

C. M. Menéndez · A. E. Hall · P. Gepts

A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred, domesticated lines

Received: 16 September 1996 / Accepted: 25 April 1997

Abstract We have constructed a genetic linkage map within the cultivated gene pool of cowpea ($2n = 2x = 22$) from an F_8 recombinant inbred population (94 individuals) derived from a cross between the inbreds IT84S-2049 and 524B. These breeding lines, developed in Nigeria and California, show contrasting reactions against several pests and diseases and differ in several morphological traits. Parental lines were screened with 332 random RAPD decamers, 74 RFLP probes (bean, cowpea and mung bean genomic DNA clones), and 17 AFLP primer combinations. RAPD primers were twice as efficient as AFLP primers and RFLP probes in detecting polymorphisms in this cross. The map consists of 181 loci, comprising 133 RAPDs, 19 RFLPs, 25 AFLPs, three morphological/classical markers, and a biochemical marker (dehydrin). These markers identified 12 linkage groups spanning 972 cM with an average distance of 6.4 cM between markers. Linkage groups ranged from 3 to 257 cM in length and included from 2 to 41 markers, respectively. A gene for earliness was mapped on linkage group 2. Seed weight showed a significant association with a RAPD marker on linkage group 5. This map should facilitate the identification of markers that “tag” genes for pest and disease resistance and other traits in the cultivated gene pool of cowpea.

Key words *Vigna unguiculata* · RFLP · RAPD · AFLP · Linkage map

Communicated by G. E. Hart

C. M. Menéndez¹ · P. Gepts (✉)
Department of Agronomy and Range Science,
University of California, Davis, CA 95616-8515, USA
E-mail: plgepts@ucdavis.edu

A. E. Hall
Department of Botany and Plant Sciences,
University of California Riverside, Riverside, CA 92521, USA

Present address:

¹MP1Z, Carl-von-Luné-Weg 10, 50829, Köln, Germany

Introduction

Cowpea [*Vigna unguiculata* (L.) Walpers] is an important food legume in Sub-Saharan Africa, Brazil and India, where it is grown in semi-arid regions, usually intercropped with cereals (sorghum or millet) but also in rotation as a sole crop. Cowpea is valued for its high protein content and is consumed as dry seeds, fresh southernpeas, green pods, or leaves. The residues of the plant are used in animal feeding.

Cowpea is a member of the genus *Vigna* Savi., which belongs to the tribe Phaseoleae. This tribe also contains the common bean (*Phaseolus vulgaris*) and the mung bean (*Vigna radiata*) among other legumes of economic importance. The latest taxonomic treatment of *Vigna* (Maréchal et al. 1978) places cowpea in subg. *Vigna* sect. *Catiang*, a section endemic to Africa. Cultivated cowpea consists of three main cultigroups: cv-gr unguiculata (cowpea), cv-gr biflora (catjang) and cv-gr sesquipedalis (yard-long or asparagus bean).

A major finding in genetic diversity studies in cowpea has been the genetic bottleneck induced by domestication in spite of substantial variation in seed color, seed coat patterns, plant type, pod type and seed size among cultivated cowpeas (Panella and Gepts 1992; Vaillancourt et al. 1993; Panella et al. 1993). The total genetic diversity in cultivated cowpea reported from these studies was lower than that reported in many other crops (Doebley 1989). Since the first traits were described in cowpea (Harland 1919), many morphological and disease resistance loci have been identified (Fery 1980, 1985 for reviews). However, prior to 1993 only a few reports of genetic linkage in cowpea were found in the literature. A cowpea linkage map was developed from a cross between an improved cultivar and a putative wild progenitor type (*Vigna unguiculata* ssp. *dekindtiana*). This cowpea map consisted of 87 random genomic and five cDNA RFLPs, five RAPDs,

and two morphological loci/locus clusters arranged in ten linkage groups (Fatokun et al. 1993).

Construction of genetic maps based on wide crosses have the disadvantage of identifying loci that may be polymorphic only between more divergent genotypes but not between more closely related genotypes, especially the ones of interest. Molecular maps based on crosses involving wild progenitors have also little direct application in breeding programs that usually exploit intraspecific variation within cultivated forms. A genetic linkage map constructed from a cross within the cultivated gene pool would, therefore, be most desirable. The low level of polymorphism at the isozyme level within the cultivated cowpea revealed by the studies described previously, in addition to their low number, precludes the use of that type of marker in any cowpea mapping study. Although RFLP markers remain extremely useful, they have failed to detect enough polymorphism in intraspecific crosses of crops with low genetic diversity (e.g., Foolad et al. 1993). Alternative molecular markers showing higher level of polymorphisms among closely related genotypes include microsatellites (Akkaya et al. 1995), RAPDs (Williams et al. 1993), minisatellites (Sonnante et al. 1994) and, most recently, AFLPs (Vos et al. 1995).

The objective of the present study was the development of a genetic map of cowpea based primarily on RAPD and AFLP markers in a cross within the cultivated gene pool.

Materials and methods

Plant material

A recombinant inbred population of 158 individuals in the F₈ was developed by single-seed descent from a cross between two agronomically contrasting breeding lines (Table 1), "IT84S-2049" and "524B". IT84S-2049 is an advanced breeding line developed at IITA in Nigeria for multiple disease and pest resistance and has been shown to have resistance to several races of black-eyed cowpea mosaic virus (R. O. Hampton, personal communication 1994) and to virulent root-knot nematodes in California (Roberts et al. 1996). Line 524B is a California black-eyed type that shows resistance to *Fusarium* wilt and was developed by one of us (A.E.H.) at the

Table 1 Morphological traits scored in the parental lines and F₈ recombinant inbreds in the field

Trait	IT84S-2049	524B
Flower color ^a	White	Tinged
Pod color	Purple tips	Green tips
Petiole pigmentation	Intense red	Light red
Pod position	Drooping	Erect
Internode length	Long	Short
Nodal position of 1 st flower	10	5
Seed weight (100 seed)	13.2	27.1

^aPhenotypes scored in the F₈ included white, tinged, pale purple and dark purple

University of California, Riverside, from a cross between California cultivars CB5 and CB3, which encompasses the genetic variability available in cowpea in California.

Analysis of morphological/classical/biochemical traits

The recombinant inbred population, consisting of 158 individuals in the F₆ generation, was planted under wide-spaced field conditions in the summer of 1992. Parental lines and progeny were grown in rows of ten plants each, but only one plant per line was scored for flower color, pod color, nodal position of the first flower, and pod position for all 158 lines. Only data from a subset of 94 randomly chosen recombinant inbred lines were used in the linkage analysis. The weight of 100 seeds was also measured for the same subset of 94 lines. The segregation of the dehydrin protein in the subset of 94 lines was detected by immunoblotting as described by Ismail et al. (1997).

DNA extraction and RAPD analysis

Plants were maintained in dark conditions for 3 days prior to harvesting leaf tissue. Total DNA was extracted from approximately 5 g of frozen leaf tissue from 94 randomly chosen F₈ recombinant inbred lines following a modification of Dellaporta (1983) as described by Dr. N. Young (personal communication). Three-hundred and thirty two random 10-mer primers (Operon kits A, B, C, D, E, F, I, K, M, P, S, W, X, Y, Z, AD and primers G5, G19, H8, H15, J1, J9, J12, J20, N9, O15, T11, and U10) were used for PCR amplifications. The amplification reaction contained 15 ng of total genomic DNA; 1 × buffer (50 mM KCl; 10 mM Tris-HCl pH 9.0; 0.1% Triton X-100); 0.4 μM of primer; 25 μM of each dNTP; 2 mM of MgCl₂ and 1 Unit of *Taq* polymerase (Promega, Madison, Wis.) in a total reaction volume of 25 μl. DNA sequences were amplified using a 96-well Twinblock (Ericomp) thermal cycler with the following cycling parameters: 1 cycle at 94°C for 2 min; 3 cycles of: 1 min at 94°C, 1 min at 35°C, 2 min at 72°C; 32 cycles of: 10 s at 94°C, 30 s at 35°C, 1 s at 35°C, 1 min at 72°C; and 1 cycle of 5 min at 72°C. After amplification, 5 μl of gel loading buffer was added to each sample and the products (15 μl) were separated by electrophoresis at 100 V for 8–12 h on a 10% (30:0.8) polyacrylamide gel (16 × 20 × 0.1 cm) (Biorad, Hercules, Calif.), stained for 20 min (0.5 μl/ml of ethidium bromide), and photographed under UV light.

RFLP analysis

Genomic DNA from parental lines and 69 F₈ recombinant inbred lines (6–8 μg) was digested with eight restriction enzymes (*EcoRV*, *EcoRI*, *HindIII*, *HaeIII*, *BclI*, *BstNI*, *DraI* and *XbaI*) according to the manufacturer's instructions (GIBCO-BRL, New England Biolabs). Southern transfer was performed as described in Nodari et al. (1993).

Pre-hybridization and hybridization of filters with ³²P-labeled probes (Feinberg and Vogelstein 1983) were performed at 65 °C in a Robbins Scientific Incubator in a solution containing 7.5% SDS, 5% SSPE and 1/100 (v/v) of salmon sperm. Washes were done at increasing stringency: two washes of 15 min each in 2 × SSC, 0.1% SDS at room temperature, and a last wash at 60°C in 2 × SSC, 0.1% SDS for 30 min for heterologous probes (bean and mung bean genomic sequences), or two washes of 30 min each in 0.1 × SSC, 0.1% SDS for cowpea genomic probes. X-ray film was then exposed to blots for 2–12 days at –70°C with intensifying screens. Under these conditions membranes could be re-used up to eight times after stripping the previous probe by two washes at 42°C in 0.4 N NaOH and 0.1 × SSC, 0.5% SDS, 0.2 M Tris pH 7.5, respectively.

AFLP analysis

AFLP analysis was conducted on the same set of 69 lines as described by Vos et al. (1995) with the following modifications. The first selective pre-amplification reaction was performed with 5 μ l of template DNA using 75 ng each of the *EcoRI* + 1 primer and the *MseI* + 1 primer and 1 unit of *Taq* polymerase (Promega, Madison, Wis.) in a total volume of 50 μ l. The cycle profile was 30 cycles of 30 s at 94°C, 30 s at 60°C and 60 s at 72°C. Amplification products were diluted 1:8 in TE 0.1 (10 mM Tris-HCl, 0.1 mM EDTA) pH 8.

For the second amplification, 12.5 ng of an *EcoRI* primer with three selective nucleotides was end-labeled with [³²P]dATP and T4 polynucleotide kinase (Pharmacia). The amplification cocktail contained 2 μ l of template DNA from the diluted first-amplification product, 0.4 units of *Taq* polymerase (Promega), 0.4 mM of dNTPs, 6.25 ng of labeled *EcoRI* + 3 primer, 23.75 ng of unlabeled *EcoRI* + 3 primer, and 30 ng of *MseI* + 3 primer in a 20- μ l total reaction volume (in addition to the other components which were unchanged from Vos et al. 1995). A "touch-down" PCR was conducted in a PE-9600 (Perkin Elmer) thermocycler. After 1 cycle of 30 s at 94°C, 30 s at 65°C and 60 s at 72°C, the temperature was lowered 0.7°C in each cycle during 11 cycles and was followed by 24 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C. The reaction products were analyzed on 5.5% denaturing polyacrylamide (19:1) gels with 7 M urea in 1 \times TBE buffer. Electrophoresis was performed at a constant power, 70 W, for approximately 1.5 h on a 31 \times 38.5-cm gel apparatus (Gibco BRL, Alameda, Calif.). In order to increase the resolution of low-molecular-weight fragments, 2 \times TBE was used as a lower running buffer for some primer combinations. Gels were placed for 2 h at 80°C in a gel dryer (Biorad, Hercules, Calif.). This was followed by exposure of an X-ray film to the gel overnight.

Identification of clones and primers revealing polymorphisms and locus nomenclature

Primers revealing polymorphisms were identified as those consistently showing different banding patterns in at least two different amplification events. These primers were then used to amplify a set of eight recombinant inbreds lines of the IT84S-2049 \times 524B population. If the polymorphism was consistent with the parental phenotypes, the rest of the population was screened. Polymorphic RAPD fragments of decreasing molecular weight were alphabetically labeled with an additional lower-case letter. The 123-bp ladder (Gibco, BRL) and λ DNA cut with *EcoRI* and *HindIII* were used as the fragment size-markers.

Cloned DNA probes were hybridized to blots containing individual restriction enzyme-digests of total genomic DNA from the two parents. Probes revealing polymorphisms were identified as those detecting differences between the parents in banding patterns with at least one of the eight enzymes tested. When the same probe detected more than one locus, fragments of decreasing size were labeled alphabetically with an additional lower-case letter.

AFLP fragments were generated from 17 primer combinations: S02 (AAC), S05 (ACA), and C01 (AGT) as *Eco* + 3, and G01 (AAG), G04 (AGA), G06 (AGC), G19 (GTA), G14 (GAC) and G05 (AGG) on the *MseI* + 3 side (Vos et al. 1995). However, only primer combinations that showed three or more polymorphic fragments were used to screen the progeny. Polymorphic fragments detected with the same primer combination were labeled alphabetically with an additional lower-case letter. Molecular weights were calculated by running a [³²P]dATP-labeled sequencing ladder (Sequamar, Research Genetics) on the denaturing polyacrylamide gels.

Segregation and linkage analysis

Segregation of individual markers was analyzed by a chi-square test for goodness-of-fit to a 1:2:1 or 3:1 ratio. Linkage analysis of the

entire set of markers was performed using MAPMAKER 3.0b (Lander et al. 1987). To identify linkage groups, pairwise comparisons and grouping of markers were performed using the "Group" command 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 within each linkage group, the "Order" command was used based on three-point linkage data with the above mentioned linkage criteria, an initial window size of 5 and 3, and an exclusion threshold of 3.0 LOD score units. The markers placed by this procedure in each linkage group were labeled framework markers (in bold type in Fig. 1). Additional, non-framework markers were placed at a threshold LOD score between 2 and 3 (in normal type in Fig. 1). The orders were confirmed by permuting all adjacent markers by the "Ripple" function (window size of 5 and 3 for large and small linkage groups, respectively). Markers that could not be placed on a linkage group at a LOD threshold above 2.0 (but appear to be linked to this group based on two-point linkage data), were listed below the respective linkage group (Fig. 1). Recombination fractions were converted into map distances in centimorgans (cM) using the Kosambi (1944) mapping function.

Chi-square analyses to detect linkage relationships between molecular markers and morphological traits were performed using PROC FREQ of SAS (1988). Traits associated with markers at the $P < 0.001$ level were then analyzed with MAPMAKER as described above. A regression analysis of seed weight and nodes to first flower on all molecular markers was performed using the PROC GLM procedure in SAS (1988) and a significance level of $P < 0.001$. Duncan's tests were used to test for differences in means of genotypic marker classes.

Results

Segregation of markers

Three-hundred and thirty two random 10-base RAPD primers (Fig. 2) were screened in amplification reactions using DNA isolated from parents IT84S-2049 and 524B, and their F₈ recombinant inbred population consisting of 94 lines. Of these primers, 269 resulted in the amplification of a total of 1522 scorable fragments (average of 5.7 scorable fragments/primer). The level of polymorphism was low in our intraspecific cross. Only 12% of the fragments appeared initially to show differences between our parental lines and only 9% of the fragments were eventually mapped. The other fragments were discarded due to lack of reproducibility, lack of segregation, or difficulties in scoring the F₈ population (Table 2).

RAPD fragments ranged in size from 225 bp to 2 kb. The number of 'mappable' markers generated from a single primer ranged from 1 to 3 with an average of 1.4 polymorphic fragments/polymorphism-detecting primer. The average number of fragments revealed by polymorphism-detecting primers was slightly greater than in the general population of primers (7.7 fragments/primer). Segregation ratios that significantly departed from the expected ratio at the 5% level were observed in 19 loci (14%). Most RAPDs segregated in a dominant manner although a small proportion of them showed co-dominance (5.3%). Polymorphic loci identified by the same primer mapped, in general, in

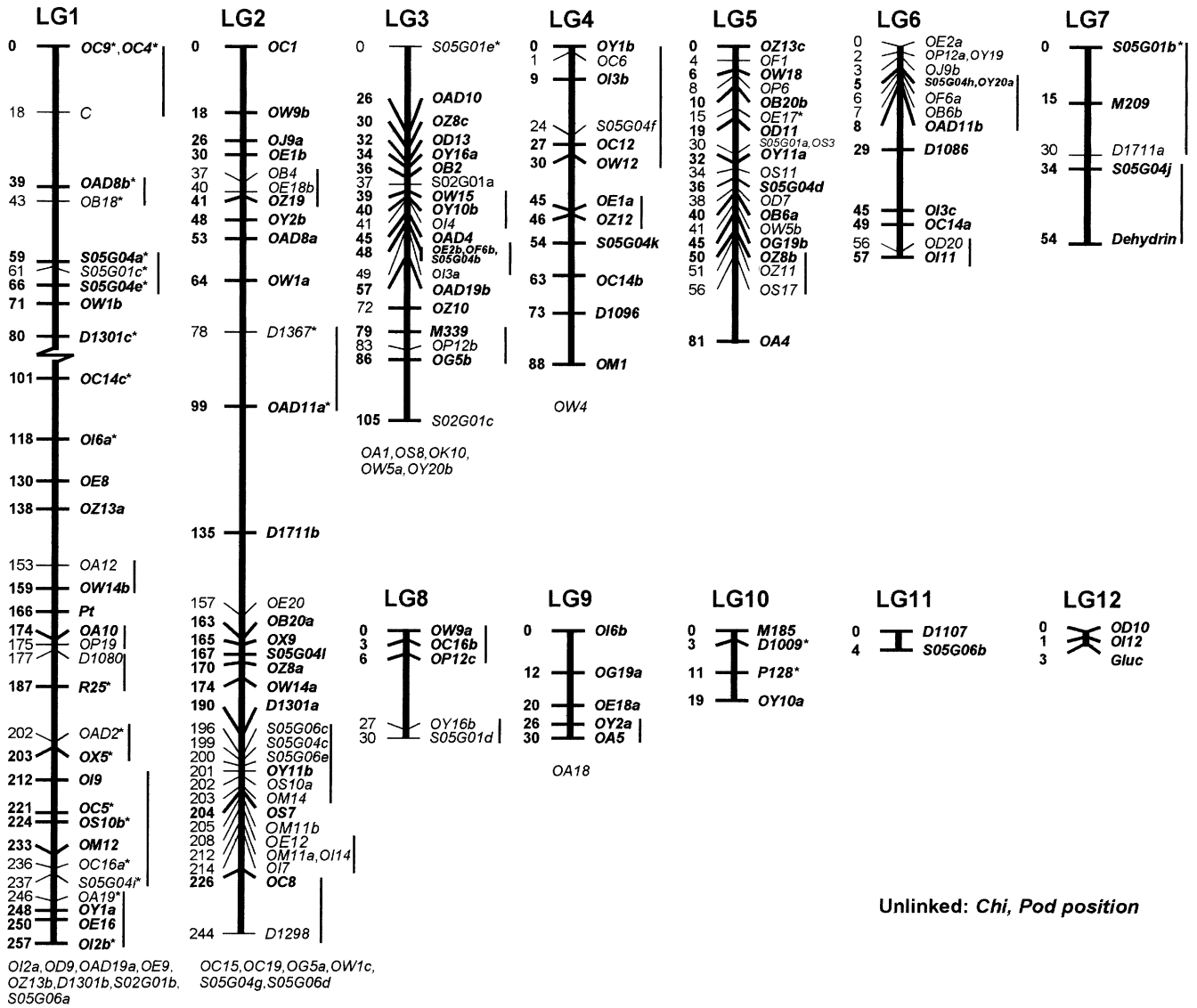


Fig. 1 Map of *V. unguiculata*. Linkage group numbers are indicated at the top. Cumulative Kosambi map distances are indicated at the left side of each marker. Vertical bars to the right of each linkage group indicate multiple orders with log-likelihood-differences less than 2.0 units. The D- and M-prefixes indicate RFLP loci detected by *P. vulgaris* and *V. radiata* genomic clones, respectively. The P-prefixes indicate RFLP loci detected by *V. unguiculata* genomic clones. The O-prefixes indicate RAPD loci detected with Operon Technologies primer. Morphological traits are Pt and Pod position. Chi represents the locus for chitinase. Markers indicated in bold represent framework markers for which a gene order with a LOD threshold value of 3.0 or above could be identified. The order of markers in normal type had a LOD value between 2.0 and 3.0. Markers listed below linkage groups appeared linked to the linkage group based on two-point linkage data, but LOD values of gene orders based on three-point data were below 2.0. "*" identify loci with distorted segregation ($P < 0.05$)

different linkage groups with four exceptions: on linkage group 1 (LG1), *OI2a* and *OI2b* and *OZ13a* and *OZ13b*; on linkage group 2: *OM11a* and *OM11b* and *OW1a* and *OW1c*.

Sixty five heterologous (47 from common bean and 18 from mung bean) and seven cowpea random genomic probes plus two additional cloned gene sequences were successfully tested on the two parental DNAs digested individually with eight restriction enzymes. Of the 92 putative loci identified by these DNA probes, 24% appeared initially to show RFLPs between the parental lines, but because of scoring difficulties only 21% were eventually mapped (Table 2). The greatest number of RFLPs were identified after digestion with *EcoRV* (Fig. 3). Of the clones that revealed polymorphism, a majority (16/20) detected a polymorphism between the parental genotypes with only one of

Fig. 2 Amplification reaction using Operon primer Z8 and parental and progeny template DNAs. The molecular-weight marker (*M*) is a 123-bp ladder and the sizes of the marker fragments (in bp) are given on the left. Parental lines correspond to the two lanes on the right: (*I*) stands for IT84S-2049 and (*B*) stands for 524B. Scored fragments are indicated by arrows on the right with their molecular weights. A negative control (without DNA) is indicated by "C-"

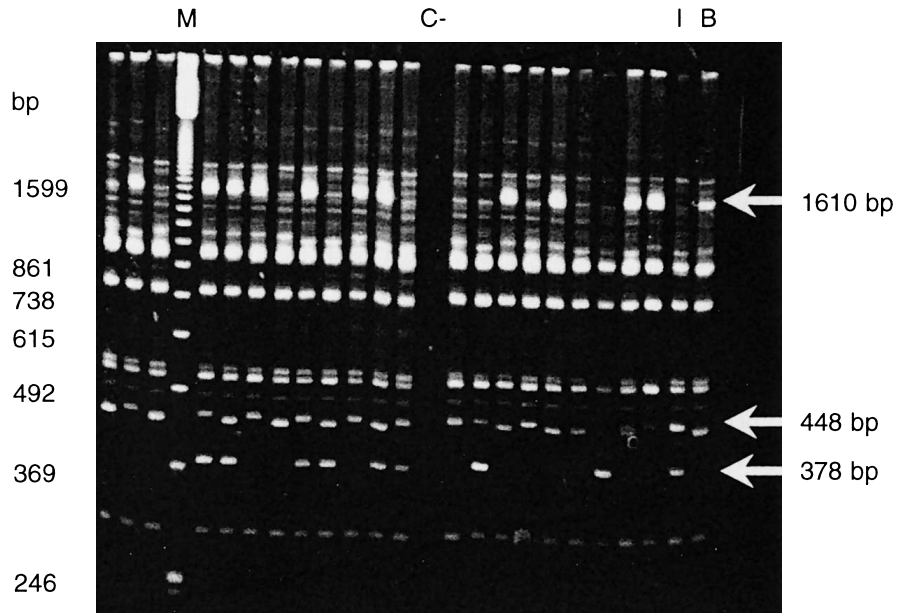


Table 2 Levels of polymorphisms detected between two inbred lines of cowpea (*V. unguiculata*) with different types of molecular markers

Type of marker	No. of probes or primers	No. of presumed loci	Proportion of polymorphic loci	Proportion mapped
RAPD	332	1522	0.12	0.09
RFLP	72	92	0.24	0.21
AFLP	17	220	0.12	0.11

the eight restriction enzymes tested. Only one probe identified polymorphisms with two restriction enzymes, and three probes detected polymorphisms with more than three enzymes. This observation suggests that point mutations at restriction sites are the primary cause of polymorphisms in cowpea and is in contrast with the situation in common bean where a majority of the RFLPs could be attributed to insertion/deletions (Nodari et al. 1992). The majority of the random genomic probes hybridized to a single fragment, as expected with single-copy clones (Nodari et al. 1993), and, with few exceptions (D1301a, D1301b, and D1711a), they segregated in a co-dominant manner.

Analysis of the fragment patterns generated with the four AFLP primer combinations (Fig. 4) gave information for 25 loci (out of a total of 220 loci assayed). The proportion of polymorphic loci was therefore similar to that for RAPDs (12%) (Table 2). AFLP fragments ranged from 65 to 600 bp in size and the number of polymorphic loci varied from 3 to 12 depending on the primer combination. The average number of fragments/primer combination was 55 with a range from 32 to 69.

Linkage analysis

Segregation analyses were conducted on 181 markers. Of these, 179 (99%) could be assigned to 12 linkage groups by two-point linkage data. Further analysis with three-point linkage data generated a framework map with 101 marker loci placed at a log-likelihood threshold of at least three units (in bold type in Fig. 1). An additional 57 marker loci were placed at log-likelihood threshold values between two and three (in normal type in Fig. 1). Based on the same three-point data, 21 marker loci could not be ordered on their respective linkage group at log-likelihood values above two, although they were attached to these linkage groups by two-point data (markers listed underneath linkage groups in Fig. 1).

Our map covers 972 cM. There are seven linkage groups of 50 cM or more, and five smaller linkage

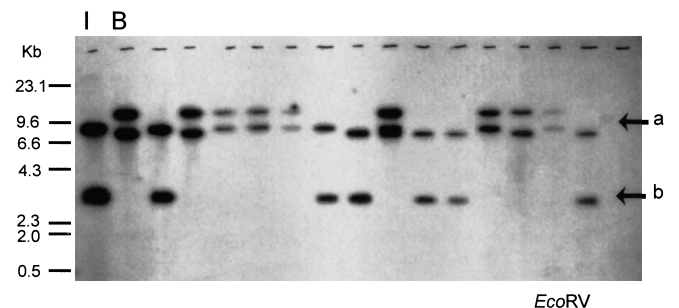


Fig. 3 Autoradiograms of Southern blots of genomic DNA, digested with *EcoRV*, of parental lines and segregating progeny of the cross of IT84S-2049 and 524B hybridized with common bean genomic clone D1711. Alleles are designated *a* and *b*

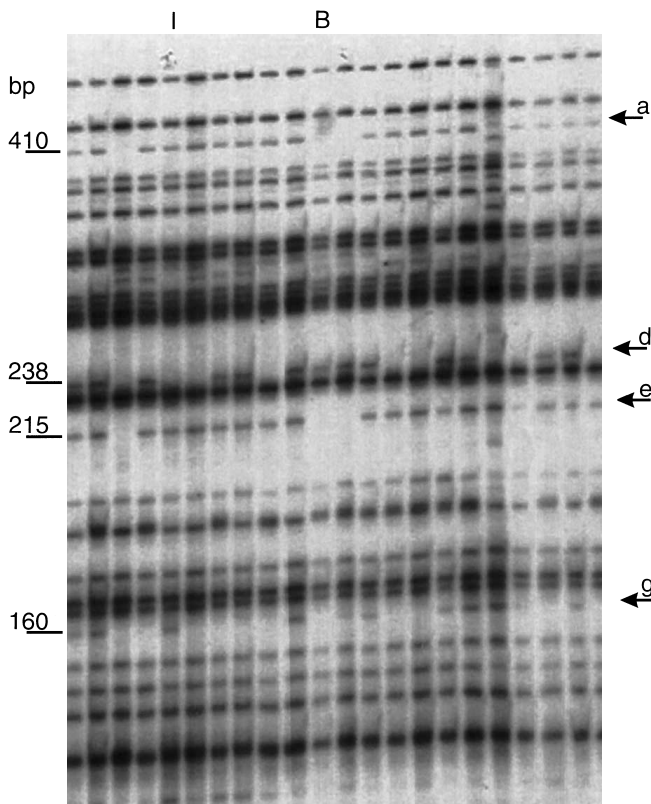


Fig. 4 AFLP fingerprint of parental lines IT84S-2049 (I) and 524B (B) and 20 F_8 RILs with primer combination S05G04 (*EcoRI* + *ACA/MseI* + *AGA*). Scored fragments are indicated by arrows on the right with their molecular weights on the left

groups ranging from 3 to 30 cM. The number of markers per linkage group ranged from 2 to 41. The longest group spans 257 cM while the shortest group's length is 3 cM. Among linkage groups the average distance between two markers varied from 2 to 14 cM. The average linkage distance between pairs of markers among all linkage groups was 6.4 cM. Approximately three quarters of the intervals were smaller than 10 cM.

The 19 RFLP loci were distributed in six linkage groups: 4 in LG1, 4 in LG2, 1 in LG3, 1 in LG4, 1 in LG6, 2 in LG7, 3 in LG10, 1 in LG11, 1 in LG12 (Fig. 1). One cloned sequence and one morphological trait, pod position, remained unassigned. This observation, together with the discrepancy between the haploid chromosome number ($n = 11$) and the current number of linkage groups (12), suggests that additional markers should be mapped to fill the gaps between linkage groups and unassigned markers.

Segregation and mapping of genes controlling morphological and biochemical traits

The presence of anthocyanin in pods is conditioned by a single gene *P* where purple pigmentation of the pods is dominant over green pods (Harland 1920). Several *P*-locus alleles have been described, among which

P_t produces a green pod with a purple tip. IT84S-2049 carries the recessive allele without pigmentation. Presence or absence of purple tips on pods segregated according to a 1:1 ratio in the F_6 generation ($\chi^2 = 2.72$, $P = 0.09$) and the *P* locus mapped on LG1 (Fig. 1). Singh and Jindla (1971) showed that erect pod attachment is dominant to drooping pod attachment and conditioned by a single gene. Fery (1980) suggested that this gene be designated *Er*. When plants are young, line 524B shows erect pods while IT84S-2049 bears drooping pods. The traditional pod-erect trait found in all members of cultigroup biflora is not present in 524B as pods tend to bend as plants mature, so we are dealing with a different locus. This locus showed distorted segregation from the expected 1:1 ratio ($\chi^2 = 6.26$, $P = 0.025$) and although it showed significant associations to markers in LG1 with the χ^2 analysis, linkage could not be confirmed using MAP-MAKER and it remains unassigned.

Flower color showed significant associations with markers on LG3 and LG1, suggesting that flower color may be conditioned by two different genes in this particular population. The presence of anthocyanin in the flowers is dependent on the presence of the general color factor *C*, which mapped on LG1 (Fig. 1). The presence and extent of development of anthocyanin are associated with seed coat color and pattern (Fery 1980). However, we did not observe the expected associations in our mapping population.

The number of nodes to first flower is an indirect measure of earliness as it is positively correlated with the number of days to first flower in cowpea for genotypes that begin flowering on the main stem (Ehlers and Hall 1996). The number of nodes to first flower was mapped on LG2 in an interval spanning 26 cM around RFLP marker locus *D1301a*, which showed the largest association with this trait (21% of the phenotypic variation observed for that trait). Seed weight is a highly heritable trait in cowpea with published heritability estimates averaging 68% (Fery 1985). Variation for seed weight (but only 9%) could be associated with RAPD marker locus *OB6a* on LG5. The consistency of the quantitative trait loci identified for both the number of nodes and seed weight needs to be investigated in further experiments.

Finally, a 35-kDa dehydrin protein was mapped at one end of LG7 (Fig. 1). This protein has been associated with chilling tolerance during the emergence of cowpea (Ismail et al. 1997). Parent 524B, a breeding line developed in temperate to subtropical climatic conditions, expresses this protein, whereas parent IT84S-2049, developed under tropical conditions, does not.

Discussion

The set of 47 bean probes were selected because they represented single-copy sequences, evenly distributed

along the *P. vulgaris* map (Nodari et al. 1993). Based on the mapping of the loci detected with these *Phaseolus* probes and the *Vigna* probes, together with the information available on the correspondence between the mungbean and common bean maps (Boutin et al. 1995), we can tentatively relate parts of the cowpea linkage map with those of the common bean map. This comparison is based only on the presence of a limited number of RFLP markers and therefore should be interpreted cautiously. The correspondence is as follows: LG1 of cowpea to D5 of common bean; LG2 to D2 and D5; LG3 to D7; LG4 to D3; and LG6 to D6. Three small linkage groups, 10, 11 and 12, correspond to common bean D4, D3 and D8 respectively.

RAPDs were relatively more efficient for mapping markers than RFLPs (Table 2). We averaged close to one polymorphism for every two RAPD primers tested (137/332) and less than one polymorphism for every four RFLP probes (16/72). AFLPs were not significantly different from RAPDs in terms of polymorphism (12%), but a more extensive AFLP study should be conducted to confirm these results. The efficiency of the AFLP screen was low (only 4 out of 17 primer combinations showed polymorphism) and similar to RFLPs, but this is clearly dependent on the primer combinations that are used because they can exhibit large differences in the level of polymorphism revealed.

The inheritance pattern of the 181 markers analyzed in the F_8 population followed the expected 1:1 ratio in 82% of the cases. A small fraction of the markers deviated significantly ($P = 0.05$) from the 1:1 ratio. The proportion of IT84S-2049 alleles was slightly higher (0.542) than that of the 524B alleles (0.458). For the 33 markers on LG1, 9 markers at one end showed an excess of 524B alleles whereas the 10 markers with distortion at the other end showed an excess of IT84S-2049 alleles. Similar proportions of distorted segregation had been found in rice (18.8%, McCouch et al. 1988), but higher proportions of distorted segregation were reported in a wild \times cultivated cowpea cross (22%, Menancio-Hautea et al. 1993) and in potato (25%, Gebhardt et al. 1989). Clustering of markers with distorted segregation had been previously reported for several crops including lettuce (Kesseli et al. 1994), common bean (Nodari et al. 1993), barley (Heun et al. 1991) and potato (Bonierbale et al. 1988).

The comparison of mapping parameters between the previous cowpea map (Fatokun et al. 1993) and the present results is conditioned by the different nature of the crosses (intra- vs inter-subspecific) and the populations used (F_2 vs F_8) which will affect mapping distances. The present cowpea map spans 972 cM vs 684 cM for the earlier map, and has an average distance of 6.4 cM instead of 7 cM. The maps differ also in the type and proportion of the different markers used. The majority of the markers mapped in the inter-subspecific cross were RFLPs (93%) and only a small proportion were RAPDs (5%). In our cross, 74% of the markers

mapped were RAPDs and this was due to the higher level of polymorphism revealed by RAPDs compared to RFLPs (only one-fourth of the RFLP probes were polymorphic versus one-half of the RAPD primers). In addition, maps developed from crosses between cultivars are most useful for breeding applications as they identify markers that are polymorphic within the cultivated gene pool, and are therefore more likely to be present in other cultivated \times cultivated crosses used by breeders. Our mapping of the *C* and *Dehydrin* loci illustrate the potential of this map for locating genes of agronomic interest. Maps based on wide crosses are also more likely to exhibit a reduction in genetic distances due to impaired pairing and recombination, and segregation distortion due to reduced viability and fertility. In addition, the quick and easy assays possible with RAPDs are a significant advantage for breeding purposes.

In conclusion, we have developed the first map for cowpea within the cultivated gene pool. The development of this map was facilitated by the adaptation of the RAPD technology to cowpea with the use of sensitive detection methods (use of polyacrylamide gels). An extensive AFLP analysis could be used to saturate the current map given the large number of loci screened per reaction. However, a prior selection of polymorphic primer combinations would be necessary to increase efficiency. This low-density map can be used to map several disease- and pest-resistance traits and other phenotypic traits segregating in this population.

Acknowledgements Financial support to C.M.M. from the Ministerio de Educación y Ciencia of Spain and the Fulbright Commission is greatly acknowledged. This research was funded by US AID Bean/Cowpea CRSP Grant DAN-1310-G-88-6008-00 to A.E.H. and P.G. We thank Richard Michelmore for his advice and support in the AFLP work, Nevin Young for providing the cowpea and mungbean genomic clones, and A. M. Ismail, M. Venegas and T. J. Close for providing the immunoblot data for the dehydrin protein. Victor Llaca, Viviana Becerra and Dean Lavelle provided helpful suggestions on marker techniques, and E. J. Rock assisted with the gels.

References

- Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB (1995) Integration of simple sequence repeat DNA markers into a soybean linkage map. *Crop Sci* 35: 1439–1445
- Bonierbale M, Plaisted R, 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
- Boutin SR, Young ND, Olson TC, Yu ZH, Shoemaker RC, Vallejos CE (1995) Genome conservation among three legume genera detected with DNA markers. *Genome* 38: 928–937
- Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA miniprep: version II. *Plant Mol Biol Rep* 1: 19–21
- Doebley J (1989) Isozymic evidence and the evolution of crop plants. In: Soltis DE, Soltis PS (eds). *Isozymes in plant biology*. Dioscorides Press, Portland, Oregon, pp 165–191
- Ehlers JD, Hall AE (1996) Genotypic classification of cowpea based on reproductive responses to heat and photoperiod. *Crop Sci* 36: 673–679

- Fatokun CA, Danesh D, Menancio-Hautea DI, Young ND (1983) A linkage map for cowpea [*Vigna unguiculata* (L.) Walp.] based on DNA markers ($2n = 22$). In: O'Brien JS (ed) Genetic maps 1992. A compilation of linkage and restriction maps of genetically studied organisms. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 6256–6258
- Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132:9–13
- Fery RL (1980) Genetics of *Vigna*. *Hort Rev* 2:311–394
- Fery RL (1985) The genetics of cowpeas: a review of the world literature. In: Singh SR, Rachie KO (eds) Cowpea research, production and utilization. John Wiley and Sons, New York, pp 25–62
- Foolad MR, Jones RA, Rodriguez RL (1993) RAPD markers for constructing intraspecific tomato genetic maps. *Plant Cell Rep* 12:293–297
- Gebhardt C, Ritter E, Debbener T, Schachtschabe V, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. *Theor Appl Genet* 78:65–75
- Harland SC (1919) Inheritance of certain characters in the cowpea (*Vigna sinensis*). *J Genet* 8:101–132
- Harland SC (1920) Inheritance of certain characters in the cowpea (*Vigna sinensis*). *J Genet* 10:193–205
- 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
- Ismail AM, Hall AE, Close TJ (1997) Chilling tolerance during emergence of cowpea associated with a dehydrin and slow electrolyte leakage. *Crop Sci* 37:1270–1277
- Kesseli RV, Paran I, Michelmore RW (1994) Analysis of a detailed genetic linkage map of *Lactuca sativa* (Lettuce) constructed from RFLP and RAPD markers. *Genetics* 16:1435–1446
- Kosambi DD (1944) The estimation of map distances from recombination values. *Ann Eugenics* 12:172–175
- 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
- Maréchal R, Macherpa JM, Stainier F (1978) Etude taxonomique d'un groupe complexe d'espèces de genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base des données morphologiques et polliniques, traitées par l'analyse informatique. *Boissiera* (Conservatoire et jardin botanique, Geneva, Switzerland) 28:1–273
- McCouch SR, Kochert G, Yu ZY, Kush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. *Theor Appl Genet* 75:815–829
- Menancio-Hautea D, Fatokun CA, Kumar L, Danesh D, Young ND (1993) Comparative genome analysis of mung bean (*Vigna radiata* L. Wilczek) and cowpea (*Vigna unguiculata* L. Walpers) using RFLP mapping data. *Theor Appl Genet* 86:797–810
- 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
- Nodari RO, Tsai SM, Gilbertson RL, Gepts P (1983) Towards an integrated linkage map of common bean. II. Development of an RFLP-based linkage map. *Theor Appl Genet* 85:513–520
- Panella L, Gepts P (1992) Genetic relationships with *Vigna unguiculata* (L.) Walp. based on isozyme analyses. *Genet Res Crop Evol* 39:71–88
- Panella L, Kami J, Gepts P (1993) Vignin diversity in wild and cultivated taxa of *Vigna unguiculata* (L.) Walp. (Fabaceae). *Econ Bot* 47:371–386
- Roberts PA, Matthews WC, Ehlers JD (1996) New resistance to virulent root-knot nematodes linked to the *Rk* locus of cowpea (*Vigna unguiculata*). *Crop Sci* 36:889–894
- SAS (1988) SAS Users Guide: statistics. SAS Institute, Cary, NC.
- Singh KB, Jindia LN (1971) Inheritance of bud and pod color, pod attachment, and growth habit in cowpeas. *Crop Sci* 11:928–929
- Sonnante G, Stockton T, Nodari RO, Becerra Velásquez VI, Gepts P (1994) Evolution of genetic diversity during the domestication of common-bean (*Phaseolus vulgaris* L.). *Theor Appl Genet* 89:629–635
- Vaillancourt RE, Weeden NF, Barnard J (1993) Isozyme diversity in the cowpea species complex. *Crop Sci* 33:606–613
- Vos R, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 23:4407–4414
- Williams JG, Hanafey MK, Rafalski JA, Tingey SV (1993) Genetic analysis using random amplified polymorphic DNA markers. *Methods Enzymol* 218:704–740