

A Molecular Marker-Based Linkage Map of *Phaseolus vulgaris* L.

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ABSTRACT

A seed and flower color marker (*P*), nine seed protein, nine isozyme and 224 restriction fragment length polymorphism marker loci were used to construct a linkage map of the common bean, *Phaseolus vulgaris* L. ($n = 11$). The mapping population consisted of a backcross progeny between the Mesoamerican breeding line 'XR-235-1-1' and the Andean cultivar 'Calima'; the former was used as the recurrent parent. A bean *Pst*I genomic library enriched for single copy sequences (95%) was the source of DNA probes. Sixty percent of the probes tested detected polymorphisms between the parental genotypes with at least one of the four restriction enzymes used here (*Dra*I, *Eco*RI, *Eco*RV and *Hind*III). The computer software **Mapmaker** was used to determine the linkage relationships and linear order of segregating markers. These markers assorted into 11 linkage groups covering 960 cM of the bean genome. Partial linkage data were used to estimate the total length of the genome at 1200 cM. This estimate and that for the physical size of the genome yield an average ratio of 530 kb/cM. The relatively small size of the genome makes this crop species a good candidate for the isolation of genes via chromosome walking techniques.

PHASEOLUS vulgaris L., the common bean, is a diploid ($2n = 22$) legume with a relatively small genome [633 Mbp (0.66 pg)/1C] (ARUMUGANATHAN and EARLE 1991). This bean is an important source of dietary protein for over half a billion people in Africa and Latin America (PACHICO 1989), and hypocholesterolemic properties add to its dietary value (ANDERSON *et al.* 1984). However, in spite of the importance of this crop species, its genetics has been poorly characterized. For instance, only a few morphological and seed and flower color markers have been used to develop a rudimentary linkage map (BASSETT 1991). More recently, a few isozyme and protein markers have been added to the map (VALLEJOS and CHASE 1991a,b). Although cytogenetic studies have been hampered by the small size of the chromosomes (ZHENG *et al.* 1991), five primary trisomics have been characterized (ASHRAF and BASSETT 1987).

The construction of linkage maps based on DNA markers (restriction fragment length polymorphisms, RFLPs) has been accomplished for a number of species: *Arabidopsis* (CHANG *et al.* 1988; NAM *et al.* 1989), lettuce (LANDRY *et al.* 1987), maize (HELENTJARIS, WEBER and WRIGHT 1986), potato (BONIERBALE, PLASTED and TANKSLEY 1988), rice (MCCOUCH *et al.* 1988), soybean (KEIM *et al.* 1990) and tomato (BERNATZKY and TANKSLEY 1986). Extensive polymorphism between the progenitors of the mapping pop-

ulation is a prerequisite for the construction of a comprehensive linkage map. Low levels of RFLPs have been detected within each of the gene pools of common bean (CHASE, ORTEGA and VALLEJOS 1991). This problem has been solved in other species through the development of mapping populations from interspecific crosses. However, this is not a viable option for the common bean. Although interspecific crosses between *P. vulgaris* and *P. coccineus* or *P. acutifolius* can be obtained (HUCL and SCOLES 1985), the resulting progenies are far from normal and of limited value for mapping purposes. However, moderate levels of polymorphism can be detected between the Mesoamerican and Andean gene pools of the common bean (CHASE, ORTEGA, and VALLEJOS 1991). In addition, the extent of detectable polymorphisms can be increased by the inclusion of breeding lines carrying introgressions from either *P. acutifolius*, or *P. coccineus*. We report the construction of a linkage map for *P. vulgaris* based mostly on RFLP markers.

MATERIALS AND METHODS

Parental genotypes: Single inbred representatives of each of the two major *P. vulgaris* gene pools were used to generate the backcross mapping population, *viz.*, 'XR-235-1-1' for the Mesoamerican gene pool and 'Calima' for the Andean gene pool. However, the Mesoamerican breeding line 'XR-235-1-1' (FREYTAG, BASSETT and ZAPATA 1982) carries some chromosome segments from *P. coccineus*. 'XR-235-1-1' was used as the pistillate parent to obtain the F₁ progeny. The resulting F₁ plants were used to pollinate the recurrent parent, 'XR-235-1-1.' The parental genotypes differ in a large number of morphological and molecular characters. These include seed size, seed pigmentation, plant morphol-

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ogy, quantitative resistance to bacterial blight, phaseolin type and other seed proteins, isozymes, and DNA marker loci (VALLEJOS and CHASE 1991a,b; CHASE, ORTEGA and VALLEJOS 1991).

Seed color and protein markers: The *P* locus controls the development of pigmentation on the seed coat; the dominant allele is present in 'Calima,' the donor parent, and absent in 'XR-235-1-1.' Thus, the segregation at this locus was followed by recording the presence or absence of pigmentation on the seeds collected from BC₁ individuals. The segregation of seed proteins was followed using SDS-PAGE as described earlier (VALLEJOS and CHASE 1991b). The α -amylase inhibitor was monitored using a combination of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western analysis using polyclonal antibodies obtained from M. CHRISPEELS (MORENO and CHRISPEELS 1989).

RFLP marker analysis: A modified procedure of MURRAY and THOMPSON (1980) was used to isolate total DNA from 2–4 g of young leaf tissue. Briefly, a liquid nitrogen powder of this tissue was mixed with three volumes of extraction buffer (133 mM Tris·HCl (pH 7.8)/6.7 mM NaEDTA/0.95 M NaCl/1.33% Na Sarkosyl/1.33% mercaptoethanol) in a polypropylene tube and incubated at 65° for 30 min; the tubes were inverted gently every 5 min. The homogenate was then extracted with one volume of chloroform (CH₂Cl₂/octanol; 24:1) and the aqueous phase separated by centrifugation. DNA was precipitated from the aqueous phase by the addition of 2/3 volumes of isopropanol; the precipitate was transferred with a glass hook (made from a Pasteur pipet) to the first washing solution (76% ethanol/0.2 M Na acetate) and held for 30 min to a few hours. This washing step eliminated flavonoid pigments and some oligosaccharides that coprecipitated with the DNA. The DNA was finally transferred to the second washing solution (76% ethanol/10 mM ammonium acetate) for 30 sec. After eliminating most of the liquid by pressing the pellet against the tube walls, the pellet was transferred to a new tube where it was dried and the DNA was dissolved in TE buffer. DNA was quantitated by electrophoresing undigested aliquots in agarose gels along with undigested lambda DNA standards. Concentrations were estimated by comparing the fluorescence intensities of the ethidium bromide stained samples against those of the standards.

DNA samples were digested with restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV and *Hind*III) under conditions recommended by the manufacturer (Life Technologies, Inc., Gaithersburg, Maryland). DNA restriction fragments (1.5–2.0 μ g/lane) were separated in 0.9 or 1.0% agarose gels using a modified TAE buffer (100 mM Tris·acetate/1 mM NaEDTA/pH 8.1; MURRAY and THOMPSON 1980). DNA in the gels was stained with ethidium bromide and visualized under UV light. DNA in the gels was depurinated with 0.25 M HCl for 10 min, and then denatured in 0.4 N NaOH/1.5 M NaCl for 30 min. SOUTHERN (1975) blots were obtained by alkaline transfer (20 mM NaOH/0.6 M NaCl) overnight. Membranes (Nytran; Schleicher & Schuell, Keene, New Hampshire) were then washed in 2 \times SSPE (1 \times SSPE = 150 mM NaCl/10 mM NaH₂PO₄, pH 7.4 (NaOH)/1 mM EDTA), air dried for 30 min over filter paper, and then UV cross-linked in a stratabox at a setting of 1200 (Stratagene, La Jolla, California). This procedure allowed the reprobing of blots for at least 8–10 times.

Probes and hybridizations: A *Pst*I genomic library enriched for single copy sequences was used as the source of probes. The construction of this library has been described elsewhere (CHASE, ORTEGA and VALLEJOS 1991). The

mungbean cDNA of the chlorophyll *a/b* binding protein was also included (THOMPSON *et al.* 1983).

Inserts for hybridizations were obtained via the polymerase chain reaction (SAIKI *et al.* 1988). Wells of a microtiter plate were loaded with 100 μ l of LB containing ampicillin (50 μ g/ml). Each well was inoculated with a different clone, and the plate was incubated overnight at 37° on a rotary shaker. Overnight cultures were transferred to 500- μ l microcentrifuge tubes and the cells pelleted for 2 min at 11,000 \times *g*. The supernate was removed and the cells were resuspended in 100 μ l of distilled water. The cell suspension was freeze-thawed and then spun at 16,000 \times *g* for 15 min. The aqueous supernate was transferred to a new tube and stored at –20°. Amplification of insert DNA was carried out in 100- μ l reaction volumes containing: 10 mM Tris·HCl (pH 8.8), 50 mM KCl, 15 mM MgCl₂, 0.1% Triton X-100, 200 μ M of each dNTP, 100 nM of each primer, 0.5 unit of *Taq* DNA polymerase (Promega, Madison, Wisconsin), and 5 μ l of the aqueous supernate from the freeze-thawed bacterial cells. Primers were synthesized at the DNA Synthesis Core Laboratory, University of Florida, and corresponded to the 18-base sequences up- and downstream from the *Pst*I cloning site of the plasmid pTZ18R. Amplification was carried out in a thermocycler (Coy Laboratory Products, Inc., Ann Arbor, Michigan). The initial denaturation was at 94° for 2 min followed by 10 cycles of 1 min at 94°/1 min at 55°/3 min at 72°, 25 cycles of 1 min at 94°/1 min at 55°/2.5 min at 72°, 10 cycles of 1 min at 94°/1 min at 55°/3 min at 72°. Amplified insert DNA was precipitated in ethanol and later resuspended in TE buffer. DNA concentration was estimated in ethidium bromide stained agarose gels. Insert DNA was labeled with [³²P]dCTP using a random primer kit (Boehringer Mannheim Biochemicals, Indianapolis, Indiana).

Hybridizations of Southern blots with ³²P-labeled probes were carried out in glass bottles mounted on the rotisserie of a Hyb-Aid oven (Hyb-Aid, Middlesex, United Kingdom). The hybridization solution contained 5 \times SSPE, 5 \times Denhardt's solution, 1% SDS, 100 ng/ml of carrier DNA, and 5–10 ng/ml labeled insert DNA (1 \times Denhardt's solution = bovine serum albumin 0.02%/Ficoll 0.02%/polyvinylpyrrolidone 0.02%). Hybridizations were carried out at 65° for 16–20 hr. Blots were washed twice in 2 \times SSPE/0.1% SDS for a few seconds followed by three washes with 2 \times SSPE/0.1% SDS at 65° for 15 min each and a final wash with 0.5 \times SSPE/0.1% SDS for 15 min at 65°. The membranes were wrapped with polyethylene film and the hybridizing fragments were visualized by autoradiography on X-Omat film (Kodak) using intensifying screens (Lightening Plus, Du Pont). Segregation data for the DNA markers was collected with high efficiency by carrying simultaneous hybridizations with three to five probes. Typically, 12 hybridizations were carried out in a single day with a total of 36 to 45 probes. For single probe hybridizations labeled DNA was used directly without purification. On the other hand, for multiple probe hybridizations, a mixture of selected probes were labeled in a single vial, and then cleaned by spun column dialysis (SAMBROOK, FRITSCH and MANIATIS 1989). This step was taken to avoid high background on the blots.

The library was screened to identify restriction enzyme-probe combinations that detected length polymorphisms between 'Calima' and 'XR-235-1-1.' Thus, test blots containing parental DNA digested with the four restriction enzymes were hybridized with each probe. Probes that detected polymorphic fragments with the same restriction enzyme on nonoverlapping areas of the blot were mixed to probe Southern blots of the segregating population. Multi-

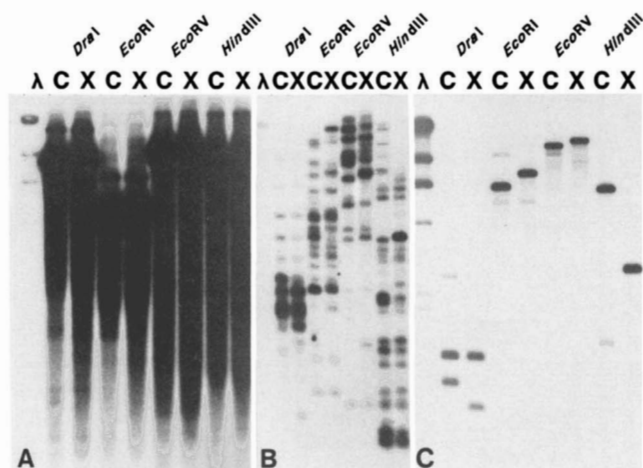


FIGURE 1.—Southern hybridizations of test blots used to screen the *Pst*I genomic library for single copy sequences that detected RFLPs between the parental genotypes. From left to right: lambda DNA (*Hind*III digest), 'Calima' (C) and 'XR-235-1-1' (X) digested with *Dra*I, *Eco*RI, *Eco*RV and *Hind*III. Hybridization patterns of: **A**, highly repetitive DNA; **B**, multigene family sequences; and **C**, single copy sequences.

point linkage analysis was conducted using **Mapmaker** (LANDER *et al.* 1987).

RESULTS

Library screen: Four restriction enzymes were used to screen 362 clones for their ability to detect polymorphisms between the parental genotypes. These clones had insert sizes ranging between 500 and 4000 bp. Eight (2.2%) of these clones produced hybridization patterns typical of highly repetitive DNA (Figure 1A), while nine (2.5%) clones yielded hybridization patterns expected of multigene families (Figure 1B). The hybridization pattern of the remaining 345 clones was typical of single copy sequences (Figure 1C). RFLPs between the parental genotypes were detected with 227 clones, although 11 of these clones were redundant. Redundancy was assumed when two clones yielded identical segregation, displayed identical hybridization patterns with the four enzymes, and had the same insert sizes. Thus, only 216 unique clones (60%) were used in this study. The four restriction enzymes differed in their ability to reveal polymorphisms between 'Calima' and 'XR-235-1-1': *Dra*I (42%), *Hind*III (53%), *Eco*RI (62%) and *Eco*RV (64%).

Monogenic segregation ratios: Ratios distorted from the expected 1:1 were detected at 19 loci in three different linkage groups (Table 1). The nine loci located centrally in group C, and a distal locus (*Bng*35) in group K displayed an excess of homozygotes. Examination of raw data indicated that the deviation detected for *Bng*35 may be an artifact. In the four plants that were missing data for *Bng*35, all loci within 25 cM from *Bng*35 were heterozygous. Thus, if we assume that these plants were also heterozygous at the *Bng*35 locus, then, no significant devia-

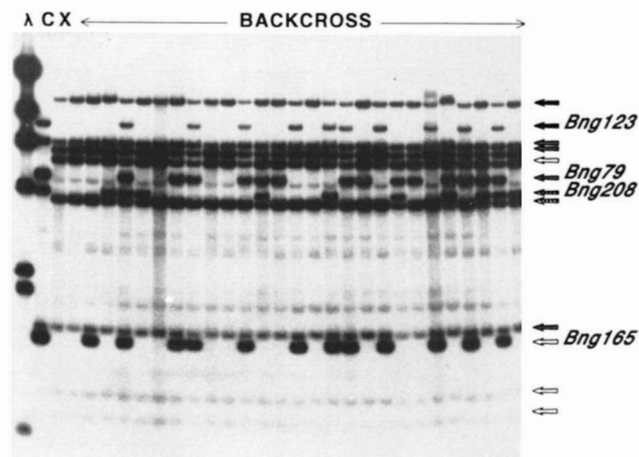


FIGURE 2.—Southern blot displaying the segregation of four RFLP loci. Lambda and bean genomic DNAs were digested with *Hind*III. From left to right: lambda fragments, 'Calima' (C), 'XR-235-1-1' (X) and 27 backcross individuals. The blot was probed simultaneously with radiolabeled inserts for *Bng*79, *Bng*123, *Bng*165, and *Bng*208. Restriction fragments detected with the same probe are marked with arrows of a single shading pattern. Arrows with a label are pointing at segregating fragments, whereas those without a label are pointing at recurrent or nonsegregating allelic fragments.

tions would be found at this locus. In contrast, the block of nine distal loci in group H showed a highly significant excess of heterozygotes.

Linkage analysis: The linkage relationships of the segregating markers were analyzed with the multi-point linkage analysis software **Mapmaker** version 1.9 (LANDER *et al.* 1987). Segregation data were collected efficiently by carrying simultaneous hybridizations with multiple probes (Figure 2). After obtaining all the two-point linkage data, the markers were sorted into distinct groups using an LOD of 4.0 and a distance of 25 cM (Haldane function) as default linkage criteria. This procedure resulted in the formation of 12 groups and six isolated markers; two of these markers showed no recombination between them and were linked to a third one. The three-point linkage data were obtained next. A **framework order** for each group was obtained using an LOD of 3.0 and a distance of 25 cM as linkage criteria. A three-point LOD exclusion threshold of -3 was also used for this process. Loci included in the framework are marked on the map (Figure 3). Linkage between the six isolated markers and the 12 linkage groups was tested relaxing the distance criterion while keeping the LOD criterion at 4.0. These markers were found to assort with three of the established linkage groups and were also assigned to the **framework** (*B*: *Bng*71, *Bng*151, *Bng*160; *E*: *Bng*161, *Bng*162; *I*: *Bng*200). The remaining loci were placed on the map using the **try** command. This command determines the relative likelihoods of maps where a marker is placed at different intervals of a gene order. A place was selected for a marker only when the log-likelihood for that order was at least 100

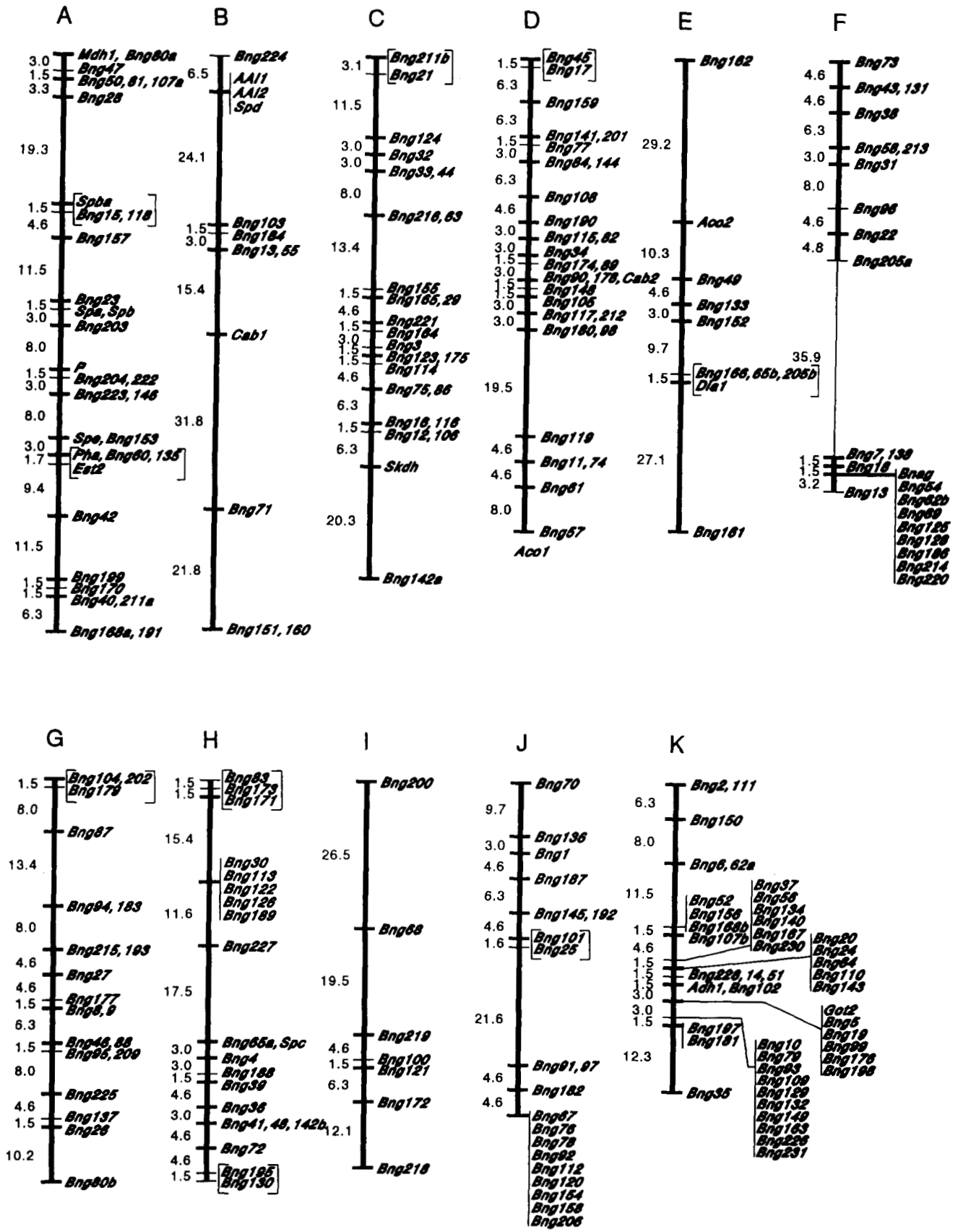


FIGURE 3.—Linkage map of *P. vulgaris* L., the common bean. With the aid of **Mapmaker** (LANDER *et al.* 1988) 244 markers were assigned to 145 loci/locus clusters on 11 linkage groups (A–K). The exact position of *Aco1* on group D could not be located due to incomplete data for this locus; *Aco1* is listed at the bottom of the group. A **framework** order was obtained after grouping the markers (LOD 4.0, 25 cM), and

TABLE 1
List of loci displaying deviations from Mendelian ratios (1:1)

Group	Locus	x/x ^a	x/c	X ²	Group	Locus	x/x	x/c	X ²
C	<i>Bng124</i>	44	24	5.88*	H	<i>Bng65a</i>	24	44	5.88*
C	<i>Bng32</i>	44	24	5.88*	H	<i>Bng4</i>	24	44	5.88*
C	<i>Bng216</i>	43	25	4.76*	H	<i>Bng188</i>	22	46	8.47**
C	<i>Bng221</i>	43	25	4.76*	H	<i>Bng39</i>	21	47	9.94**
C	<i>Bng164</i>	44	24	5.88*	H	<i>Bng36</i>	22	46	8.47**
C	<i>Bng3</i>	43	24	5.39*	H	<i>Bng41</i>	20	48	11.53**
C	<i>Bng123</i>	43	25	4.76*	H	<i>Bng72</i>	23	45	7.12**
C	<i>Bng114</i>	44	24	5.88*	H	<i>Bng195</i>	20	48	11.53**
C	<i>Bng75</i>	43	25	4.76*	H	<i>Bng130</i>	19	49	13.24**
K	<i>Bng35</i>	40	24	4.00*					

^a x/x: homozygous for XR-235-1-1 alleles; x/c: heterozygous.

times better than the second best order. Markers for which an order could not be established with a log-likelihood greater than 2 were encased by brackets to indicate this fact (Figure 3). Tests for possible linkage between two of the 12 identified linkage groups were carried out because *P. vulgaris* is known to have 11 chromosomes. The **link** command was used with all possible group pair combinations to test for possible associations. Linkage was detected between two of the smallest linkage groups, although the LOD score was of only 2.22. *Bng7* and *Bng22* have been found to be ca. 15 cM (LOD = 4.4) apart in the analysis of a smaller backcross population ($n = 34$). For these reasons, these small groups have been assigned to the same linkage group (*F*). A thin line on the map was used to indicate the low LOD score (Figure 3). The distance between these subgroups, 36 cM, was calculated using the KOSAMBI (1944) function; this function is normally used with high recombination frequencies. The final linear orders were tested with the **ripple** command; this test consists of comparing the likelihood of the current map with those of maps obtained by permuting the orders of all adjacent triplets. The isozyme locus *Aco1* was assigned to group *D* and listed at the bottom of the group. This locus could not be placed at any specific location within this group because 8 out of the 68 BC₁ plants had missing data for this locus. However, data from a different population had previously placed *Aco1* between *Bng90* and *Bng180*.

The *P* locus, 17 protein, and 226 DNA marker loci presented in this report define 963 cM of the common bean genome. These markers have assorted into 11 linkage groups ranging in size from 56 to 104 cM: *A* (104.7), *B* (104), *C* (94.6), *D* (85.8), *E* (85.5), *F* (78),

G (73.6), *H* (73.3), *I* (70.6), *J* (60.7) and *K* (56.2). The 244 markers fell into only 145 loci/locus clusters with the following distribution: 90 (62%) loci were occupied by a single marker, 40 (28%) by two markers, 8 (5.6%) by three markers, and seven clusters (4.8%) contained between 5 and 10 markers each. These clusters were located on four linkage groups and four of these clusters were detected in the smallest linkage group (*K*).

Five seed proteins (*Pha*, *Spa*, *Spb*, *Spba* and *Spe*), *Est2*, and the *P* locus were detected in the same linkage group (*A*) as previously reported (VALLEJOS and CHASE 1991b). In addition, we had also reported that three seed proteins named *Spd*, *Spf* and *Sph* were linked (VALLEJOS and CHASE 1991b). *Spf* and *Sph* have been renamed *AAI1* and *AAI2*, respectively, because they comigrated and cosegregated with two bands detected by western analysis with a polyclonal antibody produced against the bean α -amylase inhibitor (MORENO and CHRISPEELS 1989). The mung bean cDNA clone of the chlorophyll *a/b* binding protein (pMB123, THOMPSON *et al.* 1983) detected 10 *EcoRV* fragments in each of the parents, 'Calima' and 'XR-235-1-1'; these fragments ranged in size between 1.4 and 11 kb. Five of those fragments were common to both genotypes. Two fragments unique to 'Calima' (9.4 and 8.8 kb) cosegregated and were assigned to locus *Cab1* in group *B*. Three other fragments (6.4, 5.9 and 1.5 kb) also cosegregated, but assorted independently from the previous ones and were assigned to locus *Cab2* in group *D*. Eight of the 216 genomic clones detected two independent loci each and were given the letters *a* or *b* after the number to differentiate them. In all cases the homologous sequences were located on different linkage groups suggesting duplication/translocation events.

then ordering each group (LOD 3.0, 25 cM, exclusion LOD -3.0). Markers that belong to the **framework** order have been marked with thicker crossbars. Loci for which an order could not be established (LOD < 2.0) have been enclosed by brackets. Numbers on the left of a linkage group represent map distances in cM. Loci at distances that exceed 25 cM in groups *B*, *E* and *I* have an LOD score > 4.0 and have been included in the **framework**. The distance (35.9 cM) between *Bng205a* and *Bng7* (group *F*) was calculated using the Kosambi function. Although an LOD of 2.22 was found for this linkage, other independent data supports the assignment to this group.

Genome size: The length (cM) of the *P. vulgaris* genome was estimated using the method of HULBERT *et al.* (1988). This method is based on calculating the probability [$P(T, G)$] that a pair of linked markers randomly chosen from a genome of size (G) has an LOD score $\geq T$. A first-order approximation of $P(T, G)$ is given by the expression $2x/G$, where x is the distance (cM) between the markers; this distance is relatively small in relation to the size of the genome. The expected frequency (K) of locus pairs having an $\text{LOD} \geq T$ is $\binom{N}{2}P(T, G)$, where N is the number of markers. **Mapmaker** was used to determine that there were 433 locus pairs each within 25 cM and with an LOD score ≥ 4.0 . These pairs were extracted from the 145 loci identified in this study. Only a single member of a cluster was used in the calculations because inclusion of all cluster members would have artificially increased the probability estimate. With this information at hand and the relationship $G = \binom{N}{2}(2x/K)$, we have estimated the bean genome to be approximately 1,200 cM in length. Thus, the markers presented here cover 80% of the estimated bean genome.

DISCUSSION

Reassociation kinetics measurements have shown that 60% of the common bean genome contains single copy sequences (TALBOT *et al.* 1984). Thus, a genomic library of small fragments would be expected, in the best of cases, to have 60% of the clones from the slow reassociating class of DNA. However, the results from the library screen clearly demonstrated that the size selected *Pst*I fragments of bean genomic DNA were highly enriched in single copy sequences (95%). BURR *et al.* (1988) pointed out that small DNA fragments obtained with restriction enzymes sensitive to *C*-methylation, such as *Pst*I, would yield genomic libraries enriched in single copy sequences. This phenomenon has been observed previously in tomato (TANKSLEY *et al.* 1987) and maize (BURR *et al.* 1988), but not in rice (MCCOUCH *et al.* 1988). New flow cytometry measurements indicate that the bean genome is intermediate in size between rice and tomato (ARUMUGANATHAN and EARLE 1991). This observation suggests the question of whether there is a relationship between genome size and the extent of *C*-methylation in plant repetitive DNA.

We have constructed a linkage map with 145 loci/locus clusters. These loci/locus clusters are occupied by 244 markers. An interesting feature of this map is the uneven distribution of markers on the linkage groups; approximately 20% of the markers were found in 7 clusters. Groups *B*, *E* and *I* each have seven to eight loci which delimit 104, 85 and 70 cM, respectively. Groups *A* (104.7 cM) and *D* (85.8 cM) are more

densely populated and contain approximately 30–33 markers each which are distributed among 20 loci/locus clusters. Finally, group *K* is the smallest linkage group (56.2 cM), but contains 44 markers, 27 of which are organized in 4 separate clusters. Several nonexclusive explanations could account for the disparity in the distribution of these markers. If all regions of the genome were to be evenly represented in our library, then one must assume that small *Pst*I fragments (500–4000 bp) containing single copy sequences are evenly distributed throughout the bean genome and that the number of clones used to construct the linkage map was large enough to give an unbiased sample. It has been estimated that about 60,000 genes are expressed during the life cycle of a plant (KAMALAY and GOLDBERG 1980). Thus, in comparison, the small number of markers used in this project may represent a biased sample of the bean genome and be in part responsible for the uneven distribution of markers. In addition, one must consider that the library was screened for probes that detected polymorphisms between the Mesoamerican breeding line 'XR-235-1-1' and the Andean cultivar 'Calima.' Therefore, densely populated chromosome segments may correspond to regions of the genome that are variable between the two gene pools of beans, whereas sparsely populated segments may carry more conserved sequences. Differences in recombination frequencies throughout the genome may also account for marker density differences among different chromosome segments. The fact that 'XR-235-1-1' has *P. coccineus* introgression must also be taken into account. Some bias was expected towards sequences that detect polymorphisms between *P. vulgaris* and *P. coccineus*, but only to the extent by which the latter has been introgressed into 'XR-235-1-1.' Small chromosome inversions can also act as suppressors of recombination and explain the presence of clusters. Finally, the recombination frequency within those clusters may have been affected by the mixed background of the cross. For instance, the recombination frequency changes from 10 to 50% in a stretch of chromosome 2 of tomato in two different interspecific crosses (PATTERSON *et al.* (1989). It remains to be determined whether differences in recombination values in certain chromosome regions are due to the presence of *P. coccineus* chromosome segment, to the combination of Andean and Mesoamerican backgrounds, or both.

Strong distortions of Mendelian ratios detected for a distal section of group *H* have been observed previously for a marker from this group (*Spc*) in two different progenies of 'XR-235-1-1' and 'Calima' (VALLEJOS and CHASE 1991b). Significant skewings have been reported also in progenies obtained between Mesoamerican and Andean genotypes (KOENIG and GEPTS 1989), and for interspecific crosses be-

tween *P. vulgaris* and *P. coccineus* (SMARTT 1970). These distortions could be interpreted as the result of pre- or postsynthetic selection of certain allelic combinations. For instance, gametes are eliminated when certain allelic combinations are present at the *Ge* locus of tomato (RICK 1966). An alternative explanation could be found in chromosome inversions. CHENG, BASSETT and QUESENBERRY (1981) have presented evidence for chromosome inversions in interspecific crosses of *P. vulgaris* and *P. coccineus*. Indeed, inversions can also explain reduced recombination frequencies and the appearance of clusters. We are currently investigating these issues in progenies between pure *P. vulgaris* lines obtained within and between gene pools.

The linear order for the protein/isozyme loci in group A is the same as that found previously (VALLEJOS and CHASE 1991B), ALTHOUGH THE DISTANCES AROUND *Est2* are at variance with those previously obtained due mainly to missing data points for this locus. The distances for the locus pairs *Aco2-Dial1* (group E) and *Adh1-Got2* (group K) were essentially as previously reported (VALLEJOS and CHASE 1991a).

The Genetic Resources Unit of CIAT manages a large and well diversified bean germ plasm collection with over 40,000 accessions (HIDALGO 1991). It may be possible to tag many genes of agricultural importance found in that collection using the markers presented here. Many of these genes, such as those responsible for disease resistance, have eluded isolation via standard cDNA cloning approaches. Map-based cloning has been suggested as an alternative approach for the molecular cloning of these genes (TANKSLEY *et al.* 1989). The relatively small size of the bean genome makes this species a good candidate for gene isolation via chromosome walking techniques (ROMMENS *et al.* 1989). We have estimated the length of the bean genome to be approximately 1200 cM. This estimate and that of the physical size of the bean genome (637 Mbp) lead to an average estimate of 530 kb/cM. However, an accurate estimate of this relationship would be needed for a targeted region before any of these efforts are initiated in beans. For instance, a ratio of 14 kb/cM has been detected within the *bronze* locus of maize (DOONER 1986); this is about two orders of magnitude smaller than the average ratio. On the other hand, a ratio severalfold above the average has been detected around the *Tm-2a* locus of tomato (GANAL, YOUNG and TANKSLEY 1989).

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