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Linkage between isozyme markers and a locus affecting seed size in *Phaseolus vulgaris* L.*

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Summary. Backcross and F_2 progenies were produced between two bean genotypes, 'XR-235' and 'Calima,' which differ in seed weight by a factor of two. The small-seeded 'XR-235' was used as the pistillate and recurrent parent. These genotypes showed polymorphisms at nine isozyme loci and at the phaseolin locus. Seed size parameters (weight, length, width, and thickness) were determined for each BC_1 and F_2 individual, i.e., for seeds harvested from 'XR-235' after pollination with F_1 and from the F_1 after selfing, respectively. A combination of starch gel electrophoresis and enzyme activity staining was used to determine the genotype of each BC_1 and F_2 individual at the segregating loci. SDS-PAGE and Coomassie blue staining were used to determine genotype at the phaseolin locus. Tests for independent assortment using two-way contingency and maximum likelihood tables revealed three linkage pairs: *Aco-1* – 20 cM – *Dia-1*; *Adh-1* – 2 cM – *Got-2;* and *Est-2* – 11 cM – *Pha*. Statistical comparisons were made between the means of genotype classes at each segregating locus for all seed size parameters. The results from two independently obtained BC_1s and the F_2 consistently indicated that the *Adh-1*–*Got-2* segment was linked to a locus that affected seed size and overcame maternal control over seed size. This locus has been designated *Ssz-1*. This gene exhibited additive gene action and accounted for 30–50% of the seed size difference between the parents.

Key words: Beans – Seed size – QTL – Isozymes – Linkage

Introduction

Seed size (weight) is considered a component of yield in dry bean breeding programs (Al-Mukhtar and Coyne 1981; Conti 1982, 1985). Negative correlations between seed size and yield have been reported for some crosses (Coyne 1968); however, such correlations can impose limitations in breeding programs of large-seeded types. Seed size in cultivated beans has been described as polygenic (Sax 1923; Yarnell 1965) and, from crosses between cultivated and wild types, it has been estimated that there are at least ten genes involved (Motto et al. 1978). The use of genetic markers to identify genes involved in seed size control may help assess more clearly their role. Sax (1923) pointed out that the genes controlling continuous variation could be identified through linkage with qualitatively inherited characters. In fact, he identified a gene affecting seed size in beans, which was linked to a gene that controls pigmentation, the *P* locus. More recently, quantitative trait loci have been identified and mapped using isozyme loci (Tanksley et al. 1982) and DNA restriction fragment length polymorphisms (RFLPs) (Paterson et al. 1988) in tomato.

We are constructing a linkage map in beans based on isozymes and RFLPs. The general strategy has been to produce backcross and F_2 progenies from a pair of contrasting genotypes. Some of this contrast encompasses a twofold size difference in seed weight. Traditionally, the genetic analysis of bean seed weight has been carried out with progeny tests, using the average seed weight of seeds produced by each individual of a BC_1 and/or F_2 generation. This procedure is dictated by the control of the maternal tissue genotype over the size of the seed it bears (Bassett 1982). However, when we harvested F_1 seeds, i.e., seeds harvested from 'XR-235' after pollination with 'Calima,' it was noticed that these seeds had a weight

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intermediate between the parents. Furthermore, seed size variation was observed in the BC_1 seed harvested from the small-seeded parent ('XR-235') after pollination with the F_1, and in the F_2 seed harvested from F_1 plants after selfing. This variation suggested that some genes from the large-seeded 'Calima' parent could exert a direct effect on the size of the embryo and overcome the maternal effect on seed size. The objective of this project was to determine whether any of the segregating isozyme loci could be used to detect genes that affect seed size in the mode described above.

Materials and methods

Plant material

Two *Phaseolus vulgaris* L. inbreds that have allelic differences at nine enzyme loci and a twofold difference in seed size were selected to produce backcross and F_2 progenies: 'XR-235-1', a breeding line obtained from Dr. M.J. Bassett (University of Florida, Department of Vegetable Crops), which carries resistance to common bacterial blight (Freytag et al. 1982) and has an average seed weight of 275 mg, and 'Calima', a cultivar obtained from Dr. W. Roca (CIAT), which is adapted to Colombia and East Africa with an average seed weight of 500 mg. 'XR-235' was used as the pistillate parent to obtain the F_1 progeny and as the recurrent parent for the backcross progenies.

Isozyme analysis

A combination of starch gel electrophoresis and enzyme activity staining was used to detect polymorphisms between parental lines for eight enzymes: alcohol dehydrogenase (ADH) (E.C. 1.1.1.1), aconitase (ACO), (E.C. 4.2.1.3), β-N-acetylglucosaminidase (BNAG) (E.C. 3.2.1.30), diaphorase (DIA) (E.C. 1.6.4.3), esterase (EST) (E.C. 3.2.1.2), glutamate oxaloacetate transaminase (GOT) (E.C. 2.6.1.1), malate dehydrogenase (MDH) (E.C. 1.1.1.37), and shikimate dehydrogenase (SKDH) (E.C. 1.1.1.25). Enzyme activity stains were those described by Tanksley (1984) for ACO, by Vallejos (1983) for ADH, EST, GOT, MDH, and SKDH, and by Weeden (1984 and 1986) for DIA and BNAG. ADH, ACO, and MDH were resolved with a histidine buffer system: gel buffer (5 m*M* histidine · HCl, adjusted to pH 7.0 with NaOH), electrode buffer (135 m*M* TRIS, 43 m*M* citric acid, final pH 7.0). Samples were loaded in the gel at 13.3 V/cm for 15 min, the wicks were then removed and the gel was run for 3 more hours. BNAG, EST, GOT, and SKDH were resolved using a TRIS-citrate system: gel buffer (15.2 m*M* TRIS, 3.9 m*M* citric acid, pH 7.8), electrode buffer (0.3 *M* H_3BO_3 adjusted to pH 7.9 with 4 *N* NaOH). Samples were loaded for 15 min at 13.3 V/cm, the wicks were then removed and the gel was run at 13.3 V/cm for 1 h, and at 20 V/cm until the borate front reached 10 cm from the origin. Alternatively, this system can be run at 2.6 mA/cm^2 on constant current at all stages. Finally, DIA was resolved in a TRIS-citrate lithium borate system: electrode buffer (190 m*M* borate adjusted to pH 8.1 with 2 *M* LiOH), gel buffer (9 vol. of 50 m*M* TRIS adjusted to pH 8.4 with citric acid and 1 vol. of electrode buffer). Samples were loaded at 15 V/cm for 20 min and, after the wicks were removed, the gel was run at the same conditions for 4 moure hours (Sprecher and Vallejos 1989).

Seed samples were used for ADH and MDH. Seed flour was obtained by scratching the cotyledons with a scalpel blade under the raphe after partially removing the seed coat; 20 mg of seed flour was incubated in a microcentrifuge tube with 60 µl of extraction buffer (0.1 *M* TRIS · HCl, pH 7.8) for 30 min on ice. Samples were collected from the supernatant with filter paper wicks after a 2-min spin. Samples for ACO, BNAG, GOT, and SKDH were obtained by grinding petioles with 1/3 vol. of buffer, and those for DIA and EST were obtained from root tissue in a similar manner.

Phaseolin type was determined by resolving seed extracts on SDS-PAGE. Briefly, seed flour was obtained as described above, and 5 mg of seed flour was incubated with 50 µl of extraction buffer [0.0625 *M* TRIS · HCl (pH 6.8), 0.5 *M* NaCl] for 30 min. The samples were centrifuged for 5 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 37 °C for 1 h. Protein extracts were separated by SDS PAGE in 12% gels.

Seed parameters and statistical analysis

Seed measurements in the different progenies were taken on the seed of the individuals themselves, i.e., seeds harvested from 'XR-235' after pollination with 'Calima' for the F_1, from 'XR-235' after pollination with the F_1 for BC_1, and from the F_1 after selfing for F_2. Individual seed weights were measured with an analytical balance (Mettler AE 100) to 0.1 mg of resolution. Length, width and thickness of individual seeds were measured with a Vernier caliper to a resolution of 0.1 mm. All statistical analyses were performed on a mainframe computer using the Statistical Analysis System (SAS Institute, Cary/NC).

Results

Quantitative trait-seed size

The descriptive parameters of seed size (weight, length, width, and thickness) and shape (ratios of the three dimensions) for the parents and their F_1 are listed on Table 1. The mean seed weight of 'Calima' was about twice that of the breeding line 'XR-235-1', whereas the seed weight of their hybrid was intermediate (Table 1). The three one-dimensional parameters of 'Calima' seeds also exceeded those of the breeding line, whereas the dimensions of the F_1 seeds were intermediate. Regardless of size, comparisons of the ratios of the three dimensions showed that 'Calima' seeds were more elongated than 'XR-235' seeds [$(L/W)_{Cal} > (L/W)_{XR}$, and $(L/T)_{Cal} > (L/T)_{XR}$] and that they were also proportionally thinner [$(W/T)_{Cal} < (W/T)_{XR}$] (Table 1). As expected from the previous measurements the F_1 had intermediate ratios.

Seeds of the parents and F_1 used for the measurements were obtained in the greenhouse at the same time. However, those of the segregating progenies were obtained at different times and under slightly different greenhouse conditions. For this reason, direct comparisons between parents and progeny, and heritability calculations cannot be made with the available data. The two BC_1 progenies (BC_1A and BC_1B) were obtained at different times. Seed weight and sizes were slightly higher for the BC_1A than for the BC_1B, although only the differences in width and W/T ratio were significantly different (0.01 level). This difference lead to their independent analysis. On the other hand, the BC_1A and F_2 progenies could

Table 1. Seed size and shape measurements of the parents ('XR-235-1' and 'Calima'), their F_1 ('XR-235-1' × 'Calima'), two BC_1 ('XR-235' × F_1) sets (A and B), and the F_2

		'XR-235'	F_1	'Calima'		BC_1A[b]	BC_1B	F_2
Weight (mg)	x[a]	276.7	405.0	505.5	x	415.3a	383.6	518.2
	s_x	7.6	11.8	9.9	s	102.1	62.3	85.8
	n	30	25	30	n	52	38	99
Length (mm)	x	10.74	13.00	15.38	x	13.13	12.80	15.40
	s_x	0.12	0.14	0.15	s	1.39	1.41	1.22
Width (mm)	x	6.77	7.51	7.93	x	7.96**	7.63	8.21
	s_x	0.07	0.08	0.06	s	0.66	0.40	0.49
Thickness (mm)	x	4.78	5.45	6.00	x	5.48	5.51	5.87
	s_x	0.06	0.10	0.06	s	0.69	0.51	0.53
L/W[c]	x	1.59	1.73	1.94	x	1.64	1.67	1.88
	s_x	0.02	0.01	0.01	s	0.08	0.15	0.13
L/T	x	2.25	2.40	2.57	x	2.42	2.32	2.64
	s_x	0.02	0.05	0.03	s	0.20	0.21	0.21
W/T	x	1.42	1.39	1.32	x	1.46*	1.39	1.41
	s_x	0.02	0.03	0.01	s	0.15	0.12	0.13

[a] x = mean, s_x = standard error, s = standard deviation, n = sample size
[b] t-tests were used to compare the means of the two independent backcrosses; no significant differences were found between means that underlined
[c] L = length, W = width, T = thickness.
*, ** Significant differences at the 0.05 and 0.01 levels, respectively. Comparisons of BC_1A and F_2 using t-tests showed significant differences at the 0.001 level for all parameters listed

Table 2. Segregation analysis of enzyme loci backcross and F_2 progenies using the χ^2 test

Locus		Genotype			χ^2	χ^2	Locus		Genotype			χ^2	χ^2
		x/x[a]	x/c	c/c	1:1	1:2:1			x/x	x/c	c/c	1:1	1:2:1
Aco-1	BC_1A	32	20		2.77		*Est-2*	BC_1A	20	24		0.36	
	BC_1B	20	18		0.10			BC_1B	21	17		0.42	
	F_2	31	61	31		0.01		F_2	37	68			5.87*,[b]
Aco-2	BC_1A	26	23		0.18		*Got-2*	BC_1A	19	33		3.77	
	BC_1B	18	20		0.10			BC_1B	21	17		0.42	
	F_2	32	70	21		4.32		F_2	24	58	41		5.10
Adh-1	BC_1A	20	32		2.77		*Mdh-1*	BC_1A	25	27		0.08	
	BC_1B	21	17		0.42			BC_1B	20	18		0.10	
	F_2	25	59	39		3.39		F_2	25	58	40		4.06
Bnag	BC_1A	22	21		0.02		*Skdh*	BC_1A	18	23		0.61	
	BC_1B	20	18		0.10			BC_1B	13	25		3.79	
	F_2	33	57	33		0.66		F_2	38	58	27		2.36
Dia-1	BC_1A	26	23		0.18		*Pha*	BC_1A	29	23		0.69	
	BC_1B	17	21		0.42			BC_1B	18	20		0.10	
	F_2	33	65	25		1.44		F_2	31	59	29		0.07

[a] x/x and c/c are homozygous for 'XR-235' and 'Calima' alleles, respectively; x/c is heterozygous
[b] A 1:3 ratio was tested because the heterozygotes could not be distinguished from the homozygotes for 'Calima' alleles
* Significant at the 0.05 level

be compared with each other because they were obtained at the same time and under identical greenhouse conditions. One-tail t-test indicated that the means for seed weight, length, width, and thickness of the F_2 were significantly larger than those of the BC_1A (0.001 level). Finally, tests of normality using the Shapiro-Wilk statistic ($n < 51$) (Shapiro an Wilk 1965) or the Kolomogorov statistic ($n > 50$) (Stephens 1974) indicated that all of the seed weight and size parameters were normally distributed.

Table 3. Tests of independent assortment for linked isozyme loci using two-way contingency tables. Results from two independently obtained backcross progenies

Loci			Genotypes				χ^2	p[a]	$\bar{p}$
Y	Z		Parentals		Recombinants				
			$Y^{x/x}Z^{x/x}$	$Y^{x/c}Z^{x/c}$	$Y^{x/x}Z^{x/c}$	$Y^{x/c}Z^{x/x}$			
Aco-2	*Dia-1*	A[b]	19	16	7	7	8.91*	28.6	
		B	13	16	5	4	10.45**	23.7	26.1
Adh-1	*Got-2*	A	18	31	2	1	40.06**	5.8	
		B	21	17	0	0	38.00**	0.0	2.6
Est-2	*Pha*	A	20	20	4	0	30.56**	9.1	
		B	17	16	4	1	21.24**	13.1	11.1

[a] Percent recombination
[b] A and B indicate rows for data obtained with the BC_1A and BC_1B respectively
*,** Significant at the 0.005 and 0.001 levels, respectively

Table 4. Calculation of linkage by the maximum likelihood method (Allard 1956) for three pairs of linked loci using F_2 data

		Dia-1			cM
		x/x	c/c	c/c[a]	
Aco-2	x/x	23	8	1	19.7±2.9
	x/c	8	49	13	
	c/c	2	8	11	
		Got-2			
Adh-1	x/x	24	1	0	2.0±0.9
	x/c	0	56	3	
	c/c	0	1	38	
		Pha			
Est-2	x/x	28	5	3	10.8±3.2
	_/c	1	43	22	

[a] Genotypes: x/x and c/c stand for homozygotes for 'XR-235' and 'Calima' alleles, respectively; x/c stands for heterozygotes; _/c stands for a mixture of homozygotes and heterozygotes

Segregation and linkage analysis of isozyme loci

Monogenic segregations of the isozyme loci were analyzed with χ^2 tests in the backcross and F_2 progenies (Table 2). All loci tested in the backcrosses segregated in the expected 1:1 ratio. Slight but not significant skewings were observed for *Aco-1*, *Adh-1*, and *Got-2* in BC_1A, and for *Skdh* in BC_1B. No significant deviations from Mendelian ratios were detected in the F_2, with the exception of *Est-2*, for which an excess of 'XR-235' alleles was detected; a 1:3 ratio was tested at this locus because the heterozygotes could not be distinguished from the homozygotes for the 'Calima' allele. In this progeny again, a slight but not significant overabundance of 'Calima' alleles was detected at the *Adh-1*, *Got-2*, and *Mdh-1* loci, whereas the opposite was true for the *Aco-2* and *Skdh* loci.

The linkage relationships between isozyme loci were also investigated. Tests of independent assortment for all pair combinations were conducted using two-way contingency tables. These tests revealed three linked pairs (Table 3). Significant deviations from independent assortment were detected in all three progenies under study for these pairs. The average recombination obtained with the two BC_1s was 26% for the *Aco-2–Dia-1* pair, while the maximum likelihood method using F_2 data gave a linkage intensity of 19.8±2.9 cM (Table 4). The average recombination between *Adh-1* and *Got-2* estimated with BC_1 data (2.6%) was in good agreement with the linkage intensity determined with F_2 data (2±0.9 cM). Finally, an average recombination of 11% between *Est-2* and *Pha* observed with BC_1 data was confirmed with the maximum likelihood estimate from F_2 data of 10.8±3.2 cM.

Detection of QTLs for seed size

Large-seeded 'Calima' was used as the donor parent to generate the BC_1s. Thus, genes of this genotype that control embryo size by overcoming maternal control would be segregating in these progenies. In order to investigate possible linkage between these genes and isozyme loci, one-tail *t*-test were used to compare the means of seed size parameters between homozygotes and heterozygotes at all segregating enzyme loci (Table 5). The means of all seed size parameters of heterozygotes were significantly higher than those of homozygotes at the *Adh-1* and *Got-2* loci. It has already been established that these loci are 2 cM apart. Therefore, it can be concluded that there is at least one locous affecting seed size in this chromosome region. These results were confirmed with one-way analyses of variance for seed size parameters of the F_2 progeny at each segregating locus. A *t*-test

Table 5. Detection of linkage between segregating isozyme loci and a locus that affects seed size. One-tailed *t*-test was used to compare the means of homozygotes (x/x) and heterozygotes (x/c) at each segregating locus

Locus		Genotype		Weight (mg)			Length (mm)			Width (mm)			Thickness (mm)		
		x/x	x/c	x/x	x/c	*t*-T	x/x	x/c	*t*-T	x/x	x/c	*t*-T	x/x	x/c	*t*-T
Aco-1	A	32	20	396.5	445.2	*	12.8	13.6	*	7.78	8.24	*	5.42	5.58	NS
	B	20	18	376.7	391.3	NS	12.4	13.1	NS	7.56	7.70	NS	5.53	5.48	NS
Aco-2	A	26	23	441.0	405.4	NS	13.6	12.9	NS	8.09	7.96	NS	5.66	5.37	NS
	B	18	20	390.6	377.3	NS	13.0	12.5	NS	7.63	7.62	NS	5.56	5.46	NS
Adh-1	A	20	32	329.5	468.9	***	11.9	13.9	***	7.39	8.31	***	4.96	5.81	***
	B	21	17	353.5	420.8	***	12.1	13.6	***	7.50	7.79	**	5.35	5.71	*
Bnag	A	22	21	414.2	438.6	NS	13.1	13.5	NS	7.96	8.08	NS	5.50	5.64	NS
	B	20	18	394.4	371.5	NS	13.1	12.3	NS	7.68	7.57	NS	5.56	5.45	NS
Dia-1	A	26	23	424.4	424.1	NS	13.3	13.2	NS	7.95	8.12	NS	5.63	5.41	NS
	B	17	21	380.0	386.5	NS	13.0	12.6	NS	7.62	7.64	NS	5.50	5.52	NS
Est-2	A	20	24	443.1	416.9	NS	13.4	13.3	NS	8.02	8.10	NS	5.74	5.40	NS
	B	21	17	367.7	403.2	*	12.4	13.2	NS	7.55	7.72	NS	5.44	5.59	NS
Got-2	A	19	33	324.5	467.5	***	11.8	13.9	***	7.33	8.31	***	4.99	5.77	***
	B	21	17	353.5	420.8	***	12.1	13.6	***	7.50	7.79	**	5.35	5.71	*
Mdh-1	A	25	27	407.9	422.1	NS	13.0	13.3	NS	7.91	8.00	NS	5.49	5.48	NS
	B	20	18	384.3	382.8	NS	13.0	12.5	NS	7.58	7.68	NS	5.50	5.52	NS
Skdh	A	18	23	424.6	425.6	NS	13.4	13.2	NS	8.04	8.00	NS	5.50	5.60	NS
	B	13	25	385.4	382.6	NS	12.8	12.7	NS	7.66	7.61	NS	5.44	5.49	NS
Pha	A	29	23	413.4	417.6	NS	13.0	13.3	NS	7.85	8.09	NS	5.55	5.41	NS
	B	18	20	377.3	389.2	NS	12.5	13.0	NS	7.56	7.70	NS	5.55	5.46	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 levels, respectively
NS – not significant

Table 6. Results of the analyses of variance and Duncan's tests (0.05 level) used to compare the means of the F_2 genotypes at each isozyme locus

Locus	Genotypes			Weight (mg)			Length (mm)			Width (mm)			Thickness (mm)		
	x/x	x/c	c/c	x/x	x/c	c/c	x/x	x/c	c/c	x/x	x/c	c/c	x/x	x/c	c/c
Aco-1	24	48	27	518	514	524	15.1	15.4	15.7	8.18	8.18	8.30	5.80	5.94	5.80
Aco-2	24	57	18	511	523	511	15.4	15.4	15.0	8.13	8.25	8.20	5.87	5.85	5.91
Adh-1	16	49	34	471*, b	517a, b	541a	14.9*, b	15.3a, b	15.8a	8.17	8.25	8.17	5.58**, b	5.81a, b	6.08a
Bnag	26	49	24	513	535	487	15.3	15.6	15.0	8.27	8.30	7.27	5.93	5.92	5.70
Dia-1	24	52	23	514	516	526	15.3	15.5	15.3	8.14	8.20	8.30	5.89	5.84	5.91
Est-2(d	31	62		506	521		15.2	15.4		8.12	8.23		5.98	5.82	
Got-2	15	48	36	467a, b	514a, b	544a	14.8*, b	15.2a, b	15.8a	8.17	8.25	8.19	5.59**, b	5.79b	6.09a
Mdh-1	24	44	31	499	517	534	14.9*, b	15.4a, b	15.8a	8.14	8.23	8.24	5.94	5.82	5.87
Skdh	31	44	24	525	518	507	15.6	15.7	15.2	8.24	8.24	8.14	5.94	5.86	5.77

*, ** Significant at the 0.5 and 0.005 levels respectively; '*F*' tests for the main effect
a, b No significant differences were detected between means bearing the same letters
(d A *t*-test was used at this locus because only two electrophoretic phenotypes could be distinguished

was used for the *Est-2* locus, because the heterozygotes could not be distinguished from the homozygotes for 'Calima' alleles. These results showed that the chromosome segment marked by *Adh-1–Got-2* had significant effects on all seed size parameters, with the exception of width (Table 6). Duncan tests were used to evaluate the difference between the genotype means at these loci. In both cases, the two homozygote classes were significantly different from each other, but each was not significantly different from the heterozygote class. However, the mean of the heterozygote class was always intermediate, suggesting additivity at this locus. Noteworthy are the contrasting sensitivities of the statistical tests for the BC_1 ($P=0.001$) and the F_2 ($P=0.05$) progenies.

Marginal significant differences were detected only in BC_1A at *Aco-1* for weight, length, and width. Similar results were obtained for the seed weight of BC_1B at the *Est-2* locus. However, these results were proven to be artifacts under closer scrutiny. Two-way factorial analyses of variance between *Got-2* and all the other loci showed that the chromosome segment marked by *Got-2* was the only source of variation; when the variation at the *Got-2* locus was accounted for, variation at the other loci was not detected. Furthermore, inspection of the two-way contingency tables showed slight deviations for the pair combinations of both *Aco-1* (in BC_1A) and *Est-2* (in BC_1B) with *Got-2*; these anomalies can account, at least in part, for the artifacts observed. Interestingly, the two-way factorial analysis between *Adh-1* and *Got-2* in BC_1A suggested that the latter was closer to the locus responsible for seed size. However, this observation cannot be considered conclusive. The tight linkage between these loci resulted in only one individual in one recombinant class and two in the other (Table 3); the excessive disproportion in class sizes seriously reduced the usefulness of this analysis of variance. Significance for seed length at the *Mdh-1* locus was detected in the F_2. Several interpretations are possible for this results. If a seed size gene in this chromosome segment had a detectable effect only in the F_2 seed and not in the BC_1 seed, then a recessive allele would be expected; however, the homozygous class for the 'Calima' allele was not significantly different from the heterozygous class. Another explanation is the possible interaction between the *Mdh-1*- linked locus with other factors not detected in this study. Finally, this result may be due to the observed underrepresentation of the double homozygote class for 'XR-235' alleles for *Got-2* and *Mdh-1*. At best, the role of this chromosome segment cannot be assessed conclusively in this study.

Discussion

The normal distribution detected in the BC_1 and F_2 progenies underscored the polygenic nature of the seed size character, as others have previously reported (Motto et al. 1978; Sax 1923; Yarnell 1965). Two lines of evidence indicate that the principal mode of action of these polygenes is additivity. Seeds of the F_1 progeny had intermediate weights between the progenitors. The mean seeds weight of the BC_1A was closer to the small-seeded recurrent parent than the mean seed weight of the F_2 progeny. Additive gene action for genes involved in seed size has also been reported previously (Hamblin and Morton 1977; Conti 1985).

The twofold difference in seed size between the progenitors suggested that either a large number of polygenes with similar effects or at least one major and a few others with minor effects would segregate in the progenies. With a limited number of markers, one can expect to detect minor genes with tight linkages or major genes with moderate linkage. The consistent results obtained with two independently obtained backcrosses and the F_2 strengthened the validity of the linkage between (*Adh-1*-2cM-*Got-2*) and the locus that contributes to seed size. Inspection of the mean seed weights of the three genotypes at either the *Got-2* or *Adh-1* loci indicates that the mode of action of this gene is additive. The mean seed weight of heterozygotes was intermediate to the two homozygote classes. This locus is assigned the symbol *Ssz-1* to indicate seed size. An interesting feature of this gene is its ability to overcome the maternal control of seed size, which is normally observed in beans.

The contrasting sensitivities of the statistical analyses observed between the BC_1 and F_2 progenies makes for an interesting observation. The much higher level of significance detected with the BC_1 indicates clearly that this gene can easily overcome the maternal control of the recurrent parent genotype (XR-235). On the other hand, the marginal level of significance observed with the F_2 suggests two nonexclusive explanations. First, a greater variation is expected in each class of the F_2 relative to each class of the BC_1. For a given number of genes (n) involved in a character, the number of genotypes per class at each locus will be a power base higher in the F_2 than in the BC_1 [$(F_2)\ 3^{n-1} > (BC_1)\ 2^{n-1}$]. The second and more interesting possibility is that this gene has a smaller effect when it is present in seeds coming from the hybrid genotype (XR-235 × Calima). The fact that there are not many reports on this type of gene action for seed size leads to the question of whether this effect would be more frequently observed in compatible crosses between the two gene pools of beans. These results also suggest that there may be specific interactions between genes expressed in the embryo and genes expressed in the maternal plant. These interactions could be more noticeable in wide crosses. We have begun to investigate this possibility.

Parental and progeny seed sizes were obtained from seeds produced under different greenhouse conditions and direct comparisons cannot be made. However, with the available data it is possible to obtain a first approximation of the contribution to seed weight by this gene. The smallest difference (67 mg) between heterozygotes and homozygotes at the *Got-2* locus was detected in BC_1B. This weight difference represents 50% of the variation in seed weight between the F_1 and the small-seeded progenitor. On the other hand, the difference between the two homozygous classes in the F_2 at the *Got-2* locus is about 33% of that of the parents. Therefore, this appears to be a major gene that can explain from 33 to 50% of the variation in seed size. These results also mean that there

must be other genes responsible for the remainder of the seed size variation besides environmental effects.

Tagging of *Ssz-1* provides a unique opportunity to study the mechanism by which this gene controls seed size. Does *Ssz-1* control the extent of cell division in the developing embryo or the final cell size? For instance, these results indicate that *Ssz-1* affects lenght to a greater extent than it does the other two dimensions, suggesting in turn that this gene also affects seed shape. What effect does this gene have on source-sink relationships during embryo development? The availability of tags for genes that control seed size can be useful in studies of bean evolution. In addition to phaseolin type, seed size is one of the major differentiating characters between mesoamerican and andean *Phaseolus vulgaris* beans (Gepts 1988). The availability of genetic markers for genes involved in seed size control in beans will certainly aid physiological and evolutionary studies centered around this important agronomic character.

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