Korean J. Crop Sci. 48(4): 297~302(2003)

Construction of Genetic Linkage Map for Korean Soybean Genotypes using Molecular Markers

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ABSTRACT : Genetic linkage maps serve the plant geneticist in a number of ways, from marker assisted selection in plant improvement to map-based cloning in molecular genetic research. Genetic map based upon DNA polymorphism is a powerful tool for the study of qualitative and quantitative traits in crops. The objective of this study was to develop genetic linkage map of soybean using the population derived from the cross of Korean soybean cultivar 'Kwangkyo, and and wild accession 'IT182305'. Total 1,000 Operon random primers for RAPD marker, 49 combinations of primer for AFLP marker, and 100 Satt primers for SSR marker were used to screen parental polymorphism. Total 341 markers (242 RAPD, 83 AFLP, and 16 SSR markers) was segregated in 85 F_2 population. Forty two markers that shown significantly distorted segregation ratio (1:2:1 for codominant or 3:1 for domimant marker) were not used in mapping procedure. A linkage map was constructed by applying the computer program MAPMAKER/EXP 3.0 to the 299 marker data with LOD 4.0 and maximum distance 50 cM. 176 markers were found to be genetically linked and formed 25 linkage groups. Linkage map spanned 2,292.7 cM across all 25 linkage groups. The average linkage distance between pair of markers among all linkage groups was 13.0 cM. The number of markers per linkage group ranged from 2 to 55. The longest linkage group 3 spanned 967.4 cM with 55 makers. This map requires further saturation with more markers and agronomically important traits will be joined over it.

Keywords **:** *soybean, genetic map, molecular marker, linkage*

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 and tomato due to the inherent difficulty, tediousness associated with hybridization, and a lack of cytogenetic markers. The soybean classical genetic linkage map is composed of morphological, physiological and protein markers, has 46 loci localized on 20 linkage groups, but the linkage distances only sum to 440 cM (Devine *et al.*, 1991; Muehlbauer et al., 1989; Palmer and Kiang, 1990; and Palmer *et al.*, 1992). Keim et al. (1990) constructed a genetic map comprised of 150 RFLP markers in an F_2 population from an interspecific cross. Using a mapping population derived from an interspecific *G. max*×*G. soja* cross, Shoemaker and Olson (1993) developed a molecular genetic linkage map that consisted of 25 linkage groups with about 365 RFLP, 11 RAPD, 3 classical markers, and 4 isozyme loci. Shoemaker and Specht (1995) created a linkage map comprised of 13 classical and seven isozyme loci along with 110 RFLP and 8 RAPD markers in a soybean mapping population derived from a mating between near-isogenic lines of the cultivars Clark and Harosoy. Akkaya *et al.*, (1995) mapped a total of 40 PCR-based SSR markers in a soybean mapping population that consisted of 60 F_2 plants from a cross between near isogenic lines of the cultivars Clark and Harosoy. Keim et al. (1997) reported a summed distance of 3,441 cM for a map of 840 mostly dominant AFLP markers in the F_6-derived RIL population. A total of 606 SSR loci were mapped in one or more of three populations: the USDA/Iowa State *G. max* x *G. soja* F_2 population, the Univ. of Utah 'Minsoy'×'Noir1' recombinant inbred population, and the Univ. of Nebraska 'Clark' ×'Harosoy' F_2 population (Cregan *et al.*, 1999). Each SSR was mapped to a single locus in the genome, with a map order that was essentially identical in all three populations. Thus, 20+ linkage groups that correspond to the 20 pairs of soybean chromosomes were derived from each of the three populations. Ferreira *et al.*, (2000) reported a total distance of 3,275 cM for a map with 106 RAPD and 250 RFLP markers in a F_6-derived RIL population. Yamanaka et al. (2002) recently reported a 2,909 cM map of RFLP-SSRP markers in a F_2 population.

On the other hand, only a few soybean genetic maps based on DNA marker have been reported for korean soybean gene pool. Lee *et al.*, (1997) constructed a molecular genetic linkage map consisted of 18 linkage groups with 72 RFLP markers. Kim *et al.*, (2000) constructed genetic map com-

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prised of 25 linkage groups with 7 RFLP, 79 RAPD, 24 SSR, and 3 morphological markers in a 89 F_5 lines derived from the cross of 'Pureunkong' and 'Jinpumkong2'. The molecular genetic linkage map was not created to study quantitative trait locus using the population derived from the cross of cultivar and wild type soybean. The objective of this study was to develop soybean genetic linkage map and to locate QTLs using the F_2 population from a mating between Korean soybean cultivar 'Kwangkyo' and wild type soybean 'IT182305'.

MATERIALS AND METHODS

Plant Material

Mapping population was derived from a mating between soybean cultivar 'Kwangkyo' (female parent) and wild type soybean 'IT182305' (male parent). F_1 hybrids were made in the greenhouse and F_2 seed were planted in the field on May, 2001. Young leaves were collected from the 85 individual F_2 plants and parent plants. Genomic DNA was extracted from finely ground leaf tissue by means of a modified CTAB procedure (Saghai Maroof *et al.,* 1984).

RAPD Analysis

For the analysis of random amplified polymorphic DNA (RAPD) markers, one-thousand 10-mer oligonucleotide primers were obtained from Operon Technologies (Alameda, USA). 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 min 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 , and 70 second at 72°C, before ending with 1 cycle of 5 min 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.

AFLP Analysis

Approximately 60 ng of genomic DNA was double-digested using restriction enzymes *Eco*RI and *Tru*9I. *Eco*RI digestion was carried out in a final volume of 50 [EcoRI 5 unit, BSA 1 μl, 10x buffer 5 μl, ddH_2O 43.5 μl, genomic DNA (20 ng/) 3] at 37°C for 12 h, and *Tru*9I digestion was carried out in a final volume of 50 μl[*Tru*9 I 5unit, ddH_2O 44.5 μl, 10x buffer 5 μl, BSA 0.5 μl] at 65°C for 12 h. *Eco*RI and *Mse*I adaptors were subsequently ligated to DNA fragments by adding the digestion products to 20 μl adaptor ligation solution [*Eco*RI adaptor1 1 μl, *Eco*RI adaptor2 1 μl, *Mse*I adaptor1 1 μl, *Mse*I adaptors 1 μl, 10x buffer 2 μl, T_4 DNA ligase 5 unit, ddH_2O 13 μl]. The ligation was incubated for 12 h at 14°C. The adaptor-ligated DNA fragments were used as templates for the pre-amplification reaction. The *Eco*RI, *Mse*I adaptors, and primers with three selective nucleotides were synthesized by Bioneer Inc. (Korea). Adaptors and selective primer pairs used and their sequences are listed in Table 1. Pre-amplification reactions were performed in a 30 volume containing [ddH_2O 15.9 μl, 10x buffer 2.5 μl, dNTP (1.25mM/) 4 μl, *Eco*RI primer 1.2, *Mse*I primer 1.2 μl, ligated DNA 5 μl, tag polymerase (Promega, USA) 1 unit, DNA 5 μl]. Samples were covered with 15 μl of light mineral oil. The PCR amplification was carried out in a PTC-200 Thermocycler using 29 thermal cycles of 94°C for 30 sec, 60°C for 60 sec, 72°C for 60 sec. Selective amplification reactions were performed in a 30 volume containing [ddH_2O 15.9 μl, 10X buffer 2.5, dNTP (1.25 mM/μl) 4 μl, *Eco*RI anchor 1.2 μl (10 mM/μl), *Mse*I anchor 1.2 μl (10mM/μl), 1 unit tag polymerase (Promega), DNA 5 μl (2.5 μg/μl)]. Samples were covered with 15 μl of light mineral oil. The PCR profile was 1 cycle of 94°C for 30 s, 65°C for 30 s, and

Table 1. Nucleotide sequences of adapters and primers used for AFLP analysis in this study.

Adaptors/Primers	Sequences
*Mse*I adaptors	5'-GACGATGAGTCCTGAG-3'
	3'-TACTCAGGACTCAT-5'
*Eco*RI adaptors	5'-CTCGTAGACTGCGTACC-3'
	3'-CTGACGCATGGTTAA-5'
*Mse*I primers	5'-GATGAGTCCTGAGTAA-3'
*Eco*RI primers	5'-ACTGCGTACCAATTC-3'
*Mse*I primer + 3 primer	5 '-GATGAGTCCTGAGTAAgtg-3'
	5'-GATGAGTCCTGAGTAAgag-3'
	5'-GATGAGTCCTGAGTAAggc-3'
	5'-GATGAGTCCTGAGTAAgaa-3'
	5'-GATGAGTCCTGAGTAAagt-3'
	5'-GATGAGTCCTGAGTAAagg-3'
	5'-GATGAGTCCTGAGTAAacg-3'
*Eco*RI primer+3 primer	5'-ACTGCGTACCAATTCcaa-3'
	5'-ACTGCGTACCAATTCcgt-3'
	5'-ACTGCGTACCAATTCctc-3'
	5'-ACTGCGTACCAATTCctg-3'
	5'-ACTGCGTACCAATTCcac-3'
	5'-ACTGCGTACCAATTCcag-3'
	5'-ACTGCGTACCAATTCacc-3'

72°C for 1 min, followed by 9 cycles lowering the annealing temperature from 65°C to 56°C at -0.1°C steps for each cycle, and finally 29 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. The products were held at 4°C until used.

SSR Analysis

All SSR primers used were described by Cregan *et al.*,(1999). Satt primers were synthesized by Bioneer, Inc. (Korea). SSR amplification reactions were performed in a 10 volume of reaction [2 μl genomic template DNA (20 ng/μl), 2 μl Satt primer (10mM/μl), 5 unit Tag polymerase, 0.4 μl dNTP (1.25 mM/μl), 2.2 μl 5X reaction buffer, 3.8 μl ddH_2O]. Samples were covered with 10 μl of light mineral oil. The PCR amplification was carried out in a PTC-200 Thermocycler using 39 thermal cycles of 92°C for 45 sec, 47°C for 45 sec, 68°C for 45 sec, and finally 72°C for 5 min. The products were held at 4°C until used.

Gel Electrophoresis

AFLP or SSR products (6 μl) were mixed with 4 μl of 3x loading dye[10mM NaOH 4.9%, Formamide 95%, Bromophenol 0.05%, Xylene cyanole FF 0.05%] and denatured at 100°C for 5 min and cooled on ice before 6 was loaded to each lane of a 5% deanturation polyacrylamide sequencing gel. DNA fragments were separated in a vertical electrophoresis system (35×45 cm; Owl Scientific, USA) using a 5% polyacrylamide gel[15 ml 40% acrylamide, 12 ml 10x TBE buffer, 50.4 g urea, 51 ml ddH_2O, 25 g TEMED, 0.025 g ammonium persulfate] and 1X TBE buffer at 80 W, 1800 V constant power for 2 h. DNA bands were visualized by silver staining (Bassam *et al.*, 1991). The gel was first fixed in 10% acetic acid for 20 min, and then washed for 2 min with a large quantity of distilled water 3 times. The gel was transferred to a silver impregnation solution[1.5g/l $AgNO_3$, 1.5 ml 37% formaldehyde solution/l] for 30 min, followed by a 5 sec rinse with distilled water. The image development was performed with manual agitation for 1 to 2 min in a developer solution [30 g/L Na_2CO_3, 1.5 ml 37% formaldehyde solution/l, 200 μl sodium thiosulfate/l], followed by 1 min fixation with 10% acetic acid solution. The gel was then rinsed briefly in distilled water and dried at room temperature. Silver stained dry gels were photographed by exposing the gels to Promega automatic processor compatible (APC) paper about 40 sec of fluorescent ceiling light.

Map Construction

All molecular marker data were subjected to Chi-square analysis to test the goodness of fit for observed to expeced ratios (1:2:1 for codominant or 3:1 for dominant markers). Only these loci fitting (P=0.05) the Chi-square test were used in mapping procedures. The software program Mapmaker/Exp 3.0b was used to detect probable linkages at a LOD value of 4.0 and Max distance 50 cM(Lander et al., 1987). After markers were assigned to a linkage group, compare and ripple commands were used to develop linkage groups and established most likely gene order. Genetic distance was described by centi-morgan (cM) using Kosambi (1944) mapping function.

RESULTS AND DISCUSSION

Of the 1,000 RAPD primers tested on the two parents of cultivar 'Kwangkyo' and wild type 'IT182305', 242 polymorphic primers were identified. Approximately 24% primers produced polymorphic DNA fragment differences between two parents. Of the 100 SSR primer tested, 54

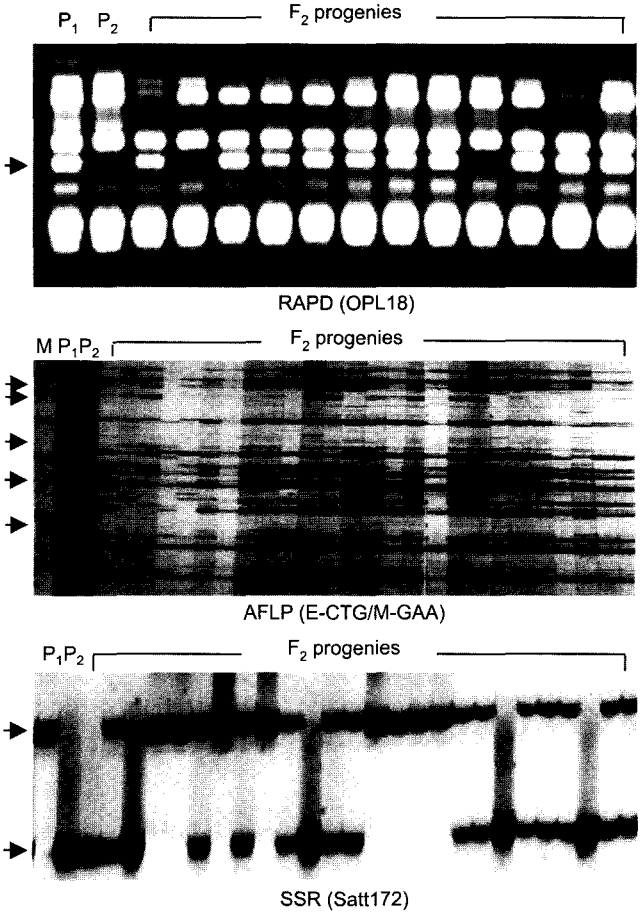

Fig. 1. Patterns of segregating DNA fragment for RAPD (OPL18), AFLP (CTG/GAA), SSR (Satt172) marker in F_2 population. P_1 is Kwangkyo (Cultivar) and P_2 is IT182305 (Wild type). Arrows indicate polymorphic fragments.

revealed a parental polymorphism and 31 AFLP markers among 49 combiantions of selective primers tested produced polymorphic fragments on the two parents. About 54% and 63 % among SSR and AFLP primers used respectively produced parental polymorphism. The degree of parental polymorphism from RAPD, SSR, and AFLP results was the higher than other researcher. Kim *et al.*, (2000) reported that 19.1 and 32.1 % of RAPD and SSR primers tested produced

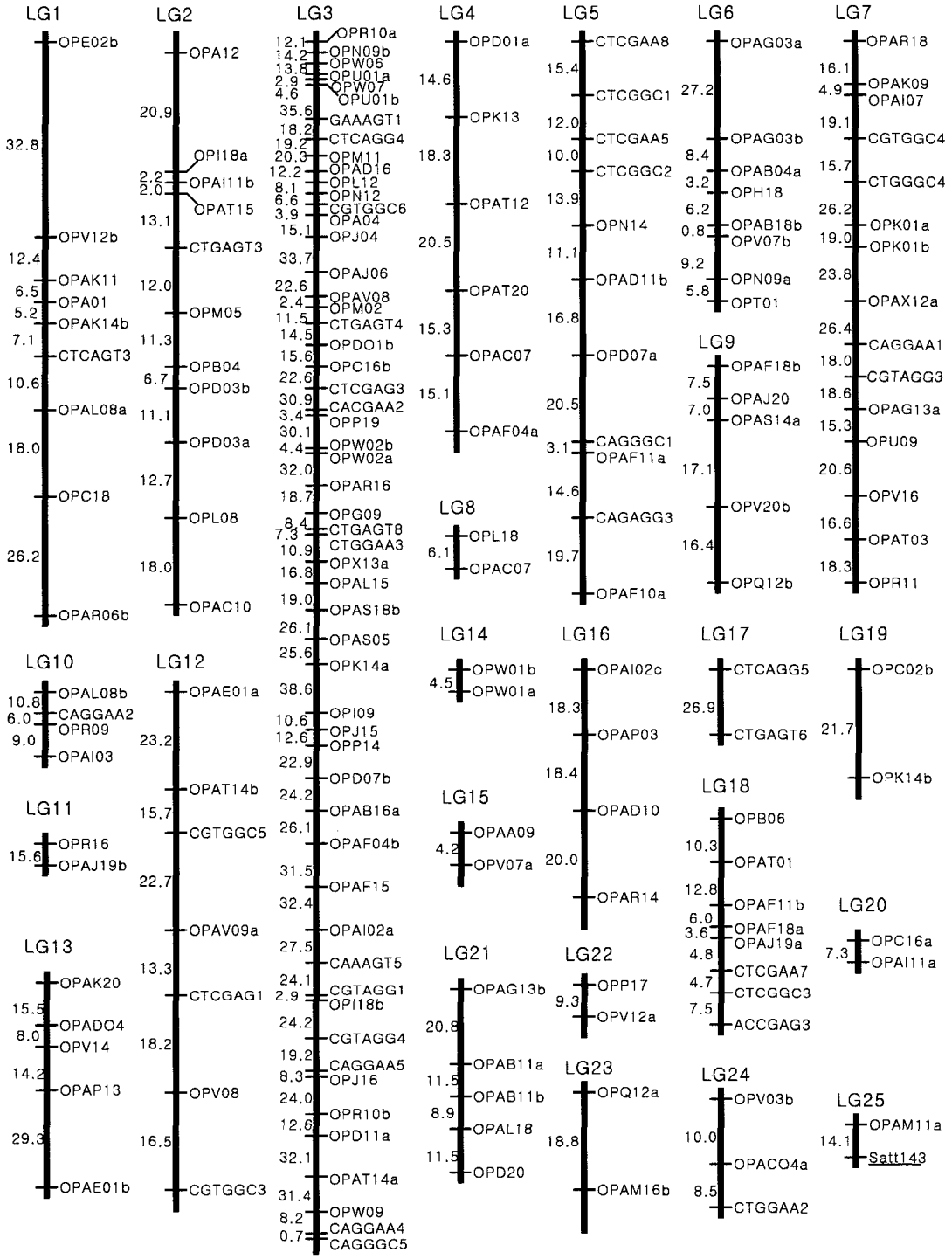

Fig. 2. Genetic linkage map of soybean consisted of 176 molecular markers that segregated in an F_2 population mating of a derived from Kwangkyo (Cultivar) and IT182305 (wild type soybean). Linkage maps were constructed using MAPMAKER with LOD 4.0 and maximum distance 50 cM. Marker loci names are on the right and Kosambi map distances are on the left of the linkage groups which are numbered from 1 to 25. Bold letters are AFLP markers and underlined is SSR marker.

polymorphic fragments for two cultivars 'Pureunkong' and 'Jinpumkong2'. The difference of polymorphic level in two studies might be due to the different genetic distance between parents used in two research. Total 327 markers (242 RAPD, 54 SSR, and 31 AFLP) were chosen in parental polymorphism screen. Segregation data were obtained for 327 markers in 85 F_2 mapping population. Total 341 segregated markers were observed and segregating scores were subjected to Chi-square analysis to test the goodness of fit for the observed to expected ratio (1:2:1 for codominant or 3:1 for dominant markers in F_2 population). Forty two markers (12.3%) of 341 segregating markers were not fitted (P=0.05) and not used in mapping procedures. Two hundred ninety markers (219 RAPD, 73 AFLP, and 7 SSR markers) were used in construction for genetic map. Fig. 1 represents some examples of segregating DNA fragment for RAPD (OPL18), AFLP (CTG/GAA), SSR (Satt172) markers in parents and F_2 population. All RAPD bands were dominant marker alleles and SSR bands were codominant marker alleles. Among AFLP bands, only one band was codominant marker allele. Keim *et al.,* (1997) observed that 87% of AFLP bands were dominant marker alleles in mapping population developed from a mating of cultivar BSR101 and unadapted plant introduction PI437.654. The frequency of codominant AFLP marker identified in this study was very low compared to 13% reported by Keim et al. (1997). This difference would be due to a relatively small number of marker tested and genetic diversity of parents.

A genetic map was constructed from the 299 segregating markers. Of the 299 markers, 176 (139 RAPD, 37 AFLP, and 1 SSR markers) were found to be genetically linked. These markers coalesced into 25 linkage groups (Fig. 2). The linkage map spanned 2,292.7 cM across all 25 linkage groups. The average linkage distance between pair of markers among all linkage groups was 13.0 cM in Kosambi map units. The number of markers per linkage group ranged from 2 to 55. Our linkage groups have been designated LG1-LG25 to avoid confusing them with linkage groups from maps of other populations (e.g., Shoemaker and Specht, 1995), and to emphasize the fact that, as yet, our map has not been merged with any other maps. Ten linkage groups was linked on only two loci. The longest group 3 spans 967.4 cM with 55 markers. Linkage group 25 contained one SSR locus. One hundred twenty three markers were unlinked. This is likely due to the relatively small population size and the inability to differentiate between homozygotes and heterozygotes in those F_2 genotypes. Recently, Chung *et al.,* (2003) reported a total distance of 2,943 cM for a 35 linkage map comprised of 329 RAPD, 103 SSR, one CAPs marker, one seed protein mobility variant, and two genes for pod and seed pigmentation in a 76 F_5-derived RIL population. On the basis of SSR homology, 35 linkage groups created were aligned with the 20 known soybean molecular genetic maps (Cregan *et al.,* 1999). Thirty four RAPD markers mapped in this study were positioned into the map by Chung et al. (2003). LG12 (OPAT14 and OPV08 markers), LG22 (OPP17 marker), and LG18 (OPAF11 marker) were correlated with MLG G (Cregan *et al.,* 1999). Also, LG6 (OPAB04 marker), LG19 (OPC02 and OPK14b markers) were matched with MLG D1a. LG9 (OPQ12 marker) and LG23 (OPQ12 marker) were same to the MLG D2. Linkage group 3 reported here was separated into MLG I, E, B2, D2, D1a, and N. Linkage group 25 was matched with MLG L by SSR marker Satt143 homology (Cregan et al. 1999). Correspondence between additional linkage groups was limited because of the lack of common markers. Linkage maps identified here contains more markers and genetic distances compared with the maps constructed by the Lee *et al.,* (1997) and Kim *et al.,* (2000). When compare genetic map constructed in this study with another genetic map, more markers should be added. also, because the haploid chromosome number of *G. max* is known to be 20, several linkage groups are likely submembers of a larger linkage group.

A high-density genetic map represents a tool to assist map-based cloning and efficient marker assisted selection for the entire soybean research and improvement. Map-based cloning efforts require very fine resolution mapping in the region of the target genes and the highest marker density to shorten chromosome walks. Marker assisted selection is most effective when the markers are tightly linked to the gene of interest. More SSR markers will be added in this map and agronomically important traits will be joined in future work.

ACKNOWLEDGEMENTS

This work was supported by a grant (CG3132) from Crop Functional Genomics Center (CFGC), 21C Frontier R & D Project, Ministry of Science & Technology.

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