Theor Appl Genet (1986) 71:847–855

Bean arcelin

2. Genetic variation, inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris* L.

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Received September 17, 1985; Accepted October 17, 1985
Communicated by D. von Wettstein

Summary. Crude proteins from seeds of wild bean accessions of Mexican origin were analyzed by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE). Several accessions had electrophoretic patterns showing unique protein bands. When analyzed by two-dimensional isoelectric focusing (IEF)-SDS/PAGE, four protein variants which had electrophoretic mobilities similar to each other but different from the other major seed proteins, phaseolin and lectin, were observed. All four variants, which have not been described in cultivated beans, were tentatively named arcelin proteins and designated as arcelin 1, 2, 3 and 4. Arcelins 3 and 4 had polypeptides that comigrated on two-dimensional gels and these variants occurred in accessions that were collected in the same location. Analysis of single F_2 seeds from crosses among arcelin-containing lines and from crosses between cultivated beans lines without arcelin and arcelin-containing lines revealed that differences in arcelin polypeptide expression were inherited monogenically. The alleles for different arcelin variants were codominant to each other and dominant to the absence of arcelin. The gene(s) controlling arcelin proteins were unlinked to those controlling phaseolin expression and tightly linked to genes controlling the presence of lectin proteins ($< 0.30\%$ recombination). The possible origins of arcelin genes and their potential role in bruchid resistance are discussed.

Key words: *Phaseolus vulgaris* – Seed protein – Arcelin – Inheritance – Linkage – Bruchidae

Introduction

Wild forms of *Phaseolus vulgaris* L., indigenous to Middle America and South America, are potential sources of protein variants that can be used for the genetic improvement of bean cultivars. In the accession PI 325690, several seeds were found to contain a novel protein previously unreported in common bean (Romero Andreas 1984). This protein, named arcelin, co-isolated with the globulin-2 fraction and had a molecular weight intermediate to phaseolin and lectin proteins. In the progeny of a cross 'Sanilac' × PI 325690-3, the expression of arcelin was inherited monogenically, with presence of arcelin being dominant. In F_2 and F_3 seeds, reduced amounts of phaseolin were associated with the presence of arcelin (Romero Andreas 1984).

Among 106 wild bean accessions screened by one-dimensional SDS/PAGE for seed protein electrophoretic variability (Gepts 1984), some were found to contain novel protein bands which appeared to be arcelin protein. This study describes electrophoretic variability and inheritance of arcelin proteins, and the linkage relationships between the genes controlling arcelin, phaseolin and lectin proteins.

Materials and methods

Plant materials

Original seeds of the wild bean accessions used in this study were obtained from the *Phaseolus* world collection at CIAT,

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Table 1. Summary of four arcelin variants and the wild bean accessions with arcelin containing seeds

Arcelin type	Accessions with arcelin-containing seeds[a]	Proportion of seeds with arcelin
Arcelin 1	G12882 (= PI325690)	1/5
	G10999	1/5
Arcelin 2	G12866	5/5
Arcelin 3	612891	4/5
	G12922–G12935	5/5
Arcelin 4	G12949	5/5
	G12952	5/5
	G12953	5/5

[a] 'G' numbers are designation given to accessions held at CIAT

Cali, Columbia and the Western Regional Plant Introduction Station, Pullman, Wash., USA (Table 1). Selected seeds from these accessions and F_1 and F_2 seeds resulting from several crosses were grown in the greenhouse under natural light during the fall and winter seasons.

Sample preparation

Bean flour samples were prepared by removing the seed coat from the raphe end of single seeds and scraping the cotyledon tissue into a test tube using a razor blade. Care was taken to avoid injuring the embryonic axis so that seeds could be germinated subsequently. Crude proteins were extracted from the flour samples by suspending the bean flour in 0.5 M NaCl for 30 min at room temperature. After centrifugation, an aliquot was removed, mixed with an equal volume of cracking buffer (0.625 M Tris HCl, pH 6.8; 2 mM EDTA; 2% (w/v) SDS; 40% (w/v) sucrose; 1% w/v 2-mercaptoethanol; and 0.01% (w/v) bromophenol blue) and placed in a boiling water bath for 5 min. These samples were submitted to electrophoresis and the remaining portions of the samples used for hemagglutination assays.

Electrophoresis

One-dimensional SDS/PAGE was performed according to the method of Laemmli (1970) as modified by Ma and Bliss (1978) using 0.75 mm thick, 15% (w/v) polyacrylamide slab gels. Two-dimensional IEF-SDS/PAGE was carried out as described by Brown et al. (1981b) using 15% (w/v) polyacrylamide slab gels for the SDS dimension.

Hemagglutination tests

The presence or absence of lectin was determined by testing single parental and F_2 seeds for rabbit erythrocyte agglutination activity. Erythrocytes in a mixture of rabbit blood and Alsevers solution (1 : 1) were washed three times with phosphate buffered saline (PBS), pH 7.0 and resuspended in PBS to give a 3% solution. Equal volumes of this suspension and solutions containing bean flour in 0.5 M NaCL were mixed and incubated for 30 min at room temperature. After incubation, the presence or absence of hemagglutination was determined macroscopically.

Analysis of segregating populations

The goodness of fit of observed segregation ratios to expected ratios was determined using analysis of chi square by orthogonal function (Mather 1951). An estimate of maximum possible linkage distance was obtained using the method of maximum likelihood (Mather 1951).

Results

Electrophoretic variants

Seeds from several accessions contained heavily staining proteins with molecular weights between 31.0 and 45.0 kd. Five putative electrophoretic variants were observed among 22 accessions (Fig. 1, lanes 1–5).

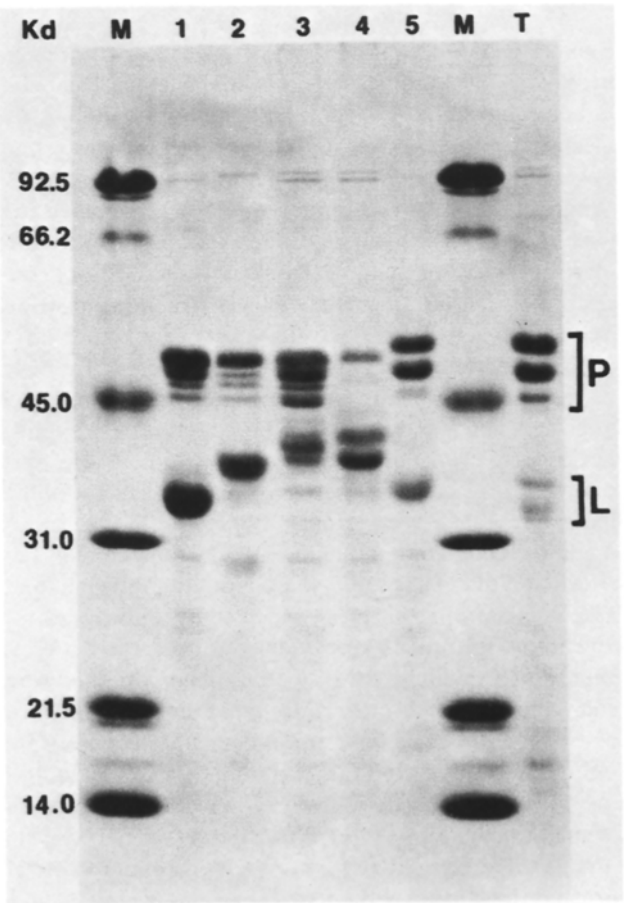

Fig. 1. One-dimensional SDS/PAGE of crude seed proteins from five wild bean accessions and one bean cultivar. *1* G12866-1 (arcelin 2); *2* G12882-1 (arcelin 1); *3* G12891-1 (arcelin-3); *4* G12949-1 (arcelin 4); *5* G10019-1; T 'Tendergreen'. The electrophoretic positions of phaseolin (*P*) and lectin (*L*) are indicated. Molecular weight standards are in lanes *M:* (from highest to lowest *MW*) phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysosyme

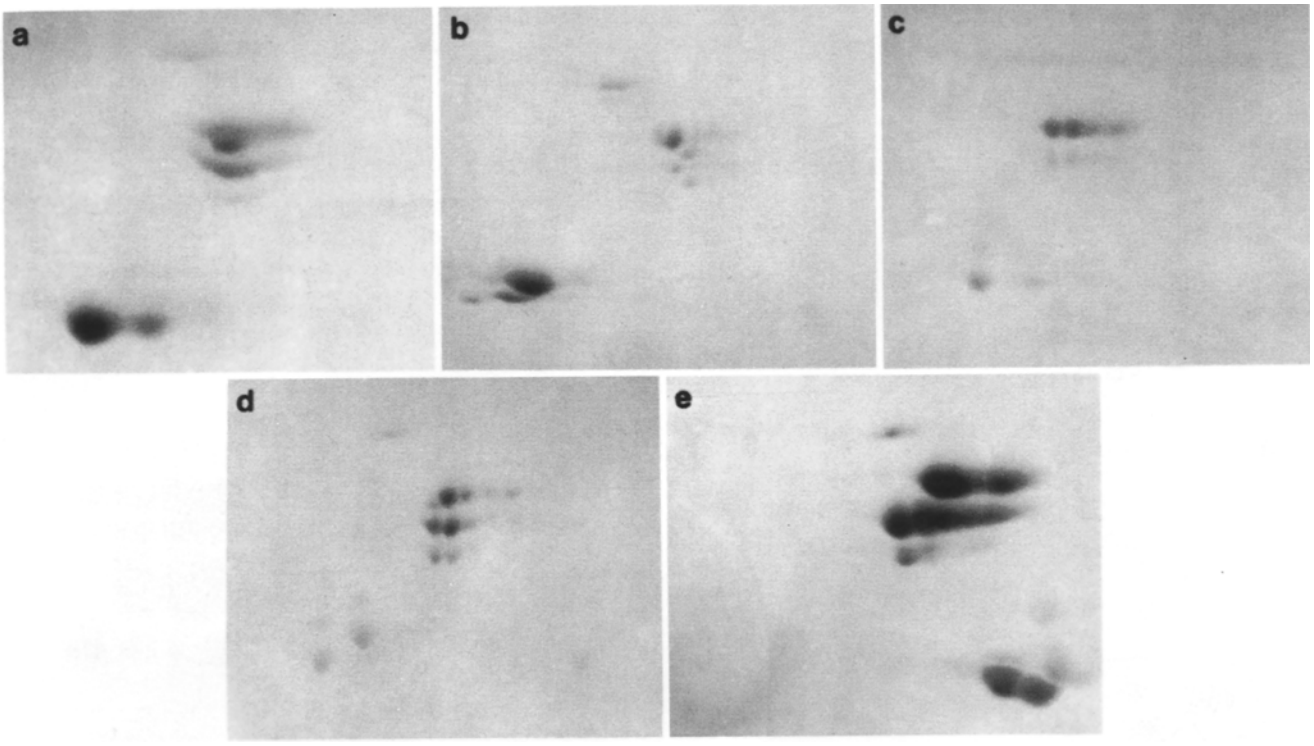

Fig. 2a–e Two-dimensional IEF-SDS/PAGE of crude seed proteins from wild bean accessions. **a** G12866-1 (arcelin 2); **b** G12882-1 (arcelin 1); **c** G12949-1 (arcelin 4); **d** G12891-1 (arcelin 3); **e** G10019-1. The horizontal dimension represents separation by isoelectric focusing with the left side being basic and the right side being acidic. The vertical dimension represents separation by molecular weight using SDS/PAGE. The major protein group in the center of each gel is phaseolin. The lightly staining protein group to the lower right side of phaseolin is lectin. Arcelin migrates to the lower left side of phaseolin

Crude protein samples from seeds containing the five electrophoretic variants were submitted to two-dimensional IEF-SDS/PAGE (Fig. 2). In four of the samples (Fig. 2a–d), novel polypeptides corresponding in molecular weight (35,000–42,000) to the ones observed by one-dimensional electrophoresis were observed to the basic (left) side of the phaseolin polypeptides. The four samples also contained small amounts of lectin polypeptides to the acidic (right) side of the phaseolin polypeptides. The fifth sample (Fig. 2e) contained no proteins to the basic side of phaseolin, but did contain a relatively large amount of lectin proteins accounting for the heavily staining band seen on one-dimensional gels.

Based on electrophoretic mobility, these four protein variants represent a new class of bean seed proteins not described previously in cultivated beans. One of these variants (Fig. 1, lane 2 and Fig. 2b) was reported previously in a wild bean accession, PI 325690 (Romero Andreas 1984). This protein was given the name arcelin after Arcelia, the town in Guerrero, Mexico, where the accession had been collected. We are tentatively naming the other three variant proteins arcelins and have designated them as arcelin 1 (Fig. 1, lane 2 and Fig. 2b) arcelin 2 (Fig. 1, lane 1 and Fig. 2a), arcelin 3 (Fig. 1, lane 3 and Fig. 2d) and arcelin 4 (Fig. 1, lane 4 and Fig. 2c).

Crude seed proteins from an accession containing arcelin 4 were mixed with crude seed proteins from accessions containing arcelins 1, 2 and 3 and submitted to two-dimensional electrophoresis (Fig. 3). Electrophoresis of the mixture containing arcelins 2 and 4 (Fig. 3a) demonstrated that these two variants contain no polypeptides in common. This was also true of arcelins 1 and 4 (Fig. 3b). However, arcelins 3 and 4 were observed to contain polypeptides in common (Fig. 3c); the two most basic polypeptides in these arcelin variants comigrated. By comparing Figs. 3a–c, it was hypothesized that arcelins 1 and 2 and 3 have no polypeptides in common, but this was not demonstrated on a single gel separation.

A summary of the accessions containing the four electrophoretic variants is presented in Table 1. One of the accessions containing a seed with arcelin 1, G12882, is another source of PI 325690, which was reported previously to contain seeds with arcelin (Romero Andreas 1984). All seeds in accessions G12922 through G12935 had identical crude protein banding patterns

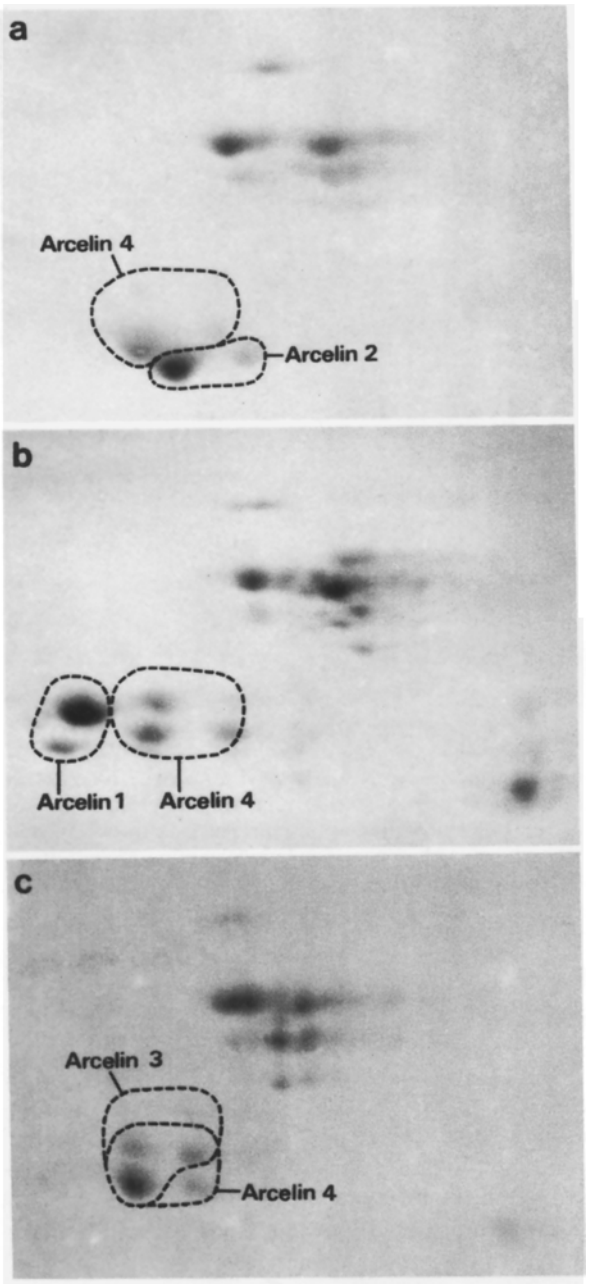

Fig. 3a–c. Two-dimensional IEF-SDS/PAGE of mixtures of crude seed proteins from arcelin-containing lines. **a** G12949-1 (arcelin 4) and G12866-1 (arcelin 2); **b** G12949-1 (arcelin 4) and G12882-1 (arcelin 1); **c** G12949-1 (arcelin 4) and G12891-1 (arcelin 3)

and, therefore, may represent the same genotype (arcelin 3). This was also true of G12949, G12952 and G12953 (arcelin 4).

Inheritance and linkage relationships

The inheritance of arcelin variants was investigated by analyzing the protein composition of individual F_1 and F_2 seeds from crosses between various lines on one-dimensional gels (Table 2). In crosses 1 und 4, all F_1 seeds contained only a combination of the arcelin polypeptides present in the parents. Since seeds resulting from self-pollination of G12882-1 later showed this plant to be heterozygous for arcelin 1, not all F_1 seeds from crosses 2 and 3 contained both arcelin phenotypes contributed by the parents. The F_2 seeds from confirmed heterozygous F_1's of crosses 2 and 3 and from all F_1's of crosses 1 and 4 contained either one or the other of the parental arcelin patterns or a combination of the two patterns (Figs. 4a and b, Table 2). We are

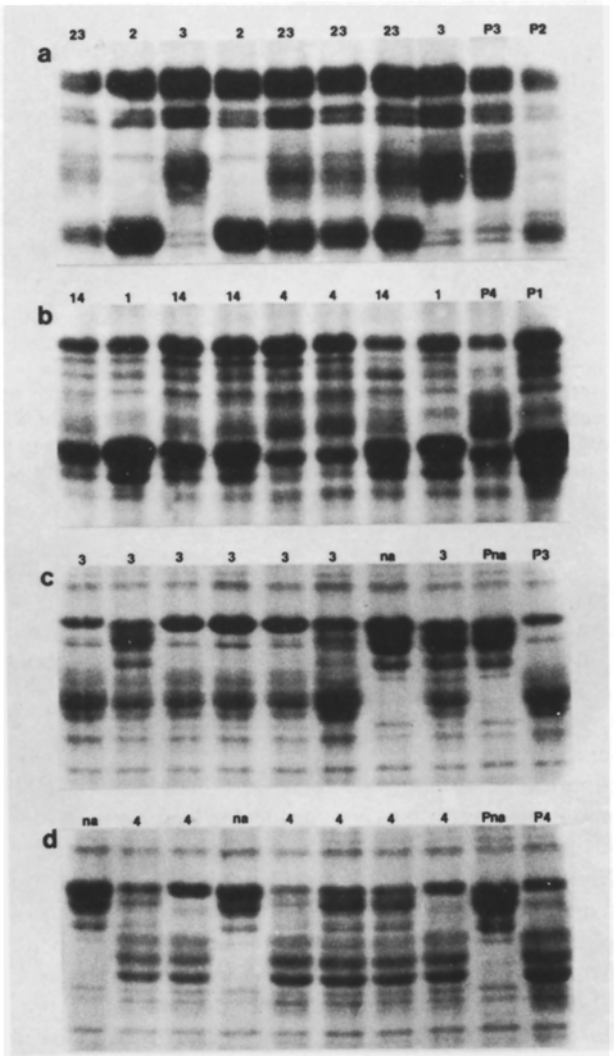

Fig. 4a–d. One-dimensional SDS/PAGE of crude seed proteins from single F_2 and parental seeds. **a** *cross 1*, G12891-1 (arcelin 3)×G12866-1 (arcelin 2); **b** *cross 2*, G12949-1 (arcelin 4) ×G12882-1 (arcelin 1); **c** *cross 10*, L50×G12891-4 (arcelin 3); **d** *cross 11*, L50×G12949-1 (arcelin 4). *Lanes P1, P2, P3, P4* and *Pna* are the protein patterns of the parents having arcelin 1, 2, 3, 4 and no arcelin, respectively. F_2 seeds with the *P1, P2, P2* and *P3, P1* and *P4* or *Pna* arcelin protein patterns are designated by *1, 2, 3, 4, 23, 14*, or *na*, respectively.

Table 2. Segregation for the genes controlling arcelin in F_2 progenies derived from crosses between arcelin-containing accessions

Cross	Parents and their arcelin types	Proposed genotypes and observed segregation of F_2 seeds			χ^{2a}	P
1	G12891-1 × G12866-1	Arc^3/Arc^3	Arc^3/Arc^2	Arc^2/Arc^2	0.20	0.905
	arcelin-3 arcelin-2	26	53	29		
2	G12949-1 × G12882-1	Arc^4/Arc^4	Arc^4/Arc^1	Arc^1/Arc^1		
	arcelin-4 arcelin-1	33	46	30	2.82	0.244
3	G12882-1 × G12922-1	Arc^1/Arc^1	Arc^1/Arc^3	Arc^3/Arc^3		
	arcelin-1 arcelin-3	9	31	7	4.84	0.089
4	G12891-1 × G12949-1	Arc^3/Arc^3	Arc^3/Arc^4	Arc^4/Arc^4		
	arcelin-3 arcelin-4	15	36	21	1.00	0.607

[a] Tested against expected 1 : 2 : 1 ratios

Table 3. Segregation for the alleles controlling arcelin, lectin and phaseolin proteins in F_2 progenies derived from crosses between cultivated bean lines and arcelin-containing accessions

Cross	Parents and their seed protein genotype		F_2 segregation (no. of seeds)					
			Arcelin genotypes	Lectin Genotypes		Phaseolin genotypes		
				Lec/–	*lec*/*lec*	$Pha^{T\text{ or }S}/Pha^{T\text{ or }S}$	$Pha^{T\text{ or }S}/Pha^{M}$	Pha^{M}/Pha^{M}
5	'BBL240'	× PI325690-3	Arc^1/–	–	–	1	9	3
	arc/*arc*, *Lec*/*Lec*, Pha^T/Pha^T	Arc^1/Arc^1, *Lec*/*Lec*, Pha^M/Pha^M	*arc*/*arc*	–	–	2	3	2
6	L50[a]	× G10999-5	Arc^1/–	78	0	21	57	
	arc/*arc*, *lec*/*lec*, Pha^S/Pha^S	Arc^1/*arc*, *Lec*/*Lec* Pha^M/Pha^M	*arc*/*arc*	0	22	6	16	
7	'Pinto UI 111'	× PI325690-3	Arc^1/–	70	0	–	–	–
	arc/*arc*, *lec*/*lec* Pha^S/Pha^S		*arc*/*arc*	0	38	–	–	–
8	'GN UI 1140'	× PI325690-3	Arc^1/–	76	0	–	–	–
	arc/*arc*, *lec*/*lec* Pha^S/Pha^S		*arc*/*arc*	0	32	–	–	–
Crosses 6, 7 and 8 pooled			Arc^1/–	224	0	–	–	–
			arc/*arc*	0	92	–	–	–
9	L50	× G12866-1	Arc^2/–	76	0	17	39	20
		Arc^2/Arc^2, *Lec*/*Lec*, Pha^M/Pha^M	*arc*/*arc*	0	24	9	9	6
10	L50	× G12891-4	Arc^3/–	73	0	19	33	21
		Arc^3/Arc^3, *Lec*/*Lec*, Pha^M/Pha^M	*arc*/*arc*	0	27	8	14	5
11	L50	× G12949-1	Arc^4/–	77	0	13	39	25
		Arc^4/Arc^4, *Lec*/*Lec* Pha^M/Pha^M	*arc*/*arc*	0	23	8	11	4

[a] L50 is a breeding line derived by backcrossing the 'lec' allele from 'Pinto UI 111' into 'Sanilac' (see Osborn and Bliss 1985)

proposing the allelic designations presented in Table 2 for the four arcelin variants.

Single F_2 seeds from crosses between accessions containing arcelins and cultivated bean lines having no arcelin were analyzed by one-dimensional electrophoresis and hemagglutination assays to determine; 1) the inheritance of arcelin, and 2) linkage relationships between the gene(s) controlling arcelin and the genes controlling phaseolin and lectin proteins. In all crosses, the cultivated bean was used as the female parent and the arcelin-containing accession as the male parent. All F_1 seeds from crosses 5 and 7–11 contained the arcelin variant present in the male parent, confirming that they were in fact hybrids (Table 3). Since the arcelin 1-containing parent, G10999-5 was heterozygous, only F_1 seeds containing arcelin 1 were used to produce F_2 progenies (Table 3, cross 6).

Initially, 20 F_2 seeds from the cross of 'BBL 240' × PI 325690-3 were analyzed by one-dimensional electrophoresis for the presence or absence of arcelin 1 and for segregation of phaseolin type (Table 3, cross 5). Only the two parental phaseolin types and the combination of the two types were observed; and both presence and absence of arcelin 1 occurred with all three phaseolin phenotypes. Plants were grown from these seeds and ten F_3 seeds from each F_2 plant were analyzed by one-dimensional electrophoresis. The segregation of F_3 progenies confirmed the genotypes assigned to F_2 plants: Pha^T/Pha^T F_2's had only progenies with T-type phaseolin; Pha^M/Pha^M F_2's had only progenies with M-type phaseolin; Pha^T/Pha^M F_2's produced progenies which segregated for the three phaseolin patterns observed in F_2 seeds. F_2 seeds with arcelin produced either all progeny with arcelin 1 or progeny which segregated for the presence or absence of arcelin 1, and F_2 seeds without arcelin produced all progeny without arcelin (data not shown). The genotype *arc/arc* was designated for the phenotype showing no arcelin.

Single F_2 seeds from crosses of cultivated bean lines containing no arcelin (*arc/arc*), no lectin (*lec/lec*) and S-type phaseolin (Pha^S/Pha^S) with accessions containing arcelin 1 (Arc^1/Arc^1) or 2 (Arc^2/Arc^2), 3 (Arc^3/Arc^3) or 4 (Arc^4/Arc^4), lectin (*Lec/Lec*) and one of the M-type phaseolins found in wild bean accessions (Pha^M/Pha^M) were analyzed for the presence or absence of arcelin and for phaseolin type by one-dimensional electrophoresis and for the presence or absence of lectin by hemagglutination assays. The F_2 segregation ratios for these crosses are presented in Table 3 (crosses 6–11). In each cross, F_2 progenies segregated for the presence or absence of arcelin and F_2 seeds that contained arcelin contained the same arcelin variant present in the parent (Fig. 4c, d). The F_2 progenies also segregated for the presence or absence of lectin. Furthermore, only progeny with the parental combination of either both arcelin and lectin or no arcelin and no lectin were observed. No recombinant F_2 seeds either with arcelin and without lectin or with lectin and without arcelin were found. In crosses where the parental phaseolin patterns could be distinguished in F_2 seeds (crosses 9–11), both the presence and absence of arcelin were associated with the three phaseolin patterns (Figs. 4c, d, Table 3). In cross 6, the heterozygous Pha^S/Pha^M phaseolin pattern could not be distinguished from the homozygous Pha^M/Pha^M phaseolin pattern. Seeds with and without arcelin 1 were observed in this combined phaseolin group as well as in the Pha^S/Pha^S phaseolin group.

Analysis of chi squares by orthogonal function was used to test segregation ratios of individual protein loci in F_2 progenies for significant deviations from expected

Fig. 5. Map of Mexico showing the collection locations of arcelin-containing lines

Table 4. Chi square values from analyses of segregation data presented in Table 3

Cross	Parents	Arcelin or lectin[a]	Phaseolin	Arcelin, lectin linkage	Arcelin, phaseolin linkage[b]
5	'BBL240' × PI325690-3	1.07[c] (0.301)	1.20 (0.549)	– –	1.20 (0.549)
6	L50 × G10999-5	0.48 (0.488)	0.21 (0.647)	84.64 (<0.001)	0 (>0.999)
7	'Pinto UI 111' × PI325690-3	5.98 (0.014)	–	174.63 (<0.001)	–
8	'GN UI 1140' × PI325690-3	1.23 (0.267)	–	136.31 (<0.001)	–
6, 7, 8 $\Sigma\chi^2$	–	7.69 (0.053)	–	389.14 (<0.001)	–
6, 7, 8 Pooled	–	2.85 (0.091)	–	389.14 (<0.001)	–
6, 7, 8 Heterogeneity	–	4.84 (0.089)	–	6.44 (0.040)	–
9	L50 × G12866-1	0.05 (0.823)	0.16 (0.923)	94.76 (<0.001)	2.29 (0.318)
10	L50 × G12891-4	0.21 (0.647)	0.38 (0.827)	110.95 (<0.001)	1.14 (0.566)
11	L50 × G12949-1	0.21 (0.647)	1.28 (0.527)	89.62 (<0.001)	3.89 (0.142)

[a] Segregation ratios and χ^2 values for arcelin and lectin genes were identical
[b] χ^2 values for lectin, phaseolin linkage are the same as those for arcelin, phaseolin linkage
[c] χ^2 value with probability of a greater χ^2 in parentheses

values and to test pairs of loci for significant deviation from independent assortment (Table 4). Segregation ratios for the presence or absence of either arcelin or lectin were tested for significant deviation from an expected 3 : 1 ratio. Segregation ratios for the three phaseolin patterns in crosses 5 and 9–11 were tested for significant deviation from an expected 1 : 2 : 1 ratio. In cross 6, segregation ratios for the two phaseolin patterns were tested for significant deviation from an expected 1 : 3 ratio. The data from crosses 6–8 were also combined for analysis, since the parents used contained the same arcelin, phaseolin and lectin genotypes. From the combined data of crosses 6–8, an estimate of maximum possible linkage distance between the genes controlling arcelin and lectin was found to be $0.30 \pm 0.31\%$ recombination.

Discussion

Two-dimensional electrophoretic analyses of protein extracts from seeds of wild bean accessions from Mexico revealed four different electrophoretic variants of heavy staining protein having polypeptides with more basic isoelectric points than phaseolin polypeptides and molecular weights ranging from 35,000 to 42,000. Major bean seed proteins with these properties have not been reported previously in common bean cultivars. We have tentatively classified all variants as arcelin proteins. Seeds that contained arcelin had large quantitites of this protein in addition to the other two major salt soluble seed proteins, phaseolin and lectin. Therefore, arcelin appears to represent a third important bean seed protein that is soluble in salt solution.

Two of the arcelin variants, arcelin 3 and 4, had polypeptides that comigrated on two-dimensional electrophoretic gels, and accessions that contained seeds with these two variants had been collected at sites in close proximity to each other in eastern Jalisco, Mexico (Fig. 5). Two accessions collected at widely separated locations contained the same variant, arcelin 1. This variant may have originated independently in these two locations, since wild bean accessions collected at sites between these locations did not contain arcelin 1. Alternatively, arcelin 1 may have been introduced into one of the two arcelin 1-containing accessions by a chance outcross during field increases of the original collections at the repository sites.

Analyses of F_2 progenies from crosses between different arcelin-containing accessions revealed a genetic relationship among the different arcelin variants. All F_2 seeds analyzed contained arcelin and F_2 progenies did not deviate significantly ($P > 0.05$) from an expected 1 : 2 : 1 segregation ratio of parental : combination : parental arcelin patterns. Therefore, the gene(s) controlling the arcelin polypeptides of each variant are inherited as a single unit and are codominant. Although all combinations of arcelin variants were not analyzed in these crosses, arcelin 3 was analyzed in combination with the other arcelin variants and, therefore, allelism of the genes controlling all four arcelin variants can be inferred. Although two different accessions containing arcelin 3 were used as parents in these crosses, both were collected at the same location and probably have a common ancestry.

The inheritance of the four arcelin variants was also investigated by analyzing crosses between arcelin-containing accessions and cultivars containing no arcelin. One F_2 progeny was analyzed for each of three arcelin variants, arcelin 2, 3 and 4, and segregation ratios did not deviate significantly ($P > 0.05$) from a 3 : 1 ratio for the presence : absence of arcelin. Four F_2 progenies derived from crosses between arcelin 1-containing parents and different bean cultivars were analyzed (crosses 5–8). Although the segregation ratio in one progeny deviated significantly ($P < 0.05$) from a 3 : 1 ratio for the presence : absence of arcelin, the combined segregation ratio for crosses 6–8 did not deviate significantly ($P > 0.05$) from a 3 : 1 ratio and the chi square value for heterogeneity among crosses 6–8 was nonsignificant ($P < 0.05$). These segregation ratios of F_2 progenies support the hypothesis of single gene inheritance of arcelin polypeptides, with presence being dominant to absence of arcelin. The segregation of phaseolin and lectin proteins also followed the patterns expected for single gene inheritance.

Phaseolin and lectin electrophoretic variants were reported previously to be controlled by codominant alleles inherited monogenically (Brown et al. 1981a). The presence of lectin was found to be dominant to absence of lectin (Brucher et al. 1969; Jaffe et al. 1972; Osborn and Bliss 1985). The data presented here and in a previous report on the inheritance of arcelin 1 (Romero Andreas 1984) indicate that the arcelin electrophoretic variants are inherited monogenically, that the alleles are codominant when present with other arcelin alleles and that presence of arcelin is dominant to the absence of arcelin.

The genes controlling phaseolin and lectin polypeptides were shown previously to be unlinked (Brown et al. 1981a). Our analyses confirmed these results. The segregation of genes controlling lectin proteins was analyzed using hemagglutination and, therefore, our method of determining lectin genetic segregation was not identical to that of Brown et al. (1981a). However, the presence or absence of hemagglutinating activity was correlated exactly with the presence or absence of lectin polypeptides in bean cultivars (Brown et al. 1982).

The genes controlling presence or absence of the four arcelin variants were not linked to genes controlling phaseolin electrophoretic variants. The genes controlling arcelin, however, were tightly linked to those controlling lectin proteins.

In a previous study, analysis of seed proteins in F_2 progenies from crosses of bean cultivars revealed a close linkage between genes controlling lectin polypeptides and those controlling a minor group of seed proteins, F polypeptides (Brown et al. 1981a). The two-dimensional electrophoretic mobility of F polypeptides was similar to the mobility of arcelin polypeptides observed in this study. However, F_2 recombinants containing nonparental combinations of lectin and F polypeptides were observed at frequencies of 5 and 9% in two F_2 progenies (Brown et al. 1981a). In our study, an estimate of the maximum possible linkage distance between arcelin 1 and lectin genes was calculated to be $0.30 \pm 0.31\%$ recombination.

The results presented in this study raise three important questions. The first deals with the chemical composition of arcelin proteins. Two-dimensional electrophoresis of arcelin polypeptides revealed that they have a distinct electrophoretic mobility compared to phaseolin and lectin polypeptides. However, a tight linkage between the genes controlling arcelin and those controlling lectin proteins was found. Therefore, does arcelin represent a type of protein that is chemically unrelated to lectin and by coincidence genetically linked, or are arcelin polypeptides rare variants of lectin polypeptides that have altered amino acid compositions causing different electrophoretic mobilities?

The second question deals with the origin of the genes coding for arcelin polypeptides. If arcelin polypeptides are unusual lectin variants, then the genes controlling arcelin could have arisen by duplication and diversification of existing lectin genes. If arcelin is chemically unrelated to lectin, three alternative hypotheses for the origin of arcelin genes seem most likely. The first is that arcelin genes existed in their present genomic location prior to expression as seed protein genes and by a mutation or localized genomic rearrangement they began to be expressed at a high level as seed protein genes. In this case 'silent' arcelin genes that are linked to lectin genes may exist in non-arcelin-containing line. The second hypothesis is that 'silent' arcelin genes pre-existed in another genomic location(s) and by duplication and/or genomic rearrangement copies of the genes were translocated to sites near the lectin genes and expressed as seed protein genes. With this hypothesis, 'silent' arcelin genes that are unlinked to lectin genes may exist in non-arcelin-containing lines and arcelin-containing lines may also have 'silent' arcelin genes in other genomic locations. The third hypothesis is that arcelin genes arose de novo in arcelin-con-

taining lines, in which case non-arcelin-containing lines would not have 'silent' arcelin genes. Evidence for or against these hypotheses could be obtained by probing the DNA of arcelin and non-arcelin-containing lines and their progenies with molecular clones of arcelin genes. It is also possible that the arcelin genes in these accessions may have resulted from introgression with another *Phaseolus* species.

The third question concerns the implications of the presence of the arcelin protein relative to resistance to insects (Coleoptera, Bruchideae) which cause extensive damage to dry bean seeds during storage. No satisfactory levels of resistance to bruchids have been identified among cultivated beans (Schoonhoven et al. 1983). The seeds of most accessions with the highest resistance levels contained arcelin: G12866 (arcelin 2); G12891 (arcelin 3); G12949, G12952 and G12953 (arcelin 4) (Schoonhoven et al. 1983; and present results). This apparent relationship between bruchid resistance and the presence of arcelin raises the possibility that arcelin is involved as a resistance mechanism. Experiments are underway to transfer genetically the different arcelin types into cultivated beans to determine the effects of arcelin on bruchid resistance and on human nutrition.

Acknowledgements. The authors gratefully acknowledge the helpful comments of Dr. Jeanne Romero Andreas (University of Wisconsin-Madison) on this manuscript and the assistance of Ken Kmiecik in conducting this research. Funds for these investigations were provided by the Graduate School and the College of Agricultural and Life Sciences, University of Wisconsin-Madison and ARS of the USDA under grant no. 81-C RCR-1-0604 of the Competitive Grants Office.

References

Brown JWS, Bliss FA, Hall TC (1981a) Linkage relationships between genes controlling seed proteins in French bean. Theor Appl Genet 60:251–259

Brown JWS, Ma Y, Bliss FA, Hall TC (1981b) Genetic variation in the subunits of globulin-1 storge protein of French bean. Theor Appl Genet 59:83–88

Brown JWS, Osborn TC, Bliss FA, Hall TC (1982) Bean lectins. 1. Relationships between agglutinating activity and electrophoretic variation in the lectin-containing G2/albumin seed proteins of French bean (*Phaseolus vulgaris* L.). Theor Appl Genet 62:263–271

Brucher O, Wecksler M, Levy A, Palozzo A, Jaffe WG (1969) Comparison of phytohemagglutinin in wild beans (*Phaseolus aborigineus*) and in common bean (*Phaseolus vulgaris*) and their inheritance. Phytochemistry 8:1739–1743

Gepts P (1984) Nutritional and evolutionary implication of phaseolin seed protein variability in common bean (*Phaseolus vulgaris*). Ph D Thesis, University of Wisconsin, Madison

Jaffe WG, Brucher O, Palozzo A (1972) Detection of four types of specific phytohemagglutinins in different lines of beans. Z Immunitätsforsch 142:439–447

Laemmli VK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 22:680 to 685

Ma Y, Bliss FA (1978) Seed proteins of common bean. Crop Sci 18:431–437

Mather K (1951) The measurement of linkage in heredity. Wiley and Sons, New York, pp 32–55

Osborn TC, Bliss FA (1985) Effects of genetically removing lectin seed protein on horticultural and seed characteristics of common bean (*Phaseolus vulgaris* L.). J Am Soc Hortic Sci 110:484–488

Romero Andreas J (1984) Genetic variability in the seed phaseolin of non-domesticated bean (*Phaseolus vulgaris* var '*aborigineus*') and the inheritance and physyiological effects of arcelin, a novel seed protein. Ph D Thesis, University of Wisconsin, Madison

Schoonhoven AV, Cardona C, Valor J (1983) Resistance to the bean weevil and the Mexican bean weevil (*Coleoptera: Bruchidae*) in non-cultivated common bean accessions. J Econ Entomol 76:1255–1259