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Bean lectins

5. Quantitative genetic variation in seed lectins of *Phaseolus vulgaris* L. and its relationship to qualitative lectin variation

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Summary. Seeds of forty bean cultivars having different lectin types based on two-dimensional isoelectric focusing-sodium dodecyl sulfate polyacrylamide gel electrophoresis (IEF-SDS/PAGE) were analyzed for quantities of lectin, phaseolin and total protein. Significant differences were found among groups of cultivars with different lectin types for the quantity of lectin and phaseolin. Cultivars with more complex lectin types based on IEF-SDS/PAGE tended to have higher quantities of lectin and lower quantities of phaseolin than cultivars with simple lectin types. An association between lectin type and the quantity of lectin and phaseolin was found also in the seeds of F_2 plants that segregated in a Mendelian fashion for two lectin types. Seeds from plants with the complex lectin type had more lectin and less phaseolin than seeds from plants with the simple lectin type. Therefore, the genes controlling qualitative lectin variation also may influence the quantitative variation of lectin and phaseolin. The results of this study are related to other studies on the quantitative variation for seed proteins and to the possible molecular basis for variation in the quantity of lectins in beans.

Key words: *Phaseolus vulgaris* – Lectin – Phaseolin – Quantitative variation – Immunoelectrophoresis

Introduction

Nutritional improvement of the grain legumes may be advanced by a better understanding of the genetic control of quantitative variation of seed storage proteins. In common bean, *Phaseolus vulgaris* L., lectins or phytohemagglutinins are of particular interest since they usually constitute a substantial proportion of total seed protein. However, they also contribute to the toxicity of raw beans (Liener 1974; Pusztai 1980) and contain little or no methionine (Moreira and Perrone 1977), the limiting amino acid in bean protein. The genetic regulation of lectin quantity is of interest since their accumulation may influence the accumulation of other nutritionally important bean seed proteins.

Bean lectins are known for their agglutinating and mitogenic activities, and the chemical and physical properties of lectins have been well characterized (reviews: Liener 1976; Goldstein and Hayes 1978; Lis and Sharon 1981). Qualitative genetic variation for lectins has been investigated, and recently, extensive variation in lectin type was reported for cultivated beans (Brown et al. 1982a). In a survey of 107 bean lines, eleven genetic variants and the absence of lectin were detected by two-dimensional electrophoretic patterns. The lectin variants showed two-dimensional polypeptide patterns ranging in complexity from 4 to 8 subunits. Most variants could also be distinguished by their non-denatured electrophoretic patterns (Osborn et al. 1984).

The quantity of seed lectin in bean cultivars and segregating lines has been determined indirectly by physical extraction of the lectin-containing G2 and albumin protein fractions (Ma and Bliss 1978), and by hemagglutinating activity toward rabbit erythrocytes (Felsted et al. 1981; Pusztai et al. 1979). Immunological methods have also been used to quantify lectin in the seeds of 13 bean cultivars (Cuperlovich et al. 1982). Although the presence or absence of lectin was shown to be inherited as a single gene (Jaffe et al. 1972), the above studies demonstrated that quantitative differences exist among lectin-containing lines.

Traits that vary quantitatively are often assumed to be controlled by many genes with small and equal effects (Mather 1941). However, studies of quantitative variation for seed proteins of beans, maize and barley suggest that single genes can have a major influence on the quantity of some protein fractions. In maize and barley, endosperm mutants

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controlled by single genes were compared to non-mutant lines and found to have reduced quantities of one protein fraction and increased quantities of other protein fractions (Misra et al. 1972; Shewry et al. 1978). In beans, differences in the quantity of the major storage protein, phaseolin, was associated with two allelic variants for phaseolin type (Sullivan and Bliss 1983; Hartana 1983).

In previous studies, we have shown that qualitative variation for lectins exists among bean cultivars based on two-dimensional (Brown et al. 1982a) and non-denaturing (Osborn et al. 1984) electrophoresis. In the current study, we have used rocket immunoelectrophoresis, a rapid and sensitive technique for protein quantification, to determine the amount of lectin and phaseolin in the seeds of bean cultivars and F_2 plants having different lectin types. We relate the observed quantitative genetic variation in seed lectin to the variation in lectin type reported previously and to variation in the quantity of phaseolin.

Materials and methods

Plant materials

In a previous study (Brown et al. 1982a), 107 bean cultivars were classified according to the two-dimensional electrophoretic pattern of their lectin polypeptides. For the present study, forty of those cultivars (Table 1) representing ten of the eleven

Table 1. Quantity of lectin, phaseolin and percentage protein in the seeds of bean cultivars and lines having different lectin types

Cultivar on line	Lectin type[a]	mg Lectin 100 mg flour	mg Phaseolin 100 mg flour	% protein
'Protop P-1'	Pr	2.80	10.91	26.9
'PI 312041'	312	2.46	10.18	25.5
'PI 310878'	310	2.43	9.87	25.0
'Bunsi'	B	2.19	9.19	22.9
'Ver 36'	B	2.32	11.02	25.2
'PI 224730'	B	2.31	11.40	25.4
'Ex Rico 23'	B	2.26	9.19	22.3
'Red Peanut Bean'	B	2.59	9.70	25.4
'15R-148'	B	2.86	9.92	27.6
'Mex 309'	B	2.54	11.12	25.2
'PI 313888'	B	2.05	9.45	23.9
'PI 195401'	B	2.35	11.12	27.3
'Sanilac'	S	2.60	8.88	22.1
'Bonita'	S	2.49	10.72	25.8
'Rufus'	S	2.21	8.87	19.9
'Red Mexican UI 36'	S	2.05	9.30	22.2
'Big Bend'	S	2.27	9.06	20.7
'Black Turtle Soup'	S	2.39	9.35	23.3
'PI 207227'	S	2.59	10.32	24.9
'Light Red Kidney'	T	1.62	13.13	25.9
'Canario Divex'	T	2.98	10.19	23.9
'Red Kote'	T	2.08	14.36	27.2
'Protop W-2'	T	2.31	11.49	24.4
'Cornell 2101'	T	2.23	12.17	25.3
'California Small White'	K	2.15	9.58	23.2
'M-1'	M	2.06	13.82	27.1
'Jamapa'	M	1.80	9.37	23.5
'Cornell 49-242'	M	1.86	9.20	24.0
'Viva'	V	1.62	8.70	21.8
'Puebla 152'	V	2.09	9.66	23.5
'P498'	V	1.92	8.94	23.0
'Honduras 46'	V	1.48	11.85	26.8
'MSU 61371'	V	1.68	12.18	23.9
'MSU 61834'	V	1.64	10.12	22.4
'Sutter Pink'	V	1.76	8.56	21.7
'Porrillo 70'	Po	1.51	14.87	26.4
'Porrillo Sintetico'	Po	1.48	12.75	25.2
'Pinto UI 111	Pi	0	10.18	21.6
'White Half Runner'	Pi	0	13.93	25.1
'PI 368737'	Pi	0	10.18	21.9

[a] Lectin type determined previously by two dimensional electrophoresis (Brown et al. 1982a) and non-denaturing electrophoresis (Osborn et al. 1984)

observed lectin types plus the absence of lectin were chosen to be analyzed for quantity of lectin, phaseolin and total protein in the seed. The cultivars were grown in the field at the Hancock, Wisconsin Experimental Station in 1979. Plants were grown in 10-plant row plots, using a randomized complete block design with 10 cm between plants and 90 cm between rows. Two replications of each line were used for this study. Seeds from F_2 plants derived from a cross between the low lectin-containing cultivar 'Porrillo 70' and a high lectin-containing breeding line 15R-148 were also analyzed. The F_2 plants were grown in rows 90 cm apart with 15 cm between plants at Hancock in 1981. Seed from cultivars and F_2 plants was harvested at maturity, threshed and weighed. Samples of 50 seeds were weighed and ground in a Udy Cyclotec mill. Protein percentage was determined using a Neotec Grain Quality Analyzer.

Tandem crossed immunoelectrophoresis

The immunological relationships among the different lectin types were determined using tandem crossed immunoelectrophoresis as described by Kroll (1973). This was necessary in order to determine whether antibody to a single lectin type could be used to quantify all lectin types. Lectin purified from the albumin fraction of the bean line PI 312041 (312AL) (Osborn et al. 1984) was injected into rabbits (50 g/injection) to obtain antiserum as previously described (Sun et al. 1978). This lectin was chosen because it contains most of the the polypeptide subunits found in other lectin types (Brown et al. 1982a). Therefore, antibody against 312AL (anti-312AL) should recognize most other lectin types. Crude proteins from eleven lines with different lectin types ('Bunsi', B; 'California Dark Red Kidney', C; 'Kerman', K; 'M-1', M; 'Porrillo 70', P; 'Protop P-1', Pr; 'Sanilac', S; 'Tendergreen', T; 'Viva', V; PI 310878, 310; PI 312041, 312) were extracted by stirring 2 gm flour in 5 ml of a solution containing 0.5 M NaCl, 0.5 M glycine, 0.025% NaN_3, pH 2.4 (with (HCl) for 2 h at room temperature. After centrifugation (30,000 g, 30 min 5 °C) the protein extracts were carbamylated by mixing equal volumes of supernatant with 2 M KOCN and heating to 50 °C for 20 min. Each protein extract from the first ten lines listed above as electrophoresed in tandem with the extract from PI 312041. Aliquots (4 µl) of the two carbamylated samples were applied to 2.5 mm diameter wells spaced 15 mm apart in gels composed of 1% agarose (Bio-rad) in 74 mM Tris, 24 mM barbital, 0.34 mM Ca lactate and 3 mM NaN_3 (pH 8.6). After 1 h of electrophoresis at 8 volts/cm gel using the Trisbarbital-lactate buffer as electrolyte, agarose strips containing the two samples were cut from the gel, transferred to a second gel containing anti-312AL and electrophoresed for 15 h at 2 volts/cm. Gels were dried, stained with 1.5% Coomassie brilliant blue in 45% methanol, 45% H_2O and 10% acetic acid and destained in the same solution without stain.

Rocket height versus intensity of staining

To further confirm that rocket height was an accurate measure of lectin quantity, the height of rockets formed by anti-312AL was correlated to the intensity of rocket staining. Crude proteins were extracted from flour samples of the eleven cultivars with different lectin types plus one cultivar with no lectin ('Pinto U.I. 111', Pi) and electrophoresed in triplicate against anti-312AL as described under rocket immunoelectrophoresis (below). After drying, staining, and destaining the rocket heights were measured. An equal area of agarose including the precipitated rockets was cut from each sample and extracted overnight in 3 ml methanol. This removed most of the Coomassie blue stain absorbed by the lectin-antibody precipitation peak. The optical density of the methanol solution was measured with a Hitachi Spectrophotometer at 580 nm using as the blank the methanol extract from 'Pinto U.I. 111', which produced no rocket peak. The correlations between rocket heights and optical density of methanol extracts were determined using mean values for each cultivar.

Rocket immunoelectrophoresis

The quantities of phaseolin and lectin in crude proteins extracted from bean seed flour were each determined by rocket immunoelectrophoresis (Laurell 1966; Weeke 1973). Proteins were extracted and carbamylated as described for tandem crossed immunoelectrophoresis except that 0.1 g flour was extracted in 5 ml of buffer. Repeated extractions showed that nearly all lectins were solubilized in the first extraction. Phaseolin quantity was determined using the procedure described previously (Sun et al. 1978). Lectins were quantified by the same procedure as phaseolin using anti-312AL and lectin purified from the albumin fraction of 'Protop P-1' (PrAL) (Osborn et al. 1984) as the standard for cultivars with B, K, M, P, Pr, S, V, 310, and 312 lectin types. Antibody was also raised against lectin purified from albumin and globulin fractions of 'Tendergreen' (Osborn et al. 1984) by injecting rabbits with a 2:1 mixture of the albumin and globulin lectins (TL). The TL anti-body (anti-TL) and TL standard were used to quantify lectin in cultivars with T-type lectin. The protein percentage in PrAL and TL standards was determined (Lowry et al. 1951) using BSA as the standard. In calculating lectin quantity, the concentrations of the lectin standards were adjusted by these values so that the percentage of lectin protein determined by the two antibodies and standards could be reported. The lectin standards were dissolved in the same buffer used for flour samples (1 mg/ml), carbamylated, and a dilution series (0.5 mg/ml, 0.375 mg/ml, 0.25 mg/ml and 0.125 mg/ml) was applied (4 µl) to each gel. Lectin quantity was determined from this standard curve.

The quantities of lectin and phaseolin in seed of bean cultivars were determined separately by incorporating either anti-312AL or antibody made against purified phaseolin (anti-phaseolin) in the agarose gel. The quantities of lectin and phaseolin in the seeds from F_2 plants were determined in the same rocket gel run by incorporating both anti-312AL and anti-phaseolin in the same agarose gel and running both purified standards. This gave the same result as when phaseolin and lectin rockets were precipitated separately, since lectin and phaseolin show no immunological cross reaction.

Hemagglutinating tests

Eleven seeds from each of 48 F_2 plants of 'Porrillo 70' × 15R-148 were tested individually for hemagglutinating activity toward rabbit erythrocytes. The 'Porrillo 70' lectin type (P) does not agglutinate rabbit erythrocytes, whereas the lectin type from 15R-148 (B) does agglutinate rabbit erythrocytes (Brown et al. 1982a). The seed coat was removed from the end distal to the plumule and a few mg of cotyledon flour was scraped into a test tube. Rabbit blood in Alsevers solution (1:1) was washed three time with phosphate buffered saline, pH 7.0 (PBS) and erythrocytes were suspended in PBS to give a 1.5% solution. To each flour sample, 0.3 ml of the erythrocyte suspension was added. After incubating for 15 min at room temperature, the erythrocytes were scored for the presence or absence of agglutination.

Statistical analysis

Expected mean squares and cross products from analysis of variance for lectin and phaseolin quantities in bean cultivars

with different lectin types are shown in Table 2. The data were analyzed using a mixed model with lectin type assumed to be a fixed variable and cultivars within lectin type a random variable. The components of covariance due to lectin type (COV_L) and cultivars within lectin type (COV_C) for the quantity of lectin and phaseolin were derived from the cross products (Table 2).

Results

Immunological relationship of lectin types

The immunological relationships between the 312 lectin type and other lectin types were determined by tandem crossed immunoelectrophoresis using anti-312AL. Examples of the precipitation arcs observed are shown in Fig. 1 a–d. Interpretations of some of these patterns were based on explanations given by Axelsen et al. (1973). A reaction of partial identity, similar to the one shown in Fig. 1 a for PI 312041 and 'Bunsi' lectins, was also obtained for PI 312041 lectin and lectins from 'Kerman', 'M-1', 'Protop P-1', 'Sanilac', 'Viva', and PI 310878. A slight spur extending from the PI 312041 lectin arc into the 'Bunsi' lectin arc indicates the presence of lectins in PI 312041 that are absent in the 'Bunsi' lectin. No spurs can be seen from the 'Bunsi' lectin arc, however, indicating that all 'Bunsi' lectins are immunologically identical to lectins in PI 312041. A

Table 2. Expected mean squares and expected cross products from a mixed model analysis of variance with lectin types fixed and cultivar within lectin type random

Source	df[a]	Expected	
		Mean squares[b]	Cross products
Blocks	b–1	–	–
Lectin type	l–1	$\sigma^2_E + b\sigma^2_C + bc\,\kappa^2_L$	$COV_E + bCOV_C + bcCOV_L$
Cultivar/lectin type	(c–1)l	$\sigma^2_E + \sigma^2_C$	$COV_E + bCOV_C$
Error	(b–1)[(l–1)+(c–l)l]	σ^2_E	COV_E

[a] b, l, and c refer to the number of blocks, lectin types and cultivars within lectin type, respectively
[b] L, C, and E refer to sources of variation due to lectin type, cultivar within lectin type and error, respectively; κ^2 is the analog of σ^2 for fixed effects

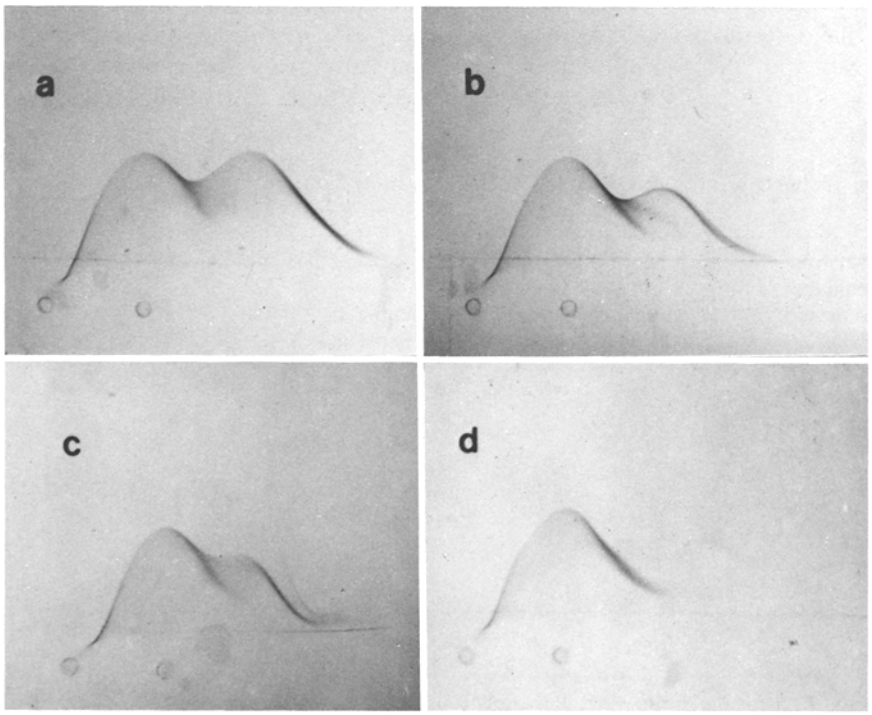

Fig. 1 a–d. Tandem crossed immunoelectrophoretic patterns of PI 312041 lectin (*left hand peak* in **a, b, c** and **d**) with 'Bunsi' lectin (**a**), 'Porrillo 70' lectin (**b**), 'Tendergreen' lectin (**c**) and 'California Dark Red Kidney' lectin (**d**) (*right hand peaks*) using antibody made against purified PI 312041 lectin

slightly different reaction of partial identity was observed for lectins from PI 312041 and 'Porrillo 70' (Fig. 1 b). Two spurs were seen extending from the PI 312041 lectin arc, but none were observed from the 'Porrillo 70' lectin arc. The reaction between the lectins of PI 312041 and 'Tendergreen' was more complex (Fig. 1 c). Two 'Tendergreen' lectin arcs can be seen; one being very faint. In other gels, this arc passed through the PI 312041 lectin arc, indicating the presence of a 'Tendergreen' lectin that is immunologically different from the PI 312041 lectins. The lectin from 'California Dark Red Kidney' was very weakly identified by anti-312AL, producing a short faint peak (Fig. 1 d), suggesting that it is also immunologically different than the lectin from PI 312041.

The validity of using rockets heights formed by anti-312AL to quantify lectin types having immunological identity with PI 312041 lectin was substantiated by correlating rocket height with intensity of rocket staining. Measuring the intensity of staining provides a different estimate of lectin quantity, since the amount of stain absorbed is proportional to the amount of protein precipitated by the antibody regardless of the rocket height. When all eleven lectin types were included in the correlation, a coefficient of $r=0.38$ was obtained. When the two lectin types (T and C) that were immunologically different than the 312 lectin type were deleted, the correlation coefficient increased to $r=0.97$. Since only one cultivar had the C lectin type, it was excluded from the remainder of this study. Anti-TL was used to quantify cultivars with the T lectin type and anti-312AL was used for cultivars with the remaining lectin types.

Variation among bean cultivars

The average values for quantities of lectin and phaseolin and the protein percentages for bean cultivars are shown in Table 1. Three cultivars, 'Pinto UI 111', 'White Half Runner' and PI 368737, had no detectable lectin. Among lectin-containing cultivars, the quantity of lectin ranged from 1.48 mg/100 mg flour in 'Porrillo Sintetico' to 2.98 mg/100 mg flour in Canario Divex. All cultivars contained phaseolin in quantities ranging from 8.56 mg/100 mg flour in 'Sutter Pink' to 14.87 mg/100 mg flour in 'Porrillo 70'. The quantities of lectin and phaseolin in mg/100 mg were divided by the protein percentage and multiplied by 100 to obtain quantities in mg/100 mg protein.

The data collected on bean cultivars were analyzed according to the model in Table 2 to determine if lectin types and cultivars within lectin types were significant sources of variation for total protein percentage, mg lectin/100 mg flour, mg lectin/100 mg protein, mg phaseolin/100 mg flour, and mg phaseolin/100 mg protein. Variation due to cultivars within lectin types was significant ($P<0.01$) for all traits. Lectin types were a significant source of variation for mg lectin/100 mg flour ($P<0.01$), mg lectin/100 mg protein ($P<0.01$), mg phaseolin/100 mg flour ($P<0.05$), and mg phaseolin/100 mg protein ($P<0.01$). Mean lectin values for each lectin type were compared using Duncan's multiple range test and significantly different groupings were found (Table 3). Furthermore, a positive relationship was evident between the quantity of lectin and the complexity of the lectin type as determined by the number of lectin polypeptides in the two-

Table 3. Number of lectin polypeptides, number of cultivars screened and mean lectin quantity in the seeds of cultivars with different lectin tpyes

Lectin tpye	No. of lectin polypeptides[a]	No. of cultivars screened	Mean lectin quantity[b]	
			mg lectin 100 mg flour	mg lectin 100 mg protein
Pr	6	1	2.80a	10.04a
312	8	1	2.46ab	9.63ab
310	7	1	2.43ab	9.75ab
B	7	9	2.39ab	9.57ab
S	5	7	2.37ab	10.50a
T	5	5	2.25abc	8.94ab
K	7	1	2.15bc	9.27ab
M	5	3	1.91bcd	7.69bc
V	5	7	1.74cd	7.55bc
P	5	2	1.49d	5.79c
Pi	0	3	0e	0d

[a] Number of lectin polypeptides determined by two dimensional gel electrophoresis, from Brown et al. (1982)

[b] Mean lectin values with different letters are significantly different based on Duncan's Multiple Range Test

dimensional electrophoretic patterns (Brown et al. 1982a). In general, cultivars with more complex lectin types also has more lectin.

The relationship between lectin type and quantity is shown by a rocket gel (Fig. 2) of cultivars representing a range of lectin quantities within five lectin types (P, V, M, S, and B) and the absence of lectin (Pi). Comparison of rocket heights demonstrates that within each lectin type there was a limited range of lectin quantity in bean fluor of the cultivars. The faint peaks observed for cultivars without lectin (Fig. 2, lanes 1, 2 and 3) are probably due to contamination with lectin containing seeds during seed harvest or processing, since other seed sources of these cultivars showed no precipitation peaks. Frequency distributions of cultivars for mg lectin/100 mg protein are shown in Fig. 3. For all cultivars screened (Fig. 3, A) those with and without lectin had separate distributions, and as expected for a

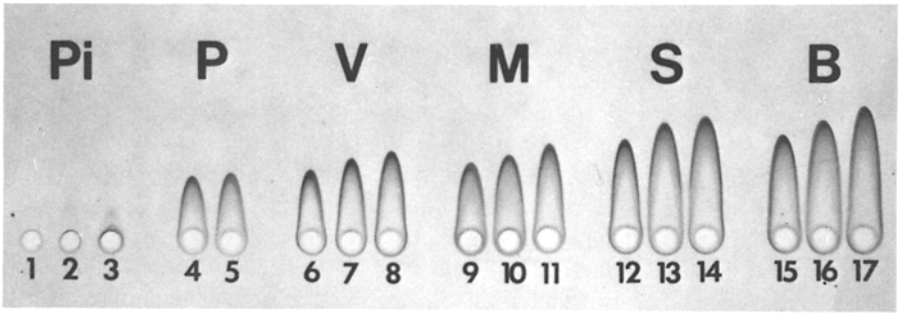

Fig. 2. Rocket gel showing quantity of lectin in cultivars with no lectin (*Pi*) and different lectin types (*P, V, M, S, B*). *1* 'Pinto U.I. 111'; *2* 'White Half Runner'; *3* PI 368737; *4* 'Porrillo Sintetico'; *5* 'Porrillo 70'; *6* 'Honduras 46'; *7* 'Viva'; *8* 'Puebla 152'; *9* 'Cornell 49-242'; *10* 'Jamapa'; *11* 'M-1'; *12* 'Rufus'; *13* 'Bonita'; *14* 'Sanilac'; *15* 'Bunsi'; *16* 'Red Peanut Bean'; *17* 15R-148

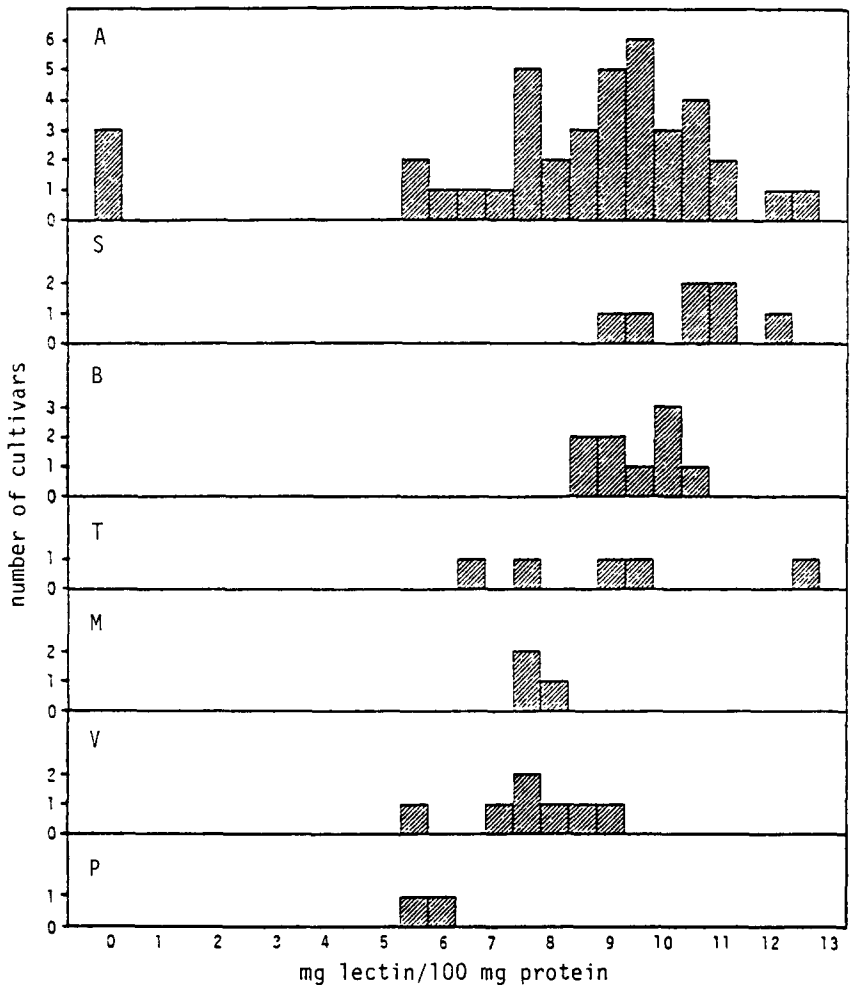

Fig. 3. Frequency distributions of all cultivars analyzed (*A*) and subsets of those cultivars having different lectin types (*B, S, T, M, V,* and *P*) for mg lectin/100 mg protein

quantitative trait, variation among lectin-containing cultivars appeared normally distributed. With the exception of the T lectin type (Fig. 3, T), cultivars within each lectin type (Fig. 1, S, B, M, V, P) were clustered around different means and were not randomly distributed across the total range of variation. Therefore, lectin type was a factor contributing to the total variation for lectin quantity.

The relationship between the quantities of lectin and phaseolin in cultivars was tested by correlating lectin and phaseolin quantities using either mean values for cultivars ($n=40$) or mean values for lectin type ($n=11$). In both cases, a non-significant negative correlation was found when lectin and phaseolin were measured as mg/100 mg flour ($r=-0.26$, $n=40$, and $r=-0.37$, $n=11$). Significant negative correlations ($P<0.05$) were found when lectin and phaseolin were measured as mg/100 mg protein ($r=-0.55$, $n=40$, and $r=-0.70$, $n=11$).

The covariation for lectin and phaseolin quantity was partitioned into components due to lectin type and to cultivars within lectin type to determine the relative contribution of these factors to the observed negative relationship (Table 4). The negative covariance due to lectin type was substantially greater than that due to cultivars with lectin type whether lectin and phaseolin were measured as mg/100 mg flour or mg/100 mg protein. Therefore, most of the negative relationship between lectin and phaseolin quantity could be attributed to variation due to lectin type.

Table 4. Lectin type, cultivar within lectin type and error components of covariance for lectin and phaseolin quantities

Component	COV for lectin and phaseolin quantitiy	
	mg/100 mg flour	mg/100 mg protein
Lectin type	−0.5074	−13.3234
Cultivar (lectin type)	−0.0590	− 1.7275
Error	−0.0082	− 0.1740

Variation among F_2 plants

The association between lectin type and the quantities of lectin and phaseolin was tested further by relating lectin and phaseolin quantity to the lectin type of seeds from F_2 plants of 'Porrillo 70' × 15R-148. 'Porrillo 70' had the P lectin type (Brown et al. 1982a) and 1.51 mg lectin/100 mg flour, while 15R-148 had the B lectin type (Brown et al. 1982a) and 2.86 mg lectin/100 mg flour. The lectin type of 11 seeds from each F_2 plant was determined by rabbit erythrocyte agglutination. All seeds from each plant contained either the P or B lectin type or segregated for both types, indicating a P/P, B/B, or P/B genotype for the F_2 plant. The ratio of P/P : P/B : B/B F_2 plants was 9 : 28 : 11, consistent with a 1 : 2 : 1 segregation ratio expected for a single gene trait ($\chi^2 = 1.50$).

Lectin and phaseolin in the flour of seeds from 48 F_2 plants was quantified by rocket immunoelectrophoresis (Fig. 4). Using variation within lectin genotypes

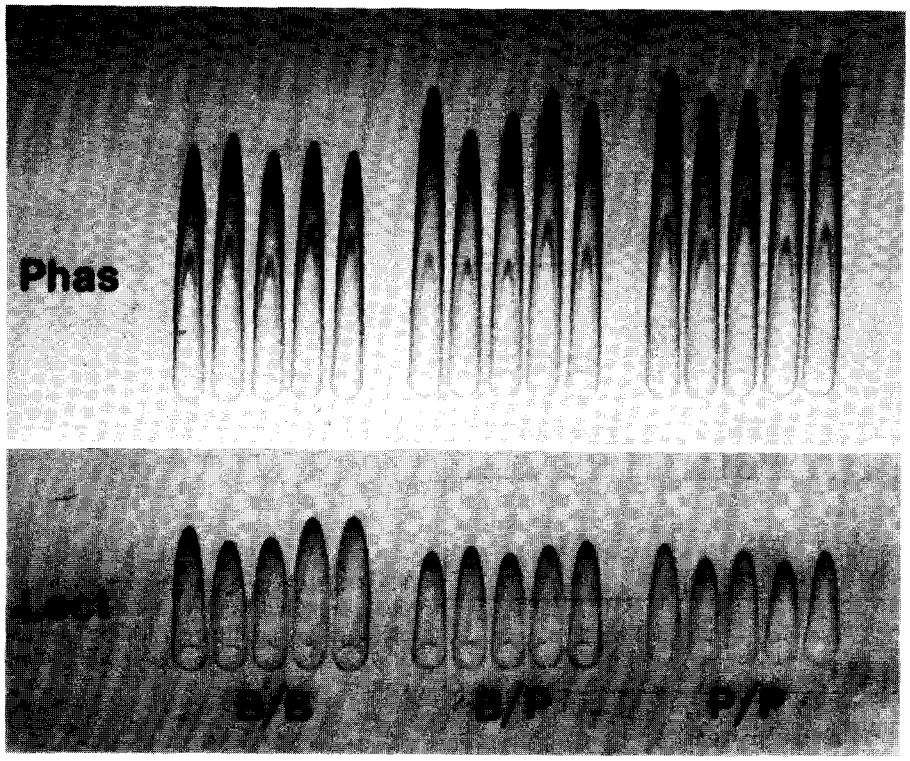

Fig. 4. Rocket gels showing quantities of phaseolin (*Phas*) and lectin (*Lect*) in seeds of 'Porrillo 70' × 15R-148 F_2 plants having three lectin genotypes (*P/P*, *P/B* and *B/B*). The five F_2 plants of each genotype were chosen at random from the 48 F_2 plants analyzed. Rockets in the same position in each gel are from the same sample

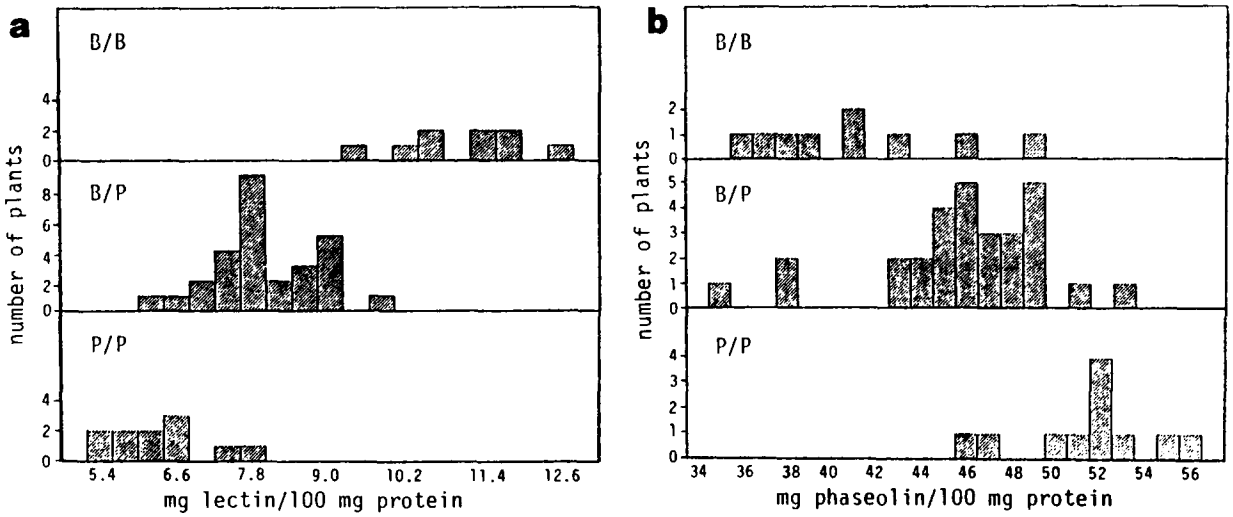

Fig. 5a, b. Frequency distributions of 'Porrillo 70' × 15R-148 F_2 plants having three lectin genotypes (*P/P, P/B* and *B/B*) for quantity of lectin (**a**) and phaseolin (**b**) measured as mg/100 mg protein

as an error term in the analysis of variance, significant differences ($P < 0.01$) were found among the three lectin genotypes for both lectin and phaseolin quantities measured as either mg/100 mg flour or mg/100 mg protein. Mean values for each genotype were compared using Duncan's multiple range test, and each genotype had a significantly different quantity of lectin and phaseolin with P/P < P/B < B/B for lectin quantity and P/P > P/B > B/B for phaseolin quantity. The effect of lectin genotype on lectin and phaseolin quantity is illustrated by frequency distributions of plants with the three lectin genotypes (Fig. 5).

Discussion

The objectives of the studies presented in this paper were, first, to evaluate the variation for lectin content in different bean cultivars using rocket immunoelectrophoresis, and second, to determine whether relationships existed between lectin type and lectin quantity (i.e. between qualitative and quantitative genetic variation) and between lectin quantity and phaseolin quantity. Previously, quantitative variation for lectins in different bean cultivars was shown by physical measurement of the lectin-containing G2 fraction (Ma and Bliss 1978) and by variation in agglutination activity towards rabbit erythrocytes (Felsted et al. 1981; Pusztai et al. 1979). However, the G2 fraction is a heterogeneous fraction containing both lectin and nonlectin proteins and therefore the observed variation cannot be attributed solely to lectin. Also, although differences in agglutination activity towards rabbit erythrocytes may reflect differences in the quantity of rabbit erythrocyte agglutinins, not all bean lectins agglutinate rabbit erythrocytes and the degree of agglutination varies with lectin type (Brown et al. 1982a). Variation for lectin quantity was observed also in the seeds of thirteen unidentified bean cultivars using immunological methods (Cuperlovich et al. 1982). However, those authors assumed that all of the cultivars had the same lectin type, e.g. that reported by Miller et al. (1975) which is the same as the T lectin type (Brown et al. 1982b). Therefore, in order to employ an immunological technique to quantify the lectin content of different bean cultivars containing different lectin types it was necessary to demonstrate complete immunological recognition of the lectin variants by the antibody to be used. For phaseolin, immunological identity for the different genetic variants was demonstrated previously (Mutschler 1979).

The immunological relationship between a single, complex lectin type (312) and the other ten lectin variants was determined here by tandem crossed immunoelectrophoresis. Antibody made against the 312 lectin type fully recognized eight of the ten lectin variants. These variants are also related to the 312 lectin type by the polypeptide composition as seen on two-dimensional electrophoretic gels (Brown et al. 1982a). Two lectin variants, T and C, contained lectins that were weakly recognized by the antibody. These lectins were very different from the 312 lectin type based on two-dimensional electrophoretic patterns (Brown et al. 1982a). When rocket heights were compared to the intensity of staining for the eleven lectin types using anti-312AL, a high correlation was obtained after eliminating the samples having T and C lectin types. Therefore, the height of rockets precipitated by anti-312AL estimated accurately the lectin quantity for nine of the lectin types (B, K, M, P, Pr, S, V, 310, and

312), and was used to determine lectin quantity in cultivars having those lectin types. Anti-TL was used to quantify lectin in cultivars with T lectin type.

The range of lectin quantities we found in analyzing forty bean cultivars was similar to that reported by Cuperlovich et al. (1982) using a different immunological technique. Among the forty cultivars, three were found to have no immunologically recognized lectin. These cultivars were shown previously to have no hemagglutinating activity and no lectin polypeptide on two-dimensional electrophoretic gels (Brown et al. 1982 a).

Relationship between lectin quantity and lectin type

In the analysis of bean cultivars for quantitative variation in lectin content, significant differences were found among cultivars with different lectin types. In general, cultivars with more complex lectin types had more lectin. The relationship between lectin type and lectin quantity was demonstrated further by analyzing seeds from F_2 plants. Segregation ratios indicated single gene inheritance for the parental lectin types. The three F_2 lectin genotypes had significantly different lectin quantities; the least amount of lectin was produced by genotypes having the simplest lectin type (P/P), the most by the genotypes having the most complex lectin type (B/B) and the heterozygotes were intermediate. Therefore, in this F_2 population the genes controlling different lectin types were associated with different quantities of lectin.

Although quantitative variation for other seed storage proteins has been studied in the common bean (Ma and Bliss 1978; Mutschler and Bliss 1981) and wheat (Cole et al. 1981; Fullington et al. 1980), it has not been related to qualitative genetic variation. Cole et al. (1981) suggested that the range of quantitative variation they observed by scanning SDS polyacrylamide gels of tetraploid wheat could be explained by the combination of different qualitative components from the diploid species. They could not, however, directly relate quantitative variants to qualitative variants. In a study of a population of inbred backcross lines of common bean in which the recurrent and donor parents had different phaseolin types, three lines contained significantly more phaseolin and total protein than all other lines (Sullivan and Bliss 1983). Two of these three lines had the phaseolin type of the donor parent. The authors noted that there was a very low probability of this occuring at random and suggested that genes for increased phaseolin were linked to the structural gene for phaseolin. Hartana (1983) compared backcross lines with the two phaseolin types and also found significant differences in the quantity of phaseolin and total protein.

The data presented in this study indicate a relationship between the genes controlling variation in lectin polypeptide composition, that is qualitative lectin variation, and the genes controlling quantitative lectin variation. Evidence from a previous study suggested that the genes controlling the polypeptides of the S and T lectin types are closely linked and segregate as alleles at a single locus (Brown et al. 1981). The F_2 data presented here support allelism for the P and B lectin types, and several lectin types have been found to be allelic to the absence of lectin (Osborn, unpublished data). The different lectin types apparently represent allelic alternatives for the lectin structural gene(s). The relationship between lectin type and lectin quantity reported here may be due simply to differences in the number of lectin structural genes in the different allelic variants, as cultivars with more complex lectin types generally contained more lectin. However, this relationship may also be due to linkage between lectin structural genes and regulatory loci or to different promotor activities giving higher transcription or translation rates.

Relationship between quantity and type of lectin and phaseolin quantity

In analyzing the data on bean cultivars, we found a significant negative correlation between the quantities of lectin and phaseolin when measured in mg/100 mg protein.

Similar negative relationships have been observed between the quantities of different storage proteins in mutant lines of corn (Misra et al. 1972), barley (Shewry et al. 1978) and soybean (Kitamura and Kaizumo 1981). In each case, mutant genotypes having reduced quantities of a particular protein fraction had corresponding increases in one or more of the other fractions. In studies that have dealt with the natural range of genetic variation, however, this type of negative correlation has not been reported. In fact, significant positive correlations have been reported between the quantities of phaseolin and G-2 (Ma and Bliss 1978; Mutschler 1979) and between phaseolin and the alkali-soluble fraction and the prolamine and alkali-soluble fractions (Ma and Bliss 1978) in segregating progenies of beans. For some of these correlations, protein fractions were measured as a percentage of bean flour and were positively correlated with total protein. This might account for positive correlations among protein fractions.

Since lectin quantity was related to lectin type, and quantities of lectin and phaseolin were related inversely, it follows that phaseolin quantity should be associated with lectin type. This association was observed in three separate ways: 1) lectin type was a significant source of variation for phaseolin quantity in bean cultivars, 2) most of the negative covariance between lectin and phaseolin quantities was due to lectin type, and 3) plants with the three lectin genotypes in the F_2 population had separate distributions for phaseolin quantity. Therefore, it appears that the genes controlling lectin type influence phaseolin accumulation possibly by determining the proportion of available substrate that goes into lectin synthesis. Whatever the mechanism, it is evident that qualitative genetic variation for lectin affects the protein composition of bean seed by influencing the accumulation of both lectin and phaseolin.

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