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Towards an integrated linkage map of common bean 2. Development of an RFLP-based linkage map

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Summary. A restriction fragment length polymorphism (RFLP)-based linkage map for common bean (*Phaseolus vulgaris* L.) covering 827 centiMorgans (cM) was developed based on a F_2 mapping population derived from a cross between BAT93 and Jalo EEP558. The parental genotypes were chosen because they exhibited differences in evolutionary origin, allozymes, phaseolin type, and for several agronomic traits. The segregation of 152 markers was analyzed, including 115 RFLP loci, 7 isozyme loci, 8 random amplified polymorphic DNA (RAPD) marker loci, and 19 loci corresponding to 15 clones of known genes, 1 virus resistance gene, 1 flower color gene, and 1 seed color pattern gene. Using MAPMAKER and LINKAGE-1, we were able to assign 143 markers to 15 linkage groups, whereas 9 markers remained unassigned. The average interval between markers was 6.5 cM; only one interval was larger than 30 cM. A small fraction (9%) of the markers deviated significantly from the expected Mendelian ratios (1:2:1 or 3:1) and mapped into four clusters. Probes of known genes belonged to three categories: seed proteins, pathogen response genes, and *Rhizobium* response genes. Within each category, sequences homologous to the various probes were unlinked. The *I* gene for bean common mosaic virus resistance is the first disease resistance gene to be located on the common bean genetic linkage map.

Key words: RFLP – RAPD – Linkage map – Bean Common Mosaic Virus resistance – Segregation distortion

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Introduction

The current linkage map of common bean (*Phaseolus vulgaris;* 2n = 2x = 22) is poorly developed. It consists of a small number of linkage groups that include genes controlling mostly morphological and pigmentation traits such as flower and seed color or color patterning (reviewed by Bassett 1991; Gepts 1988). Many of these traits are subject to epistatic, pleiotropic, and environmental effects, and with the exception of the locus for phaseolin seed protein and loci for ribosomal RNA, none has been assigned to a particular chromosome (Schumann et al. 1990; Durante et al. 1977).

Restriction fragment length polymorphisms (RFLPs) can be used as genetic markers to construct a saturated linkage map (Botstein et al. 1980). In the past 5 years, RFLP linkage maps have been developed for *Arabidopsis thaliana* (Reiter et al. 1992) and several crops such as barley (Heun et al. 1991), *Brassica napus* (Landry et al. 1991), *Brassica rapa* (Song et al. 1991), lettuce (Kesseli et al. 1990), maize (Coe et al. 1990), potato (Bonierbale et al. 1988; Gebhardt et al. 1989), rice (McCouch et al. 1988), soybean (Diers et al. 1992), and tomato (Paterson et al. 1991). The advantages of RFLP markers over morphological and biochemical markers include their relatively high number, absence of pleiotropic and epistatic effects, and higher frequency of polymorphism.

Preliminary studies with a set of approximately 60 random *Pst*I clones hybridized to genomic DNA digested with three restriction enzymes revealed high levels of RFLP in common bean. These levels reach 80–90% between the Middle American and Andean gene pools and 50–60% within these gene pools (Nodari et al. 1992). In the work presented here we have used RFLPs to develop a linkage map of common bean for use in evolutionary, genetic, and breeding studies. We also identified linkages

between RFLP loci and other molecular markers including RAPDs, isozymes, and seed proteins. We mapped sequences involved in responses to pathogens and *Rhizobium*. Furthermore, we mapped the *I* gene for bean common mosaic virus resistance.

Material and methods

Plant material

The two parents of the F_2 mapping population were selected because they had divergent evolutionary origins and exhibited contrasting disease responses. BAT93 is a breeding line developed at the Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia) and was derived from a double cross involving four Middle American genotypes (Veranic 2, PI 207262, Jamapa, and Great Northern Tara). Jalo EEP558 was a selection from the Andean landrace Jalo obtained at the Estação Experimental de Pato de Minas (Minas Gerais, Brazil). Phaseolin and isozyme profiles of BAT93 and Jalo EEP558 confirmed their Middle American and Andean evolutionary origins, respectively (Koenig et al. 1989 b; Singh et al. 1991 b). BAT93 is resistant to bean common mosaic virus (BCMV), rust (*Uromyces phaseoli*), common bacterial blight (*Xanthomonas phaseoli*), and anthracnose (*Colletotrichum lindemuthianum*), but is susceptible to angular leaf spot (*Phaeoisariopsis angularis*). Jalo EEP558 exhibits opposite reactions to the same pathogens (Schwartz et al. 1982; S. Singh, personal communication; R. Stavely, personal communication). Jalo EEP558 develops a large number of nodules after inoculation with *Rhizobium tropici*, whereas BAT93 develops few nodules (Tsai et al. unpublished results). The two parents also differ in morpho-agronomic traits such as seed size, flower color, presence of the seed corona, and length of the fifth internode (Singh et al. 1991 a). An F_2 mapping population of 75 greenhouse-grown individuals was obtained after selfing of the F_1 generation under insect-free conditions.

Markers: source and nomenclature

Genomic clones and probes of known genes

Segregations for RFLPs detected by genomic clones and probes of known genes were determined in the F_2 population of 75 individuals. Two genomic libraries, *Eco*RI-*Bam*HI (clones numbered from GUC001 to GUC999) and *Pst*I (clones numbered from GUC1001 to GUC1862), were constructed as described in Nodari et al. (1992). Single-copy clones were selected after hybridization of the insert with total radiolabeled bean genomic DNA as described in Nodari et al. (1992). The following sequences were also used for RFLP mapping: nuclear ribosomal RNA gene (pHA2; Jorgensen et al. 1987), α-amylase inhibitor (pHU20; Moreno and Chrispeels 1989), seed lectin or phytohemagglutinin (pMcPHA; M. Chrispeels, personal communication), phaseolin (pPhasMC31; Slightom et al. 1983), cellulase (pBAC10; Tucker and Milligan 1991), chitinase (pCH18, Broglie et al. 1986), glucanase (pG101; Edington et al. 1991), chalcone isomerase (pCHI1; Mehdy and Lamb 1987), chalcone synthase (pCHS1; Ryder et al. 1984), phenylalanine ammonia-lyase (pPAL5; Edwards et al. 1985), pathogenesis-related proteins 1 and 2 (pEIT23 and pEIT14a; Walter et al. 1990), glutamine synthetase (pCPGS2; DPS Verma, personal communication), sucrose synthetase (nodulin-100; Fuller et al. 1983; Thummler and Verma 1987), and uricase (pNOD35; Nguyen et al. 1985). When the same probe detected more than one polymorphic band, fragments of decreasing size were labeled alphabetically with an additional lower case letter. The same rule was followed for RAPD markers.

Random amplified polymorphic DNA (RAPD)

DNA sequences from the parents and 54 randomly chosen plants of the F_2 mapping population were amplified with randomn 10-mer primers (Kit A, Operon, Alameda, CA) in a thermal cycler (Ericomp). The cycling parameters (40 cycles) for denaturation, annealing and extension were: 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C, respectively. One last cycle of 10 min at 72 °C was performed to complete the reaction. Amplification was carried out with 25 ng of total genomic DNA in a 25 µl solution containing 1 × buffer (10 × = 100 m*M* TRIS-HCl pH 8.3, 500 m*M* KCl, 15 m*M* $MgCl_2$, 0.01% gelatin), 0.1 m*M* of each dNTP, 0.45 m*M* $MgCl_2$, 0.4 µ*M* primer, and 1 U *Taq* DNA polymerase (Promega). One-half of the volume was electrophoresed on a 1.5% agarose gel (containing 0.5 µg/ml ethidium bromide), and the RAPD polymorphisms were scored as the presence or absence of a specific band. Each amplification run included two negative controls (without DNA, without polymerase) and the two parents to verify the repeatability of the experiment. The scored bands were labeled with an R (for RAPD), followed by an O (Operon), an A (Kit A from Operon) and by the number of the primer in the kit. When the same probe detected more than 1 locus, fragments of decreasing size were labeled alphabetically with an additional lower case letter.

Phaseolin and isozymes

Polyacrylamide and starch gel electrophoresis assays were performed as described by Koenig and Gepts (1989 a, b). Seeds from 70 individuals of the mapping population were analyzed for seed-protein phaseolin type. Leaf and root tissues from the same individuals were scored for seven polymorphic isozyme systems: aconitase (ACO, E.C. 4.2.1.3), diaphorase (DIAP, E.C. 1.6.4.3), leucine aminopeptidase (LAP, E.C. 3.4.11.1), malic enzyme (ME, E.C. 1.1.1.40), methyl-umbelliferyl esterase (MUE), the small subunit of ribulose bisphosphate carboxylase (RBCS, E.C. 4.1.1.39), and shikimate dehydrogenase (SKDH, E.C. 1.1.1.25). The stain recipes and genetic control for all enzymes except aconitase have been reported previously (Koenig and Gepts 1989 a, b; Garrido et al. 1991). Aconitase was stained as described by Gepts et al. (1992) and showed two bands of activity. The most cathodal of these was polymorphic, with BAT93 and Jalo EEP558 carrying the $Aco\text{-}2^{100}$ and $Aco\text{-}2^{102}$ alleles, respectively.

Morphological and agronomic traits

Three morpho-agronomic traits were evaluated: flower color, corona, and reaction to bean common mosaic virus (BCMV). Flower color (pink versus white) was evaluated in 75 F_2 plants, whereas the presence or absence of corona (*Cor;* Lamprecht 1961) was evaluated in F_3 seeds of the same 75 F_2 plants. The presence (hypersensitivity as revealed by "black root" necrosis symptoms in leaves and/or whole plants) or absence (typical common mosaic symptoms) of the dominant *I* allele, which confers resistance to non-necrosis-inducing strains of bean common mosaic potyvirus (BCMV: Drijfhout et al. 1978), was tested in 10 individuals of 70 F_3 families by mechanically inoculating primary leaves with NL-3, a necrosis-inducing strain of BCMV (Morales and Castaño 1987). The inoculation of 10 individuals per F_3 family allowed us to distinguish at the $P = 0.95$ level between F_2 plants homozygous or heterozygous for the dominant allele at the *I* locus.

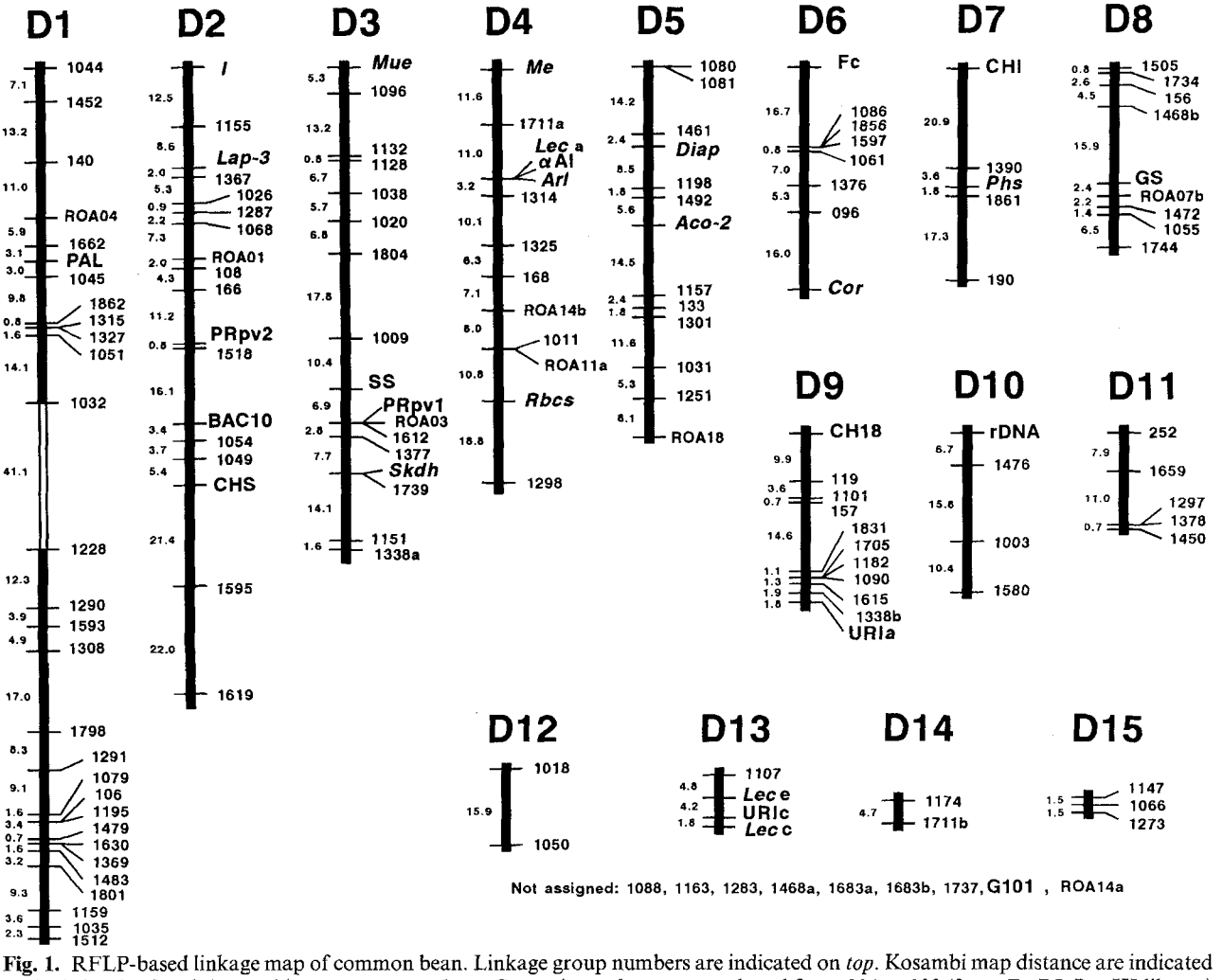

Fig. 1. RFLP-based linkage map of common bean. Linkage group numbers are indicated on *top*. Kosambi map distance are indicated at the *left side* of each interval between two markers. Genomic markers are numbered from *001* to *999* (from *Eco*RI-*Bam*HI library) and from *1001* to *1862* (from *Pst*I library) (the GUC prefix was removed for the sake of simplicity). *Aco-2*, *Diap*, *Lap-3*, *Me*, *Mue*, *Rbcs*, and *Skdh* are isozyme loci. RAPD marker loci are designated by *ROA* followed by a *number* (see text for explanations). Morpho-agronomic traits are *C*, *Cor*, and *I* (see text for explanations). The following markers represent sequences coding for products of known function: *Arl*, *αAI*, BAC10, CHI, CHS, CH18, GS, *Lec*, PAL, *Phs*, PRPv1, PRPv2, rDNA, SS, and URI (see text for explanations). The *open bar* in linkage group D1 represents an interval with a LOD score above 3.0 but with a recombination frequency above 30%

DNA extraction, digestion, and electrophoresis

Total genomic DNA from frozen leaf tissue (5–7 g) that had been harvested prior to flowering was extracted as described by Nodari et al. (1992). Genomic DNA samples (5–10 µg) from parents and F_1 and F_2 plants were digested with either *Eco*RI, *Eco*RV, *Hae*III, or *Hin*dIII restriction enzymes because these enzymes revealed the highest levels of polymorphism between BAT93 and Jalo EEP558 (Nodari et al. 1992). Restriction digestions (4–6 U enzyme/µg of genomic DNA) were performed according to the manufacturers' recommendations (Bethesda Research Laboratories and New England Biolabs) for 4–6 h at 37 °C. The digested DNA was electrophoresed in 0.8% agarose gels (prepared with TAE: 40 m*M* TRIS-acetate pH 7.4, 1 m*M* EDTA) for 16–20 h (1 V/cm of gel) in TAE running buffer.

Southern blotting, hybridization, and autoradiography

Southern hybridization was performed according to the Zetabind protocol (AMF-CUNO, Meriden, Conn.). Approximately 30–40 ng of the insert of each clone, except pHA2, were radiolabeled with α-[^{32}P]dCTP (Amersham) by the random primer method (Feinberg and Vogelstein 1984). The pHA2 plasmid was radiolabeled with the same isotope by nick translation (Rigby et al. 1977). Hybridizations were performed at 42 °C in 50% formamide. Washes after hybridization were performed according to recommendations of the manufacturer (2 × SSC, 0.1% SDS at room temperature; 0.1 × SSC, 0.1% SDS at room temperature and twice at 60 °C for 30 min each). Kodak X-Omat X-ray film was exposed to the membranes for a few hours to a week depending on the intensity of the signal.

Linkage analysis

Linkage analysis was performed with MAPMAKER (Lander et al. 1987) and LINKAGE-1 (Suiter et al. 1983). To identify the linkage groups, pairwise comparisons and grouping of markers were performed with MAPMAKER under the following conditions: (1) recombination frequency below 30% and (2) LOD

score equal to or above 3.0. To establish the most likely order of markers within each group, the "orders" command was used with the above-mentioned linkage criteria and with a three-point exclusion threshold of −2.0 LOD score units. Additional markers were located in these groups using the "try" command with the aforementioned linkage criteria. Kosambi map distances among linked markers were used to generate the map (Fig. 1). Pairwise comparisons to confirm detected linkage relationships between markers and goodness-of-fit tests to detect distorted segregation ratios were performed with LINKAGE-1.

Results and discussion

Segregation of markers

The majority of the random *Pst*I and *Eco*RI-*Bam*HI genomic clones (75%) hybridized to a single band and, with few exceptions, segregated in a co-dominant manner. Four clones – GUC1338, GUC1468, GUC1683, and GUC1711 – detected two bands, which, in all four cases, segregated independently and represent duplications. The remaining clones displayed a hybridization pattern with more than two bands. However, in all cases just one segregating locus could be scored. Probes representing cloned genes in general gave more complex hybridization patterns than random genomic clones. With the exception of BAC10 (cellulase), G101 (glucanase), and CHI (chalcone isomerase), which hybridized to a single band, all other gene clones hybridized to multiple bands. For example, seven and six segregating bands were observed after hybridization with CHS (chalcone synthase) and *Lec* (seed lectin) sequences, respectively. In the case of chalcone synthase (CHS), all segregating bands mapped to the same locus. For seed lectin (*Lec*), four bands (*Leca*, *Lecb*, *Lecd*, and *Lecf*) co-segregated at one locus in linkage group D4 and the other two (*Lecc* and *Lece*) mapped in linkage group D13 (see below). The presence of these multiple bands points to the existence of multigene families. Evidence of Ryder et al. (1987) suggests that CHS is represented by a family of six to eight genes, some of which are tightly linked. Hoffman and Donaldson (1985) identified two lectin genes 4 kb apart and tightly to the the loci coding for the homologous seed proteins arcelin and α-amylase inhibitor (Osborn et al. 1986; Moreno and Chrispeels 1989). RAPD markers were scored for the presence of absence of specific bands of high intensity that distinguished the two parents; hence, these markers segregated as dominant markers. Isozymes and phaseolin seed protein segregated as co-dominant markers as reported previously (Koenig and Gepts 1989a; Garrido et al. 1991; Gepts et al. 1992).

The inheritance pattern of the 152 markers analyzed in the segregating population of 75 individuals followed the expected F_2 co-dominant (1:2:1) or dominant (3:1) Mendelian ratios in 91% of the cases. A small fraction (9%) of the markers deviated significantly ($P<0.05$) from the ratios mentioned above. A similar proportion of distorted segregation (10%) was recently reported in lettuce (Kesseli et al. 1990) and in barley (Heun et al. 1991). Higher proportions of distorted markers have been detected in rice (18.8%; McCouch et al. 1988) and potato (25.5%; Gebhardt et al. 1988). The proportions of BAT93 and Jalo EEP558 alleles were almost identical (0.492:0.508). The 14 markers with distorted segregation were clustered in four regions of the bean genome. Seven of them mapped adjacent to the PAL locus in linkage group D1 (GUC140, ROA04, GUC1662, GUC1045, GUC1862, GUC1315, and GUC1327); 1 marker was located in linkage group D2 (BAC 10); 2 markers were placed 2.4 cM apart in linkage group D8 (GS and ROA07b), and the other 4 constituted linkage group D13 (*Lecc*, *Lece*, URIc, and GUC1107). The clustering of markers with distorted segregation has been observed previously in barley (Heun et al. 1991) and potato (Bonierbale et al. 1988). Possible causes for these segregation distortions include small population size and/or, genetic factors affecting the transmission of the genes. Of the 7 markers in linkage group D16 showed an excess of the Jalo EEP558 alleles. The 7th marker showed an excess of heterozygotes. The 4 markers in linkage group D13 showed a distortion favoring the BAT93 alleles. The number of markers and the consistency in the direction of the deviation among the markers in these two regions suggest a possible genetic origin for the deviation on the linkage groups D1 and D13.

Linkage mapping of markers

Of the 152 markers 143 (94%) could be assigned into 15 linkage groups, comprising a total of 827 cM (Fig. 1 and Table 1). These linkage groups are temporarily labeled D (as in Davis) followed by a number pending further genetic and cytogenetic confirmation. The number (and in parentheses the percentage) of markers per linkage group ranged from 2 (1.4%) to 29 (20.6%). Almost the same order of magnitude was found for variation in length of the linkage groups (3.0–191.5 cM). Among linkage groups the average distance between 2 markers varied from 1.5 cM to 15.9 cM. The average linkage distance between pairs of markers among all linkage groups was 6.5 cM. Only one interval between 2 markers was larger than 30 cM, and it was located in linkage group D1; however, the LOD score for this interval was above the threshold of 3.0. The majority of the intervals (74%) were smaller than 10 cM.

The 7 segregating isozyme loci were distributed in 4 linkage groups. *Me* (malic enzyme) and *Rbcs* (locus for the small subunit of ribulose bisphosphate carboxylase) had been found previously to be linked (Weeden 1984; Koenig and Gepts 1989a). We confirmed the linkage

Table 1. Size distribution of linkage groups in the common bean RFLP map

Linkage group	Markers		Length		Average distance (cM)
	Number	% of total number or probes	cM	% of total	
D1	29	20.3	191.5	23.6	6.6
D2	18	12.6	129.1	15.6	7.6
D3	17	11.9	99.9	12.1	6.2
D4	13	9.1	87.0	10.5	7.2
D5	13	9.1	76.3	9.2	6.4
D6	8	5.6	45.9	5.6	6.6
D7	5	3.5	43.5	5.3	10.9
D8	9	6.3	36.4	4.4	4.6
D9	11	7.7	35.0	4.2	3.5
D10	4	2.8	32.9	4.0	11.0
D11	5	3.5	19.7	2.4	4.9
D12	2	1.4	15.9	1.9	15.9
D13	4	2.8	10.8	1.3	3.6
D14	2	1.4	4.7	0.6	4.7
D15	3	2.1	3.0	0.4	1.5
Total	143	100	826.6	100	
Average					6.5

between *Me* and *Rbcs* by identifying several intermediate markers in linkage group D4, including the tightly linked seed lectin (*Lec*) and the α-amylase inhibitor (*αAI*) loci. Although the arcelin locus was not mapped here, previous studies by Osborn et al. (1986) showed that *Lec* and *Arl* were also tightly linked. Two other isozyme loci, *Aco-2* and *Diap-1*, were located 16 cM apart in linkage group D5, confirming the observations of Vallejos and Chase (1991). The remaining two allozyme loci, *Skdh* and *Mue*, mapped to the same linkage group D3, although they were separated by a distance of more than 50 cM.

Cloned gene sequences used for RFLP mapping belonged to three broad categories. Disease response genes included chitinase (CH18), glucanase (G101), chalcone synthase (CHS), chalcone isomerase (CHI), phenylalanine ammonialyase (PAL), and pathogenesis-related proteins 1 and 2 (PRPv1 and PRPv2). *Rhizobium* nodulation response genes consisted of glutamine synthetase (GS), sucrose synthetase (SS), and uricase (URI). Seed proteins included phaseolin (*Phs*), seed lectin or phytohemagglutinin (*Lec*), and α-amylase inhibitor (*αAI*). Within each of these three categories, sequences homologous to the various probes were unlinked (Fig. 1).

We were able to map one locus for the ribosomal RNA genes located in linkage group D10. However, *in situ* hybridizations revealed at least 3 loci for rRNA genes (Durante et al. 1977). This discrepancy can be attributed to the lower level of polymorphism associated with rDNA sequences in population BAT93 × Jalo EEP558 compared to that of other sequences. Of the four restriction enzymes (*Eco*RI, *Eco*RV, *Hae*III, and *Hin*dIII) used in this study, only *Eco*RV revealed a polymorphism for rRNA genes between the two parents. The use of additional restriction enzymes may identify additional polymorphisms for the rDNA sequences and should allow us to map additional rRNA loci.

Eight genomic clones and 3 RAPD marker remained unassigned to linkage groups. A small proportion of loci have been reported as unassigned for other RFLP maps [e.g., 10% in potato (Gebhardt et al. 1988) and 13% in *Brassica napus* (Landry et al. 1991)]. This observation, together with the discrepancy between the haploid chromosome number (n = 11) and the current number of linkage groups, suggest that additional markers should be mapped to fill the gaps between some linkage groups and between linkage groups and currently unassigned markers. Genetic walking experiments based on bulked segregant analysis of RAPD markers as described by Michelmore et al. (1991) should facilitate this objective.

Segregation and mapping of morpho-agronomic traits

The *Cor* (corona) gene induces a colored ring on the outside of the hilum ring, which surrounds the seed hilum of the seed (von Lamprecht 1934; Leakey 1988). Jalo EEP558 and BAT93 carry the dominant and recessive *Cor* alleles, respectively, of this gene. Presence or absence of the corona segregated according to a 3:1 ratio in the F_2 generation ($\chi^2 = 3.74$; $P = 0.053$). Flower color (provisionally labeled Fc) also segregated according to a 3:1 ratio ($\chi^2 = 1.69$; $P = 0.19$) and appeared to be linked to the *Cor* locus in linkage group D6 (Fig. 1). The gene actually involved in flower color determination in this cross is not known. The *P*, *T*, and *V* loci (Prakken 1970; Leakey 1988) have been shown to control flower color. The *P* and *T* genes can be excluded because they also induce a complete or partial absence, respectively, of pigments in the seeds, which was not observed in this population. The *V* locus has, so far, not been shown to be linked to the *Cor* locus. On the other hand, the *C* locus has been shown to be linked to *Cor* (Lamprecht 1961). It is possible that flower color in this cross is controlled by the *C* pigmentation locus because the mapping distance of 45 cM between *Cor* and the flower color locus identified here corresponds to a recombination value of 25% that is very close to the recombination value of 23% detected previously by Lamprecht (1961). Test crosses are needed to identify the gene responsible for flower color segregation.

The *I* locus conditioning dominant resistance to the non-necrosis-inducing strains of BCMV and hypersensitivity to the necrosis-inducing strains (Drijfhout et al.

Table 2. Comparison of RFLP maps of common bean and other species

Species	C (pg/haploid genome)[a]	Number of markers	Number of linkage groups/ haploid chromosome number	Length (cM)	Average distance (cM)	Amount of DNA/map unit (kbp/cM)[b]	Reference
Arabidopsis thaliana	0.15	320	5/5	630	2	230	Reiter et al. (1992)
Barley	5.05	157	7/7	1,096	7.3	4,843	Heun et al. (1991)
Brassica napus	1.20	120	19/19	1,413	14.0	820	Landry et al. (1991)
Brassica rapa	0.53	280	10/10	1,850	6.9	276	Song et al. (1991)
Common bean	0.65	143	15/11	827	6.5	758	Present results
Lettuce	2.73	147	16/9	1,404	10.7	1,876	Kesseli et al. (1990)
Maize	~2.8	261	10/10	1,776	7.1	1,521	Coe et al. (1990)
Potato	~1.8	141	12/12	690	5.3	2,517	Gebhardt et al. (1989)
Potato	~1.8	134	12/12	606	5.0	2,866	Bonierbale et al. (1988)
Rice	~0.45	135	12/12	1,389	11.3	313	McCouch et al. (1988)
Soybean	1.15	252	31/20	2,147	8.5	517	Diers et al. (1992)
Tomato	0.95	800	12/20	1,480	1.9	619	Paterson et al. (1991)

[a] Arumuganathan and Earle (1991)
[b] 1 pg = 965 mbp (Arumuganathan and Earle 1991)

1978) segregated according to a 3:1 ratio ($\chi^2 = 0.60$; $P = 0.44$) as reported previously (Ali 1950) and mapped to linkage group D2.

Comparison with RFLP maps in other crops

Table 2 provides a comparison of mapping parameters for various published RFLP maps. Caution should be exercised in comparing these various maps because recombination rates will vary depending on the biological material (e.g., inter- versus intraspecific crosses) and the environmental conditions. In addition, recombination rates, and, hence, the amount of DNA per map unit, vary widely within a genome. Of particular interest is the average interval between 2 adjacent markers, which ranged from approximately 2.0 cM (*A. thaliana* and tomato) to 14.0 cM (*Brassica napus*). The average interval in common bean (6.5 cM) is sufficiently small to warrant mapping of quantitative trait loci. The average physical distance per unit of genetic distance ranged from 230 kbp/cM in *A. thaliana* to 4,843 kbp/cM in barley. The current average physical distance (expressed as amount of DNA per map unit) in common bean is only 3.2 times that of *A. thaliana*, which raises the possibility of map-based cloning of selected genes provided additional tightly linked markers can be identified that lie within a relatively short physical distance around genes of interest. Genes controlling agronomic traits of interest segregating in this mapping population include genes for disease resistance and genes for *Rhizobium* nodulation intensity.

In conclusion, we have developed a low-density genetic linkage map for the common bean genome. Markers used to develop this map included RFLPs, RAPDs, isozymes, and morphoagronomic traits, including 1 virus resistance gene. The development of this map was facilitated by the high levels of RFLP observed in *P. vulgaris*. Additional markers need to be mapped in order to increase its density and to reduce the number of linkage groups to 11, the haploid chromosome number of common bean. We will use this map to study the genetic control of morpho-agronomic traits in order to improve our understanding of the genetics of this species. Furthermore, some of the markers located on this map may serve as starting points for map-based cloning.

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References

Ali M (1950) Genetics of resistance to the common bean mosaic virus (bean virus 1) in the bean (*Phaseolus vulgaris* L.). Phytopathology 40:69–79

Aramuganathan K, Earle DE (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Bassett MJ (1991) A revised linkage map of common bean. HortScience 26:834–836

Bonierbale M, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Botstein D, White RL, Skolnick M, Davis RV (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Broglie KE, Gaynore JJ, Broglie RM (1986) Ethylene-regulated gene expression: molecular cloning of the genes encoding an endochitinase from *Phaseolus vulgaris*. Proc Natl Acad Sci USA 83:6820–6824

Coe EH, Hoisington DA, Neuffer MG (1990) Linkage map of corn (maize) (*Zea mays* L.) (2n=20). In: O'Brien SJ (ed) Genetic maps. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp 639–667

Diers BW, Keim P, Fehr WR, Shoemaker RC (1992) RFLP analysis of soybean seed protein and oil content. Theor Appl Genet 83:608–612

Drijfhout E, Silbernagel MJ, Burke DW (1978) Differentiation of strains of bean common mosaic virus. Neth J Plant Pathol 84:13–26

Durante M, Cionini PG, Avanzi S, Cremonini R, D'Amato F (1977) Cytological localization of the genes for the four classes of ribosomal RNA (25S, 18S, 5.8S, and 5S) in polytene chromosomes of *Phaseolus coccineus*. Chromosoma 60:269–282

Edington BV, Lamb CJ, Dixon RA (1991) cDNA cloning and characterization of a putative 1,3-β-D-glucanase transcript induced by fungal elicitor in bean cell suspension cultures. Plant Mol Biol 16:81–94

Edwards KE, Cramer CL, Bolwell GP, Dixon RA, Schuch W, Lamb CJ (1985) Rapid transient induction of phenylalanine ammonia-lyase mRNA in elicitor-treated bean cells. Proc Natl Acad Sci USA 82:6731–6735

Feinberg AP, Vogelstein B (1984) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Fuller F, Kunstner PW, Nguyen T, Verma DPS (1983) Soybean nodulin genes: analysis of cDNA clones reveals several major tissue-specific sequences in nitrogen-fixing root nodules. Proc Natl Acad Sci USA 80:2594–2598

Garrido B, Nodari R, Debouck DG, Gepts P (1991) *Uni*-2, a dominant mutation affecting leaf development in *Phaseolus vulgaris*. J Hered 82:181–183

Gebhardt C, Ritter E, Debener T, Schachtschabel V, Wakemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78: 65–75

Gepts P (1988) Provisional linkage map of common bean. Annu Rep Bean Improv Coop 31:20–25

Gepts P, Llaca V, Nodari RO, Panella L (1992) Analysis of seed proteins, isozymes, and RFLPs for genetic and evolutionary studies in *Phaseolus*. In: Linskens HF, Jackson JF (eds) Modern methods of plant analysis (new series): seed analysis. Springer, Berlin Heidelberg New York pp 63–93

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Hoffman LM, Donaldson DD (1985) Characterization of two *Phaseolus vulgaris* phytohemagglutinin genes closely linked on the chromosome. EMBO J 4:883–889

Jorgensen RA, Cuellar RE, Thompson WP, Kavanagh TA (1987) Structure and variation in ribosomal RNA genes of pea. Plant Mol Biol 8:3–12

Kesseli RV, Paran I, Michelmore RW (1990) Genetic linkage map of lettuce (*Lactuca sativa*, 2n=18). In: O'Brien SJ (ed) Genetic maps. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, N.Y., pp 6100–6102

Koenig R, Gepts P (1989a) Segregation and linkage of genes for seed proteins, isozymes, and morphological traits in common bean (*Phaseolus vulgaris*). J Hered 80:455–459

Koenig R, Gepts P (1989b) Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of diversity. Theor Appl Genet 78:809–817

Lamprecht H (1961) Weitere Kopplungsstudien an *Phaseolus vulgaris* mit einer Übersicht über die Koppelungsgruppen. Agric Hortic Genet 19:319–332

Lamprecht H von (1934) Zur Genetik von *Phaseolus vulgaris*. VII. Zwei weitere Gene für die Sameneigenschaft, *Cor* und *Fast*. Hereditas 19:163–176

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Hubert N, Etoh T, Harada JJ, Lincoln SE (1991) A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome 34:543–552

Leakey CLA (1988) Genotypic and phenotypic variation in common bean. In: Gepts P (ed) Genetic resources of *Phaseolus* beans. Kluwer, Dordrecht, the Netherlands, pp 245–327

McCouch SR, Kochert G, Yu ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 75:815–829

Mehdy MC, Lamb CJ (1987) Chalcone isomerase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection. EMBO J 6:1527–1533

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Morales FJ, Castaño M (1987) Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. Plant Dis 71:51–53

Moreno J, Chrispeels MJ (1989) A lectin gene encodes the α-amylase inhibitor of the common bean. Proc Natl Acad Sci USA 86:7885–7889

Nguyen T, Zelechowska M, Foster V, Bergmann H, Verma DPS (1985) Primary structure of the soybean nodulin-35 gene encoding uricase II localized in the peroxisomes of uninfected cells of nodules. Proc Natl Acad Sci USA 82:5040–5044

Nodari RO, Koinange EMK, Kelly JD, Gepts P (1992) Towards an integrated linkage map of common bean. I. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theor Appl Genet 84:186–192

Osborn TC, Blake T, Gepts P, Bliss FA (1986) Bean arcelin. 2. Genetic variation, inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris*. Theor Appl Genet 71:847–855

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv Agron 46:39–90

Prakken R (1970) Inheritance of colour in *Phaseolus vulgaris* L. II. Critical review. Meded Landbouwhogesch Wageningen 72-23:1–38

Reiter RS, Williams JGK, Feldmann KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477–1481

Rigby PWJ, Dieckman M, Rhodes C, Berg O (1977) Labeling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase. J Mol Biol 113:237–251

Ryder TB, Cremer CL, Bell JN, Robbins MP, Dixon RA, Lamb CJ (1984) Elicitor rapidly induces chalcone synthase mRNA in *Phaseolus vulgaris* cells at the onset of the phytoalexin defense response. Proc Natl Acad Sci USA 81:5724–5728

Ryder TB, Hedrick SA, Bell JB, Liang X, Clouse SD, Lamb CJ (1987) Organization and differential activation of a gene family encoding the plant defense enzyme chalcone synthase in *Phaseolus vulgaris*. Mol Gen Genet 210:219–233

Schumann K, Baumann A, Nägl W (1990) Localization of phaseolin genes in the polytene chromosomes of *Phaseolus coccineus* (Leguminosae). Genetica 83:73–76

Schwartz HF, Pastor Corrales MA, Singh SP (1982) New sources of resistance to anthracnose and angular leafspot of beans (*Phaseolus vulgaris* L.). Euphytica 31:741–754

Singh SP, Gutiérrez JA, Molina A, Urrea C, Gepts P (1991a) Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. Crop Sci 31.23–29

Singh SP, Nodari R, Gepts P (1991b) Genetic diversity in cultivated common bean. I. Allozymes. Crop Sci 31:19–23

Slighton JL, Sun SM, Hall TC (1983) Complete nucleotide sequence of a French bean storage protein gene: phaseolin. Proc Natl Acad Sci USA 80:1897–1901

Song KM, Suzuki JY, Slocum MK, Williams PH, Osborn TC (1991) A linkage map of *Brassica rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci. Theor Appl Genet 82:296–304

Suiter KA, Wendel JF, Case JS (1983) LINKAGE-1, a PASCAL computer program for the detection and analysis of genetic linkage. J Hered 74:203–204

Thummler F, Verma DPS (1987) Nodulin-100 of soybean is the subunit of sucrose synthase regulated by availability of free heme in nodules. J Biol Chem 262:14730–14736

Tucker ML, Milligan SB (1991) Sequence analysis and comparison of avocado fruit and bean abscission cellulases. Plant Physiol 95:928–933

Vallejos CE, Chase CD (1991) Linkage between isozyme markers and a locus affecting seed size in *Phaseolus vulgaris* L. Theor Appl Genet 81:413–419

Walter MH, Liu J, Grand C, Lamb CJ, Hess D (1990) Bean-pathogenesis-related (PR) proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens. Mol Gen Genet 222:353–360

Weeden NF (1984) Distinguishing among white-seeded bean cultivars by means of allozyme genotypes. Euphytica 33:199–208