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Incorporation of RAPD linkage Map Into RFLP Map in *Glycine max* (L.) Merr

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Abstract

The incorporation of RAPD markers into the previous classical and RFLP genetic linkage maps will facilitate the generation of a detailed genetic map by compensating for the lack of one type of marker in the region of interest. The objective of this paper was to present features we observed when we associated RAPD map from an intraspecific cross of a *Glycine max*×*G. max*, 'Essex'×PI 437654 with the public RFLP map developed from an interspecific cross of *G. max*×*G. soja*. Among 27 linkage groups of RAPD map, eight linkage groups contained probe/enzyme combination RFLP markers, which allowed us the incorporation of RAPD markers into the public RFLP map. Map position rearrangement was observed. In incorporating L.G.C-3 into the public RFLP linkage group a1 and a2, both pSAC3 and pA136 region, and pA170/EcoRV and pB170/HindIII region were in opposite order, respectively. And, pK400 was localized 1.8 cM from pA96-1 and 8.4 cM from pB172 in the public RFLP map, but was localized 9.9 cM from i locus and 18.9 cM from pA85 in our study. A noticeable expansion of the map distances in the intraspecific cross of Essex and PI 437654 was also observed. Map distance between probes pA890 and pK493 in L.G.C-1 was 48.6 cM, but it was only 13.3 cM in the public RFLP map. The distances from the probe pB32-2 to pA670 and from pA670 to pA668 in L.G. C-2 were 50.9 cM and 31.7 cM, but they were 35.9 cM and 13.5 cM in the public RFLP map. The detection of duplicate loci from the same probe that were mapped on the same or/and different linkage group was another feature we observed.

Key words – Soybean, RFLP, RAPD, Genetic map, Comparison

Introduction

The efficiency of mapping traits of agronomic importance depends on the level of polymorphisms in segregating populations. A large number of polymorphic markers in a single segregating population are needed for the identification of linkages between markers and quantitative trait loci (QTLs). Until recently, virtually all progress in breeding has relied on a phenotypic assay of genotype[29]. Morphological markers are easily distinguishable and have advantages of speed and low cost of detection. This allows a large number of progeny to be screened, increasing the likelihood of linkage detection. But, morphological markers have disadvantages of being influenced by environmental factors and may not represent true genetic potential. Moreover, the utility of phenotypic markers is restricted because only a few markers are available in any single cross and consequently linkage can be detected in only a small fraction of the genome[32].

Biochemical genetic markers are advantageous in that

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they represent only genetic variation and are not subject to environmental influence. In addition, biochemical genetic markers may also be used to compare genetic diversity within and among accessions and to monitor changes in allele frequencies during maintenance and regeneration of genetic stocks[1]. Many of the complications of a phenotype-based assay can be mitigated through direct identification of genotype with a DNA-based diagnostic assay[3,4]. For this reason, DNA-based genetic markers are being integrated into several genetic systems, and are expected to play an important role in the future of plant breeding[29].

Soybean [*Glycine max*(L.) Merr.] is a crop of major importance in the world. However, development of soybean genetic maps proceeded slowly relative to genetic maps of other major crops such as maize and rice. This has been due largely to inherent difficulties in performing sexual crosses, a lack of cytogenetic markers, and a lack of genetic variation in the genetic stocks[12, 23]. The large genome size of soybean (1.29×109bp 10) to 1.81×109bp 9) for 1N DNA content) might be another impedement for the development of a saturated genetic maps. The classical genetic map contains only 63 morphological, pigmentation or isoenzyme markers in 19 linkage groups[19].

With the application of DNA-based genetic markers such as Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD), the elucidation of soybean genetic maps has been accelated in a short period. Since Keim et al.[12] reported a genetic map consisting of 26 linkage groups, which defined about 1200 cM, several groups have reported the development of genetic maps. Diers et al.[7] expanded the map to include 252 markers in 31 linkage groups covering 2147 cM. Lark et al.[16] reported a 1550 cM genetic map in 31 linkage groups consisting of 132 RFLP, isozyme, morphological, and biochemical markers. We[6] reported the construction of 27 linkage groups that were comprised of 137 markers including 104 RAPD markers, 31 RFLP probes, and 2 morphological traits (*i* and *t* locus). The linkage map defined 1,096.3 cM, and the average map distance between two adjacent markers was 8.12 cM. The current public soybean RFLP linkage map covers approximately 3,000 cM[22].

To make full use of the potential of a genetic map it is necessary to integrate conventional markers into molecular maps. Shoemaker and Specht[24] reported the map integration in soybean using the data from a *G. max*×*G. max* mapping population, a near-isogenic line (NIL) of the cultivar Clark and a NIL of the cultivar Harosoy, which segregates for 20 classical markers and 120 molecular markers into the current public soybean RFLP map and the classical linkage map. As a result, about half of the 19 soybean classical linkage groups were associated with corresponding molecular linkage groups.

The objective of this paper was to describe features we observed when we compared the RAPD map from an intraspecific cross of a *G. max*×*G. max*, 'Essex'×PI 437654 with the public soybean RFLP map developed from an interspecific cross a *G. max*×*G. soja*.

Materials and Methods

Plant materials

An intraspecific cross of soybean between Essex and PI 437654 was made and seventy-nine $F_{2:3}$ lines were derived. Cultivar Essex is maturity group V, and is characterized by yellow seed coat color (*iiii*), buff hilum color (*rr*), and gray pubescence (*tt*)[26]. It has desirable agronomic traits such as high yields, excellent standability, and good seed quality, but is extremely susceptible to all known races of soybean cyst nematode (SCN). PI 437654 is a plant introduction maintained in the USDA Soybean Germplasm Collection (Univ. of Illinois, Urbana, Illinois). Originally from China, it was introduced from USSR in 1980[18]. PI 437654 is characterized by black seed coat color (*ii*), black hilum color

(*RR*), and tawny pubescence (*TT*). It has undesirable agronomic traits including low yields, poor standability, and seed shattering, but is resistant to all known SCN races.

RFLP analysis

Total genomic DNA was extracted using the CTAB method[22] with modifications for soybeans as outlined by Keim et al.13). Purified DNA was digested with five restriction endonucleases; EcoRI, EcoRV, DraI, TaqI, and HindIII. Digested DNA was size fractioned through electrophoresis at 0.8% agarose gels in TBE (Tris Borate EDTA) at 35 V for 18h. The DNA was then transferred 27) to nylon membrane (Hybond-N, Amersham). Random primer generated ($dCTP^{32}$) DNA soybean RFLP probes 14) were used for hybridization of genomic DNA [8]. Membranes were hybridized in siliconized glass bottles at 65^0C using a hybridization incubator (Robbins Scientific Model 310) for 16 hrs. and washed three times each in 6.0×, 3.0×, and 0.5×SSC and 0.1% SDS. The membranes were exposed to X-ray film (Kodak X-OmatAR) for at least 72 hrs. at -78^0C.

RAPD analysis

The 10-base nucleotide primers from Operon Technologies, Inc. were used in PCR. Approximately 60 ng DNA was used as a template in a 25 μl reaction volume that contained 4.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.0, 200 μM dNTPs (Pharmacia LKB Biotech.), 0.4 (m primer, and 0.5 units Ampli-Taq Polymerase (Perkin-Elmer). Amplifications (45 cycles of 1 min. at 94^0C, 1 min. at 36^0C, and 2 min. at 72^0C) were performed in a Perkin-Elmer Cetus DNA Thermal Cycler. Amplified products were separated electrophoretically on 1.4% agarose gel at 65 V for 4h and visualized by staining with ethidium bromide. Polymorphisms were scored based on the presence or absence of DNA bands.

Statistical analysis

Chi-square analysis to test the goodness of fit for a 3:1 or 1:2:1 ratio was performed using Linkage-1[28] and PC-SAS version 6.0[21]. MAPMAKER/EXP (version 3.0*b*) [15,17] was used to create the linkage map of markers. Linkage was declared at a minimum LOD threshold of 3.0 and recombination frequency of 0.50.

Results and Discussion

We used three different RAPD marker selection strategies: (1) selection of RAPD markers transmitted from PI 437654 to cv. Hartwig[25], (2) selection of RAPD markers using comparative screening of bulks of cultivars with sources for resistance to SCN 5), and (3) selection of polymorphisms between Essex and PI 437654 6). From these marker selection strategies, 164 RAPD markers (96 polymorphisms between Essex and PI 437654, 18 polymorphisms from transmission analysis, and 50 polymorphisms from comparisons of RAPDs in PI 437654 with bulks of cultivars susceptible and resistant to SCN races 3, 5, and 14) were selected. In our experiment, the selection of RAPD markers for segregation analysis was biased toward markers which differed between susceptible and resistant germplasms (e.g., bulks of resistant vs susceptible cultivars) and differed among resistant sources for a specific race (e.g., comparison of Peking, PI 88788, and PI 437654).

In addition to RAPD marker selections, RFLP analysis was employed to associate the RAPD linkage map developed in our study with the public soybean RFLP map [22]. In RFLP analysis, 123 probes on 18 linkage groups of the public soybean RFLP map were hybridized with DNA digested with each of five restriction enzymes (Fig. 1). Twenty-one polymorphic probe/enzyme combination markers and 20 probe/enzyme non-combination markers were detected[6]. Based on RAPD and RFLP analyses, a total of 207 markers consisting of 41 RFLP probes, 164 RAPD markers, and 2 morphological traits [seed coat color (*i* locus) and pubescence color (*t* locus)] were selected and analyzed for the segregation ratio. One

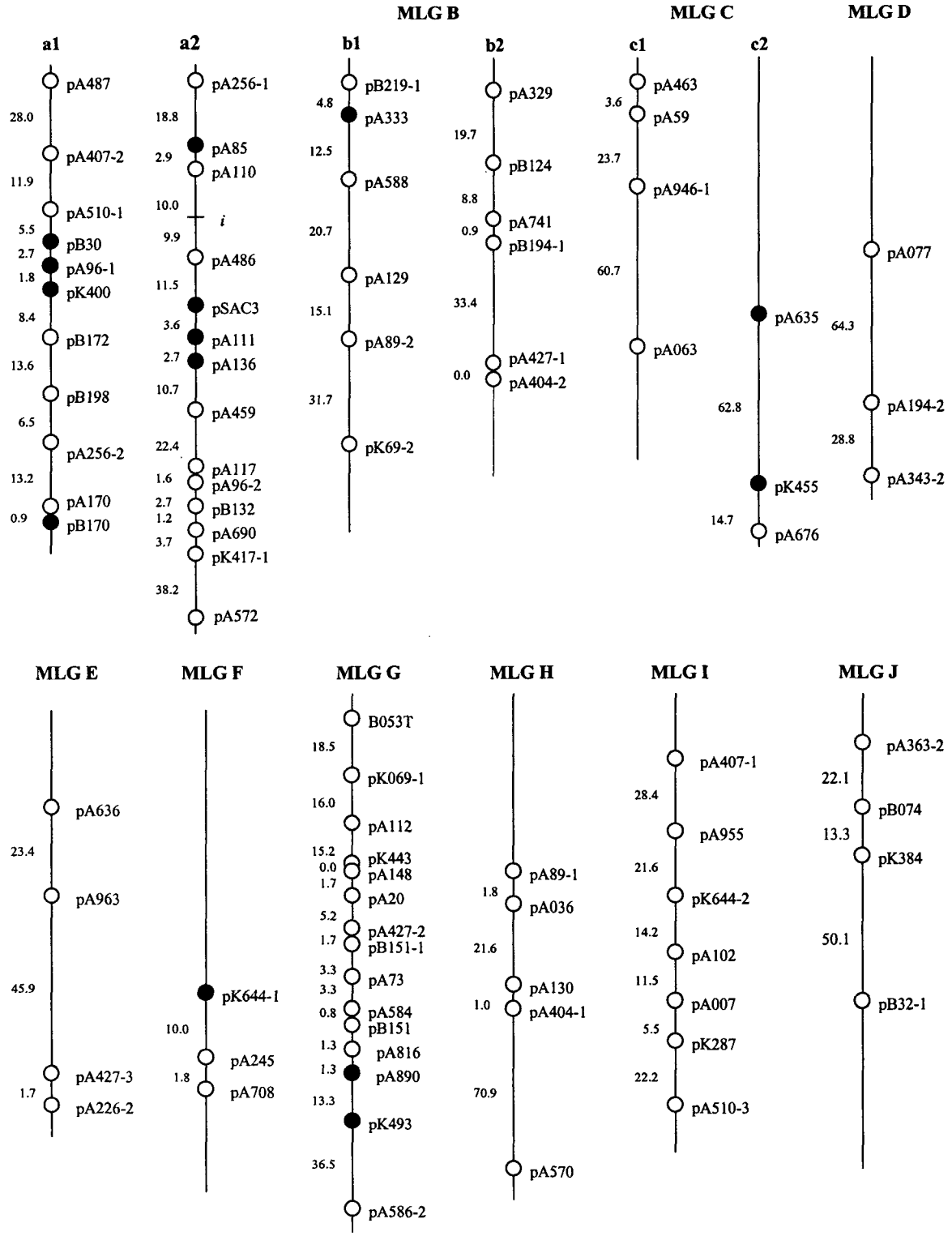

Fig. 1. Restriction fragment length polymorphism analysis in parents, Essex and PI 437654 using probes from the public soybean RFLP map (Shoemaker and Olson, 1993). Black circles represent the probe/enzyme combination polymorphic probes, and white circles representmono morphic probes. Map distances are in centimorgans.

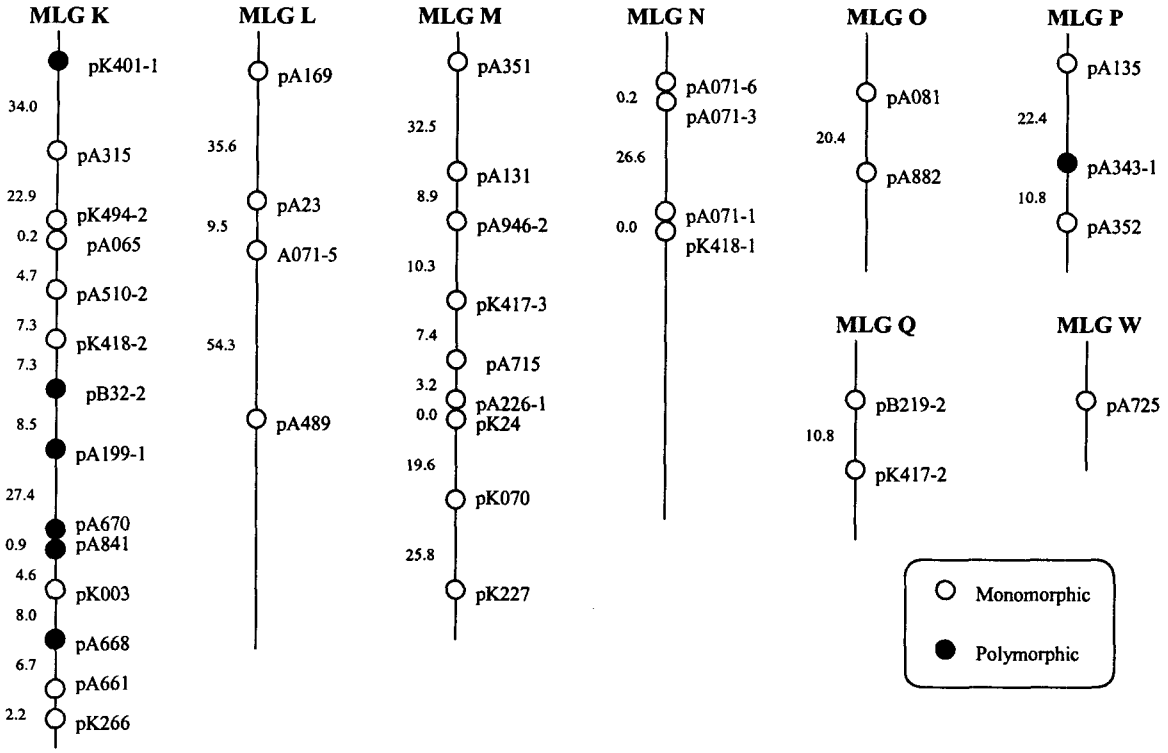

Fig. 1. continued

hundred thirty-seven markers including 104 RAPD markers, 31 RFLP probes, and 2 morphological traits (*i* and *t* locus) were mapped into 27 linkage groups and 46 markers remained unlinked at a minimum LOD score of 3.0 6). The linkage map defined 1,096.3 cM of the soybean genome, and the average map distance between two adjacent markers is 8.12 cM 6).

Linkage assignments

Eight linkage groups (L.G.C-1, C-2, C-3, C-4, C-11, C-18, C-21, and C-24) contained RFLP markers from probe/enzyme combinations. Thus, the incorporation of RAPD linkages into the public soybean RFLP map was possible.

Linkage group L.G.C-3 is part of linkage group A in the molecular linkage groups (MLG) (Fig. 2). Four RFLP probe/enzyme markers and 1 morphological trait for seed coat color (*i* locus), which has already been linked to this group, were mapped. However, the order of the markers has been changed ; (1) both pSAC3 and pA136 region, and pA170/EcoRV and pB170/HindIII region were in opposite order, respectively, and (2) pK400 was localized 1.8 cM from pA96-1 and 8.4 cM from pB172 in MLG, but was localized 9.9 cM from *i* locus and 18.9 cM from pA85 in our study. One probe/enzyme combination, pB170, was linked with the X14 polymorphism in L.G.C-18 (Fig. 2). The X14-pB170 region could be incorporated if additional markers would facilitate the expanded distance.

Map position rearrangement was observed in two regions in merging an intraspecific cross of *G. max*×*G. max*, Clark×Harosoy, linkage group into its corresponding *G. max*×*G. soja* linkage group 24). At first, probe pA816-1 was mapped between pA112-1 and pA121-2 in Clark×Harosoy Group14, but was localized 21.7 cM away from pA121-2 in MLG. Secondly, probes pA588-1 and pA702-2 were adjacent with a map distance of 9.9 cM in MLG, but, these probes were mapped at the

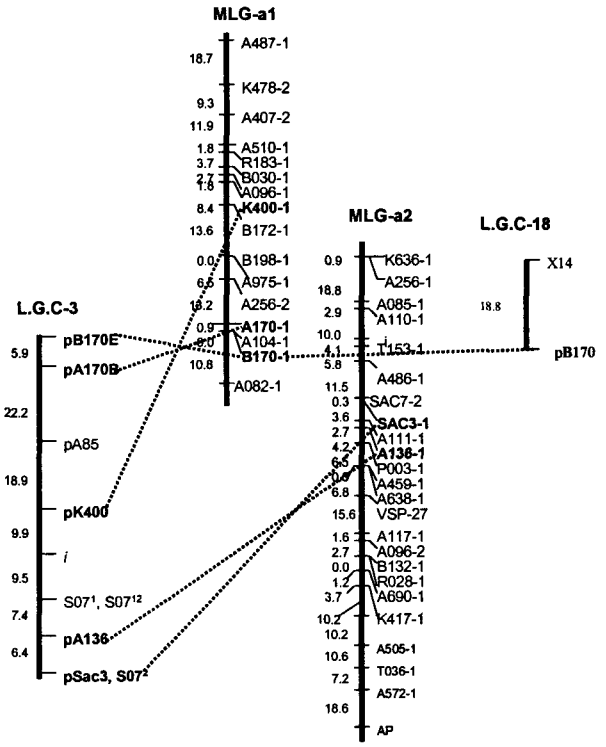

Fig. 2. Incorporation of L.G.C-3 and L.G.C-18 into the MLG-a1 and MLG-a2.

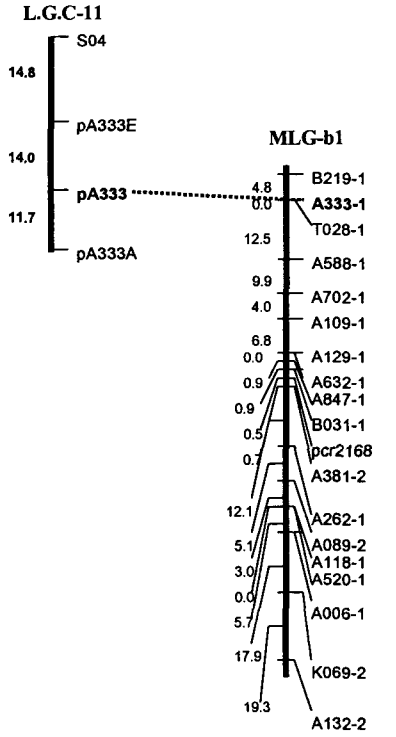

Fig. 3. Incorporation of L.G.C-11 into the MLG-b1.

ends of two different linkage groups, pA702-2 in Clark× Harosoy group1 and pA588-1 in Clark×Harosoy group10. In merging these 2 probes into molecular linkage group b1, the positions of these probes were in reverse direction[24].

Linkage group L.G.C-11 contains probe pA333 which is localized in linkage group B of MLG (Fig. 3). It also includes two probe/enzyme non-combination markers of the same probe which were digested with restriction enzymes EcoRI and HindIII as well. The map distance between probe/enzyme combination marker and non-combination markers were 11.7 cM and 14.0 cM, respectively.

Linkage group L.G.C-4 includes probe/enzyme combination probe pA635, which is localized in linkage group C in MLG (Fig. 4). The incorporated region also includes probe/enzyme non-combination duplicate loci pK418E$_2$ and pK418E$_3$, morphological trait for pubescence

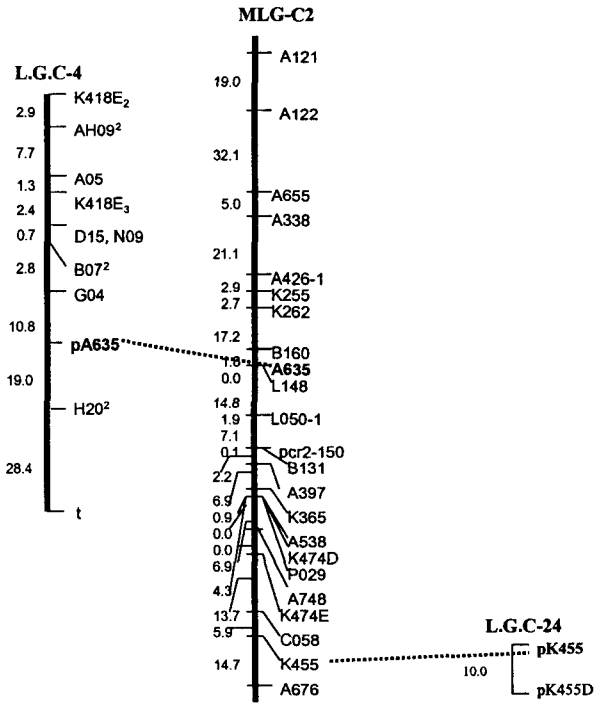

Fig. 4. Incorporation of L.G.C-4 and L.G.C-24 into the MLG-C2.

color (*t* locus), and 7 RAPD markers. The t locus, which mapped on the largest classical linkage group, linkage group-01[19], was localized to the linkage group C2 of MLG and in Clark×Harosoy linkage group 6 with pA635[24]. Another probe/enzyme combination probe from linkage group C in MLG, pK455, was mapped to L.G.C-24 at a distance of 10 cM from non-combination marker pK455/TaqI (Fig. 4). The separation of these two markers might be caused by the expansion of map distances in this study.

Linkage group L.G.C-1 is a fragment of the linkage group G of MLG (Fig. 5). The diagnostic probes for linkage group G, pA890 and pK493, were mapped to this linkage group. Three markers for probe/enzyme non-combination markers of pA112 digested with EcoRI, EcoRV, and HindIII, and 2 markers for the probe/enzyme non-combination markers of pA890 digested with DraI were also mapped with 8 RAPD markers.

Linkage group L.G.C-2 matches the linkage group K of MLG (Fig. 6). Three probe/enzyme combination markers pA668, pA670, and pB32-2 were arranged in the same order, but map distances between molecular markers in this study showed expansion compared with distances on the public RFLP map. One probe/enzyme non-combination marker pB32/EcoRV was localized next to pB32-2 toward the probe pA670 showing map distance 6.7 cM from pB32-2. Five RAPD markers, A162, A201, H051, T022, and T141, were assigned to this linkage group. One probe/enzyme combination marker in linkage group K of MLG, pA199-1, was mapped into L.G.C-21 with 2 RAPD markers, $B07^1$ and F103 (Fig. 6).

A probe/enzyme non-combination probe pA226, digested with restriction endonuclease EcoRV, was mapped to linkage group L.G.C-7 with 5 RAPD markers (Fig. 7).

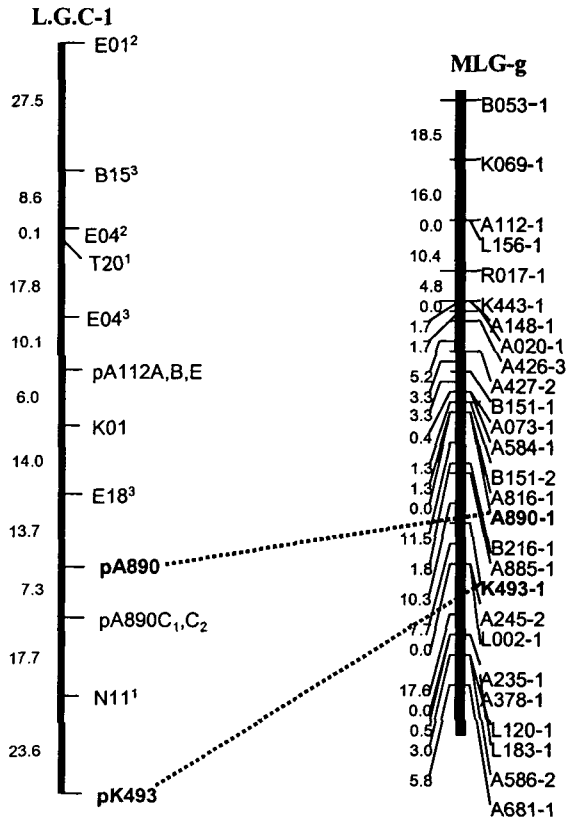

Fig. 5. Incorporation of L.G.C-1 into the MLG-g.

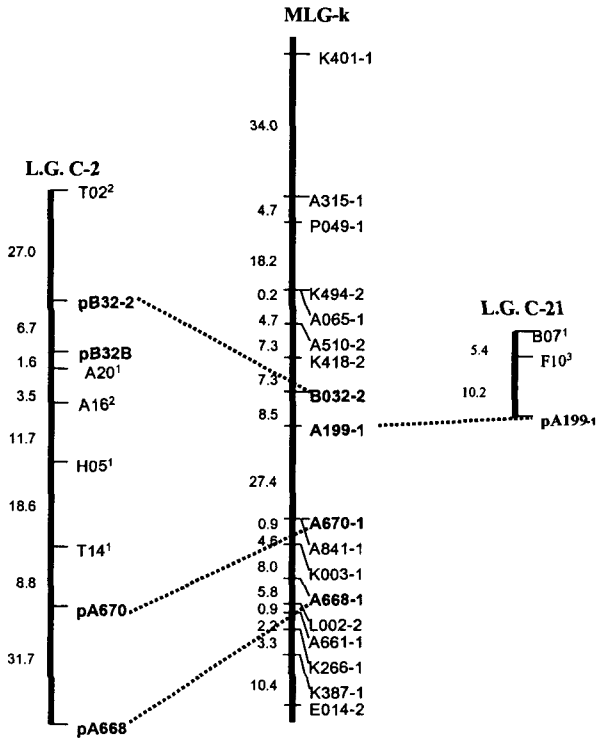

Fig. 6. Incorporation of L.G.C-2 and L.G.C-21 into the MLG-k.

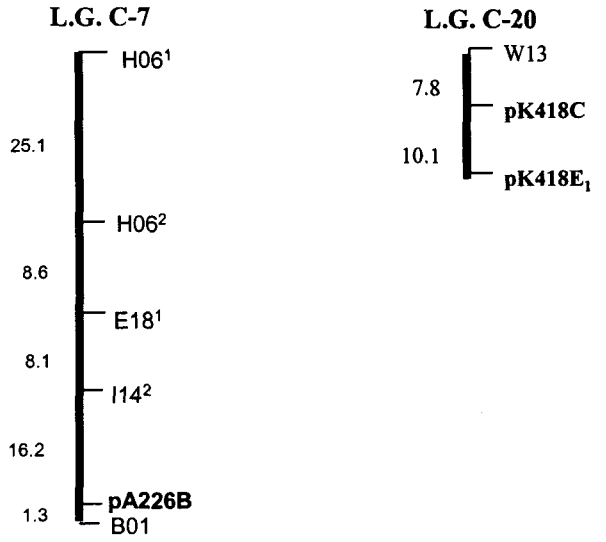

Fig. 7. Two linkage groups(L.G.C-7 and L.G.C-20) that were not incorporated into MLG yet.

The probe/enzyme combination for pA226 is on linkage group M in MLG. Another linkage group L.G.C-20 is composed of probe/enzyme non-combination markers pK418C and pK418E$_1$ with 1 RAPD marker W13 (Fig. 7). Assignment of these RAPD and RFLP polymorphisms into the corresponding segements of MLG requires further mapping study.

In our mapping experiment, a noticeable expansion of the map distances in the intraspecific cross of Essex and PI 437654 in comparison with MLG was observed. For example, the map distance between probes pA890 and pK493 in L.G.C-1 was 48.6 cM, but it was only 13.3 cM in MLG (Fig. 5). The distances from the probe pB32-2 to pA670 and from pA670 to pA668 in L.G. C-2 were 50.9 cM and 31.7 cM, but they were 35.9 cM and 13.5 cM in MLG (Fig. 6). Map distance expansion was obvious in the other intraspecific cross of *G. max*×*G. max* linkage map as well[24]. This might be explained by the suppression of recombination in the interspecific cross that was used for estimation of genetic distances[12,22].

Multiple polymorphic loci were detected from RFLP analysis. DNA probes that showed duplicate loci were from probe/enzyme non-combinations. Probe pK418, digested with restriction enzyme HindIII (probe/enzyme combination for TaqI digestion), showed triplicate loci ; one locus was mapped in linkage group L.G.C-20 (Fig. 7), and other two loci were mapped in L.G.C-4 with map distance between these two loci 11.9 cM (Fig. 4). Duplicate loci from the same probe that mapped to the same linkage group were detected in another intraspecific cross of *G. max*×*G. max* 24). In Clark×Harosoy linkage group 4, pK644-2 and pK644-3 were localized next to each other with map distance of 22.5 cM. Four markers were mapped to Clark×Harosoy group 21, of these, 2 markers were duplicate loci from the probe pK002 (pK002-1 and pK002-2) at map distance of 32.6 cM, and other 2 markers were duplicate loci from the probe pK644 (pK644-1 and pK644-4) at a close distance of 3.6 cM.

Duplicate loci might be the evidence of a tetraploid state in a diploid organism's evolutionary past[11,31]. According to Keim et al.[12], in most cases (with the exception of pA-256a and b, and pK474d and e), the duplicate RFLP markers occur in independent linkage groups in soybean. Similar observations have been attributed to the existence of ancient homologous chromosomes in maize[11]. Furthermore, Keim et al.[12] found that duplicate loci linked in one group do not occur again in another single linkage group and explained that these duplicate loci might originate by mechanisms other than polyploidy.

In this paper, we presented differences we observed in comparing results of our study with the MLG such as map distance expansion in RFLP probes, map position exchange in incorporating markers into the MLG, and duplicate loci in the same or/and different linkage groups. The differences might be caused by the fact that the intraspecific cross of Essex and PI 437654 differed in genomic regions that were not genetically diverse between the two parents of the *G. max*×*G. soja*. This might be explained by the consistency of the results of this study with another intraspecific cross between Clark

and Harosoy[24].

The best way to saturate the genetic linkage maps is to identify a large number of markers to score from a single segregating population. In order to be used in a plant breeding program from any single genetic map, one must have a sufficient number of markers to be able to distinguish between any two genomes of interest at a minimum map resolution of 20 cM. The total number of mapped markers must exceed 1300 to have a standard genetic map that provides an average map resolution of 20 cM between markers for any two inbreds[30]. This requires tremendous effort when one considers that about 20% of the polymorphisms are generated from the probes tested in the interspecific cross of *G. max*×*G. soja* 2), and about one third of probes mapped in the interspecific cross are useful for mapping in intraspecific cross of *G. max*×*G. max* 24). In this regard, the incorporation of RAPD markers into the previous classical and RFLP genetic linkage maps will facilitate the generation of a detailed genetic map by compensating for the lack of one type of marker in the region of interest.

References

1. Andersen, W. R. and D. J. Fairbanks. 1990. Molecular markers: Important tools for plant genetic resource characterization. *Diversity* **6,** 51-53.
2. Apuya, N., B. L. Frazier, P. Keim, E. J. Roth and K. G. Lark. 1988. Restriction length polymorphisms as genetic markers in soybean, *Glycine max* (L.) *Merr. Theor. Appl. Genet.* **75,** 889-901.
3. Beckman, J. S. and M. Soller. 1983. Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. *Theor. Appl. Genet.* **67,** 35-43.
4. Burr, B. S., V. Evolva and F. A. Burr. 1983. The application of RFLP to plant breeding. In: J. K. Sketlow and A. Hollander (eds.) Genetic engineering: Principles and Methods. pp. 45-59. Plenum, New York.
5. Choi, I. S. and H. T. Skorupska. 1995. RAPD marker selection using DNA composite of cultivars for differential response to soybean cyst nematode races 3, 5, and 14. *Soybean Genetics Newsletter* **250,** 245-250.
6. Choi, I. S., Y.C. Kim and H. T. Skorupska. 1997. Random Amplified Polymorphic DNA map for intraspecific cross of Essex×PI 437654 in *Glycine max*(L.) *Merr. Korean J. Breeding* **29,** 270-281.
7. Diers, B. W., P. Keim, W. R. Fehr and R. C. Shoemaker. 1992. RFLP analysis of soybean seed protein and oil content. *Theor. Appl. Genet.* **83,** 608-612.
8. Feinberg, A. P. and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137,** 266-267.
9. Goldberg, R. B. 1978. DNA sequence organization in the soybean plant. *Biochemical Genetics* **16,** 45-68.
10. Gurley, W. B., A. G. Hepburn and J. L. Key. 1979. Sequence organization of soybean genome. *Biochem. Biophys. Acta* **561,** 167-183.
11. Helentjaris, T., D. Weber and S. Wright. 1988. Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. *Genetics* **118,** 353-363.
12. Keim, P., B. W. Diers, T. C. Olson and R. C. Shoemaker. 1990. RFLP mapping in soybean: Association between marker loci and variation in quantitative trait. *Genetics* **126,** 735-742.
13. Keim, P., B. , T. Olson and R. C. Shoemaker. 1988a. A rapid protocol for isolating soybean DNA. *Soy. Genet. Newsl.* **15,** 151-152.
14. Keim, P., B. and R. C. Shoemaker. 1988b. Construction of a random recombinant DNA library that is primarily single copy sequence. *Soy. Genet. Newsl.* **15,** 147-148.
15. Lander, E., P. Green, J. Abrahamson, A. Barlow, M. Daley, S. Lincoln and L. Newburg. 1987. MAPMAKER: An Interactive Computer Package for Constructing Primary Genetic Linkage Maps of Experimental and Natural Populations. *Genomics* **1,** 174-181.
16. Lark, K. G., J. M. Weisemann, B. F. Mathews, R. Palmer, K. Chase and T. Macalma. 1993. A genetic map of soybean [*Glycine max* (L.)] using an intraspecific cross of two cultivars: 'Minosoy' and 'Noir 1'. *Theor. Appl. Genet.* **86,** 901-906.
17. Lincoln, S., M. Daly and E. Lander. 1992. Con-

structing Genetic Maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report. 3rd edition.

18. Nelson, R. L., Amdor, P. J., Ofr, J. H. and Cavins, J. F. 1988. Evaluation of the USDA soybean germplasm collection: maturity groups 000 to IV (PI 427.136 to PI 445.845). USDA-ARS Tech. Bull. 1726.
19. Palmer, R. G. and B. R. Hedges. 1993. Linkage map of soybean (*Glycine max* L. Merr.). pp. 6.139-6.148. *In* S. J. O'Brien (ed.) Genetic Maps: Locus maps of complex genomes. Cold Spring Harbor Laboratory Press, New York.
20. Rogers, S. O. and A. G. Bendich. 1985. Extraction of DNA from milligram amount of fresh, herbarium and mummified plant tissue. *Plant Mol. Biol.* **5,** 69-76.
21. SAS. 1990. SAS/STAT User's Guide, version 6.0. 3rd ed. SAS Institute Inc. SAS Campus Drive, Cary, NC.
22. Shoemaker, R. C. and T. C. Olson. 1993. Molecular linkage map of soybean [*Glycine max* (L.) Merr.]. In: Genetic maps, 6th ed. S. J. O'Brien (ed.) New York: Cold Spring Habor Laboratory Press. **6**.131-6.138.
23. Shoemaker, R. C., T. C. Olson, K. M. Polzin, L. L. Lorenzen and J. E. Specht. 1996. Molecular genetic mapping of soybean. *In*:Soybean: Genetics, Molecular Biology and Biotechnology. (eds.) D. P. S. Verma and R. C. Shoemaker. CAB INTERNATIONAL pp. 37-56.
24. Shoemaker, R. C. and J. E. Specht. 1995. Integration of the Soybean Molecular and Classical Genetic Linkage Groups. *Crop Sci.* **35,** 436-446.
25. Skorupska, H. T., A. Warner and I. S. Choi. 1995. Identification of PCR-generated DNA variation in transmission analysis with PI 437654. *Soybean Genetics Newsletter* **250,** 240-244.
26. Smith and H. M. Camper. 1973. Registration of Essex. *Crop Sci.* **13,** 495.
27. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98,** 503-517.
28. Suiter, K. A., J. F. Wendel and J. S. Case. 1983. Linkage-1: A Pascal computer program for the detection and analysis of genetic linkage. *J. Hered.* **74,** 203-204.
29. Tingey, S. V., J. A. Rafalski and J. G. K. Williams. 1992. Genetic analysis with RAPD markers. In: Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series. Nov. 1, 1992, Minneapolis, MN. pp. 3-8.
30. Tingey, S. V., J. A. Rafalski and J. G. K. Williams, and S. Sebastian. 1991. Soybean genome analysis: DNA polymorphisms are identified by oligonucleotide primers of arbitrary sequence. *In*: Plant Molecular Biology 2. (eds.) R. G. Herrmann and B. Larkins. Academic Press, New York. pp. 263-268.
31. Wendel, J. F., C. W. Stuber, M. M. Goodman and J. B. Beckett. 1989. Duplicated plastid and triplicated cytosolic isozymes of triosephosphate isomerase in maize (*Zea mays* L.). *J. Hered.* **80,** 218-228.
32. Williams, J. G. K., R. S. Reiter, R. M. Young and P. S. Scolnik. 1993. Genetic mapping of mutations using phenotypic pools and mapped RAPD markers. *Nucleic Acids. Research* **21,** 2697-2702.

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초록 : 콩의 RAPD 연관지도를 RFLP 연관지도와 합병

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RAPD 연관지도를 RFLP 연관지도와 합병을 하는 것은 각각의 유전 marker들의 단점을 서로 보완하여 세밀화된 유전자 지도작성을 용이하게 할 수 있다. 본 연구는 Essex와 PI 437654의 F_2 및 F_3 후대계통들을 재료로 하여 작성된 RAPD 연관지도를 콩의 RFLP 연관지도와 합병을 함에 있어서 나타난 몇가지 특징들을 기술하고자 함을 목적으로 하는 바 그 특징들은 아래와 같이 요약된다.

1. RAPD 연관지도상에서의 RFLP probe들의 위치가 RFLP 연관지도상에서의 위치와 부분적으로 변동된 현상이 나타났다. RAPD 연관그룹 L.G.C-3을 RFLP 연관그룹 a1 및 a2와 합병하는 과정에서 pSAC3와 pA136, 그리고 pA170/EcoRV와 pB170/HindIII이 서로 반대방향으로 위치하였다. pK400은 RFLP 연관지도상에서는 pA96-1과 pB172의 사이에 위치한 반면 RAPD 연관지도상에서는 i locus와 pA85 사이에 위치하였다.

2. RAPD 연관지도상에서의 두 marker들간의 간격이 RFLP 연관지도상에서의 간격보다 멀어진 현상이 두드러지게 나타났다. pA890과 pK493간의 간격은 RAPD 연관그룹 L.G.C-1에서는 48.6 cM이었던 반면 RFLP 연관그룹상에서는 단지 13.3 cM으로 나타났다. 또한 pB32-2와 pA670, pA670과 pA668 사이의 간격은 RAPD 연관그룹 L.G.C-2에서는 50.9 cM과 31.7 cM이었던 반면, RFLP 연관지도상에서의 간격은 각각 35.9 cM과 13.5 cM으로 나타났다.

3. 하나의 RFLP probe로부터 두개 이상의 다형화 현상을 나타낸 marker들이 동일한 연관그룹이나 다른 연관그룹에 위치하는 현상이 나타났다. 제한효소 HindIII로 절단된 probe pK418은 세개의 marker를 나타내었는데, 그 중 하나는 L.G.C-20에 위치하였으며, 다른 두개는 L.G.C-4에 위치하였다.

위에 나타난 특징들은 RAPD 연관지도는 intraspecific cross의 후대계통들을 재료로 하여 작성된 반면 RFLP 연관지도는 interspecific cross의 후대계통들을 재료로 하여 작성된 결과에서 비롯된 차이점 때문인 것으로 추측된다.