KJPR Korean J.Plant.Res. 10(3) 241~246(1997)

Construction of Linkage Map Using RAPD and SSR Markers in Soybean (*Glycine max*)

Jongil Chung[1] and James E. Specht[2]
[1]Dept. of Agronomy, Gyeongsang Natl. Univ., Chinju 660-701, Korea
[2]Dept. of Agronomy, Univ. of Nebraska-Lincoln, Lincoln, NE 68583-0915

ABSTRACT

Linkage maps based on molecular markers are valuable tools in plant breeding and genetic studies. A population of 76 RI lines from the mating of A3733 and PI437.088 was evaluated with Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) markers to create soybean molecular linkage map. 302 RAPD and 21 SSR markers were genetically linked and formed forty linkage groups. These linkage groups spanned a genetic distance of 1,775 cM. The average distance between markers was 5.5 cM.

Key word: soybean, linkage, RAPD, SSR, marker.

INTRODUCTION

Molecular markers have become fundamental tools for research involving plant genomes. This includes fingerprinting varieties, establishing phylogenies, tagging desirable genes (to assist their introgression into new varieties), determining similarities among inbreds (to maximize heterosis in hybrids), and linkage mapping.

Genetic maps are ordinarily constructed by observing the segregation patterns of genes in the progeny or offspring derived from crossing two inbred parental organisms with contrasting alleles at many loci. Genetic maps based upon DNA polymorphism is a powerful tool for the study of qualitative and quantitative traits and ultimately can be used to facilitate the cloning of genes of interest. Linkage mapping of the soybean genome has developed rather slowly, in comparison to the mapping progress that has been achieved in corn (Helentjaris, 1987) and tomato (Bernatzky and Tanksley, 1986) due to the inherent difficulty and tediousness an associated with hybridization, and a lack of cytogenetic markers. Keim et al. (1990a) and Diers et al. (1992a) constructed a genetic map for soybean using RFLP markers. Shoemaker and Specht (1995) created a linkage map comprised of 13 classical and seven isozyme loci along with 110 RFLP and eight RAPD markers in a soybean mapping population derived from a mating between near-isogenic lines of the cultivars 'Clark' and 'Harosoy' . So far, only a few RAPD markers were used in soybean linkage study. This study was conducted to create soybean linkage map using many RAPD markers.

MATERIALS AND METHODS

Materials and DNA extraction

The study was conducted with a 76 RI lines derived from a mating between the cultivar A3733 and plant introduction PI437.088. Total genomic DNA was isolated from the 76 RI lines that emerged in the field. Young leaves were collected from the each 76 RI lines and parent plant. DNA was isolated from finely ground leaf tissue by means of a modified CTAB procedure (Saghai Maroof et al., 1984).

RAPD and SSR marker assay

For the analysis of random amplified polymorphic DNA (RAPD) markers, one-thousand 10-mer oligonucleotide

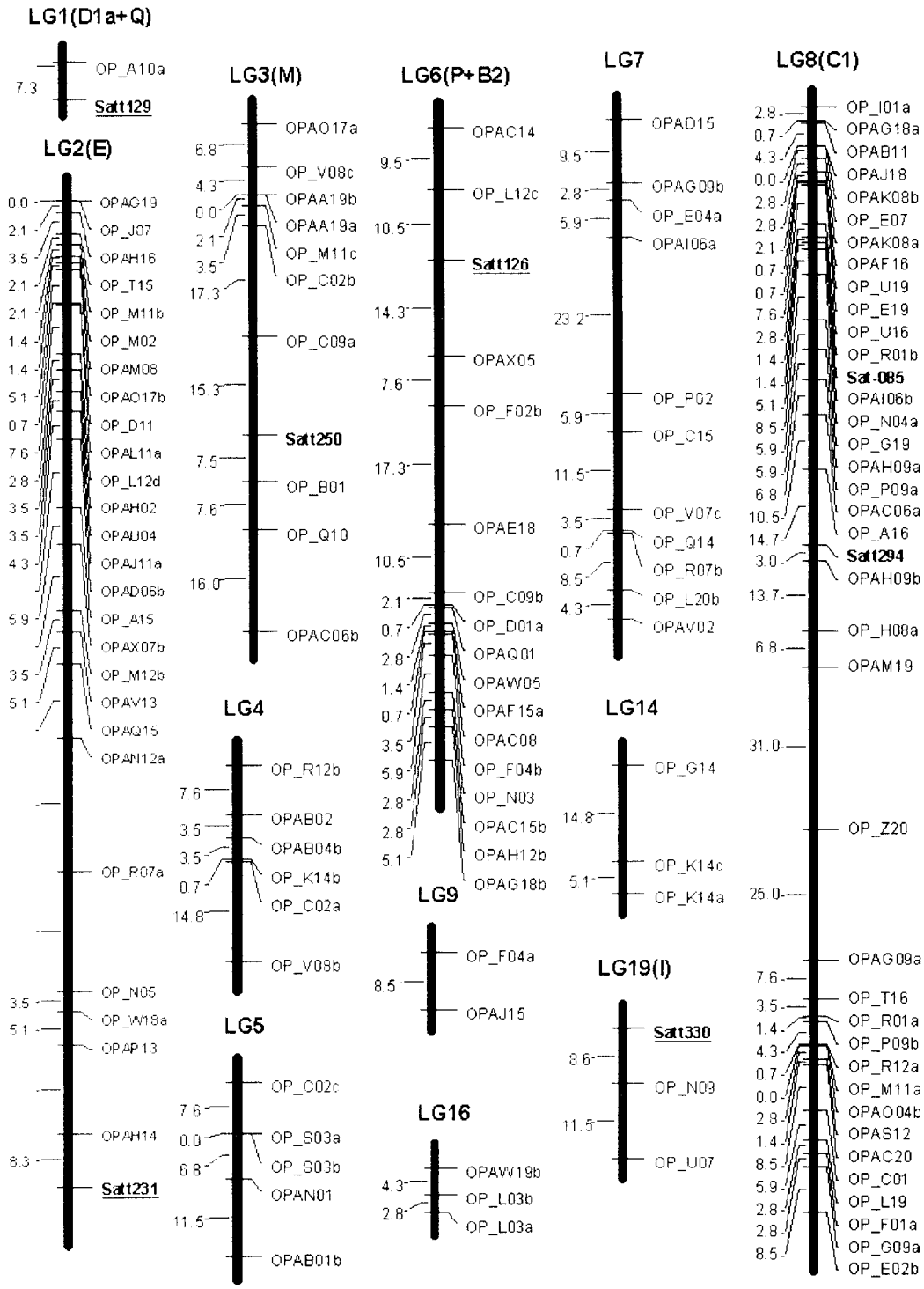
primers were obtained from Operon Technologies, INC. (Alameda, Calif.). The 1,000 primers consisted of 50 sets of 20 primers (i.e., Operon kits A01-20 to Z01-20, and AA01-20 to AX01-20). The RAPD-PCR protocol followed that described by Williams et al. (1990), except for minor modifications in the thermocycler temperatures and times that were needed to optimize the amplification of soybean DNA. The PCR reaction was performed in a MJ Research PTC - 100 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 38 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. For the analysis of simple sequence repeat (SSR) markers, one hundred twenty 20-primer pairs were obtained from Perry B. Cregan (USDA-ARS, Soybean and Alfalfa Research Lab., Beltsville, MD). The SSR-PCR protocol followed that described by Akkaya et al. (1995), except that after the final cycle the reaction mixture was cooled to 4° C and maintained at this temperature until gel electrophoresis. Amplification products were electrophoresed in 1.2 % TBE agarose gels for RAPD markers, or in 2.5% TBE superfine resolution agarose gels for SSR markers. The gels were stained with ethidium bromide to reveal DNA segments (amplicons) of varying sizes (i.e., number of base pairs) that had been amplified by the PCR. Gels were photographed under transmitted UV light. RAPD and SSR primers were first tested on the two parental DNAs; those primers producing parental polymorphisms were then tested on the 76 RI lines. For RAPD marker nomenclature, the locus was given the kit letter, followed by the number within that kit. When more than one segregating amplicon was identified for a given RAPD primer, a small case letter (a, b, or c) was suffixed to the kit number to indicate the multi-locus nature of that primer. For SSR markers, the locus name consists of the first letter of the names of di- or tri- bases in the nucleotide repeat, followed by a numerical designation.

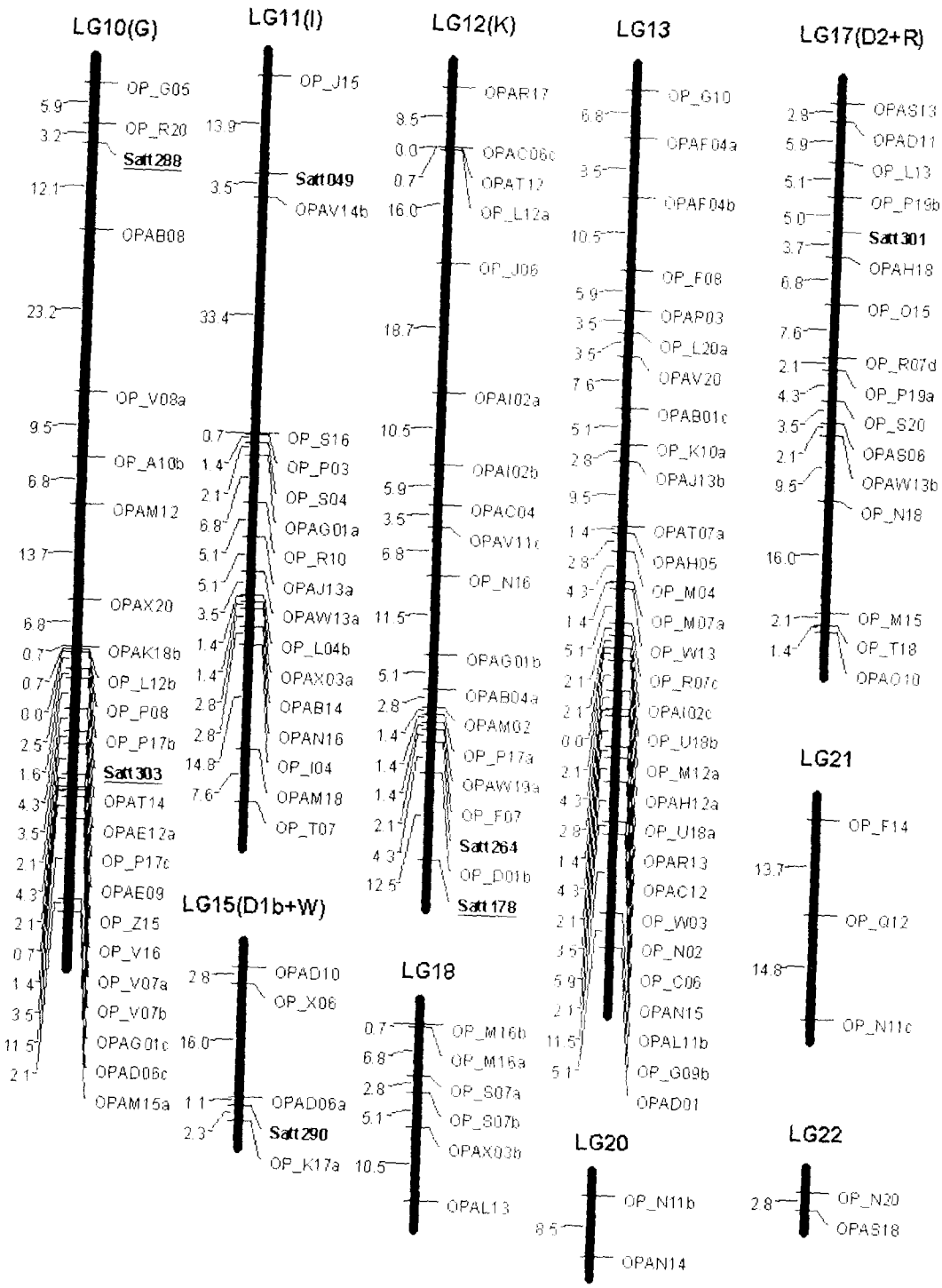
Construction of linkage map

A linkage map of RAPD and SSR markers was constructed by applying the computer program MAPMAKER v. 3.0 (Lander et al., 1987) to the marker data obtained from 76 RI lines. Markers were assigned to groups using the "Group" command, with a LOD (logarithm of the odds ratio) score of 4.0 and maximum recombination distance of 50 cM. Once markers were assigned to a given linkage group, the most likely marker order within the group was determined using the "Compare" command. Marker orders within each linkage group were ascertained by use of Mapmaker' s ripple commands. Map distances (centiMorgans) were computed using the Kosambi mapping function.

RESULTS AND DISCUSSION

Of the 1000 RAPD primers tested on the two parents, 310 RAPD markers were identified. Of the 120 SSR primers tested, 24 revealed a parental polymorphism and produced a scorable 1:1 segregation pattern in the progeny. For many of the SSR primers, the size differences between the two parental amplicons was too small to be resolved on an agarose gel system. Only 302 of the 310 segregating RAPD markers and 21 of the 24 segregating SSR markers were found to be genetically linked. These markers coalesced into 40 linkage groups (Figure 1). The linkage map spanned 1,775 cM, with 323 molecular markers separated by an average distance of about 5.5 cM in Kosambi map units. Because SSRs are single locus markers, they can be used to identify which linkage groups are homologous with those of the public soybean map published by Shoemaker and Specht (1995). In the soybean, several studies over the last 7 years have utilized RFLP markers to construct linkage map. Keim et al. (1990) reported twenty-six genetic linkage groups in a map that spanned ca. 1200 cM and contained 150 RFLP markers. Diers et al. (1992) updated the foregoing map to 31 linkage groups that spanned 2147 cM and contained 252 RFLP markers. The greater marker density decreased the average distance between adjacent marker loci to 8.5 cM. Using the same mapping population, Shoemaker and Olson (1993) significantly updated the map to the extent if now contains about 365 RFLP markers, 11 RAPD markers,


LG1(D1a+Q)
7.3
OP_A10a
Satt129
LG2(E)
0.0
OPAG19
2.1
OP_J07
3.5
OPAH16
2.1
OP_T15
2.1
OP_M11b
1.4
OP_M02
1.4
OPAM08
5.1
OPAO17b
0.7
OP_D11
7.6
OPAL11a
2.8
OP_L12d
3.5
OPAH02
3.5
OPAU04
4.3
OPAJ11a
OPAD06b
5.9
OP_A15
OPAX07b
3.5
OP_M12b
5.1
OPAV13
OPAQ15
OPAN12a
OP_R07a
3.5
OP_N05
5.1
OP_W18a
OPAP13
OPAH14
8.3
Satt231
LG3(M)
6.8
OPAO17a
4.3
OP_V08c
0.0
OPAA19b
2.1
OPAA19a
3.5
OP_M11c
17.3
OP_C02b
OP_C09a
15.3
Satt250
7.5
OP_B01
7.6
OP_Q10
16.0
OPAC06b
LG4
7.6
OP_R12b
OPAB02
3.5
OPAB04b
3.5
OP_K14b
0.7
OP_C02a
14.8
OP_V08b
LG5
7.6
OP_C02c
0.0
OP_S03a
6.8
OP_S03b
OPAN01
11.5
OPAB01b
LG6(P+B2)
9.5
OPAC14
OP_L12c
10.5
Satt126
14.3
OPAX05
7.6
OP_F02b
17.3
OPAE18
10.5
2.1
OP_C09b
0.7
OP_D01a
2.8
OPAQ01
1.4
OPAW05
0.7
OPAF15a
3.5
OPAC08
5.9
OP_F04b
2.8
OP_N03
2.8
OPAC15b
5.1
OPAH12b
OPAG18b
LG9
OP_F04a
8.5
OPAJ15
LG16
OPAW19b
4.3
OP_L03b
2.8
OP_L03a
LG7
OPAD15
9.5
OPAG09b
2.8
OP_E04a
5.9
OPAI06a
23.2
OP_P02
5.9
OP_C15
11.5
OP_V07c
3.5
OP_Q14
0.7
OP_R07b
8.5
OP_L20b
4.3
OPAV02
LG14
OP_G14
14.8
OP_K14c
5.1
OP_K14a
LG19(I)
Satt330
8.6
OP_N09
11.5
OP_U07
LG8(C1)
2.8
OP_I01a
0.7
OPAG18a
4.3
OPAB11
0.0
OPAJ18
2.8
OPAK08b
2.8
OP_E07
2.1
OPAK08a
0.7
OPAF16
0.7
OP_U19
7.6
OP_E19
2.8
OP_U16
1.4
OP_R01b
1.4
Sat-085
5.1
OPAI06b
8.5
OP_N04a
5.9
OP_G19
5.9
OPAH09a
6.8
OP_P09a
10.5
OPAC06a
14.7
OP_A16
3.0
Satt294
OPAH09b
13.7
OP_H08a
6.8
OPAM19
31.0
OP_Z20
25.0
OPAG09a
7.6
OP_T16
3.5
OP_R01a
1.4
OP_P09b
4.3
OP_R12a
0.7
OP_M11a
0.0
OPAO04b
2.8
OPAS12
1.4
OPAC20
8.5
OP_C01
5.9
OP_L19
2.8
OP_F01a
2.8
OP_G09a
8.5
OP_E02b


LG10(G)
OP_G05
5.9
OP_R20
3.2
Satt288
12.1
OPAB08
23.2
OP_V08a
9.5
OP_A10b
6.8
OPAM12
13.7
OPAX20
6.8
OPAK18b
0.7
OP_L12b
0.7
OP_P08
0.0
OP_P17b
2.5
Satt303
1.6
OPAT14
4.3
OPAE12a
3.5
OP_P17c
2.1
OPAE09
4.3
OP_Z15
2.1
OP_V16
0.7
OP_V07a
1.4
OP_V07b
3.5
OPAG01c
11.5
OPAD06c
2.1
OPAM15a
LG11(I)
OP_J15
13.9
Satt049
3.5
OPAV14b
33.4
OP_S16
0.7
OP_P03
1.4
OP_S04
2.1
OPAG01a
6.8
OP_R10
5.1
OPAJ13a
5.1
OPAW13a
3.5
OP_L04b
1.4
OPAX03a
1.4
OPAB14
2.8
OPAN16
2.8
OP_I04
14.8
OPAM18
7.6
OP_T07
LG12(K)
OPAR17
8.5
OPAC06c
0.0
OPAT12
0.7
OP_L12a
16.0
OP_J06
18.7
OPAI02a
10.5
OPAI02b
5.9
OPAC04
3.5
OPAV11c
6.8
OP_N16
11.5
OPAG01b
5.1
OPAB04a
2.8
OPAM02
1.4
OP_P17a
1.4
OPAW19a
1.4
OP_F07
2.1
Satt264
4.3
OP_D01b
12.5
Satt178
LG13
OP_G10
6.8
OPAF04a
3.5
OPAF04b
10.5
OP_F08
5.9
OPAP03
3.5
OP_L20a
3.5
OPAV20
7.6
OPAB01c
5.1
OP_K10a
2.8
OPAJ13b
9.5
OPAT07a
1.4
OPAH05
2.8
OP_M04
4.3
OP_M07a
1.4
OP_W13
5.1
OP_R07c
2.1
OPAI02c
2.1
OP_U18b
0.0
OP_M12a
2.1
OPAH12a
4.3
OP_U18a
2.8
OPAR13
1.4
OPAC12
4.3
OP_W03
2.1
OP_N02
3.5
OP_C06
5.9
OPAN15
2.1
OPAL11b
11.5
OP_G09b
5.1
OPAD01
LG17(D2+R)
OPAS13
2.8
OPAD11
5.9
OP_L13
5.1
OP_P19b
5.0
Satt301
3.7
OPAH18
6.8
OP_O15
7.6
OP_R07d
2.1
OP_P19a
4.3
OP_S20
3.5
OPAS06
2.1
OPAW13b
9.5
OP_N18
16.0
OP_M15
2.1
OP_T18
1.4
OPAQ10
LG21
OP_F14
13.7
OP_Q12
14.8
OP_N11c
LG15(D1b+W)
OPAD10
2.8
OP_X06
16.0
OPAD06a
1.1
Satt290
2.3
OP_K17a
LG18
OP_M16b
0.7
OP_M16a
6.8
OP_S07a
2.8
OP_S07b
5.1
OPAX03b
10.5
OPAL13
LG20
OP_N11b
8.5
OPAN14
LG22
OP_N20
2.8
OPAS18

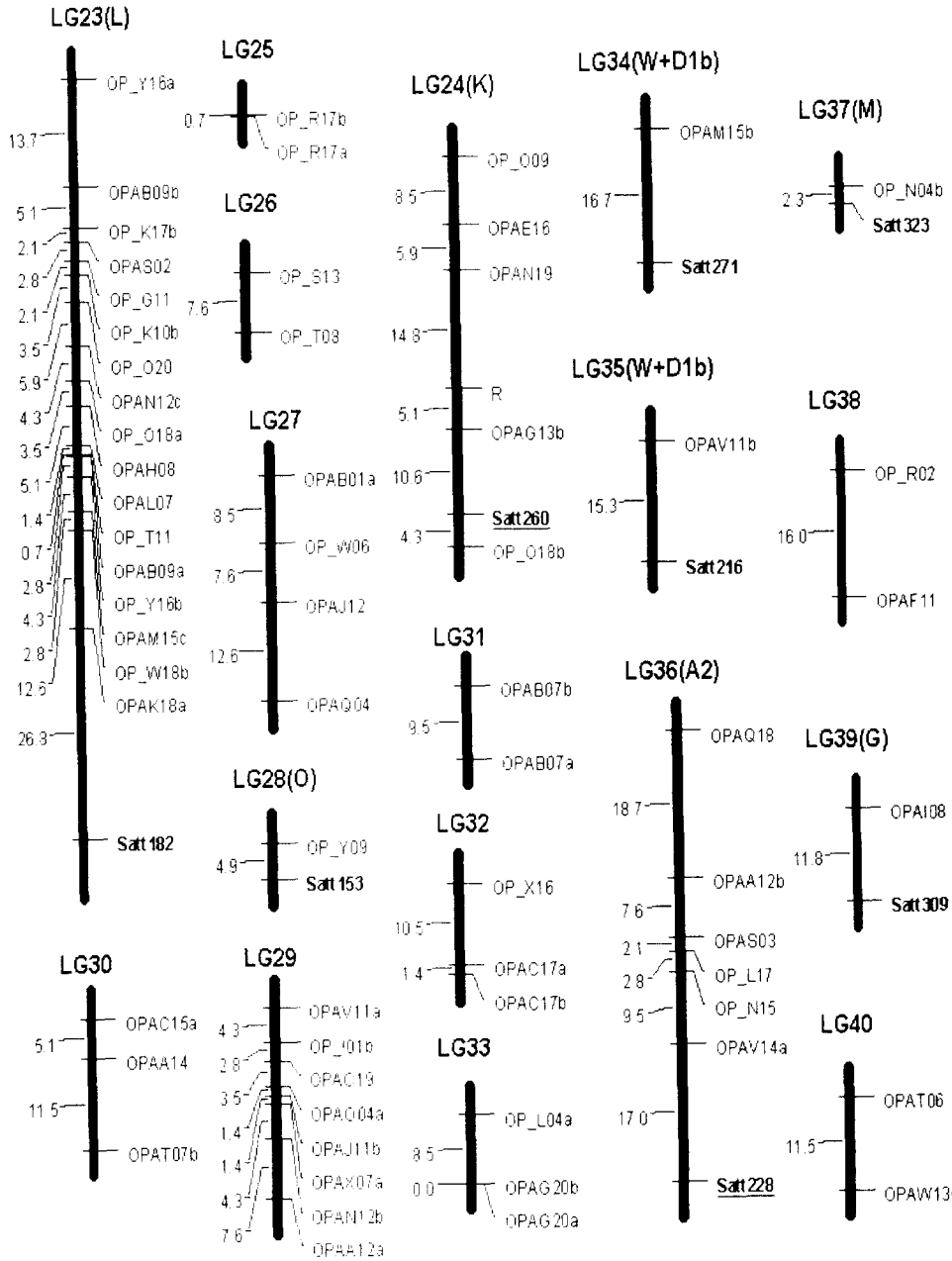


Figure 1. Soybean linkage map comprised of 302 RAPD and 21 SSR markers. The arbitrarily assigned linkage group names (LG1 to LG40) are listed at the top of each group, with map distance shown on the left and marker positions on the right. The SSR markers are underscored. The parenthetic letters indicate the corresponding linkage group in the public soybean map (Shoemaker and Specht, 1995).

three classical loci and four isozyme loci. The PCR-mediated RAPD marker technology is relatively simple, and the time needed to obtain results is very short, when compared with RFLP analysis. RAPDs have been shown to be useful in linkage analysis and for the construction of genetic maps (Klein-Lankhorst et al., 1991; Michelmore et al., 1991; Quiros, 1991). We demonstrated the potential of RAPD markers as genetic markers in soybean molecular biology.

LITERATURE CITED

Akkaya M.S., Shoemaker R.C., Specht J.E., Bhagwat A.A., and Cregan P.B. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage group. Crop SCi. 35:1439-1445.

Bernatzky R., and Tanksley S.D. 1986. Towards a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898.

Diers B.W., Mansur L., Imsnade J., and Shoemaker R.C. 1992. Mapping phytophthora resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci. 32:377-383.

Helentjaris T. 1987. A genetic linkage map for maize based on RFLPs. Trends in Genetics 3:217-221.

Keim P., Diers B.W., and Shoemaker R.C. 1990a. Genetic analysis of soybean hard seededness with molecular markers. Theor. Appl. Genet. 79:465-469.

Klein L. R.M., Vermunt A., Weide R., Liharska T, Zabel P. 1991. Isolation of molecular markers for tomato (*Lycopersicon esculentum*) using radom amplified polymorphic DNA (RAPD). Theor. Appl. Genet. 83:108-114.

Lander E.S., Green P., Abrahamson J., Barlow A., Daly M.J., Lincoln S.E., and Newburg L. 1987. Mapmaker: an interactive computer package for constructing primary genetic linakge maps of experimental and natural populations. Genomics 1:174-181.

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

Quiros C.f., Hu J., This P., Chevre A.M., and Delseny M. 1991. Development and chromosomal localisation of genome-specific markers by polymerase chain reaction in Brassica. Theor. Appl. Genet. 82:627-632.

Saghai Maroof, A.A., Soliman K.M., Jorgenson R., and Allard R.W. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA 81:8014-8018.

Shoemaker R.C., and Olson T.C. 1993. Molecular linkage map of soybean (Glycine max L. Merr). P. 6.131-6.138. In S.J. O' Brien (ed.) Genetic Maps: Locus maps of complex genomes. Cold Spring Harbor Laboratory Press, New York.

Shoemaker R.C., and Specht J.E. 1995. Integration of the soybean molecular and classical genetic linkage groups. Crop Sci. 35:436- 446.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.