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Linkage Relationships Between Genes Controlling Seed Proteins in French Bean

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Summary. The inheritance of phaseolin[1] and globulin-2 (G2)/albumin polypeptides was investigated in crosses involving varieties which exhibited the three electrophoretic banding patterns of phaseolin found in French bean. 'Total' seed protein extracts of single seeds of the F_1 and F_2 generations from the crosses: 'Sanilac' × 'Contender', 'BBL 240' × 'Contender', and 'Sanilac' × 'BBL 240' were analyzed by two-dimensional electrophoresis. Segregation of the genes controlling phaseolin and G2/albumin polypeptides, and those controlling a further five groups of seed proteins (A, B, D, E, and F) were observed. No recombinant electrophoretic phenotypes were seen for phaseolin or G2/albumin polypeptides suggesting that the genes controlling each of these groups of polypeptides are closely linked and segregate like single Mendelian genes. The phaseolin genes and G2/albumin genes were not linked to each other. The group of genes controlling phaseolin polypeptides were linked to those controlling group B proteins, and those controlling G2/albumin polypeptides were linked to those controlling group F proteins.

Key words: *Phaseolus vulgaris* – Phaseolin – Seed proteins – Electrophoresis – Linkage

Introduction

Phaseolin protein constitutes some 40% of the total seed protein of *Phaseolus vulgaris* L. (Ma and Bliss 1978) and because of its importance for improved protein content and composition, it has received considerable attention (Sun et al. 1974, 1975; Sun and Hall 1975; Romero et al. 1975; Hall et al. 1977; Ma and Bliss 1978). The globulin-2 (G2) protein fraction as described by McLeester et al. (1973) and Sun and Hall (1975) constitutes between 5% and 12% of the total seed protein (Ma and Bliss 1978). Haemagglutination properties have been reported for G2 proteins (Bollini and Crispeels 1978) suggesting that at least some of these proteins are lectins and, consequently, may affect the digestibility and nutritional value of beans.

Studies of the seed storage proteins of many plants have suggested that the numerous storage protein subunits, distinguishable by molecular weight and isoelectric point, are the products of different but evolutionarily related genes clustered at a few genomic sites (Wrigley and Shepherd 1973; Mecham et al. 1978). This hypothesis is supported by the considerable amino acid sequence homology which has been shown for the gliadins of wheat (*Triticum aestivum* L.) (Bietz et al. 1977) and for the zeins of maize (*Zea mays* L.) (Bietz et al. 1979). Some homology has also been shown for the B hordeins of barley (*Hordeum vulgare* L.) by peptide mapping (Miflin and Shewry 1979). Genetic analyses (Soave et al. 1978; Valentini et al. 1979) and in situ hybridisation (Viotti et al. 1980) have shown that the structural genes controlling zein polypeptides are clustered in three or four sites on the maize genome. Those controlling gliadin and glutenin subunits of wheat are located on the homoeologous chromosomes of groups 1 and 6 (Wrigley and Shepherd 1973; Mecham et al. 1978; Brown et al. 1979, 1981b). The Hor-1 and Hor-2 genes controlling the hordeins of barley are located on chromosome 5 (Shewry et al. 1980). Finally, the genes controlling the α^M-legumin polypeptides in one pea (*pisum sativum* L.) variety appear to be linked (Casey et al. 1979) and the structural genes for α-legumin subunits in peas are located on chromosome 7 (Davies and Bedford 1979). In the bean, the phaseolin polypeptides were shown to be similar in molecular weight and pI (Brown et al. 1981a) and by peptide mapping (Ma et al. 1980). The G2 and albumin fractions both contain haemagglutinating lectin polypeptides (Brown et al. 1981c) and extensive amino acid sequence homology has been shown for them (Miller et al. 1975). Such homologies suggest that the polypeptides of phaseolin and of G2 lectins each have a common genetic origin and each group may be encoded for by a cluster of genes.

The inheritance of phaseolin polypeptide banding patterns has been studied in a cross between cultivars 'BBL 240' and 'PI 229815' (Romero et al. 1975; Hall et al. 1977). Both studies used electrophoretic analysis of single seeds of the F_1 and F_2 progenies and showed that the gene(s) controlling the highest molecular weight band of

1 Phaseolin is now the preferred trivial designation for the globulin-1 (Gl) protein (Buchbinder 1980)

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each pattern were inherited in a Mendelian fashion (Romero et al. 1975) and that in reciprocal crosses, both maternal and paternal genes were expressed (Hall et al. 1977). In this paper, genetic variation for 'total' seed protein composition is described for several cultivars. The inheritance of phaseolin polypeptides and polypeptides of other seed protein groups is studied in crosses between cultivars exhibiting the Tendergreen, Sanilac, and Contender phaseolin banding patterns as described by Brown et al. (1981a). Linkage relationships both within and between the protein groups are estimated from F_2 analyses.

Materials and Methods

Plant Materials

The cultivars of *P. vulgaris* studied were 'Tendergreen', 'PI 302542', 'BBL 240', 'Greensleeves', 'Sanilac', 'PI 229815', 'Mecosta', 'Pinto 111', 'Contender', 'Gina', and 'Nikos'. Seeds of these cultivars and of the F_1 and F_2 generations of the following crosses were obtained from the stocks held by Dr. F.A. Bliss: 'BBL 240' X 'Contender', 'BBL 240' X 'Gina', 'Sanilac' X 'Contender', 'Sanilac' X 'Gina', and 'Sanilac' X 'BBL 240'. Seed of 'Greensleeves' was obtained from W. Atlee Burpee Co., Warminster, PA.

Single Seed Protein Extraction

The non-germ end was dissected and ground in a mortar and pestle. 'Total' protein was extracted from the flour with 1 ml of a freshly-made solution of 2 M Urea, 0.5% (w/v) SDS, 0.6% (v/v) β-mercaptoethanol for 30 min at room temperature. 100 μl of the supernatant fraction was removed and dissociated as described previously (Brown et al. 1981a).

Two-dimensional Electrophoresis

Proteins were separated in the first dimension by isoelectricfocusing (IEF) as described by O'Farrell (1975), except that the sample overlay solution was 15% sucrose, 1% ampholines (LKB) (comprised of 0.67% pH range pH 5.0-8.0 and 0.33% pH range pH 3.5-10.0). Proteins were separated by molecular weight in the second dimension utilising the SDS-polyacrylamide gel system described by Brown et al. (1981a). Electrophoresis was carried out in the 0.75 mm thick slab gels at 22.5 mA/gel through the stacking gel and 35 mA/gel through the running gel. Molecular weights were determined as in Brown et al. (1981a).

Results

Polypeptide Subunit Composition of 'Total' Seed Protein

The Urea-SDS-β-mercaptoethanol buffer extracted the two main groups of protein subunits: phaseolin and G2/albumin (consisting of the major subunits of the G2 and

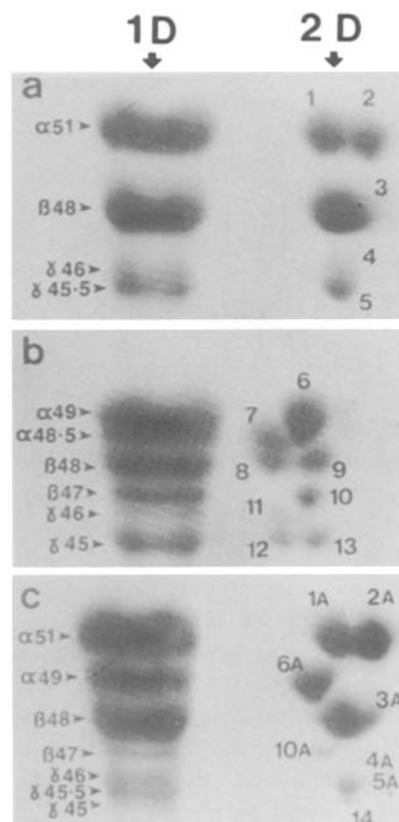

Fig. 1a-c. One- and two-dimensional electrophoretic patterns of the major phaseolin polypeptides of the parental cultivars. a 'BBL 240' – Tendergreen type; b 'Sanilac' – Sanilac type; c 'Contender', 'Gina' – Contender types. 40 μg phaseolin protein loaded per separation

albumin fractions of beans) (Brown et al. 1981c), along with many other proteins. The electrophoretic patterns of the polypeptide subunits of the three phaseolin banding types, Tendergreen, Sanilac, and Contender (Brown et al. 1981a) are shown in Fig. 1. The electrophoretic patterns of the major subunits of G2/albumin fell into two types, Tendergreen (T_{G2}) and Sanilac (S_{G2}) (Figs. 2, 4) and are described by Brown et al. (1981c). In addition to these major protein groups, five other groups (Fig. 2, groups A, B, D-F) were studied because their protein components segregated in the F_2 populations.

Protein groups A_1 and A_2 with isoelectric points of pH 5.4 and apparent molecular weights of 75,000 and 73,000 daltons respectively (Fig. 2) each consisted of a pair of polypeptide subunits. Protein groups B_1 and B_2 with isoelectric points around pH 6.1 and molecular weights of 58,000 and 56,000 daltons respectively each contained three subunits. The isoelectric points of protein groups D, E, and F lay in the range of pH 6.8-7.0. Each group consisted of a pair of subunits: D_1, D_2, E_1, E_2, F_1, and F_2 with apparent molecular weights of 44,000,

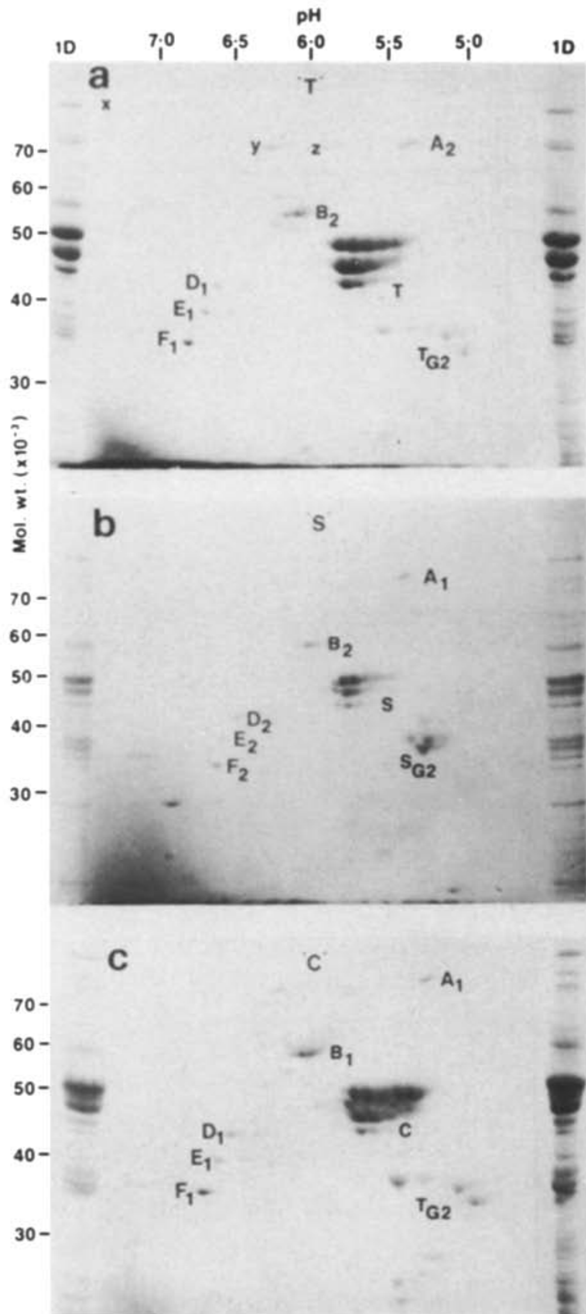

Fig. 2a-c. One- and two-dimensional electrophoretic patterns of 'total' seed protein extracts from parental cultivars. **a** 'BBL 240' (T); **b** 'Sanilac' (S); **c** 'Contender' (C). The gels show the allelic variants of the seven protein groups: phaseolin (T, S, and C), G2/albumin (T_{G2} and S_{G2}), A (A_1 and A_2), B (B_1 and B_2), D (D_1 and D_2), E (E_1 and E_2) and F (F_1 and F_2) given in table 1 and described in the text

43,000, 40,000, 39,000, 34,000, and 33,000 daltons respectively (Fig. 2).

The 'total' protein extract also contained a doublet of protein bands of molecular weights 82,000 and 81,000 daltons. These were clearly seen in the one-dimensional separation but could only be discerned in the 2D separation as a 'streak' of protein towards the basic end of the gel (Fig. 2a; x). Two protein groups of isoelectric points of pH 5.7 and pH 6.3 and molecular weights similar to A_2 were also seen in all the separations (Fig. 2a; y, z). The group of protein subunits with pI's > pH 7.0 and molecular weight lying between 30,000 and 40,000 daltons and the group of lower molecular weight proteins (< 30,000 daltons; pI's between pH 5.0 and pH 7.0) correspond to the minor components of the G2 and albumin fractions described by Brown et al. (1981c). No segregation of the genes controlling the proteins described immediately above was observed in the F_1 or F_2 generations.

Variation for 'Total' Seed Proteins in Bean Cultivars

The phenotypes of the eleven cultivars studied for their phaseolin, G2/albumin, A, B, D, E, and F proteins and their seed types are given in Table 1. The cultivars are grouped according to their phaseolin banding patterns (Brown et al. 1981a). Both within and between phaseolin group variation was observed for the other protein groups.

Three cultivars having Tendergreen type phaseolin, 'Tendergreen', 'BBL 240', and 'Greensleeves' had identical phenotypes for all seven protein groups while 'PI 302542' contained S_{G2} and F_2 instead of T_{G2} and F_1. There was greater variation for other proteins within the Sanilac types than among the Tendergreen types. 'Mecosta' and 'PI 229815' had T_{G2}, unlike 'Sanilac', and 'Pinto 111' contained no major G2/albumin subunits (Brown et al. 1981c). 'Sanilac', 'PI 229815' and 'Pinto 111' possessed the A_1 and B_2 phenotypes while 'Mecosta' had A_2 and B_1. 'Mecosta' and 'PI 229815' contained D_1, E_1, and F_1, like most of the Tendergreen and Contender types, which differed from 'Sanilac', which contained D_2, E_2, and F_2. 'Pinto 111' had D_2 and E_2 like 'Sanilac', but contained neither F_1 nor F_2 but a similar polypeptide of molecular weight 34,000 daltons and isoelectric point of pH 7.1. The three Contender types were identical for all the protein groups except for 'Nikos' which contained E_2 and F_2, instead of E_1 and F_1 found in 'Contender' and 'Gina'.

There was no apparent relationship between qualitative protein composition and seed type. Also, there appeared to be no correlation between phaseolin type and G2/albumin types in the Tendergreen and Sanilac phaseolin groups. All the Tendergreen phaseolin types had the A_2 and B_2 phenotypes, all the Contender phaseolin types had A_1 and B_1, and the Sanilac phaseolin types were intermediate, containing either A_1 and B_2 or A_2 and B_1. There was some variation between cultivars in groups D, E, and F, but the phenotype D_1, E_1, and F_1 predominated in all three phaseolin classes.

Table 1. Cultivar phenotypes for total seed protein and seed type

Cultivar	Seed type	Phaseolin	G2	A	B	D	E	F
'Tendergreen'	Buff kidney	T	T_{G2}	A_2	B_2	D_1	E_1	F_1
'BBL 240'	White kidney	T	T_{G2}	A_2	B_2	D_1	E_1	F_1
'PI 302542'	White kidney	T	S_{G2}	A_2	B_2	D_1	E_1	F_2
'Greensleeves'	White kidney	T	T_{G2}	A_2	B_2	D_1	E_1	F_1
'Sanilac'	White pea	S	S_{G2}	A_1	B_2	D_2	E_2	F_2
'Mecosta'	Red kidney	S	T_{G2}	A_2	B_1	D_1	E_1	F_1
'PI 229815'	Red kidney	S	T_{G2}	A_1	B_2	D_1	E_1	F_1
'Pinto 111'	Pinto	S	–	A_1	B_2	D_2	E_2	F[a]
'Contender'	Red kidney	C	T_{G2}	A_1	B_1	D_1	E_1	F_1
'Gina'	White kidney	C	T_{G2}	A_1	B_1	D_1	E_1	F_1
'Nikos'	Brown kidney	C	T_{G2}	A_1	B_1	D_1	E_2	F_2

[a] 'Pinto 111' had neither subunit F_1 nor F_2, but had a similar subunit of 34,000 daltons and pI of pH 7.1

Single Seed Analysis of Intervarietal Crosses

The 'total' seed protein compositions of the parental varieties 'BBL 240', 'Sanilac', and 'Contender' are shown in Fig. 2. Since the phenotypes of 'Contender' and 'Gina' were identical, the F_2 results were pooled where either of these cultivars was used as a parent.

'BBL 240' × 'Contender' or 'Gina'

The phaseolin of 'BBL 240' and 'Contender' had five subunits in common (Fig. 1a, nos. 1-5; Fig. 1c, nos. 1A-5A). F_1 and F_2 seeds where, therefore, homozygous for the genes controlling these proteins, and segregation was expected only for those genes controlling subunits 6A, 10A, and 14 (Fig. 1c). The F_1 progeny showed the intermediate T/C pattern (Figs. 3a, 5a). In the F_2 generation, only three phaseolin banding patterns were observed: the two parental types and the intermediate F_1 pattern. F_2 segregation ratios are given in Table 2.

The phenotypes of 'BBL 240' and 'Contender' were both T_{G2}, D_1, E_1, and F_1 (Table 1) and, therefore, no segregation was observed for these protein groups (Fig. 5a, Table 2). The F_1 progeny exhibited intermediate banding patterns for protein groups A and B: A_1A_2 and B_1B_2, and the F_2 segregation ratios for these proteins are shown in Table 2.

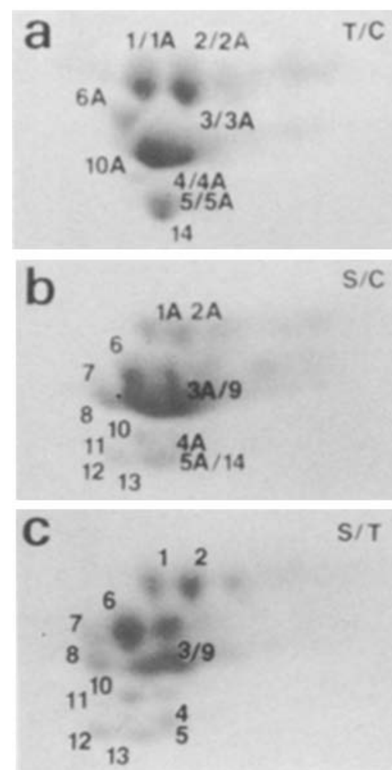

Fig. 3a-c. Enlargements of the intermediate two-dimensional electrophoretic patterns of phaseolin from the F_1 progenies. **a** T/C – F_1 ('BBL 240' × 'Contender'); **b** S/C – F_1 ('Sanilac' × 'Contender'); **c** S/T – F_1 ('Sanilac' × 'BBL 240'). The subunit numbers correspond to those of Fig. 1

'Sanilac' × 'Contender' or 'Gina'

The phaseolin of 'Sanilac' and 'Contender' had two subunits in common (Fig. 1b, nos. 6 and 10 and Fig. 1c, nos. 6A and 10A), and full expression of these proteins was seen in both the F_1 and F_2 progenies. Any recombinant phenotypes should, therefore, have intermediate combinations of subunits 1A, 2A, 7, and 5A ($\alpha51$, $\alpha48.5$, and $\gamma45.5$ bands) and of subunits 3A, 8, and 9 ($\beta48^T$ and $\beta48^S$), 4A and 11 ($\gamma46^T$ and $\gamma46^S$), and 12, 13, and 14 ($\gamma45^S$ and $\gamma45^C$) (Fig. 1).

The F_1 progeny from this cross had the intermediate S/C phaseolin pattern (Figs. 3b, 5b). Only three electro-

Table 2. Genetic analyses of the polypeptide composition of the F_2 progeny of 'BBL 240' X 'Contender'

Protein group[a]	Progeny banding pattern			χ^{2*}
	Parental	Intermediate	Parental	
Phaseolin	T	T/C	C	
	22	64	22	3.67 n.s.
A	A_2A_2	A_2A_1	A_1A_1	
	23	59	26	1.09 n.s.
B	B_2B_2	B_2B_1	B_1B_1	
	19	64	25	4.37 n.s.

n.s. = not significant
[a] There is no segregation for groups G2/albumin, D, E, or F
* $p < 0.05$

Table 3. Genetic analyses of the polypeptide composition of the F_2 progeny of 'Sanilac' X 'Contender'

Protein group[a]	Progeny banding pattern			χ^{2*}
	Parental	Intermediate	Parental	
Phaseolin	S	S/C	C	
	18	49	22	1.27 n.s.
G2/albumin	S_{G2}	S_{G2}/T_{G2}	T_{G2}	
	21	47	21	0.42 n.s.
B	B_2B_2	B_2B_1	B_1B_1	
	14	45	29	5.12 n.s.
D	D_2D_2	D_2D_1	D_1D_1	
	12	46	16	4.81 n.s.
F	F_2F_2	F_2F_1	F_1F_1	
	19	36	19	0.06 n.s.

[a] There is no segregation for group A proteins. Group D and F phenotypes were scored on one-dimensional gels and group E phenotypes could not be scored for this cross
* $p < 0.05$

Table 4. Genetic analyses of the polypeptide composition of the F_2 progeny of 'Salinac' X 'BBL 240'

Protein group[a]	Progeny banding pattern			χ^{2*}
	Parental	Intermediate	Parental	
Phaseolin	S	S/T	T	
	19	47	29	2.12 n.s.
G2/albumin	S_{G2}	S_{G2}/T_{G2}	T_{G2}	
	20	49	26	0.85 n.s.
A	A_1A_1	A_1A_2	A_2A_2	
	20	45	30	2.36 n.s.
D	D_2D_2	D_2D_1	D_1D_1	
	26	40	14	3.60 n.s.
E	E_2E_2	E_2E_1	E_1E_1	
	26	38	16	2.70 n.s.
F	F_2F_2	F_2F_1	F_1F_1	
	23	46	11	5.40 n.s.

[a] There is no segregation for group B proteins
* $p < 0.05$

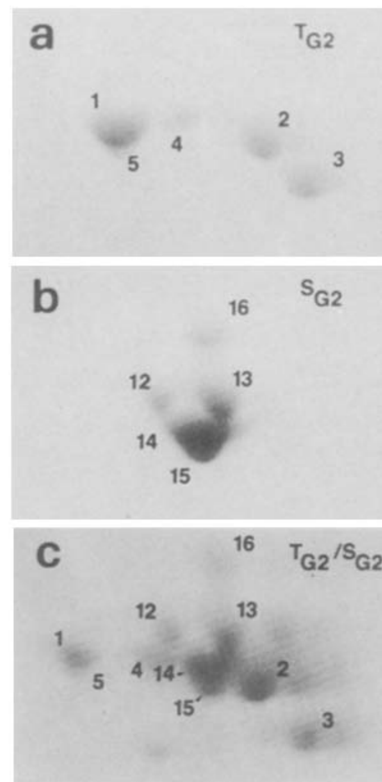

Fig. 4a-c. Enlargements of the two-dimensional electrophoretic patterns of the G2/albumin fractions from the parental cultivars and the F_1 progenies. **a** T_{G2} – 'BBL 240', 'Contender', and F_1 ('BBL 240' X 'Contender'); **b** S_{G2} – 'Sanilac'; **c** T_{G2}/S_{G2} – F_1 ('Sanilac' X 'Contender') and F_1 ('Sanilac' X 'BBL 240'). The subunit numbers correspond to those of Brown et al. (1981c)

phoretic phenotypes were observed in the F_2: the two parental patterns and the intermediate F_1 pattern. F_2 segregation ratios are given in Table 3.

The G2/albumin patterns of 'Sanilac' and 'Contender' had no subunits in common (Brown et al. 1981c) and the F_1 showed the intermediate T_{G2}/S_{G2} electrophoretic pattern (Figs. 4c, 5b) combining those of the parental cultivars (Figs. 4a, b). In the F_2 only this pattern and the two parental patterns were seen. The F_1 showed the heterozygous condition for protein groups B, D, E, and F (Fig. 5b), and F_2 segregation ratios for these proteins are presented in Table 3. 'Sanilac' and 'Contender' produced only A_1 proteins and no segregation was observed for this group.

'BBL 240' x 'Sanilac'

The phaseolin patterns of 'BBL 240' and 'Sanilac' showed only subunits 3 and 9 with a similar molecular weight and isoelectric point (Figs. 1a, b). Recombinant F_2 types should, therefore, show the absence of one or more of

subunits 1-13 from the intermediate S/T pattern observed in the F_1 progeny (Figs. 3c, 5c). The F_2 analysis showed only the two parental types and the intermediate F_1 pattern. F_2 segregation ratios are given in Table 4.

For G2/albumin polypeptides only the two parental patterns, T_{G2} and S_{G2} (Figs. 2a, b; 4a, b), and the F_1 intermediate pattern, T_{G2}/S_{G2} (Figs. 4c, 5c) were seen. Heterozygous phenotypes were also observed in the F_1 for protein groups A, D, E, and F (Fig. 5c). 'BBL 240' and 'Sanilac' both produced the B_2 phenotype. F_2 segregation ratios for these proteins are shown in Table 4.

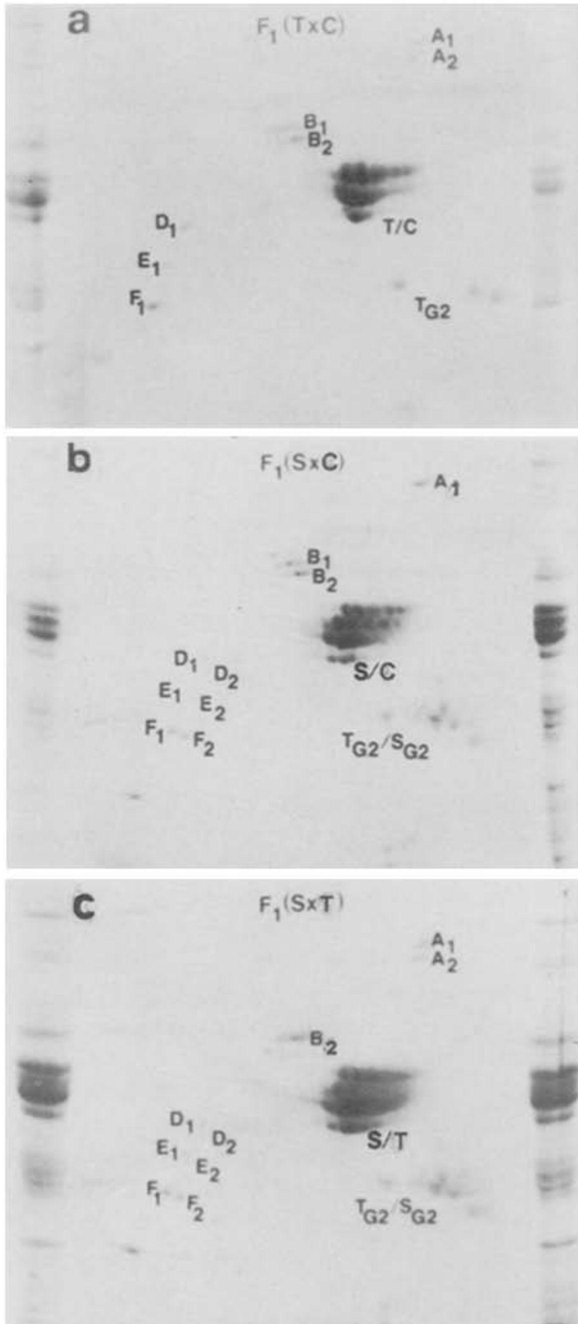

Fig. 5a-c. One- and two-dimensional electrophoretic patterns of 'total' seed protein extracts from F_1 progeny of the three intervarietal crosses. **a** F_1 'BBL 240' × 'Contender' (T × C); **b** F_1 'Sanilac' × 'Contender' (S × C), **c** F_1 'Sanilac' × 'BBL 240' (S × T)

Linkage Relationships Between Protein Groups

Chi-square tests for independent segregation were carried out on the observed segregation ratios of combination of pairs of the seven protein groups scored in the F_2 generations. These results for the F_2 populations of the crosses 'BBL 240' × 'Contender', 'Sanilac' × 'Contender', and 'Sanilac' × 'BBL 240' are shown in Tables 5, 6, and 7 respectively. Values for % recombination between the linked loci are shown in Table 8.

Table 5. Chi-square values for linkage of seed protein groups in the F_2 generation of 'Sanilac' × 'Contender'

Protein group	Phaseolin	A
B	39.70*	12.49
A	5.99	

* $p < 0.05$

Table 6. Chi-square values for linkage of seed protein groups in the F_2 generation of 'Sanilac' × 'Contender'

Protein group	Phaseolin	G2/albumin	B	D
F	8.28	125.41*	7.84	13.19
D	9.29	15.13	10.31	
B	51.44[a]	10.45		
G2/albumin	8.39			

* $p < 0.05$

Table 7. Chi-square values for linkage of seed protein groups in the F_2 generation of 'Sanilac' × 'BBL 240'

Protein group	Phaseolin	G2/albumin	A	D	E
F	8.55	112.55*	4.05	8.00	12.05
E	7.05	7.90	13.65	12.85	
D	8.80	9.65	7.65		
A	12.36	3.57			
G2/albumin	7.05				

* $p < 0.05$

Table 8. Values for % recombination between the pairs of linked loci

Cross	Linkage Group (%)	
	Phaseolin/B	G2/albumin/F
T × C	34	–
S × C	32	5
T × S	–	9

Discussion

Single Seed Analysis of Phaseolin Polypeptides

The electrophoretic banding patterns for the major polypeptide subunits of phaseolin protein have been described by Brown et al. (1981a) and are shown in Figure 1. Other phaseolin polypeptides, especially towards the acidic ends of the IEF gels, have been reported previously (Brown et al. 1980, 1981a). In the present experiments much higher sample loadings were required to see clearly the patterns of the other protein groups in the seed (Figs. 2, 5). The amount of phaseolin, therefore, greatly exceeded that loaded in the previous studies ($\sim$ 40 μg) (Brown et al. 1980, 1981a) and, therefore, the phaseolin polypeptides towards the acidic end stand out very clearly. In many cases it was necessary to electrophorese smaller amounts of 'total' extract to clearly discern the polypeptide patterns of the major phaseolin subunits.

In the three crosses studied the phaseolin banding patterns of the F_1 progenies contain the phaseolin subunits from both parental cultivars. Screening of the 292 F_2 progeny from the three crosses showed no recombinant phaseolin protein patterns and a 1:2:1 ratio of parental: F_1 intermediate:parental banding patterns (Tables 2-4). These results suggest that the genes controlling the major protein subunits of phaseolin are tightly linked and inherited in a block like a single Mendelian gene, and that the alleles are co-dominant. The ability to distinguish between the phaseolin patterns by two-dimensional analysis facilitated this detailed investigation of the inheritance of the genes controlling phaseolin polypeptide production which extends the findings of Romero et al. (1975) and Hall et al. (1977).

Single Seed Analysis of G2/Albumin Polypeptides and Other Protein Groups

The subunit composition of the major G2/albumin polypeptides was described by Brown et al. (1981c). The F_1 progeny of the crosses 'Sanilac' × 'BBL 240' and 'Sanilac' × 'Contender' had an intermediate electrophoretic pattern (T_{G2}/S_{G2}) combining those of the parental cultivars (Fig. 4). In the F_2 populations no recombinant types were seen and a 1:2:1 ratio of parental:intermediate:parental patterns was obtained (Tables 3, 4). These results suggest that the genes controlling the five major G2/albumin polypeptides of 'BBL 240' and 'Contender' and the five major polypeptides of 'Sanilac' (Brown et al. 1981c) are inherited as a block of genes in a simple Mendelian fashion and that the alleles are co-dominant.

Protein groups A, B, D, E, and F each consisted essentially of a pair of protein subunits. The genes controlling these were assumed to be allelic because each member of the F_2 populations had either a parental phenotype or the intermediate pattern reflecting the heterozygous condition. It is possible that each pair of proteins could be controlled by two unlinked genes and the absence of progeny lacking both proteins to be due to the lethality of the condition. However, the 1:2:1 ratios of parental (homozygote): heterozygote:homozygote (Tables 2-4) suggest that each pair of proteins is controlled by a pair of co-dominant alleles.

Variation for Seed Protein Composition in Bean Cultivars

Ten of the eleven cultivars studied were previously classified into three groups on the basis of their phaseolin electrophoretic patterns (Brown et al. 1981c). The cultivar 'Greensleeves' was included to allow direct comparison with the work of Bollini and Crispeels (1978) and in agreement with their report, the phaseolin of 'Greensleeves' was shown to contain three major bands (a Tendergreen type). There appears to be an underlying conservation of variation for qualitative seed protein composition as seen for phaseolin polypeptides (Brown et al. 1981a), G2/albumin polypeptides (Brown et al. 1981c), and groups A-F in Table 1.

From earlier studies of phaseolin polypeptide composition, the Contender types were postulated to have arisen as an aberrant genetic product of a cross between a Tendergreen and a Sanilac phaseolin type (Brown et al. 1981a). From the data presented here both parental types must have had the T_{G2} phenotypes and the D_1, E_1, and F_1 phenotypes to produce 'Contender' and 'Gina'. Both types must carry both, or at least one, of either the A_1 or B_1 phenotypes to produce the A_1 and B_1 phenotypes seen in the three Contender types. This suggests that variation within the phaseolin groups for other protein groups may be greater than that described here. Initial screening of other cultivars has suggested that more G2/albumin variants exist but that the T_{G2} and S_{G2} types predominate (Brown and Osborn, unpublished data). Also, 'Pinto 111' has no major G2/albumin polypeptide subunits, apparently due to either a deletion of the structural genes or a mutation inhibiting their transcription or translation (Brown et al. 1981c).

Linkage Relationships Between Genes Controlling Seed Proteins

The results in Tables 5, 6, and 7 showed clearly that the genes controlling phaseolin polypeptides were linked to

those controlling the protein subunits of group B and that those controlling the G2/albumin polypeptides were linked to the locus controlling group F polypeptides. The linkage between G2/albumin and group F proteins was also seen in Table 1 where the T_{G2} and F_1, and S_{G2} and F_2 phenotype combinations were always observed. However, for the phaseolin-group B situation, two cultivars 'Sanilac' and 'PI 229815' showed phenotypes outside our expectations. The relatively lower % recombination values obtained for this linkage group as compared to the G2/albumin and F linkage group (Table 8) suggests a more distant physical linkage between the genes controlling phaseolin and group B subunits, such that crossovers are more common and intermediate phenotypes are observed for some cultivars. All the other combinations of the protein groups showed independent segregation and, therefore, the genes controlling these seed proteins were located in five different areas of the bean genome.

The linkage of the genes controlling phaseolin and B polypeptides was observed in both the 'Sanilac' × 'Contender' and 'BBL 240' × 'Contender' crosses, and the % recombination values were similar (Table 8). Therefore, although 'BBL 240' and 'Sanilac' have no identical polypeptides in their phaseolin patterns (Brown et al. 1981a), the genes controlling their phaseolin polypeptides were allelic. Similarly, the genes controlling the major G2/albumin polypeptides of 'BBL 240' and 'Sanilac' which also had no subunits in common (Brown et al. 1981c) were allelic because linkage to group F proteins is shown in both 'Sanilac' × 'BBL 240' and 'Sanilac' × 'Contender' crosses, and the % recombination values are again very similar (Table 8).

The linkage of the genes controlling phaseolin polypeptides is consistent with the idea of similar storage proteins evolving from a single ancestral gene which has amplified and accumulated mutations (Wrigley and Shepherd 1973; Mecham et al. 1978). The small isoelectric point differences between the phaseolin polypeptides may be due to *in vivo* deamidation of glutamine or asparagine residues or to single point mutations in the base sequences of the genes. The molecular weight differences may be due to deletions, internal duplications or insertions, differences in mRNA processing, translation, or post-translational modifications. Some of the phaseolin variation can be explained in terms of mRNAs of different molecular weights (Sun et al. 1975; Hall et al. 1978) which reflect the phaseolin polypeptide differences of the three different phaseolin banding types (Hall et al. 1980). Further phaseolin variation is due to differential glycosylation of the polypeptides (Hall et al. 1978; Hall et al. 1980) and this may reflect point mutations which have changed the amino acid sequence of the proteins to cause the loss or gain of post-translational glycosylation sites.

The lectin polypeptides are contained in the G2/albumin patterns, T_{G2} and S_{G2} (Brown et al. 1981c) and, therefore, the genes controlling their production are linked as expected from their amino acid sequence homology (Miller et al. 1975). However, not all the G2/albumin polypeptides have lectin properties (Brown et al. 1981c). Also, the 41,000 dalton polypeptide (Fig. 4b, no. 16) of 'Sanilac' has different solubility characteristics than the other four major polypeptides (Fig. 4b, nos. 12-15) of the S_{G2} pattern. It is only found in the water-soluble albumin fraction, while polypeptides 12-15 are found in both the albumin and globulin fractions (Brown et al. 1981c). Thus, either the genes controlling the production of lectin polypeptides are linked to unrelated genes producing different polypeptides or the G2/albumin polypeptides are the products of a family of genes with a common ancestral origin but accumulated mutations have given rise to polypeptides with somewhat different properties and, possibly, functions. The linkage of the genes controlling the lectin polypeptides is consistent with the presence of agglutinin in bean cultivars being due to a single dominant factor (Brücher 1968; Jaffé et al. 1972).

The lack of major varietal variation and the general homology of the phaseolin polypeptides (Brown et al. 1981a; Ma et al. 1980) suggests that functional constraints on the diversity of these proteins exist. Phaseolin polypeptides have pH-dependent reversible association and dissociation properties (Sun et al. 1974). This property may have functional implications in the packaging of polypeptides for storage which impose limitations on the positions in the base sequence of phaseolin genes where mutations can be tolerated. Indeed, Thanh and Shibasaki (1978) have shown that the association of polypeptides into oligomers is non-random for β-conglycinins, the globulin proteins of soybean (*Glycine max* L.). Righetti et al. (1977) also pointed to apparent selection mutations giving rise to increased basic amino acid composition in zeins which would affect their hydrophobicity and their packaging into protein bodies.

The use of two-dimensional electrophoretic analysis of single seeds has enabled a detailed study of the inheritance of the genes controlling the phaseolin polypeptides to be made. The linkage among the phaseolin genes, together with the structural similarities of the polypeptides, extends our understanding of these genes at the molecular level. The finding that the genes controlling the phaseolin polypeptides are not linked to those controlling the G2/albumin polypeptides may be important to breeding programs. The removal of the lectinaceous G2 polypeptides without affecting phaseolin composition may reduce the necessity for heating to inactivate lectins in commercial practices. The genetic data presented will contribute to the production of a linkage map for *P. vulgaris*.

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