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Molecular Marker Analysis for Resistance of Soybean Cultivars to Soybean Cyst Nematode

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ABSTRACT: Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is an important soybean pest and the use of resistant cultivars is the effective method to reduce or eliminate SCN damage. However, breeding for SCN resistance is difficult and expensive by the oligogenic nature of the resistance and genetic variability in the pathogen. Fortunately, SCN resistance loci, *rhg1* and *Rhg4* are generally accepted as a necessity for the development of resistant genotypes using any source of resistance. In this study, resistance of 44 Korean soybean cultivars to SCN was tested using two molecular markers. Seonheukkong and Pokwangkong were the homozygous to *rhg1* locus. Seven cultivars were susceptible to SCN based on Satt309 marker linked *rhg1* locus. All Korean cultivars estimated in this study were recessive homozygous to *Rhg4* locus and were susceptible in the PCR reaction using primer 548/563 linked to the *Rhg4* locus conferring resistance to SCN race 3. Among 44 cultivars estimated, seven cultivars were susceptible to SCN in both Satt309 and primer 548/563 markers. Based on both Satt309 and primer 548/563 markers, there is no resistant cultivar to SCN in Korea. Therefore, SCN resistant cultivars need to be developed in the future. These two markers can be used for improving SCN resistant cultivars.

Keywords **:** soybean, molecular marker, SCN.

The soybean cyst nematode (*Heterodera glycines* Inchinoe; SCN) is one of the most economically destructive pathogens of soybean. SCN causes root necrosis, reduced nodulation and decreased shoot vigor. Multiple races of SCN exist (Riggs and Schmitt, 1988). 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). 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, 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).

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. But, so far, intensive genetic and breeding studies were not well done in Korea. In the present study, we test the resistance of Korean soybean cultivars to SCN using molecular markers to obtain the basic knowledge for improving SCN resistant soybean cultivars.

MATERIALS AND METHODS

Plant materials and DNA extraction

Seeds of four SCN resistant genotypes (Peking, PI89772, PI90763, and PI437654) and two susceptible genotypes (PI548667 and PI518664) were obtained from USDA soybean germplasm collection center. Four resistant genotypes are an important source of resistance to SCN giving resistance to all known races. Two susceptible genotypes are sus-

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Table 1. SCN resistant gene, primer linked, forward (F) and reverse (R) sequence for Satt309, and primer 548/563 sequence.

Gene	Primer linked	Sequence (5' to 3')
rhg1	Satt309 (F)	TTCTGCGGTTAAACTTCCGCG
rhg1	Satt309 (R)	TCAAAGCCCAAAATAAATTCCGCG
Rhg4	548	GCAGATATCAACAGTTGGGAC
Rhg4	563	GGAATGGACAGCTCGTAAAGCC

ceptible to all known SCN races. Seeds of 44 Korean soybean cultivars were obtained from National Crop Experiment Station, RDA. All genotypes were grown in the greenhouse. Young leaves were harvested and genomic DNA extracted using the CTAB method.

Marker selection and PCR

Among many molecular markers linked to the SCN resistance locus, Satt309 marker linked tightly to the *rhg1* resistance locus that has been demonstrated to control more than 50% of the variation for resistance was selected (Cregan *et al.*, 1999). And, primer 548/563 marker linked to the *Rhg4* locus conferring resistance SCN race 3 was selected (Matthews *et al.*, 1998). The DNA sequence for both markers is presented in Table 1. Both primers were synthesized and used. PCR reactions for primer 548/563 marker were performed using the method of Williams *et al.* (1990). Reactions of 10 μl contained 20-25 ng of genomic DNA, 1.5 mM Tris-HCl, pH 8.3, 6 mM KCl, 2.75 mM $MgCl_2$, 100 μM dNTPs, 0.8 μM of primer 548 and primer 563, and 1.8 units of Taq polymerase. Reations were heated for 2 min and amplified for 35 cycles at 94°C for 1 min, 57°C for 30 sec and 72°C for 1 min. Amplified DNAs were separated on a 2 agarose gel containing TBE and stained with ethidium bromide. The amplification protocols for Satt309 marker were similar to those used by Akkaya *et al.* (1992). The PCR reaction mix contained 50 ng of genomic DNA, 0.1M of each of the paired primers, 5X reaction buffer (250 mM Tris), 0.6 unit DNA polymerase, and 2.5 M of each of the four dNTPs. Each sample was subjected to 31 cycles of denaturing (25 sec at 94°C); annealing (25 set at 47°C); and extension (25 sec at 68°C) in a thermocycler, followed by a final extension step (3 min at 72°C) and incubation at 4°C.

Silver staining

The PCR products for Satt309 marker were mixed with an equal volume (25 μl) of loading buffer containing 98% formamide, 10 mM EDTA, 0.01% (w/v) bromo phenol blue, 0.01% (w/v) xylene cyanol. The mixture was incubated in boiling water for 3 minutes and cooled on ice before 6 μl was loaded to each lane of a 5% denaturing polyacrylamide sequencing gel (8 M urea). The gels were run at 80 watts constant power for 90 min. The gels were first fixed in 7.5% acetic acid for 30 min. It was then washed three times with a large quantity of ddH_2O water for 5, 3 and 2 min, respectively. The gel was transferred to a silver impregnation solution (1.5 g/L $AgNO_3$, 0.056% formaldehyde) for 30 min, followed by a 5 sec rinse with ddH_2O water. All of the above steps were done with slow agitation on an automatic shaker. The image development step was done with manual agitation for 1 to 2 min in a developer solution (30 g/L Na_2CO_3, 0.056% formaldehyde, 400 μg/L sodium thiosulfate) followed by a 1 min fix in 7.5% acetic acid. The gel was then rinsed in ddH_2O water and dried at room temperature. Photography of silver stained gels was made by exposing the gel to Promega APC paper for 45 sec on fluorescent ceiling light.

RESULTS AND DISCUSSION

Satt309 marker was previously mapped (LG G) in three populations as part of an effort to develop and map approximately 600 soybean SSR loci (Cregan et al. 1999). Analysis of SCN susceptible and resistant soybean genotypes revealed four SSR alleles at the Satt309 locus (Fig. 1). All resistant genotypes, PI89772 (lane 1), PI90763 (lane 2), PI548402 (lane 3), and PI437654 (lane 4) produced allele 3 and 4. However, allele 1 and 2 was produced in only two susceptible genotypes, PI548667 (lane 5) and PI518664 (lane 6). Bulked genomic DNA of four resistant genotypes (lane 1 to 4) produced allele 3 and 4. Also, allele 1 and 2 was identified in the bulked genomic DNA of two susceptible genotypes (lane 5 and 6). Therefore, allele 3 and 4 identified by Satt309 primer is the SCN resistant molecular marker

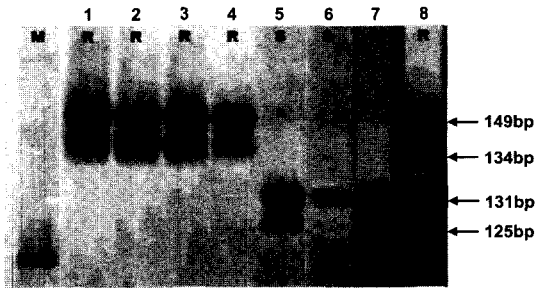

Fig. 1. Four alleles at the Satt309 SSR primer linked to the *rhg1* locus in SCN resistant (lane 1 to 4) and susceptible (lane 5 and 6) genotypes. M is marker. Lane 1, PI89772 (R); 2, PI90763 (R); 3, PI548402 (R); 4, PI437654 (R); 5, PI548667 (S); 6, PI518664 (S); 7, bulked DNA of susceptible lane 5 and 6; 8, bulked DNA of resistant lane 1 to 4. R is resistant and S is susceptible. Allele 1 and 2 is susceptible and allele 3 and 4 is resistant fragments.

and allele 1 and 2 is the susceptible marker. This result was consistent with the results of earlier studies. Using Satt309 primer, Cregan *et al.* (1999) identified four alleles from SCN resistant and susceptible genotypes and cultivars. Allele 3 and 4 presented in most resistant genotypes and allele 1 and 2 presented in susceptible genotypes.

The PCR reaction of primer 548 and 563 using genomic DNA of SCN resistant and susceptible genotypes produces several DNA fragments (Fig. 2). The polymorphic band (1150 bp) was identified between resistant and susceptible genotypes. All resistant genotypes, PI89772 (lane 1), PI90763 (lane 2), PI548402 (lane 3), and PI437654 (lane 4) produced the fragment. However, no fragment was produced in only two susceptible genotypes, PI548667 (lane 5) and PI518664 (lane 6). Also, bulked genomic DNA of four resistant genotypes (lane 1 to 4) produced polymorphic band. But, it was not in the bulked genomic DNA of two susceptible genotypes (lane 5 and 6). Therefore, polymorphic fragment by primer 548 and 563 was approved to identify the SCN resistant and susceptible genotypes. Matthews *et al.* (1998) identified the polymorphic band between SCN resistant and susceptible genotypes using primer 548 and 563. This banding patterns was readily distinguished in all cultivars examined.

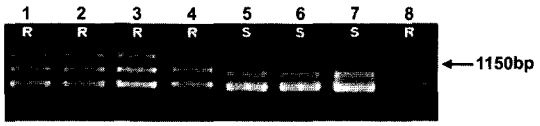

Fig. 2. The fragments amplified by primer 548 and 563 linked to the *Rhg4* locus. Lane 1, PI89772 (R); 2, PI90763 (R); 3, PI548402 (R); 4, PI437654 (R); 5, PI548667 (S); 6, PI518664 (S); 7, bulked DNA of susceptible lane 5 and 6; 8, bulked DNA of resistant lane 1 to 4. R is resistant and S is susceptible. Resistant fragment is indicated by arrow.

Table 2. Soybean cultivar response to Satt309 marker located 0.4 cM proximal to the *rhg1* locus and 548/563 primer linked to the *Rhg4* locus (resistance to SCN race 3).

Cultivar	Marker		SCN response	Cultivar	Marker		SCN response
	Satt309	Primer 548/563			Satt309	Primer 548/563	
PI 89772	R	R	R	Myeongjunamulkong	M	S	S
PI 90763	R	R	R	Seokryangputkong	M	S	S
PI 548402	R	R	R	Muhankong	M	S	S
PI 437654	R	R	R	Jangsukong	M	S	S
PI 548667	S	S	S	Hwankgeumkong	M	S	S
PI 518664	S	S	S	Sinpaldalkong	M	S	S
Jangwonkong	M	S	S	Pureunkong	M	S	S
Kwanggyo	M	S	S	Hwaeomputkong	M	S	S
Sowonkong	S	S	S	Danbaekkong	S	S	S
Dawonkong	M	S	S	Geomjeongkong 1	M	S	S
				Jinyulkong	M	S	S
Sodamkong	M	S	S				
Sinrokkong	M	S	S	Milyangkong	M	S	S
Baekunkong	M	S	S	Jangbaegkong	M	S	S
Sinpaldalkong 2	M	S	S	Deokyukong	M	S	S
Geomjeongkong 2	M	S	S	Saealkong	M	S	S
Seonheukkong	R	S	S	Jangkyeongkong	M	S	S
Taekwangkong	M	S	S	Eunhakong	S	S	S
Daewonkong	M	S	S	Dankyungkong	M	S	S
Jangyeopkong	M	S	S	Pangsakong	M	S	S
Somyeongkong	S	S	S	Jangyeopkong	S	S	S
Gwangankong	S	S	S	Bukwangkong	M	S	S
Jinpumkong 2	M	S	S	Baekcheonkong	M	S	S
Jinpumkong 1	M	S	S	Danyeopkong	S	S	S
				Samnamkong	M	S	S
Bokwangkong	R	S	S				
Manrikong	M	S	S	Namcheonkong	M	S	S

R: resistant in case of both markers, S; susceptible in case of one marker.

The result for PCR reaction of genomic DNA from 44 Korean cultivars using Satt309 and primer 548/563 is presented in Table 2.

Two cultivars, Seonheukkong and Pokwangkong produced allele 3 and 4 in the PCR reaction using Satt309 primer. This result indicates that these two cultivars are the homozygous to rhg1 locus and are resistant to SCN. Seven cultivars, Sowonkong, Somyeongkong, Kwangankong, Danbaekkong, Eunhakong, Jangyeopkong, and Danyeopkong produced allele 1 and 2 that indicates susceptible to SCN. 35 cultivars produced combination of allele 1 to 4 such as allele 2 and 3, 2 and 4, 1 and 3. This result indicates that most Korean cultivars are more diverse in Satt309 locus and are medium to SCN resistance. However, all Korean cultivars estimated in this study did not produced polymorphic band (1150 bp) in the PCR reaction using primer 548/563 linked to the *Rhg4* locus conferring resistance to SCN race 3. Based on primer 548 and 563 marker result, all korean cultivars are recessive homozygous to *Rhg4* locus and are susceptible. Among 44 cultivars tested, seven cultivars were susceptible in both Satt309 and primer 548/563 markers., A major goal of DNA marker analysis is to provide a foundation for marker-assisted breeding. Thus allelic differences at the Satt309 and primer 548/563 locus allow complete discrimination between resistant and susceptible genotypes. Selection using these markers is particularly useful for discarding susceptible genotypes and greatly reducing the number of lines that must be further tested in the greenhouse and field for resistance. In considering both Satt309 and primer 548/563 markers, there is no resistant cultivar to SCN in Korea. Therefore, SCN resistant cultivars needed to be developed in the future. These two markers can be used for improving SCN resistant cultivars.

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REFERENCES

Akkaya, M. A., A. Bhagwat, and P.B. Cregan. 1992. Length polymorphism of simple sequence repeat DNA in soybean. *Genetics* 132 :1131-1139.

Caldwell, B. E., C. A. Brim, and J. P. Rose. 1960. Inheritance of resistance of soybeans to the cyst nematode, *Heterodera glycines*. *Agron. J.* 52 : 635-636.

Caviness, C. E. 1992. Breeding for resistance to soybean cyst nematode. In: Riggs RD, Wrather JA (eds) Biology and management of the soybean cyst nematode. APS press, st. Paul, Minn., pp. 143-156.

Concibido, V. C., R. L. Denny, S. R. Boutin, R. Hautea, J. H. Orf, and N. D. Young. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). *Crop Sci* 34 : 240-246.

Concibido, V. C., R. L. Denny, D. A. Lange, J. H. Orf, and N. D. Young. 1996. RFLP mapping and Marker-assisted selection of soybean cyst nematode resistance in PI209332. *Crop Sci* 36 : 1643-1650.

Concibido, V. C., D. A. Lange, R. L. Denny, J. H. Orf, and N. D. Young. 1997. Genome mapping of soybean cyst nematode resistance genes inpeking, PI90763, PI88788 using DNA markers. *Crop Sci* 37 : 258-264.

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, J. Chung, and J. E. specht. 1999. An integrated genetic linkage map of the soybean genome. *Crop Sci.* 39 : 1464-1490.

Cregan, P. B., J. Mudge, E. W. Fickus, D. Danesh, R. Denny, and N. D. Young. 1999. Two simple sequence repeat markers to select for soybean cyst nematode resistance conditioned by the *rhg1* locus. *Theor Appl Genet.* 99 : 811-818.

Kim, D. G., J. K. Lee, and Y. K. Lee. 1999. Distribution of Races of Soybean Cyst Nematode in Korea. Korean *J. Appl. Entomol.* 38(3) : 249-253.

Matson, A. L., and L. F. Williams. 1965. Evidence of a fourth gene for resistance to the soybean cyst nematode. *Crop Sci.* 5 : 477.

Myers, G. O., and S. C. Anand. 1991. Inheritance of resistance and genetic relationships among soybean plant introductions to races of soybean cyst nematode. *Euphytica.* 55 : 197-201.

Mahalingam, R., and H. T. Skorupska. 1995. DNA markers for resistance to *Heterodera glycines* race3 in the soybean cultivar peking. *Breed. Sci.* 45 :435-443.

Matthews, B. F., M. H. MacDonald, J. D. Gebhart. and T. E. Devine. 1998. PCR markers residing close to the *Rhg4* locus conferring resistance to the soybean cyst nematode race3 on linkage gorup A_2 of soybean. *Theor Appl Genet.* 97 : 1047-1052.

Niblack, T. L., and D. C. NOrton. 1992. Soybean yield losses due to *Heterodera glycines* in lowa. *Plant Dis* 76 : 943-948.

Riggs, R. D., and D. P. Schmitt. 1988. Complete characterization of the race scheme for *Heterodera glycines*, *J. Nematol.* 20 : 392-395.

Rao-Arelli, A. P, S. C. Anand, and J. A. Wrather. 1992. Soybean resistance to soybean cyst nematode Race 3 is conditioned by an additional dominant gene. *Crop Sci.* 32 : 862-864.

Rao-Arelli, A. P. 1994. Inheritance of resistance to *Heterodera glycines* Race 3 in soybean accesions. *Plant Dis* 78 : 898-900.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by a arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 18 : 6531-6535.

Webb, D. M., B. M. Baltazar, A. P. Rao-Arelli, J. Schupp, P. Kelim, K. Clayton, A. R. Ferrira, T. Owens, and W. D. Beavis. 1995. Genetic mapping of soybean cyst nematoce race3 resistance loci in the soybean PI437654. *Theor Appl Genet* 91 : 574-581.