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

FOR AID USE ONLY
Batch 91
ARDA

1. SUBJECT CLASSIFICATION
A. PRIMARY: Serials
B. SECONDARY: Food production and nutrition--Plant breeding--Beans

Y-AF30-1410-0000

2. TITLE AND SUBTITLE
Improving yield and quality of bean seed protein; progress report, June,1978

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

4. DOCUMENT DATE: 1978
5. NUMBER OF PAGES: 67p.
6. ARC NUMBER: ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Wis.

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

9. ABSTRACT
A Project: To improve quantity and quality of bean seed protein.
Duration:
Developments: Because of the major role of grain legumes in the diets of many people, world-wide improvement in legume availability and digestability could have considerable impact on nutrition. The project identified strains of common beans which are consistently high in seed proteir and confirmed that protein variability is a heritable trait. Researchers established that selection programs should consider crop yield and proteir content simultaneously for maximum effectiveness. The project demonstrated the feasibility of cooperative research with other programs. Strains and breeding lines with superior protein characteristics were made available to other programs for tests and evaluation. The report identified problems for future research and development.

10. CONTROL NUMBER
PN-AAG-033

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Beans

13. PROJECT NUMBER
931060400

14. CONTRACT NUMBER
AID/ta-C-1173 Res.

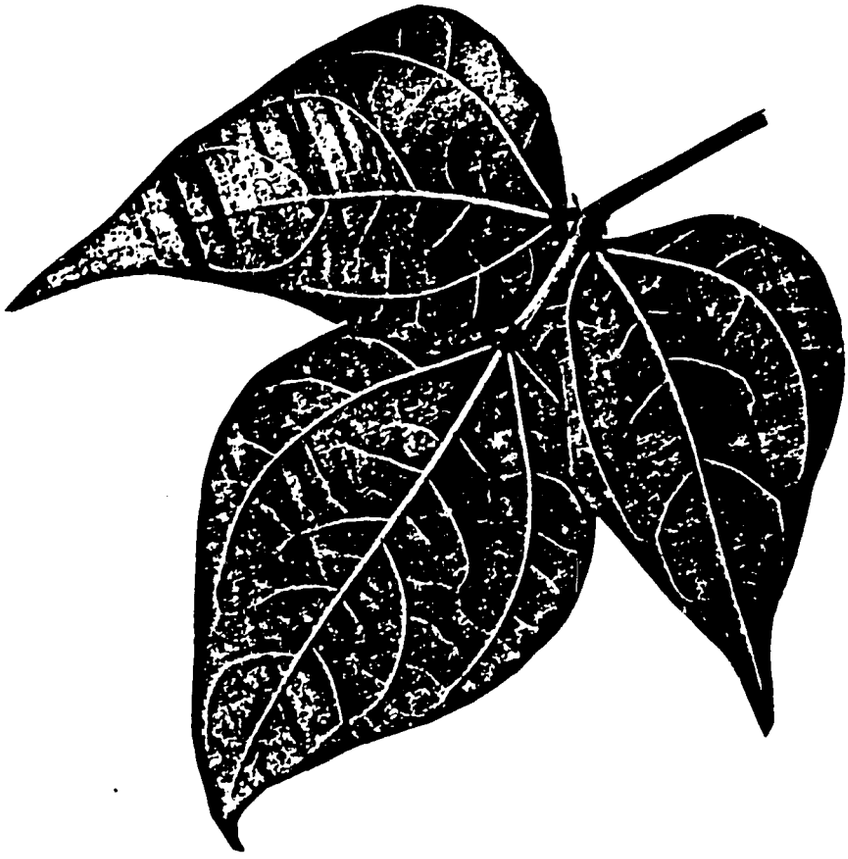
15. TYPE OF DOCUMENT

AID 590-1 (4-74)

Return

AID/ta-C-1173 PN-AAG-033

Project Dec.



IMPROVING the

Quantity and Quality of

BEAN SEED PROTEIN

A Progress Report

F.A. Bliss

FORWARD

There has been intensified interest in improving quantity and quality of bean seed protein since about 1970, when variability for these traits was identified. Because of the major role of grain legumes in the diets of many people worldwide substantial improvement in availability, intake and digestibility of bean protein could have considerable impact on nutrition if coupled with yield increases.

The support made available through a grant from the Agency for International Development has allowed us to focus on the identification and development of superior germplasm. However, without support from various other sources and the diligent work of staff and students this research would not have been possible.

I have been fortunate for the opportunity to interact with Dr. T. C. Hall, Professor of Horticulture, Dr. S. M. Sun, and R. C. McLeester. Without the methods, procedures and apparatus they have developed and their knowledge of bean seed protein this work would have progressed slowly at best. The ideas and research of current and former students Dr. J. D. Kelly, Dr. Yu Ma, Dr. G. E. Tolla, Martha Mutschler and Joe Sullivan; and research technician Lynn Hummel as well as numerous student assistants were essential to this project. The necessity for and value of an integrated research program should be readily apparent.

Although the results are promising, future success will depend on the production of high yielding cultivars with enhanced growth-promoting properties. The results we report must be used and expanded if this goal is to be reached.

June 16, 1978

F. A. Bliss
Dept. of Horticulture
Univ. of Wisconsin
Madison, WI 53706

TABLE OF CONTENTS

	Page
SUMMARY OF FINDINGS	1
ACCOMPLISHMENTS	3
FUTURE RESEARCH AND DEVELOPMENT	4
INTRODUCTION	5
GENETIC VARIABILITY OF PROTEIN QUANTITY AND QUALITY	6
VARIATION IN PERCENTAGE PROTEIN	6
VARIATION IN PROTEIN COMPOSITION	9
Genetic Variability for Seed Protein Fractions	9
Variation in Amino Acid Composition	14
HERITABILITY OF PROTEIN VARIATION	18
Total Seed Protein	18
Methionine Content	18
VARIATION IN ELECTROPHORETIC PATTERNS OF THE G1 SUBUNITS	21
VARIATION IN TANNIN CONTENT	23
RELATIONSHIPS BETWEEN PROTEIN AND OTHER TRAITS	26
Yield and Protein	26
Percentage Protein and Methionine	26
UTILIZATION OF GENETIC VARIABILITY	28
BI-DIRECTIONAL SELECTION FOR PERCENTAGE PROTEIN	28
POPULATION IMPROVEMENT USING RECURRENT SELECTION	30
IDENTIFICATION OF GENE(S) THAT ALTER THE PROTEIN EXCHANGE OF PLANT MATERIALS	35
EXCHANGE OF PLANT MATERIALS FOR MULTIPLE EVALUATION	37

Table of Contents - cont.

	Page
ANALYTICAL PROCEDURES	49
QUANTITATIVE ESTIMATION OF PERCENTAGE PROTEIN	49
EXTRACTION AND SEPARATION OF PROTEIN FRACTIONS	51
QUANTITATIVE ESTIMATION OF G1 PROTEIN USING IMMUNOELECTROPHORESIS	53
CHARACTERIZATION OF GLOBULIN PROTEINS USING GEL ELECTROPHORESIS	55
ESTIMATION OF TANNIN CONTENT	58
LITERATURE CITED	60

IMPROVING THE QUANTITY AND QUALITY OF BEAN SEED PROTEIN

SUMMARY OF FINDINGS

1. Substantial genetic variability for total seed protein exists within *Phaseolus vulgaris*, and is found in a wide range of phenotypes.
2. The heritability of quantitative differences in total seed protein is moderate, usually in the range .30 to .70.
3. Small negative correlations (0 to -.30) between yield and percentage protein are usually found, but they should not impede progress toward developing high yielding, high protein lines provided suitable selection strategies are followed.
4. Recurrent selection which includes intercrossing the best genotypes offers a promising means of combining several sources of superior germplasm upon which to practice selection.
5. Selection strategies that allow identification of high protein individuals within high yielding families appear to be most promising.
6. Substantial progress has been made toward increasing the percentage protein of adapted strains which also have acceptable yield. Successful selection strategies must consider improving yield and protein simultaneously.
7. Alteration of total protein content of the bean seed is a consequence of altering the amounts of specific protein fractions. The major components are the globulin-1 fraction (35 to 50%) and the alkali-solution fraction (20-30% of the total).
8. The amino acid profiles of the constituent fractions differ substantially. The different levels of methionine in the globulin-1 (.88%) and the alkali-soluble (1.98%) fractions suggest that methionine

content of the whole seed can be altered substantially by changing the relative amounts of these two major fractions.

9. Nutritional quality of beans involves more than percentage seed protein and methionine content. Several quality suppressing factors may be present.

10. Tannins found in the testae of colored-seeded strains are related to reduced nutritional value.

11. There is considerable variability in tannin content within the different seed color types studied. Tannin levels do not appear to be closely associated with color intensity.

12. Tannin content is highly heritable (.50 to .90), suggesting that levels can be altered substantially by selection.

13. Since pest resistance is sometimes associated with colored-seeded types, these relationships should be studied before genetic alteration of tannin levels is recommended without reservation.

14. Practical breeding programs should continue to give highest priority to increased productivity and pest resistance, but should include selection for superior protein quantity and quality among the promising breeding lines.

ACCOMPLISHMENTS

1. Identified strains of common bean, Phaseolus vulgaris, which are consistently high in seed protein.
2. Confirmed that protein variability is a heritable trait, and that strains having high protein and satisfactory yield levels can be developed using suitable breeding procedures. Protop W-1, Protop W-2, and Protop P-1 are lines available for testing.
3. Established that selection programs should consider yield and protein simultaneously for maximum effectiveness.
4. Demonstrated substantial differences in relative amounts of constituent protein fractions and that these fractions have different amino acid compositions.
5. Established that there are substantial differences in tannin content among lines, and that it is a highly heritable trait only loosely related to seed coat color.
6. Demonstrated the feasibility of cooperative research with other programs, which allows for exchange of materials for protein analyses in our lab and assessment of pest resistance and adaptability in tropical environments.
7. Provided strains and breeding lines that have superior protein characteristics to other programs for testing and evaluation.

FUTURE RESEARCH AND DEVELOPMENT

1. Continued identification of genotypes having superior protein levels and quality, particularly those with outstanding yield potential.
2. Identification and characterization of genes and gene blocks that control synthesis and accumulation of constituent protein fractions and starch accumulation.
3. Recombination of diverse strains having superior yield, resistance and protein enhancing genes into populations that can be subjected to selection using efficient breeding procedures.
4. Selection for improvement of the ratio of methionine-rich to methionine-poor constituent fractions, as well as total protein and yield increases.
5. Estimation of the effects of seed protein modification on protein quality and utilization in diets.
6. Determination of the effect of modifying tannin levels on other traits such as protein quality and pest resistance.
7. Breeding of adapted, high yielding bean strains with improved nutritional value.
8. Increased distribution of improved strains to other research and testing programs.

INTRODUCTION

Food legumes provide about one-fifth of the protein available to humans on a worldwide basis, and in many less developed countries, they are the sole protein source for nearly 50% of the population. Where the grain legumes are readily accepted and consumed, they have an excellent potential for providing increased calorie and protein intakes as well as improving protein quality. If this potential is to be realized, the productivity, availability, intake and nutritional value of beans must be increased.

The quest for greater yields and productivity of common bean, *Phaseolus vulgaris*, includes the incorporation of pest resistance, alteration of plant type to provide more efficient conversion of soil nutrients, sunlight, CO_2 and atmospheric nitrogen, and improvement of yield potential *per se*. The presence of substantial genetic variability for traits affecting protein content, constituent fractions, amino acid composition and nutritional-depressing factors suggests that selection to improve these traits can proceed simultaneously with selection for higher yields.

GENETIC VARIABILITY OF PROTEIN QUANTITY AND QUALITY

VARIATION IN PERCENTAGE SEED PROTEIN.

The amount of protein, measured as a percentage of the total seed weight shows substantial variation due to both genetic and environmental factors. Often the variation reported for the same cultivar grown under different conditions may be nearly as large as the variation between different cultivars grown in uniform trials. Thus it is essential that test plants be grown in uniform replicated trials if valid estimates of genetic differences are to be obtained and breeding and genetic studies conducted.

There have been numerous reports of common bean strains that have either high or very low seed protein levels. Too often these differences are not observed when comparisons are obtained between materials grown in uniform trials. Consequently, we have found relatively few strains that consistently produce seeds that are high in protein. Considerable effort has been made to identify and study genotypes that are stable over different environments. We have found that two strains, BBL 240 and PI 229815, which were identified by Leleji (1971) have consistently produced high protein seeds (Tables 1 & 2). Unfortunately both are horticultural types which were selected for their edible pods, and they produce poor seed yields. Nevertheless they have been useful parents, and the breeding lines PROTOP W-1, PROTOP W-2 and PROTOP P-1, which are being tested further in 1978, are derived from the cross BBL 240 x PI 207227 (Table 3). These 3 selections demonstrate that high percentage protein can be transferred and combined with acceptable levels of seed yield through breeding.

Table 1. Variation in percentage protein among standard bean cultivars grown in Wisconsin for three years.

Strain	1975	1976	1977
	%	%	%
Sanilac	23.1	22.3	21.4
BBL 240	27.3	28.0	26.6
PI 229815	28.1	--	27.6
BTS	24.4	22.7	23.3

Table 2. Variation in percentage protein among standard cultivars grown in Wisconsin, 1972 and Colombia, S.A., 1973.

Strain	Wisconsin	Colombia
	%	%
PI 207227	21.5	22.4
PI 302542	25.1	26.1
BBL 240	28.1	28.0
PI 229815	31.9	28.4

TABLE 3. Characteristics and Performances of Three Superior Wisconsin Breeding Lines Chosen for Advanced Trials in 1978, and Their Comparisons to Standard Cultivars.

Designation	Seed		Plant	1976		1977			
				Hill Plots		Hill Plots		Row Plots	
	Color	1977 wt. gm/100 seeds	Type	Protein	Yield	Protein	Yield	Protein	Yield
				%	gm/plot	%	gm/plot	%	gm/plot
Protop-W1	White	31.5	3	28.3	183	29.3 ± .4	246 ± 13	27.5 ± 0	639 ± 157
Protop-W2	White	37.5	2	28.2	205	28.0 ± .1	222 ± 17	27.4 ± .6	656 ± 22
Protop-P1	Pinto	38.5	2	28.8	163	28.7 ± .5	132 ± 21	28.2 ± .9	749 ± 74
Sanilac	White	20.0	2	22.3	96	21.4 ± .5	144 ± 11	21.3 ± .9	649 ± 21
BBL 240	White	37.5	1	28.0	182	26.6 ± .5	158 ± 14	25.5 ± 0	553 ± 76
Bl. Turtle Soup	Black	23.0	2			23.3 ± .7	340 ± 38	22.4 ± .1	942 ± 227

Although some genotypes grown in certain environments produce up to 35% protein, these high levels are usually due to non-genetic factors. Among the materials studied to date, it appears, that genotypic differences account for a range in protein of approx. 17 to 30%. The protein levels of well-adapted cultivars having acceptable yields similar to those of cv. Sanilac are in the range of 27 to 28.5%. However, the amount of genetic variability available suggests that intercrossing high protein parents followed by appropriate selection should produce progenies with somewhat higher levels of seed protein. The performances during 1976 and 1977, of several strains that are promising sources of genes to enhance protein are shown in Table 4.

VARIATION IN PROTEIN COMPOSITION.

Genetic variability for seed protein fractions. Separation of the bean seed protein of 5 homozygous lines indicates variability in the relative amounts of protein fractions (Table 5). Ten F_5 lines resulting from bi-directional selection within populations from PI 207227 x BBL 240 were analyzed to determine the constituent fractions (Ma and Bliss, 1978b). G-1 was found to be the major fraction, accounting for 34 to 51% of the total seed protein; and the alkali-soluble fraction was also important (20 to 29% of the total) (Table 6). G1, G2, alkali-soluble and free amino acid fractions were positively correlated to total cotyledon proteins (Table 7). It appears that additional G1 protein constitutes the largest proportional increase among the fractions in high protein strains developed through selection. The alteration of specific protein fractions as a consequence of selection for total protein suggests that there is genetic variability for genes controlling each fraction. Therefore selection should be directed at altering specific protein fractions.

Table 4. Yield and percentage protein of bean strains identified as promising sources of genes that modify seed protein accumulation and/or storage. Hancock, Wis. 1976 and 1977.

Strain	Origin	1976 Yield	1976 Protein	1977 Yield	1977 Protein
		gm	%	gm	%
		- - - - - - High Protein - - - - - -			
BBL 240	U.S.	182	28.0	158 ± 14	26.6 ± .5
PI 229805	U.S.			135 ± 42	27.6 ± .2
Bonita	Puerto Rico	85	28.0	69 ± 28	27.3 ± 1.0
Endogava Z.N.	Canada			96	26.9
15R-148	Puerto Rico	160	27.5	160 ±12	28.1 ± .5
PI 311975	Mexico		27.7	261 ± 53	27.2 ± .4
PI 312041	Mexico		27.8	249 ± 49	26.8 ± 1.6
Protop W-1	U.S. (Wisc.)	183	28.3	246 ± 13	29.3 ± .4
Protop W-2	U.S. (Wisc.)	205	28.2	222 ± 17	28.0 ± .1
Protop P-1	U.S. (Wisc.)	163	28.8	132 ± 21	28.7 ± 5
		- - - - - - Low Protein - - - - - -			
Swedish Brown	Canada	156	21.3	136 ± 105	19.1 ± 1.0
Pirol			18.4	126 ± 9	17.8 ± .7
		- - - - - - Standard Lines - - - - -			
Sanilac		96	22.3	144 ± 11	21.4 ± .5
Black Turtle Soup			22.7	340 ± 38	23.3 ± .7

Table 5. Constituents of seed storage protein in the cotyledons of five homozygous strains of common bean (*Phaseolus vulgaris* L.).

Fraction		PI 207227	PI 302542	BBL 240	PI 229815	Sanilac
Albumin	(1)†	31.7 ± .1‡	39.0 ± .5	43.2 ± 1.9	42.0 ± 1.3	35.5
	(2)	12.4	14.5	15.5	15.0	14.0
Globulin-1	(1)	93.3 ± 2.3	103.3 ± 2.5	123.5 ± 2.4	129.4 ± 2.2	114.1
	(2)	36.4	38.6	44.3	46.1	45.1
Globulin-2	(1)	19.1 ± .3	23.4 ± .7	20.6 ± .5	14.7 ± 1.1	30.9
	(2)	7.5	8.7	7.4	5.2	12.2
I-fraction	(1)	100.4 ± 4.0	89.7 ± 1.3	74.8 ± .2	82.2 ± 3.0	62.8
	(2)	39.2	33.5	26.8	29.3	24.8
Free amino acids	(1)	11.4 ± .1	12.3 ± .1	16.6 ± .4	12.3 ± .3	10.7
	(2)	4.5	4.6	6.0	4.4	4.2
Total Cotyledon protein	(1)	256.0	259.0	277.0	280.0	254.0
Percentage recovery§		100.0	103.3	100.6	100.2	98.4

† (1) mg protein/g cotyledon; (2) percentage of each fraction among total proteins.

‡ Results are mean and standard error of mean values of triplicate determination except Sanilac.

§ Percentage recovery = sum of individual fractions/total cotyledon protein (independent estimate).

From: Ma and Bliss, 1978b.

Table 6. Constituents of seed storage protein in the mature cotyledons of 10 F_5 lines of bean from the cross PI 207227 x BBL 240. (From: Ma and Bliss, 1978b)

Line		Total protein	Protein fraction							Recovery[§]		Seed yield (g/plant)
			Albumin	G-1	G-2	Prolamine	Alkali-soluble	Residue	Free a.a.	Total (mg)	Percentage	
742015	(1)[†]	275.0	32.0[‡]	131.9	21.2	8.3	59.6	7.5	26.7	287.2	104.4	25.1
	(2)		11.1	45.9	7.4	2.9	20.8	2.6	9.3			
742016	(1)	240.0	33.0	117.7	11.1	7.0	56.7	6.4	12.3	231.4	96.4	27.8
	(2)		14.3	40.8	4.8	3.0	24.5	2.8	5.3			
742032	(1)	322.0	37.8	166.8	19.4	7.1	64.0	6.1	24.8	326.0	101.2	37.7
	(2)		11.6	51.2	6.0	2.2	19.6	1.9	7.6			
742047	(1)	191.0	36.8	64.0	9.4	6.3	51.9	6.3	11.2	185.9	97.3	32.5
	(2)		19.8	34.4	5.1	3.4	27.9	3.4	6.0			
742065	(1)	247.0	38.2	104.2	18.2	6.4	55.1	7.0	15.4	244.5	99.0	26.7
	(2)		15.6	42.6	7.4	2.6	22.5	2.9	6.3			
742066	(1)	189.0	33.5	65.0	8.9	7.0	55.9	6.9	12.0	189.2	100.1	28.8
	(2)		17.7	34.4	4.7	3.7	29.5	3.6	6.3			
742069	(1)	214.0	32.5	84.7	16.1	7.4	52.3	8.0	11.2	212.2	99.2	28.0
	(2)		15.3	39.9	7.6	3.5	24.6	3.8	5.3			
742071	(1)	262.0	32.5	112.0	15.4	6.9	58.0	5.8	16.6	247.2	94.4	27.2
	(2)		13.1	45.2	6.2	2.8	23.5	2.3	6.7			
742077	(1)	295.0	39.7	115.3	16.6	8.5	70.2	5.5	24.0	279.8	94.8	20.4
	(2)		14.2	41.2	5.9	3.4	28.4	2.0	8.6			
742090	(1)	199.0	28.1	76.6	8.3	5.7	55.5	5.5	11.3	191.0	96.0	20.4
	(2)		14.7	40.1	4.3	3.0	29.1	2.9	5.9			

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

[‡] Results are mean values of duplicate determinations.

[§] Total recovery = sum of individual fractions; Percentage recovery = sum of individual fractions/total cotyledon protein (independent estimate).

Table 7. Phenotypic correlations between total cotyledon protein and constituent fractions of 10 F_5 lines from the cross PI 207227 x BBL 240.

Trait	Total cotyledon protein	Albumin	G-1	G-2	Prolamine	Alkali-soluble	Residue
Albumin	.485						
G-1	.941**	.312					
G-2	.803**	.381	.766**				
Prolamine	.586	.324	.457	.617			
Alkali-soluble	.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

From: Ma and Bliss, 1978b.

Variation in amino acid composition. The amino acid composition of the constituent fractions of bean protein varies greatly (Table 8). The methionine content of the G1 and alkali-soluble fractions is particularly important since the sulfur amino acids are usually limiting and these two fractions constitute a major portion of the total protein. Methionine makes up about 1.1% of the cotyledon protein. The G1 is relatively poor in methionine (.88%), while the alkali-soluble fraction is quite rich (1,99%). This suggests that major alteration of the relative amounts of these fractions should have a large effect on the methionine content of the whole bean flour, since cotyledon protein and methionine are correlated to the protein and methionine in the alkali-soluble fraction (Table 9). It was believed initially that the alkali-soluble fraction was composed of glutelin, however it was found to contain alkali-soluble albumins also. A screening procedure to detect genotypes with high protein and a large amount of alkali-soluble protein has been suggested by Ma and Bliss (1978b).

Substantial variation in the methionine content of seed flower has been reported (Kelly, 1971; Kelly and Bliss, 1975; Leleji, 1971). This variation has been shown to affect rate of gain and Protein Efficiency Ratio (PER) (Kelly and Bliss, 1975). Analyses of 15 strains show a range of from .91 to 1.44% methionine per unit of protein. Similar differences based on methionine per seed and per unit of bean flour exist (Table 10). The availability of rapid, accurate methionine assays should allow successful alteration of this amino acid as well as total seed protein. We now have a clear basis for understanding the relationships between seed protein and methionine.

Table 8. Amino acid composition of the cotyledon protein and different protein fractions based on the average values of 10 F_5 lines (mg/100 mg of protein). Data corrected to 100% recovery.

AMINO ACID	Cotyledon	Albumin	Globulin-1	Globulin-2	Prolamine	Alkali-soluble	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
Actual recovery	102.2	107.0	103.7	96.1	96.6	92.4	100.2

† Values including their amide form.

From: Ma and Bliss, 1978b.

Table 9. Phenotypic correlations regarding protein and methionine of the total cotyledon and Alkali-soluble fraction of 10 F_5 lines.

Traits correlated	r value
Total cotyledon protein (mg/g flour) vs Alkali-sol protein (mg/g flour)	.813**
Total cotvledon protein (mg/cotyledon) vs Alkali-sol protein (mg/g cotyledon)	.950**
Total cotyledon methionine (mg/g flour) vs Alkali-sol methionine (mg/g flour)	.747*
Total cotyledon methionine (mg/cotyledon) vs Alkali-sol methionine (mg/cotyledon)	.929**
Protein ratio ($\frac{\text{Alkali-sol protein}}{\text{cotyledon protein}}$) vs methionine ratio ($\frac{\text{Alkali-sol methionine}}{\text{cotyledon methionine}}$)	.766**

* significant at 5% level.

** significant at 1% level.

From: Ma and Bliss, 1978b.

Table 10. Protein and methionine composition of the seed flour and the I-fractions (ascorbate-NaCl insoluble proteins) of 15 pure lines of common bean (*Phaseolus vulgaris* L.), expressed as (a) mg/g of seed flour, and (b) mg/seed.

Line		Protein (mg)			Methionine				
					I-fraction		Seed		
		I-frac.	Seed	Ratio‡	(%)§	(mg)	(%)§	(mg)	Ratio¶
BBL 240	a.	73.7†	273.0	0.27	2.01	1.48	1.44	3.93	0.38
	b.	29.5	109.2			.59		1.57	
PI, 229815	a.	90.5	281.0	0.32	1.70	1.54	1.11	3.12	0.49
	b.	23.6	73.3			.40	...	.81	
Sanilac	a.	68.8	231.0	0.30	1.36	.94	1.19	2.75	0.34
	b.	18.5	62.1			.25		.74	
WCBT-713938	a.	81.0	275.0	0.30	1.33	1.08	1.06	2.92	0.37
	b.	21.8	74.0			.29		.79	
Rust Diff. -765	a.	85.5	231.0	0.37	1.37	1.18	1.01	2.33	0.50
	b.	24.9	67.2			.34		.68	
H 2222	a.	74.4	177.0	0.42	1.37	1.02	1.09	1.93	0.54
	b.	31.0	73.8			.43		.80	
Black Turtle Soup	a.	77.8	244.0	0.32	1.55	1.21	1.01	2.46	0.49
	b.	24.6	77.1			.38		.78	
State Half Runner	a.	74.0	266.0	0.28	1.51	1.12	1.06	2.82	0.39
	b.	24.0	86.5			.38		.92	
Tui	a.	78.7	268.0	0.29	1.38	1.09	.96	2.57	0.42
	b.	21.8	74.2			.30		.71	
Imperial Pea	a.	76.9	244.0	0.32	1.56	1.20	.98	2.39	0.50
	b.	22.5	71.2			.35		.70	
Alberta Brown	a.	73.7	249.0	0.30	1.24	.91	.99	2.47	0.37
	b.	27.5	92.0			.34		.92	
Norway Brown	a.	67.6	235.0	0.29	1.31	.89	.98	2.30	0.39
	b.	24.7	86.0			.33		.84	
Swedish Brown	a.	61.8	194.0	0.32	1.70	1.05	.91	1.77	0.59
	b.	22.0	69.1			.37		.63	
Endogava Z.N.	a.	82.4	273.0	0.30	1.41	1.16	1.05	2.87	0.40
	b.	25.8	85.7			.36		.90	
O.S.U. 58	a.	64.1	276.0	0.23	1.20	.77	1.14	3.15	0.24
	b.	22.8	98.0			.27		1.12	

† Results are mean values of triplicate determinations.

‡ mg I-fraction protein/mg seed protein.

§ mg I-fraction or seed methionine/100 mg I-fraction or seed protein, respectively.

¶ mg I-fraction methionine/mg seed methionine.

From: Ma and Bliss, 1978b.

HERITABILITY OF PROTEIN VARIATION.

Total seed protein. The heritability of variation in bean seed protein (percentage protein) is usually in the low to medium range, a consequence of large effects of non-genetic factors such as the environment. The magnitudes of the estimates vary depending on the population studied, sites used for estimation and the method of estimation. Most estimates range from .25 to .50 (Leleji et al., 1972; Porter, 1972), with occasional values to .75. Kelly (1975) used variance estimates from segregating generations and parent/offspring regressions to estimate heritability. These are summarized in Table 11.

Realized heritability estimates from populations resulting from bi-directional selection in the progeny of BBL 240 x PI 207227 ranged from .30 to .69, depending on the generation and direction of selection. Although the magnitudes of estimates vary, heritability is large enough to permit improvement by hybridization and selection.

Methionine content. A major problem in studying sulfur amino acid content is the lack of adequate assays. Estimates have been based on determinations of total sulfur, methionine and cystine using an automatic amino acid analyzer, microbial assays and various lab animal growth tests. Variation in the amount of sulfur-containing amino acids, particularly methionine, is of interest since it is often considered to be the primary limiting amino acid of legume protein. Kelly (1971) reported differences in methionine content of bean seeds. Although there are few estimates, heritability of seed methionine is usually low to medium. The heritability estimates of methionine differences obtained by Kelly (1975) are summarized in Table 12.

The demonstration by Ma and Bliss (1978b) that various protein fractions differ substantially in methionine content should allow greater precision in determining the magnitude of genetic differences, heritability and optimum breeding strategies.

Table 11. Heritability estimates for percentage seed protein in several populations of common bean.

Population	Magnitude of heritability			
	Broad sense		Narrow sense	
	Wisc.	Colombia	Wisc.	Colombia
	- - - - Segregating generations - - - -			
BBL 240 x PI 207227	.69	.46		
BBL 240 x PI 302542	.32	.16		
BBL 240 x PI 229815	.71	.73		
	- - - Parent/offspring regression - - -			
BBL 240 x PI 207227				
F_3/F_2			.36	.79
F_4/F_3			.69	.32
BBL 240 x PI 229815				
F_3/F_2				.63
F_4/F_3				.61

From: Kelly, J. D., 1975.

Table 12. Heritability estimates for percent methionine (gm/100 gm protein) in several popualtions of common bean.

Population	Magnitude of heritability			
	Broad sense		Narrow sense	
	Wisc.	Colombia	Wisc.	Colombia
	- - - - Segregating generations - - - -			
BBL 240 x PI 207227	.38	.64		
BBL 240 x PI 302542	.39	.54		
BBL 240 x PI 229815	.60	.73		
	- - - Parent/offspring regression - - -			
BBL 240 x PI 207227				
F_3/F_2				.81
F_4/F_3				.51
BBL 240 x PI 229815				
F_3/F_2				.85
F_4/F_3				.81

From: Kelly, J. D., 1975.

VARIATION IN ELECTROPHORETIC PATTERNS OF THE G1 SUBUNITS

Globulin-1 (G1) protein reversibly dissociates between a tetrameric form at pH 4 (Mol. wt. approx. 600,000), a monomeric form at neutral pH (Mol. wt. 150,000) and free polypeptide subunits at pH values above 11.5 (Mol. wt. approx. 50,000). The G1 fraction of cv. Tendergreen is resolved into 3 subunits (α mol. wt. 53,000, β mol. wt. 47,000 and γ mol. wt. 43,000) (Sun et al., 1974). Analyses of the electrophoretic patterns of the G1 protein from other genotypes of *P. vulgaris* suggest genetic variation in the banding pattern, particularly with regard to the α subunit (Fig. 1).

Variation in the α subunit is such that in strains PI 207,227, PI 229,815 and Sanilac, it migrates differently indicating a smaller Mol. wt. of about 50,500 (" α 50.5"). Genetic analyses of F_1 seeds from BBL 240 x PI 229,815 showed a banding pattern in which both parental types were expressed suggesting co-dominant alleles. The segregation for subunit pattern in F_2 seeds suggested that control was due to a single Mendelian gene (Romero et al., 1975). Subsequent studies involving the variant polypeptide have shown that there is equal expression of maternal and paternal alleles in the progeny of parents differing genetically for the " α 53" and the " α 50.5" subunits (Hall et al., 1977).

The effects of these subunit differences on quantitative and qualitative expression of the G1 protein is not known. Earlier studies suggested a relationship between genotypes having the " α 53" subunit and high levels of methionine, but this has not yet been confirmed.

G1 banding patterns of other *Phaseolus* species are considerably different from *P. vulgaris* and can be used for species identification.

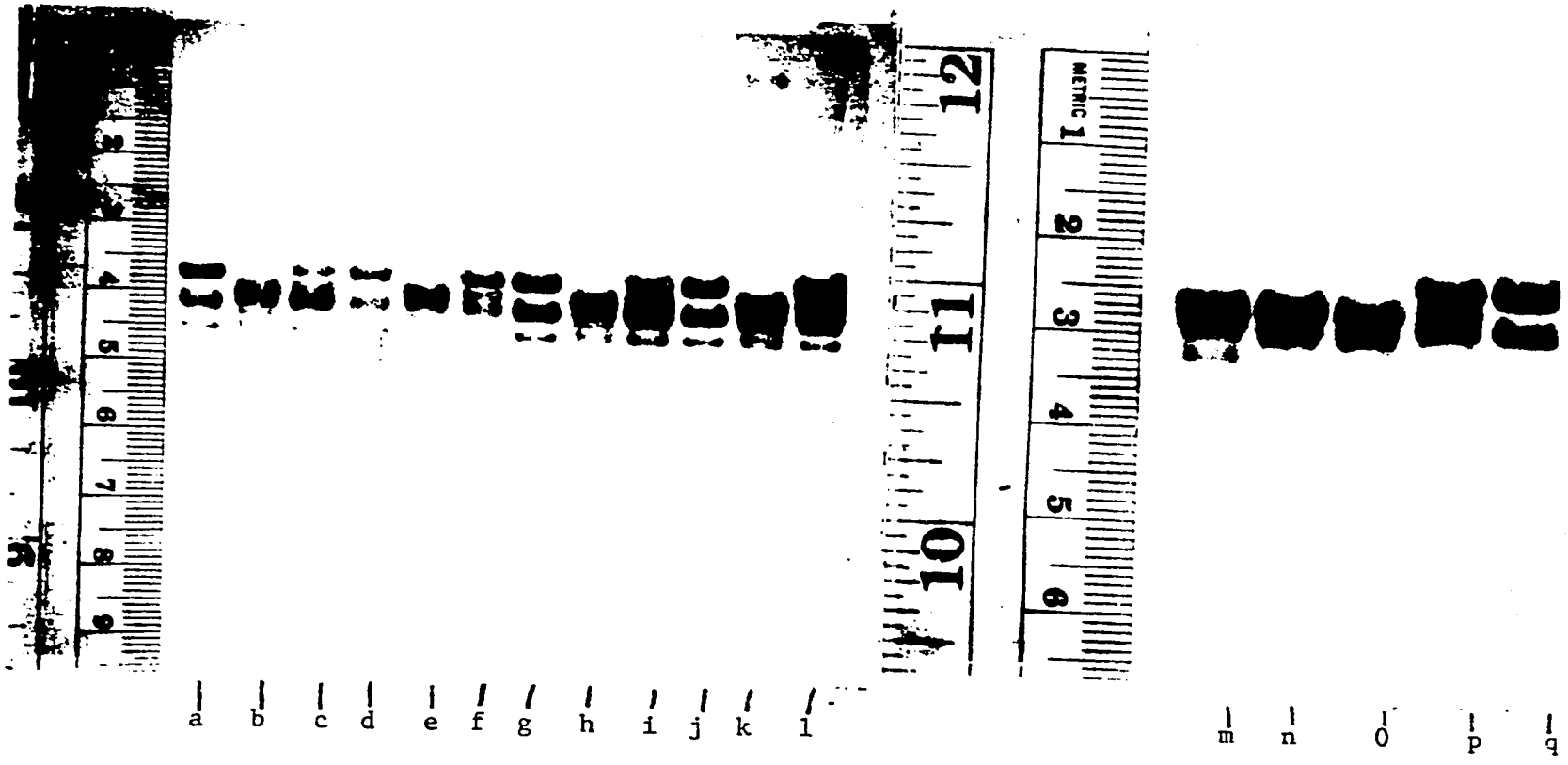


Fig. 1. Polypeptide subunits of the G1 protein from several strains of Phaseolus vulgaris separated on discontinuous SDS slab gels. Patterns are shown for: (a) BBL 240; (b) PI 229815; (c) F_1 (PI 229815 x BBL 240); (d) BBL 240; (e) PI 229815; (f) 'Contender'. Protein was at a conc. of 1 µg/µl .5 M NaCl, 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.

VARIATION IN TANNIN CONTENT.

Tannins in bean seeds are found in the testae of colored-seeded types (Ronnenkamp, 1977; Ma and Bliss, 1978a). Analyses of white-seeded strains and colored-seeded strains with the testae removed indicated little or no tannin in the cotyledon tissue. Although tannin is associated with seed color, there appears to be a range of tannin levels within each color class (Table 13). Ma and Bliss (1978) and Ronnenkamp (1977) have reported heritable differences in tannin content, and although tannin is present in colored segregates, it appears possible to select for most seed coat colors with either high or low tannin levels (Table 14).

The reduction in nutritional value associated with presence of tannin (Ronnenkamp, 1977) suggests that it should be eliminated through breeding. However, before proceeding too far it is necessary to determine the effects of reducing tannin levels on disease and insect resistance. Many colored-seeded strains are known to have higher resistance levels, but again there is much variation among colored-seeded types with regard to almost any pest resistance.

Table 13. Seed protein and tannin content of twenty-nine common bean (*Phaseolus vulgaris* L.) strains. Tannin was extracted using 100% methanol.

Line	Testa color	Percentage protein	Catechin equivalent (C.E.) value (mg/g flour)	
			Cotyledons W/O testa	Whole seed
--- Seed grown in Wisconsin ---				
Sanilac	White	24.5	2.04± .14	2.48± .06
PI 207227	Black	21.5	2.40± .34	18.86± .92
PI 302542	White	25.1	2.40± .10	3.26± .10
BBL 240	White	28.1	2.42± .16	2.72± .26
PI 229815	Buff	31.9	3.26± .14	2.76± .10
74-2015	Dark Bronze	27.5	1.44± .10	8.16± .32
74-2016	Dark Bronze	24.0	2.78± .70	8.14± .36
74-2032	White	32.2	2.02± .66	1.98± .00
74-2047	Bronze	19.1	1.90± .34	7.10± .32
74-2065	Brown/White patches	24.7	1.64± .06	2.40± .10
74-2066	Black	18.9	2.40± .04	2.52± .32
74-2069	Black	21.4	2.16± .40	2.48± .24
74-2071	White	26.2	2.20± .06	1.50± .28
74-2077	White	29.5	1.98± .16	1.92± .10
74-2090	Black	19.9	1.66± .12	2.76± .18
--- Seed grown in Puerto Rico ---				
Bonita	Ivory-white	24.5	0.44± .16	2.40± .20
French Hort.	Mosaic red	19.3	0.72± .14	14.10± .24
Venzuela 36	Black	22.3	0.26± .00	3.40± .00
Ecuador 299	Red	20.4	2.22± .26	7.18± .00
Mexico 309	Black	20.3	2.40± .44	5.50± .20
Porillo	Black	19.6	0.56± .04	5.92± .16
50600	Black	22.1	0.98± .16	5.44± .06
LaVega	Black	19.7	0.62± .16	3.42± .00
51052	Black	21.0	1.34± .48	4.60± .18
Mexico 27R	Red	19.6	0.50± .20	13.86± .54
15-R-55	Black	22.3	1.08± .24	10.60± .00
Jamapa	Black	20.7	0.74± .04	4.76± .00
71-1R-113	Black	20.8	0.80± .14	4.04± .00
Colorado de Pais	Mosaic red	21.0	0.62± .28	15.10± .10

From: Ma and Bliss, 1978a.

Table 14. The mean, standard error of mean, and variance of each generation and the broad sense heritability for each cross.

Year grown	Pedigree	Testa color‡	Generation	C.E. values $\bar{x}$ ± S.E.	s^2 Variance	H_b†
1971	PI 207227	Bl	P_1	49.2 ± 3.3	87.10	.48
	x					
	BBL 240	Wh	P_2	.9 ± .1	.12	
			F_1	18.8 ± .6	4.24	
			F_2	10.5 ± .8	83.80	
1973	PI 207227	Bl	P_1	35.2 ± .6	3.72	.97
	x					
	PI 229815	Bu	P_2	3.3 ± .2	.22	
			F_2	20.3 ± 1.8	171.71	
1976	PI 224730	Bl	P_1	35.8 ± 1.3	12.58	.96
	x					
	PI 311917	Bu	P_2	6.1 ± .1	.08	
			F_2	15.3 ± 1.7	166.16	
	PI 311991	Bl	P_1	20.8 ± 1.3	20.61	.84
	x					
	PI 309726	Gr-Bu	P_2	3.4 ± .1	.09	
			F_2	10.9 ± 1.3	65.22	

† H_b = Broad sense heritability = $s^2_{F_2} / [(s^2_{P_1} + s^2_{P_2})/2]$.
‡ Bl = black, wh = white, Bu = buff, Gr-Bu = greenish-buff.

From: Ma and Bliss, 1978a.

RELATIONSHIPS BETWEEN PROTEIN AND OTHER TRAITS.

Yield and protein. Most studies involving bean seed protein have shown negative correlations between percentage protein and seed yield. These have been relatively small, usually in the range 0 to -.30 (Kelly, 1975). Although the small magnitude suggests that improvement of both traits should be possible, certain precautions should be followed during selection. The results of bi-directional selection for high and low percentage protein without regard to seed yield clearly show that high protein selections produced less seed of generally poorer quality (Bliss et al., 1974). Plants selected for low percentage protein were generally higher yielding but the seed quality of some of these selections was also poor. Selection for higher levels of protein should be done using procedures that allow for consideration of seed size and total seed yield, to insure that increases in percentage protein are not merely a decrease in seed starch accumulation (ie. shriveled seed).

Although definitive studies are not completed it appears that the accumulation of nitrogen begins and terminates earlier than accumulation of starch in the seed. Thus factors that reduce starch accumulation (and therefore yield) may have less effect on protein since it is already present. Reductions in yield are associated with an increase in percentage nitrogen, but there is no increase in the amount of protein per seed. Thus a negative correlation results.

Percentage protein and methionine. The relationship between percentage protein and methionine content/unit protein varies depending on the strains studied. Adams (1975) reported a negative correlation based largely on pure lines, while Hackler and Dickson (1973) found no correlation and

Kelly (1975) reported a positive correlation of +.33 between percentage protein and percentage methionine. If these values are representative, they suggest that raising both the quantity of protein and methionine should be possible.

Knowledge of the constituent protein fractions and the methionine content of each offers a likely explanation of the observed relationships. Since globulin-1 (G1) is the major fraction but is low in methionine, large relative increases in this fraction will result in a lower percentage methionine of the total protein. Since high protein is associated with increases in the G1, this probably accounts for situations in which negative correlations appear. Other possibilities which are more favorable may also occur. In lines which have large amounts of fractions other than globulins, higher protein levels may be associated with high methionine since the albumins and the alkali-soluble proteins are rich in methionine. It appears that improvement of methionine is possible and that significant alteration will depend on the ability to differentially regulate synthesis and accumulation of the constituent fractions. This is not surprising since similar situations exist in the cereals such as corn, barley and sorghum.

UTILIZATION OF GENETIC VARIABILITY

BI-DIRECTIONAL SELECTION FOR PERCENTAGE PROTEIN.

Broad sense heritability estimates of percentage protein in the F_2 of BBL 240 x PI 207227 were .69 and .73 when the populations were grown in Wisconsin and Colombia, South America, respectively. Beginning in the F_2 and through the F_5, the 10% of the plants producing seeds with the highest, and 10% with the lowest percentage protein were selected as parents for the following generation. The resulting populations diverged significantly, with the mean of the low population being 22.3% and, the mean of the high population 30.2% (Table 15). Of considerable interest was the correlated response of seed yield/plant. Mean seed yield of the high protein population was only about 60% of that of the low protein population. This shows that selection for high protein *per se* without regard to seed yield produced an undesirable effect, at least in this population. During the course of selection some plants were observed to produce good yields with relatively high (but not the highest) percentage protein. These were selected for several generations and three lines, Protop W-1, Protop W-2 and Protop P-1, which combine high percentage protein with good yields of large, high quality seeds have now been developed (Table 3).

To further compare gain from selection in this population, 140 lines were advanced from the F_2 through F_5 generations by Single Seed Descent (SSD). F_5 families were grown in replicated trials and the 14 lines having highest percentage protein and 14 lines with lowest percentage protein were selected. F_6 seeds from these selections and from the 14 high and 14 low selections of the bi-directional experiment were increased in the field in 1977. Uniform comparisons will be made in the field in 1978, to determine actual gain from selection and correlated yield response for both modified pedigree selection (SSD) and standard pedigree selection.

Table 15. 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		Seed Yield (gm/plant)
		Actual	Predicted	
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	

POPULATION IMPROVEMENT THROUGH RECURRENT SELECTION

A genetically variable population formed by intercrossing diverse genotypes provides materials that can be improved by recurrent selection. It is desirable to have a high population mean, and necessary to have substantial genetic variance for the trait(s) undergoing selection, as well as a high frequency of favorable alleles as possible, if genetic gain is to be realized. The contribution to genetic variance and the number of controlling loci are related to the breeding value of each parent, while the frequency of the favorable allele(s) at each locus depends on the genotype of the parents and the number of parents containing the favorable allele(s).

The initial composite population was formed in 1974 by intercrossing 37 different strains in various combinations. These represented a wide array of materials from the U.S. and Latin America and included commercially-grown cultivars. All entries had not been evaluated in uniform trials, thus the phenotypic value of each regarding yield and percentage protein was not known precisely. Most had been evaluated in separate trials and only 5 were considered to be superior for seed protein. Two of the 5 were snap-bean cultivars which produced low seed yield. Consequently the frequency of superior "protein genes" in this population was low as was the genotypic variance for percentage protein. Nevertheless selection for yield and percentage protein was begun since this was the best material available.

Tolla (1978) reported that populations resulting from 2 cycles of recurrent selection using several procedures showed little if any improvement over the initial composite population for either trait, when the selected populations were grown in a uniform trial. However, the yield

levels and percentage protein of the selected and unselected populations compared favorably to 3 check cultivars, Black Turtle Soup, Sanilac and BBL 240. The mean of the best 5 families selected in 1976 was higher than those selected in 1975 (Table 16). But since, cycle 1 and cycle 2 selections were made in different years, the effects due to years and to cycles of selection were confounded. Comparisons of the performances of the best 3 families from cycles 1 and 2 to the unselected original population showed small differences in Selection Procedure 1, but these differences were not statistically significant (Table 17). Although results were inconclusive, the Selection Procedure 1 that provided for identification of the highest yielding families based on their mean performance followed by selection of the high protein individuals within these families appeared promising. Selection was also practiced for yield and percentage protein under field conditions where combined nitrogen was either added or withheld in addition to nitrogen fixation. Interestingly those populations developed without added nitrogen, performed better, even when combined nitrogen was present in uniform comparisons (Table 18). Seed increases of the selections made in each cycle are planned for 1978, with uniform comparisons to be made in 1979.

Tolla (1978) suggested that in addition to more precise comparisons of the selected families, the original composite should be made up of parents selected for their yielding ability, that the frequency of superior genes controlling protein should be higher and that selection for yield be based on replicated family performance followed by selection of high protein individuals. To follow this suggestion we have made up a composite from diallel crosses among 6 selected white-seeded parents, Bunsi, Bonita, Nep 2, Sanilac, BBL 240 and M-1. Selection will be

Table 16. 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.

Table 17. Yield (gm/plant) and seed protein (%) of the three best S_3 families resulting from two cycles of selection with two selection procedures and evaluated in a uniform trial,[z] 1977.

Entry	Selection procedure 1[y] Yield	Selection procedure 1[y] Protein	Selection procedure 2[x] Yield	Selection procedure 2[x] Protein	Checks Yield	Checks Protein
	gm	%	gm	%	gm	%
C_0 (unselected)	23.7	24.5	23.7	24.5		
C_1 (1st cycle)	26.3	23.2	22.9	24.8		
	20.2	23.4	18.2	25.2		
	19.1	24.0	16.7	27.7		
Mean	21.9	23.5	19.3	25.9		
C_2 (2nd cycle)	25.6	24.5	18.5	26.1		
	23.8	24.4	16.4	24.6		
	21.7	25.1	12.1	27.3		
Mean	23.7	24.7	15.7	26.0		
Sanilac					21.5	19.6
Bunsi					15.3	25.6
Bl. Turtle Soup					28.5	22.4

[z]N Fertilizer was not applied but an alfalfa crop was incorporated prior to planting the trial.

[y]Selection between S_1 family means for yield and within the highest yielding S_1 families for percentage protein.

[x]Selection between S_1 family means for yield and between S_1 family means for percentage protein.

From: Tolla, 1978

Table 18. Yield (gm/plant) and seed protein (%) for two cycles of recurrent selection with and without added N and evaluated with and without N, 1977.

Evaluation condition	Cycle	Recurrent selection procedure: Without N Yield	Without N Protein	With N Yield	With N Protein	Check cultivars	Yield	Protein
		gm	%	gm	%		gm	%
Without N	C_0	20.2	23.4	20.2	23.4	Sanilac	8.9	19.8
	C_1	18.5	23.8	22.0	24.0	Bunsi	13.8	24.5
	C_2	20.4	23.8	19.0	24.1	BBL 240	8.0	27.9
						Bl. Turtle Soup	20.4	23.2
With N	C_0	30.1	23.8	30.1	23.8	Sanilac	17.2	19.9
	C_1	29.4	22.8	26.1	23.8	Bunsi	18.8	22.7
	C_2	33.6	22.5	20.3	24.6	BBL 240	22.1	26.7
						Bl. Turtle Soup	28.9	23.0

From: Tolla, 1978.

initiated beginning in 1978. We anticipate introgressing germplasm from other outstanding materials that become available.

Although our results from recurrent selection to date are inconclusive, we feel that genetic gain will result after making the modifications described. It is expected that greater progress will be made in populations that have better initial parents. Some breeders have suggested intercrossing large populations of diverse material with little regard for parental superiority of yield and other important traits. It is unlikely that such an approach will show much progress, at least in the short run.

IDENTIFICATION OF GENES THAT ALTER PROTEIN.

The segregation patterns in F_2 populations of the crosses studied by Kelly (1975) suggest that relatively few genes account for the protein differences between the parents. The frequency distributions appear to fit a normal distribution, suggesting quantitative inheritance. Our knowledge of the constituent fractions of the seed protein suggest that there are not likely to be "protein genes" *per se*, but rather genes that alter specific protein fractions either singularly or concurrently.

To test the possibility that there are genes with discernible effects that alter quantitative amounts of G1 and other fractions we are using the backcross/selfing procedure described by Wehrhahn and Allard (1965), to develop a series of near isogenic lines in 'Sanilac' germplasm that should differ by as few as a single gene if such genes exist and alter G1 or other fractions. Four high protein parents, BBL 240, PI 229815, PI 302542 and 15R-148 and the low parents H2222 and Swedish Brown have been used as putitive donors of genes which regulate protein fraction synthesis. The F_1 plus 2 backcross and 3 self generations are being completed to provide populations of about 60 backcross/inbred lines in each of the 6 populations. The procedure shown in Fig. 2, as proposed by Wehrhahn and Allard (1965) is being followed here. The first analyses will be made in 1978 and 1979.

Generation	Crossing procedure	Genetic expectation
1	$P_1 \times P_2$	aa x AA
2	$P_1 \times F_1$	aa x Aa
3	$P_1 \times 256\ B_1$	aa x (128Aa:128aa)
4	$P_1 \times 256\ B_{11}$	aa x (64Aa:192aa)
5	$256\ B_{111}$ selfed 3-5 generations	

R = 3 backcrosses.

Fig. 2. A backcross/selfing procedure for detecting and measuring the effects of individual genes involved in the inheritance of a quantitative trait (after Wehrhahn and Allard, 1965).

EXCHANGE OF PLANT MATERIALS FOR MULTIPLE EVALUATION.

The most useful germplasm for improvement programs is material that not only possesses improved levels of protein but also has other desirable attributes such as yield potential, wide adaptability and pest resistance. To increase the potential for identifying such strains we have developed a program to exchange materials with Dr. G. Freytag and Dr. N. Vakili at the Mayaguez Institute for Tropical Agriculture (MITA), and some of our selections have been evaluated for yield potential and disease resistance by Dr. .'. Temple of the Centro International Agricultura Tropical (CIAT) and Dr. G. Hernandez-Bravo, formerly at CIAT. This exchange of material with evaluation for different traits at various locations has proved useful for identifying promising lines that are now being further tested.

Protein Analyses of MITA Lines. The 15 genotypes used by Dr. Freytag as parents of a composite population were grown at Hancock, Wisconsin, in 1976. Only one entry failed to yield as well or better than 'Sanilac' the standard check (Table 19). Two entries, Bonita (white-seeded) and 15R-148 (red-seeded) had higher percentage protein values than Sanilac, but had relatively low yields compared to other entries. 15R-148 has high protein values, it also possesses some resistance to rust and Rhizoctonia root rot and is only mildly susceptible to bacterial blight in Puerto Rico. Its potential as a high protein parent is indicated by the performance of mixed-colored selections (Entry nos. 35-49) shown in Table 20. All entries except No. 35 and 42 contain 15R-148 as a parent (ie. No. 8 in the pedigree). The mean of the mixed colored selections is 25.4; compared to the black seeded 23.1; and white seeded 23.4. Since all were grown in the same replicated trials

Table 19. 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 20 Percentage seed protein of 4 check varieties and 44 advanced breeding lines grown at 3 locations in Puerto Rico by Dr. G. F. Freytag, MITA. Seed analyzed at University of Wisconsin, Madison.

Entry No.	Pedigree	Isabella	Fortuna	Limani	
	- - - - - - - Check varieties - - - - - - -				
1	La Vega	22.1 ± .71[z]	23.3 ± .46	22.8 ± .53	22.7
2	Mex 309	20.7 ± 1.31	23.3 ± .59	25.5 ± .43	23.2
3	Ecuador 299	18.4 ± .76	19.4 ± .83	25.4 ± 1.32	21.1
4	Bonita	22.8 ± 1.21	24.7 ± .54	25.8 ± .19	24.4
	- - - - - - - - Black-seeded - - - - - - -				
5	(2x6)-B47-BK	20.9 ± .81	24.0 ± .59	24.7 ± .92	23.2
6	(9x5)-B365-BK	20.2 ± .78	23.9 ± .55	24.2 ± .68	22.8
7	(5x3)-B257-BK	21.1 ± .94	23.7 ± 1.11	25.9 ± .60	23.6
8	(2x6)-B48-BK	20.0 ± 1.14	22.6 ± .65	24.2 ± .66	22.3
9	(9x3)-B354-BK	22.8 ± 1.22	24.1 ± 1.09	27.1 ± .26	24.7
10	(5x9)-B281-BK	21.1 ± .57	22.9 ± .90	23.9 ± .38	22.6
11	(5x2)-B242-BK	21.3 ± .84	23.5 ± .46	26.3 ± .67	23.7
12	Jamapa-B433-BK	20.4 ± .94	23.0 ± .35	21.6 ± 1.10	21.7
13	(2x6)-B57-BK	20.2 ± 1.07	23.7 ± .90	25.8 ± 1.06	23.2
14	(3x5)-B128-BK	22.0 ± .74	23.5 ± .64	23.9 ± .77	23.1
15	(4x5)-B190-BK	20.0 ± .85	21.5 ± .32	24.9 ± .43	22.1
16	(9x2)-B351)-BK	21.4 ± .48	23.0 ± .87	23.1 ± .29	22.5
17	(3x6)-B113-BK	22.2 ± 1.57	24.8 ± .33	25.8 ± .67	24.3
18	(2x6)-B50-BK	20.9 ± 1.02	23.7 ± .72	25.6 ± 1.13	23.4
19	(1x9)-B8-BK	21.9 ± .79	22.3 ± .54	22.9 ± .43	22.4
20	(2x6)-B54-BK	20.9 ± .74	24.7 ± .90	25.3 ± .40	23.6
				Black mean - -	23.1
	- - - - - - - White-seeded - - - - - - -				
21	(4x9)-W41-BK	21.2 ± .72	22.6 ± .56	25.9 ± .56	23.2
22	(9x3)-W117-BK	22.2 ± .39	24.2 ± .53	24.3 ± .49	23.6
23	(9x2)-W105-BK	22.1 ± 1.16	24.2 ± 1.02	25.3 ± .41	23.9
24	(9x8)-W126-BK	22.3 ± 1.65	24.8 ± .51	24.1 ± .90	23.7
25	(9x4)-W119-BK	--	--	--	--

Table 20 continued

Entry No.	Pedigree	Isabella	Fortuna	Limani	
26	(1x9)-W27-BK	20.3 ± .39	23.4 ± 1.74	23.6 ± 1.03	22.4
27	(9x5)-W128-BK	20.7 ± 1.05	24.6 ± .05	24.2 ± .50	23.2
28	(9x5)-W142-BK	21.2 ± 1.01	22.6 ± 1.66	23.8 ± .30	22.5
29	(8x9)-W66-BK	22.6 ± 1.03	24.8 ± .50	26.6 ± 1.36	24.7
30	(1x9)-W7-BK	20.6 ± 1.30	23.5 ± .40	24.8 ± .18	23.0
31	(9x5)-W143-BK	21.1 ± 1.39	24.1 ± 1.02	26.4 ± .68	23.9
32	(9x2)-W99-BK	23.1 ± 1.19	24.6 ± .48	25.2 ± 1.37	24.3
33	(1x9)-W22-BK	20.9 ± .76	22.7 ± .89	23.3 ± .54	22.3
34	(9x6)-W132-BK	20.6 ± .45	24.4 ± .69	23.7 ± .55	22.9
				White mean - -	23.4
	- - - - - - - Mixed color - - - - - - -				
35	(3x7)-M30-BK	23.1 ± 1.71	23.1 ± 1.33	24.0 ± .72	23.4
36	(8x5)-M101	24.4 ± 1.00	26.4 ± .47	26.8 ± .83	25.9
37	(8x3)-M92-BK	22.6 ± 1.02	25.8 ± .84	27.0 ± .74	25.1
38	(8x9)-M84-BK	22.9 ± 1.14	26.4 ± .52	29.9 ± .47	26.4
39	(6x8)-M82-BK	24.3 ± 1.29	27.0 ± .44	28.2 ± .05	26.5
40	(8x5)-M100-BK	22.8 ± .98	25.1 ± 1.15	26.0 ± .77	24.6
41	15R-148-M187-BK	25.0 ± 1.38	26.6 ± .77	30.1 ± .13	27.2
42	(11x3)-M148-BK	20.9 ± 1.12	24.5 ± .93	24.3 ± .41	23.2
43	(5x8)-M67-BK	23.1 ± 1.03	26.4 ± .70	26.9 ± .59	25.5
44	(8x6)-M168-BK	21.1 ± .70	24.0 ± .32	27.1 ± .83	24.1
45	(8x6)-M102-BK	22.3 ± 1.21	25.9 ± .45	29.1 ± .80	25.8
46	(6x8)-M81-BK	21.7 ± .91	24.5 ± 1.24	28.8 ± .98	25.0
47	(8x1)-M88-BK	25.3 ± 1.24	27.0 ± .63	27.7 ± .70	26.7
48	(8x5)-M99-BK	24.0 ± .48	25.2 ± .34	26.3 ± .47	25.2
49	(8x6)-M103-BK	22.1 ± .98	25.7 ± .32	29.5 ± 1.03	25.8
				Mixed color mean - -	25.4

[z] Percentage protein values are the means and standard errors from 4 reps of field grown material

the superior protein values may indicate that 15R-148 is an outstanding parent. Similar high protein values are shown for 2 F_3 bulks (Nos. 22 and 24) (Table 21) having 15R-148 as a parent and grown at the same 3 locations in Puerto Rico. 15R-148 is being studied extensively in several crosses. Some of the lines with superior performance in Puerto Rico were also promising when grown in Wisconsin (Table 22).

Analyses of white-seeded cultivars grown in Puerto Rico by N. Vakili also identified a promising selection, M-1, which has a higher protein value than widely grown standards (Table 23). Although there are large location effects, M-1 is clearly a primising line. It is well adapted to tropical conditions and contains multiple disease resistance (N. Vakili, 1977; personal communication).

The relatively short time required to determine the percentage protein of seed samples allows many selections to be analyzed. Since these promising lines carry other superior traits, exchange of germplasm provides an effective means of surveying much more material than could be grown and analyzed by one program.

Performance of Wisconsin Breeding Lines in Tropical Areas. Early generation selections from the recurrent selection program at Wisconsin have shown mixed performances at CIAT and MITA (Tables 24,25,26). Some lines show promise for both yield and percentage protein, but growth habit, maturity and disease reactions have generally been poor and the performance of the lines much inferior to the best selections from those research programs. The promising materials that are now being identified, such as 15R-148 and M-1 are being incorporated into the composite populations and it is expected that the overall performance as well as the yield and protein of future Wisconsin selections will be better.

Table 21. Percentage seed protein of breeding lines growth in Puerto Rico by G. Freytag, MITA, and analyzed at the University of Wisconsin.

Entry No.	Identity	Isabella	Fortuna	Limani	Entry mean
1	Testigo local #1	21.5 ± .81	24.8 ± .41	26.7 ± .37	24.3
2	Testigo local #2	22.0 ± .53	24.4 ± .52	25.6 ± .29	24.0
3	Testigo local #3	24.5 ± .75	25.3 ± .56	28.0 ± .35	25.9
4	Testigo local #4	23.7 ± .82	25.8 ± .65	27.1 ± .67	25.5
5	La Vega	23.0 ± 1.12	23.4 ± .48	25.1 ± .59	23.8
6	Mex. 309	19.1 ± .48	22.6 ± .92	28.7 ± .37	23.5
7	Ecuador 299	19.3 ± .23	22.2 ± .22	29.1 ± 2.59	23.5
8	Bonita	23.2 ± .64	24.7 ± .58	29.1 ± .42	25.7
	- - - - F_3 Bulks - - - -				
9	(La Vega x Bonita)	22.3 ± .67	24.6 ± .46	25.6 ± .28	24.1
10	Mex. 309 x Bonita	22.0 ± .73	25.7 ± .40	28.5 ± .32	25.4
11	50600 x La Vega	22.3 ± .80	23.7 ± .33	24.9 ± .21	23.6
12	Mex. 309 x La Vega	20.0 ± .33	23.4 ± .37	27.6 ± 1.10	23.7
13	50600 x 15R-55	21.6 ± .58	23.6 ± .42	26.2 ± .39	23.8
14	50600 x Mex. 309	20.1 ± .31	23.1 ± .48	24.6 ± .39	22.6
15	Mex. 309 x Bonita	21.0 ± .49	25.1 ± .48	27.7 ± 1.23	24.6
16	Jamapa x Mex. 309	20.5 ± 1.24	23.5 ± .63	27.8 ± .32	23.9
17	Mex. 309 x 15R-87	20.1 ± .62	25.3 ± .54	29.4 ± .76	24.9
18	Ecuador 299 x 50600	20.6 ± .43	22.6 ± .58	24.3 ± .65	22.5
19	Ecuador 299 x 50600	19.5 ± .51	23.1 ± .57	24.2 ± .43	22.3
20	15R-55 x Ecuador 299	20.2 ± .78	23.1 ± .54	25.0 ± .31	22.8
21	La Vega x Ecuador 299	22.2 ± 1.42	22.5 ± .44	25.1 ± .81	23.3
22	La Vega x 15R-148 + recip	23.7 ± .90	26.2 ± .57	28.6 ± .57	26.2
23	15R-148 x Ecuador 299	20.5 ± .69	24.6 ± .56	29.9 ± .76	25.0
24	Mex. 27R x 15R-148 + recip	24.1 ± .42	26.8 ± .50	28.9 ± .99	26.6
25	50600 x Ecuador + recip	20.1 ± .65	22.6 ± .21	24.3 ± .56	22.3

Table 22. Grain yield and percentage protein of MITA lines grown at Hancock, WI, 1977.

Entry No.	Pedigree	Protein %	Yield gm/plot
95	15R-148	28.8 ± .15	140 ± 2
96	Venezuela 36	25.4 ± 1.05	286 ± 29
97	(2x6)-B47-BK	23.4	233 ± 22
98	(9x5)-B364-BK	24.1 ± 2.44	251 ± 38
99	(5x3)-B257-BK	24.3 ± .70	268 ± 45
100	(2x6)-B48-BK	23.3 ± .54	222 ± 39
101	(9x3)-B354-BK	25.8 ± .10	151 ± 40
102	(5x9)-B281-BK	21.8 ± .75	277 ± 15
103	(5x2)-B242-BK	23.7 ± .40	291 ± 35
104	Jamapa-B433-BK	24.0 ± 1.07	230 ± 68
105	(2x6)-B57-BK	22.4 ± .05	227 ± 26
106	Black Turtle Soup	23.3 ± .80	342 ± 65
107	(3x5)-B128-BK	25.5 ± 1.20	229 ± 27
108	(4x5)-B190-BK	22.7 ± .05	362 ± 22
109	(9x2)-B351-BK	24.3 ± 1.29	288 ± 22
110	(3x6)-B113-BK	26.4 ± 1.15	235 ± 31
111	(2x6)-B50-BK	22.3 ± 1.20	200 ± 6
112	(4x9)-W41-BK	26.8 ± 1.25	151 ± 38
113	(9x3)-W117-BK	26.7 ± 1.50	96 ± 53
114	(9x2)-W105-BK	24.9 ± 1.60	191 ± 51
115	(9x8)-W126-BK	26.0 ± .50	93 ± 15
116	(1x9)-W27-BK	25.1 ± .55	316 ± 22
117	(9x5)-W128-BK	25.4 ± .34	153 ± 33
118	(9x5)-W142-BK	21.8 ± 1.05	329 ± 32
119	(8x9)-W66-BK	27.0 ± 0	261 ± 48
120	(9x2)-W99-BK	25.9 ± .65	216 ± 12
121	(1x9)-W22-BK	24.5 ± 1.14	322 ± 51
122	(9x6)-W132-BK	25.6 ± 1.65	168 ± 6
123	(3x7)-M30-BK	25.3 ± 1.65	411 ± 122
124	(8x5)-M101	27.9 ± 1.95	186 ± 36

Table 22. Continued

Entry No.	Pedigree	Protein %	Yield gm/plot
125	(8x3)-M92-BK	25.4	120 ± 26
126	(8x9)-M84-BK	27.5 ± 1.50	85 ± 15
127	(6x8)-M82-BK	26.9 ± .50	97 ± 48
128	Sanilac	20.4	131 ± 2
129	(8x5)-M100-BK	23.6 ± .35	241 ± 12
130	15R-148-M187-BK	29.6 ± .71	137 ± 6
131	(11x3)-M148-BK	25.2	234
132	(5x8)-M67-BK	25.7 ± .35	305 ± 76
133	(8x6)-M168-BK	27.3	
134	Nep 2	24.6 ± 1.55	209 ± 27
135	(8x6)-M102-BK	27.8 ± .05	116 ± 35
136	(6x8)-M81-BK	27.6 ± 1.05	132 ± 8
137	(8x1)-M88-BK	26.4 ± .85	210 ± 23
138	(8x5)-M99-BK	25.0 ± .55	152 ± 13
139	(8x6)-M103-BK	28.3 ± .15	75 ± 9
141	Bunsi	23.4 ± .15	242 ± 4
144	Cornell 49-242	22.5 ± .90	253 ± 22

Mean values are of 2 reps of 9-plant hill plots.

Table 23. Percentage protein of seeds of white bean strains grown in Puerto Rico by N. Vakili, 1977. Analyses at Univ. of Wisc.

Strain	Location		
	Mayaguez	Isabela	Limani
Bonita	23.1 ± .84	24.1 ± .26	31.3 ± .40
Blanca	20.5 ± .83	23.0 ± .48	--
M-1	27.6 ± .88	28.4 ± .73	--
Nep 2	22.6 ± .75	25.8 ± .25	26.7 ± .37
B. del P.	23.4 ± .65	25.4 ± .41	25.1 ± .97
#643	20.0 ± 1.32	20.5 ± .54	22.8 ± .65
Sanilac	23.3 ± .64	27.1 ± .37	--
Location means	23.0	24.9	26.1

Table 24. 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 25. Performances of Wisconsin selections from the 2nd cycle of recurrent selection in observation trials at CIAT, 1976.

Line No.	Protein	Yield	No. Plants	Growth habit
	%	gms/plot		
WISC. 763093	27.2	465	48	2,3
WISC. 763113	27.2	380	56	3
WISC. 763338	25.2	495	55	2,3
WISC. 763462	26.5	386	48	3
Black Turtle Soup	26.3	745	55	2
WISC. 763704	27.4	400	53	3
WISC. 763809	24.9	472	51	3
WISC. 763140	27.6	495	49	2
WISC. 763775	27.4	330	48	2
WISC. 763940	27.3	450	55	3
WISC. 764080	27.6	430	50	3
WISC. 764105	27.5	360	48	3
WISC. 764244	26.2	470	52	2,3
WISC. 764285	28.1	360	49	2
WISC. 753295	26.5	438	52	2
WISC. 753371	29.4	362	47	3
WISC. 753426	29.5	361	49	2,3
WISC. 752950	27.1	555	52	2
WISC. 754222	29.3	158	51	3
WISC. 754236	27.8	380	55	1,2,3

Values are of one observation only.

Table 26. Mean yield of 16 Wisconsin breeding lines and other entries grown in Puerto Rico, 1976. Values are from 2 reps at each of 2 locations.

Entry	Plot Yield			No. of Selections
	Isabela	Limani	Entry mean	
	gm	gm	gm	
WISCOP-02	348	278	313	
WISCOP-012	289	396	342	
WISCOP-013	274	130	201	
WISCOP-020	273	114	193	
WISCOP-021	325	102	213	
WISCOP-032	378	217	298	
WISCOP-048	236	410	323	3
WISCOP-016	246	172	209	1
WISCOP-036	310	448	379	
WISCOP-023	382	205	293	
WISCOP-031	252	288	270	
WISCOP-040	220	561	390	1
Black Turtle Soup	302	164	232	
WISCOP-024	340	505	422	2
WISCOP-025	290	337	313	3
WISCOP-046	323	117	220	
74-36 (Wisc.)	313	460	386	1
4210-12 (Mich.)	187	286	236	
(9x11) MITA	186	201	193	
(4x1) MITA	224	670	447	
La Vega (Check)	333	507	420	
Location Mean	287	312		

ANALYTICAL PROCEDURES

QUANTITATIVE ESTIMATION OF PERCENTAGE PROTEIN.

Use of the Grain Quality Analyzer (GQA) allows rapid measurement of percentage protein in bean seed flour to the same degree of precision obtained using standard laboratory procedures such as the Kjeldahl method. Results are obtained in 3 steps by (1) grinding each grain sample with a suitable mill (In our studies a Udy Cyclone Sample Mill was used), (2) placing the flour in the GQA sample drawer and closing the drawer, and (3) ten seconds later the digital meter displays the percentage protein. The GQA measures the amount of certain wavelengths of light that are abosrbed. The light reflected off the grain sample is determined and a built-in computer solves the equations related to protein content of the sample. The machine is standardized against the Kjeldahl protein percentages of 20 to 30 standard samples of bean flour processed similarly to experimental samples. The range of protein should be as wide or preferably wider than the range expected for experimental samples.

Considerable time saving is realized by using the GQA. Previously, 2 trained people were required each day to analyze approximately 30 samples for Kjeldahl nitrogen. Using the GQA, one person can analyze 100 samples per day with the same accuracy (Table 27). Other instruments that measure percentage protein using the same principle are available. Mention of the GQA in this report does not imply superiority of this instrument, but rather the suitability of its performance.

Table 27. 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

EXTRACTION AND SEPARATION OF PROTEIN FRACTIONS.

Since the storage proteins are primarily 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 (Danielsson, 1949). However, this procedure has been shown to yield globulin fractions that either contain other proteins or are a mixture of several globulins (Blagrove and Gillespie, 1975; Sun, 1974), seriously limiting its use for quantitative analyses or studies of genetic control of protein synthesis. McLeester et al. (1973) 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 globulin-1 (G1) and globulin-2 (G2) were shown to be completely separated from each other, with a high proportion of each fraction being extracted (Sun and Hall, 1975).

This procedure has been modified to provide a method for quantitatively estimating the protein fractions (Ma and Bliss, 1978b). The outline of sequential fractionation is shown in Figure 3.

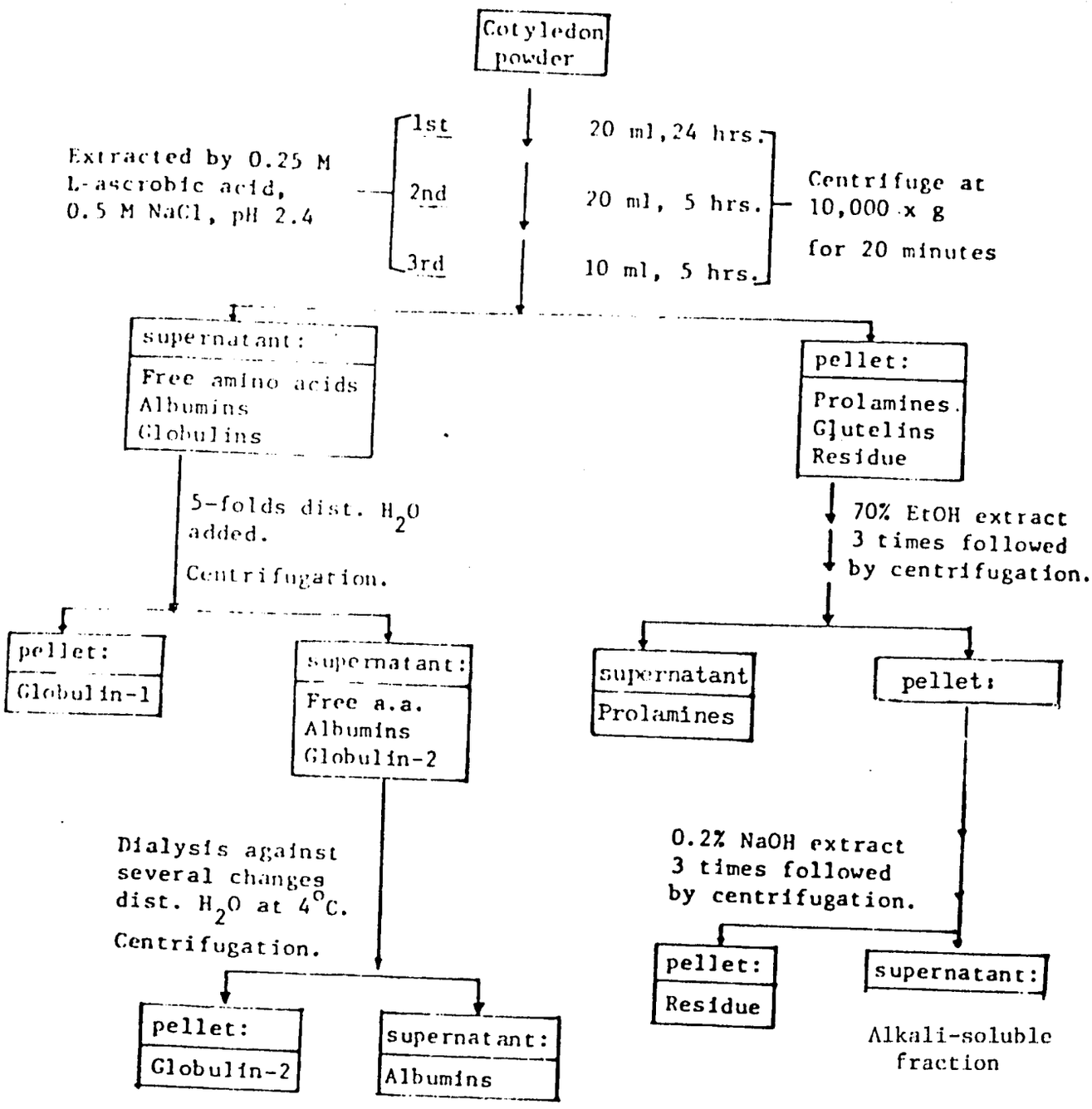


Figure 3. Outline of sequential fractionation of bean cotyledon proteins.

QUANTITATIVE ESTIMATION OF G1 PROTEIN USING IMMUNOELECTROPHORESIS.

The following procedure described Sun et al. (1978) has been used in our studies. "The G1 content of total extracts was determined by rocket immunoelectrophoresis (Laurell, 1967; Laurell, 1972; Weeke, 1973) using 1% agarose (BioRad) in a buffer containing 74 mM tris, 24 mM barbital, 0.34 mM Ca lactate and 3 mM NaN_3 (pH 8.6). To prepare gels, 40 ml of buffered agarose was melted, the temperature lowered to 47 C, and about 0.7 ml of rabbit anti-G1 serum added. The mixture was injected into a mold to give an 18 x 10 x 0.15 cm gel. Sample wells (32, each 2.4 mm diameter and spaced 3 mm apart) were punched out at least 1.5 cm distant from gel edges. Lyophilized purified G1 protein was dissolved (5 mg/ml) in the glycine buffer as described above for sample extraction. The samples and standard were carbamylated by heating to 50 C for 20 min with an equal volume of 2 M KOCN. An aliquot (4 µl) of sample or of dilutions of a standard (containing from 2.5 to 0.25 mg G1/ml) were applied to the wells, the standards being distributed across the gel to minimize any errors due to position. The gel was run at 90 v (25-15 mA) for about 15 hr using the tris-barbital-lactate buffer as electrolyte, then pressed, dried, and stained with Coomassie brilliant blue. The heights of the resulting rockets were measured, and the G1 content calculated from a standard curve obtained from the reference samples. Values given are the mean of at least 3 extracts, with replication within extracts" (Figure 4).

The quantification of G1 protein by rocket immunoelectrophoresis using a single antiserum assumes that the G1 of all samples will be equally reactive with the anti-G1 produced with 'Sanilac' G1 as the immunogen. The G1 of two genotypes tested in our lab differed in

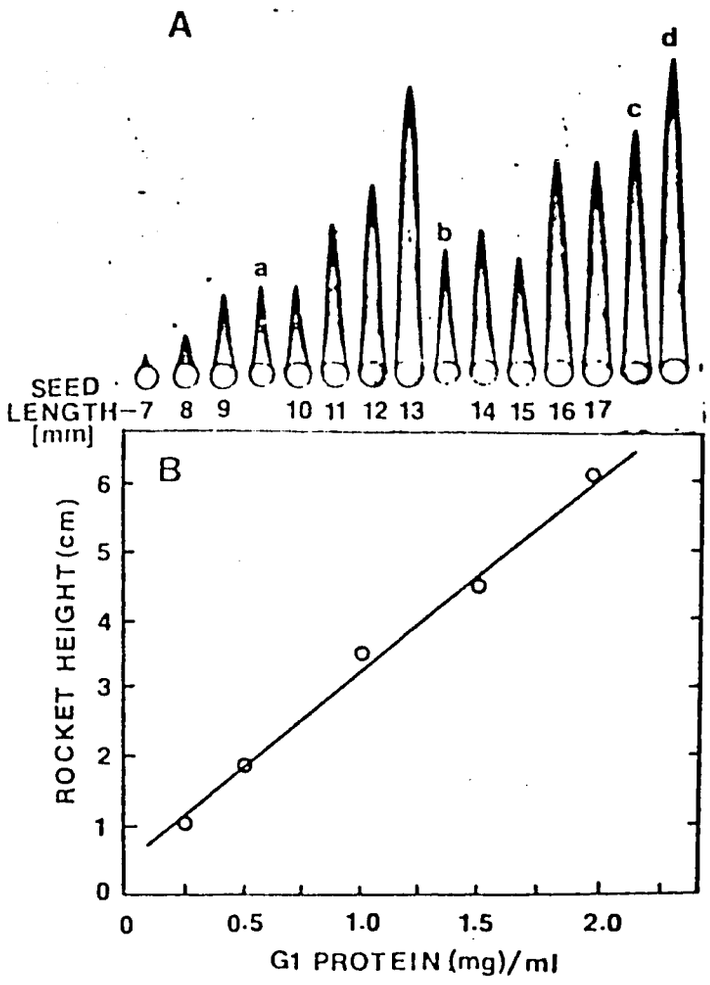


Fig. 4. Determination of G1 protein content by rocket immunoelectrophoresis.

A: Protein samples from cotyledons of each seed size (7 to 17 mm) were run in wells as indicated by the numbers below the rockets. These protein samples were obtained by extraction of 30, 20, 20, 20, 15, 10, 7, 2, 1, 1, and 1 cotyledons (with 2 ml buffer) for 7 to 17 mm seeds respectively. Equal volumes (4 µl) were applied to each well. Samples of standard G1 solutions of 0.25, 0.5, 1.0, and 1.5 mg/ml were run in wells marked a, b, c, and d respectively. B: A typical standard curve for G1 protein determination (Sun et al., 1978).

SDS-acrylamide gel banding patterns and so presumably are not strictly identical. The possible patterns for cases of non-identity, partial-identity and full-identity are shown in Figure 5. The G1 peaks of the innunograms clearly show complete identity. The G2 peak of the 'Tendergreen' sample shows that there is no identity between G1 and G2. Full identity reactions have been seen using other lines showing different G1 banding patterns on acrylamide gels. Since these results support the assumption of full identity, we feel that the measurement of G1 content by immunoelectrophoresis is valid, and that little if any error due to genotype differences in reactivity to the serum will be encountered in quantification.

CHARACTERIZATION OF GLOBULIN PROTEINS USING GEL ELECTROPHORESIS.

Globulin protein fractions free from cross-contamination can be obtained using an acidic extraction procedure, which permits characterization of the G1 and G2 globulin fractions (Sun and Hall, 1975). Although the G2 fraction gives a single boundary sedimenting at 6.6S in the analytical centrifuge (Sun et al., 1974), it is not homogeneous, and contains several growth inhibiting factors. A major component, glycoprotein I, possesses hemagglutinating properties (Pusztai, 1966). The G1 fraction is a homogeneous nonhemagglutinating globulin identical to that described as glycoprotein II by Pusztai and Watt (1970).

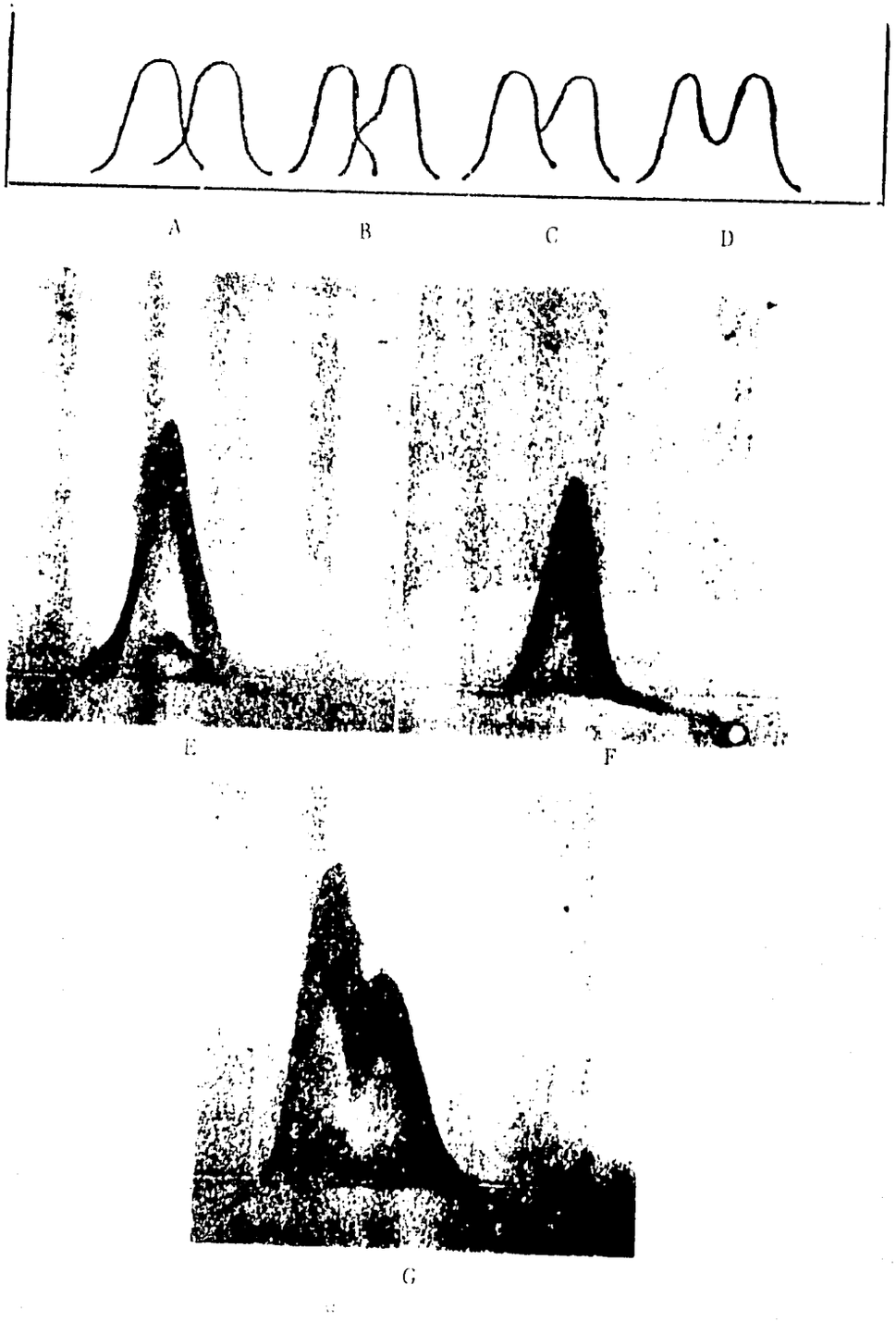


Figure 5. Globulin 1 seed proteins examined by crossed immunoelectrophoresis. Schematic diagrams showing (A) no identity; (B) slight identity causing deflection; (C) partial identity causing a spur; (D) full immunobiological identity. Actual electrophoresis of (E) cv. Tendergreen G1 + trace of G2; (F) cv. Sanilac G1; and (G) Tandom of Tendergreen G1 (left) and Sanilac G1 (right).

For the electrophoretic separation and identification the following procedures are used. Testas are removed from the seeds and the cotyledons are ground prior to protein extraction. The flour is washed into a centrifuge tube with a measured volume of 0.5 M NaCl containing 0.25 M ascorbic acid, pH 2.4. Two ml of solution are used for each 100 mg of seed flour. The protein is extracted with intermittant stirring for 3 hr at 25 to 27 C, then centrifuged at 20,000 rpm for 15 min. The supernatant is decanted immediately into 5 volumes of distilled H_2O (4 C) when a white precipitate is obtained. This is pelleted by centrifugation (20,000 rpm for 15 min at 4 C) then redissolved in 0.5 M NaCl and washed free of ascorbate by another cycle of centrifugation. The G1 pellet is finally dissolved in 0.5 M NaCl. The volume being one-fifth that of the original extraction solution. Purity and concentration of the samples are determined and the purified samples are adjusted with 0.5 M NaCl to a protein concentration of 5 mg/ml.

The G1 protein is dissociated into its polypeptide subunits by adding an equal volume of a solution containing 9 M urea, 3% (w/v) SDS, and 3% (v/v) 2-mercaptoethanol and heating for 3 min in a boiling water bath. The subunits are electrophoretically separated on 5% (w/v) SDS-urea acrylamide gels following the procedure described by Sun and Hall (1974).

Globulin 1 (G1) fraction of common bean protein consists of three subunits with molecular wts of about 50,000 each. In cv. Tendergreen these three subunits have molecular wts of approximately 43,000 (subunit α), 47,000 (subunit β) and 53,000 (subunit γ) (Fig. 1). G2 protein is seen in the gel profile as distinct bands.

ESTIMATION OF SEED TANNIN CONTENT.

Tannin in these studies refers to any naturally-occurring phenolic compound of molecular weight between 500 and 3,000 and containing a sufficiently large number of phenolic hydroxyl or other suitable groups (1 to 2 per 100 MW) to enable it to form effective cross linkages to proteins and other macromolecules. Since the amount of phenolic material bound to proteins by hydrogen bonding may be as much as one-third of the protein weight, this may account for inactivation and precipitation of enzymes or other proteins. Tannin has been found to alter the nutritional quality of plant products (Schaffert et al., 1974) including beans (Ronnenkamp, 1977) and other legumes (Lindgren, 1975).

Tannin content has been determined colorimetrically using the Vanillin-HCl method of Burns (1963, 1971), which was modified for estimation in beans. Extraction using 50% methanol has been found to give satisfactory results, since there are both alcohol and water-soluble phenols (Fig. 9). Seeds are ground and a 500 mg sample (20 mesh or finer) placed in a Sorvall 16 ml plastic centrifuge tube. Ten ml of 50% methanol is added to the tube, stoppered and stirred 24 hours. Centrifugation is carried out at 10,000 x g for 15 minutes. The supernatant is transferred into another container for assay and estimation of Catechin Equivalents (mg of C.E./unit wt) is determined from O.D. readings at 535 mμ (Ma and Bliss, 1978a).

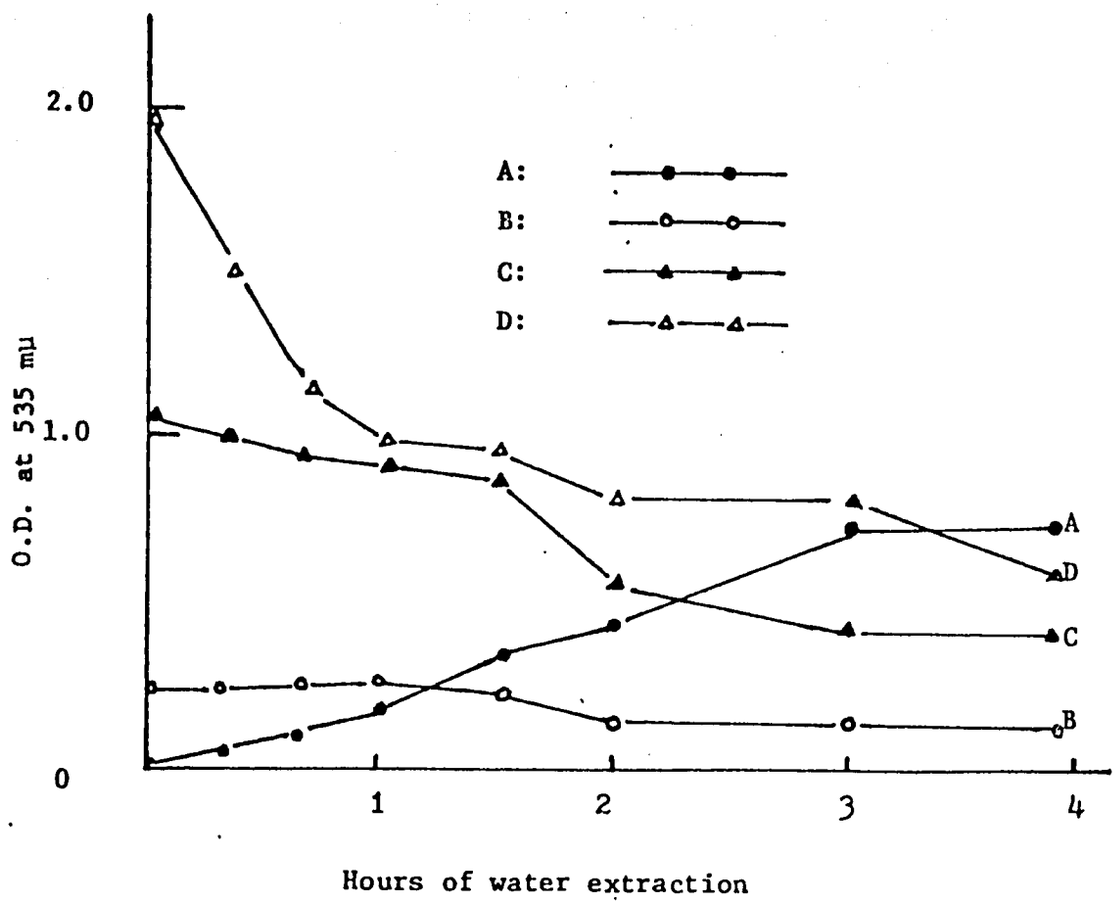


Figure 6. The tannin extracted by different solvent systems after presoaking whole seeds in distilled water for different lengths of time; (A) water extractable tannin, (B) 100% methanol extractable tannin, (C) 50% methanol extractable tannin, (D) 50% ethanol extractable tannin.

LITERATURE CITED

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

2. Bliss, F. A., J. D. Kelly and T. C. Hall. 1974. Selection for percent seed protein in a population of common bean (Phaseolus vulgaris L.). Agron. Abst. 1974.

3. Burns, R. E. 1963. Methods of tannin analysis for forage crop evaluation. Tech. Bull. N.S. 32, Georgia Agric. Exp. Stn., Athens.

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

5. Hackler, L. R. and M. H. Dickson. 1973. A comparison of the amino acid and nitrogen content of pods and seeds of beans (Phaseolus vulgaris L.) Search 3:1-6 N.Y.S. Agric. Expt. Sta., Geneva, N.Y.

6. Hall, T. C., S. M. Sun, Y. Ma, R. C. McLeester, J. W. Pyne, F. A. Bliss and B. U. Buchbinder. The major storage protein of French bean seeds: Characterization in vivo and translation in vitro. In: "The Plant Seed: Development, preservation and Germination." Academic Press (In press).

7. Kelly, J. F. 1971. Genetic variation in the methionine levels of mature seeds of common bean (Phaseolus vulgaris L.) J. Amer. Soc. Hort. Sci. 96:561-563.

8. Kelly, J. D. 1975. Genetic modification of protein quantity and quality in beans, Phaseolus vulgaris L. Ph.D. Thesis, University of Wisconsin, Madison.

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

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

11. Laurell, C. B. 1967. Quantitative estimation of proteins by electrophoresis in antibody-containing agarose gel. In: H. Peters (Ed.) Proteins in Biological Fluids. Amstedon.

12. Laurell, C. B. 1972. Electroimmuno assay. Scand. J. Clin. Lab. Invest. Vol 2, supplement 124:21-37.

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

14. Leleji, O. I., M. H. Dickson and L. R. Hackler. 1972. Effect of genotype on microbiologically available methionine content of bean seeds. HortScience 7:227-228.

15. Lindgren, E. 1975. The nutritive values of peas and field beans for hens. Swedish J. Agric. Res. 5:159-161.

16. Ma, Y. and F. A. Bliss. 1978a. Tannin content and inheritance in common bean (Phaseolus vulgaris L.). Crop Sci. 18:201-204.

17. Ma, Y. and F. A. Bliss. 1978b. Seed proteins of common bean (Phaseolus vulgaris L.). Crop Sci. (In press).

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

19. Pusztai, A. 1966. The isolation of two proteins, glycoprotein I and a trypsin inhibitor from the seeds of kidney bean (Phaseolus vulgaris). Biochem J. 101:379-384.

20. Pusztai, A. and W. B. Watt. 1970. Glycoprotein II: The isolation and characterization of a major antigenic and non-hemagglutinating glycoprotein from Phaseolus vulgaris. Biochim. Biophys. Acta 207:413-431.

21. 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.

22. Ronnenkamp, R. R. 1977. The effect of tannins on nutritional quality of dry beans. Ph.D. Thesis, Purdue University.

23. Schaffert, R. E., V. L. Lechtenberg, D. L. Oswalt, J. D. Axtell, R. C. Pickett, and C. L. Phykerd. 1974. Effect of tannin on in vitro dry matter and protein disappearance in sorghum grain. Crop Sci. 14:640-643.

24. Sun, S. M., R. C. McLeester, F. A. Bliss and T. C. Hall. 1974. Reversible and irreversible dissociation of globulins from Phaseolus vulgaris seed. J. Biol. Chem. 249:2118-2121.

25. Sun, S. M. and T. C. Hall. 1975. Solubility characteristics of globulins from Phaseolus seeds in regard to their isolation and characterization. J. Agr. Food Chem. 23:184-189.

26. Tolla, G. E. 1978. Effect of post-bloom nutrient applications and recurrent selection on seed yield and seed protein in common bean (Phaseolus vulgaris L.). Ph.D. Thesis, University of Wisconsin, Madison.

27. Weeks, B. 1973. Rocket immunoelectrophoresis. Scand. J. Immunol. Vol 2, supplement 1:37-46.

28. Wehrhahn, C. and R. W. Allard. 1965. The detection and measurement of the effects of individual genes involved in the inheritance of a quantitative character in wheat. Genetics 51:109-119.