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SSR Marker Linked to *f* Locus in Soybean

Ki-Chul Nam, Myung-Sik Kim, Woo-Hyeun Jeong, Seok-Hyeon Kim, and Jong-Il Chung†

Division of Applied Life Science, Agronomy major, Research Institute of Life Sci., Gyeongsang National University, Chinju 660-701, Korea

ABSTRACT Soybean has a morphological type with a broadened and flattened stem. Fasciation has been suggested as a new gene for soybean research. SSR marker linked to the *f* locus that controls fasciation phenotype has not identified within 10 cM. A mapping population consisting of 94 F_2 progenies was derived from a cross between wild type Clark (*FF*) and fasciation mutant C32 (*ff*). The phenotype of F_2 individual plants was recorded at R2 and R3 growth stage from field. One-thousand 10-mer oligonucleotide RAPD primers and 29 SSR primers selected from the D1b+W of the soybean molecular linkage map were used. A genetic map was constructed from the segregating 35 RAPD, four SSR markers and one phenotypic (wild type/fasciation) marker. The segregation ratios of 3 : 1 observed in the F_2 population and the Chi-square values strongly suggest that the fasciation trait is controlled by a single recessive gene. Satt537 marker was linked to *f* locus at a distance of 9.6 cM. Assignment of the *f* locus to linkage group D1b+W and identification of markers can be used as an initial step for fine mapping of the *f* gene.

Keywords : fasciation, mutant, molecular marker, SSR marker, soybean

In many vascular plants, fasciation includes changes of stem shape and phyllotaxy. The basic cause of fasciation is a disturbed metabolism in abnormal, and unpredictable tissue production usually detrimental to the plant (White, 1948). Fasciated soybean plants exhibit a broadened and flattened stem. Albertsen *et al.* (1983) reported three fasciated soybean genotypes (PI243541, PI83945-4, and Keito-mame) as having the same allele for fasciation inherited as a single recessive gene *f*.

Nonbranching fasciation has been illustrated as an example of a potentially new gene for soybean improvement to reduce machine harvest losses and to maximize seed yields at increased plant densities. Leffel *et al.* (1993) reported that selection for seed yield apparently decreased the penetrance and expressivity of the fasciated gene in the development of near- isogenic lines for fasciation. Fasciated lines were generally taller, more susceptible to lodging, and exhibited poorer quality seed as compared with commercial cultivars.

DNA markers have become fundamental tools for research involving soybean improvement programs. Several molecular maps have been generated using restriction fragment length polymorphism (RFLP), amplified restriction fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD) markers (Apuya *et al.*, 1988; Lark *et al.*, 1993; Akkaya *et al.*, 1995; Shoemaker and Specht, 1995; Keim *et al.*, 1997; Cregan *et al.*, 1999) in soybean. Microsatellites or simple sequence repeat (SSR) markers are highly polymorphic, abundant, and distributed throughout the genome (Cregan *et al.*, 1999). With the development and public release of SSR primers, SSR markers have become available on molecular soybean linkage group (Cregan *et al.*, 1999). Many SSR markers associated with loci that control important agronomical traits were reported in soybean (Cregan *et al.*, 1999; Kim *et al.*, 2004, 2006).

The *f* locus that control soybean fasciation was localized on the LG11 of the soybean conventional genetic map (Hedges *et al.*, 1990). The LG 11 of the classical soybean map containing the *f* locus was integrated with LG D1b+W of the soybean molecular map (Cregan *et al.*, 1999). Karakaya *et al.* (2002) reported that four SSR markers were linked with the *f* locus using the 70 F_2 progenies derived from a cross between Clark63 and the fasciation

†Corresponding author: (Phone) +82-55-751-5421
(E-mail) jongil@gnu.ac.kr <Received September 21, 2006>

mutant. SSR marker linked to the *f* locus within 10 cM was not identified. The objective of this research was to identify SSR markers linked to the *f* locus on the D1b+W of the soybean molecular map.

MATERIALS AND METHODS

Plant genotype and DNA isolation

The genotype of wild type Clark and fasciation mutant C32 (Clark- derived near isogenic line) were used. The genotype of Clark and C32 at the *F* locus was *FF* and *ff*, respectively. Clark and C32 genotype have the almost same genetic constituent except for *F* gene. Seeds of Clark and C32 were planted at greenhouse in March 2004. Cross of Clark and C32 was made and F_1 seed was obtained. F_1 seeds was planted at greenhouse in August 2004 and F_2 seeds was harvested. The seeds of parent and F_2 were planted at the field in May 2005. A mapping population consisting of 94 F_2 progenies was derived from a cross between Clark and C32. The phenotype of F_2 individual plants was recorded at R2 and R3 growth stage from field. Young leaves were collected from the 94 individual F_2 plants and parent plants. Genomic DNA was extracted from finely ground leaf tissue using a modified 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℃, 22 second at 42℃, and 70 second at 72℃, followed by 39 cycles of 16 second at 92℃, 22 second at 42℃, and 70 second at 72℃, before ending with 1 cycle of 5 minutes at 72℃. 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. For the analysis of simple sequence repeats (SSR) markers, a total of 29 SSR primers were selected from the D1b+W of the soybean molecular linkage map (Cregan *et al.*, 1999). Satt primers selected were synthesized by Bioneer, Inc. (Korea). SSR amplification reactions were performed in a 10 ㎕ volume of reaction [2 ㎕ genomic template DNA (20 ng/㎕), 2 ㎕ Satt primer (10 mM/㎕), 5 unit Tag polymerase, 0.4 ㎕ dNTP (1.25 mM/㎕), 2.2 ㎕ 5X reaction buffer, 3.8 ㎕ ddH_2O]. Samples were covered with 10㎕ of light mineral oil. The PCR amplification was carried out in a PTC-200 Thermocycler using 39 thermal cycles of 92℃ for 45 sec, 47℃ for 45 sec, 68℃ for 45 sec, and finally 72℃ for 5 min. The products were held at 4℃ until used. 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 using the computer program MAPMAKER v. 3.0 (Lander *et al.*, 1987) from the marker and phenotype (wild type/fasciation) data obtained from 94 F_2 progenies. Markers were assigned to group using the "Group" command, with a LOD score of 4.0 and maximum recombination distance of 50 cM. Map distance (cM) were computed using the Kosambi (Kosambi, 1944) mapping function.

RESULTS

The phenotype of wild type and the fasciation type was segregated in F_2 population (Fig. 1). Seventy F_2 plants showed the phenotype of wild type and twenty four F_2 plants showed fasciation phenotype. The summarized data

Fig. 1. Phenotype of the wild type (left) and the fasciation type (right).

Table 1. Observed and expected segregation of F_2 plants from the cross Clark (*FF*) × C32 (*ff*) for the fasciation phenotype.

Parent-cross	Number of F_2 plants		Expected	Chi-square	P
	Wild type	Fasciation			
Clark	10				
C32		10			
F_1	5				
F_2	70	24	3 : 1	0.01	0.90-0.95

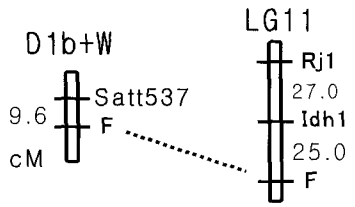

Fig. 2. Molecular linkage map D1b+W (Cregan *et al.*, 1999) of *f* locus defined using 94 F_2 plants derived from the cross of Clark (*FF*) and C32 (*ff*). Marker orders in D1b+W and LG11 were similar to those from the results by Cregan *et al.* (1999). Map was constructed using MAPMAKER/EXP (LOD 4.0 maximum distance 50 cM). Marker loci names are on the right and Kosambi map distances are on the left. LG11 is classical soybean linkage map.

for the observed and expected distribution of F_2 plants are shown in Table 1. Segregation of the fasciation phenotype gave a good fit to the 3 : 1 ratio based on F_2 genotypes (wild type : mutant = 70 : 24, Chi-square = 0.01, $0.90 < P < 0.95$). Of the 1,000 RAPD primers tested on the two parents of clark (*FF*) and C32 (*ff*), 35 polymorphic primers were identified. Approximately 3.5% primers produced polymorphic DNA fragment differences between the parents. These primers was segregated in the F_2 population. Among 29 SSR primers selected from the D1b+W of the soybean molecular linkage map, four primers showed parental polymorphism.

A genetic map was constructed from the segregating 35 RAPD, four SSR markers and one phenotypic (wild type/ fasciation) marker. Only Satt537 marker was linked to *f* locus at a distance of 9.6 cM (Fig. 2).

DISCUSSION

The segregation ratio of 3 : 1 was observed in the F_2 population and the Chi-square value strongly suggest that the fasciation trait is controlled by a single recessive gene (Table 1). Previous studies observed that fasciation phenotype in soybean was inherited as a single recessive gene (Albertsen *et al.*, 1983). The lower polymorphism (3.5%) between wild type parent Clark and C32 (Clark derived near isogenic line) estimated with RAPD markers might be due to the near isogenic nature of the parents. Unfortunately, all polymorphic RAPD markers were not linked to *f* locus that causes fasciation phenotype. Because RAPD marker is not codominant, it is quite possible that there are several RAPD markers tightly linked to the f locus among the 35 RAPD markers. Only four SSR primer (Satt141, Satt290, Satt296, Satt537) among 29 SSR primer selected from the D1b+W of the soybean molecular linkage map (Cregan *et al.*, 1999) that contains *F* locus showed parental polymorphism. However, only Satt537 primer was linked to the *f* locus at a distance of 9.6 cM (Fig. 2). Karakaya *et al.* (2002) reported identification of four SSR primers (Satt005, Satt141, Satt600 and Satt703) to the f locus using the 70 F_2 progenies. Among four SSR markers, Satt141 marker was more closer to the *f* locus. But, exact genetic distance between Satt141 marker and *f* locus was not reported. Satt141 marker was not linked to *f* locus in this study. Although many markers did not show parental polymorphism, SSR marker that was linked to the *f* locus at exact genetic distance was firstly identified in this experiment. Linkage of the *f* locus and Satt537 on the molecular linkage map D1b+W suggests further investigations.

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