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Extended map for the phaseolin linkage group of *Phaseolus vulgaris* L*

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Summary. The linkage relationship of 11 bean (*Phaseolus vulgaris*) seed proteins (including phaseolin), 9 enzyme loci, and the *P* locus were analyzed in backcross and F_2 progenies by use of the software package "Mapmaker." The progenies were obtained by crossing the breeding line 'XR-235-1' and the cultivar 'Calima'. Allelic differences for seed protein loci were detected with SDS-PAGE and those for enzyme loci with starch gel electrophoresis and activity stains. The seed coat color of 'Calima' is a red/beige mottled pattern and that of 'XR-235-1' is white. Segregation at the *P* locus was followed by recording the phenotype of the BC_1S_1 and F_3 seed. A linkage group comprising ca. 90 cM was detected with the following gene order: *Est-2* – 11 – *Pha* – 8 – (*Spe*/*Spg*) – 24 – *P* – 9 – (*Spa*/*Spb*) – 16 – *Spba* – 22 – *Mdh-1*. In addition, another linkage group was detected: (*Spd*/*Spf*/*Sph*) – 5 – *Spca*. Therefore, the seed proteins appear to be organized in clusters in the bean genome.

Key words: Common bean – Seed proteins – Isozymes – Mapmaker

Introduction

Limited linkage information is currently available for *Phaseolus vulgaris* L. Only nine small linkage groups have been identified and they are defined by as few as two or as many as six morphological marker loci (Bassett 1988). The paucity of linkage data in common bean is due to reliance on morphological markers provided by natural and induced mutations; bean geneticists have found it difficult to work with lines that carry more than two recessive mutations, because of reduced vigor and fertility (Bassett 1988).

The use of molecular markers (isozymes and other proteins) for linkage analysis in common bean has been introduced in more recent years. Brown et al. (1981 b) reported that the gene encoding the major seed storage protein (phaseolin, *Pha*) was linked to genes encoding other seed storage proteins. Weeden (1984) reported linkage between the small subunit of Rubisco and malic enzyme; later, Weeden and Liang (1985) detected linkage between *Est-2* and the *P* locus. The *P* locus is essential for the expression of pigmentation in common bean (Leakey 1988). We have recently reported (Vallejos and Chase 1991) three pairs of linked isozyme/protein loci: *Adh-1* – *Got-2* (2 cM), *Aco-2* – *Dia-1* (20 cM), and *Est-2* – *Pha* (11 cM). We report here an extended linkage map of the latter group, which spans ca. 90 cM and includes six seed storage proteins, two enzyme loci, and the *P* locus.

Materials and methods

Plant material

Two inbred lines of beans were used to generate the segregating progenies: 'Calima' and 'XR-235-1.' 'Calima' is a large-seeded cultivar which belongs to the phaseolin type C group. The seed coat has a red/beige mottled pattern; this line is homozygous dominant (*PP*) at the *P* locus. 'XR-235-1' is a breeding line derived from an interspecific cross with *P. coccineous* (Freytag et al. 1982); this is a small, white-seeded (*pp*) bean which belongs to the phaseolin type S group. This breeding line was used as the pistillate parent to produce both the F_1 and the backcross (BC_1) progeny; and F_2 progeny was also produced. All BC_1 and F_2

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plants were grown to maturity and selfed to produce the BC_1S_1 and F_3 progenies, respectively.

Isozymes

Preparation of samples, electrophoretic separation in starch gels, and enzyme activity staining have been described elsewhere (Vallejos and Chase 1991). Segregation for the following enzyme loci was recorded: *Adh-1, Aco-1, Aco-2, Bnag, Dia-1, Est-2, Got-2, Mdh-1,* and *Skdh.*

Seed storage proteins

After removing the seed coat under the raphe, seed flour was obtained by carefully scraping the cotyledons with a scalpel. Five milligrams of seed flour was incubated with 50 µl of extraction buffer (0.0625 *M* TRIS · HCl, pH 6.8) for 30 min on ice. The samples were centrifuged for 2 min at 16,000 × *g* and 5 µl of the supernatant was mixed with 195 µl of SDS sample buffer (Laemmli 1970) and incubated at 65 °C for 1 h. Protein extracts were separated by SDS-PAGE (13%). Five microliter of each SDS sample was loaded in gels that were 16 cm long and 1 mm thick (Protean II; Bio-Rad, Richmond/CA). Phaseolin bands were visualized by staining with Coomassie brilliant blue. These were the only protein bands that were clearly visible in the gel at these protein concentrations. Under these conditions, it was possible to clearly identify the three *Pha* genotypes (*SS, SC,* and *CC*) in the F_2 progeny. In order to visualize the other seed proteins, the gels were destained and then silver stained using the procedure of Morrissey (1981), with the following modifications: gels were fixed overnight in ethanol (30%)/acetic acid (10%) and then incubated in glutaraldehyde (1.5%); finally, the gels were incubated in acetic acid (10%) after development of the silver stain.

Seed proteins of the parental genotypes were extracted and separated into G1, G2, albumin, and prolamine fractions according to the procedures of Brown et al. (1981a). The fractions were further separated by SDS-PAGE (Laemmli 1970) and visualized by silver staining as described above. This procedure allowed for further characterization of the protein bands that showed polymorphism between the selected parents.

Segregation and linkage analysis

Statistical analyses were carried out on a mainframe computer using the Statistical Analysis System (SAS Institute, Cary/NC). Linkage analysis was performed using the computer package "MAPMAKER" (Lander et al. 1987), which allows for a simultaneous multipoint linkage analysis. This program uses the Lander and Green (1987) algorithm to calculate the "best" map for a given order of loci.

Results

Polymorphisms

Mdh-1 alleles were detected as doublet bands. The 'Calima' allele was ca. 5 mm more advanced than the 'XR-235-1' allele, and the three possible genotypes could be distinguished in the F_2 progeny. On the other hand, the EST banding patterns of 'Calima' and XR-235 were almost identical, except that the second most anodal band of 'Calima' stained more intensely than that of 'XR-235-1.' It was not possible to distinguish between heterozygotes and 'Calima' homozygotes in the F_2. For this reason the 'Calima' allele was treated as dominant. Allelic differences in staining intensity between bands of identical electrophoretic mobility have been described in tomato (Tanksley and Rick 1980). All other enzyme loci were detected as single bands of different mobility, as described earlier (Vallejos and Chase 1991).

The seed coat is maternal tissue and therefore reflects the genotype of the mother plant. For this reason, the pigmentation pattern was recorded for seeds harvested from BC_1 and F_2 progenies. Seeds of the progenies were either white (*pp*) or pigmented (*Pp* for BC_1 and *P-* for F_2). In the pigmented class the patterns varied from plain beige to varying intensities of red mottling, indicating the presence of other genes involved in mottling.

Comparisons of the seed storage protein profiles revealed size polymorphisms for at least 11 different proteins (Table 1). The two parental alleles were identified for 5 of the 12 proteins, including phaseolin (*Spa, Spb, Spf, Sph,* and *Pha*). Full segregation information was recorded for these proteins in the F_2 progeny. Of the remaining protein bands, five were unique to 'Calima' and their corresponding alleles were unidentified in 'XR-235-1' (*Spba, Spc, Spd, Spe,* and *Spg*). The segregation of these proteins was recorded according to genotype in the BC_1 and according to phenotype in the F_2 progeny. One band was unique to 'XR-235-1' (*Spca*), and its allelic counterpart was not identified in 'Calima'; the segregation of this protein was recorded only in the F_2.

Monogenic segregations

Results of the tests for Mendelian ratios in the BC_1 (1:1) are depicted in Table 2. Significant deviations were detected for only two of the loci studied: *Spba* ($P=0.05$) and *Spc* ($P=0.005$). A significant deficiency of recurrent parent (XR-235) alleles was detected at each one of these

Table 1. Characterization of seed proteins that display allelic differences between 'Calima' and 'XR-235-1': molecular weights and protein fraction

Protein		Molecular weights (kD)	
Band	Fraction	Calima	XR-235
Spa	G1	82.8	79.1
Spb	G1	58.5	53.3
Spba	G2	52.1	
Pha	G1	45.3	
		44.0	44.5
		42.4	42.4
Spc	G2	37.9	
Spca	G2		34.8
Spd	G2	31.0	
Spe	G2	20.5	
Spf	G2	17.0	17.4
Spg	G1	16.0	
Sph	G2	14.8	15.4

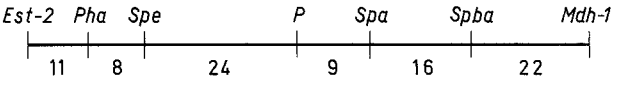

Fig. 1. Average map distances in centiMorgans for the *Pha* linkage group

Table 2. Analysis of monogenic segregation ratios in backcross (BC_1) and F_2 progenies

Locus	Backcross			F_2				
	Genotype		$\chi^2_{(1:1)}$	Genotype			$\chi^2_{(1:2:1)}$	$\chi^2_{(1:3)}$
	x/x[a]	x/c		x/x	x/c	c/c		
Est-2	41	41	0.00	37	68[b]			5.87*
Pha	47	43	0.18	31	59	29	0.07	
Spe, g	47	43	0.18	37	80[b]			2.74
P	31	37	0.37	22	101[b]			3.32
Spa, b	43	47	0.18	20	58	43	8.90*	
Spba	35	52	4.13*	30	92[b]			0.01
Mdh-1	45	45	0.00	25	58	40	4.06	
Spc	31	59	8.71**	21	101			3.94*
Spca					83	30		0.14
Spd, f, h	42	48	0.40	26	95			0.80

[a] Genotypes: x/x homozygous for XR-235 alleles; c/c homozygous for Calima alleles; x/c heterozygous

[b] When heterozygous plants could not be distinguished from homozygous for either Calima or XR-235 alleles, a 1 : 3 ratio was tested in the F_2 progeny

*, ** Significant deviation at the 0.05 and 0.005 levels, respectively

loci, although it was less pronounced for *Spba* than for *Spc*. This deficiency persisted for *Spc* in the F_2. In addition, in the F_2 progeny, two different loci displayed significant ($P=0.05$) deviations from expected ratios. One of them, *Est-2*, had an excess of homozygotes for 'XR-235' alleles, whereas the linked pair *Spa*/*Spb* showed a deficiency for the same class.

Linkage analysis

During the process of recording the segregation data, it was noticed that some of the seed proteins were inherited in clusters, i.e., only parental combinations could be detected. These clusters were made up as follows: (*Spa*/*Spb*), (*Spd*/*Spf*/*Sph*), and (*Spe*/*Spg*). One member of each group was included in the data set for linkage analysis. "Mapmaker" was used to analyze the linkage relationships among the segregating loci in both progenies (BC_1 and F_2). The linkage criteria for the analysis were such that linkage was considered only if the LOD scores were greater than 3.0 and the recombination value was less than 0.30. The maximum LOD score is the $\log_{10}$ of the ratio between the likelihood when the locus pair is at its maximum likelihood recombination fraction and when the loci are taken to be unlinked. Thus, a LOD score of 3.0 for a locus pair means that the pair is 1,000 times more likely to be linked than not linked. The analysis confirmed our previous report (Vallejos and Chase 1991) for three isozyme/protein locus pairs: (*Got-2* – *Adh-1*),

Table 3. Two-point linkage data for loci associated with the phaseolin (*Pha*) linkage group. The upper right corner contains data from the BC_1 and the lower left corner for the F_2

		Est-2	*Pha*	*Spe*	*P*	*Spa*	*Spba*	*Mdh-1*
Est-2	'p'	–	0.11	0.18	0.24	0.26	–	–
	cM	–	11.18	19.19	26.02	28.27	–	–
	LOD	–	12.36	7.74	4.17	4.42	–	–
Pha	'p'	0.11	–	0.07	0.19	0.22	–	–
	cM	10.87	–	6.74	20.47	23.86	–	–
	LOD	13.57	–	17.52	5.85	6.39	–	–
Spe	'p'	0.16	0.10	–	0.21	0.18	–	–
	cM	16.25	10.03	–	22.26	18.61	–	–
	LOD	9.75	15.29	–	5.25	8.80	–	–
P	'p'	–	0.18	0.25	–	0.05	0.17	–
	cM	–	18.50	28.14	–	4.51	17.37	–
	LOD	–	7.33	3.79	–	14.85	6.95	–
Spa	'p'	–	0.31	–	0.13	–	0.13	–
	cM	–	36.91	–	13.31	–	13.84	–
	LOD	–	4.04	–	10.26	–	11.51	–
Spba	'p'	–	–	–	0.26	0.18	–	0.21
	cM	–	–	–	29.37	18.61	–	22.75
	LOD	–	–	–	3.20	8.37	–	6.75
Mdh-1	'p'	–	–	–	–	0.29	0.21	–
	cM	–	–	–	–	33.88	22.14	–
	LOD	–	–	–	–	4.95	7.02	–

'p': recombination fraction; cM: centiMorgan distance; LOD: maximum LOD score

(*Aco-2 – Dia-1*), and (*Est-2 – Pha*). A linkage cluster was detected for a group of seed proteins: (*Spd/Spf/Sph*) – 5 cM – *Spca*. Furthermore, five additional seed proteins, the *P* locus, and *Mdh-1* were found in the linkage group of the *Pha* locus. The two-point linkage results from both progenies (BC_1 and F_2) for the members of this linkage group are shown in Table 3.

"Mapmaker" assigned seven loci (*Spa, Spe, Spba, Pha, P, Est-2,* and *Mdh-1*) to one linkage group based on two- and three-point linkage analyses. Inspection of these results led to the selection of the first six loci for determination of gene order. With the multipoint mapping capability, "Mapmaker" evaluates all possible gene orders and then selects the best 20 orders. To reduce the number of permutations and save computer time, the *Mdh-1* locus was not included. After the gene order was established, this locus was positioned based on the two-point linkage results. The best gene orders were identical in both the BC_1 and the F_2-*Est-2 – Pha – Spe – P – Spa – Spba*. However, it should be pointed out that in the BC_1 this order was only 2.5 times more likely than the second order, in which the positions of *P* and *Spa* were reversed. The possibility of this order reversal was not apparent in the F_2 data. The distances between loci were calculated using the simultaneous multipoint linkage capability of "Mapmaker." The Kosambi (1944) function was selected to estimate map distances. Simlar distances between loci were calculated with both progenies; however, the major discrepancy was observed for the distance between *P* and *Spa* (see Table 3). The distances between loci were averaged for the two progenies (Fig. 1).

Discussion

Two interesting observations can be made about the seed proteins described in this project. First, the polymorphisms detected by SDS-PAGE suggest that insertions and/or deletions in the coding regions may be responsible for the size differences; however, one cannot disregard the possibility of critical amino acid changes, which can alter mobility in an SDS gel (Ferl 1985). Second, some of the seed proteins appear to be organized in clusters in the bean genome. These observations raise questions about the nature of these polymorphisms and the bases for cluster organization. Proteins from a single cluster may be related and may have arisen by tandem gene duplication and subsequent divergence, as proposed for the phaseolin multigene family (Talbot et al. 1984).

The BC_1 and F_2 differed in the loci that displayed significant deviations from Mendelian ratios. Although there was an excess of 'XR-235-1' allele homozygotes at the *Est-2* locus in the F_2, defficiencies of 'XR-235-1' homozygotes were detected for *Spba* in the BC_1 and for *Spa* in the F_2. These differences suggest the possibility of specific interaction between the genomes of the two bean groups. Noteworthy is the fact that the deviations in the same direction occurred in two neighboring loci. Deviations from Mendelian ratios have been detected previously in bean progenies resulting from crosses between accessions from middle America and those of Andean origin (Koenig and Gepts 1989). Furthermore, distorted ratios have been reported for interspecific progenies between *P. vulgaris* and *P. coccineous* (Smartt 1970). This type of deviation has also been detected in interspecific progenies in *Lycopersicon* (Rick 1969; Tanksley et al. 1982; Vallejos and Tanksley 1983). Thus, in this particular cross, some of the observed skewings may involve chromosome segments from the *P. coccineous* progenitor of 'XR-235-1,' whereas others may involve interactions between the two *P. vulgaris* gene pools.

The linkage group flanked by *Est-2* and *Mdh-1* and comprising ca. 80–100 cM is the largest identified for beans with molecular markers. The greater distances between loci observed in the F_2 suggests that perhaps recombination is not uniform in male and female gametes. The use of molecular markers and the multipoint mapping capability of "Mapmaker" have made it possible to define such a linkage group. This group appears to be that identified by Bassett (1988) as Group VII. This group is also the largest group that has been identified with morphological markers.

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