Theor Appl Genet (1999) 98: 356–364

B. X. Qiu · P. R. Arelli · D. A. Sleper

RFLP markers associated with soybean cyst nematode resistance and seed composition in a 'Peking' × 'Essex' population

Received: 3 March 1998 / Accepted: 18 August 1998

Abstract Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, causes severe damage to soybean [*Glycine max* (L.) Merr] throughout North America and worldwide. Molecular markers associated with loci conferring SCN resistance would be useful in breeding programs using marker-assisted selection (MAS). In this study, 200 $F_{2:3}$ families derived from two contrasting parents, SCN-resistant 'Peking' with relatively low protein and oil concentrations, and SCN-susceptible 'Essex' with high protein and oil concentrations, were used to determine loci underlying the SCN resistance and seed composition. Three different SCN Race isolates (1, 3, and 5) were used to screen both parents and $F_{2:3}$ families. The parents were surveyed with 216 restriction fragment length polymorphism (RFLP) probes with five different restriction enzymes. Fifty-six were polymorphic and contrasted with trait data from bioassays to identify molecular markers associated with loci controlling resistance to SCN and seed composition. Five RFLP markers, A593 and T005 on linkage group (LG) B, A018 on LG E, and K014 and B072 on LG H, were significantly linked to resistance loci for Race 1 isolate, which jointly explained 57.7% of the total phenotypic variation. Three markers (B072 and K014, both on LG H; T005 on LG B) were associated with resistance to the Race 3 isolate and jointly explained 21.4% of the total phenotypic variation. Two markers (K011 on LG I, A963 on LG E) associated with resistance to the Race 5 isolate together explained 14.0% of the total phenotypic variation. In the same population we also identified two RFLP markers (B072 on LG H, B148 on LG F) associated with loci conferring protein concentration, which jointly explained 32.3% of the total phenotypic variation. Marker B072 was also linked to loci controlling the concentration of seed oil, which explained 21% of the total phenotypic variation. Clustering among quantitative trait loci (QTLs) conditioning resistance to different SCN Race isolates and seed protein and oil concentrations may exist in this population. We believe that markers located near these QTLs could be used to select for new SCN resistance and higher levels of seed protein and oil concentrations in breeding improved soybean cultivars.

Key words SCN · RFLP · QTL · Molecular marker · Soybean

Introduction

Soybean cyst nematode (SCN) is the most widespread pest infesting soybean plants. First reported in the United States in 1954 (Winstead et al. 1955), the SCN has since spread throughout most of the soybean production states. In 1988, the SCN was ranked as the number one crop disease in the southern United States (Sciumbato 1993). The estimated reduction in soybean yield in the United States in 1994 was 1.99×10^6 tons (Wrather et al. 1995, 1997), amounting to approximately $438.8 million loss to soybean producers. After field infestation with SCN, the number of nematodes may be reduced by carefully managing crop rotation, but it is difficult to completely eliminate them. The most efficient way to control the infestation of this pest is to plant SCN-resistant cultivars.

'Peking' is an important source of resistance to SCN, giving resistance to Races 1, 3, and 5. Genetic studies have shown that SCN resistance is conditioned by

Contribution from the Missouri Agriculture Experimental Station Journal Serial No. 10678

Communicated by M. A. Saghai Maroof

B. X. Qiu · P. R. Arelli (✉) · D. A. Sleper
Department of Agronomy, University of Missouri, Columbia, MO 65211, USA

multiple genes. Caldwell et al. (1960) reported that 'Peking' possessed three resistance genes, and an additional dominant gene was documented by Matson and Williams (1965). Rao Arelli et al. (1992) have verified three genes (*rgh1*, *rgh2*, and *Rhg4*) for resistance to SCN Race 3 isolate in 'Peking'. Myers et al. (1989) reported that in PI437654 there were three genes controlling resistance to SCN Race 3 isolate, which were in common with that in 'Peking'. In our previous study, we reported that resistance to isolates of SCN Races 1, 3, and 5 in 'Peking' was each controlled by three genes, one dominant and two recessive (Qiu et al. 1997). Most recently, new germplasm for resistance to SCN Races 1 and 2 has been reported (Rao Arelli et al. 1997). Screening for new resistant germplasm is important in the breeding of improved soybean cultivars, but it is tedious. The complexity of SCN multiple resistance genes in soybean and the heterogeneity of indigenous field SCN Race populations greatly slow down the efficiency in breeding resistant soybean cultivars. Identification and characterization of resistance genes in soybean by molecular markers may play an important role in tagging and introgressing these desirable genes into improved cultivars. The efficiency of marker assisted selection (MAS) has been previously documented (Lande and Thompson 1990). Hospital et al. (1997) estimated genetic gains over 12 years based on the alternate selection of markers with and without phenotypic evaluation and concluded that MAS was more efficient than phenotypic selection alone, even for traits with high heritability ($h^2 \geq 0.5$).

Molecular markers have been used for mapping resistance genes in many crops (McMullen and Louie 1991; Pineda et al. 1992; Hartl et al. 1993; Yu et al. 1996). Molecular markers were also used to map SCN resistance loci in soybean, and several molecular markers were found to be linked to resistance loci of SCN Race 3 (Concibido et al. 1994; Mahalingam and Skorupska 1995; Webb et al. 1995; Vierling et al. 1996; Chang et al. 1997). However, little is known on DNA markers conferring resistance to isolates of SCN Races 1 and 5 in soybean.

Soybean seed protein and oil concentrations are polygenic traits (Burton et al. 1987) and are highly influenced by environmental factors. Molecular markers can be used to characterize quantitative trait loci (QTLs) conditioning these traits. That may simplify genetic analysis. However, only limited information is available on the association of DNA markers and soybean protein or oil concentration (Diers et al. 1992; Mansur et al. 1993, 1996; Lark et al. 1994; Lee et al. 1996).

The objectives of the investigation presented here were to: (1) identify DNA markers associated with SCN resistance loci to isolates of SCN Races 1, 3, and 5 in 'Peking' and (2) screen DNA markers linked to QTLs controlling soybean seed protein and oil concentrations in 'Essex'.

Materials and methods

Plant materials

F_1 hybrids were obtained in 1992 from a cross between 'Peking', which is resistant to isolates of SCN Races 1, 3, and 5 and has low protein and oil concentrations, and 'Essex', which is susceptible to all known SCN Races and has high protein and oil concentrations. An F_2 population was derived from F_1 plants and further selfed to generate $F_{2:3}$ families. The $F_{2:3}$ families were divided into three subgroups, with 5 individuals from each of the $F_{2:3}$ families forming a subgroup. These subgroups have been screened against three different isolates of Races 1, 3, and 5, respectively, and used for mapping SCN resistance loci. The same $F_{2:3}$ families were also used for evaluating the association between DNA markers and seed protein and oil concentrations.

Sources of SCN race isolates

Race 1

The SCN Race 1 isolate was initially collected from a field in Cape Girardeau County, Mo. in the summer of 1994, where soybean had a history of infection by SCN. The susceptible cultivar 'Hutcheson' (Buss et al. 1988) was used for maintaining and increasing white females and cysts for several generations to achieve a near-homogeneous population. The SCN Race 1 isolate was confirmed by Race determination tests using four standard differentials ('Pickett-71', 'Peking', PI90763, and PI88788), and one susceptible check ('Hutcheson').

Race 3

The SCN Race 3 isolate was obtained from a soybean field at Ames Plantation, located near Grand Junction, Ten. (courtesy of L.D. Young, USDA-ARS, Jackson, Ten.) in the summer of 1994. This population was increased on the roots of 'Essex' for several generations. The near-homogeneous population was classified as a Race 3 isolate based on the reactions from four standard differentials (the same as ones used for the Race 1 isolate above).

Race 5

A Race 5 population was collected at the Rhoades Farm of the University of Missouri-Columbia, near Clarkton, Mo. in summer of 1994, where soybean cv 'Bedford' (Hartwig et al. 1978) was grown. The Race 5 population was reproduced and increased on the roots of soybean PI88788 to achieve a near-homogeneous population, which was classified as a Race 5 isolate based on standard Race determination tests.

SCN bioassay

The SCN bioassays were performed in the greenhouse at the University of Missouri-Columbia during the winter of 1995 following previous methods (Rao Arelli 1994). Two parents, 1000 $F_{2:3}$ individuals (200 $F_{2:3}$ families with 5 individuals each), and the standard host differentials were germinated and transplanted into micropots filled with steam-pasteurized soil, with 1 seedling in each pot, and maintained at $27^\circ \pm 1^\circ$C in a waterbath controlled by a cooling system (Forma Scientific, Inc., Marietta, Ohio). A total of

2000(±50) eggs were used to inoculate the roots of each 4-day-old seedling using an automatic pipet (Model 40A, Scientific Equipment, Baltimore, Md.) to ensure relatively accurate numbers of white females and cysts on all individual plants. Thirty days after inoculation, white females and cysts were dislodged from the roots using pressurized water, counted under a stereo-microscope, and then converted to index of parasitism (IP) values. The number of females and cysts from 5 individuals in each subgroup was averaged and used for evaluating the SCN response for its corresponding F_2 plant.

Based on the standard SCN classification systems (Golden et al. 1970; Riggs and Schmitt 1988) and previous genetic studies (Rao Arelli et al. 1992), the index of parasitism (IP) was calculated as follows, and used as bioassay data for regression analysis and MAPMAKER/QTL interval mapping.

$$\text{IP} = \frac{\text{Mean number of females in a given subfamily}}{\text{Mean number of females on susceptible 'Essex'}} \times 100$$

Seed protein and oil evaluation

The same $F_{2:3}$ families were used for seed protein and oil evaluation. These $F_{2:3}$ families were grown at the Bradford Research Center, University of Missouri, at Columbia in 1996. Twenty-five grams of dry seed from each of the $F_{2:3}$ families was used for measuring the percentage of protein and oil. These analyses were performed using a near-infrared (NIR) food and feed analyzer located at the USDA National Center for Agricultural Utilization Research, Peoria, Ill. All individuals with black seed-coat color were re-analyzed by grinding seeds into powder to avoid the misreading of extreme data by NIR due to the dark pigmentation. The original data of the seed protein and oil concentrations from each of the $F_{2:3}$ families were used for statistical analysis and QTL detection.

DNA preparation and hybridization

Standard methods were used for DNA preparation (Saghai Maroof et al. 1984). Briefly, young leaf tissues were harvested from the two parents and each of the 200 F_2 plants and used for DNA extraction and marker identification. Two-hundred and sixteen RFLP probes were used for screening both parents. The desirable clone/enzyme combinations from the screenings were selected for Southern hybridization in the 200 F_2 populations. DNA probes used in this study were initially developed by P. Keim and R. C. Shoemaker (Iowa State University, USDA-ARS, Ames, Iowa), and purchased from Biogenetic Services, Brookings, S.D. These inserts were recovered either by polymerase chain reaction (PCR) amplification (Saiki et al. 1988) or by stab-culturing and plasmid mini-preps. All DNA samples extracted from each of the 200 F_2 individuals were digested using five different restriction enzymes, *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, and *Taq*I. Digested DNA fragments were separated by 10 g/l (1.0%) agarose gel electrophoresis and transferred onto MSI magnacharge Nylon membrane (Micro Separations, Westborough, Mass.) using the method adopted by Southern (1975). Procedures for oligo-labelling were based on the methods of Feinberg and Vogelstein (1983).

Data analysis

Two-way regression analyses (SAS 1990) were performed to detect the association between restriction fragment length polymorphism (RFLP) markers and loci controlling resistance to SCN Race isolates, and QTL conferring protein and oil concentrations. Coefficient of regression (R^2) values were used for estimating the amount of phenotypic variation explained by the associated molecular markers.

Computer software MAPMAKER/EXP 3.0 (Lincoln et al. 1993) was used for determining genetic linkage and distance between different DNA markers by using maximum-likelihood analysis of the segregation for the RFLP-marker patterns in the F_2 populations. MAPMAKER/QTL 1.1 (Lincoln and Lander 1990) was used for scanning the QTLs in each linkage group of the genome. A LOD (logarithm of odd) score of 3.0 was set as the threshold in declaring linkage between a marker and a QTL. The position of the QTL relative to its nearby marker(s) was estimated based on the peaks from MAPMAKER/QTL scans.

Results

Distribution of SCN scores and seed compositions

The mean IP values of the $F_{2:3}$ families for Races 1, 3, and 5 isolates were 31.9, 41.8, and 32.7, respectively (Table 1). Based on the untransformed data, the distributions of Races 1, 3, and 5 were abnormal (Race 1: $W = 0.9449$, $P = 0.0001$; Race 3: $W = 0.9346$, $P = 0.0001$; Race 5: $W = 0.8694$, $P = 0.001$), with a slight shifting to the low cyst frequency side (Figs. 1A–C). After square-root transformation of the SCN score data, Races 1 and 3 showed a normal distribution with $W = 0.9786$, $P = 0.2463$ and $W = 0.9827$, $P = 0.6819$, respectively. However, the transformation of the SCN

Table 1 Mean and standard deviation (SD) of SCN scores and soybean protein and oil concentrations in parents and their $F_{2:3}$ individuals

SCN races and seed compositions	Peking		Essex		$F_{2:3}$	
	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD
Races[b]						
Race 1	0.60	0.89	308.25	27.02	31.88	38.90
Race 3	0.25	0.50	117.27	25.96	41.80	39.82
Race 5	1.00	1.00	201.50	20.51	32.74	25.47
Seed composition[c]						
Protein (g/kg)	405.67	2.3785	425.67	2.3725	417.35	3.7865
Oil (g/kg)	164.67	2.0946	204.77	1.9556	186.36	3.0577

[a, b] Mean IP values of SCN scores for Race isolates
[a, c] Mean seed composition,

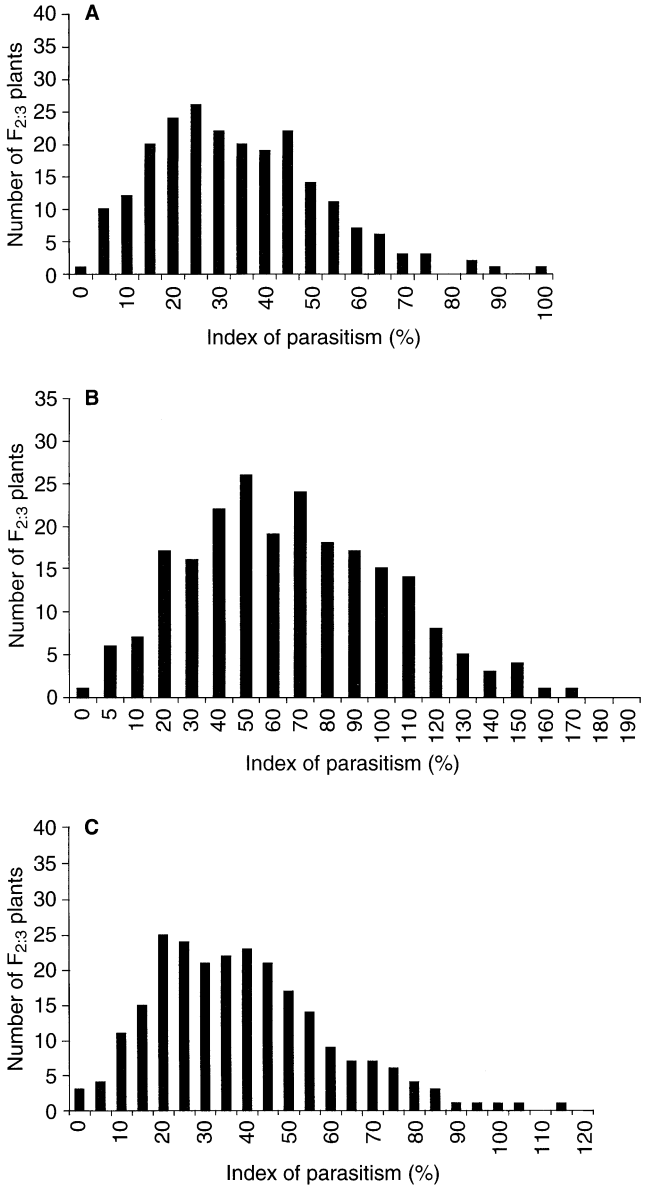

Fig. 1 A Frequency of distribution of the index of parasitism (IP) for SCN Race 1 among 200 $F_{2:3}$ families. The mean SCN score for 'Peking' was 0.60 (IP = 0.19) and for 'Essex' 79.00 (IP = 100). **B** Frequency of distribution of the index of parasitism (IP) for SCN Race 3 among 200 $F_{2:3}$ families. The mean SCN score for 'Peking' was 0.25 (IP = 0.22) and for 'Essex' 308.25 (IP = 100). **C** Frequency of distribution of the index of parasitism (IP) for SCN Race 5 among 200 $F_{2:3}$ families. The mean SCN score for 'Peking' was 1.00 (IP = 0.43) and for 'Essex' 246.00 (IP = 100)

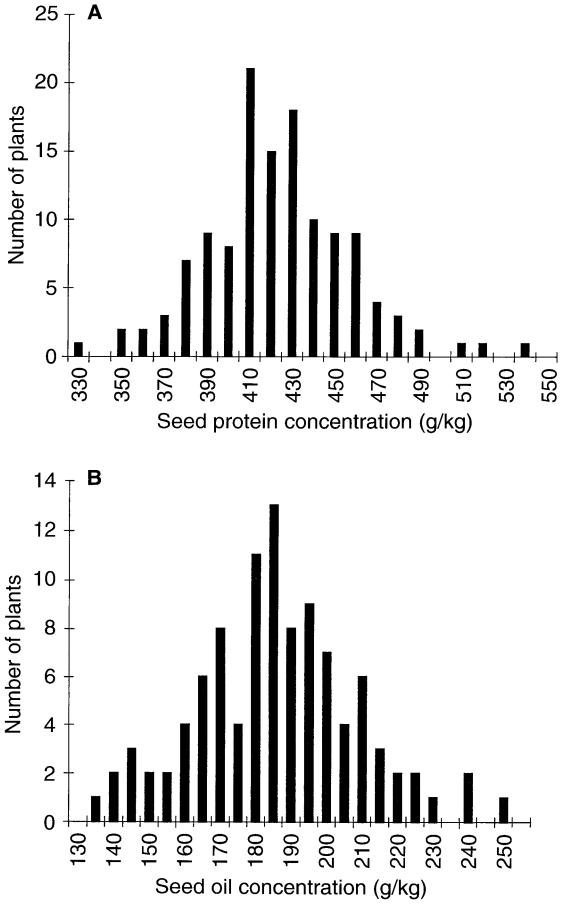

Fig. 2 A Frequency of distribution of seed protein concentration among $F_{2:3}$ families. The mean seed protein concentration for 'Peking' was 405.67 g/kg and for 'Essex' 425.67 g/kg. **B** Frequency of distribution of seed oil concentration among $F_{2:3}$ families. The mean seed oil concentration for 'Peking' was 164.67 g/kg and for 'Essex' 194.00 g/kg.

score data for Race 5 did not achieve the normalization (Fig. 1C), with significant skewness (−0.3529) and kurtosis (−0.7932) remaining and $W = 0.9387$, $P = 0.0020$.

The protein concentration data showed an approximately normal distribution (Fig. 2A), with $W = 0.9675$ and $P = 0.0898$. The frequency distribution of the original data from oil concentration was not normal with significant skewness (1.3736) and kurtosis (5.6614), $W = 0.9314$ and $P = 0.0001$ (Fig. 2B), but the square-root transformation normalized the original data, with $W = 0.9621$ and $P = 0.0329$.

RFLP linkage map

A total of 216 RFLP probes were screened, and 56 were found to be polymorphic between parental lines. These polymorphic probes were further surveyed among the 200 F_2 individuals. The computer program MAPMAKER/QTL was used for anchoring the polymorphic markers, first then the anchored RFLP markers were used to identify the possible linkage groups based on information provided by the public soybean RFLP linkage map (Shoemaker and Specht 1995). Since there were only limited polymorphic markers in this study, the linkage map was generated tentatively, and only markers that were significantly associated with resistance to SCN or seed composition are presented (Table 2 and Fig. 3).

Table 2 Molecular markers significantly ($P < 0.01$) associated with resistance loci to SCN Race 1, 3 and 5, isolates and seed composition

Loci	LG[a]	R^2	$P > F$	Sources[b]	D/A ratio	Gene actions	Allelic means[c]		
							A	H	B
Race 1									
A593	B	0.21	0.0001	Peking	− 0.12	Additive	19	30	44
A018	E	0.16	0.0001	Peking	–	–		(25)[d]	
T005	B	0.15	0.0001	Peking	− 0.10	Additive	19	28	39
B072	H	0.13	0.0022	Peking	0.07	Additive	22	29	35
K014	H	0.12	0.0023	Peking	− 0.14	Additive	13	19	27
Race 3									
B072	H	0.13	0.0030	Peking	− 0.65	Partial Dominance	41	47	75
K014	H	0.09	0.0030	Peking	− 0.21	Partial Dominance	40	51	68
T005	B	0.09	0.0009	Peking	− 0.09	Additive	43	53	65
Race 5									
K011	I	0.11	0.0010	Peking	− 0.77	Partial Dominance	25	30	70
A963	E	0.09	0.0062	Peking	–	–		(33)[d]	53
Protein									
B072	H	0.32	0.0018	Essex	0.20	Additive	46	43	41
B148	F	0.17	0.0001	Essex	0.50	Partial Dominance	45	42	41
Oil									
B072	H	0.21	0.0020	Essex	0.00	Additive	17	18	19

[a] Soybean genome linkage group
[b] Sources of favorable alleles
[c] A, Homozygous alleles for resistance to SCN and higher level of seed protein concentration or lower level of oil concentration, H, heterozygous alelles for SCN resistance or seed composition, B, homozygous alleles for susceptibility to SCN and lower level of seed protein concentration or higher level of oil concentration
[d] Including both heterozygous and homozygous alleles for resistance to SCN, and seed composition

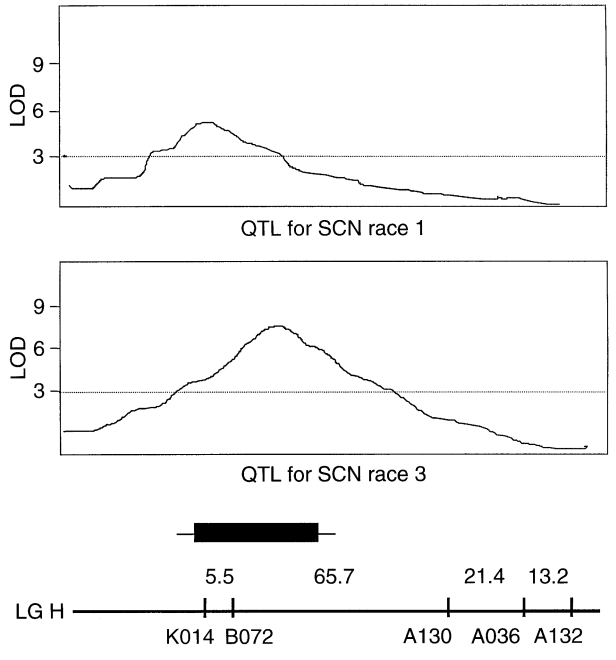

Fig. 3 MAPMAKER-QTL scans of the genomic regions on LG H covering SCN resistance loci for Races 1 and 3 isolates in a 'Peking' × 'Essex' population. *Filled rectangle* represents putative QTLs conferring SCN resistance to Races 1 and 3 isolates. Distances are given as Haldane centiMorgans (cM)

RFLP markers associated with SCN resistance

Associations between DNA markers and SCN resistance or seed composition were evaluated using both original and square-root transformed data in ANOVA and MAPMAKER/QTL analyses. Since the calculated R^2 values between the original and transformed data (data not shown) were not changed substantially, all results of the associations between DNA markers and traits using untransformed data are presented.

Five RFLP markers (A593, A018, T005, K014, and B072) were significantly associated with resistance to the SCN Race 1 isolate. Together they explained 57.7% of the total phenotypic variation (Table 3). Three markers (B072, K014, and T005) were significantly linked to resistance for Race 3 isolate (Table 2), jointly explaining 21.4% of the total phenotypic variation. Two markers (K011, and A963) were associated with resistance to SCN Race 5 isolate (Table 2), accounting for 14.0% of the total phenotypic variation. In this population it was found that all favorable alleles of resistance to SCN Races 1, 3, and 5 came from 'Peking' (Table 2), suggesting that 'Peking' is the donor of the resistance source.

On linkage group H, MAPMAKER-QTL scans showed one LOD peak near marker B072 for the QTL

Table 3 Combinations of RFLP markers and stepwise regression analysis for selected F_2 individuals for 'Peking' and 'Essex' markertypes against SCN Race 1 isolate

Combinations of markers[a]					*F*-test		
A593	A018	T005	B072	K014	*F*	$P > F$	R^2
+	+	−	−	−	19.06	0.0001**	0.2828
+	−	+	−	−	20.77	0.0001**	0.2956
+	−	−	+	−	12.27	0.0001**	0.3615
+	−	−	−	+	13.66	0.0001**	0.3664
−	+	+	−	−	16.29	0.0001**	0.2263
−	+	−	+	−	10.21	0.0001**	0.1755
−	+	−	−	+	5.69	0.0015*	0.1269
−	−	+	+	−	14.20	0.0001**	0.2831
−	−	+	−	+	10.93	0.0001**	0.2492
−	−	−	+	+	3.99	0.0110	0.1393
+	+	+	−	−	14.15	0.0001**	0.3742
+	+	−	+	−	8.38	0.0001**	0.4128
+	+	−	−	+	10.89	0.0001**	0.3764
+	−	+	+	−	8.99	0.0001**	0.4292
+	−	+	−	+	13.02	0.0001**	0.4213
+	−	−	+	+	11.13	0.0001**	0.3688
−	+	+	+	−	9.41	0.0001**	0.2758
−	+	+	−	+	8.54	0.0001**	0.2403
−	+	−	+	+	3.55	0.0042*	0.1353
−	−	+	+	+	5.73	0.0001**	0.2679
+	+	+	+	−	7.93	0.0001**	0.4569
+	+	+	−	+	8.78	0.0001**	0.6815
+	+	−	+	+	5.12	0.0001**	0.3789
+	−	+	+	+	9.18	0.0001**	0.5813
−	+	+	+	+	3.88	0.0005**	0.2585
+	+	+	+	+	6.80	0.0001**	0.5770

* Significant at $P < 0.005$; ** Significant at $P < 0.005$
[a] '+', present; '−', absent

conditioning resistance to Race 1 isolate (LOD = 5.0), and another peak on the other side of the same marker (LOD = 7.5) conditioning resistance to Race 3 isolate (Fig. 3). This indicated the locations of putative QTLs for resistance to SCN Race 1 and 3 isolates in 'Peking'.

Molecular markers that were associated with resistance to Race 1 showed additive gene action (Table 2). RFLP markers linked to Races 1 and 2 showed mostly partial dominance gene action except marker T005, which showed additive gene action.

Associated RFLP markers for protein and oil concentrations

RFLP marker B072 (LG H) was found to be significantly linked to protein concentration ($R^2 = 32\%$, $P < 0.0018$). Marker B148 (LG F) was also associated with protein concentration with a $R^2 = 17\%$ and $P < 0.0001$ (Table 2). These two markers jointly explained 33% of the total phenotypic variation. Marker B072 (LG H) was shown to be linked to loci governing oil ($R^2 = 21\%$, $P = 0.0020$) and protein concentrations (Table 1).

In this study, soybean seed protein concentration was found to be inversely correlated with seed oil concentration, with a correlation coefficient $r = -0.8860$ ($R^2 = 0.7850$, $P = 0.0001$). This finding is consistent with the results from a study by Lark et al. (1994).

Molecular markers B072 (LG H) and B148 (LG F) had additive and partial dominant gene actions, respectively, in determining protein concentration. The sources of the favorable alleles for protein and oil concentrations in this study are all from 'Essex' (Table 2), implying that 'Essex' could be a potential source for breeding high levels of seed protein and oil concentrations. Compared with the parents, some heterozygous individuals in the $F_{2:3}$ progenies had extreme phenotypes exceeding those of the parental values (Table 1). This transgressive segregation pattern suggests that both parents might have beneficial alleles at different loci controlling protein and oil concentrations, which could allow their progenies to be valuable sources of new germplasm in breeding cultivars with high protein and oil concentrations.

Discussion

Clustering of QTL

The clustering of QTLs has reported for a number of crops (Martin et al. 1993; Mansur et al. 1996;

McMullen et al. 1995; Chang et al. 1997). Clusters of QTLs may also exist in this population. We found that five RFLP markers, located in LG B, E and H, were associated with loci controlling resistance to the SCN Race 1 isolate (Table 2). Among these 5 markers, three (B072 and K014 on LG H, T005 on LG B) were also linked to loci conferring resistance to the Race 3 isolate but not to the Race 5 isolate. The marker B072 on LG H was associated with both resistance loci to SCN (Races 1 and 3 isolates) and QTLs governing protein and oil concentrations (Table 2). The genomic region in LG H may contain a cluster of unique, but closely adjacent QTLs conferring traits. The possibility of pleiotropism among loci for SCN resistance to different Races was not eliminated in this population. In fact, MAPMAKER/QTL scans showed that the locations of QTLs conferring SCN resistance to both Races 1 and 3 isolates traversed across marker B072 (Fig. 3), suggesting the possible existence of a pleiotropic effect of a single gene for resistance to both Races 1 and 3 isolates. Data from regression analysis between mean IP scores from Race 1 and Race 3 isolates supported both the hypotheses of pleiotropism and clustering of QTLs ($R^2 = 8.05\%$, $P = 0.0001$).

Genetic variation of SCN QTLs

Several DNA markers have been reported to be associated with SCN Race 3 isolate in LGs A, G, and M in different populations (Webb et al. 1995; Concibido et al. 1996), including populations involving cv 'Peking' (Mahalingam and Skorupska 1995) or Forrest (Chang et al. 1997), a 'Peking'-derived SCN-resistant cultivar. Our results showed that DNA markers linked to resistance loci for SCN Races 1 and 3 isolates were involved in LGs B, E, and H. The different locations for the SCN resistance QTLs we identified in this population may indicate the existence of unique resistance loci in our soybean lines.

The genetic variation among QTL for resistance to SCN may vary in different populations. Molecular marker A006 (LG B) was found to be highly linked to a locus conferring the SCN Race 3 inbred isolate ($R^2 = 91\%$, $P = 0.0001$) in a population 'Williams × Hartwig' (Vierling et al. 1996). Concibido et al. (1996) showed that marker Bng122 (LG G) was significantly linked to resistance for SCN Race 3 ($R^2 = 28.1\%$, LOD = 6.94) in a population derived from 'Evans × Peking'. However, these two markers (A006 and Bng122) were not polymorphic in our population when screened using five different enzymes, *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, and *Taq*I. Mahalingam and Skorupska (1995) reported that RFLP marker A136 on LG A2 ($R^2 = 12.5\%$, $P = 0.0001$) and pA635 ($R^2 = 8.0\%$, $P = 0.0001$) were significantly linked to resistance loci for SCN Race 3 using an $F_{2:3}$ population from a cross between 'Peking' and 'Essex'. However, these RFLP markers did not reach significant levels in our population. The resistant 'Peking' used in each population could be different since genetic diversity among different sources of 'Peking' has been reported (Skorupska et al. 1994). These variations could also result from environmental factors, such as SCN screening in the greenhouse or genetic variation among SCN populations.

Mansur et al. (1993) reported that RFLP marker K1 was linked to loci controlling oil concentration ($R^2 = 11\%$, $P = 0.02$), and that marker L48 was associated with loci underlying soybean protein concentration ($R^2 = 20\%$, $P = 0.004$) in a F_5 population derived from a cross between 'Minsoy' (Bernard et al. 1988) and 'Noir1'. We used a different population, derived from a cross between 'Peking' and 'Essex', and found two different RFLP markers on LG H (B072) and LG F (B148) which were associated with loci controlling seed protein concentration. Marker B072 was also associated with loci conditioning oil concentration (Table 2). Data showing different molecular markers in different populations may indicate the existence of several different QTLs controlling seed protein and oil traits. Genome or population specificity of QTLs for soybean seed protein and oil concentrations has also been reported in another study (Diers 1992; Lee et al. 1996).

Marker-assisted selection

In breeding improved SCN-resistant cultivars using marker-assisted selection, it is necessary to choose the best marker combinations. We estimated the amount of phenotypic variations explained by different marker combinations for the Race 1 isolate. The efficiency for selecting resistant genotypes was evaluated using various combinations of five selected molecular markers in this study (Table 3). The 5 markers associated with resistance to the SCN Race 1 isolate could appear in 26 different combinations. Of these 26 combinations 23 statistically reached the $P < 0.001$ level, and two combinations reached the $P < 0.005$ level in regression analysis. The R^2 values ranged from 12.7% to 36.6% in the various two-marker combinations, 13.5% to 42.9% in the three-marker combinations, and 25.9% to 68.2% in different four-marker combinations (Table 3), indicating that when different marker combinations are used, different efficiencies in marker-assisted selection will likely result. A two-marker combination (e.g. A593 and K014) selected a total of 38 individuals in terms of parental genotypes, 20 with 'Peking'-type RFLP patterns and 18 with 'Essex'-type patterns, which explained 36% of the total phenotypic variation in the SCN responses. When a three-marker combination (A593, T005, and B72) was used as a selection tool, a total of 37 parental types were selected, explaining 42% of the total phenotypic variation. With a

four-marker combination (A593, A018, T005 and K014), approximately 68% of the total phenotypic variation to SCN reactions was explained. When all five markers (A593, A018, T005, B072 and K014) were used as a marker-assisted selection tool, 57% of the total phenotypic variation was explained (Table 3). These data show that different marker combinations could have different efficiencies in marker-assisted selection. In theory, the more markers used the more reliable marker-assisted selection would be because various markers could simultaneously select desirable soybean genotypes based on all these different markers. This may eliminate the selection of false positives based on only one marker. In breeding practices, consideration has to be given to the number of markers employed and the efficiency of different marker combinations. The comparison of efficiencies of different marker combinations may provide valuable information on choosing desirable marker combinations for optimizing marker-assisted selection in soybean breeding programs.

Acknowledgments We thank J. A. Wilcox for his assistance in preparing greenhouse SCN bioassays. We acknowledge the services of personnel located at the USDA's National Center for Agricultural Utilization Research, Peoria, Illinois for conducting the protein and oil analyses. We also thank Drs. M. D. McMullen and E. A. Lee (USDA-ARS) for their valuable advice and assistance. This study was partially supported by a grant from the Missouri Soybean Merchandising Council. All experiments performed in this research comply with the current laws of the USA.

References

Bernard RL, Juvick GA, Hartwig EE, Edwards CJ Jr (1988) Origins and pedigree of public soybean varieties in the United States and Canada. US Dep Agric Tech Bull No. 1748

Burton JW (1987) Quantitative genetics: results relevant to soybean breeding. *In*: Wilcox JR (ed) Soybeans: improvement, production, and use. (Agronomy, series, vol. 16). American Society of Agronomy, Madison, Wis., pp 211–247

Buss GR, Camper HM Jr, Roane CW (1988) Registration of 'Hutcheson' soybean. Crop Sci 28:1024

Caldwell BE, Brim CA, Ross JP (1960) Inheritance of resistance of soybeans to the cyst nematode, *Heterodera glycines*. Agron J 52:635–636

Chang SJC, Doubler TW, Kilo VY, Abu-Thredeih J, Prabhu R, Freire V, Suttner R, Klein J, Schmidt ME, Gibson PT, Lightfoot DA (1997) Association of loci underlying field resistance to Soybean Sudden Death Syndrome (SDS) and Cyst Nematode (SCN) Race 3. Crop Sci 37:965–971

Concibido VC, Denny RL, Boutin SR, Hautea R, Orf JH, Young ND (1994) DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Sci 34:240–246

Concibido VC, Young ND, Lange DA, Denny RL, Danash D, Orf JH (1996) Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. Theor Appl Genet 93:234–241

Diers BW, Shoemaker R (1992) Restriction fragment length polymrophism analysis of soybean fatty acid content. J Am Oil Chem Soc 69:1242–1244

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction fragments to high specific activity. Anal Biochem 132:6–13

Golden AM, Epps JM, Riggs RD, Duclos LA, Fox JA, Bernard RL (1970) Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis Rep 54:544–546

Hartl L, Weiss H, Zeller FJ, Jahoor A (1993) Use of RFLP markers for the identification of alleles of the *Pm3* locus conferring powdery mildew resistance in wheat (*Triticum aestivum* L.). Theor Appl Genet 86:959–963

Hartwig EE, Epps JM (1978) Registration of Bedford soybean. Crop Sci 18:915

Hospital F, Moreau L, Lacoudre F, Charcosset A, Gallais A (1997) More on the efficiency of marker-assisted selection. Theor Appl Genet 95:1181–1189

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756

Lark KG, Orf J, Mansur LM (1994) Epistatic expression of quantitative trait loci (QTL) in soybean [*Glycine max* (L.) Merr.] determined by QTL association with RFLP alleles. Theor Appl Genet 88:486–489

Lee SH, Bailey MA, Mian MAR, Carter Jr TE, Shipe ER, Ashley DA, Parrott WA, Hussey RS, Boerma HR (1996) RFLP loci associated with soybean seed protein and oil content across populations and locations. Theor Appl Genet 93:649–657

Lincoln SE, Lander ES (1990) MAPMAKER/QTL. Whitehead Institute of Biomedical Research, Cambridge, Mass

Lincoln SE, Daly MJ, Lander ES (1993) MAPMAKER/EXP. Whitehead Institute of Biomedical Research, Cambridge, Mass.

Mahalingam R, Skorupska HT (1995) DNA markers for resistance to *Heterodera glycines* I. Race 3 in soybean cultivar 'Peking'. Breeding Sci 45:435–443

Mansur LM, Lark KG, Kross H, Oliveira A (1993) Interval mapping of quantitative trait loci for reproductive, morphological, and seed traits of soybean (*Glycine max* L.). Theor Appl Genet 86:907–913

Mansur LM, Orf JH, Chase K, Jarvick T, Cregan PB, Lark KG (1996) Genetic mapping of agronomic traits using recombinant inbred lines. Crop Sci 36:1327–1336

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spirey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring resistance in tomato. Science 262:1432–1435

Matson AL, Williams LF (1965) Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Sci 5:477

McMullen MD, Louie R (1991) Identification of a gene for resistance to wheat streak mosaic virus in maize. Phytopathology 81:624–627

McMullen MD, Simcox KD (1995) Genomic organization of disease and insect resistance genes in maize. MPMI 8:811–815

Myers GO, Anand SC, Rao Arelli AP (1989) Resistance to *Heterodera glycines* in soybean PI 437654. In: Pascale AJ (ed.) Proc 4th World Soybean Res Conf. Buenos Aires, Argentina, pp 1230–1234

Pineda O, Bonierbale MW, Plaisted RL (1992) Identification of RFLP markers linked to the *H1* gene conferring resistance to the potato cyst nematode *Globodera rostochiensis*. Genome 36:152–156

Qiu BX, Sleper DA, Rao Arelli AP (1997) Genetic and molecular characterization of resistance to *Heterodera glycines* Races 1, 3, and 5 in 'Peking'. Euphytica 96:225–231

Rao Arelli AP (1994) Inheritance of resistance to *Heterodera glycines* Race 3 in soybean accessions. Plant Dis 78:898–900

Rao Arelli AP, Wrather JA, Anand SC (1992) Soybean resistance to soybean cyst nematode Race 3 is conditioned by an additional dominant gene. Crop Sci 32:862–864

Rao Arelli AP, Wilcox JA, Myers O Jr, Gibson PT (1997) Soybean germplasm resistant to Races 1 and 2 of *Heterodera glycines*. Crop Sci 37:1367–1369

Riggs RD, Schmitt DP (1988) Complete characterization of the Race scheme for *Heterodera glycines*. J Nematol 20 : 392–395

Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81 : 8014–8018

Saiki RK, Gelfand DH, Stoffel S, Scharf S, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239 : 487–491

SAS Institute (1990) SAS/STAT user's guide, version 6.0, 3rd edn. SAS Institute, Cary, N.C.

Sciumbato GL (1993) Soybean disease loss estimates for the southern United States during 1988–1991. Plant Dis 77 : 954–956

Shoemaker RC, Specht JE (1995) Integration of the soybean molecular and classical genetic linkage groups. Crop Sci 35 : 436–446

Skorupska HT, Choi IS, Rao Arelli AP, Bridges WC (1994) Resistance to soybean cyst nematode and molecular polymorphism in various sources of 'Peking' soybean. Euphytica 75 : 63–70

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98 : 503–517

Vierling RA, Faghihi J, Ferris VR, Ferris JM (1996) Association of RFLP markers with loci conferring broad-based resistance to the soybean cyst nematode (*Heterodera glycines*). Theor Appl Genet 92 : 83–86

Webb DM, Baltazar BM, Rao Arelli AP, Schupp J, Clayton K, Keim P, Beavis WD (1995) Genetic mapping of soybean cyst nematode Race 3 resistance loci in the soybean PI 437654. Theor Appl Genet 91 : 574–581

Winstead WW, Skotland CB, Sasser JW (1955) Soybean cyst nematode in North Carolina. Plant Dis Rep 39 : 9–11

Wrather JA, Chambers AY, Fox JA, Moore WF, Sciumbato GL (1995) Soybean disease loss estimates for the southern United States, 1974 to 1994. Plant Dis 79 : 1076–1079

Wrather JA, Anderson TR, Arsyad DM, Gai J, Ploper LD, Porta-Puglia A, Ram HH, Yorinori JT (1997) Soybean Disease Loss Estimates for the Top 10 Soybean Producing Countries in 1994. Plant Dis 81 : 107–110

Yu YG, Saghai Maroof MA, Buss GR (1996) Divergence and allelomorphic relationship of a soybean virus resistance gene based on tightly linked DNA microsatellite and RFLP markers. Theor Appl Genet 92 : 64–69