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Construction of Molecular Genetic Linkage Map Using RAPD Markes in Cowpea

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ABSTRACT: Molecular markers have become fundamental tools for crop genome study. The objective of this study was to construct a genetic linkage map for cowpea with PCR-based molecular markers. Five hundred and twenty random RAPD primers were screened for parental polymorphism. Ninety RAPD markers from sixty primers was segregated in 75 F2 mapping population derived from the cross of local cultivars GSC01 and GSC02. 70 RAPD markers were found to be genetically linked and formed 11 linkage groups. Linkage map spanned 474.1 cM across all 11 linkage groups. There are six linkage groups of 40 cM or more, and five smaller linkage groups range from 4.9 to 24.8 cM. The average linkage distance between pairs of markers among all linkage groups was 6.87 cM. The number of markers per linkage group ranged from 2 to 32. The longest group 1 spans 190.6 cM, while the length of shortest group 11 is 4.9 cM. This map is further needed to be saturated with the various markers such as RFLP, AFLP, SSR and more various populations and primers. In addition, morphological markers and biochemical markers should be united to construct a comprehensive linkage map.

Keywords **:** cowpea, linkage map, genetic marker, RAPD

Cowpea [*Vigna unguiculata* (L) Walpers] is an important food legume. Cowpea is valuable for its high protein content and is consumed as dry seeds, fresh southernpeas, green pods, or leaves. Cowpea is a member of the genus *Vigna* Savi., which belongs to the tribe Phaseoleae. This tribe also contains the common bean (*Phaselous vlgaris*) and the mungbean (*Vigna radiata*) among other legumes of economic importance.

Molecular markers have become fundamental tools for research involving plant genomes. A well saturated genomic map is a necessity for a breeding program based on marker assisted selection. Menancio-Hautea *et al.* (1993) published cowpea linkage map from F_2 population derived from the mating of cultivar and wild type. The cowpea map consisted of 87 random genomic and 5 cDNA RFLPs, 5 RAPDs, and 2 morphological loci arranged in 10 linkage groups. Ten linkage groups spanned 684 cM of the cowpea genome. The average distance between adjacent markers was 7 cM. Menendez *et al.* (1997) constructed a genetic linkage map from 94 RILs derived from a cross between the two inbreds. The map consisted of 181 loci, comprising 133 RAPDs, 19 RFLPs, 25 AFLPs, 3 morphological/classical markers, and a biochemical markers. These markers identified 12 linkage group spanning 972 cM with an average distance of 6.4 cM between markers. Fatokun *et al.* (1992) identified ten RFLP loci that were linked to QTL for seed weight in cowpea. RFLP and AFLP markers was mainly used to construct molecular genetic linkage map in cowpea. The objective of the present study was to develop genetic linkage map of cowpea based primarily on RAPD markers in a cross within the cultivated gene pool.

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MATERIALS AND METHODS

This study was conducted with a population derived from a mating between cultivar GSC01 (female parent) and cultivar GSC02 (male parent). GSC01 has a short pod length (average 15 cm), whereas GSC02 has a long pod length (average 27 cm). F_1 hybrids were made in the greenhouse and F_2 seed were planted in the field on May 24, 2000. Genomic DNA was isolated from 75 individual F_2 young leaf by means of a modified CTAB method.

For the analysis of random amplified polymorphic DNA (RAPD) markers, 520 10-mer oligonucleotide primers were obtained from Operon Technologies, INC. (Alameda, Calif.). The PCR protocol followed that described by Williams *et al.* (1990), except for minor modifications in the thermocycler temperatures and times. The PCR reaction was performed in a MJ Research PTC-200 Thermocycler. The thermal profile consisted of 2 cycles of 1 minute at 92°C, 22 second at 42°C, and 70 second at 72°C, followed by 39 cycles of 16 second at 92°C, 22 second at 42°C, and 70 second at 72°C, before ending with 1 cycle of 5 minutes at 72°C. Amplification products were electrophoresed in 1.2% TBE agarose gels and were stained with EtBr to reveal DNA segments of varying sizes. Gels were photographed under transmitted UV light.

Primers that showed parental polymorphism were used in

F2 progeny test. A linkage map of RAPD markers was constructed using the computer program MAPMAKER v. 3.0 (Lander *et al.*, 1987) from the marker data obtained from 75 F_2 progenies. Markers were assigned to group using the "Group" command, with a LOD score of 3.0 and maximum recombination distance of 50 cM. Once markers were assigned to a given linkage group, the most linkage marker order within the group was determined using the "Compare" command. Marker orders within each linkage group were ascertained by use of "Ripple" command. Map distance (cM) were computed using the Kosambi (Kosambi, 1944) mapping function .

RESULTS AND DISCUSSION

Five hundred and twenty random RAPD primers were screened for parental polymorphism. Of these primers, sixty six primers showed polymorphism. The frequency of primer showed parental polymorphism was 12.7%. Ninety RAPD markers from sixty six primers were segregated in 75 F_2 mapping population. Example of the amplification products obtained with the Operon primer OPC13 is shown in Fig. 1.

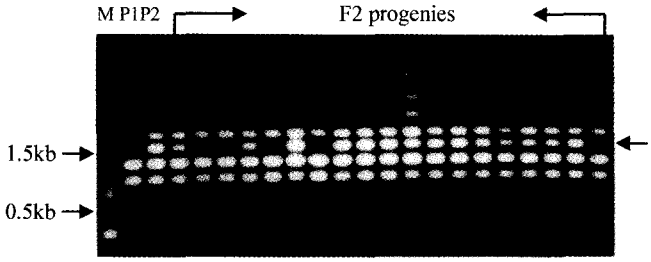

Fig. 1. DNA amplification patterns obtained with RAPD primers OPC13. M is molecular weight, P1 and P2 is parents. The polymorphic DNA fragments are indicated by arrow.

Most of the polymorphic markers were inherited in a Mendelian fashion. However, forty of the markers displayed segregation distortion at the 5% probability level. A genetic map was constructed from the 90 segregation markers. Of

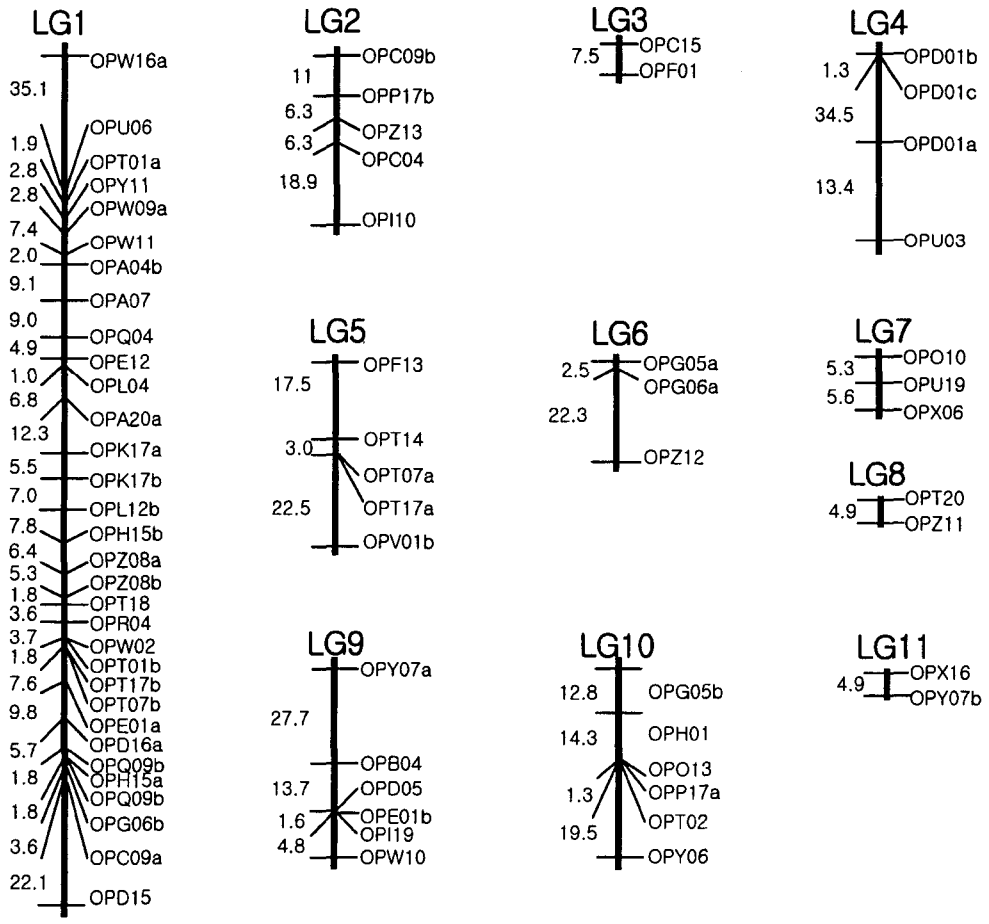

Fig. 2. Genetic linkage map of cowpea based upon a linkage analysis of 70 RAPD markers that segregated in a F2 population. Linkage maps of RAPD markers were constructed using MAPMAKER (LOD 3.0, 50 cM) Marker loci names are on the right and Kosambi map distances are on the left of the linkage groups which are numbered from 1 to 11.

Table 1. Linkage group number, number of loci, total distance and average distance among 70 RAPD loci.

Linkage group	Number of markers	Total distance (cM)	Average distance between loci (cM)
1	32	190.6	6.15
2	5	42.5	10.63
3	2	7.5	-
4	4	49.3	16.43
5	5	43.0	10.75
6	3	24.8	12.40
7	3	10.9	5.45
8	2	4.9	-
9	6	47.9	9.58
10	6	47.8	9.56
11	2	4.9	-
Total	70	474.1	6.87

the 90 markers, 70 RAPD markers were found to be genetically linked and formed 11 linkage groups, with 20 markers unlinked (Fig. 2).

Linkage map spanned 474.1 cM across all 11 linkage groups. There are six linkage groups of 40 cM or more, and five smaller linkage groups range from 4.9 to 24.8 cM. The average linkage distance between pairs of markers among all linkage groups was 6.87 cM. The number of markers per linkage group ranged from 2 to 32. The longest group 1 spans 190.6 cM while the length of shortest group 11 is 4.9 cM. Approximately three quarters of each distances of intervals were smaller than 10 cM (Table 1).

In the whole molecular map, the pattern of linkage group is strikingly similar to result of Menendez *et al.* (1997). In the result of Menendezet *et al.* (1997), linkage group 1 is also longer than the rest 11 linkage groups even though the total number of linkage group is twelve. In the whole linkage group, the result of linkage group 1 included many markers agrees to our linkage map. The rest linkage groups strikingly shorten also agree to our linkage map. We can estimate that many genes is located in a specific chromosome as compare to both results. However, each linkage group number identified in this result was not same to the linkage group number published from Menendez *et al.* (1997).

Maps developed from crosses between cultivars are most useful for breeding applications. In addition the quick and easy assays possible with RAPDs are a significant advantage for breeding purposes. The pattern of genome is similar to mungbean, pea, common bean (Menancio-Hautea *et al.* 1993). Therefore a comparative map should construct to get more information. This map is needed to be saturated with the various markers such as RFLP, AFLP, SSR and more various populations and primers to construct detailed map. In addition, morphological markers and biochemical markers should unite to get an available map.

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