Identification of AFLP Marker Linked to a SCN Resistant Gene in Soybean

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ABSTRACT

The soybean cyst nematode (*Heterodera glycines* Inchinoe; SCN) is a devastating pest of soybean and is responsible for significant losses in yield. The use of resistant cultivars is the effective method to reduce or eliminate SCN damage. The objective of this research is to identify AFLP markers linked to the SCN resistant genes. Bulked genomic DNA was made from resistant and susceptible genotypes to SCN and a total of 19 primer combinations were used. About 31 fragments were detected per primer combination. The banding patterns were readily distinguished in resistant and susceptible bulked genotypes. Polymorphic fragments were detected between resistant and susceptible bulked genotypes in the primer combination of CGT/GGC, CAG/GTG and CTC/GAG. In primer combinations of CGT/GGC and CAG/GTG, bulked resistant genotype produced a polymorphic bands. However, in primer of CTC/GAG, bulked susceptible genotype produced a polymorphic fragments. Three AFLP markers identified as a polymorphic fragments between bulked genomic DNA were mapped in 85 F2 population. Among them, only two markers, CGT/GGC and CTC/GAG, was linked and was mapped. Broad application of AFLP marker would be possible for improving resistant cultivars to SCN.

Key Words : AFLP, soybean, molecular marker, SCN AFLP Marker to SCN in Soybean

INTRODUCTION

The soybean cyst nematode (*Heterodera glycines* Inchinoe; SCN) is a devastating pest of soybean and is responsible for significant losses in yield. SCN causes root necrosis, reduced nodulation and decreased shoot vigor. The use of resistant cultivars is very effective in reducing crop loss to SCN. Genetic studies have demonstrated that resistance to SCN is oligogenic (Caldwell et al., 1960; Matson and Williams, 1965; Myers and Anand, 1991; Rao-Arelli et al., 1992; Rao-Arelli, 1994). Multiple races of SCN exist (Riggs and Schmitt, 1988). Breeding for resistance has been complicated by the genetic heterogeneity of SCN populations (Niblack, 1992) and the oligogenic nature of resistance (Caviness, 1992). Several genes involved in resistance to specific races have been identified (Caldwell et al., 1960; Matson and Williams, 1965). These include *rhg1, rhg2, rhg3, and Rhg4.* A number of sources of genetic resistance have been identified and used. These include Peking (PI548402), PI88788,

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Table 1. Phenotypic characteristics of soybean genotypes used in this study.

Genotype	Mat[a]	Traits[b]						SCN Response[c]
		1	2	3	4	5	6	
PI 548402	IV	D	W	T	Br	B1	Bl	R
PI 88788	III	N	W	T	Br	B1	Bl	R
PI 89772	IV	N	P	T	Br	B1	Bl	R
PI 90763	IV	N	P	Lt	Tn	B1	Bl	R
PI 438489B	IV	N	P	T	B1	Bl	Bl	R
PI 548667	V	D	P	G	Tn	Y	Ib	S
PI 518664	V	D	W	G	Tn	Y	Bf	S

a) Maturity group

b) Traits

1. Stem termination: Determinate, INdeterminate
2. Flower color: Purple, White
3. Pubescence color: Tawny, Light tawny, Gray
4. Pod color: Black, Brown, Tan
5. Seed coat color: Black, Yellow
6. Hilum color: Black, Imperfect black, Buff

c) R: resistant, S: susceptible

PI437654, PI90763, PI 89772 and PI209332. Also, these are an important sources of resistance to SCN, giving resistance to Races 1, 3, and 5. The allele for partial resistance at the *rhg1* resistance locus has been demonstrated to controll more than 50% of the variation for resistance (Concibido et al., 1996, 1997) and appears to effectively control a number of SCN races (Concibido et al., 1997; Webb et al., 1995).

Molecular markers have become fundamental tools for research involving plant genomes. Recently, molecular mapping has been used to enhance our understanding of SCN resistance in soybean (Concibido et al., 1994, 1996; Mahalingam and Skorupska, 1995; Webb et al., 1995). Although resistance to SCN is a complex trait, a quantitative trait locus (QTL) that explains more than 50% of resistance was mapped to linkage group G (Concibido et al., 1996). This QTL is present in several resistance sources, including PI209332, PI88788, PI90763, PI437654 and Peking, and confers partial resistance to several races of SCN. Cregan et al. (1999) identified two simple sequence repeat markers linked to the *rhg1* locus. These markers were located 0.4 cM proximal to the *rhg1*, soybean cyst nematode resistance locus on soybean linkage group G. Matthews et al. (1998) identified PCR primer linked closely to the *Rhg4* locus conferring resistance to race 3 of the SCN. Kim et al. (1999) reported that SCN races 1, 3, 5 and 6 are important in soybean field of Korea. Intensive genetic and breeding studies were not well done in Korea. Molecular markers linked to the SCN resistant genes would be used broadly to develop SCN resistant cultivars. The objective of this research is to identify AFLP markers linked to the SCN resistant genes and is to map markers identified.

MATERIALS AND METHODS

Plant materials and DNA extraction

Seeds of five SCN resistant genotypes (PI548402, PI89772, PI90763, PI88788 and PI438489B) and two susceptible genotypes (PI548667 and PI518664) were

Table 2. Nucleotide sequences of adapters and primers used in this study.

adapters/primers	sequence
MseI Adapters	5'-GACGATGAGTCCTGAG-3'
	3'-TACTCAGGACTCAT-5'
EcoRI Adapters	5'-CTCGTAGACTGCGTACC-3'
3'-CTGACGCATGGTTAA-5'	
MseI primers	5'-GATGAGTCCTGAGTAA-3'
EcoRI primers	5'-ACTGCGTACCAATTC-3'
MseI primer +3primer	CAA/CGT/CTC/CTG/CAC/CAG/ACC
EcoRI primer +3primer	GTG/GAG/GGC/GAA/AGT/AGG/ACG

obtained from USDA soybean germplasm collection center. Resistant genotypes are an important source of resistance to SCN giving resistance to all known races. Two susceptible genotypes are susceptible to all known SCN races. Agronomic traits of 7 genotypes used in this study are presented in Table 1. All genotypes were grown in the greenhouse. Young leaves were harvested. Two susceptible genotypes were bulked and five resistant genotypes were also bulked. Genomic DNA of bulked samples was extracted using the CTAB method.

AFLP Analysis

The EcoRI, MseI adapters and primers with three selective nucleotides were synthesized by Bioneer, INC (Korea). Sequence of adapter and primer is presented in Table 2. Restriction and ligation were done concurrently by adding 2.5㎍ genomic DNA to 60㎕ buffer[10mM Tris-HCl, pH7.5, 10mM Mg-acetate, 50mM K-acetate, 10mM EcoRI adapter, 10mM MseI adapter, 1 unit T4 DNA ligase and 0.1uM ATP. The mixture was incubated at 37℃ for 6 hrs. A 25㎕ PCR reaction contained 2.5㎕ of the re-suspended DNA sample, 10mM of each +3 primer, 10X reaction buffer 2.5㎕, 1.5mM MgCl2, 0.5 unit of Ampli tag DNA polymerase (Promega), and 250 uM of dNTP. Samples were covered with 15㎕ of light mineral oil and subjected to 30 thermal cycles of 94℃(denature) for 30s, 60℃(annealing) for 60s, 72℃(extension) for 60s. The PCR reaction was performed in a MJ research PTC-200 Thermocycler.

Selective PCR and silver staining

A 30㎕ master mix solution[ddH2O 25.9㎕, 10X buffer 2.5㎕, dNTP(1.25mM/㎕) 4㎕, EcoRI anchor 1.2㎕(10mM/㎕), MseI anchor 1.2㎕(10mM/㎕), 1 unit tag polymerase (Promega), DNA 4㎕ (2.5㎍/㎕)]. Samples were covered with 15㎕ of light mineral oil andsubjected to 30 thermal cycles of 94℃(denature) for 30s, 65℃(annealing) for 30s, 72℃(extension) for 60s, -1℃ cycle go to step 1, 9 cycle, 94℃ for 30s, 56℃ for 30s, 72℃ for 60s, go to step 5, 29 cycle 72℃ for 10 min. The PCR products were mixed with an equal volume(30㎕) of loading 3X buffer containing 98% formamide, 10mM EDTA, 0.01%(w/v) bromo phenol blue, 0.01%(w/v) xylene cyanole. The mixture was incubated in boiling water for 5 minutes 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 using a 5% polyacryamide gel (8M urea), and 1X TBE buffer at 80W constant power for 2 hrs. The gel was pre-warmed for 20 to 30 minutes before loading the samples. DNA bands were visualized by silver staining. The gel were first fixed in 10% acetic acid for 20 minutes. The gel were then washed with a large quantity of distilled water 2 min for 3 times,

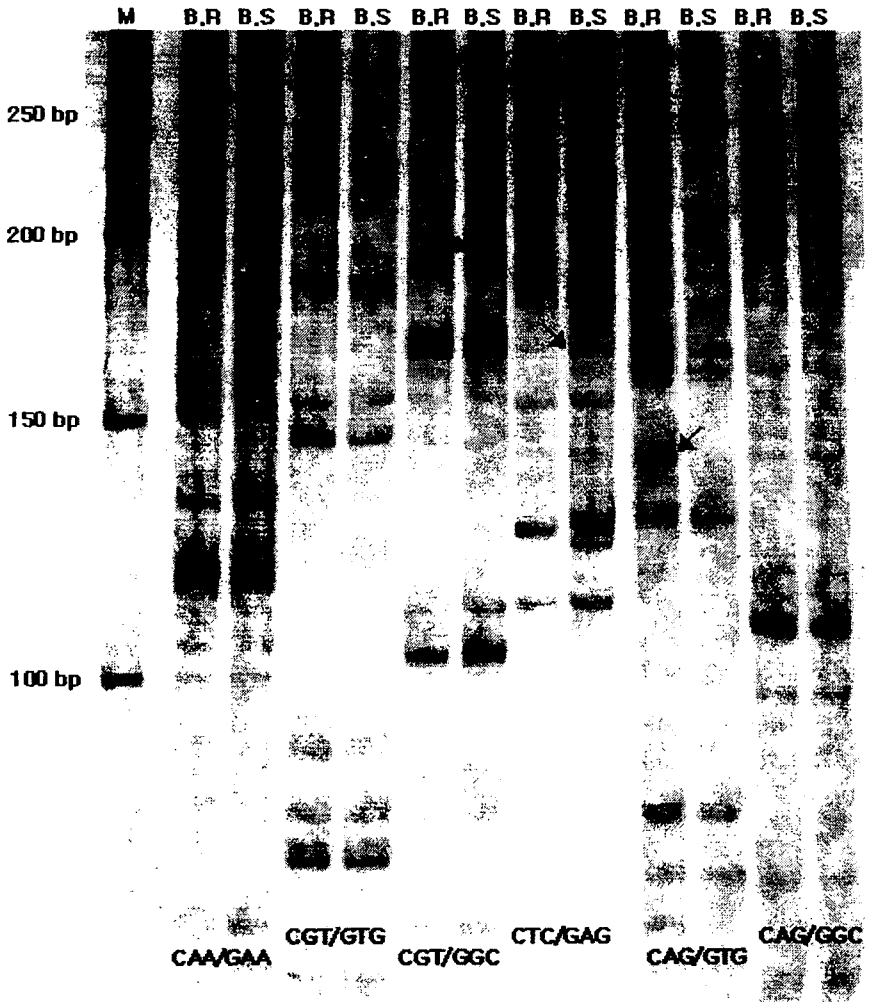

Fig. 1. Part of the amplified fragment length polymorphism autodiogram of CAA/GAA, CGT/GTG, CGT/GGC, CTC/GAG, CAG/GTG, and CAG/GGC primer combinations. M is molecular marker. B.R. is bulked resistant genotype and B.S. is bulked susceptible genotype. Arrows indicate polymorphic fragments.

respectively. The gel was transferred to a silver in pregnation solution(1.5g/L AgNO3, 1.5ml 37% formaldehyde solution/L) for 30 min, followed by a 5 sec rinse with distilled water. The image development step was done with manual agitation for 1 to 2 min in a developer solution (30g/L Na2 CO3, 1.5ml 37% formaldehyde solution, 400 ug/L sodium thiosulfate), and was followed by a 1 min fixation 10% acetic acid solution. The gel was then rinsed briefly in distilled water and dried at room temperature. Photography for silver stained dry gels was done by exposing the gels to Promega automatic processor compatibl (APC) film for 40 sec of fluorescent ceiling light.

Construction of linkage map

85 individual F2 progenies derived from the cross of cultivar Kwangkyo (female) and wild type IT182305 (male) were used to map AFLP markers identified. The software program mapmaker/Exp 3.0b was used. Markers were assigned to groups using the "Group"

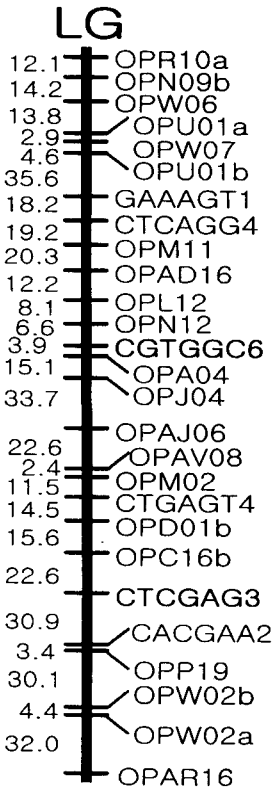

Fig. 2. Linkage group of CGT/GGC and CTC/GAG markers constructed using MAPMAKER (4.0 50) in 85 F2 progenies. Marker loci names are on the right and Kosambi distances are on the left.

command, with a LOD score of 4.0 and maximum recombination distance of 50 cM. Map distances were computed using the Kosambi mapping function.

RESULTS AND DISCUSSION

A total of 19 primer combinations were used. About 31 fragments were detected per primer combination. The number of fragments produced between genomic DNA of bulked resistant and susceptible genotypes were very similar. PCR-amplification pattern of resistant and susceptible bulked genomic DNA is presented in Figure 1. The banding patterns were readily distinguished in resistant and susceptible bulked genotypes. Polymorphic fragments were detected between resistant and susceptible bulked genotypes in the primers of CGT/GGC, CAG/GTG and CTC/GAG. In primer combinations of CGT/GGC and CAG/GTG, bulked resistant genotype produced a polymorphic bands. However, in primer of CTC/GAG, bulked susceptible genotype produced a polymorphic fragments. Therefore, AFLP markers identified polymorphism

between SCN-susceptible and -resistant soybean genotypes and would be used in the breeding program to SCN. Many types of molecular markers were identified in resistant and susceptible genotypes to SCN. Four independent partial SCN resistance loci were uncovered at $P < 0.0002$ (probability per locus) by the Concibido et al. (1997). Two RFLP markers, pA85 and pB32, were found to be significantly associated with SCN disease response, together accounting for 51.7% of total phenotypic variation (Concibido et al., 1994). Meksem et al. (2001) produced a high-density genetic map for the intervals carrying *Rhg1* and *Rhg4* using bulked segregant analysis with 512 AFLP primer combinations and microsatellite. Chang et al. (1997) identified two RAPD markers, OI03 and OW15, linked to the SCN race 3 resistant loci. Also, Wang et al. (2001) detected three significant QTLs for SCN race 3 resistance. Two microsatellites, BARC-Satt038 and BARC-Satt130 flanked the major SCN resistance locus on molecular linkage group G (Mudge et al., 1997). Concibido et al. (1996) reported that a major partial resistance locus on linkage group G near RFLP marker C006V was effective against SCN race 1, 3, and 6. Prabhu et al. (1999) used two molecular markers, BLT65 and Satt038 to identify SCN resistant genotypes to race 3. Cregan et al. (1999) reported two SSR markers, Satt309 and Sat_168, cosegregated to *rhg1* locus. Matthews et al. (1998) identified molecular markers closed to the *Rhg4* locus conferring resistance to soybean cyst nematode race 3 on linkage group A of

soybean. Three AFLP markers identified as a polymorphic fragments between bulked genomic DNA were mapped in 85 F2 population. Among them, only two markers, CGT/GGC and CTC/GAG, was mapped (Figure 2). this linkage group including two markers was not matched to the public soybean genome linkage map from USDA/Iowa State University contains 1004 markers (486 microsatellite, 501 RFLP, ten RAPD, four isozyme and three classical markers) in 20 linkage groups (Cregan et al., 1999). Therefore more marker should be added. Broad application of AFLP marker would be possible for improving resistant cultivars to SCN.

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