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A Genetic Linkage Map of Soybean with RFLP, RAPD, SSR and Morphological Markers

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ABSTRACT : The objective of this study was to develop a linkage map of soybean under the genetic background of Korean soybean. A set of 89 F_5 lines was developed from a cross between 'Pureunkong', which was released for soybean sprout, and 'Jinpumkong 2', which had no beany taste in seed due to lack of lipoxygenase 1, 2, and 3. A linkage map was constructed for this population with a set of 113 genetic markers including 7 restriction fragment length polymorphism (RFLP) markers, 79 randomly amplified polymorphic DNA (RAPD) markers, 24 simple sequence repeat (SSR) markers, and 3 morphological markers. The map defined approximately 807.4 cM of the soybean genome comprising 25 linkage groups with 98 polymorphic markers. Fifteen markers remained unlinked. Seventeen linkage groups identified here could be assigned to the respective 13 linkage groups in the USDA soybean genetic map. RFLP and SSR markers segregated at only single genetic loci. Fourteen of the 25 linkage groups contained at least one SSR marker locus. Map positions of most of the SSR loci and their linkages with RFLP markers were consistent with previous reports of the USDA soybean linkage groups. For RAPD, banding patterns of 13 decamer primers showed independent segregations at two or more marker loci for each primer. Only the segregation at opY07 locus was expressed with codominant manner among all RAPD loci. As the soybean genetic map in our study is more updated, molecular approaches of agronomically important genes would be useful to improve Korean soybean improvement.

Keywords : linkage map, genetic marker, RFLP, RAPD, SSR, genome, soybean.

Recombination and selection of genes of agronomic importance are the major backbone for improvement of crop cultivars. It is virtually impossible to directly manipulate favorable genes in the field by the basic breeding methods. With development of molecular tools based on the novel recombinant DNA technology, at least the qualitatively inherited genes could be easily tagged and traced in the breeding population for their identification and selection.

As for the breeding for quantitative traits with the aid of molecular markers, a highly saturated linkage map is required to cover the whole genome. This is because phenotypes of quantitative traits as viewed in the field should be genetically dissected for a few genes with major and minor effects over the genome. With extensive multidisciplinary efforts to develop and saturate genetic linkage maps of cereal crops using DNA markers as well as conventional markers, some quantitative trait loci (QTL) of agronomic importance could have been identified and utilized for selection in breeding programs.

In soybean, construction of genetic linkage maps has been continuously improved in the past 10 years. The original public molecular genetic map was developed from a F_2 population derived from a cross between a breeding line A81-356022 (*Glycine max* L.) and a wild type PI468916 (*G. soja*) (Shoemaker & Olson, 1993). Different versions of frame map were also constructed from intraspecific crosses. A genetic map defining 1,550 cM of 31 linkage groups and 132 RFLP, isozyme, morphological markers was developed from a cross between two soybean cultivars, 'Minsoy' and 'Noir 1' (Lark *et al.*, 1993). Shoemaker & Specht (1995) joined 20 classical loci including seven pigmentation, six morphological and seven isozyme loci, and 8 RAPD loci with the previously mapped 110 RFLP loci. Keim *et al.* (1997) proved the validity of AFLP markers for generating soybean linkage map, and defined an high density map including 650 AFLP markers using a 300 recombinant inbred line population from 'BSR-101' × PI437654. Since Akkaya *et al.* (1995) mapped a total of 40 simple sequence repeat (SSR) DNA markers to a soybean map, numerous microsatellite loci have been evaluated over the soybean genome and developed as markers by Dr. P.B. Cregan's group in the ARS, USDA. A set of 606 SSR marker loci were mapped to more than 20 homologous linkage groups derived from one or more of three populations: the F_2 population of *G. max* × *G. soja* from the USDA/Iowa State, the recombinant inbred population of 'Minsoy' × 'Noir 1' from the Univ. of Utah, and the F_2 population of 'Clark' × 'Harosoy' from the Univ.

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of Nebraska (Cregan *et al.*, 1999).

On the other hand, only a few DNA marker studies have been reported for Korean soybean gene pool. Lee *et al.* (1997a) reported that more polymorphisms were observed in the Korean soybean cultivars than the U.S.-developed cultivars in the molecular level. They constructed a RFLP genetic map by integration of two independent maps derived from two F_2 populations, 'Suwon157' × 'Danbaekkong' and 'Pureunkong' × 'Jinpumkong 2' (Lee *et al.*, 1997b).

We aimed to develop more saturated genetic map of soybean under the genetic background of Korean soybean. The resulting map will be useful for identification of putative loci of the agronomically important qualitative and quantitative traits.

MATERIALS AND METHODS

Eighty-nine F_5 lines were developed from a cross between 'Pureunkong' which was released for soybean sprout, and 'Jinpumkong 2' which had no beany taste in seed due to lack of lipoxygenase 1, 2, and 3. Other major agronomic traits contrasted between two parental cultivars are also shown in Table 1.

Soybean DNA was extracted from greenhouse-grown plants by a modified CTAB procedure (Keim and Shoemaker, 1988). Pulverized leaf tissue was incubated for one hour at 63~65°C with CTAB extraction buffer (2% CTAB, 1.4M NaCl, 0.2M EDTA, 0.1M Tris-HCl pH 8.0, 1% 2-mercaptoethanol). The aqueous phase was then extracted twice with chloroform:isoamyl alcohol [24:1(v/v)] and the total genomic DNA was precipitated with ice-cold 95% alcohol. For RFLP analysis, 5~7 μg of genomic DNA of each line was digested with *Hin*dIII, *Eco*RI and *Eco*RV. The procedures for restriction enzyme digestion, gel electrophoresis, Southern blotting, and hybridization were followed by Kim and Diers (2000). Seven soybean genomic DNA clones were selected among the polymorphic clones for the two parental cultivars and used as probes for RFLP analysis. Map positions of these probes are already known in the USDA frame map and used as anker markers to allocate DNA markers to individual linkage groups.

Table 1. Differences of morphological and agronomic traits between Pureunkong and Jinpumkong 2.

	Pureunkong	Jinpumkong 2
Growth type	Indeterminate	Determinate
Plant height (cm)	101	68
100-seed weight (g)	13.5	22.0
Flower color	White	Purple
Hypocotyl color	Green	Purple
Pubescence color	Tawny (Brown)	Gray
Seed coat color	Green	Yellow
Soybean Mosaic Virus	Resistant	Susceptible
Lipoxygenase	Lx-1, Lx-2, Lx-3	lx-1, lx-2, lx-3

Randomly amplified polymorphic DNA (RAPD) analysis was done as described in Kim and Diers (2000). Random decamer primers obtained from Operon Technologies Inc., Alameda, CA. were used for polymerase chain reaction (PCR). DNA amplification was done by using PTC-100™ Programmable Thermal Controller (MJ Research, Inc., Watertown, MA). PCR products were separated on a 1.4% agarose horizontal gel and visualized by ethidium bromide staining.

Simple sequence repeat (SSR) DNA marker analysis was also done as described in Kim and Diers (2000). A set of 81 forward/reverse (3' & 5' end) primers (Research Genetics, Inc., MAPPAIRS™, Huntsville, AL) was used for SSR DNA marker analysis. PCR products were separated on 1.0% agarose plus 1.0% Synergel (Diversified Biotech, West Roxbury, MA) horizontal gels and visualized by ethidium bromide staining.

The linkage tests among the DNA markers and such qualitative traits such as flower color, hypocotyl color and pubescence color was done with the computer program Mapmaker/Exp 3.0 (Lander *et al.*, 1987). A minimum LOD (Likelihood of odds) of 3.0 and maximum distance of 50 cM was used for testing linkages among markers. The Kosambi centimorgan function was used to indicate the distance between markers.

RESULTS AND DISCUSSION

In order to identify polymorphic markers between 'Pureunkong' and 'Jinpumkong 2', a set of 445 decamer-primers and eighty-one 3' & 5' end 20mer-primers were screened for RAPD and SSR analyses, respectively. Eighty-five decamer-primers (19.1%) revealed polymorphic bands for two cultivars and 63 of them were selected for RAPD analysis. SSR marker loci were more diverse than RAPDs and twenty-six 3' & 5' primers (32.1%) were polymorphic for two cultivars and 24 of them were selected for SSR analysis. Primers which produced unscorable polymorphic bands were ignored in our studies.

Segregation of markers at each locus of RFLP, RAPD, SSR and morphological traits were scored to construct linkage groups for the 'Pureunkong' × 'Jinpumkong 2' population. Fig. 1 illustrates some examples of segregation of DNA markers among parents and F_5 lines for RFLP, SSR, and RAPD. Each of RFLP probes and SSR primers mapped to a respective single marker locus. Segregation of RAPD markers was observed at 79 loci derived from 63 primers. Banding patterns of 13 decamer primers showed indepen-

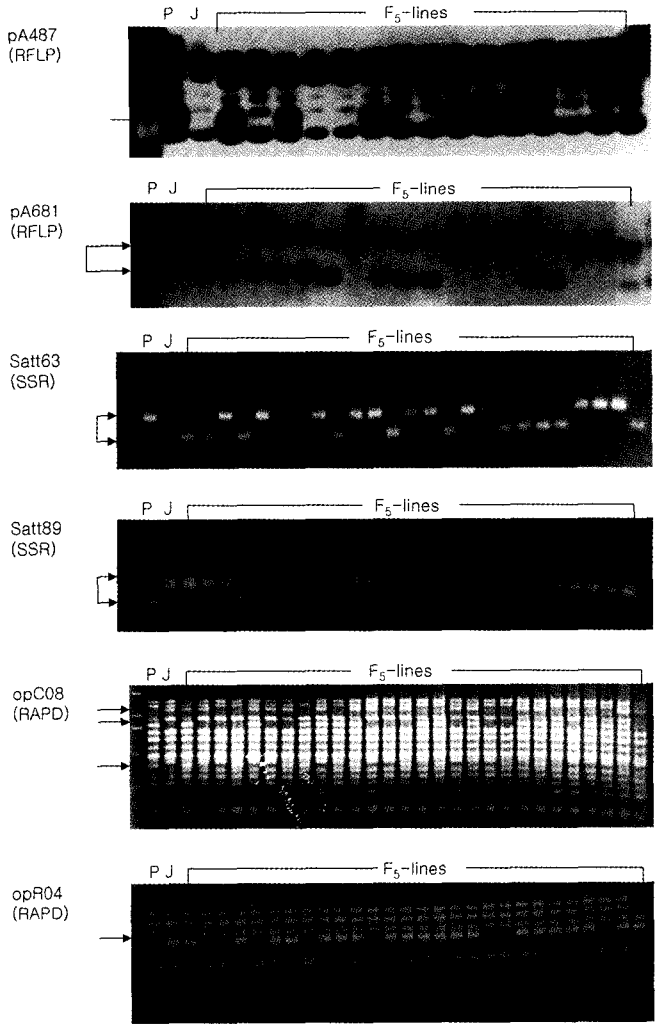

Fig. 1. Segregation of DNA markers of RFLP, SSR, and RAPD among parents and F_5 lines. RFLP and SSR markers show cosegregation of DNA bands at a single locus, respectively (P-'Pureunkong', J-'Jinpumkong 2').

dent segregations at two or more RAPD marker loci for each primer. In particular, opA04, opC08 and opT14 showed marker segregation at three independent RAPD loci, respectively (data for opA04 and opT14 not shown). Only the segregation at opY07 locus was expressed with codominant manner among all RAPD loci (data not shown).

Linkage analysis with Mapmaker 3.0b yielded 25 linkage groups with a total length of 807.4 centimorgan(cM) (Fig. 2). Fifteen DNA markers including pA668, pA963, Sat38, and Satt1 remained unlinked. Eight linkage groups could not be assigned to the linkage groups in the USDA soybean genetic map (Shoemaker *et al.*, 1996) due to the lack of anker markers with known map positions.

Fourteen of the 25 linkage groups contained at least one SSR marker locus. Map positions of most of these SSR loci could be confirmed by agreement with previous reports of soybean map with SSR markers (Akkaya *et al.*, 1995; Cregan *et al.*, 1999). The SSR loci reported on the same USDA soybean genetic linkage groups were also linked together in our study, such as Satt63 and Sat67 (LG5, B2), Satt100 and Sat76 (LG8, C2), Satt5, Satt41 and Sat69 (LG10, D1b), Sat1 and Sat22 (LG11, D2), and Satt9 and Satt88 (LG17, N). Linkages between RFLP and SSR loci with known map positions on LG14(G) and LG17(N) were also consistent with those in the USDA linkage map.

Some linkage groups such as LG2 and LG3, LG5 and LG6, and LG15 and LG16 were not assigned to each of the corresponding USDA linkage groups, respectively. For example, a RFLP marker locus, pA111, and a SSR marker locus, Satt89, were not linked in our map even though they were on the same LG A2 in the USDA map. These linkage groups are expected to be joined as more informative ankering DNA markers will be developed and link the markers on these linkage groups together.

Analysis of segregation for three pigmentation loci resulted in discovery of linkage between those classical markers and previously mapped DNA markers (Fig. 2). The pleiotrophic effect of a single gene locus was reconfirmed for flower and hypocotyl colors with the cosegregation observed between these two traits. The locus controlling flower color (*W1/w1*) and hypocotyl color was linked to Sat39 and opA04-1200 with 7.5 and 2.5 cM on LG12, respectively. The locus for pubescence color (*T/t/t'*) was mapped to Satt100 with 5.5 cM on LG8. This *T* locus was also flanked with two RAPD markers, opC09-700 and opQ11-650 with 0.6 and 3.1 cM, respectively. A few classical markers of pigmentation and morphological loci have been integrated into the soybean molecular genetic map (Keim *et al.*, 1990; Shoemaker & Specht, 1995; Cregan *et al.*, 1999). Shoemaker & Specht (1995) and Cregan *et al.* (1999) mapped the genetic loci controlling pubescence color (*T*) and flower color (*W1*) on LG C2 and LG F, respectively. Our result consistent with those of Shoemaker & Specht (1995) and Cregan *et al.* (1999), suggested there were typical segregations of markers over the genome occurred in the 'Pureunkong' × 'Jinpumkong 2' population.

Recently, many soybean breeders and geneticists have a great interest in molecular breeding and development of strategies for marker assisted selection (MAS). However, most studies using molecular markers have been limited to evaluation of genetic diversity only within Korean germplasm pool (Lee *et al.*, 1997a; Yoon *et al.*, 1998; Han & Abe 1999; Han *et al.*, 1999). The data from those studies would be valuable for selection of genetically distant breeding parents to increase genetic variation in their progeny generations and for evolutionary studies of soybean genomes. On

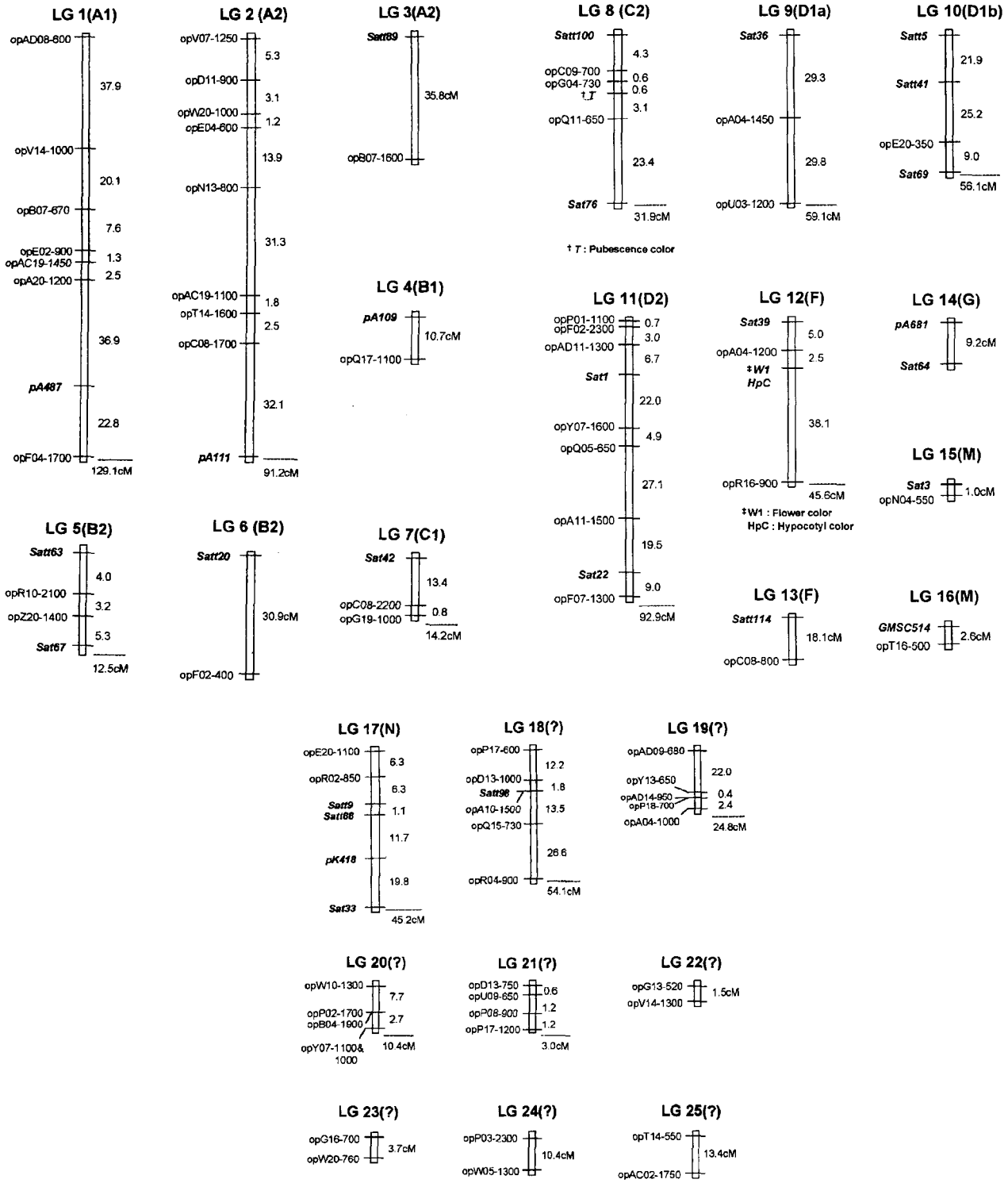

Fig. 2. Twenty-five linkage groups in the soybean genetic map developed from F_5 lines of 'Pureunkong' × 'Jinpumkong 2' and RFLP, SSR, RAPD, and morphological markers. For RAPD markers, the estimated molecular weight values (base pairs) of bands are indicated following decamer primer names.

the other hand, gene tagging and QTL analysis using linkage map based on Korean soybean pool have just started. Lee *et al.* (1997b) constructed RFLP linkage map joined from two different populations and suggested this map might be useful for identification and comparison of genomic regions associated with end-use quality and other agronomically

important traits. However, there are some problems to be solved for practical use of their RFLP map for MAS. Map distance between markers may be biased during joining maps of different populations by using a certain algorithm (Stam, 1993). In addition, RFLP markers using radioactives are not easy to manipulate as compared to PCR-based DNA markers. Marker segregation data from such early generation as F_2 are enough to develop genetic linkage map, but are not optimum for QTL analysis because 50% of genomic loci are theoretically heterozygous and values of field/greenhouse performance for individual lines are not fixed yet.

Our genetic linkage map reported here is not completed yet. It needs to be updated with more DNA markers such as SSR and AFLPs for further genetic studies. As reported by Akkaya *et al.* (1995) and Cregan *et al.* (1999), SSR markers were randomly distributed over the soybean genome. On the other hand, many RAPD markers were not informative because they had a trend to join together in a specific linkage group. As more SSR markers are assigned to our linkage map, a further progress is expected regarding saturation of linkage map and its application for MAS. In particular, one of the geographical origins for soybean is Korea, suggesting that Korean soybean germplasm pool may have a great level of genetic diversity. This feature is especially beneficial for genetic mapping studies of Korea-originated materials because more genomic regions of interest may be polymorphic among the two parents and random distribution of segregating markers may be expected over the soybean genome.

Our putative linkage map on the genetic basis of Korean soybean may attribute to improve the ability to locate and characterize genes of interest and to manipulate genes in the domestic soybean breeding program. As shown in Table 1, Pureunkong and Jinpumkong 2 have many different agronomic traits in terms of morphology, disease resistance and end-use quality. Kim *et al.* (2000) identified QTLs associated with soybean seed size and weight using the F_6 seeds of population from the cross between these two parents. In addition, a putative resistant gene of soybean mosaic virus (SMV) disease was identified with linkage to DNA markers in the LG 13(F) after analysis of SMV (G5-H strain) level among parents and population (communicated with Mr. Jung-Kyung Moon, Upland Crop Division at National Crop Experiment Station in Suwon, Korea). As the soybean genetic map in our study is getting more updated, there will be more possibility of molecular approaches useful for the improvement of Korean soybean.

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