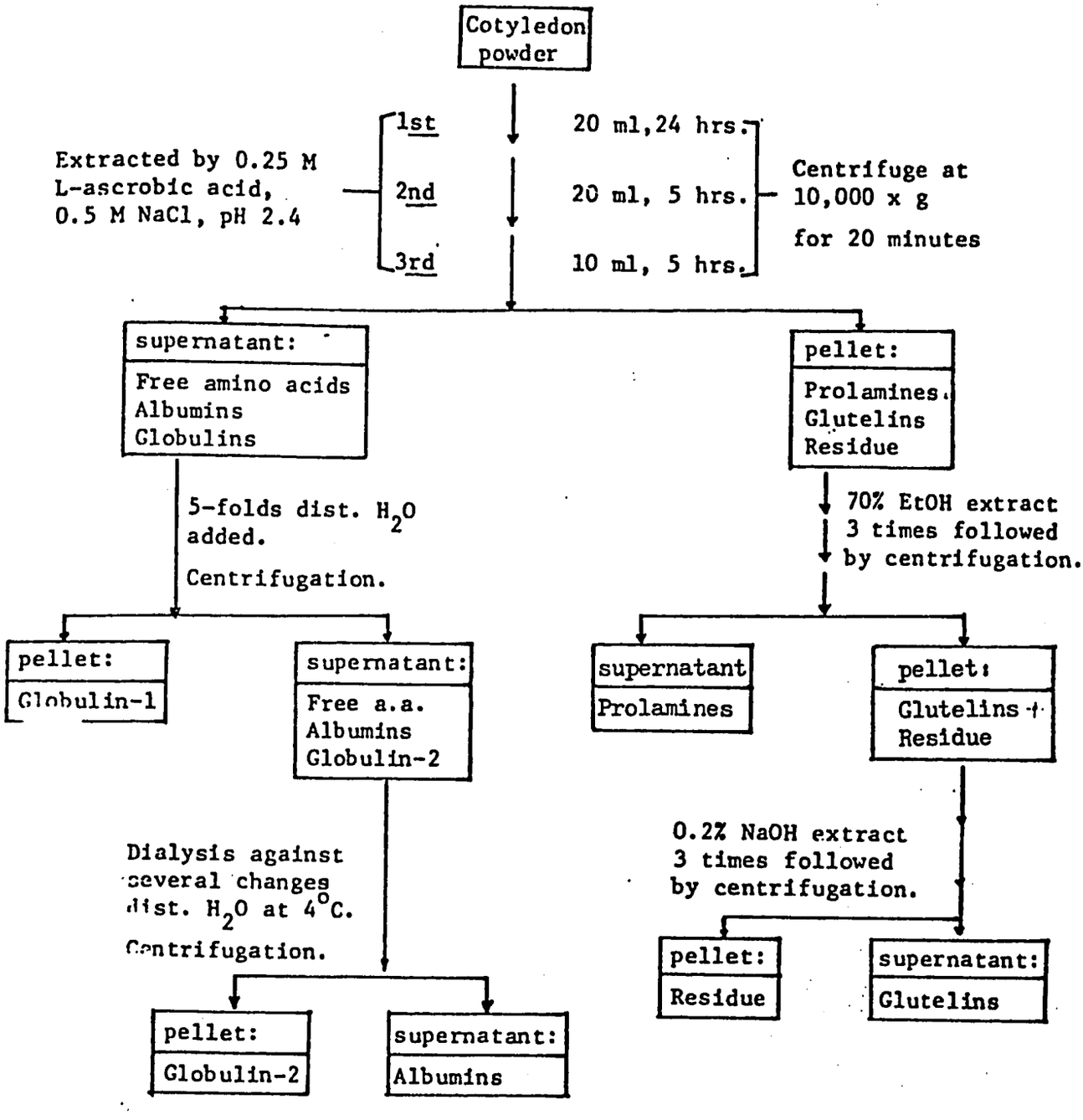


Figure 1. Outline of sequential fractionation of bean cotyledon proteins.

Table 1. Constituents of seed storage protein in the cotyledones of 10 F_5 lines of bean resulting from the cross PI 207227 x BBL 240, determined using sequential fractionation.

Strains		Total Protein Cotyledon	Protein fraction							Total recovery	Seed yield (g/plant)
			Albumin	G-1	G-2	Prolamine	Glutelin	Residue	Free a.a.		
742015	(1)[z]	275.0	32.0[y]	131.9	21.2	8.3	59.6	7.5	26.7	287.2	25.1
	(2)		11.1	45.9	7.4	2.9	20.8	2.6	9.3	104.4	
742016	(1)	240.0	33.0	117.7	11.1	7.0	56.7	6.4	12.3	231.4	27.8
	(2)		14.3	40.8	4.8	3.0	24.5	2.8	5.3	96.4	
742032	(1)	322.0	37.8	166.8	19.4	7.1	64.0	6.1	24.8	326.0	37.7
	(2)		11.6	51.2	6.0	2.2	19.6	1.9	7.6	101.2	
742047	(1)	191.0	36.8	64.0	9.4	6.3	51.9	6.3	11.2	185.9	32.5
	(2)		19.8	34.4	5.1	3.4	27.9	3.4	6.0	97.3	
742065	(1)	247.0	38.2	104.2	18.2	6.4	55.1	7.0	15.4	244.5	26.7
	(2)		15.6	42.6	7.4	2.6	22.5	2.9	6.3	99.0	
742066	(1)	189.0	33.5	65.0	8.9	7.0	55.9	6.9	12.0	189.2	28.8
	(2)		17.7	34.4	4.7	3.7	29.5	3.6	6.3	100.1	
742069	(1)	214.0	32.5	84.7	16.1	7.4	52.3	8.0	11.2	212.2	28.0
	(2)		15.3	39.9	7.6	3.5	24.6	3.8	5.3	99.2	
742071	(1)	262.0	32.5	112.0	15.4	6.9	58.0	5.8	16.6	247.2	27.2
	(2)		13.1	45.3	6.2	2.8	23.5	2.3	6.7	94.4	
742077	(1)	295.0	39.7	115.3	16.6	8.5	70.2	5.5	24.0	279.8	20.4
	(2)		14.2	41.2	5.9	3.4	28.4	2.0	8.6	94.8	
742090	(1)	199.0	28.1	76.6	8.3	5.7	55.5	5.5	11.3	191.0	20.4
	(2)		14.7	40.1	4.3	3.0	29.1	2.9	5.9	96.0	

[z] (1) mg/g cotyledon flour, (2) percentage of each protein fraction of the total protein.

[y] Results are mean values of duplicate determinations.

An amino acid profile of the cotyledon flour and each constituent fraction was determined for the 10 F_5 lines using the amino acid auto analyzer (Table 2). For several amino acids there are major differences between fractions, as would be expected. The composition of the cotyledon flour reflects the amino acid profile and the relative amount of each fraction. Major differences exist with regard to methionine, the primary limiting amino acid in most legumes. The glutelin and prolamine fractions are much richer in methionine (1.99 mg met - and 1.55 mg met/100 mg protein) than the globulin and albumin fractions. The importance of the glutelin fraction is shown in Table 3. In each case the majority of the methionine (measured as ug met/g cotyledon flour) comes from the glutelin, since this fraction makes up 20-29% of the total and is rich in methionine.

Percentage cotyledon protein was positively correlated to G-1, G-2, glutelin and free amino acid fractions (Table 4). Thus it appears that selection for high protein is associated with increases in these fractions. As our earlier analyses suggested, the largest increase appears to be in the G-1 fraction. Since this is a methionine-poor fraction, a negative correlation between percentage seed protein and methionine as percentage of protein might be expected and indeed has often been observed.

Based on these data the greatest improvement in methionine content would result by developing genotypes in which the G-1 fraction is reduced along with a concommitant increase in the glutelin fraction. A procedure based on selection of high

Table 2. Average values of amino acids in cotyledon and different protein fractions of the 10 selected F_5 strains expressed as mg/ 100 mg of protein.

AMINO ACIDS	Cotyledon	Albumin	Globulin-1	Globulin-2	Prolamine	Glutelin	Residue
Aspartic Acid*	13.59	14.21	13.07	13.84	9.51	11.23	10.21
Threonine	4.42	7.44	3.02	6.81	3.92	4.61	4.41
Serine	4.61	5.53	4.73	5.78	4.75	4.30	4.31
Glutamic Acid*	17.42	12.79	18.64	9.06	12.39	14.52	12.38
Proline	4.26	5.02	2.65	3.32	9.09	4.03	3.84
Glycine	3.79	4.67	3.21	4.02	5.19	4.35	5.34
Alanine	3.86	4.91	2.96	4.49	4.72	4.88	5.45
Cystine	.12	.23	.18	.17	.09	--	.08
Valine	5.95	4.90	6.03	6.16	7.87	6.57	7.09
Methionine	1.10	1.01	.88	.34	1.55	1.99	1.20
Isoleucine	5.75	4.32	6.10	5.43	5.68	6.19	6.36
Leucine	9.99	6.62	10.71	10.32	10.12	11.42	10.90
Tyrosine	3.67	3.52	3.97	5.14	2.33	2.83	2.20
Phenylalanine	4.65	4.04	6.33	9.72	7.36	5.43	9.69
Lysine	7.56	10.85	7.98	8.49	5.87	8.09	6.96
Histidine	3.20	3.45	3.35	1.28	2.43	3.50	3.12
Arginine	6.03	6.47	5.62	5.62	7.13	6.05	6.45

Data corrected to 100 percent recovery.
* Values including their amide form.

Table 3. The methionine content in the protein fractionof the 10 F_5 lines expressed as (a) mg/100 mg protein. (b) ug/ g. of cotyledon flour. (c) % of total methionine recovered.

Strain		Albumin	G-1	G-2	Prolamine	Glutelin	Residue	Total	Cotyledon
742015	a.	1.35	1.00	.21	1.15	2.12	1.26		1.21
	b.	432	1319	45	95	1264	95	3250	3352
	c.							97.0	
742016	a.	1.02	.97	.33	1.75	2.10	1.58		1.16
	b.	337	1136	37	123	1191	101	2925	2900
	c.							100.9	
742032	a.	1.28	.79	.37	1.01	2.16	.89		1.13
	b.	484	1318	72	72	1382	54	3382	3629
	c.							92.9	
742047	a.	.65	.90	.39	1.80	2.22	1.47		1.23
	b.	239	576	37	113	1152	93	2210	2349
	c.							94.1	
742065	a.	.99	.84	.16	1.48	2.09	1.56		.96
	b.	378	875	29	95	1152	109	2638	2410
	c.							109.4	
742066	a.	.85	.91	.35	1.92	2.09	.90		1.24
	b.	285	592	31	134	1168	62	2272	2480
	c.							91.6	
742069	a.	.75	.86	.62	1.25	1.44	1.22		1.10
	b.	244	728	100	93	753	98	2016	2211
	c.							91.2	
742071	a.	1.14	.86	.30	1.92	1.87	.97		1.01
	b.	371	963	46	132	1085	56	2653	2646
	c.							100.3	
742077	a.	1.35	.75	.33	1.40	1.74	1.47		1.03
	b.	536	865	55	119	1221	81	2877	3039
	c.							94.7	
742090	a.	.74	.87	.38	1.82	2.08	.94		1.12
	b.	208	666	32	104	1154	52	2216	2229
	c.							99.4	
$\bar{X}$	a.	1.01	.87	.34	1.55	1.99	1.22		1.12

Table 4. Phenotypic correlations among proteins in the 10 F_5 selections from the crosses between PI 207227 and BBL 240.

Trait	Total cotyledon protein	Albumin	G-1	G-2	Prolamine	Glutelin	Residue
Albumin	.485						
G-1	.941**	.312					
G-2	.803**	.381	.766**				
Prolamine	.586	.324	.457	.617			
Glutelin	.813**	.480	.649*	.464	.653*		
Residue	-.210	-.113	-.100	.326	.242	-.476	
Free amino acids	.890**	.390	.809**	.800**	.711*	.803**	-.091

* significant at 5% level

**significant at 1% level

protein, high glutelin lines should improve both percentage protein and methionine. A rapid, economical method for quantitatively estimating glutelin is not currently available. We are considering an indirect estimation, which will be discussed later.

We determined whether an estimation of the total fraction (I-fraction) which is non-extractible in ascorbate-NaCl buffer (glutelin, prolamine, residue) would be satisfactory for identifying methionine-rich lines (Table 5). The same relationships as appear in Table 4, were seen but the correlations were smaller.

b. Quantitative estimation of the globulin-1 (G-1) fraction.

Since the G1 fraction is of major importance in the seed protein and there is evidence that it can be altered through selection, we are studying genetic control of G1 synthesis. Professor T. C. Hall and associates of the Department of Horticulture have developed techniques for isolating pure G1 protein. This has been injected into rabbits to produce antiserum for use in immunoelectrophoretic analyses. Rocket immunoelectrophoresis provides a rapid, accurate measure of the amount of G1 protein in a crude protein sample. Using this method, Martha Mutschler (Research Assistant), is studying the genetic control of G1 synthesis in various high and low protein strains we have identified.

c. Variation in the polypeptide subunit structure of the G-1 protein.

The G-1 polypeptide subunit composition of P. vulgaris genotypes is largely unaffected by non-genetic factors. In addition

Table 5. Phenotypic correlation regarding protein and methionine of the total seed protein and I-fractions of 15 pure lines of bean.

Traits correlated	r values
Total seed protein(mg/g flour) vs I-fraction protein(mg/g flour)	.396
Total seed protein(mg/seed) vs I-fraction protein(mg/seed)	.529*
Total seed methionine(mg/g flour) vs I-fraction methionine(mg/g flour)	.433
Total seed methionine(mg/seed) vs I-fraction methionine(mg/seed)	.625*
Protein ratio $\left(\frac{\text{I-fraction protein}}{\text{seed protein}}\right)$ vs Methionine ratio $\left(\frac{\text{I-fraction methionine}}{\text{seed methionine}}\right)$	.765**

* significant at 5 % level

** significant at 1 % level

to providing excellent identification of different genera, species and cultivars, these individual subunits combine to makeup the G-1 protein fraction. It is possible that variations in the polypeptides may affect quantitative and qualitative properties of the G-1 and have considerable effect on total seed protein and digestibility.

Earlier studies (Romero et al., 1976) showed that variation in electrophoretic mobility of the large subunit was controlled by co-dominant alleles at a single locus. Subsequent work has provided evidence for variation in other polypeptides as well (Fig. 2). The variant alleles are being backcrossed into 'Sanilac' to allow precise determination of their effects on G-1 quantity and quality.

d. Estimation of seed tannins.

The tannin content has been found to vary considerably between various bean strains (see 1st annual report of this project). Nearly all tannin is located in the testa, as was shown by removal from the remainder of the seed. Modifications in the standard estimation procedure have been made to extract water soluble - as well as alcohol soluble tannins. This provides a more accurate estimate of the total tannin content, although few changes in relative values between strains resulted from using this improved procedure.

Genetic analyses of segregating F_2 populations resulting from crosses between parents having different tannin levels have shown the inheritance is complex (Yu Ma, Research Assistant). It appears that relatively few genes are involved and

Fig. 2. Polypeptide subunits of the G1 protein from several strains of Phaseolus vulgaris separated on discontinuous SDS slab gels. Patterns are shown for: (a) BBL 240; (b) PI 229815; (c) F_1 (PI 229815 x BBL 240); (d) BBL 240; (e) PI 229815; (f) 'Contender'. Protein was at a conc. of 1 μg/μl .5 M NaCl, with 1 μl applied to each gel. (g) through (l) is same sequence of genotypes, but 2 μl applied per gel; (m) 'Sanilac'; (n) 'Zach 54A'; (o) PI 207227; (p) 'Contender'; (q) 'Metis Eclypsy'. 2 μl/gel.

that low levels of tannins can be obtained with different testa colors. Although reduced tannin levels are probably desirable from the standpoint of improved nutritional value, the relationship of tannin to disease and insect resistance must also be considered.

e. Additional high protein germplasm.

The acquisition of the Grain Quality Analyzer (GQA) has greatly simplified estimation of percentage seed protein. Comparison of this procedure to estimation of protein using the standard Kjeldahl method shows that both provide a similar degree of accuracy (Table 6). However, the GQA is much faster and economical. Previously 2 people were required to analyze approx. 30 samples for Kjeldahl nitrogen each day. Using the GQA, 1 part-time student can easily analyze 100 samples per day.

One hundred strains were grown in the protein uniformity trial at the Hancock, Wis. Experimental Station (Table 7). Many of these (the PR. No's.) were materials received from Dr. Nader Vakili, MITA and are of additional interest since they may also possess multiple disease resistance. The high protein check (BBL 240) contained slightly lower protein than usual (27.0%), however Sanilac was as expected (25.6%). Entries which were similar to or better than BBL 240 were PR 8, PR 11, PR 21, PR 22, PR 30, PR 31, PR 33, PR 40, PR 46, PR 53, P-6-C, PI 309726, PI 311975, 15R-55, WI 742487, and PI 312041. These lines will be grown in advanced yield/protein trials in 1977, and also tested for various disease and insect resistance. This is a potentially important group of lines particularly for use in tropical areas.

Table 6. Percentage seed protein estimated using the Grain Quality Analyzer (GQA) and the Kjeldahl (K) nitrogen procedure. Seeds produced at CIAT, 1976.

Line identification	Percentage protein					
	Rep 1		Rep 2		Mean	
	GQA	K	GQA	K	GQA	K
753151	27.4	28.2	28.7	28.2	28.0	28.2
753235	25.1	24.2	24.2	23.6	24.6	23.9
753250	24.7	25.3	25.2	24.9	24.9	25.1
753382	29.9	29.1	30.1	29.8	30.0	29.5
754068	27.4	27.9	27.9	28.3	27.6	28.1
753569	29.3	31.2	29.0	30.1	29.1	30.7
753609 (BTS)	30.2	27.8	28.2	27.3	29.2	27.6
Mean	27.7	27.7	27.6	27.5	27.6	27.6
Standard dev.	2.22	2.32	2.13	2.42	2.12	2.37
Sanilac (standard sample)		24.8		24.1		24.5

Table 7. Percentage protein of entries grown in the protein uniformity trial to screen for sources of improved bean protein. Hancock, Wisc., 1976.

Identification	Percentage protein	Identification	Percentage protein
WCBT 713938	23.5	PR 30	27.4
PR 1	26.4	PR 31	30.8
PR 2	26.2	PR 32	25.6
PR 5	25.5	PR 33	29.5
PR 6	25.3	PR 34	23.8
PR 7	No seed	PR 35	25.6
PR 8	26.9	PR 36	24.8
PR 10	26.1	PR 37	26.4
PR 11	26.7	PR 38	25.1
PR 14	22.3	PR 39	22.9
PR 15	24.5	PR 40	28.1
PR 16	25.5	PR 41	26.6
PR 17	23.1	PR 42	24.5
PR 18	23.4	PR 43	24.2
PR 19	24.4	PR 44	23.7
PR 20	24.9	PR 45	24.1
PR 21	27.2	PR 46	28.8
PR 22	26.6	PR 47	24.1
PR 23	25.6	PR 48	23.1
PR 24	24.7	PR 49	25.9
PR 25	22.8	PR 50	23.8
PR 26	23.8	PR 53	27.3
PR 27	24.5	PR 54	26.0
PR 28	22.4	PR 56	26.2
PR 29	21.4	PR 58	25.4
PR 59	23.2	PI 312043	23.6
PR 60	No seed	PI 319606	No seed
PR 61	20.9	15R-55	26.9
PR 62	21.7	Pirol	18.4
PR 63	25.4	Pico	23.7
La Vega (PR 66)	24.3	Greencrop	21.0
PR 67	24.2	742487	28.9
PR 68	25.0	742672	26.4
PR 69	25.0	Sanilac	25.6
Santana	22.2	Pearlgreen	24.1
W740555	22.8	Michelite (N-K)	25.5
P-6-C	27.3	Blue Lakes (FM-1)	25.8
P-459-C	21.8	Blue Lakes (Bl. seed)	26.6
Mexico 80R	25.1	PI 319596	No seed
PI 224730	24.5	PI 319597	No seed
PI 309726	26.7	PI 319598	No seed
PI 309801	No seed	PI 319599	No seed
PI 311975	27.7	PI 319600 (1 rep)	27.3
PI 311987	No seed	PI 319601 (1 rep)	25.5
PI 311989	No seed	PI 319602	No seed
Black Turtle Soup	22.7	PI 319603 (1 rep)	22.9

Table 7. Continued

Identification	Percentage protein	Identification	Percentage protein
PI 311991	26.2	BBL 240	27.0
PI 312028	24.5	750829 (1 rep)	25.7
PI 312033	23.9	750830 (1 rep)	24.1
PI 312041	27.8	Cornell 49-242 (1 rep)	25.4
740249 (1 rep)	26.6	750791 (1 rep)	23.8

The parent genotypes used by Dr. George Freytag in population studies at MITA were grown in field trials at Hancock, WI, for estimation of yield and % protein (Table 8). 'Bonita', which we found to have high protein in earlier analyses was again promising, although under Wisconsin conditions it was somewhat low yielding. 15R-148, having red seed, was superior for % protein although also low yielding. However, both strains yielded as well as Sanilac the local check and were considerable yield potential, with Jamapa being the best. Venzuela 36 is of particular interest since it also possesses some tolerance to Golden Mosaic Virus (G. Galvez, personal communication).

Uniformity trials are necessary to allow accurate comparisons between lines, since there is usually a large environmental effect on both seed yield and percentage protein. The inclusion of standard checks allows some comparisons to be made between different trials, but observations over several years are necessary to establish the superiority of a genotype.

f. Population development and improvement.

Development and maintenance of a variable composite population which can be improved through introgression of promising alleles and selection of superior gene combinations are necessary to provide a source of families for testing and use as cultivars. Efficient management of such populations greatly facilitates their use by many breeders for a multitude of purposes in different regions.

Two cycles of recurrent selection for seed yield and percentage protein produced substantial gain for both traits (Table 9).

Table 8. Seed yield and percentage protein of bean strains received from George Freytag, M.I.T.A., Puerto Rico. Seed produced at Hancock, WI., summer, 1976.

Strain	Yield	Protein
	(gm/plot)	(%)
50600	232	20.3
15R-42	239	22.5
15R-55	255	23.1
Mex 309	242	23.8
La Vega	248	22.7
Jamapa	277	21.7
Venz. 36	239	23.1
Porrillo	225	23.5
Colo. de Pais	160	22.5
Mex 27-R	247	24.3
51052	257	23.5
71-1R-113	239	22.3
15R-148	160	27.5
French's Hort.	185	18.5
Bonita	130	25.7
Sanilac (ck)	167	22.9
lsd .05	57.5	1.7
lsd .01	76.9	2.2

Entries grown as 9-plant hill plots, each replicated 4 times.

Table 9. Performance of Superior S_1 Families Selected During Two Cycles of Recurrent Selection within the Composite Open Population. Hancock, Wisconsin, 1975 and 1976.

Family	Mean[z] seed yield (gm/plant)	Percentage protein: Family $\bar{x}^z$	Family range	Select. individuals $\bar{x}^y$	Select. individuals range
		1975			
COP-0-2	75.2	20.9	18.7-24.7	23.3	22.4-24.7
COP-0-12	73.0	22.6	19.9-25.6	24.8	24.0-25.6
COP-0-13	78.6	23.3	21.2-27.5	25.4	24.5-27.5
COP-0-20	75.7	21.1	18.2-23.3	23.0	22.5-23.3
COP-0-32	69.9	23.6	19.7-26.5	26.0	25.6-26.5
1975 Mean	74.5	22.3		24.5	
BTS	70.5	26.1			
Sanilac	13.5	23.7	Standard checks		
BBL 240	27.4	27.6			
		1976			
COP-1-8	109.4	23.2	20.3-27.1	24.9	23.3-27.1
COP-1-10	105.8	23.0	20.8-26.2	25.0	24.2-26.2
COP-1-12	111.8	23.3	21.5-26.7	25.3	24.0-26.7
COP-1-39	105.9	25.4	22.8-26.8	26.4	26.2-26.8
COP-1-29	88.3	24.0	22.3-26.5	25.6	24.6-26.5
1976 Mean	104.2	23.8		25.5	
BTS	56.4	22.5			
Sanilac	19.8	21.1	Standard checks		
BBL 240	22.8	25.8			

[z] Mean values are from 20 plants grown in 2 reps of 10 plants each, in a RCB design.

[y] Mean values are from the 4 plants selected within each family as having the highest percentage protein values.

Fifty families each containing 10-12 single plants grown in a hill plot design for segregating populations (Bliss, 1976), were replicated twice in the field. Three standard checks were included. The best 5 families were selected on the bases of superior mean yield per plant. Seed from each plant of the selected families was analyzed for percentage protein, with the best 2 plants being chosen from each rep. Seeds from these selected plants were sown in the greenhouse (fall and 50 pair-wise crosses made among the resulting plants. F_1 seeds were then planted (spring) to produce S_1 seeds for planting the next summer. Rapid protein estimation and efficient use of the greenhouse (3 generations per year) allows completion of 1 complete cycle of recurrent selection per year, a situation as efficient as working in the tropics. Thus, gain per cycle equals gain per year.

Perhaps more important than population performance in Wisconsin, is the performance of the selections in tropical areas of Latin America. Strains resulting from the 1st cycle of recurrent selection performed well in field trials at CIAT, compared both to 'Black Turtle Soup' and local checks (Table 10). Several selections found to be promising for both yield and percentage protein have been intercrossed into the population prior to beginning the 3rd cycle of selection. S_2 families were also sent to MITA for evaluation against several diseases (Table 11). Several selections made by George Freytag appear to carry resistance to several diseases and these have been intercrossed into the 3rd cycle of the Composite Open Population.

Table 10. Mean Yields and percentage protein of S_2 breeding lines derived from S_1 families selected for high yield and high percentage protein in Wisconsin. Trial conducted at CIAT, Colombia, 1976.

Identification	Seed yield (gm/plot)	% protein (GQA values)
- - - - - - Experimental S_2 families - - - - - - - - - -		
753844	150	27.2
753137	62	28.8
754110	41	28.8
753151	249	28.0
753152	117	27.5
754196	140	24.8
753235	185	24.6
753239	123	25.9
753240	114	27.2
754202	128	26.7
753245	158	26.6
753250	108	24.9
754291	98	26.2
753888	195	25.3
753980	195	26.6
753379	96	30.0
753382	199	30.0
754068	274	27.6
753426	59	28.0
753569	189	29.1
- - - - - - Check lines - - - - - - - - - - - - - - - -		
Black Turtle Soup (Wisc)	262	29.2
P459 (Jamapa-Venz) (CIAT)	5	24.9
P569 (CIAT)	110	26.2
P416 (CIAT)	79	25.9
lsd .05	97	1.4

Table 11

SINGLE PLANT SELECTIONS FROM WISCONSIN MATERIAL

Code No.	Wisconsin No.	Row No. & Planting	Disease and Agronomic Information
VW-12	74-36	761278-1 Limani	Seed-dull, white; prostrate vine; 5 branches Rust-Susc., Virus-MR, Isoriopsis-S; At harvest-root dead, Rhizoc. & Fusarium; 76 pods of 7 seed, 50.7 g yield ($0.25m^2$)
M-167	WISCOP-040	760268-1 Isabela?	Seed-dull, pinto; Semi-vine, semi-erect, 7 branches spreading; Rust-R, Virus-MS, Bact.-R; at harvest - root dead, Rhis. & Fus.; 40 pods of 7 seed, 35.2 g. yield.
W-148	WISCOP-016	760214-1 Isabela?	Seed-shiny, white; semi-vine, erect; 4 branches; Rust-R, virus - MS, Bact.-R; at harvest-root dead, Macrophomina; 22 pods of 7 seed, 10.5 g. yield
W-149	WISCOP-024	760244-1 ?	Seed-dull, white; semi-vine, semi-erect, 4 branches; Rust-MR, Virus-MS, Isoriopsis-S, Scholetotrichum-S; at harvest- root green; 30 pods of 7 seed, 21.9 g yield.
W-150	WISCOP-024	760244-2 ?	Seed-dull, white; long semi-vine, semi-erect, 5 branches; Rust-MS, Virus-MS; Isoriopsis-S, Scholetotrichum-S; at harvest - root green, Rhizoc. & Fus.; 24 pods of 8 seed, 19.8 g yield.
W-152	WISCOP-025	760235-1	Seed-shiny, white; semi-vine, erect, 6 branches; Rust-R, Virus-MR, Bact.-MR; at harvest root dead - no disease; 18 pods of 8 seed, 15.7 g yield.
W-153	WISCOP-025	760235-2	Seed-dull, white; determinate, erect; 6 branches; Rust-R, Virus-MS, Bact.-MS; at harvest, root green; 19 pods of 6 seed, 11.3 g yield.
W-154	WISCOP-025	760235-1	Seed-shiny, white; determinate, erect; 7 branches; Rust-R, virus-MS, Bact.-S; at harvest, root dead, Macrophomina and Fus.; long inflorescence; 35 pods of 7 seed, 26.2 g yield.
W-155	WISCOP-048	760220-1	Seed-dull, white; determinate, erect; 6 branches; Rust-R, virus-R, Bact.-R; at harvest, root dead, Macrophomina and Fus.; 25 pods of 6 seed, 9.5 g yield.
W-156	WISCOP-048	760220-2	Seed-dull, white; determinate, erect; 7 branches; Rust-MR, virus-MR, Bact.-R; at harvest, root dead, Macrophomina and Fusarium; 21 pods of 7 seed, 8.9 g yield.
W-157	WISCOP-048	760245-1	Seed-shiny, white; determinate, erect; 5 branches; Rust-MS, Virus-MS, Isoriopsis-S; at harvest, root dead, Macrophomina and Fus.; 39 pods of 6 seed, 22.7 g

Another important criterion for evaluating the population is the amount of genetic diversity maintained. A detailed analysis of the pedigree of each family contained in the 3rd cycle shows that 13 of the original 50 families are still represented and that they provide considerable pest resistance (Table 12). Several additional sources have been intercrossed into the 3rd cycle. It is likely that 10-13 families are the minimum stable number that will comprise the Composite Open Population. This base will be broadened with each cycle as new germplasm is introgressed. Intercrossing prior to the initial cycle of selection would have provided a broader genetic base, but it would have also delayed gain from selection for a least 1 year.

The derivation of useful lines from this population appears to be relatively easy, using Modified Pedigree Selection and Single Seed Descent. The genetic variability that is present among superior S_1 families allows great flexibility for breeders to further select and maintain heterogeneity for many different and particular objectives. A system for integrating this population approach into a breeding scheme suited to LDC's will be discussed in Section E.

g. Developing root rot resistant populations.

Recurrent selection for root rot resistance is being initiated in a black bean population, in which the objective is to select for high yield, root rot resistance, high % protein, and low seed tannin. This work is being conducted by Steve Beebe (Research Assistant) currently at CIAT, and was initiated by intercrossing some 18-20 promising black bean genotypes.

Table 12. Summary of the disease reactions of germplasm represented in the Composite Open Population, 1976 and 1977.

Germplasm	Protein[z]	R. root[y]	Rust	Web blight	Anthracnose	Common blight	BCMV	BGMV
Lines in 1976 population (COP_1)								
Rim de Porco (Brazil)	M							
State Half Runner (U.S.)	M	T						
PI 203958 (Mexico, U.S.)	H	R						
Gloria (U.S.)	M	T					R	
Mulatinho (Brazil)	M		S	S	I	S		
R. Mexican (U.S.)	L	S						
Guali (U.S.)	L		I	S	S	S	S	
Sanilac (U.S.)	M	S	S	S		S	R	
Gratiot (U.S.)	M	S	S	S		S	R	
BBL 240 (U.S.)	H	S					R	
California Small White (U.S.)	M	S	I	R		T	S	
Kerman (Australia)	M							
Pinto III (U.S.)	L	S						
Additional lines incorporated into 1977 population (COP_2)								
Cornell 49-242 (U.S.	M		S		R	S	S	
15R-55 (Puerto Rico)	M	R	R			T	R	
Black Turtle Soup (Venz., U.S.)	M	T	S				M	
Endogava ZN (Canada)	H							
WCBT 71-3938 (U.S.)	M	S	T			T		
PI 150414 (El Salvador)	M	S			T			
PI 229815 (U.S.)	H							
Chimbolo (Mexico)	M							
Mexico 80R (Mexico)	H							
Wisc 720219 (U.S.)	H							

[z] H = High; M = medium; L = low % protein.

[y] R = resistant; T = tolerant; S = susceptible; M = mixed reaction to the disease inciting pathogen.

E. Dissemination and Utilization of Research Results.

1. Information generated from this research has been disseminated largely through recognized professional journals and lecture presentations. As more data become available a research report for cooperators and interested research personnel will be compiled.

Exchange of plant material is as important to this project as is dissemination of written information. Routine exchange of promising lines is made with Puerto Rico (MITA) and Colombia (CIAT), and to some extent Brazil. Occasional distribution is made to others upon request and depending upon seed availability. Seed of promising new lines to be included in this study is received from many sources.

a. Bibliography list and a list of known usages of materials are presented in Appendix 3.

2. Development of a program for utilization of improved germplasm.

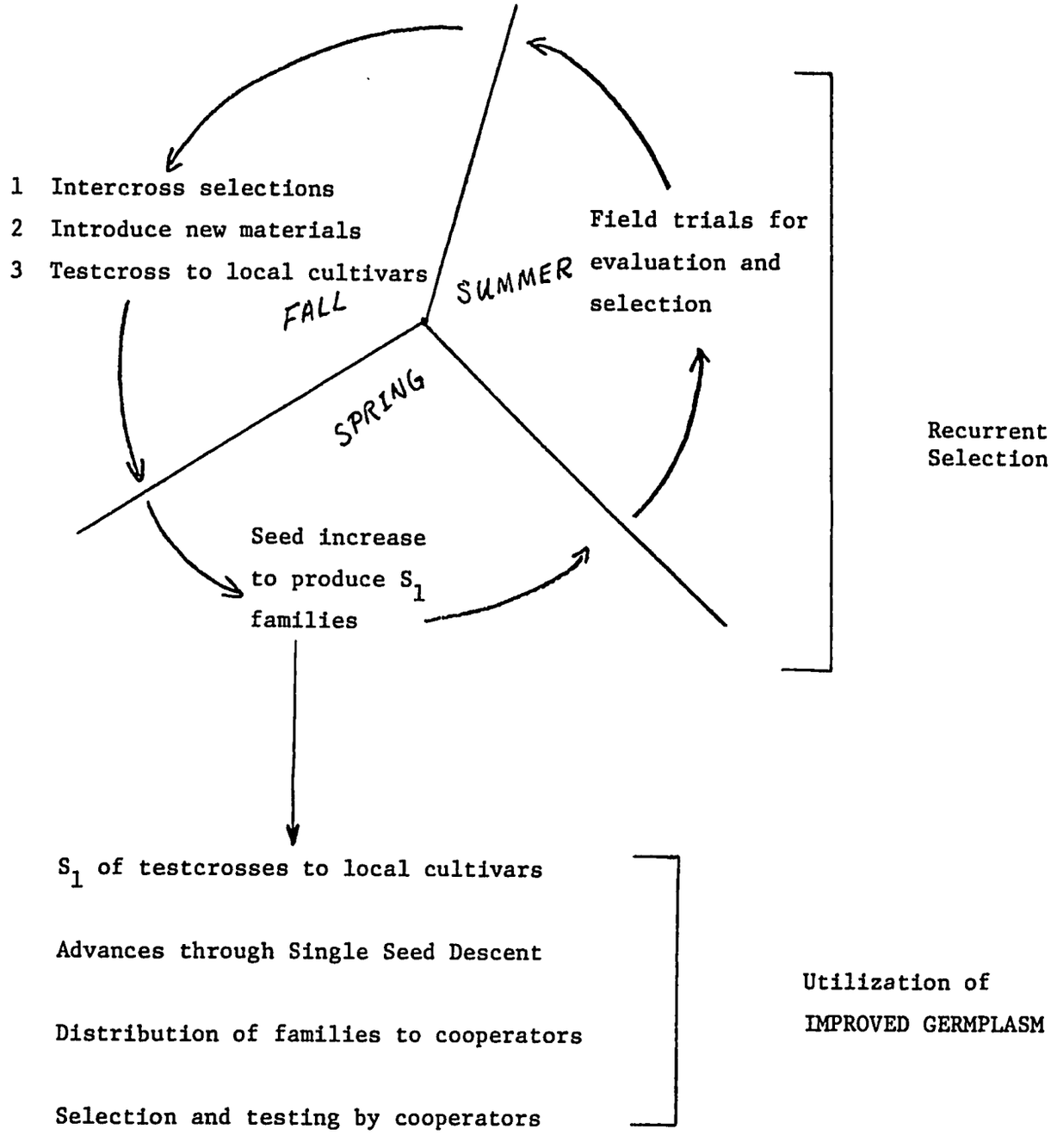
The concept of developing genetically diverse populations which are being improved through introgression of new genes followed by recurrent selection, and the utilization of improved germplasm from these central populations have received only limited attention, particularly in self-pollinated crops. The traditional approach has been to develop numerous singular breeding programs at various locations where staff and facilities are sufficient to allow program development. Little thought is given to establishing complementary programs that share ideas and plant materials, except on an informal basis. Often programs, particularly those in LDS's, are rather discontinuous because of rapid turnover in scientists or insufficient support, with the results being rather minimal.

It is clear that individual scientists in each country or region should play a major role in selecting and developing new cultivars to fit particular objectives. They are likely to be closer to individual problems faced by the growers and consumers. However, limited facilities and expertise may preclude the development of comprehensive programs in all locations that could benefit from new varietal development. Thus it seems desirable to emphasize selection and varietal development at the local level where it must ultimately be done, and to emphasize the identification of new genes, development of superior populations, hybridization to local cultivars and the fundamental support work at central locations having adequate support. If conducted in a joint, complementary manner many small programs can benefit from large, continually improving central resource populations.

An effective central population must offer a means for improvement to breeders who sample the population with high expectations of recovering favorable genes. The population must be diverse enough to contain superior genes or genotypes for resistance to pests, wide adaptability, high general combining ability and nutritional excellence. The derived materials must be superior in a wide array of situations. There must be sufficient flexibility in population improvement to allow easy introgression of new genes without disruption of the overall objectives (Fig. 3).

Three steps are basic to optimal population improvement and utilization of improved germplasm: 1) Development of the central population and introgression of new superior genes as they become

Figure 3. POPULATION IMPROVEMENT AND UTILIZATION OF IMPROVED GERMPLASM.



available, 2) improvement of the population through intercrossing and increasing the frequency of favorable alleles by recurrent selection, and 3) retrieval of superior genotypes and utilization of improved germplasm by the breeder. Since this project has been concerned largely with population improvement through recurrent selection to this point, we must now consider the utilization of germplasm.

Procedures:

I. Functions performed at the central population.

   A. Population Improvement.

   B. Test crosses between the improved, central population and the best local cultivars provided by cooperators from various countries.

      1. Utilization of year-around facilities such as mild climate, greenhouses, biotron, etc. to allow climate modification (i.e. daylength) as needed.

   C. Seed increase of S_1 and S_2 families by Single Seed Descent to produce a wide array of lines for cooperators.

      1. These families would derive ½ of their germplasm from the local cultivar and a sample of favorable genes for improvement from the improved central population.

   D. Certain tests for population and families performances e.g.,

      1. pest resistance

      2. nutritional quality.

   E. Combined analyses of families performance obtained from data sent by cooperators

II. Functions performed by cooperating breeders.

A. Receipt of segregating populations derived from crosses of local cultivars which they chose and sent to the central population.

B. Increase of seed to the level of homozygosity they desire.

C. Selection for traits they feel are important and use of breeding methods and procedures they choose.

D. Evaluation of selections under local conditions for their farmers.

E. Release of new varieties which they test and develop. They receive due credit.

F. Return data and samples of superior selections to the central population for further crossing.

The intended objective of this approach is to make maximum use of all available personnel and resources and to have maximum input at the local level.

F. Statement of Expenditures and Obligations and Contractor resources.

Line Item	AID's obligated funds	Expended to date	Estimated Budget 1/1/77-12/31/77
Travel & per diem	5,665.00	1,121.37	4,543.63
Consultants	900.00	300.00	600.00
Equipment & materials	12,500.00	10,488.07	2,011.93
Other direct costs	5,650.00	1,210.71	4,439.29
	24,715.00	13,120.15	11,594.85

G. Work Plan and Budget Forecast for Coming Year.

1. Anticipated accomplishments for the coming year.

a. Identify and describe bean genotypes that are genetically superior with regard to seed yield, percentage protein, methionine content and seed tannins.

b. Develop and use a screening technique that will allow identification of genotypes that contain high levels of glutelin and/or low levels of globulin-1 (G-1) protein.

c. Identify genes controlling synthesis of G-1 protein and quantification of their effects using rocket immunoelectrophoresis.

d. Determine the effects of seed tannins on availability and digestibility of seed proteins.

e. Select for improved yield and protein within the Composite Open Population; addition of new germplasm, completion of the 3rd cycle of recurrent selection, and utilization of superior selections.

f. Condust comparative trials using the best selections from cycles 1 and 2 to determine the efficiency of the selection procedure.

g. Initiate development of a method for Utilization of Improved Germplasm through crossing adapted local strains to the Composite Open Population and development of advanced derived lines using Single Seed Descent and Modified Pedigree Selection.

h. Distribute potentially superior selections to cooperators for testing and utilization.

i. Provide protein analyses of advanced lines sent by cooperators.

j. Conduct cooperative Biotron research with Dr. Peter Graham, Director of Bean Research, CIAT who will spend Aug. 1, 1977 to July 15, 1978, at the Univ. of Wisconsin, Madison.

2. Procedures to be used and expected activities.

a. Use of a protein uniformity field trial to identify crosses high yielding/high protein genotypes.

b. Standard genetic analyses of segregating progenies from crosses of promising genotypes x adapted strains.

c. Estimation of total seed protein using The Grain Quality Analyzer.

d. Estimation of G-1 protein using rocket immunoelectrophoresis.

e. Polyacrylamide slab gel electrophoresis for analysis of polypeptide subunits of various protein fractions.

f. A suitable combination of the above methods to indirectly estimate the glutelin fraction of promising genotypes, and biochemical separation to confirm promising results.

g. Recurrent selection for improved yield and protein content in The Composite Open Population.

h. Estimation of seed tannin levels with extraction procedures using H_2O and methyl alcohol.

i. Development of a program for Utilization of Improved Germplasm from The Composite Open Population.

3. Significant factors affecting accomplishments.

a. Use of the Grain Quality Analyzer for rapid protein analysis.

b. Ability to develop a suitable screening method to detect changes in the glutelin/G-1 protein ratio.

c. Use of rocket immunoelectrophoresis to estimate G-1 protein.

d. Continued cooperative exchange of materials with programs at MITA and CIAT.

e. Identification of research workers in LDC's who wish to begin participating in the program for Utilization of Improved Germplasm.

4. Dissemination and utilization of expected results.

a. Publication of technical articles in appropriate journals with distribution of reprints to people requesting articles.

b. Presentation of results at meetings and seminars.

i. annual meeting - Crop Science Society of America.

ii. annual meeting - American Society of Hort. Science.

iii. Biennial meeting - Bean Improvement Coop.

c. Distribution of seeds of promising selections to cooperators.

d. Provide seed protein analyses of strains found by cooperating investigators to be outstanding for other traits, i.e. yield, pest resistance, etc.

e. Make crosses that may be requested by breeders in LDC's to the Composite Open Population for initiating a program for Utilization of Improved Germplasm.

5. Planned expenditures, Jan. 1, 1977 - Dec. 31, 1977.

Line Item	Estimated expenditure
Travel & per diem	4,543.63
Consultants	600.00
Equipment & materials	2,011.93
Other direct costs	4,439.29
	11,594.85

APPENDIX I. Literature cited.

Bliss, F. A. 1976. Use of hill plots for genetic and breeding studies of bean. J. Amer. Soc. Hort. Sci. 10:77-80.

Romero, J., S. M. Sun, R. C. McLeester, F. A. Bliss and T. C. Hall. 1975. Heritable variation in a polypeptide subunit of the major storage protein of the bean Phaseolus vulgaris L. Plant Physiol. 56:776-779.

APPENDIX II.

Personnel associated with, but not funded by the project.

Department of Horticulture.

Prof. F. A. Bliss (Project Leader)
Mr. Moses Adenihum (Research Assistant - Nigeria)*
Ms. Jeanne Romero-Andreas (Research Assistant)*
Mr. Steve Beebe (NSF Fellow)*
Mr. Yu Ma (Research Assistant - Taiwan)*
Ms. Martha Mutschler (Research Assistant)*
Mr. Greg Tolla (Research Assistant)*

Cooperating Staff (Department of Horticulture)**

Professor T. C. Hall
Mr. R. C. McLeester (Specialist)
Dr. S. M. Sun (Research Associate)
Dr. J. Pyne (Research Associate)

* Graduate students funded from other sources, but whose work contributes to this research program.

** Staff members who are not directly associated with this program, but whose counsel and expertise contributes substantially.

APPENDIX III.

A. Bibliographic list of papers and publications presented in association with the project.

Bliss, F. A. 1976. Use of hill plots for genetic and breeding studies of bean. J. Amer. Soc. Hort. Sci. 10:77-80.

Bliss, F. A. and T. C.Hall. 1977. Food Legume-Compositional and Nutritional Changes Induced by Breeding. Cereal Foods World 22:106-113.

Boomstra, A. G., F. A. Bliss and S. E. Beebe. 1977. New sources of Fusarium root rot resistance in Phaseolus vulgaris L. J. Amer. Soc. Hort. Sci. 11(in press).

Boomstra, A. G. and F. A. Bliss. 1977. Inheritance of resistance to Fusarium solani f. sp. phaseoli in beans (Phaseolus vulgaris L.) and breeding strategy to transfer resistance. J. Amer. Soc. Hort. Sci. 11(in press).

Hall, T. C., F. A. Bliss, D. S. Ryan and S. M. Sun. 1977. The subunit structure and cell-free synthesis of the major storage protein from bean (Phaseolus vulgaris L.) seeds. In: Nucleic Acids and Protein Syntehsis in Plants, Roy Baker, Ed. Plenum Press, London (in press).

Hall, T. C., R. C. McLeester and F. A. Bliss. 1977. Equal expression of the maternal and paternal loci for the polypeptide subunits of the major storage protein of the bean, Phaseolus vulgaris L. Plant Physiol. (submitted).

Mohamed, Ali K. 1976. Some Cultural and Environmental Factors Affecting Snap Bean (Phaseolus vulgaris L.) Growth and Root Rot Incidence. Ph.D. Thesis, University of Wisconsin, Madison.

Invited Papers & Symposia:

F. A. Bliss. 1976. Breeding Systems in Self-Pollinated Crops. Campbell Research Institute, Camden, New Jersey, Feb. 16-17, 1976.

F. A. Bliss. 1976. Nutritional Quality Improvement of Food Legumes. Univ. of Kentucky, Graduate Improvement Seminars, March 9-10, 1976.

F. A. Bliss. 1976. Concepts for Genetic Improvement in Self-pollinated Crops and Breeding for Improved Nutritional Value. Univ. of Nebraska Research Council Seminar, March 22-23, 1976.

Hall, T. C., F. A. Bliss, D. S. Ryan and S. M. Sun. 1976. The subunit structure and cell-free synthesis of the major stroage protein from bean (Phaseolus vulgaris L.) seeds. "Nucleic Acids and Protein Synthesis in Plants", Strasbourg, July 15-24, 1976.

B. Abstracts of Publications.

1. Food Legumes—Compositional and Nut

F. A. Bliss and T. C. Hall
Dept. of Horticulture
Univ. of Wisconsin
Madison, WI 53706

The desirable combination of ability to biologically fix nitrogen and the capacity to accumulate abundant seed protein makes the food legumes attractive at a time when nitrogen fertilizer costs are high and there is increasing demand for protein. The pulses (food legumes) include several genera and are distributed worldwide. However, their importance varies greatly between localities, often depending on the per capita income of consumers. Roberts (1) has pointed out that protein from food legumes comprises nearly all the dietary protein for up to 50% of the people in many less developed countries.

The efficiency of protein production (ratio of energy input to protein output) in food legumes compares favorably with other major food crops such as potato, corn, rice and wheat. However, if the food legumes are to become even more important protein sources, improvement in the yielding ability and nutritional value of the seed protein must be realized. A comprehensive understanding of the controls which limit the types and amounts of seed protein synthesized and of constraints which must exist in the chemistry of the storage proteins is necessary if these limitations are to be alleviated or removed by biological manipulation (2).

Serious consideration will probably be given to oilseed meals as a direct source of dietary protein, and the production and use of fish protein concentrate, single cell protein and extractable leaf protein as food sources will be explored. Fortification of these sources with synthetic amino acids is another important aspect. However, genetic enhancement of productivity and protein quality in traditionally accepted crop plants deserves particular attention, since by following this approach people requiring better sources of protein need not be induced to adopt new foods and no new economic infrastructure for processing, fortification and distribution is essential (3).

Legume Seed Proteins

Seed proteins are of two types—metabolic and storage. Legume seeds contain large amounts of storage proteins, the majority of which are globulin in nature, being soluble in dilute salt solutions and insoluble in water (2). Despite the preponderance of globulins, the albumin (metabolic) fraction has also been shown to be important for increasing the nutritional value of cultivars through breeding (4,5).

The storage proteins of *Vicia* spp. and *Pisum sativum* were originally named legumin and vicilin (6). However, these terms are sometimes used indiscriminately and we have questioned the wisdom of their broad usage to indicate similarities that do not exist. We have found that the major globulin fractions from *Phaseolus vulgaris* L., which have the solubility characteristics of the original definitions of legumin and vicilin, are not identical to legumin and vicilin from *Vicia* (7). Millerd (2) suggests that experimental evidence clearly shows these terms should be reserved for genera in the Fabeae and Trifolieae.

Since the storage proteins are globulins, it is customary to use the properties of their solubility in dilute salt solution and insolubility in water to separate them from the metabolic proteins (8). After extraction with buffered sodium chloride and precipitation with ammonium sulphate, salt is removed by dialysis; the resulting precipitate is assumed to be only legumin and vicilin. The two fractions are then separated by the low solubility of legumin at its isoelectric point, pH 4.7.

However, this procedure has been shown to yield globulin fractions that either contain metabolic proteins or are a mixture of several globulins (9, 10), thus seriously limiting its use for quantitative studies. Millerd (2) concludes that if purification procedures similar to the Danielsson method are used to prepare proteins, data on subunit composition should be treated with caution.

McLeester *et al.* (7) have described a procedure for the extraction and preparation of two globulin fractions from *Phaseolus vulgaris* L. seeds with an ascorbate-NaCl medium. The two preparative fractions which we refer to as G1 and G2 globulins were shown to be completely separated from each other, with a high proportion of each fraction being extracted (11). This procedure has been modified to provide a method for quantitatively estimating the protein fractions in seeds of *P. vulgaris* genotypes differing in percentage seed protein (Yu Ma and F. A. Bliss, *unpublished data*). If genetic control of seed protein synthesis and accumulation is to be utilized for modifying protein quantity and quality through breeding, analytical procedures for obtaining pure protein species are essential.

Glycoproteins are widespread among the pulses and include substances that either directly or indirectly affect the nutritional value of the seeds. They have been found in the globulin protein fraction of beans, where they constitute the largest single portion of storage protein as in the case of glycoprotein II, isolated and described by Pusztai and Watt (12).

According to Millerd (2) the 8S protein isolated from *Phaseolus aureus* by Ericson and Chrispeels (13) corresponds to glycoprotein II. Racusen (14) found a similar fraction to constitute 35% of the total seed protein of *P. vulgaris*. Recent evidence (10) suggests that G1

[1]Presented at the AACC 60th Annual Meeting, Kansas City, MO, Oct. 26-30, 1975.

Copyright© 1977 American Association of Cereal Chemists, Inc., 3340 Pilot Knob Road, St. Paul, MN 55121. All rights reserved.

2. ABSTRACT

SOME CULTURAL AND ENVIRONMENTAL FACTORS AFFECTING SNAP BEAN (PHASEOLUS VULGARIS L.) GROWTH AND ROOT ROT INCIDENCE

Ali K. Mohamed

Under the supervision of Professor Fredrick A. Bliss

Root rot of bean occurs worldwide and the severity varies depending largely on the prevailing cultural and environmental conditions. In the irrigated central sand area of Wisconsin, snap beans are grown extensively and root rot has become progressively worse. They are sensitive to many environmental and cultural factors that predispose the plant to root rot infection. The provision of optimum conditions for plant growth sometimes enables the plants to outgrow the pathogen and produce a substantial crop yield in spite of the disease.

Preliminary greenhouse experiments to test the effects of seed size, planting depth and herbicides on root rot severity were conducted 1) in soil or nutrient culture solutions artificially inoculated with Fusarium solani (Mart.) Appel and Wr. f. sp. phaseoli (Burk.), Snyd. and Hans., and 2) in soil naturally infested with root rot inciting

3. INHERITANCE OF RESISTANCE TO *FUSARIUM SOLANI* f.sp. *PHASEOLI* IN BEANS (*PHASEOLUS VULGARIS* L.) AND BREEDING STRATEGY TO TRANSFER RESISTANCE[1]

A. G. Boomstra[2] and F. A. Bliss

Dept. of Horticulture, University of Wisconsin, Madison, WI 53706

Additional index words: nutrient culture, recurrent selection.

Abstract. Susceptibility to root rot in beans *Phaseolus vulgaris* L., incited by *Fusarium solani* f.sp. *phaseoli* was dominant in crosses between resistant PI 203958 and susceptible cultivars 'Cascade', 'Falcon', 'State Half Runner' and 'California Small White'. A quantitative inheritance pattern for disease reaction was observed. Selection of F_2 plants showing slight disease symptoms resulted in F_4 populations with a lower disease index than the unselected F_2 population, indicating substantial gain. The results suggested that breeding methods such as recurrent selection, which are more suitable for improving quantitative traits than are backcrossing or

---

[1] Received for publication September 13, 1976. Research supported by the College of Agricultural and Life Sciences. This investigation is a portion of a thesis, submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree.

[2] Present address: Institute De Haaff, Foundation for Agricultural Plant Breeding, Wageningen, The Netherlands.

[3] Burke, D. W. 1974. Naming and release of the Red Mexican bean cultivar 'Rufus'; Naming and release of two pink bean cultivars, 'Viva' and 'Roza'; USDA-ARS and Wash. Agric. Expt. Sta., Pullman; Naming and release of the pink bean cultivar, 'Gloria'. USDA-ARS and the Wash. Agric. Expt. Sta., Pullman and Calif. Agric. Expt. Sta., Davis.

4. New Sources of Fusarium Root Rot Resistance in *Phaseolus vulgaris* L.[1]

A. G. Boomstra[2], F. A. Bliss and S. E. Beebe

Dept. of Horticulture, University of Wisconsin, Madison, WI 53706

Additional index words: Beans, *Fusarium solani* f.sp. *phaseoli*, hypocotyl lesions.

Abstract. A procedure using nutrient culture media was developed to screen bean seedlings on an individual plant basis for reaction to *Fusarium solani* f.sp. *phaseoli*. From over 800 accessions, 18 plant introductions and several cultivars of *Phaseolus vulgaris* were found to be either resistant or tolerant. Susceptible plants, grown with ammonium nitrate as the nitrogen source (210 ppm N) showed reduced symptoms compared to plants grown on nitrate-N only. Ammonium as the sole nitrogen source was toxic to bean

---

[1] Received for publication September 13, 1976. Research supported by the College of Agricultural and Life Sciences. This investigation is part of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree.

[2] Present address: Instituut De Haaff, Foundation for Agricultural Plant Breeding, Wageningen, The Netherlands.

[3] Burke, D. W. 1974. Naming and release of the Red Mexican bean cultivar 'Rufus'; Naming and release of two pink bean cultivars, 'Viva' and 'Roza'. USDA-ARS and The Wash. Agric. Expt. Sta., Pullman; Naming and release of the pink bean cultivar, 'Gloria'. USDA-ARS and The Wash. Agric. Expt. Sta., Pullman and Calif. Agric. Expt. Sta., Davis.

APPENDIX IV.

Travel by personnel associated with the project.

A. National.

1. F. A. Bliss attended operators workshop at Neotec Crop., Silver Spring, Md., January, 1976, to learn operation procedures of Grain Quality Analyzer.

2. F. A. Bliss presented lecture at Campbell Soup Co., Cinnaminson, New Jersey, February, 1976.

3. F. A. Bliss presented lecture at University of Kentucky, Lexington, Ky., March, 1976.

4. F. A. Bliss presented lecture at University of Nebraska, Lincoln, Nebr., March, 1976.

5. F. A. Bliss participated in workshop concerning Puerto Rico Grain Legume Project, Mayaguez, P.R., August, 1976.

6. Steve Beebe (Research Assistant) participated in World Food Conference, Ames, Iowa, June, 1976.

7. Greg Tolla and Martha Mutschler (Research Assistants) attended International Conference on Quantitative Genetics, Ames, Iowa, August, 1976; and visited with Professor Hanway, Iowa State University concerning foliar application of elements to grain legumes.

APPENDIX V.

Recipients of bean seeds.

Dr. George Freytag, MITA.
Breeding lines from recurrent selection.

Dr. Steven Temple, CIAT.
20 Superior S_2 lines for field trials.

Dr. Roger Schmitt, Del Monte Corp.
18 Mexican PI's resistant to root rot.

Dr. Mark Bassett, University of Florida, Gainesville.
BBL 240

Dr. M. H. Dickson, Geneva, N.Y.
18 Mexican PI's resistant to root rot.

Dr. D. T. Edwards, Yates Seeds, Sydney, Australia.
18 Mexican PI's resistant to root rot.

Mr. C. M. Binnenkijk, Enza-Zaden, Enkhuizen, Holland.
18 Mexican PI's resistant to root rot.

APPENDIX VI.

Visitors to the Project.

Name	Address
Keith Byergo (Sept., 1976)	Bureau for Technical Assistance USAID/Washington
M. H. Dickson (Aug., 1976)	Bean breeder, Geneva, N.Y.
Steve Magnuson (Aug., 1976)	Bean breeder, FMC Corp.
Dr. D. P. Mohan (Oct., 1976)	Bean research group Virginia State College Petersburg, VA
Dr. John Thomson	C.S.I.R.O. Canberra City, Australia
Dr. J. M. Gillespie	Chief Research Scientist Division of Protein Chemistry C.S.I.R.O. Parkville, Victoria Australis