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DNA restriction fragment length polymorphisms correlate with isozyme diversity in *Phaseolus vulgaris* L.

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Summary. Genetic variation in *Phaseolus vulgaris* L. (*P. vulgaris*) was investigated at the isozyme and DNA levels. We constructed a library of size-selected *Pst*I clones of *P. vulgaris* nuclear DNA. Clones from this library were used to examine 14 *P. vulgaris* accessions for restriction fragment length polymorphisms (RFLPs). DNAs from each accession were analyzed with three restriction enzymes and 18 single copy probes. The same accessions were also examined for variability at 16 isozyme loci. Accessions included four representatives of the T phaseolin group and five representatives each of the C and S phaseolin groups. One member of the S group (the breeding line XR-235-1-1) was derived from a cross between *P. vulgaris* and *P. coccineus*. Isozymes and RFLPs revealed very similar patterns of genetic variation. Little variation was observed among accessions with C and T phaseolin types or among those with the S phaseolin type. However, both isozyme and RFLP data grouped accessions with S phaseolin separately from those accessions with C or T phaseolin. The highest degree of polymorphism was observed between XR-235-1-1 and members of the C/T group. RFLP markers will supplement isozymes, increasing the number of polymorphic loci that can be analyzed in breeding, genetic, and evolutionary studies of *Phaseolus*.

Key words: Common bean – Molecular markers – Phaseolin – *Phaseolus coccineus* – Gene pools

Introduction

Restriction fragment length polymorphisms (RFLPs) provide a powerful tool for studies of plant genetics and evolution (Beckman and Soller 1986; Helentjaris and Burr 1989; Helentjaris et al. 1985). Application of this technology to the study of a species requires polymorphism at the DNA level, and different higher plant species exhibit different levels of DNA variability. While inbred lines of maize exhibit RFLPs with a high percentage of probes, RFLPs among lines of cultivated tomato are rare. RFLPs are readily detected, however, among *Lycopersicon* species which can be crossed with the cultivated tomato (Helentjaris et al. 1985). The level of DNA polymorphism in these species correlates with the level of polymorphism observed through isozyme analysis (Helentjaris et al. 1985).

In *P. vulgaris*, variability at the protein level has been well documented (Weeden 1984; Bassiri and Adams 1978 b; Koenig and Gepts 1989). Isozyme analysis (Koenig and Gepts 1989) and the analysis of phaseolin seed storage proteins (Gepts and Bliss 1985; Gepts et al. 1986) demonstrate two genetically distinct groups of *P. vulgaris*. Accessions from Mesoamerica are primarily of the S phaseolin type (with some exhibiting the M phaseolin pattern). Accessions from the Andes are primarily of the T phaseolin type, with some accessions exhibiting C, H, A, J, or I types. *P. vulgaris* cultivars include members of both the S and the C/T phaseolin groups, suggesting multiple domestication events (Brown et al. 1982; Gepts et al. 1986).

P. vulgaris has been successfully hybridized with other *Phaseolus* species (Coyne 1964; Smartt 1970), and interspecific variation has been reported for isozymes (Bassiri and Adams 1978 a) and seed proteins (Sullivan and Freytag 1986). Therefore, lines developed from interspecific crosses may also provide useful variants for DNA analysis.

Our interest in constructing a molecular marker-based linkage map of the *Phaseolus vulgaris* genome led

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us to investigate the frequency of DNA polymorphisms within and between the genetically distinct groups. The assessment of genetic variation at the DNA level is an essential prerequisite to the design of evolutionary, breeding, and genetic studies employing DNA probes. Accessions with the S, C, and T phaseolin types were analyzed. The breeding line XR-235-1-1, which was developed from a *P. vulgaris* × *P. coccineus* cross (Freytag et al. 1982), was included to determine whether introgression of DNA from close relatives of *P. vulgaris* can contribute to variation at the DNA level. DNAs were examined with 18 single-copy genomic probes. Accessions were also analyzed for polymorphisms at 16 isozyme loci, to allow direct comparison of DNA and isozyme variability.

Materials and methods

Plant materials

With the exception of XR-235-1-1 and Sprite, seeds of all accessions used in this study were provided by J. Tohme and W. Roca, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. Seeds of XR-235-1-1 and Sprite were provided by M. Bassett, University of Florida, Gainesville/FL. The accessions analyzed for isozyme and DNA polymorphisms are listed in Table 1. The breeding line Ica Pijao (CIAT No. G05773) was included as a source of reference alleles in the isozyme studies.

Isozyme analysis

A combination of starch gel electrophoresis and enzyme activity staining was used to screen for polymorphisms of: aconitase (ACO) (E.C. 1.6.4.3); alcohol dehydrogenase (ADH) (E.C. 1.1.1.1); acid phosphatase (APS) (E.C. 3.1.3.2); Beta-N-acetylglucosaminidase (BNAG) (E.C. 3.2.1.30); diaphorase (DIA) (E.C. 1.6.4.3); glutamate oxaloacetate transaminase (GOT) (E.C. 2.6.1.1); malate dehydrogenase (MDH) (E.C. 1.1.1.37); 6-phosphogluconate dehydrogenase (6PGDH) (E.C. 1.1.1.44); phosphoglucoisomerase (PGI) (E.C. 5.3.1.9); phosphoglucomutase (PGM) (E.C. 2.2.5.1); and shikimate dehydrogenase (SKDH) (E.C. 1.1.1.25). These analyses were performed as described previously (Vallejos and Chase 1991).

Table 1. *Phaseolus vulgaris* accessions analyzed for isozyme and restriction fragment length polymorphisms

CIAT Number	Name	Phaseolin	Type
G15416	XR-235-1-1	S	Breeding line
G03645	Jamapa	S	Landrace
G04489	Culiapa 72	S	Landrace
G04493	Ica Bunsi	S	Breeding line
G04459	Nep 2	S	Mutant
G04435	Diacol Calima	C	Breeding line
G09011	Taylor Cramberry	C	Breeding line
G12172	Unnamed	C	Landrace
G12207	Canario	C	Landrace
G17668	Cran 28	C	Breeding line
G00051	Swedish Brown	T	Landrace
G01293	Algarrobo	T	Landrace
G03668	Sangretorro	T	Landrace
G14192	Horoz Fasulyesi	T	Landrace

Enzymes and reagents

Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories (BRL). All enzyme reactions were performed as recommended by the supplier in buffers provided by the supplier. [Alpha-^{32}P]dCTP (3,000 Ci/mmol) was purchased from Dupont/New England Nuclear Research Products.

Genomic library construction

Nuclear DNA was isolated by the procedure of Kislev and Rubenstein (1980) from 7-day-old etiolated seedlings of the cultivar Sprite. *Pst*I-digested DNA was layered onto a 10-ml, 10–30% (w/v) linear sucrose gradient. (Sucrose was dissolved in 10 m*M* TRIS, 1 m*M* EDTA, pH 8.) Gradients were centrifuged in an SW 41 rotor at 30,000 rpm for 8 h. Fractions of 250 µl were collected. Aliquots of each fraction were visualized following agarose gel electrophoresis in parallel with molecular weight standards. Gradient fractions containing DNA fragments of 0.5–2.5 kb were identified and combined. DNA was recovered by ethanol precipitation, rehydrated in sterile distilled water, and ligated with *Pst*I-digested plasmid vector (pTZ18R purchased from Pharmacia). The ligation reaction was used to transform the *Escherichia coli* strain TB1 by the procedure of Hanahan (1983). Recombinant colonies were identified by blue/white colony selection on LB-X-gal-ampicillin agar (Vieria and Messing 1982).

Probe preparation

Plasmid DNAs were prepared by the triton lysis method (Lonsdale et al. 1981). GeneClean (BIO 101) was used (as directed by the manufacturer) to recover the cloned *Pst*I inserts from agarose gels. The concentrations of insert DNAs were estimated by visualization; 10% of each insert preparation was visualized following agarose gel electrophoresis in parallel with DNAs of known concentrations. Random priming (Feinberg and Vogelstein 1984) DNA labeling kits (Boehringer, Mannheim) were used to label 100 ng of insert DNA in the presence of 50 µCi [alpha-^{32}P]dCTP.

Total nucleic acid preparations

Young trifoliolate leaves (one-third to one-half expanded) were harvested from a single plant of each accession. Leaf samples of 1 g were wrapped in foil, frozen in liquid nitrogen, and stored at −80 °C. Total nucleic acids were purified from 1 g leaf samples by the method of Dellaporta et al. (1983).

Restriction, electrophoresis, and hybridization of nucleic acids

Samples containing 27 µg of nucleic acid (DNA and RNA) were digested with 20 units of restriction enzyme according to the supplier's instructions. Digested samples were loaded onto 0.8% agarose gels, which were run at 2 V/cm for 18.75 h. Molecular size standards (*Pst*I fragments of bacteriophage lambda-DNA) were included on each gel. Gels were stained for 1 h in ethidium bromide (0.5 µg/ml) and photographed over a UV (302 nm) transilluminator.

Following photography, DNAs were blotted to Hybond-N+ nylon membranes (Amersham Corp.) as described by Southern (1975). DNAs were fixed to membranes by incubation for 20 min on Whatman 3-mm chromatography paper soaked in 0.4 *M* NaOH. Each 20 × 20 cm blot was hybridized with 100 ng of heat-denatured, radiolabeled insert DNA prepared as de-

scribed above. Blots were prehybridized, hybridized, and washed as described by Church and Gilbert (1984). X-ray films (Kodak XAR-5) were exposed under Lightning Plus intensifier screens (Dupont) to radiolabeled blots for 5 days. Following autoradiography, probes were removed from blots by placing the blots in a boiling solution of 0.5% w/v SDS and allowing the solution to cool to room temperature.

Cluster analysis

Cluster analysis was accomplished through use of the BIOSYS-I computer program, version 1.6 (D. L. Swofford and R. B. Selander, University of Illinios at Urbana-Champaign). Isozyme and RFLP banding patterns were converted to allele frequencies. Pair-wise distances (Nei 1973) between accessions were computed based upon comparisons at the 18 DNA or 16 isozyme loci. Dendrograms based upon genetic distances were constructed by the unweighted paired group method (Sneath and Sokal 1973).

Results

The results of isozyme analysis are summarized in Table 2. Of the 16 isozyme loci examined, 9 revealed polymorphisms. Relatively little variation was observed among members of the C/T phaseolin group. Only two loci (*Aco-1* and *Mdh-1*) distinguished any members of this group, and only one member was distinguished in each case. The S group was slightly more variable, with four loci (*Adh-1*, *Bnag*, *Got-2*, and *Dia-2*) revealing differences. However, two of the four loci distinguished only XR-235-1-1 (accession G15416) from the other members, and XR-235-1-1 is unique in that it was derived from a *P. vulgaris* × *P. coccineus* cross. All members of the S phaseolin group differed from all members of the C and T phaseolin groups with respect to *Aco-2*, *Dia-1*, and *Skdh*. Maximum variation was observed between XR-235-1-1 and members of the C/T phaseolin group, with 6 of 16 isozyme loci revealing differences.

For the investigation of DNA polymorphism, we constructed a library of size-selected *Pst*I clones of *P. vulgaris* nuclear DNA. Others report this type of library to be enriched in single-copy sequences, because single-copy sequences include those which are actively expressed and therefore undermethylated. These sequences are preferentially digested by methylation-sensitive enzymes such as *Pst*I (Burr et al. 1988; Helentjaris et al. 1988). This strategy was highly effective for *P. vulgaris*, as 95% of cloned inserts shorter than 2.5 kb gave simple (one to three band) hybridization patterns expected for single-copy probes. Representative hybridization data are shown in Fig. 1.

DNAs prepared from the 14 different accessions were digested with each of three different restriction enzymes (*Eco*RI, *Eco*RV, and *Hin*dIII) and probed with 18 different probes, for a total of 54 different probe-enzyme combinations. Results of the *Eco*RI hybridizations are summarized in Table 3. Each of the three restriction enzymes resulted in useful polymorphism. Polymorphisms were revealed in *Eco*RI digests by five different probes, in *Eco*RV digests by five probes, and in *Hin*dIII digests by seven probes. With four exceptions, polymorphisms revealed with one probe-enzyme combination were not revealed by the same probe in combination with another enzyme. In three of the four exceptions, the polymorphic individual was XR-235-1-1.

Variation observed at the DNA level matched that detected by isozyme analysis (Table 2; Koenig and Gepts

Table 2. Zymotypes[a] of *P. vulgaris* accessions

Accession	Phaseolin	Enzyme locus								
		Aco-1	*Aco-2*	*Adh-1*	*Bnag*	*Dia-1*	*Dia-2*	*Got-2*	*Mdh-1*	*Skdh*
G15416	S	+	+	a_4	a_{10}	+	c_{30}	r_7	+	+
G03645	S	+	+	+	+	+	c_{30}	+	+	+
G04459	S	+	+	+	+	+	+	+	+	+
G04489	S	+	+	+	+	+	+	+	+	+
G04493	S	+	+	+	a_5	+	c_{30}	+	+	+
G04435	C	r_2	a_1	+	+	a_5	c_{30}	+	a_{10}	r_5
G09011	C	+	a_1	+	+	a_5	c_{30}	+	+	r_5
G12172	C	+	a_1	+	+	a_5	c_{30}	+	+	r_5
G12207	C	+	a_1	+	+	a_5	c_{30}	+	a_{10}	r_5
G17668	C	+	a_1	+	+	a_5	c_{30}	+	+	r_5
G00051	T	+	a_1	+	+	a_5	c_{30}	+	+	r_5
G01293	T	+	a_1	+	+	a_5	c_{30}	+	+	r_5
G03668	T	+	a_1	+	+	a_5	c_{30}	+	a_{10}	r_5
G14192	T	+	a_1	+	+	a_5	c_{30}	+	+	r_5

[a] Electrophoretic mobilities were determined relative to reference alleles of Ica Pijao (G05773); + indicates mobility equivalent to that of the reference allele; a_x indicates mobility advanced x mm relative to the reference allele; r_x indicates mobility retarded x mm relative to the reference allele; c_x indicates cathodal mobility x mm relative to the origin while mobility of the reference allele was anodal. The accessions showed no variability at the *Aps-1*, *Aps-2*, *Got-1*, *Mdh-2*, *6Pgdh*, *Pgi-1*, or *Pgm-1* loci

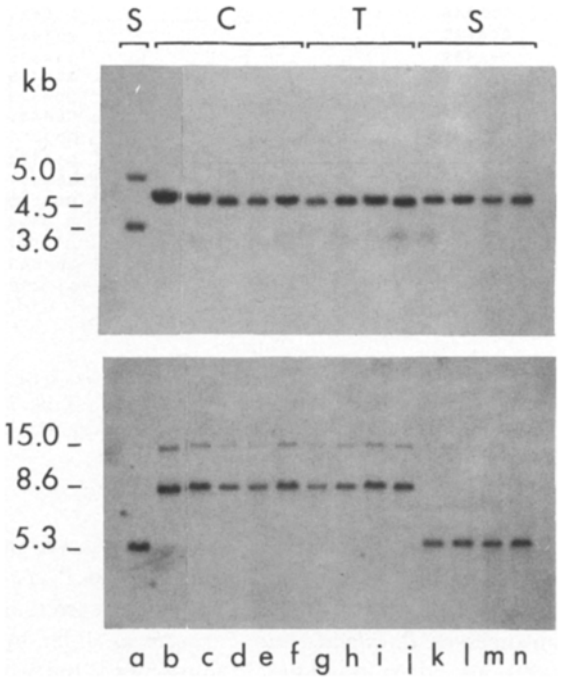

Fig. 1. Autoradiographs of *P. vulgaris* DNAs hybridized with genomic probes. The autoradiograph in the top panel showns *Hin*dIII-digested DNA hybridized with probe 2F5; this probe-enzyme combination distinguished XR-235-1-1 (accession G15416) from the other accessions. The autoradiograph in the *bottom panel* shows *Eco*RI-digested DNA hybridized with probe 2F7; this probe-enzyme combination distinguished all accessions with S phaseolin from those with C or T phaseolin. DNAs were prepared from accessions: G15416 (*a*), G04435 (*b*), G09011 (*c*), G12172 (*d*), G12207 (*e*), G17668 (*f*), G00051 (*g*) G01293 (*h*), G03668 (*i*), G14192 (*j*), G03645 (*k*), G04459 (*l*), G04489, (*m*), and G04493 (*n*). *S*, *C*, and *T* indicate the phaseolin types of the accessions

Table 3. Polymorphic *Eco*RI fragments[a] of *P. vulgaris* DNA detected by hybridization with genomic probes

Accession	Phaseolin	Probe					
		2C11	2E7	2F3	2F7	2F12	4A9
G15416	S	4.6	3.7	4.8	5.3	7.3	9.1
G03645	S	4.6/3.3	3.4	4.8	5.3	7.3	9.1
G04459	S	4.6/3.3	3.7	4.8	5.3	7.3	9.1
G04489	S	4.6/3.3	3.7	4.8	5.3	7.3	9.1/7.4
G04493	S	4.6/3.3	3.7	4.8	5.3	7.3	9.1
G04435	C	4.6/3.1	3.7	2.6	15/8.6	10.5	5.0
G09011	C	4.6/3.1	3.4	2.6	15/8.6	10.5	5.0
G12172	C	4.6/3.1	3.4	2.6	15/8.6	10.5	5.0
G12207	C	4.6/3.1	3.4	2.6	15/8.6	10.5	5.0
G17668	C	4.6/3.1	3.4	2.6	15/8.6	7.3	5.0
G00051	T	4.6/3.1	3.4	2.6	15/8.6	10.5	5.0
G01293	T	4.6/3.1	3.4	2.6	15/8.6	10.5	5.0
G03668	T	4.6/3.1	3.4	2.6	15/8.6	10.5	5.0
G14192	T	4.6/3.1	3.4	2.6	15/8.6	10.5	5.0

[a] Sizes of fragments are given in kilobase pairs (kb). No variation was observed among these accessions when *Eco*RI digest were probed with 2B12, 2C6, 2E9, 2E10, 2F5, 2F6, 2F8, 2F9, 2H4, 2H9, 4B10, or 4C2

1989) and by the analysis of seed storage proteins (Gepts et al. 1986; Gepts and Bliss 1985). Relatively little variation was revealed within each of the genetically distinct groups defined by phaseolin type. Of the 18 probes used, only 4 distinguished any of the C/T accessions from the others, and no more than 3 of the 8 C/T accessions were differentiated by any single probe. Members of the S group were more variable than those of the C/T group; 9 of the 18 probes distinguished one or more members of the S group. However, 4 of the 9 probes distinguished only XR-235-1-1 from the others. Variation was readily detected between the two groups; 6 of the 18 probes distinguished all members of the S group from all members of the C/T group. Maximum variation was observed between XR-235-1-1 and members of the C/T group, with 12 of the 18 probes revealing polymorphisms.

Agreement of the isozyme and RFLP data was apparent in dendrograms based upon genetic distances calculated from these data. Dendrograms based upon *Eco*RI, isozyme, and combined *Eco*RI and isozyme data are shown in Fig. 2a, b, and c, respectively. Features common to these dendrogramms were also present in dendrograms (not shown) generated from *Eco*RV or *Hin*dIII data. All data sets grouped the five accessions with S phaseolin separately from those accessions with C or T phaseolin, and showed XR-235-1-1 (G15416) to be the most divergent accession of the S phaseolin group. Other subgroups within the S or the C/T phaseolin groups varied from dendrogram to dendrogram, and there was no clear separation of accessions with the C phaseolin from those with the T phaseolin type.

Discussion

The limited genomic variation observed within each of the genetically divergent groups of *P. vulgaris* was comparable to that observed in cultivated tomato, where only 2 of 22 probes revealed polymorphisms between two lines (Helentjaris et al. 1985). No more than 1 of the 18 probes used in our study distinguished any pair of accessions with the C or T phaseolin types or any pair of accessions with the S phaseolin type (excluding XR-235-1-1 from the analysis). This is in contrast to the highly polymorphic nature of maize inbreds (Evola et al. 1986; Helentjaris et al. 1985). Helentjaris et al. (1985) noted that DNA variation in maize and tomato correlates with variation observed at isozyme loci. Our results (Tables 2–3, Fig. 2), taken with the results of others (Gepts et al. 1986; Koenig and Gepts 1989), demonstrate that DNA variation is well correlated with isozyme variation in *P. vulgaris*.

Helentjaris et al. (1985) suggested a number of factors that could account for differences in genomic variability; these include differences in mating systems, breeding systems, and domestication events. Studies of natural plant

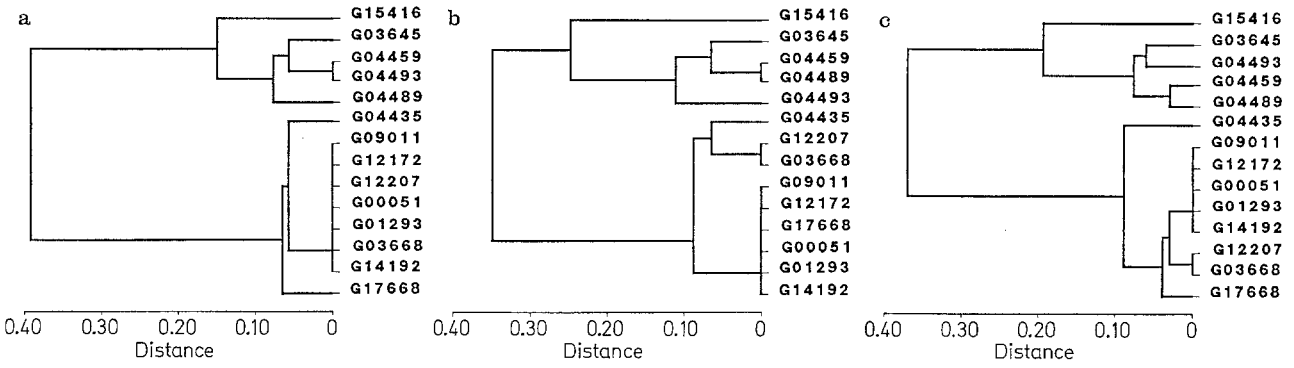

Fig. 2a–c. Dendrograms of *P. vulgaris* accessions based upon comparisons at isozyme and RFLP loci. Dendrogram **a** was based upon comparison of the accessions at DNA loci detected by probing EcoRI digests with the 18 probes described in Table 3. Dendrogram **b** was based upon comparisons at the 16 isozyme loci described in Table 2. The data used to generate dendrograms **a** and **b** were combined to generate dendrogram **c**

populations demonstrate high variability at isozyme loci in cross-pollinating populations and low levels of variability in self-pollinating populations (Hamrick et al. 1979; Rick et al. 1977, 1979). The two genetically distinct groups of *P. vulgaris* are believed to originate from two distinct wild gene pools – Andean and Mesoamerican (Gepts and Bliss 1985; Gepts et al. 1986; Koenig and Gepts 1989). *P. vulgaris* therefore provides an example of a naturally self-pollinating species that exhibits low levels of DNA and isozyme variation within gene pools.

Self-pollination, however, also results in increased genetic variability between gene pools (Loveless and Hamrick 1984). Variability between the two major genetic groups of *P. vulgaris* has been documented through analysis of seed storage proteins (Gepts and Bliss 1985; Gepts et al. 1986) and isozymes (Koenig and Gepts 1989). We have extended these analyses to the DNA level. RFLP data, considered independently from phaseolin or zymotype data, group accessions in a manner consistent with isozyme and seed storage protein groupings (Fig. 2).

Additional variability is present in accessions of wild *P. vulgaris*. Novel phaseolin types have been identified in wild populations of Mexico (Romero-Andreas and Bliss 1985), Colombia (Gepts and Bliss 1986), and Andean South America (Gepts et al. 1986). RFLPs may be useful in investigating the relationships among accessions with novel phaseolins and those with C, T, and S types. RFLPs may also be useful in investigating the genetic diversity of *P. vulgaris* accessions from the Colombia-Peru region, a minor center of genetic diversity and domestication for common bean (Gepts and Bliss 1986; Koenig and Gepts 1989).

XR-235-1-1 contributed a high degree of variability to the S phaseolin group in this study and was distinguished from members of the C/T group by more probes and isozymes than any other S type accession examined. This high degree of variation may result from the *P. coccineus* genome contributions to the genome of XR-235-1-1. High pollen abortion has been observed in F_2 plants from crosses between *P. vulgaris* and *P. coccineus*. While Cheng et al. (1981) reported evidence that chromosomes of *P. vulgaris* and *P. coccineus* differ by two inversions, others found the chromosomes of the two species to be very similar if not identical (Shii et al. 1982). This discrepancy could be due to the different plant accessions analyzed. Most of the DNA polymorphisms observed in this study were probably the result of single base changes, as polymorphisms revealed by a given probe-enzyme combination were generally not revealed by the same probe in combination with other restriction enzymes. We note that in three of the four exceptions to this generalization the polymorphic individual was XR-235-1-1. This may be indicative of minor structural differences (short insertions, deletions, or inversions) between the genomes of the two species.

Construction of a linkage map (based upon RFLP and isozyme markers) of the *P. vulgaris* genome is in progress. The breeding lines XR-235-1-1 (G15416) and Diacol Calima (G04435) were selected as appropriate mapping parents exhibiting maximum levels of polymorphism. Maximum variation is an obvious advantage in linkage studies. However, application of RFLPs to investigations of *P. vulgaris* will not necessarily require materials generated by interspecific crosses, as one-third of our probes revealed polymorphisms between two genetic groups of this species. RFLPs will therefore supplement isozymes, increasing the number of polymorphic loci that can be analyzed in breeding, genetic, and evolutionary studies of *Phaseolus*.

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