Korean Journal of Life Science
Vol. 13. No. 4. 375~382, 2003

Identification of Quantitative Trait Loci for Resistance to Soybean Cyst Nematode Race 14

In-Soo Choi[1]* and Yong Chul Kim[1]

[1]*Dept. of Plant Resources, Miryang National University, Miryang 627-702, Korea*

Abstract

A direct and precise explanation of soybean resistance to soybean cyst nematode will be possible only when the individual gene(s) involved in the resistance are tagged. This study was conducted; (1) to identify and localize quantitative trait loci for resistance to soybean cyst nematode race 14 on RAPD map, (2) to identify the magnitude and mode of inheritance for each quantitative trait loci, and (3) to identify the best combinations of quantitative trait loci for resistance to soybean cyst nematode race 14. Thirty markers (29 RAPD and 1 RFLP) showed significant association with resistance to soybean cyst nematode race 14. From MAPMAKER/QTL analysis, we identified two regions (linkage group C-7 and linkage group C-9) for resistance to soybean cyst nematode race 14. The first quantitative trait loci that was localized at 6.0 cM from $H06^1$ on linkage group C-7 showed a dominant inheritance mode. However, we can not exclude the possibility of additive inheritance mode. The second quantitative trait loci that was localized between $B15^2$ and $E01^1$ on linkage group C-9 also showed a dominant mode of inheritance. One pair of flanking markers ($H06^1$ and $H06^2$) and $B15^2$ were used for multiple regression analysis. Marker combination that included 2 markers, $B15^2$ and $H06^1$, explained the highest total variance (22.9%) for resistance to soybean cyst nematode race 14. Further localization of genes for resistance to soybean cyst nematode race 14 and examination of interaction between quantitative trait loci will accelerate the exploitation of resistance to soybean cyst nematode.

Key words – soybean, soybean cyst nematode, race 14, quantitative trait loci

Introduction

The limitation in effectiveness of controlling the soybean cyst nematode (SCN) using current management practices and environmental awareness necessitate the development of soybean cultivars resistant to SCN. However, breeding effort to incorporate resistance to SCN into elite lines is complicated by the quantitative nature of the trait. The existence of biological races within the nematode species makes it more complex[10]. Explanation of the complicated nature of soybean resistance requires detailed study of the resistance mechanisms and knowledge of the individual genes contributing resistance to SCN.

Resistance to SCN in soybean is expressed as a quantitative trait. Quantitative traits are the result of the interactions of many segregating genes. Each gene contributes a small amount of expression to the phenotype, so that the individual effects of genes are not discernible by Mendelian methods. Caldwell et al.[4] reported that resistance in Peking was conditioned by three recessive genes, which were designated as *rhg1*, *rhg2*, and *rhg3*. Matson & Williams[12] suggested that the dominant SCN resistance allele, *Rhg4*, is closely linked to *i* locus for black seed-coat color in cultivar Peking. Rao-Arelli et

*To whom all correspondence should be addressed
Tel : 82-55-350-5365, Fax : 82-55-350-5360
E-mail : ichoi@mnu.ac.kr

al.[16] reported a dominant gene, *Rhg4*, and two recessive genes for resistance to SCN race 3 in Peking. Sugiyama & Katsumi[21] proposed the black seed trait was linked with recessive gene for resistance in Peking. PI 88788 is another major source of resistance to SCN races 3 and 14. Resistance to race 3 in PI 88788 is conditioned by one recessive and two dominant genes[15]. Of the 141 F_2 progeny of the cross of Peking x PI 88788, 113 progenies were resistant and 28 were susceptible. In F_2 progenies of PI 88788 x PI 438496B cross, they observed the segregation ratio of 47 resistant and 2 susceptible, which gave a good agreement to the 15:1 ratio for a two gene segregation pattern.

In recent years, studies on resistance to SCN using molecular markers have been intensively developed in soybean. Weisemann et al.[22] detected two molecular markers, pBLT24 and pBLT65 that were closely linked to *i* locus, and suggested the association of these two markers with SCN resistance gene, *Rhg4*. Boutin et al.[3] reported the association of RFLP marker, pK69, with resistance to SCN race 3, and tentatively mapped pK69 to USDA/ISU linkage group B and G. Concibido et al.[8] detected two unlinked RFLP markers, pA85 (LGA) and pB32 (probably LGK), which showed significant association with SCN disease response using 56 F_2 lines from a cross between M83-15 and M85-1430. Mahalingam & Skorupska [11] identified the dominant QTL locus and localized this locus 0.6 cM from *i* locus in the molecular map of 90 $F_{2:3}$ progenies of the cross of Essex x Peking. We[7] reported the identification of 2 dominant and 1 recessive genes for resistance to SCN race 3.

The plant introduction PI 437654 has been reported to be resistant to all known races of SCN[1]. Myers & Anand[13] conducted SCN resistance study in the cross of PI 437654 x Essex. In bioassay for race 14, they observed 2 resistant and 121 susceptible plants, proposed one dominant and two recessive genes for resistance to SCN race 14. However, Anand et al.[2] suggested that PI 437654 must have more genes since it rarely had any cysts when infected with different populations of SCN.

Present knowledge is insufficient to surely and rapidly resolve the resistance to single interactions[10]. A direct and precise explanation of resistance will be possible only when the individual gene(s) involved in the resistance are tagged. If all the loci of the quantitative trait were tagged, the QTL genotypes could be obtained by scoring all the marker loci genotypes. This would reduce breeding for quantitative traits to selection of individual loci[20].

The objectives of this study were; (1) to identify and localize QTLs for resistance to SCN race 14 on RAPD map developed in our previous study[6], (2) to identify the magnitude and mode of inheritance for each QTL, and (3) to identify the best combinations of QTLs for resistance to SCN race 14.

Materials and Methods

We have reported plant materials, RAPD and RFLP analyses, strategies for RAPD marker selection, and construction of RAPD linkage map in previous papers [5, 6, 19]. Seventy-nine $F_{2:3}$ progenies derived from the cross of Essex x PI 437654 were used for this study. One hundred sixty-four RAPD markers, which have been selected by RAPD marker selection strategies, and 41 RFLP markers identified from 123 probes, were utilized for mapping of resistance to SCN.

Soybean cyst nematode bioassay

A homogeneous isolate of SCN race 14 was obtained from Dr. Riggs (University of Arkansas, AR) and reproduced on Essex. The isolates were maintained and increased in the growth chamber and greenhouse for 12 generations, and tested with differential lines (Peking, Pickett, PI 88788, PI 90763, PI 437654, and Essex) for race classification.

SCN bioassays were conducted in the greenhouse where temperature was maintained at 28°C. Pregerminated

seedlings (72 hrs at 27°C) were transplanted into plastic cups filled with sterile sand. Seven seedlings of each $F_{2:3}$ mapping population, differential lines for race classification, and both parents (Essex and PI 437654) were included in bioassay. The seedlings were well watered before inoculation. In about 2-3 days after transplanting, each seedling was inoculated with approximately 2000 nematode eggs. Irrigation was suspended for 24 hours post-inoculation to prevent the draining of the inoculum. Subsequently, the plants were watered 1 to 2 times a day depending on weather conditions. Thirty days after inoculation, the roots were carefully dislodged and washed with a powerful jet of water. Cysts were collected on a 75㎛ sieve and counted under the stereoscope. Female Index (FI) was used to estimate plant response to SCN race 14. FI was calculated for each $F_{2:3}$ genotype by average number of cysts on each genotype devided by the average number of cysts on susceptible check Essex, and it was expressed as a percentage.

Statistical analysis

Genotypic classes of molecular and morphological markers were contrasted with phenotypic variation (FI values) of SCN race 14. Association between markers and resistance to SCN race 14 was tested by linear regression analysis and analysis of variance (ANOVA) using the general linear fixed effect model. The homogeneity within- and between-genotypes was estimated by analysis of variance. Statistical analysis was conducted using PC-SAS version 6.0[18].

MAPMAKER/QTL (version1.1)[9,14] was used to identify the association of markers with phenotypic variation at each marker position. Map distances, orders of each linkage group, and phenotypic data were the input for MAPMAKER/QTL analysis. Each linkage group was scanned at every 2.0 cM for the detection of presence of QTL. A LOD threshold of 2.0 was used to declare the presence of a putative QTL in a given genome region. "QTL likelihood plots" was performed using the MAPMAKER/QTL covering the entire genome to estimate the following information: (1) regions in the genome which are likely to contain putative QTLs, (2) the strength of the data supporting the hypothesis that particular QTL exists, and (3) the likely position of putative QTLs. All possible modes of inheritance (dominant, additive, and recessive) were tested at each QTL position, and compared with the mode of inheritance in genetics free model. The mode of inheritance at each QTL that had similar 2 value, log-likelihood, and variance-explained in comparison with genetics free model was determined as a possible mode of inheritance for the QTL. Interactions among flanking markers and identification of the best marker combinations for resistance to SCN race 14 were estimated by multiple regression analysis and analysis of variance.

Results and Discussion

Response to SCN race 14.

The nematode isolate used for bioassays was confirmed as SCN race 14. When considering the average number of cysts on susceptible Essex as 100%, PI 88788 that is the only resistance source among race classification differential lines in response to SCN race 14 had FI of 1.1% (Table 1). The FI values of susceptible genotypes to

Table 1. Reaction to soybean cyst nematode race 14 of parental lines Essex and PI 437654, and SCN race classification differential lines

Genotype	Race 14	
	Range[1]	F. I.[2] (%)
Essex	115-204	100
PI437654	0	0
Peking	28-74	26.4
PI88788	0-4	1.1
Pickett	29-103	36.1
PI90763	27-62	25.6

[1]Number of cysts
[2]Female Index

SCN race 14 were 26.4%, 36.1%, 25.6% for Peking, Pickett, and PI 90763, respectively. PI 437654 showed complete resistance to race 14. It might be possible to suggest the presence of gene/genes for susceptibility on the production of small number of cysts(1.1%) on PI88788.

The distribution of FI values in response to SCN race 14 in $F_{2:3}$ progenies in Essex x PI 437654 is shown in Fig. 1. One genotype had complete resistance, and one genotype had FI value of 125%. The mean FI value of the $F_{2:3}$ population was 31.6 (Table 2), and 17 (22.7%) genotypes were resistant (FI<10%).

Analysis of variance to estimate the homogeneity between- and among-genotypes is presented in Table 3. Variance between F_2 progenies was highly significant (P = 0.0001), indicating genotypic differences in segregating population. F-value was 6.25. This result was expected in consideration of the wide range of FI of $F_{2:3}$ progeny, 0 - 125.0 (Table 2). Analysis of variance within genotypes showed no significance. However, the probability of 0.061 (Table 3) indicated that there were meaningful differences in female indices among plants of the same genotype. The standard deviation 75.6% (23.9/31.6) of mean FI supports this hypothesis (Table 2).

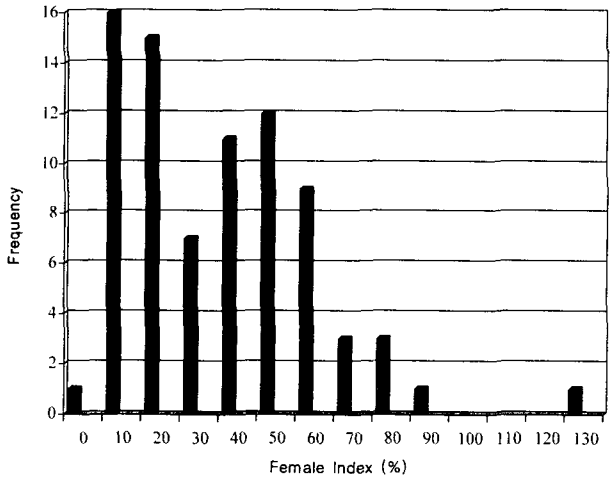

Fig. 1. Distribution of Female Index of response to soybean cyst nematode race14 in F2:3 progenies of Essex x PI 437654.

Table 2. Mean value and distribution of female index (FI) in response to soybean cyst nematode race 14 in $F_{2:3}$ progenies of Essex x PI 437654 cross

SCN race	Mean	SD[1]	F. I. value	
			minumum	maximum
race 14	31.6	23.9	0	125

[1]Standard deviation

Molecular marker association with SCN.

To identify associations of selected RAPD markers and unlinked markers for resistance to SCN race 14, univariate linear regression analysis using PC-SAS[18] was performed. Thirty markers (29 RAPD and 1 RFLP) showed significant association (Table 4). Eleven markers showed highly significant association. The highest variation-explained was 12. 9% by the RAPD marker H06[1].

Thirty markers that showed significant associations with resistance to SCN race 14 exceeded the number of suggested loci. Some reasons can cause the high number of detected markers. As we described in previous paper [7], the occurrence of Type 1 error can be one of the reasons for overestimating number of association. Another reason can be explained by that some of these markers might provide information for the same chromosomal region. Our preference was to detect more markers that can be confirmed in subsequent study than to ignore possibility of any molecular marker/SCN association. We used relatively relaxed significance level (P<0.05) for identification of marker/SCN association in univariate

Table 3. Analysis of variance within- and between- genotypes in response to soybean cyst nematode race 14 in $F_{2:3}$ progenies of Essex x PI 437654 cross

	Mean value of female index; 49.969 cysts/plant		
	d. f.	F-value	Pr>F
Between genotypes	74	6.25	0.0001**
Within genotypes	6	2.03	0.061

**Significant at 99% confidence level

Table 4. RAPD markers and RFLP markers that significantly associated with resistance to soybean cyst nematode race 14 from linear regression analysis in $F_{2:3}$ progenies of Essex x PI 437654 cross

Marker		L. G†	Select†	F-value	Pr>F	R^2value
RAPD	A021	C-12		4.09	0.047*	0.050
	A022	Group 1	S	7.58	0.007**	0.090
	A071	Group 2	S	6.81	0.011*	0.081
	A073	Group 2	S	5.62	0.020*	0.069
	A111	unlinked		5.21	0.025*	0.063
	A114	Group 2	S	5.29	0.024*	0.064
	A12	Group 1	S	4.43	0.039*	0.054
	A161	unlinked		5.47	0.022*	0.066
	B121	C-27		5.37	0.023*	0.065
	B151	Group 1	S	5.29	0.024*	0.064
	B152	C-9		8.77	0.004**	0.102
	C085	Group 1	S	7.14	0.009**	0.085
	D16	Group 2	S	4.83	0.031*	0.060
	E023	Group 2	S	5.80	0.018*	0.070
	G101	unlinked		4.37	0.040**	0.054
	G102	Group 2	S	7.58	0.007**	0.090
	G104	Group 2	S	7.58	0.007**	0.090
	G132	Group 2	S	7.58	0.007**	0.090
	G15	Group 2	S	7.58	0.007**	0.090
	H041	unlinked		6.68	0.012*	0.081
	H042	Group 2	S	7.67	0.007**	0.091
	H061	C-7		11.28	0.001**	0.129
	H062	C-7		5.67	0.020*	0.071
	H071	Group 2	S	6.54	0.013*	0.078
	H15	Group 1	S	5.93	0.017*	0.072
	N063	C-22		4.75	0.032*	0.058
	W03	Group 2	S	7.58	0.007**	0.091
	AW15	C-14		5.22	0.025*	0.067
	AW192	unlinked		4.52	0.037*	0.058
RFLP	pK418E1	C-20		4.57	0.036*	0.059

†Linkage group
†Markers from RAPD marker selection strategies
*Significant at 95% confidence level
**Significant at 99% confidence level

regression analysis to compare molecular marker association with those identified by MAPMAKER/QTL analysis. Furthermore, this strategy might minimize the possible Type II error.

Identification of QTL for resistance to SCN race 14. MAPMAKER/QTL analysis was performed to detect associations of molecular marker regions with resistance to SCN. We identified two regions for resistance to SCN race 14 and these two regions were mapped on LGC-7 and LGC-9 (Fig. 2). Diagnostic primers for these QTLs were; H061 and H062 on LGC-7, and B152 and E011 on LGC-9. (Table 5). The QTLs were assigned at LOD 3.80 and 2.44, and explained 42.2% and 16.7% of variation,

Table 5. Analysis of gene action at the QTL loci for resistance to SCN race 14

(1) LGC-7

Interval: $H06^1$ - $H06^2$
Length: 25.1
QTL-POS: 6.0
X^2 = 17.512 (2 d. f.) log-likelihood = 3.80
mean = 1.805 variance-explained = 42.2%
σ^2 = 0.106

Genetics	mean	X^2	log-likelihood	variance-explained (%)
Dominant	1.790	15.570	3.38	41.9
Recessive	1.595	11.294	2.45	16.3
Additive	1.711	13.702	2.98	26.6

(2) LGC-9

Interval: $B15^2$ - $E01^1$
Length: 7.8
QTL-POS: 2.0
X^2 = 11.217 (2 d. f.) log-likelihood = 2.44
mean = 1.562 variance-explained = 16.7%
σ^2 = 0.147

Genetics	mean	X^2	log-likelihood	variance-explained (%)
Dominant	1.555	9.388	2.04	12.8
Recessive	1.373	0.167	0.04	0.3
Additive	1.483	4.702	1.02	6.7

Unit; centimorgan (cM)
QTL position

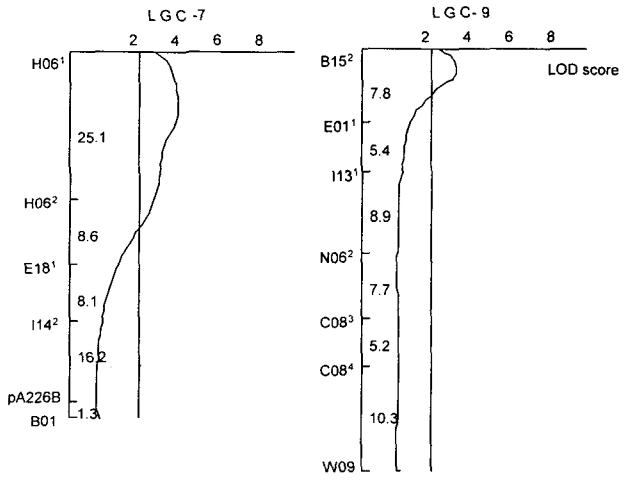

Fig. 2. Localization of quantitative trait loci for resistance to soybean cyst nematode race 14 in F2:3 progenies in 'Essex' x PI 437654.

respectively (Table 5).

Mode of inheritance at QTLs for resistance to SCN race 14.

The first QTL that was localized at 6.0 cM from $H06^1$ showed a dominant inheritance mode. The values of LOD and variation-explained of dominant inheritance mode gave the best fit to the genetics free model (Table 5). However, LOD and variation-explained for additive inheritance exhibited relatively small decrease. Comparison of LOD between dominant and additive inheritance mode, $10^{(3.38-2.98)} = 2.5$, showed the log-likelihood ratio 2.5 : 1 favoring the dominance mode over the additive mode (Table 5). Even though we suggest this QTL as dominant

inheritance, we can not exclude the possibility of additive inheritance mode. The second that was localized between $B15^2$ and $E01^1$ also showed a dominant mode of inheritance. The dominant inheritance mode showed similar values of LOD and variation to genetics free model (Table 5). The values for both recessive and additive modes were low, indicating that this QTL is not inherited in recessive or additive mode.

Molecular marker combinations for SCN.

The combined effects of QTL loci and an estimate of the total variation for SCN response were explained by multiple regression analysis. One pair of flanking markers ($H06^1$ and $H06^2$) and $B15^2$ were used. Four sets of marker combination are presented in Table 6. Three marker combination sets were significant at 99.9% confidence level and the remaining one marker combination set was significant at 99.0% confidence level. Marker combination, which included 2 markers, $B15^2$ and $H06^1$, explained the highest total variance (22.9%) for resistance to SCN race 14 (Table 6).

Myers & Anand[13] proposed one dominant and two recessive genes for resistance to SCN race 14. Anand et al.[2] suggested that PI 437654 must have more genes since it rarely had any cysts when infected with different populations of SCN. And, Riggs & Schmitt[17] have suggested that more than 10 genes might be needed for resistance to SCN. In this paper, we presented the identification and localization of two dominant QTLs for resistance to SCN race 14. Our results necessitate identification of the remaining QTLs for resistance to SCN race 14 in order to explain larger portion of phenotypic variation and further research on genotypic and environmental components of phenotypic variation. The further localization of genes for resistance to SCN race 14 and examination of interaction between QTLs will accelerate the exploitation of resistance to SCN.

Table 6. Molecular marker combinations for multiple regression analysis for resistance to soybean cyst nematode race 14 in $F_{2:3}$ progenies of Essex x PI 437654 cross

Set	$B15^2$	$H06^1$	$H06^2$	F	Pr>F	R^2
1	+	+	-	11.01	0.0001***	0.229
2	+	-	+	7.57	0.001**	0.174
3	-	+	+	5.70	0.005**	0.137
4	+	+	+	6.83	0.0004***	0.224

**Significant at 99 % confidence level
***Significant at 99.9 % confidence level

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(Received January 22, 2003; Accepted July 30, 2003)

초록 : 콩시스트 선충 race14에 대한 저항성 유전자좌 구명

최인수[1] · 김용철[1]
([1]밀양대학교 식물자원학과)

본 연구는 콩 cyst 선충 race 14에 대한 저항성 QTLs 구명을 목적으로 한 바 결과를 요약하면 다음과 같다.

1. 회귀분석 결과 30개의 marker들(29 RAPD, 1 RFLP)에서 cyst 선충 race 14의 저항성에 대한 유의성이 인정되었다.

2. MAPMAKER/QTL 분석 결과 2개의 QTL들이 구명되었는데, 이 QTL들은 2개의 linkage groups (LGC-7와 LGC-9)에 위치하였으며, 모두 우성유전 양상을 나타내었다.

3. 다중회귀분석 결과 2개의 marker들(B15[2]와 H06[1])로 구성된 조합에서 가장 높은 표현적 변이의 값(22.9%)을 나타내었다. 콩 cyst 선충 race 14에 대한 표현적 변이를 충분히 설명하기 위해서는 지속적인 QTL 구명 연구가 요구된다.