Segregation and Linkage of Genes for Seed Proteins, Isozymes, and Morphological Traits in Common Bean (*Phaseolus vulgaris*)

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Gene segregation and linkage relationships between biochemical and morphological markers were analyzed in F_2 populations derived from crosses between wild and cultivated *Phaseolus vulgaris* accessions from Mesoamerica and the Andean region. Biochemical markers included the seed proteins phaseolin and lectin and the isozymes diaphorase, leucine amino-peptidase, malic enzyme, malate dehydrogenase, ribulose bisphosphate carboxylase, and shikimic acid dehydrogenase. The morphological markers were seed shininess, flower color, and banner petal spot. All crosses showed reciprocal differences in the segregation of some markers. With the exception of two hybrids ('Pinto UI 114' × G07469 and 'Black Turtle Soup' × Peru 34), the crosses displayed skewed segregation ratios. The phaseolin locus was not linked to any of the isozyme loci or the lectin locus, indicating that these loci are in different regions of the bean genome. Linkages were suggested between the genes for the small subunit of ribulose bisphosphate carboxylase, seed lectin, and malic enzyme (in that order) and between phaseolin and seed shininess.

The genetic linkage map of common bean (*Phaseolus vulgaris* L., Fabacae; $2n = 2x = 22$) is partially developed and consists mainly of morphological markers distributed over eight linkage groups.[2,21] None of the linkage groups has been mapped to chromosomes.[2] Many of the morphological markers used in previous inheritance studies involved color-specifying genes with pleiotropic effects or genes that were not seedling markers; therefore, the populations required much time and effort to score.[17,22,29,36] Partially to remedy this situation, Nagata and Bassett[28] used artificial mutagenesis to induce nine morphological seedling mutants. They found that two of the marker genes mapped to the previously known linkage group VII and that three others formed a new linkage group.

In common bean, Brown et al.[4] examined the segregation and linkage relationships of several seed proteins, including phaseolin and lectin. They found that phaseolin and lectin are unlinked and that both are inherited as single Mendelian factors. Linkages were identified between the genes coding for the small subunit of ribulose bisphosphate carboxylase and malic enzyme and between those coding for white flower color and esterase-2.[42] With the exception of the latter study, no study has examined genetic linkages between morphological and biochemical markers.

We examined the linkage relationships of genes for two seed proteins (phaseolin and lectin), six isozymes [ribulose bisphosphate carboxylase (its small subunit), shikimic acid dehydrogenase, malic enzyme, malate dehydrogenase, diaphorase, and leucine amino-peptidase], and three simply inherited morphological traits (flower color, banner petal spot, and seed shininess).

Materials and Methods

Plant Materials

We analyzed F_2 populations of the following crosses: L13 × G07469 (and reciprocal), 'Pinto UI 114' × G07469 (and reciprocal), NB 1401 × L13 (and reciprocal), PI319441 × G07469, and 'Black Turtle Soup' (BTS) × Peru 34. Parents L13, G07469, and PI319441 are wild *P. vulgaris* accessions obtained from J. G. Waines of the University of California at Riverside. L13 and PI319441 originated in Mexico, and G07469 originated in the Andes. NB 1401, BTS, Peru 34, and Pinto UI 114 are common bean cultivars that were obtained from J. G. Waines. NB 1401 and Peru 34 are large-seeded genotypes that originally were domesticated in the Andean region.[12] BTS, a small-seeded genotype, and Pinto UI 114, a medium-seeded genotype, both were domesticated in Mesoamerica.[12] The geno-

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Table 1. Genotypes of the common bean lines used in this study

Seed identification	Evolutionary origin[b]	Seed proteins		Isozymes[a]						Morphological traits		
		Phs[c]	*Lec*[d]	*Rbcs*	*Lap-3*	*Skdh*	*Diap-1*	*Mdh-1*	*Me*	*V*	*Mf*	*J*
L13	M	M	NS[e]	100	100	100	95	100	100	NS	NS	NS
PI319441	M	S	NS	100	103	103	100	100	100	NS	NS	NS
Pinto UI 114	M	S	NS	100	103	103	95	100	102	NS	NS	NS
Black Turtle Soup	M	Sb	B	100	100	103	95	100	100	V	Mf	j
G07469	A	T	NS	98	103	100	100	100	98	NS	NS	NS
NB1401	A	T	NS	98	103	100	100	103	98	NS	NS	NS
Peru 34	A	T	P	98	103	100	100	103	98	v[lae]	mf	J

[a] A = Andean region; M = Mesoamerican region.

[b] Phaseolin type. M = Mesoamerican phaseolin; S = 'S' phaseolin; Sb = 'Sb' phaseolin; T = 'T' phaseolin (Gepts et al.[13] and Koenig et al., unpublished observations).

[c] Lectin type: B = lectin of BTS; P = lectin of Peru 34.

[d] Isozyme variants, according to anodal migration and frequency (see text).

[e] NS = not scored.

types are shown in Table 1. Some of the genotypes in Table 1 were not scored because they did not segregate in the particular populations (isozymes or morphological markers) or did not reveal clearly distinguishable segregating classes (lectin).

Crosses were made in the fall of 1986, and the F_1 plants were grown in the spring of 1987 in a greenhouse to prevent insect cross-pollination. F_2 seed were stored at room temperature until analyzed in the winter of 1987–1988. Before seed protein analysis, we removed a small part of each seed at the raphe end to analyze seed proteins (see below). Seed were germinated, and at the primary leaf stage we took tissue samples for isozyme analysis (see below). For the cross BTS × Peru 34, plants were grown to maturity to evaluate the three morphological markers included in this study. Flowers segregated for purple versus pink color and for presence versus absence of a darker blotch at the basis of the banner petal.[38] Seed segregated for opaqueness versus shininess.

Polyacrylamide Gel Electrophoresis of Phaseolin and Lectin Seed Proteins

Sixty seeds from each F_2 population were individually analyzed for seed proteins by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE). We prepared a flour sample by removing the seed coat and scraping the endosperm from the raphe end of each seed. The sample was suspended for at least 30 min in a buffered 0.25 M NaCl solution [0.3125 M Tris-HCl, pH 6.8; 1 mM EDTA; 1% (wt/vol) SDS; 30% (wt/vol) sucrose; 0.5% (vol/vol) 2-mercaptoethanol; and 0.005% (wt/vol) bromophenol marker dye).[4] The sealed mixture was heated at 100°C for 5 min and then centrifuged at 15,000 *g* for 5 min at room temperature. Five-microliter samples of the supernatant were subjected to one-dimensional SDS/PAGE, following the method of Laemmli[16] as modified by Ma and Bliss.[24] Electrophoresis was carried out in 0.75-mm-thick, 15% (wt/vol) polyacrylamide slab gels (height, 13 cm; width, 16 cm). Samples were layered in the wells and subjected to 10 mA per gel at loading (approximately 30 min) and thereafter to 30 mA per gel until the separation was complete. Proteins were stained with Coomassie Brilliant Blue 250 dissolved in 5 parts water : 5 parts methanol : 1 part glacial acetic acid; gels were destained in 5 parts water : 5 parts methanol : 1 part glacial acetic acid; and phaseolin (PHS) and lectin (LEC) proteins were identified by the method of Brown et al.[4]

Starch Gel Electrophoresis of Isozymes

The 60 seeds used for seed protein analysis were sown in vermiculite, and plant tissues were analyzed at the first true-leaf stage (V2 stage),[8] approximately 10 days after sowing. A crude tissue homogenate was produced by grinding the primary leaf or root apex (depending on the enzymes assayed) in a solution of 3.75% reduced glutathione and 1% polyvinyl pyrrolidone (PVP) adjusted to pH 7.6 with 1 M Tris. The homogenate was absorbed onto paper wicks that were loaded on 10% starch gel and subjected to electrophoresis according to the procedures of Weeden.[40] Preliminary studies indicated the plant tissue with maximum enzyme expression and the appropriate buffer system. Ribulose biphosphate carboxylase (RBCS), leucine amino-peptidase (LAP), and shikimate dehydrogenase (SKDH) were analyzed in leaf extracts separated in Tris citrate/lithium borate, pH 8.1[32] or the histidine citrate, pH 6.5[5] gels. Malic enzyme (ME) and malate dehydrogenase (MDH) were analyzed in root extracts separated in Tris citrate/lithium borate, pH 8.1, gels. NADH-dependent diaphorase (DIAP) was analyzed in root extracts subjected to electrophoretic separation in histidine citrate, pH 6.5, gels. Gels were stained for LAP by the method of Shaw and Parasad;[33] for RBCS by the method of Weeden;[40] for SKDH, MDH, and DIAP by the method of Vallejos;[37] and for ME by the method of Brewer.[3]

Loci were labeled sequentially, with those migrating farthest from the cathodal end being designated 1. The most common allozyme was designated 100, and all other allozymes were measured in millimeters from the standard, as described by Kesseli and Michelmore.[15] The designation of the most common allozyme was based on data from the *P. vulgaris* collection at the University of California, Davis, which includes over 500 landraces and bred cultivars with a wide range of geographical origins (S. P. Singh et al., unpublished observations). In each gel, the cultivars 'ICA-Pijao' and 'California Dark Red Kidney' (CDRK) were included as standards. ICA-Pijao has the following genotype: *Rbcs*^{100}, *Skdh*^{103}, *Me*^{100}, *Mdh-1*^{100}, *Diap-1*^{95}, *Diap-2*^{105}, and *Lap-3*^{100}. CDRK exhibits the following genotype: *Rbcs*^{98}, *Skdh*^{100}, *Me*^{98}, *Mdh-1*^{103}, *Diap-1*^{100}, *Diap-2*^{100}, and *Lap-3*^{103}. Goodness-of-fit and linkage analyses were conducted for each F_2 family using LINKAGE-1.[35]

Results

Segregation

We studied the segregation of biochemical and morphological markers in eight F_2 populations, including three reciprocal crosses (Table 2). The genetic control of phaseolin (*Phs*), lectin (*Lec*), RBCS, SKDH, ME, DIAP, flower color (*V*), banner petal blotch (*Mf*), and seed shininess (*J*) has been described.[4,18,20,34,38,39,41] DIAP is a tetrameric enzyme that is coded by two tightly linked genes, *Diap-1* and *Diap-2*.[34] No

recombinants between these two loci were observed in our study. Consequently, segregation and linkage data for *Diap-1* only are presented here. The markers *Mdh-1* and *Lap-3* have not been described previously in the common bean. *Lap-3* codes for the most cathodal of three LAP isozymes. The more common slow allozyme migrates to 100 mm, and the fast allozyme migrates to 103 mm. *Mdh-1* codes for the most anodal of two MDH isozymes. The more common slow allozyme migrates to 100 mm, and the fast allozyme migrates to 103 mm.

Segregation ratios for a high percentage of the markers, whether biochemical or morphological, deviated significantly from the expected ratios in most of the crosses (Table 2). Among the 50 segregations analyzed in our study, 18 (36% of the total) deviated significantly from the expected ratios. *Phs* and *Diap-1* had a high percentage of distorted segregations (50% and 62.5%, respectively). In comparison, other loci, such as *Rbcs* and *Me* (25% and 14.3%, respectively), showed a lower percentage of distorted segregations in the crosses we examined (Table 2).

Normal Mendelian ratios for all loci were obtained in the F_2 generation of crosses between Pinto UI 114 and G07469 and crosses between Black Turtle Soup and Peru 34. The majority of the crosses examined in this study, however, had a high proportion of skewed segregations. For example, four of five segregating loci in the F_2 generation of cross G07469 × L13 and four of six loci examined in the F_2 generation of cross L13 × NB1401 had distorted ratios. For a given cross, distortion resulted from a deficiency of either the maternal or the paternal allele. For example, in the cross L13 × NB1401 the observed frequencies of maternal alleles for the *Me* locus and of paternal alleles for the *Lap-3, Diap-1,* and *Mdh-1* loci were significantly lower than expected.

We observed reciprocal cross differences at several loci. Two patterns of distortion were found. In the first pattern, one cross fitted the expected ratio while the reciprocal cross deviated significantly from the expected ratio. For example, in cross L13 × G07469, *Lap-3* exhibited a normal segregation ratio ($P = .98$), whereas in the reciprocal cross, *Lap-3* exhibited a deficiency in the paternal (*Lap-3*^{100}) allele (Table 2). In the second pattern, both crosses deviated significantly from the expected ratio. For example, the *Diap-1*^{100} allele was deficient in both reciprocal crosses involving parents L13 and G07469 (Table 2).

Table 2. Single-locus segregation data

F_2 family (P1P1 × P2P2)	Segregation classes			Goodness of fit[a]	
	P1P1	P1P2	P2P2	χ^2	P
L13 × G07469					
Phs	14	39	4	11.25	.004
Rbcs	13	24	13	0.80	.96
Lap-3	12	23	11	0.43	.98
Me	9	24	18	3.35	.19
Diap-1	18	31	2	12.41	.002
G07469 × L13					
Phs	7	38	15	6.40	.041
Rbcs	18	15	19	9.35	.009
Lap-3	11	39	2	16.12	<.001
Me	15	26	11	0.62	.74
Diap-1	1	29	23	18.73	<.001
L13 × NB1401					
Phs	21	28	11	3.60	.17
Rbcs	15	25	18	1.41	.49
Lap-3	20	29	7	6.11	.047
Me	7	30	23	8.53	.014
Mdh-1	24	31	4	13.71	.001
Diap-1	24	24	12	7.20	.027
NB1401 × L13					
Phs	9	27	24	8.10	.017
Rbcs	18	27	13	1.14	.57
Lap-3	16	25	17	1.14	.57
Me	16	32	9	2.58	.28
Mdh-1	1	30	25	20.86	<.001
Diap-1	8	25	27	13.70	.001
Pinto UI 114 × G07469					
Phs	15	36	9	3.60	.17
Rbcs	7	27	12	2.48	.29
Lap-3	14	22	10	0.78	.68
Me	9	23	15	1.55	.46
Diap-1	11	23	13	0.19	.91
Skdh	16	24	6	4.43	.11
G07469 × Pinto UI 114					
Phs	8	36	12	5.14	.08
Rbcs	14	27	8	1.98	.13
Lap-3	11	25	12	0.12	.94
Me	11	20	18	3.65	.16
Diap-1	13	26	12	0.06	.97
Skdh	9	31	8	4.12	.13
PI319441 × G07469					
Phs	12	23	25	8.90	.012
Rbcs	12	17	20	7.20	.027
Lap-3	18	19	11	4.12	.13
Me	8	30	12	2.64	.27
Mdh-1	6	30	14	4.56	.10
Diap-1	4	13	32	42.80	<.001
Skdh	13	25	11	0.18	.91
BTS × Peru 34					
Phs	27	36	14	4.71	.10
Lec	21	34	22	1.08	.58
Rbcs	22	32	20	1.46	.48
Me	15	38	23	1.68	.43
Mdh-1	17	39	20	0.29	.87
Diap-1	14	15	9	3.00	.22
Skdh	17	26	21	0.49	.78
V	..... 49[b]		13	0.54	.46
Mf	..... 47[b]		16	0.01	.94
J	20	..... 44[b]		1.33	.25

[a] Loci *Phs, Lec, Rbcs, Lap-3, Me, Mdh-1, Diap-1,* and *Skdh* were tested against a 1:2:1 ratio; loci *V, Mf,* and *J* were tested against a 3:1 ratio.

[b] Numbers in the dominant class.

Linkages

We investigated linkages between loci whose segregation fitted the expected ratios. *Lec* was linked to both *Rbcs* ($r = 0.29$) and *Me* ($r = 0.23$) in the cross BTS × Peru 34. The recombination value between *Rbcs* and *Me* was $r = 0.36$; therefore, the likely gene order is *Rbcs-Lec-Me.* Linkage between *Rbcs* and *Me* also was observed in the progeny of cross PI319441 × G07469

($r = 0.32$); in the crosses G07469 × Pinto UI 114 and NB1401 × L13, we also observed linkages between these two loci, although at lower significance levels ($r = 0.35$, $P = .12$ and $r = 0.33$, $P = .07$, respectively). In the other progenies included in this study, no statistically significant linkages between *Rbcs* and *Me* were observed. In the cross BTS × Peru 34, linkage was found between *Phs* and *J* ($r = 0.19$). We observed a similar recombination value in an additional cross.[1] We did not find linkage between *Phs* and any isozyme locus or *Lec*, indicating that these loci characterize different regions of the bean genome.

Discussion

The crosses we examined involved a number of both wild and cultivated genotypes from the Andes and Mesoamerica. Cultivars from the Andean region on average have larger seeds than do Mesoamerican cultivars. In addition, larger seeds are correlated in most cases with larger leaves and longer internodes, resulting in an overall effect on plant growth habit.[6,7,9,10] Cultivated bean accessions from Mesoamerica exhibit the 'S' phaseolin types, in contrast to those from the Andean region, which have 'T,' 'C,' 'H,' and 'A' phaseolin types.[11,12] Allozyme data (R. Koenig and P. Gepts, manuscript in preparation; S. P. Singh et al., manuscript in preparation) indicate two distinct clusters—Mesoamerican and Andean—based on allelic similarities. The F_1 hybrid weakness observed in crosses involving Andean and Mesoamerican cultivars suggests that geographical isolation between the two regions might have led to genetic divergence and the establishment of two distinct gene pools.[10]

The high frequency of segregation distortion may be a result of genetic divergence between Mesoamerican and Andean *P. vulgaris* genotypes. A study by Zamir and Tadmor[43] of the genera *Lens, Capsicum,* and *Lycopersicon* revealed that 54% of the loci in the progeny of interspecific crosses deviated from the expected segregation ratios, whereas 13% of the loci deviated from the expected ratios in the progeny of intraspecific hybrids. In comparison, our data are intermediate, indicating that the progeny result from crosses between genetically divergent parents. To confirm this genetic divergence, more segregation data are needed on intragene pool crosses (i.e., Mesoamerican × Mesoamerican or Andean × Andean) and interspecific crosses such as *P. vulgaris* × *P. coccineus.*

Distorted segregations of specific loci occurred in one direction or both directions of a reciprocal cross. An example of the former is provided by the segregation of *Lap-3* in the reciprocal crosses involving L13 and G07469. We found distorted segregation ratios when G07469 was used as the female parent. This lack of reciprocity suggests a nuclear–cytoplasmic interaction. The deficient allele $Lap\text{-}3^{100}$ of the Mesoamerican parent may be linked to genes that cause the genotypes that carry them to be at a disadvantage in the Andean cytoplasm at the sporophytic level or the gametophytic level. These interactions assume the existence of differences between the maternal and paternal cytoplasmic genomes and unipaternal inheritance of cytoplasmic genomes; data supporting these assumptions are lacking in the common bean.

Distorted segregations of specific loci that occur in both directions of a reciprocal cross presumably result from nuclear interactions. Examples of such reciprocally distorted segregations were provided at the *Diap-1* and *Mdh-1* loci in the cross involving NB1401 and L13 (Table 2). In both cases, the Andean allele was present at a lower-than-expected frequency irrespective of the cytoplasmic background (Table 2). No observations of a deficient number of Mesoamerican alleles were made in our study.

The elimination of specific alleles or weak progeny associated with certain genetic combinations that result in altered segregation ratios has been widely observed.[13] Distorted segregation ratios arise from a wide array of processes, including preferential segregation,[14] certation or pollen tube competition,[25] pollen lethal factors,[23,27,30] preferential fertilization,[19,31] and selective elimination of the zygote.[26] Which of these, among others, is responsible for the distorted segregation ratios observed in our study must await further experimentation.

We observed linkage between *Rbcs* and *Me* in only some of the progenies. A similar observation was made by Weeden.[39,41] Both the genetic background and the environment are known to influence recombination frequencies and may account for the variability in linkage values observed between two distant loci such as *Rbcs* and *Me.* The identification of an intermediate locus (*Lec*) to which both *Rbcs* and *Me* are linked supports the existence of *Rbcs–Me* linkage.

There are several implications of our results to breeding programs. Based on molecular markers, crosses involving Mesoamerican × Andean genotypes were found to be the most divergent intraspecific crosses and may generate more significant progress from selection. The transfer of polygenic traits such as the high yield potential of small-seeded Mesoamerican types to large-seeded Andean types may, however, be a problem, because distorted segregations make it more difficult to recover certain recombinants. This may explain in part why it has been difficult to transfer quantitative traits between cultivars of Mesoamerican and Andean origin.

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