Korean J. Crop Sci. 51(2): 169~172(2006)

Identification of Molecular Markers Linked to *Lf2* Locus in Soybean

Myung Sik Kim*, Min Jung Park*, Woo Hyeun Jeong*, Ki Chul Nam*, and Jong Il Chung*†

**Department of Agronomy, Research Institute of Life Sci., Gyeongsang National University, Chinju 660-701, Korea*

ABSTRACT: Leaflet number of soybean controlled by *Lf2* locus is the important trait in photosynthesis and plant type. The objective of this research was to identify molecular markers linked to the *lf2* locus. A total of 115 F_2 plants were derived from a cross between normal three-leaflet type Sinpaldalkong (*Lf2Lf2*) and seven-leaflet mutant type T255 (*lf2lf2*). All leaflet counts of parents and F_2 individual plants were made in the field on fully expanded leaves on the main stem when terminal growth of the main stem had ceased. One-thousand 10-mer oligonucleotide RAPD primers and 664 SSR primers were used. The segregation ratios of 3 : 1 were observed in the F_2 population and the Chi-square values strongly suggested that the seven-leaflet was controlled by a single recessive gene. A genetic map was constructed from the 15 segregating markers (9 RAPDs, 5 SSRs, 1 *lf2* locus). OPAD03 and OPAI13 RAPD markers were linked to the *lf2* locus that controlled seven-leaflet type at a distance of 20.5 and 23.5 cM, respectively. Molecular markers identified in this study linked with *lf2* locus will be helpful to locate *lf2* locus on the public soybean molecular linkage map and would be useful for tagging the *lf2* locus that controls seven-leaflet trait.

Keywords: leaflet number, seven-leaflet, molecular marker, soybean, linkage

Leaflet number is an important trait in photosynthesis and plant type. Most soybeans [*Glycine max* (L.) Merr.] have trifoliolate leaves. Soybean genotypes with a high frequency of leaves with five leaflets were first observed and these multifoliolate leaves were controlled by a single major gene (Takahashi and Fukuyama, 1919). Fehr (1972) observed a plant with seven-leaflets per leaf in the cultivar "Hawkeye", resulting from a spontaneous mutation and reported this seven-leaflet character was controlled by a single major gene (*lf2*). Devine (2003) reported that *Pd2* locus, controlling dense pubescence was linked to *lf2* locus with a recombination frequency of 12.1 ± 2.2%.

RFLP, RAPD, AFLP, and SSR markers have become fundamental tools for genetics and genetic variation research involving soybean improvement programs. Cregan *et al.* (1999) described the efforts to integrate the classical genetic map with the molecular map. They reported that of the estimated more than 250 named genes controlling traits identified without molecular technology, 68 had been associated in 20 classical linkage groups or linkage fragments. The *lf2* locus that controls seven-leaflet trait in soybean was localized on classical linkage group 16 (Devine, 2003). Classical linkage group 16 was not integrated with soybean molecular map (Cregan *et al.*, 1999). Also, any molecular markers linked with *lf2* locus in soybean were not identified. The objective of this research was to identify molecular markers linked to the *lf2* locus.

MATERIALS AND METHODS

Plant genotype and DNA isolation

Normal three-leaflet cultivar Sinpaldalkong and seven-leaflet mutant genotype T255 was used as parents. The genotype of Sinpaldalkong (female) was *Lf2Lf2* and that of T255 (male) was *lf2lf2*. The leaflet phenotype of both parents is presented in Fig. 1. Cultivar Sinpaldalkong was crossed with T255 and F_1 seeds were planted to produce F_2 seeds in the greenhouse. Parents and F_2 seeds were planted in the field on May, 2004. Total 115 F_2 individual plants and parents were grown. All leaflet counts of parents and F_2 individual plants were made in the field on fully expanded leaves on the main stem when terminal growth of the main stem had ceased. Chi-square was partitioned to test for mode of single gene inheritance. Total 94 individual F_2 plants were used in mapping. Young leaves were collected from the 94 individual F_2 plants and parent plants. Genomic DNA was extracted from finely ground leaf tissue by means of a modi-

Fig. 1. Phenotype of normal three-leaflet parent type, Sinpaldalkong (left) and the seven-leaflet mutant type, T255 (right).

†Corresponding author: (Phone) +82-55-751-5421 (E-mail) jongil@gnu.ac.kr
<Received March 16, 2006>

fied CTAB procedure (Saghai Maroof *et al.*, 1984).

RAPD and SSR marker analysis

For the analysis of random amplified polymorphic DNA (RAPD) markers, one-thousand 10-mer oligonucleotide primers were obtained from Operon Technologies (Alameda, U.S.A). 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 seconds at 42 °C, and 70 seconds at 72 °C, followed by 39 cycles of 16 seconds at 92 °C, 22 seconds at 42 °C, and 70 seconds at 72 °C with 1 cycle of 5 minutes at 72 °C before ending. Amplified 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. For the analysis of simple sequence repeats (SSR) markers, total 664 SSR primers were used (Cregan *et al.*, 1999). SSR amplification reactions were performed in a 10 volume of reaction [2 μl genomic template DNA (20 ng/μl), 2 μl Satt primer (10 mM/μl), 5 unit Taq 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 analyzed. Silver staining method was used to separate the PCR product.

Genetic linkage analysis

Primers that distinguished the parents were tested on the entire F_2 population. A linkage map of markers was constructed with the data of markers and phenotypes (normal three-leaflet type/mutant seven-leaflet type) obtained from 94 F_2 progenies using the computer program MAPMAKER v. 3.0 (Lander *et al.*, 1987). Markers were assigned to group using the "Group" command, with a LOD score of 3.0 and maximum recombination distance of 50 cM. Map distances (cM) were computed using the Kosambi (Kosambi, 1944) mapping function .

RESULTS AND DISCUSSION

The normal three-leaflet and seven-leaflet type were segregated in F_2 population (Fig. 2). Ninety four F_2 plants showed the normal three-leaflet type as Sinpaldalkong and twenty one F_2 plants showed seven-leaflet type as T255. The summarized data for the segregation of F_2 plants are shown in Table 1. Segregation of the seven-leaflet type gave a good fit to the 3 : 1 ratio based on F_2 genotypes (normal : mutant = 94 : 21, Chi-square value = 2.79 P>0.05).

Fig. 2. Segregation of leaflet type in F_2 generation. *Lf2_* genotype shows normal three-leaflet and *lf2* genotype presents seven-leaflet type.

Table 1. Observed and expected segregation of F_2 plants from selfed F_1 soybean plants from the cross Sinpaldalkong (*Lf2Lf2*) x T255 (*lf2lf2*) for the leaflet type.

	Number of F2 plants				
Parent-cross	Normal	Mutant	Expected	Chi-square (3 : 1)	P
Sinpaldalkong	all				
T255		all			
F_1	all				
F_2	94	21	86.3 : 28.8	2.79	0.05-0.10

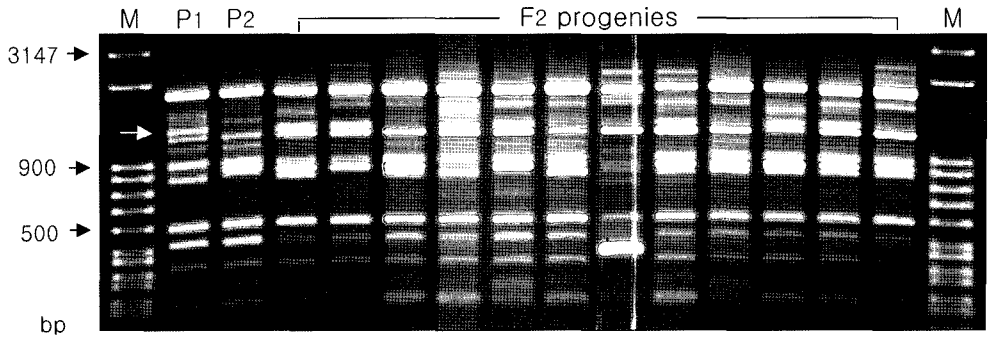

Fig. 3. Patterns of segregating DNA fragments for RAPD primer OPAD03 in parents and F_2 population. P1 is Sinpaldalkong (*Lf2Lf2*) and P2 is T255 (*lf2lf2*). M is marker and white arrow is polymorphic band.

The segregation ratio of 3 : 1 observed in the F_2 population and the Chi-square values strongly suggested that the seven-leaflet character was controlled by a single recessive gene (Table 1). Previous studies observed that seven-leaflet trait in soybean was inherited as a single recessive gene (Fehr, 1972). Of the 1,000 RAPD primers tested on the two parents of Sinpaldalkong (*Lf2Lf2*) and T255 (*lf2lf2*), 66 polymorphic primers were identified. Approximately 6.6% primers produced polymorphic DNA fragment differences between the parents. Among 66 polymorpic primers, 9 primers that showed polymorphism in preliminary test consisting of both parents and 10 normal three-leaflet F_2 plants and 10 seven-leaflet F_2 plants was used to obtain segregation ratio in 94 F_2 population. Fig. 3 represents some examples of segregating DNA fragments for RAPD markers (OPAD03) in parents and F_2 population. Eighty four SSR primers were identified in parental polymorphic test. Among 84 polymorpic primers, 5 primers that showed polymorphism in preliminary test consisting of both parents and 10 normal three-leaflet F_2 plants and 10 seven-leaflet F_2 plants were used to obtain segregation ratio in 94 F_2 population.

A genetic map was constructed from the 15 segregating markers (9 RAPDs, 5 SSRs, and a *lf2* locus). Of the 15 markers, 7 markers (3 RAPDs, 3 SSRs, and a *lf2* locus) were found to be genetically linked. These markers coalesced into 2 linkage groups. Linkage group 1 showed that RAPD primers OPAD03 and OPAI13 was linked to *lf2* locus that controlled seven-leaflet type in soybean at a distance of 20.5 and 23.5 cM, respectively (Fig. 4).

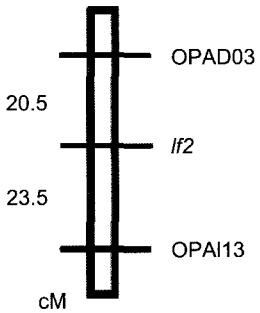

Fig. 4. Linkage group of lf2 locus that controlled seven-leaflet trait identified using 94 F2 population derived from the cross of Sinpaldalkong (Lf2Lf2) and T255 (lf2lf2). Map was constructed using MAPMAKER/EXP (3.0, 50 cM). Marker loci names are on the right and Kosambi map distances are on the left.

Unfortunately, SSR markers were not linked to the *lf2* locus in this study although 84 SSR primers were identified in parental polymorphism test. So far, only Devine (2003) reported that *Pd2* locus, controlling dense pubescence was linked to *lf2* locus with a crossover frequency of 12.1 ± 2.2 %. *lf2 locus* has not been identified with any molecular linkage group of Cregan *et al.* (1999). The *lf2* allele is of interest for potential in increasing leaf tissue in forage soybean. Molecular markers identified in this study linked with *lf2* locus will be helpful to locate *lf2* locus on the public soybean molecular linkage map.

ACKNOWLEDGEMENTS

This work was supported by a grant (CG3122) from Functional Genomics Center (CFGC), 21C Frontier R & D Project, Ministry of Science & Technology.

REFERENCES

Cregan, P. B., T. Jarvik, A. L. Bush, R. C. Shoemaker, K. G. Lark, A. L. Kahler, N. Kaya, T. T. VanToai, D. G. Lohnes, Jongil Chung, and J. E. Specht. 1999. An integrated genetic linkage map of the soybean genome. Crop Sci. 39 : 1464-1490.

Devine, T. E. 2003. The Pd2 and Lf2 loci define soybean linkage group 16. Crop Sci. 43 : 2028-2030.

Fehr, W. R. 1972. Genetic control of leaflet number in soybean. Crop Sci. 12 : 221-224.

Kosambi, D. D. 1944. The estimation of map distance from recombination values. Ann. Eugen. 12 : 172-175.

Lander, E., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive

computer package for constructing primary genetic linkage maps of experiment and natural populations. Genomics 1 : 174-181.

Saghai Maroof, M. A., K. M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984.

Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. 81 : 8014-8018.

Takahashi, Y. and J. Fukuyama. 1919. Morphological and genetic studies on the soybean. Hokkaido Agr. Exp. Sta. Rept. No. 10 : 1-138.