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9. ABSTRACT

The ability of a bean strain, BBL 240, to transmit high percentage protein to its offspring is shown by the significant modification of this trait that has been realized in 3 generations of selection. Comparisons between high and low protein selections indicate that the primary effect of selection is manifested through changes in the amount of G1 globulin fraction. This fraction is the major constituent of bean seed protein, but it is poor in methionine. Therefore, selection for high protein often results in accompanying low percentage methionine levels. There are exceptions to this negative association, as best exemplified by BBL 240, which is high in both percentage of protein and methionine and by promising selections. Some differences are shown in the structure of one of the polypeptides that constitute the G1 globulin that are due to a single gene (likely a structural gene). The effects of this gene on the quality (amino acid composition) of the G1 fraction are being studied, since BBL (high methionine) carries a variant allele. Considerable variability in tannin levels of different strains have been found. High protein/methionine strains have been intercrossed to high yielding, adapted, and disease resistant germ plasm to form the first generation of a Composite Open Population that includes approximately 50 accessions. One cycle of recurrent selection for high yield (family basis) and high protein (individual plants) has been completed, with the selected lines having; 1) intercrossed to form the second Composite Open Population generation and; 2) sent to cooperators in Puerto Rico, Colombia, and Brazil for further evaluation.

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

February 5, 1975 thru December 31, 1975

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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.
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B. Narrative Summary of Accomplishments and Utilization.

The ability of a bean strain, BBL 240, to transmit high percentage protein to its offspring is shown by the significant modification of this trait that has been realized in 3 generations of selection. Comparisons between high and low protein selections indicate that the primary effect of selection is manifested thru changes in the amount of Gl globulin fraction. This fraction is the major constituent of bean seed protein, but it is poor in methionine. Therefore, selection for high protein often results in accompanying low percentage methionine levels. There are exceptions to this negative association, as best exemplified by BBL 240, which is high in both percentage protein and methionine and by promising selections. We have shown that some differences in the structure of one of the polypeptides that constitute the Gl globulin are due to a single gene (likely a structural gene). We are studying the effects of this gene on the quality (i.e. amino acid composition) of the Gl fraction, since BBL 240 (high methionine) carries a variant allele.

We have found considerable variability in tannin levels between different strains. White-seeded types have very low levels and there appears to be no strong association between seed color intensity and tannin content in types with colored seed coats.

High protein/methionine strains have been intercrossed to high yielding, adapted and disease resistant germ plasm to form the first-generation of a Composite Open Population that includes approximately 50 accessions. One cycle of recurrent selection for high yield (family basis) and high protein (individual plants) has been completed, with the selected lines having been (1) intercrossed to form the 2nd Composite Open Population generation and (2) sent to cooperators in Puerto Rico (MITA), Colombia (CIAT), and Brazil for further evaluation.

Exchanges of plant materials have been made with Puerto Rico (MITA), Colombia (CIAT), and Brazil (Embrapa) and promising lines.are being evaluated.

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 been 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 thru 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 thruout 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 co-operative 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.

There is no indication that the project objectives as stated should be changed. We have, as suggested by Drs. Litzenburger and Desrosiers, begun to assess the presence and possible significance of tannin in bean seeds.

D. Accomplishments to Date:

1. Findings:

The data presented herein and the results of other independent studies show that differences for seed protein percentage and methionine content are moderately heritable, depending on the population, method of estimation and the environment in which plants are grown. Levels of methionine present in genotypes such as BBL 240 are high enough to show biological significance in small-animal tests.

Negative correlations between seed yield and percentage protein are low, and improvement of both traits will likely result only if selection procedures consider both. We first select for seed yield based on mean family performance then for percentage protein based on individual plant phenotype within the selected families. The development and improvement of the Composite Open Population and the use of superior selections provides a procedure for developing populations with a broad genetic base for use in many regions for different purposes.

Biochemical analyses of families selected for high and low percentage protein indicate that changes in total protein result largely from modification of the Gl globulin fraction by genetic alteration of regulator genes controlling Gl synthesis. Changes in the globulin/albumin ratio can affect the methionine content, since albumin is richer in methionine than globulin. Heritable differences in polypeptide subunit structure are the result of a single structural gene. The effects of different alleles at this locus on the amino acid content of the Gl are being studied as a possible approach to altering methionine levels.

Bean lines vary considerably in tannin content of the seed coat, with white seeds having little or none and colored seeds varying without relation to color intensity. There is little relationship between tannin content and root rot resistance among some 20 resistant plant introductions studied.

Based on a study of 3 high-, medium-and low-protein strains grown in the Biotron, no differences in nitrogen uptake and transport to reproductive structures were found. Low percentage protein was found

in seeds that were produced on plants having high yields. Occurrence of recombinant lines that combine high yield and relatively high % protein demonstrates the feasibility of breeding strains having a combination of these desirable traits, if proper selection is practiced.

2. Interpretation of data and supporting evidence.

a. The nature of heritable differences for protein quantity and quality.

Percentage protein, percentage available methionine and available methionine as percent of protein are moderately heritable traits and gene action appears to be largely additive (Tables 1 and 2) (Kelly and Bliss, 1975). Genetic improvement of these traits can be obtained, provided suitable selection methods are employed. Inverse correlations have been reported for seed yield vs. percentage protein and for percentage protein vs. methionine as % of protein (Adams, 1973). We have found such correlations to be small (Kelly and Bliss, 1975), and it appears that the basis for this relationship is the ratio of globulin to albumin.

b. The effects of directional selection for percentage protein.

Three generations of directional selection (the 4th generation is being analyzed) for high and low percentage protein has resulted in two strikingly-different populations having mean percentage protein values of 30% and 22%, respectively (Table 3). This response to selection clearly shows that populations can be modified substantially by individual plant selection, provided sufficient genetic variation exists in the base population, and a suitable experimental design such as the hill plot design (Bliss, 1976) is employed to minimize non-genetic variation.

Table I. Protein and available methionine levels in five bean strains.

Identification	Percent protein	Percent available methionine	Available methionine, percent of protein	Protein, mg per seed	Available methionine, mg per seed
PI 207227	21.5±.20	0.197±.003	0.916±.015	39.6	0.362
PI 302542	25.1±.32	0.260±.004	1.036±.014	99.7	1.032
BBL 240	28.1±.27	0.339±.007	1.206±.016	105.9	1.278
PI 229815	31.9±.36	0.272±.006	0.853±.015	101.4	0.865
Sanilac	24.5±.40	0.235±.004	0.959±.015	49.7	0.477

Table 2. Broad sense heritability estimates for several traits in three bean populations grown in Wisconsin (W) and Colombia, S.A. (C).

Population	Location	Percentage protein	Percentage available methionine	Available methionine, percentage of protein
BBL 240 x PI 207227	W	0.69	0.52	0.38
	C	0.73	0.78	0.64
BBL 240 x PI 302542	W	0.32	0.43	0.39
	C	0.16	0.40	0.54
BBL 240 x PI 229815	W	0.71	0.56	0.60
	C	0.46	0.80	0.73

Table 3. Response to selection for high and low percentage seed protein, based on individual plant phenotype, within populations resulting from the cross BBL 240 x PI 207227.

Generation	Direction of selection	Percentage protein Actual	Percentage protein Predicted	Seed Yield (gm/plant)
F_5	H		32.7	
F_4	H	30.2	31.1	18.3
F_3	H	28.4	29.6	12.8
F_2	unselected	25.8	24.8	20.9
F_3	L	25.4	24.0	28.9
F_4	L	22.3	24.1	28.0
F_5	L		21.0	

Our results also show the consequences of selection for percentage protein *per se*, at least in this particular population. Mean yields of the high protein populations were substantially lower than those of the low protein populations (Table 3). Although this is the general case, we have identified families that combine high seed yield with increased levels of percentage protein (Table 4). These results suggest that the breeder must consider the effects of a negative correlation between yield and percentage protein, but that recombinant types occur frequently enough to allow improvement of both traits. We are using an approach that we feel will produce improvement of both traits simultaneously. This procedure will be discussed in detail in the section on population development.

Plants from the F_5 generation having similar seed yields but which show wide differences in percentage protein were chosen from the populations resulting from directional selection. Proteins were extracted from the seeds and separated according to a procedure developed recently (Yu Ma and F. A. Bliss, unpublished data) that allows quantification of several protein fractions. These results show that changes in total seed protein are largely associated with changes in the Gl globulin that were due to genetic selection (Table 5). The Gl globulin has been found to be the predominant fraction (Sun, 1974), but it is poor in methionine, particularly when compared with albumins. Our data suggest that large changes in the globulin/albumin ratio may affect the available methionine as % of protein (determined by the microbial assay). Among this limited number of experimental plants, % protein and methionine as % of protein were inversely related. This finding provides a

Table 4. F_3 and F_4 families from the cross BBL 240 x PI 207,227 showing the potential for selecting high yielding, high protein genotypes.

Family	Seed yield (G/plant)	Percentage protein	G protein per plant
1973			
73-27	37.2	26.6	9.85
73-25	38.8	22.8	8.84
73-24	32.7	24.5	8.01
* 73-26	36.1	27.9	10.07 *
73-21	32.4	23.6	7.64
73-16	38.6	24.7	9.53
Sanilac	30.7	25.5	8.43
BBL 240	14.7	31.1	4.57
PI 207,227	21.0	22.5	4.73
1974			
74-16	36.5	22.2	8.10
* 74-6	39.5	27.5	10.86 *
74-28	33.5	20.3	6.81
74-21	43.5	21.5	9.40
74-24	40.9	20.9	8.55
74-19	34.5	23.8	8.21
Sanilac	29.1	23.0	6.69
BBL 240	17.3	30.6	5.29
PI 207,227	26.2	22.3	5.84

*Indicates families having high mean yields and percentage protein.

Table 5. Protein fraction composition of bean lines selected for either high or low percentage protein.

Line	% protein	Protein fraction (mg/1000 mg of cotyledon meal)							ug met/ mg pro
		Albumin	Globulin			Glutelin	Other	Glob/Albumin	
			Total	G1	G2				
742015	27.5	28.1	163.6	140.3	23.3	62.6	28.2	5.8/1	7.11
742016	24.0	28.2	140.4	124.7	15.7	56.5	19.4	4.9/1	7.28
742032	32.2	33.9	190.2	167.3	22.9	62.1	30.6	5.6/1	6.52
742047	19.1	32.3	78.9	65.5	13.4	60.0	17.8	2.4/1	9.05
742065	24.7	34.1	124.1	101.4	22.7	57.9	20.6	3.6/1	6.33
742066	18.9	30.7	76.9	64.6	12.3	59.4	20.0	2.5/1	6.44
742071	26.2	30.3	134.0	109.5	24.5	56.0	23.8	4.4/1	6.91
742069	21.4	29.4	102.3	82.7	19.6	52.3	16.9	3.4/1	8.16
742077	29.5	37.3	137.5	119.2	18.3	69.4	31.4	3.6/1	6.00
742090	19.9	25.3	83.7	71.3	12.4	61.7	16.9	3.3/1	9.10

basis for the widely observed negative correlation and shows the importance of assessing both protein quantity and quality. The negative association is not complete since one parent of this population, BBL 240 combines both high % protein and high methionine as % of protein. Further studies are necessary to identify individual plants among the progeny that are superior for both traits.

c. Inheritance of differences in the polypeptide subunit structure of the Gl globulin.

The isolation and characterization of the Gl globulin (Sun, 1974) has permitted extensive study of this major storage protein fraction. Characterization of the Gl globulins of protein-modified strains using polyacrylamide gel electrophoresis has shown that Sanilac, PI 207227, and PI 229815, possess 2 clearly-discernable polypeptide bands, while BBL 240 and PI 302542 have 3 distinct bands. We are interested in these differences since our initial results suggested that BBL 240 and PI 302542 were higher in methionine than the 2-banded types. We are currently conducting studies to determine the effects of the polypeptide structure on the amino acid composition of the Gl globulin. Nelson (1969) feels it is unlikely that changes in the amino acid composition of protein fractions caused by structural genes will be large enough to be of practical significance. Rather he feels that substantial changes will result from changes in the relative amounts of the protein fractions. We have shown that alteration of globulin/albumin ratios due to modification of Gl globulin by regulator genes are important in modifying available methionine of bean, and we are now studying the effects of modifications in the polypeptide subunits.

Genetic analysis of the cross BBL 240 (3 bands) x PI 299815 (2 bands) has shown this difference is simply inherited and controlled by a single gene (Table 6) (Romero,et al., 1975). F_1 seeds are intermediate and contain polypeptides that correspond to alleles contributed by each parent. Evidence suggests the difference to be due to a structural gene. The microbial assay for available methionine continues to be satisfactory for determining the relative amounts of this amino acid in breeding lines. However, we intend to determine the utility of a new method for measuring methionine residues chemically (Finlayson and MacKenzie, 1976). This could be very useful in studies where more precision is required.

d. Identification of additional germ plasm.

Extensive protein analyses of some 200 strains grown in uniform traits in the field in the summer of 1976, will be completed when the newly-acquired infra-red analyzer is fully operational. We are now calibrating this instrument and expect to be analyzing samples by March, 1976. However, Kjeldahl analyses of some strains have been completed and promising new sources of variability exist, such as Bonita, WCBT-713938, OSU 58 and Endogava Z.N. (Tables 7 and 8).

e. Variability in tannin content of bean seeds.

Several people have suggested there may be significant levels of tannin in the bean seed coats particularly of colored types. Tannin is known to reduce the protein values of certain grain sorghums, thus the interest in the possible effect on nutritional value. We have followed the procedure of Burns (1971)

Table 6. Genetic analysis of the polypeptide banding patterns of G1 globulin from single seeds of BBL 240, PI 229,815, F_1 crosses, reciprocal crosses, F_2 and F_3 progeny.

Population	Parental banding pattern	Progeny banding pattern[1] 2	1	3	Expected ratio 2	1	3	X^2
P_1 (BBL 240)	3	0	0	13	0	0	1	
P_2 (PI 229,815)	2	13	0	0	1	0	0	
F_1 (BBL 240 x PI 229,815)	3 x 2	0	6	0	0	1	0	
F_1 (PI 229,815 x BBL 240)	2 x 3	0	9	0	0	1	0	
F_2	1	12	31	12	1	2	1	0.891
F_3 (1 family)	2	8	0	0	1	0	0	
F_2 (11 families)	1	16	41	28	1	2	1	3.188
F_3 (3 families)	3	0	0	30	0	0	1	

[1] The banding pattern is two-, intermediate-, or three-banded. The X^2 values show no significant deviation of the observed from the expected segregation ratios.

Source: Romero, J., et al., 1975, Plant Phys. (In press).

Table 7. Protein and Tannin content evaluation of selected lines of common bean, *Phaseolus vulgaris* L.

Yu Ma and F. A. Bliss

Strain	Seed color[z]	Percentage protein	Catechin equivalent (C.E.) values (mg/g sample) Without seed coat	With seed coat
Sanilac	W	24.5	.102 ± .007	.124 ± .003
PI 207227	Bl	21.5	.120 ± .017	.943 ± .046
PI 302542	W	25.1	.120 ± .005	.163 ± .005
BBL 240	W	28.1	.121 ± .008	.136 ± .013
PI 229815	Bu	31.9	.163 ± .007	.138 ± .009
742015	D. Bron	27.5	.072 ± .008	.408 ± .016
742016	D. Bron	24.0	.139 ± .035	.407 ± .018
742032	W	32.2	.101 ± .033	.099 ± .000
742047	Bron	19.1	.095 ± .017	.305 ± .016
742065	Br/wh	24.7	.082 ± .003	.120 ± .005
742066	Bl	18.9	.120 ± .002	.126 ± .016
742069	Bl	21.4	.108 ± .020	.124 ± .012
742071	W	26.2	.110 ± .003	.075 ± .014
742077	W	29.5	.099 ± .008	.096 ± .005
742090	Bl	19.9	.083 ± .006	.138 ± .009
Bonita	Iv-W	24.6	.022 ± .008	.120 ± .010
French Hort.	Mos. Rd.	19.3	.036 ± .007	.705 ± .012
Venz. 36	Bl	22.3	.013 ± .000	.170 ± .000
Ecuador 299	Rd	20.4	.111 ± .013	.359 ± .000
Mexico 309	Bl	20.3	.120 ± .022	.275 ± .010
Porillo	Bl	19.6	.028 ± .002	.296 ± .008
50600	Bl	22.1	.049 ± .008	.272 ± .003
LaVega	Bl	19.7	.031 ± .008	.171 ± .000
51052	Bl	21.0	.067 ± .024	.230 ± .009
Mexico 27R	Rd	19.6	.025 ± .010	.693 ± .027
15R-55	Bl	22.3	.054 ± .012	.530 ± .000
Jamapa	Bl	20.7	.037 ± .002	.238 ± .000
71-1R-113	Bl	20.8	.040 ± .007	.202 ± .000
Colorado de Pais	Mos. Rd.	21.0	.031 ± .014	.755 ± .005

[z] W= white; Bl=black; Bu=buff; D. Bron=Dark bronze; Br/wh=brown with white patches; Iv-W=ivory white; Mos. Rd.=mosaic red; Rd=red.

Table 8. Percentage Protein of cultivars and introductions grown at Hancock, WI., 1975.

Identification	% Protein	Identification	% Protein
BBL 240	27.3	PI 150414	24.2
French Hort.	23.2	PI 169740	22.8
Mecosta	24.9	PI 169740	22.5
WCBT-713938	27.5*	PI 169740	21.0
Stockbridge Indian Bean	20.3	PI 226934	24.4
Aurora	22.8	PI 226934	23.3
Venzuela 54	24.4	PI 226934	22.2
Chimbalo	22.8	OSU 58	27.6*
Kerman	24.7	Male Sterile Bulk	21.6
US #13	24.6	FM-1-K	25.5
Pinto III	21.1	Cal. Small White	23.2
Rust diff. 765	23.1	Gloria	23.8
Golden Gate	24.8	Tui	26.8
Rust diff. 181	25.9	Viva	20.2
Rust diff. 780	23.3	Rufus	21.0
Rust diff. 814	22.7	Roza	20.6
Carioca	22.3	St. Andes	22.7
P97	21.9	Medusa	24.3
30-L-50	24.8	Borinqueen	24.6
Rico 23	20.9	Peter's Yellow Eye	25.2
P17	20.8	Coffey	23.2
Vagem Roxa	20.5	Dutch Brown	18.7
Sacavem	23.7	Polish White Extra	24.9
Rim de Porco	24.7	Endogava Z.N.	27.3*
Flor Roxa de Cacho	23.6	Alberta Brown	24.9
Mulatinho	23.5	Nikos	24.8
Sanilac	23.1	Swedish Brown	19.4
PI 203958		Saxa	22.4
Limelight	24.6	Norway Brown	23.5
H 2222	17.7	Metis Eclipsy	24.0
Gratiot	27.2	St. Espirit	20.3
Black Turtle Soup	24.4	Japan Tebo	22.5
Redkote	22.4	Chevroir	23.1
Guali	21.2	Polish Pea II	23.5
Bunsi	24.6	Imperial Pea	23.5
PI 229815	28.1	Finalto	21.9
PI 207227	25.2	Baurenfreudl	24.8
PI 302542	25.2	Imperial Pea	24.4
State Half Runner	26.6	Eir Starozagorski	23.6
73-Vul 66	20.2	Imperial Flat	21.7
P-5-I	23.7	GN UI 31	23.7
P-17	21.4	Norwhite	25.0
P-566-A	25.5	Simplo	21.0
73-Vul 222	22.1	Widusa	22.8
73-Vul 14	22.6	GN US 1140	21.5
73-Vul 223	23.7	73-Vul 221	25.6
73-Vul 6 ICA	25.0	Red Mexican	22.0
P-488-B	24.0	15-R-55	24.0
Nep 2	22.4		

in which tannin is measured in catechin equivalents (C. E. values). Comparisons between seeds with and without seed coats suggest that most tannin is found in the seed coat (Table 7). White-seeded types have little or no tannin, but colored-seeded types vary considerably, with little relationship between tannin and color intensity. PI 207227, the black-seed bean we have studied extensively is interesting since we found it to be quite high in tannin. Kelly (1974) also found that there was a substantial difference in total methionine and available methionine in this line as measured by an autoanalyzer and the microbial assay, respectively. It is not known whether high tannin is related to this discrepancy in methionine values. We are now studying the inheritance of differences in tannin content using populations resulting from the cross BBL 240 x PI 207227.

Since colored-seeded types are sometimes found to be more vigorous and resistant to soil-borne pathogens, we were interested in assessing any relationships among accessions we have found to be resistant to both Fusarium solani and the root rot complex. We have found little if any relationship between tannin content and root rot resistance in our most promising lines. This finding should be of considerable importance, since the breeder can apparently develop resistant lines that are also low in tannin, thus minimizing any potential deleterious effect of this substance. This is even more important to regions where black beans are favored. We expect to repeat these initial analyses and determine the importance of tannins at levels at which they occur in beans.

f. Population development and improvement.

The identification of genetic variability for a particular trait such as percentage protein is only the first step in a comprehensive improvement program. If the full potential is to be realized, a suitable procedure must be developed to incorporate the improved trait into existing cultivars or breeding materials that will eventually be used by the consumer. For this reason we have initiated a program to transfer genetic superiority for protein quantity and quality into breeding populations that also possess yield potential, wide adaptability and disease resistance. Since there are a large number of problems concerned with yield improvement and breeding for disease resistance in beans, particularly in tropical and sub-tropical regions, little emphasis is likely to be placed on improving nutritional quality. By developing populations with a broad genetic base, we feel that improved populations and promising selections can be developed and shared with breeders working in a wide range of conditions.

The small, but important negative correlation between seed yield and percentage protein affects the results of selection for either trait. Selection solely for percentage protein is likely to result in static or reduced yields, while selection for yield alone will reduce or not affect protein. To improve both traits, there must be genetic variability in the initial population and selection for both traits must be practiced.

The Composite Open Population has been developed by initially intercrossing 50 different genotypes in different combinations. Parents were chosen from various locations,

primarily in North and South America and all were superior for some characteristic, i.e. high protein, high yield, disease resistance factors, adaptability, etc. S_1 seed was produced in the greenhouse and S_1 families grown in the breeding nursery at Hancock, Wisconsin. Each S_1 family contained up to 10 single plants per replication with 2 reps. Selection for seed yield was based on family mean performance of gm seed per plant. Kjeldahl analyses were performed on seeds from each plant within the selected high yielding families. Seeds from plants having the highest % protein were planted and the resulting plants intercrossed. S_1 families are being produced for planting in the field during 1976, as the 2nd cycle of the Composite Open Population. A second procedure in which S_1 family performance is based on the bulk yield and % protein of 9-plant plots replicated twice is being compared for efficiency.

Yield differences among S_1 families were significant, with the selection differential between the mean of selected and unselected families being 36.2 gm/plant (Table 9). Within families, the mean differences between selected and unselected individuals for percentage protein range from 1.9% to 3.0%. Selection differential for percentage protein is expected to be lower than within the entire population, since only the high yielding families are considered. Nevertheless, we should expect substantial improvement of yield, and a small, but steady increase in percentage protein.

A major feature of the Composite Open Population is the flexibility of adding new superior parents and of extracting selected families for testing and evaluation. Promising new

Table 9. Performance of Superior Families selected from the First Generation Composite Open Population, Hancock, WI., 1975.

	Seed yield(gm/plt)		Percentage protein				
			Unselected plants			Selected plants	
Population	$\bar{x}$	range	n	$\bar{x}$	range	$\bar{x}$	range
COP_o^2	75.2	28.7-128.7	18	20.9	18.7-24.7	23.3	22.4-24.7
COP_o^{12}	73.0	22.3-114.5	16	22.6	19.9-25.6	24.8	24.0-25.6
COP_o^{13}	78.6	34.8-183.7	19	23.3	21.2-27.5	25.4	24.5-27.5
COP_o^{20}	75.7	50.4-115.2	20	21.1	18.2-23.3	23.0	22.5-23.3
COP_o^{21}	61.3	40.3-100.7	20	20.8	18.0-24.8	23.8	22.9-24.8
COP_o^{32}	69.9	35.4-115.2	18	23.6	19.7-26.5	26.0	25.6-26.5
Total Population	35.4	(Unselected Control)					
Sanilac	13.5	4.4-29.4					
Black Turtle Soup	70.5	37.6-105.8					

materials are being intercrossed to continually expand the genetic base in a purposeful manner. Seeds of S_2 individuals and families have been sent to Puerto Rico, Brazil and Colombia for evaluation.

A major limitation thus far has been that the Kjeldahl method is too slow to analyze numerous seed samples from individual selections. There are potentially useful families that were not selected but which may be of practical significance. The availability of the infra-red Protein Analyzer should alleviate this problem and allow other potentially superior strains to be identified.

g. Nature of the yield-protein relationship.

Although the existance of genetic variability for seed protein percentage has been varified by several investigations (Leleji, 1971; Porter, 1972; Kelly, 1974), the basis of protein differences is unknown. We have several investigations underway to determine the importance of nitrogen uptake, transport, storage and utilization as it affects seed yield and % protein.

Using the protein-modified genotypes BBL 240 and PI 207227 in addition to the standard cultivar 'Sanilac', we have found that high, medium and low protein genotypes absorb similar amounts of nitrogen from the growth media. Furthermore, about one-half the nitrogen is transported to the seed and ½ to other plant parts. However, there are striking differences in seed yield, with the result being that high seed yield is associated with low percentage protein since similar total nitrogen goes to the reproductive parts (seeds) in both cases (Table 10). This then provides a good understanding of the reason for the often-observed negative

Table 10. Accumulation and partition of nitrogen and dry matter to vegetative and reproductive tissues of protein-modified bean genotypes.

Line	Nitrogen Accumulated (mg/plant)	Portion of accumulated nitrogen in: Veg. Parts	Portion of accumulated nitrogen in: Seeds	Seed Yield (gm/plant)	Percentage protein in seeds	Total Dry Matter (g/plant)
PI 207,227	1093.2	47.0%	53.0%	23.81a	15.87c	65.9a
BBL 240	1143.5	52.8%	47.2%	10.90c	25.87a	58.4b
Sanilac	1092.3	48.4%	51.6%	16.34b	20.50b	57.4b
	n.s.	n.s.	n.s.	**	**	**

relationship between seed yield and percentage protein.

The possibility of minimizing this negative relationship is critical to the breeder seeking to develop high yielding, high protein lines. We are postulating that selection should be directed toward plants that absorb or preferably fix large amounts of N, but which store small amounts in the vegetative plant parts. The possibility of developing high yielding, high protein lines exists, since in both 1973 and 1974 we have identified such families (Table 4). These materials will be used to test the above hypothesis under well-controlled conditions in the Biotron at the University of Wisconsin.

h. Development of root rot resistant materials.

Bean strains resistant to numerous diseases are universally important. We are working extensively on the development of populations resistant to several pathogens including Fusarium solani. Several plant introductions have been found to possess useful levels of resistance (Boomstra, 1975) (Table 11). Seed of these materials has been sent to Puerto Rico, Colombia, Washington, Nebraska and other locations. We are evaluating the inheritance of this resistance and intercrossing various genotypes. We are currently intercrossing black-seeded types to construct a resistant, genetically-diverse black bean population for selection and testing in Latin America. This is expected to be ready for use in early 1977.

3. Research design.

The sole use of the Kjeldahl procedure for protein analysis is adequate for the population development work. However, it is not

Table 11. Field evaluations of plant introductions and cultivars of beans for resistance to root rot. Row plots, Hancock, Wisc., 1975.

Steve Beebe and F. A. Bliss
Horticulture Dept., Univ. of Wisc., Madison

Cultivar or P.I. No.	Seed color[b]	Percent germination	Disease rating[c] w/o non-emergers	Disease rating[c] with non-emergers
325,619	B	60[a]	2	41
311,991	B	70	4	33
311,987	B	73	5	30
311,989	B	78	7	28
312,033	B	76	7	29
312,028	B	61	7	42
224,737	W	43	7	49
312,043	B	80	11	29
311,917	PPu/Mo	51	11	36
312,077	B	68	13	41
310,607	PPu/Mo	80	15	33
309,726	PPu/Mo	75	17	37
319,606	Rpu	66	17	44
15R-55	B	77[a]	18	37
N203	B	71	19	41
73-507	W	66	21	47
309,801	BG	60	23	53
312,041	B	78	25	41
312,062	B	76	25	43
Black Turtle Soup	B	74	26	45
Cherokee Wax	B	80	27	42
State Half Runner	W	81	29	42
Falcon	W	51	29	63
224,730	W	70	34	53
73-501	W	69	35	51
BBL 290	W	61	36	60
73-510	W	71	40	57
311,975	B & Br	66	41	60
Bush Romano	TStr.	65	42	62
Provider	B	80	49	58
Cascade	W	36	43	80

[a] Values based on 1 rep. All others based on 2 reps.

[b] Colors as indicated: B = black; W = white; PPU/Mo = pink, purple mottled; Br = brown; Rpu = red, purple mottled; TStr = tan striped.

[c] Disease rating (mean of 30 plants/rep): 0 = no damage; 20 = slight hypocotyl damage and good growth; 50 = moderate damage; 80 = severe damage and stunted; 100 = hypocotyl rotted, plants dead or missing.

rapid enough to allow analysis of many other potentially useful individuals or extensive materials from cooperators. Future routine analysis of % protein will be made with an infrared gain quality analyzer. An assessment of a chemical method for determining methionine content of protein fractions and seed meal is being made to perhaps provide more accurate characterization of this amino acid than can be made using solely the microbial assay.

Other techniques and procedures employed are adequate and no major changes are anticipated.

E. Dissemination and Utilization of Research Results.

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

Dissemination of plant material is as important to this project as is dissemination of written information. Routine distribution of promising lines is made to Puerto Rico (MITA), Colombia (CIAT), Brazil and the Sudan. 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. Use of findings of this project elsewhere. Evidence of this nature is limited since the work has been in progress for less than 1 year.

3. Discussions with research personnel at various places in the U.S. and LDC's indicate the need for an expanded exchange of materials and ideas. Most institutions in LDC's have limited facilities, personnel and capabilities for extensive fundamental research to back-stop the essential practical work that is underway or which must be initiated. Increased emphasis should be placed on cooperative work among U.S. institutions and between U.S. institutions and appropriate organizations serving the needs of LDC's. Some development of a research network can be accomplished thru the provisions of this contract, but limits will soon be met, due largely to the need for more technical personnel working with the principal investigator. Service functions such as protein analyses of large numbers of samples require use of additional technical staff.

4. Efforts have been made to involve personnel in institutions that serve the needs of LDC's. The most significant contribution of people in LDC's can be thru the testing, evaluation and ultimate use of selected materials in regional and national improvement programs. The initiation of international bean trial nurseries thru the supervision of CIAT should be useful to this and other improvement programs.

5. Publications, etc. are sent under separate cover. For list see Appendix 3.

F. Statement of Expenditures and Obligations and Contractor resources.

Line Item	AID's obligated funds	Expended to date	Estimated Budget 1/1/76-12/31/76
Travel & per diem	5,665.00	613.92	2,360.00
Consultants	900.00	300.00	300.00
Equipment & materials	12,500.00	6,732.94	2,500.00
Other direct costs	5,650.00	549.39	2,650.00
	24,715.00	8,196.25	7,810.00

G. Work Plan and Budget Forecast for Coming Year.

1. Anticipated accomplishments for the coming year.

   a. Identification of additional bean genotypes that are genetically superior with respect to protein percentage and methionine content.

   b. Quantification of the effects of regulator genes responsible for the synthesis of Gl globulin on total protein, globulin/albumin ratio and methionine as percentage of protein.

   c. Determination of the extent and type of genetic control of tannin content in bean seed coats, and the effects on protein value.

d. Selection for improved yield and protein content within The Composite Open Population; addition of new parents, completion of the 2nd cycle of recurrent selection and utilization of superior selections.

e. Distribution of potentially superior lines and selections to cooperators and other research workers for testing and utilization.

2. Procedures to be used and expected activities.

We will use standard procedures for genetical analyses of promising new parents to determine number and similarity of genes, effect of genes on total protein and methionine, and heritability of these traits. The quantitative separation procedures we have developed will be used to quantify the protein fraction within the bean seeds. Polypeptide subunit structure of G1 globulin is determined by gel electrophoresis. Total protein will be estimated using the Kjeldahl procedure and the infrared protein analyzer. Methionine will be estimated using the microbial assay and the new chemical procedure, if the latter is found to be successful.

Recurrent selection for improved yield and protein content will continue in The Composite Open Population, as outline for the current year.

3. Significant factors affecting accomplishments.

a. Use of the infrared protein analyzer for rapid protein analysis.

b. Use of a chemical method for estimation of methionine to give greater precision for some uses.

c. Additional cooperative work with research personnel in LDC to provide greater opportunity for evaluation of selections.

4. Dissemination and utilization of expected results.

   a. Publication of technical articles in appropriate journals and distribution of reprints to people known to be involved in bean research in the U.S. and LDC's.

   b. Compilation and distribution of a research report during the year to include pertinent research results that may be too preliminary for journal publication.

   c. Presentation of seminars in the U.S.

      i. Campbell Research Institute, Feb., 1976.

      ii. University of Kentucky, March, 1976.

      iii. University of Nebraska, March, 1976.

   d. Distribution of seeds of promising selections to cooperators in: Brazil, Colombia, Puerto Rico and The Sudan, and others as requested.

   e. Provide protein analyses of plant materials found by other investigators to be outstanding for other traits, i.e. yield, pest resistance, etc.

5. Planned Expenditures, Jan. 1, 1976 - Dec. 31, 1976.

Line Item	Estimated expenditure
Travel & per diem	2,360.00
Consultants	300.00
Equipment & Materials	2,500.00
Other direct costs	2,650.00
	7,810.00

Appendix I.

Literature Cited

1. Adams, M. W. 1975. On the quest for quality in the field bean. In: M. Milner (Ed.) Nutritional Improvement of Grain Legumes by Breeding. Wiley and Sons, N.Y.

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

3. Boomstra, A. G. 1975. Breeding for Resistance to Fusarium Root Rot in Beans (Phaseolus vulgaris L.). Ph.D. Thesis, University of Wisconsin.

4. Burns, R. E. 1971. Method of estimation of Tannin in grain sorghum. Agron. J. 63:511-512.

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6. Kelly, J. D. 1974. Genetic modification of protein quantity and quality in beans, Phaseolus vulgaris L. Ph.D. Thesis, University of Wisconsin, Madison.

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9. Lieleji, O. I. 1971. The genetics of crude protein and its relation to physiological and agronomic factors in dry beans. Ph.D. Thesis, Cornell University.

10. Nelson, O. E. 1969. Genetic modification of protein quality in plants. Adv. Agron. 21:171-194.

11. Porter, W. M. 1972. Genetic control of protein and sulfur content in dry bean, Phaseolus vulgaris L. Ph.D. Thesis, Purdue University.

12. 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:

13. Sun, S. M. 1974. Isolation and physical and chemical characterization of globulins from Phaseolus vulgaris seed. Ph.D. Thesis, University of Wisconsin, Madison.

Appendix II. Personnel.

Department of Horticulture

Dr. F. A. Bliss (Project Leader)

Mr. Moses Adenihun (Research Assistant - Nigeria)*

Mr. Yu Ma (Research Assistant - Taiwan)*

Ms. Jeanne Romero-Andreas (Research Assistant)*

Mr. Greg Tolla (Research Assistant)*

Ms. Martha Mutschler (Research Fellow)*

Mr. Steve Beebe (NSF Fellow)*

Cooperating staff (Department of Horticulture)**

Dr. T. C. Hall (Professor)

Mr. R. C. McLeester (Specialist)

Dr. S. Sun (Post-doctorate)

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

**Staff members who are not directly related to 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. 1975. Improvement of seed protein in cowpeas for Nigeria. IN: Milner (Ed). Nutritional Improvement of Food Legumes by Breeding. Wiley and Sons, Inc., N.Y.

Bliss, F. A. and T. C. Hall. 1976. Food Legumes-Compositional and Nutritional Changes Induced by Breeding. Cereal Chemistry (In Press).

Kelly, J. D. and F. A. Bliss. 1975. Quality factors affecting the nutritive value of bean seed protein. Crop Sci. 15:757-760.

Kelly, J. D. and F. A. Bliss. 1975. Heritability estimates of percentage seed protein and available methionine and correlations with yield in dry beans, Phaseolus vulgaris L. Crop Sci. 15:753-757.

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

Tolla, G. and F. A. Bliss. 1975. Nitrogen utilization in protein-modified genotypes of beans (Phaseolus vulgaris L.). Bean Improvement Cooperative, East Lansing (Abst.).

Boomstra, A. G. 1975. Breeding For Resistance to Fusarium Root Rot in Beans (Phaseolus vulgaris L.). Ph.D. Thesis, University of Wisconsin, Madison.

Hamad, Ibnouf. 1975. Inheritance of Yield, Yield Components, Flowering Time, Plant Height and Incidence of Interlocular Cavitation in Snap Bean, Phaseolus vulgaris L. Ph.D. Thesis, University of Wisconsin, Madison.

B. Abstracts of Papers and Publications Developed with the project.

Food Legumes-Compositional and Nutritional Changes Induced by Breeding

F. A. Bliss and T. C. Hall
Department of Horticulture, University of Wisconsin, Madison

SUMMARY

The desirable combination of ability to fix nitrogen biologically and the capacity to accumulate large amounts of seed protein makes the food legumes very attractive at a time when nitrogen fertilizer costs are high and there is an increasing demand for protein at a reasonable price. Genetic improvement of yield and nutritional value of the seed protein must be accomplished if the food legumes are to maintain their roles in traditional diets and become even more important protein sources.

Glycoproteins which are found in the globulin fraction constitute a major portion of the storage protein of bean seeds. Since they are generally deficient in sulfur-containing amino acids, efforts to improve nutritional quality are likely to involve alteration of these proteins. Protease inhibitors and phytohemagglutinins, two major toxicants of legumes, often constitute a portion of the storage protein. Thus attempts to alter protein quality must consider these constituents as well as total protein and amino acid composition.

Seed protein percentage and sulfur amino acid content of legume seeds are generally found to be of low to medium heritability. Improvement of these traits through selection has been shown to be possible with appropriate breeding procedures. However, because of low negative correlations between seed yield vs. percentage protein and percentage protein vs. methionine content as percentage of protein,

selection for a particular trait should not be conducted without attention to the other affected traits. Possible approaches to the improvement of methionine content include lowering the globulin to albumin ratio and qualitatively altering the amino acid composition of specific protein fractions.

Acknowledgments

Supported in part by National Science Foundation, grant PCM74-21675, Agency for International Development contract No. AID/ta-C-1173, The Graduate School, and The College of Agricultural and Life Sciences, University of Wisconsin, Madison.

Symposium on Oilseeds and Legumes:
Factors in Production which
Influence Composition and Quality.
Amer. Assoc. Cereal Chemists
Kansas City, 1975

Appendix IV.

Travel by personnel associated with the project.

A. National.

1. F. A. Bliss visited the Mayaguez Institute for Tropical Agriculture, Feb., 1975, to discuss exchange of information and materials with Dr. Nader Vakili and Dr. George Freytag, and to establish a program for cooperative bean research.

2. F. A. Bliss attended the AID contractor's meeting at Mississippi State University, March, 1975, to participate in discussions and present a program report.

3. F. A. Bliss visited Michigan State University, June, 1975, to discuss exchange of information and materials with Dr. Wayne Adams, Al Smucker, Fred Saettler and to discuss possibilities for cooperative bean research between institutions and with Latin America.

4. F. A. Bliss visited Campbell Institute for Agricultural Research, Napolean, Ohio, to observe bean research program and discuss breeding approaches.

5. F. A. Bliss attended meeting of the American Assoc. Cereal Chemists, October, 1975, Kansas City, MO. to present an invitational paper "Food Legumes - Compositional and Nutritional Changes Induced by Breeding."

6. F. A. Bliss attended meeting of The Bean Improvement Cooperative, Nov., 1975, East Lansing, Michigan, to present invitational paper "Nitrogen Utilization in Protein-modified Genotypes of Beans (*Phaseolus vulgaris* L.).

7. Greg Tolla and Steve Beebe (Research Assistant and NSF Fellow) attended Michigan State University, Bean Field Day, East Lansing, July, 1975.
8. Jeanne Romero (Research Assistant) attended annual meeting Plant Physiology Society, Corvallis, Oregon, Aug., 1975, to present a paper "Heritable differences in Phaseolus globulin. polypeptides."
9. Steve Beebe and Yu Ma (NSF Fellow and Research Assistant) attended meeting of Bean Improvement Coop., Nov., 1975, East Lansing, Michigan.

B. International.

1. F. A. Bliss participated in International Bean Improvement Workshop, CIAT, Cali, Colombia, Oct., 1975.
2. F. A. Bliss traveled to Brazil to confer with researchers at National Center for Rice and Bean Research, Goias, Brazil, Oct., 1975.

Appendix V.

Recipients of bean seeds.

Dr. James Steadman, Dept. of Plant Pathology, University of Nebraska, Lincoln, Nebraska.

19 PI selections possessing root rot resistance.

Dr. Nader Vakili, Mayaguez Institute for Tropical Agriculture, Mayaguez, P. R.

19 PI selections possessing root rot resistance.

Dr. Doug Burke, ARS/USDA, Irrigated Agriculture Research Center, Prosser, Washington.

19 PI selections possessing root rot resistance.

Dr. G. Hernandez-Bravo, CIAT, Cali, Colombia.

19 PI selections possessing root rot resistance

40 Breeding lines

Dr. George Freytag, Mayaguez Institute for Tropical Agriculture, Mayaguez, P.R.

26 Breeding lines

Dr. M. W. Adams, Michigan State University, East Lansing, MI.

6 segregating families, 1 cultivar

Dr. Neto, CENA, Brazil.

9 Breeding lines

Appendix VI.

Visitors to the project.

Name	Address
1. D. H. Wallace	Cornell University
2. Drs. Ma. Luisa Ortega Delgada	Escuela Nacional De Agricultural, Chapingo Mexico.
3. Dr. Dermot P. Coyne (consultant)	University of Nebraska, Lincoln, Nebraska
4. Dr. A. Downey	USAID, Washington
5. R. H. Jones	P.O. Box 288 Dubbo, N.SW. Australia
6. Drs. M. J. Silbernagel and D. Burke	Irrigated Agr. Center, ARS, USDA, Prosser, WA.

Appendix VII. Cooperative agreements.

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Southern Region
Mayaguez Institute of Tropical Agriculture
P.O. Box 70
Mayaguez, Puerto Rico 00708

March 10, 1975

Subject: Cooperative Research

To: F. Bliss
University of Wisconsin
Madison, Wisconsin

R. Desrosiers
Agency for International Development
Washington, D. C.

G. Hernández Bravo
CIAT
Cali, Colombia

J. López Rosa
Agricultural Experiment Station
Rio Piedras, P. R.

F. W. Martin
Mayaguez Institute of Tropical Agriculture
Mayaguez, Puerto Rico

During the week of February 24-28, 1975 a visit was received from Dr. G. Hernández Bravo of CIAT, Colombia and Prof. F. Bliss, of the University of Wisconsin. Bean field plantings were inspected at UPR substations at Fortuna and Lajas and at the ARS locations at Mayaguez and Isabela and laboratories, greenhouses and research facilities were visited at the MITA, ARS station in Mayaguez. Consultations and meetings were held with the various research personnel of both MITA and the UPR, Edible Legume Project C-457.

As a result of these visits the following specific areas of cooperation were tentatively agreed upon:

MITA-University of Wisconsin

A. Disease resistance screening.

1. MITA will send 100 seed each of 16 lines to Wisconsin for screening for Fusarium root rot resistance.

B. Adaptability and yield testing.

1. MITA will send 1 set of 16 lines for hill plot testing at one location in Wisconsin.

2. Wisconsin will send 10 lines for resistance and adaptability testing at one location in Puerto Rico.

C. Seed protein analysis.

1. MITA will send 10 gm samples from one location of 16 parent lines of the breeding program to Wisconsin to run total protein and percent methionine analyses.

2. MITA will send 10 seed each of representative subspecies, races, groups, and several apparently introgressing lines (vulgaris-acutifolius, vulgaris coccineus, coccineus-coccineus) for electrophoretic runs of seed protein patterns.

MITA-CIAT

A. Disease resistance screening.

1. CIAT will send their best yielding materials for testing at Mayaguez for disease resistance.

B. Adaptability and yield testing.

1. MITA will send 3 sets of materials for testing at one location in Colombia. (one set of 16 lines for hill plot test, 1 set of 15 lines for single row testing, and 1 set of 14 lines of Vigna for single row testing).

N. G. Vakili
Research Plant Pathologist

G. F. Freytag
Research Geneticist

March 24, 1975

Dr. Julio Lopez Rosa
Agricultural Experiment
Station
Rio Piedras, P.R.

RE: Cooperative Research

Dear Dr. Rosa:

Based on our discussions at the AID contractors meetings at Mississippi State University, I suggest that we begin working toward cooperative research in a modest way through an initial exchange of the best parental materials with which we are now working. If our programs expand and as we more fully understand our own and each others capabilities, we may then wish to circulate improved populations or progenies as they become available.

The following specific areas could be undertaken:

(1) The AID-sponsored program at Wisconsin will provide protein and/or methionine analyses on promising parental (research) stocks being studied at Rio Piedras. This would comprise not more than 50 samples the first year.

(a) Initial attempts will be made at Wisconsin to determine the feasibility of tannin analysis, and if possible a limited number of strains from Wisconsin and Rio Piedras will be analyzed.

(2) The group at Rio Piedras will determine the disease reactions (for those diseases with which they are working) of selected parental lines that are being used in the formation of the breeding population at Wisconsin. This will number between 20-50 the first year.

Dr. Julio Lopez Rosa
March 24, 1975
Page 2

I look forward to a mutually beneficial exchange of materials and information. Please reply as to the feasibility of these suggestions.

Yours truly,

F. A. Bilas
Associate Professor

FAB/jek

cc - Russell Desrosiers