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DNA Markers for Resistance to *Heterodera glycines* I. Race 3 in Soybean Cultivar Peking

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Summary

Ninety $F_{2:3}$ progeny from a cross of resistant cultivar Peking and susceptible Essex and near-isogenic lines, NC55, and Lee, were employed in transmission, bulk, and segregation analyses to identify molecular markers associated with resistance to soybean cyst nematode (SCN) race 3. SCN bioassays were performed in a greenhouse using a race 3 isolate. Progeny response to SCN was characterized by Female Index (FI). Three morphological traits, 108 restriction fragment length polymorphisms (RFLPs), and 400 random amplified polymorphic DNAs (RAPDs) were employed. The *i* locus for seed coat color showed highly significant association with resistance to race 3 SCN ($R^2=0.25$, $P=0.0001$). Two independently inherited RFLP markers, pA 136 and pA635, (linkage groups A and C, of the USDA-ARS/ISU soybean RFLP map) and three RAPD markers, E01c (LG A), G15d (LG F), and S07a (LG A), explained 32.5 % of the total phenotypic variation for SCN response in F_2 progeny. Two-way ANOVA indicated significant interaction of *i* locus with pA136, pA635, S07a, and E01c ($P=0.0001$) suggesting that a quantitative trait locus (QTL) on LG A interacts with other QTLs for SCN resistance in the genome. The dominant QTL was localized 0.6 cM from *i* locus using MAPMAKER/QTL 1.1. Twenty-six combinations of the identified molecular markers were used in selection for SCN resistance. Combination of 4 markers (pA 136, S07a, G15d, and E01c) explained 67 % of total variation among selected resistant and susceptible individuals.

Key Words : soybean, cyst nematode, molecular markers, quantitative trait locus.

Introduction

Damage to soybean caused by cyst nematode was first observed in 1915 in Japan by Hori (1916). This nematode was described as a new species, *Heterodera glycines* (Ichinohe, 1952). In the United States, it was first reported in southeastern North Carolina (Winstead *et al.*, 1955) and has spread throughout soybean producing states. According to Riggs and Schmitt (1987) soybean cyst nematode (SCN) may eventually infest all acreage since it seems to be adapted to most or all environments where soybeans are grown.

The economic and ecological reasons for developing cultivars resistant to SCN are very compelling. When planted on fields infested with SCN race 3, susceptible soybean cultivars produced 5.7-35.8 % lower seed yields compared with resistant cultivars (Niblack and Norton, 1992). In the Southern US alone, damage due to SCN has been estimated to be 634,000 metric tons which amounts to a loss of 143.23 million dollars for the year 1991 (Sciumbato, 1993). With the cancellation of permits for the use of DBCP (1,2-Dibromo-3-chloropropane) and EDB (Ethylene di-bromide) fumigant nematicides, increased emphasis is placed on "host plant resistance" (Boerma and Hussey, 1992).

Based on conventional studies, the genetic basis of soybean resistance to *H. glycines* is complex and far from being definitive (Luedders, 1989). At least ten genes have been hypothesized to govern resistance to SCN in soybeans (Riggs and Schmitt, 1987). Plant resistance, besides being complex in nature, has been further complicated by the genetic variability of the pathogen itself.

Substantial progress has been made in determining genomic regions associated with resistance to viral, bacterial, and fungal pathogens inherited in a simple Mendelian fashion. Restriction fragment length polymorphism (RFLP) markers were employed in mapping loci conferring resistance in tomato (Young *et al.*, 1988, Sarfatti *et al.*, 1989, Martin *et al.*, 1993, Balint-Kurti *et al.*, 1994); in maize (McMullen and Louie, 1989); potato (Ritter *et al.*, 1991); barley (Hinze *et al.*, 1991); rice (Yu *et al.*, 1991); common beans (Adam-Blondon *et al.*, 1994); and wheat (Schachermayr *et al.*, 1994). Random amplified polymorphic DNA (RAPD) markers were utilized to detect loci for resistance to *Rhynchosporium secalis* in barley (Barua *et al.*, 1993), rust resistance in oats (Penner *et al.*, 1993), and resistance to downy mildew in *Lactuca sativa* (Michelmore *et al.*, 1991). Resistance to nematodes controlled by single genes, e.g., *Meloidogyne incognito* in tomato (Klein-Lankhorst *et al.*, 1989, 1991), root cyst nematode *Globodera rostachiensis* in potato (Barone *et al.*, 1990), and *Heterodera schactii* in sugar beets (Uphoff and Wricke, 1992) were tagged using the RAPD methodology.

The application of DNA markers, RFLPs and RAPDs, to identify individual loci determining complex quantitative traits (QTLs), is more difficult. However, progress in this area has been achieved. QTLs for wa-

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ter use efficiency (Martin *et al*., 1989), fruit pH and soluble solids (Patterson *et al*., 1990) in tomato; seed hardedness (Keim *et al*., 1990b), seed protein and oil content (Diers *et al*., 1992) in soybeans; and plant height in corn (Beavis *et al*., 1991) were mapped using RFLPs. In soybean, Concibido *et al*. (1994) identified RFLPs associated with resistance to SCN race 3 in plant introduction PI 209332. Wang *et al*. (1994) using RFLPs identified two qualitative resistance loci and ten partial resistance loci against the blast fungus, *Pyricularia oryzae*, in rice.

We utilized RFLP and RAPD markers to identify associations with resistance to SCN race 3 in 'Peking' cultivar. Peking, an introduction from China, has been used as a predominant source for SCN resistance in development of soybean cultivars in Northern America (Bernard *et al*., 1987). Race 3 SCN is widely spread in Northern America. The detection of loci for resistance to SCN in various resistance sources will facilitate genetic manipulation of oligogenic resistance in soybean that will have potential to be more durable in the presence of heterogeneous nematode populations and shifts in virulence genes of nematode. Molecular marker-assisted selection might effectively accelerate introgression of resistance to SCN in soybean genotypes.

Materials and Methods

Plant material

Cultivar Peking has been extensively used as a genetic source for resistance to SCN race 3 and race 5. Forty-six out of 288 public cultivars released in USA have cultivar Peking in ancestry (Lohnes and Bernard, 1991). Prior to this study, several Peking lines maintained in the USDA Soybean Germplasm Collection at Urbana-Champaign, Illinois, USA and in several USA breeding programs, have been tested for SCN resistance and characterized molecularly to assure the purity of Peking line used in the molecular mapping experiment and as a host differential in soybean cyst nematode classification system (Skorupska *et al*., 1994a).

Cultivar Essex, released in 1972 (Smith and Champer, 1973) is characterized by high seed yields, excellent standability and good seed quality; however, it is extremely susceptible to all known races of the nematode and was chosen as the susceptible parent. Essex was also used as the host for maintaining SCN populations in the greenhouse. Hybrid F_1 seeds from Peking and Essex were obtained from hybridizations made in the greenhouse in the summer of 1991. Ninety F_2 progeny formed the mapping population. The population was advanced for two more generations ($F_{2:3}$ and $F_{3:4}$ progeny lines) to confirm the F_2 genotypes for morphological characters (seed coat color, pubescence color, and flower color) and response to race 3 SCN.

The near-isogenic lines NC55 and Lee were screened for RAPD polymorphism. NC55 was derived from Lee by three backcrosses with Peking as donor parent (Bernard *et al*., 1988). The genes for SCN resistance reported to be transferred from Peking to NC55 are *Rhg4* (linked to *i* locus for seed coat color) and probably *rhg1, rhg2*, and *rhg3* (Bernard *et al*., 1988).

Bioassay for SCN female index (FI)

Race 3 SCN was obtained from Dr. Steve Lewis (Dept. of Plant Pathology and Physiology, Clemson University). Race determination tests (Riggs and Schmitt, 1988) were performed and the isolates was confirmed to be race 3 (Table 1). The differentials, Peking, PI 88788, PI 90763, Pickett, PI 437654, and the susceptible Essex were included in the bioassays. The race 3 isolate was reproduced on Essex in a growth chamber for 12 generations. The race identity was kindly confirmed by Dr. Rao-Arelli, University of Missouri, Columbia.

SCN bioassays were conducted in a greenhouse where temperature was maintained between 28-30 °C. Four-days old seedlings were transplanted into plastic cups filled with sterile sand. Each seedling was inoculated with 2,000 eggs. Thirty days after inoculation, the roots were washed with a powerful jet of water and cysts were collected on a 75 μm sieve and counted under a stereoscope. Female index (FI) was used to characterize plant response to SCN race 3. FI is the ratio of the number of mature female nematodes (cysts) on the roots of the plant to the average number of cysts on susceptible Essex. The SCN bioassays were conducted in two phases. In the first phase, $F_{2:3}$ progeny of the mapping population were assayed for race 3 resistance utilizing 10 replications per F_2 progeny. In the second phase, 22 $F_{2:4}$ progeny (15 F_4 plants per F_2 individual), which had very high or very low FIs ($FI > 1.00$ or $FI < 0.10$), were tested again to confirm biassay results in the previous generation.

RFLP and RAPD analyses

Total genomic DNA was extracted using the CTAB method (Rogers and Bendich, 1985) with modifications for soybeans as outlined by Keim *et al*. 1988. RFLP and RAPD analysis were conducted as described before (Skorupska *et al*., 1993 and Skorupska *et al*., 1994a, respectively).

The homologous soybean genomic DNA clones from *Pst*I library were obtained from Biogenetic Services

Table 1. SCN race 3 Female Index[1)] of soybean differentials used for race determination in phase 1 and phase 2 of bioassay

Differentials	Phase 1	Phase 2
Peking	0.33	0.45
Pickett	0.41	0.38
PI 88788	2.98	3.43
PI 90763	0.64	0.00
PI 437654	0.00	0.00
Essex	100.00	100.00

[1)]Female index is expressed in percentages.

Inc., Brookings, SD. The soybean DNA inserts in the plasmid vector pBS+ were amplified by the polymerase chain reaction using T3 and T7 primers (Operon Technologies, Inc., Alameda, CA). Amplifications were performed in a PCR thermocycler 480 (Perkin-Elmer, Norwalk, CT). One-hundred eight RFLP probes were screened for polymorphisms between Peking and Essex. The polymorphisms for the probe-restriction enzyme combination used for the RFLP locus assignment on the USDA-ARS/ISU soybean RFLP map (Shoemaker and Olson, 1993) were used. Twenty-two such polymorphisms were detected and used for analysis of the F_2 progeny.

Decamer primers (Operon Technologies, Inc., Alameda, CA) were employed in RAPD analysis between near isogenic lines, bulks for *i* locus, and Peking and Essex. Selection of RAPDs for screening the mapping population was based on the concordance of molecular patterns between Peking-NC55 and/or black bulk *versus* Essex-Lee and/or yellow bulk (Table 2). Three-hundred ninety-seven primers were employed to test molecular patterns of NC55 and Lee. Seventeen RAPD bands confirmed the donor (Peking) molecular patterns in NC55 and they were used to examine segregating F_2 progeny. Bulk analysis was undertaken to find markers for the *i* locus, reported to be linked with *Rhg4* (Matson and Williams, 1965). Twelve F_2 *ii* genotypes (black seed coat color) and 10 F_2 i^ii^i genotypes (homozygous yellow seed coat color) were employed for the bulk experiment. Equal volumes of each *ii* and i^ii^i genotype DNA at the concentration of 60 ng/μl were pooled to create two pools of DNA. Theoretically, the two bulks of DNA should be in linkage equilibrium for all the loci in the genome except those in the vicinity of the *i* locus. The *ii* and i^ii^i bulks were screened for polymorphisms using 400 decamer primers (Operon Technologies, Inc., Alameda, CA) and 24 polymorphic RAPD bands were selected to test their linkage with *i* locus. Additional polymorphisms for screening the Peking × Essex segregating population were obtained from a molecular survey of the Peking gene pool (Skorupska *et al*., 1994a) and comparisons of bulks of race 3 resistant and race 3 susceptible cultivars (Skorupska *et al*., 1994b). Sixty-five polymorphic bands generated by forty-five primers were used for segregation analysis of the 90 F_2 progeny.

Table 2. Transmission of the RAPD markers corresponding to SCN race 3 resistant Peking and susceptible Essex molecular patterns based on the presence (+) or absence (−) of DNA band

Marker loci	NC55	Lee	Black-bulk	Yellow-bulk	Peking	Essex
C15	+	−	+	−	+	−
D06	+	−	+	−	+	−
E01 a	+	−	+	−	+	−
K06 a	−	+	−	+	−	+
S07 a	−	+	−	+	−	+
S07 b	+	−	+	−	+	−

Statistical analyses

Kendall's tau-b coefficient, a non-parametric measure of association was computed to examine the linear relationship between phase 1 and phase 2 of SCN bioassays. The RFLP and RAPD allels were given a numerical value for statistical analyses. Sets of molecular markers following the Peking molecular pattern and those following the Essex molecular pattern were designated as Peking allelic type and Essex allelic type, respectively. Each of the 90 F_2 genotypes were described with 94 markers. The F_2 genotypic marker classes were contrasted with the bioassay FI values. We examined molecular markers association with resistance to race 3 by univariate analysis using the general linear fixed effects model. To detect interactions, markers identified as statistically significant were subjected to multiple regression and a two-factor analysis of variance. F-tests and t-tests were performed for all the marker combinations and interactions were considered significant when $P \leq 0.0001$. Statistical analysis was conducted using PC-SAS version 6.0 (SAS, 1990). Contingency frequency tables between all markers were obtained using SAS and maximum likelihood estimates of map distances were calculated using Linkage-1 program (Suiter *et al*., 1983). MAPMAKER/EXP 3.0 (Lander *et al*., 1987) and MAPMAKER/QTL 1.1 (Paterson *et al*., 1988) were used to construct a map around *i* locus region and localize QTL for SCN resistance (minimum LOD 3.0 and recombination frequency 0.50).

Results and Discussion

Seed coat color and SCN response

The FI distribution in the Peking × Essex F_2 mapping population is shown in Fig. 1. Mean FI value of the population was 47.7. The negative kurtosis value (Fig. 1) was indicated by a shift towards the low cyst frequencies. The FI distribution had a coefficient of skewness of 0.734, implying a greater scattering at higher FI values (Fig. 1). The SCN bioassays of phase 1 and phase 2 were highly correlated ($r=0.75$, $P<0.0001$). The Kendall's tau-b coefficient was 0.86 for the black seeded individuals and 0.44 for the yellow seeded individuals suggesting the response to SCN is more homogeneous in the *ii* genotypes and is not fixed in yellow seeded i^ii^i F_2 individuals.

Linear regression analysis indicated that the *i* locus was significantly associated with SCN resistance in the Peking × Essex cross (F value = 14.73, $P > F = 0.0001$ and $R^2 = 0.25$). Distribution of FI values for the i^ii^i, i^ii and *ii* genotypes are presented in Figures 2A, B, and C, respectively. In PI 209332, *i* locus was reported to be weakly associated with SCN (Concibido *et al*., 1994). Strong association in Peking between *i* locus and SCN response indicates that genotypic differences may exist

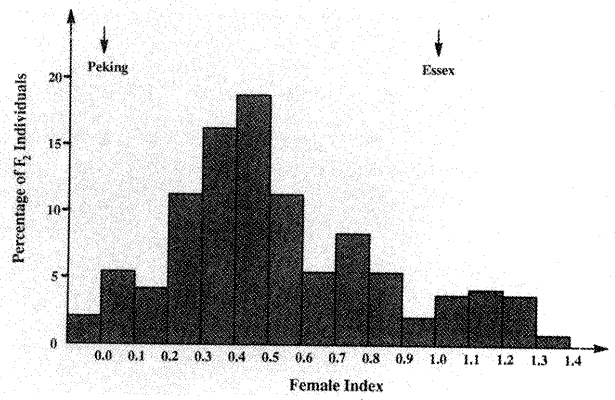

Fig. 1 Distribution of female indices (FI) in F_2 population of Peking × Essex cross. Statistics of distribution (FI × 100): ● =47.7±4.7, skewness=0.734, kurtosis=−0.030.

in different sources of resistance.

Pubescence color, flower color, and four isozyme loci (G3PDH, Aco4, Pg, and Idh2) showed no associations with the nematode response (data not shown).

DNA polymorphisms and SCN response

The localization of *i* locus (Keim *et al*., 1990a) prompted us to initiate mapping for SCN resistance on the linkage group A. A total of 26 RFLP probes from LG A were screened and 9 probes were polymorphic. Probes from the other linkage grousp were selected randomly at 20-25 cM interval. Two independently inherited RFLPs, pA136 on linkage group A and pA635 on linkage group C were significantly associated with SCN resistance and explained 12.5 % and 8.0 % variation (Table 4), respectively. Previously, probe pA85 on linkage group A was reported to be significantly associated (R^2=0.15, P =0.0041) with SCN resistance in PI 209332 (Concibido *et al*., 1994). The pA85 probe did not show this strong association with SCN response in the Peking × Essex progeny (R^2 = 0.05, P = 0.0496). In our study, probe pA136 (Fig. 3A), localized on the side of the *i* locus opposite to pA85, was found to be highly informative for SCN resisntace. The mean FI value of F_2 progeny of the genotypes heterozygous for pA136 did not differ significantly from the FI of the progeny bearing the Peking type allele (Table 3). This suggests that the SCN resistance gene associated with the probe pA136 is completely dominant. The F_2 genotypes with the Essex allele for probe pA635 had a higher FI (~44 %) than the homozygotes for the Peking allele (Table 3, Fig. 3B). There was no difference between the homozygotes and the heterozygotes for the Essex allele, suggesting that the resistance gene associated with pA635 was recessive. Probe pK2 on LG F and probe pK3 on LG K showed differences between the FI means for the Peking type allele and Essex type allele. However, these differences were not statistically significant and can be attributed to deviations in the segregation ratio of pK2 and pK3 in the mapping population (data not shown). The importance of these two LGs in SCN resistance is being examined in different popoulations.

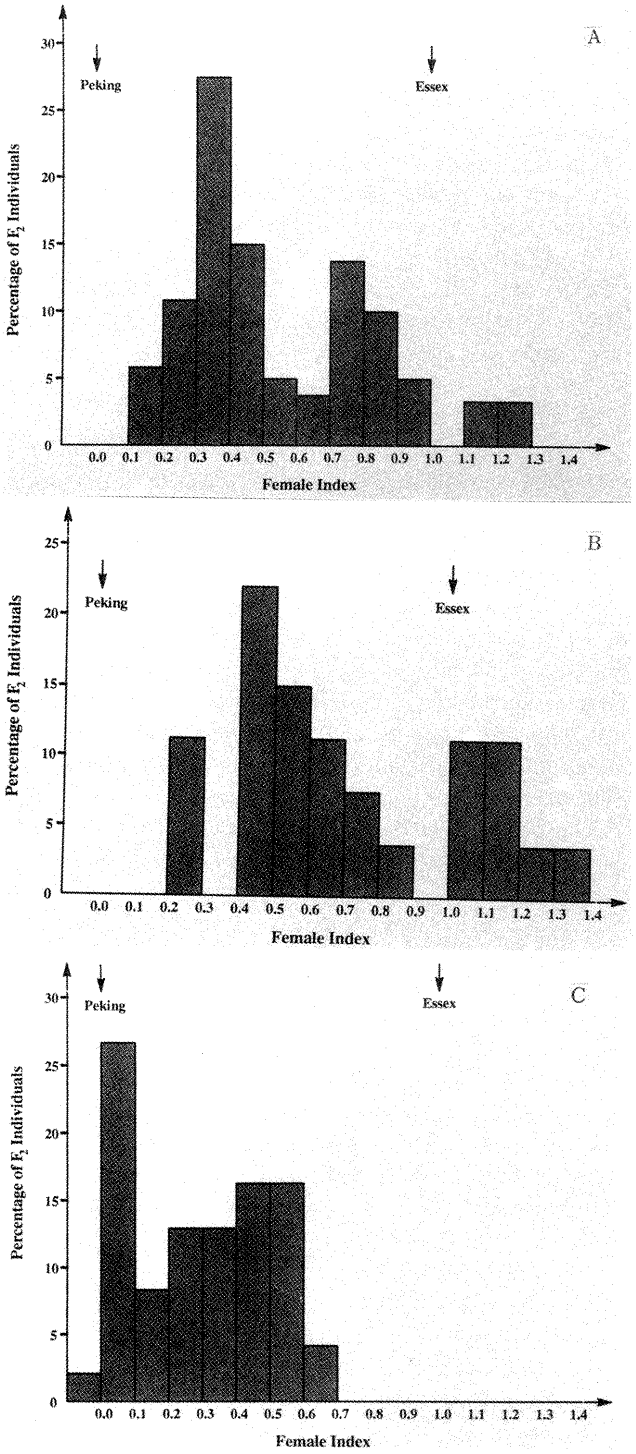

Fig. 2 Distribution of Female Indices for i^1i^1 (A), i^1i (B) and ii (C) genotypes in Peking × Essex F_2 population. Statistics of distributions (FI × 100): (A) ●=66.7±5.3, skewness=0.497, kurtosis=−0.921; (B) ●= 48.4±4.4, skewness=0.748, kurtosis=−0.351; (C) ●=25.0±5.6, skewness=0.223, kurtosis=−1.297.

Preliminary selection of RAPDs using transmission analysis increased the probability of detecting markers significantly associated with the nematode response. Three markers, E01c, G15d, and S07a showed significant association with resistance to race 3 (Fig. 4, Table

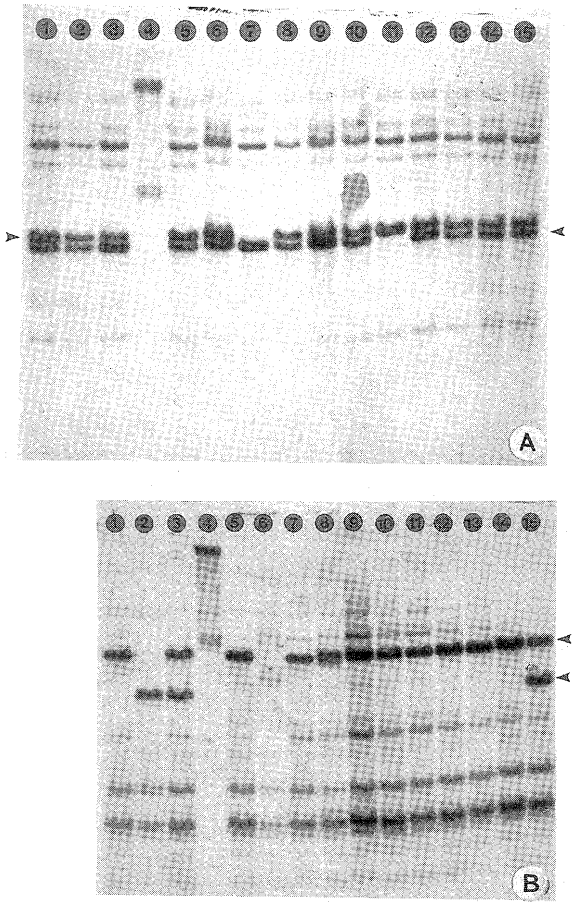

Fig. 3 A: Restriction fragment length polymorphism of *Eco* RV digested DNA hybridized with the RFLP probe pA136 in Peking × Essex F_2 population. Lane 4: *Hind* III digested λ DNA; Lane 7-Peking pattern, resistant parent; Lane 11-Essex pattern, susceptible parent; Lanes-1, 2, 3, 5, 6, 8, 9, 10, 12, 13, 14, 15-heterozygotes. B: Restriction fragment length polymorphism of *Taq* I digested DNA hybridized with the RFLP probe pA635 in Peking × Essex F_2 population. Lane 4: *Hind* III digested λ DNA; Lane 2-Peking pattern, resistant parent; Lanes 1, 5, 7, 8, 9, 10, 11, 12, 13, 14 - Essex pattern, susceptible parent; Lane 3, 15 -heterozygotes. Arrow indicates SCN associated polymorphic DNA band.

4). The marker, S07a (1.0 kb, Fig. 4C), explained 8.0 % of the total variation in the nematode response, P = 0.0094 (Table 4). S07a polymorphism mapped to LG A, 17 cM from the *i* locus (Fig. 5). G15d and E01c did not show any linkage with the *i* locus or any other probes around the *i* locus nor with the probe pA635. G15d mapped 30.0 cM from the probe pA401, a probe localized on linkage group F. E01c mapped to linkage group A, 23.5 cM from pA96a. This implies existance of a second locus with a minor effect for resistance to SCN on LG A.

In a selfing population, there is not likely to be much linkage disequilibirium between loci on different chromosomes (Lande and Thompson, 1990). Thus separate multiple regression analyses were performed for identified markers in each linkage group (Table 4). When the linked marker loci displayed significant interactions, only the marker locus with the highest R^2

Table 3. SCN race 3 Female Index of the RFLP and RAPD markers significantly associated with resistance to SCN in Peking x Essex F_2 population

Allele	Marker	FI-LSM[4]	SE-LSM	Comparison of LSM
P[1]	pA136	33.14	6.21	P vs E=28.87 (P>T=0.0008)
E[2]		62.07	5.53	E vs H=16.56 (P>T=0.0267)
H[3]		45.51	4.83	H vs P=11.37 (P>T=0.1197)
P	pA635	35.37	6.07	P vs E=14.34 (P>T=0.0087)
E		54.71	3.88	
P	E01c	40.80	4.84	P vs E=16.15 (P>T=0.0261)
E		56.95	5.22	
P	S07a	34.97	3.88	P vs E=19.21 (P>T=0.0098)
E		54.18	6.10	
P	G15d	42.76	4.89	P vs E=15.96 (P>T=0.0290)
E		58.72	5.23	

[1]Peking type allele.
[2]Essex type allele.
[3]Heterozygote type.
[4]Least square means.

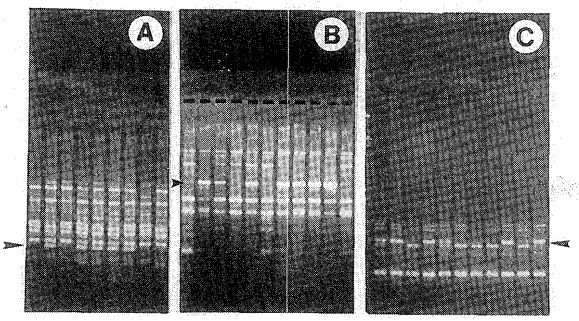

Fig. 4 Random amplified polymorphic DNAs of Peking × Essex F_2 population. A: E01 primer, B: G15 primer, C: S07 primer. Arrow indicates SCN associated polymorphic DNA band at 2.0 kb, 1.45 kb, 1.0 kb, respectively.

Table 4. Variation of SCN race 3 resistance explained by molecular markers in univariate and multivariate regression analyses in Peking x Essex F_2 population

Marker	F value	Pr>F	R^2	Linkage group
Seed color[1]	14.73	0.0001	25.00[2]	A
pA136[1]	6.22	0.0030	12.50	A
S07a[1]	7.06	0.0094	8.00	A
E01c	5.14	0.0261	6.00	A
G15d	4.96	0.0290	6.00	F
pA635	7.22	0.0087	8.00	C
			V R^2=32.50 (excluding the *i* locus contribution)	
			V R^2=45.00 (excluding the *i* locus contribution)	

[1]When linked marker loci [2]} displayed significant interactions, only the marker locus with the highest R^2 value was used in the calculation of the sum of R^2.

value in univariate regression analysis was used for calculation of R^2 in multiple regression analysis. A similar approach was adopted in a RFLP association of four disease resistance genes in *Pisum sativum* (Dirlewanger *et al*., 1994). In our research, 45 % of the total variation in the nematode response could be explained by the markers when the *i* locus is included. Excluding the *i* locus contribution, the detected molecular markers alone accounted for 32.5 % of the variation in the nematode response (Table 4).

Two-way ANOVA was performed for all the significant markers to identify interactions between the selected markers. At P=0.0001, the *i* locus showed significant interaction with pA136, S07a, E01c, and pA635. Significant interaction with G15d was aslo determined at a probability level P = 0.0003 (Table 5). This suggests that the QTL for SCN resistance on LG A in the proximity of *i* locus region interacts with other QTLs for SCN resistance localized elsewhere in the genome. We mapped this major QTL for resistance to SCN using MAPMAKER/QTL 1.1 (Paterson *et al*., 1988). In the segregating F_2 population of Essex and Peking cross, QTL for SCN resistance was assigned with a LOD 7.01 and was localized 0.6 cM from the *i* locus (Fig. 5). The QTL was dominant in action and explained 30.7 % of the variation in the resistance response. Matson and Williams (1965) first suggested the existence of a dominant gene (*Rhg4*) for SCN resistance closely linked to the *i* locus (0.35 cM). We confirmed the existence of this gene in cultivar Peking. Weisemann *et al*. (1992) found two molecular markers pBLT24 encoding a 34kDa soybean seed protein with sequence homology to thiol proteases of the papain family and pBLT65 isolated from a λgt11 cDNA library encoding aspartokinase homoserine dehydrogenase tightly linked to the *i* locus. The authors suggested that the close proximity of the loci and their position straddling the *i* locus enhances the opportunity to isolate and identify the *Rhg4* locus itself. The localization of QTL for SCN resistance in proximity to the *i* locus in our research can contribute to the physical dissection of the *Rhg4* region.

Table 5. Interaction of *i* locus and molecular markers for soybean cyst nematode resistance in Peking x Essex F_2 population

Combination	F value	R>F	R^2 value
ii * A136	4.66	0.0001	0.32
ii * A635	5.98	0.0001	0.27
ii * E01 c	6.54	0.0001	0.31
ii * G15 d	5.47	0.0003	0.28
ii * S07 a	6.45	0.0001	0.28

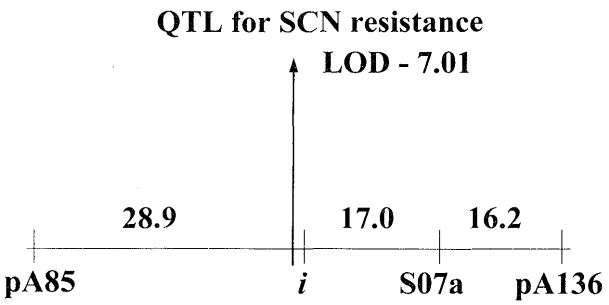

Fig. 5 Map of the region around *i* locus showing *Rhg4* (SCN resistance gene), *i* (seed coat color), RFLP and RAPD markers.

Utility of selected markers in marker assisted selection

The five molecular markers pA136, pA635, E01c, S07a, and G15d were used in all possible combinations to determine the most efficient set of markers for selecting the resistant genotypes. Twenty-six possible combinations of the selected markers are presented in Talbe 6. Five molecular marker combinations differentiated FI values of segregants of Peking molecular type and Essex molecular type at the probability level P< 0.001 and 9 marker combinations at P<0.005>0.001 (Table 7). Simultaneous detection of t-statistics at high probability level and strong associations between marker and SCN response within selected classes of F_2 segregants representing Peking and Essex molecular allelic types can be helpful for selection of marker combinations for marker-assisted selection. For example, set 4 which comprised of only two markers pA136 and G15d, selected 8 individuals as Peking molecular type and 11 individuals as Essex molecular type. These two markers explained 48 % of the variation in the nematode re-

Table 6. Molecular marker combinations for resistance to SCN race 3 in Peking x Essex F_2 population

Set	pA136	pA635	S07a	E01c	G15d
1	+	+	−	−	−
2	+	−	+	−	−
3	+	−	−	+	−
4	+	−	−	−	+
5	−	+	+	−	−
6	−	+	−	+	−
7	−	+	−	−	+
8	−	−	+	+	−
9	−	−	+	−	+
10	−	−	−	+	+
11	+	+	+	−	−
12	+	+	−	+	−
13	+	+	−	−	+
14	+	−	+	+	−
15	+	−	+	−	+
16	+	−	−	+	+
17	−	+	+	+	−
18	−	+	+	−	+
19	−	+	−	+	+
20	−	−	+	+	+
21	+	+	+	+	−
22	+	+	+	−	+
23	+	+	−	+	+
24	+	−	+	+	+
25	−	+	+	+	+
26	+	+	+	+	+

Table 7. SCN race 3 Female Indices t-test for selection of molecular marker combinations and univariate regression analyses (F-test) of F_2 individuals selected as Essex and Peking molecular types

Set[1)]	T	Pr>T	F value	Pr>F	R^2 value
1	3.8444	0.0006**	10.67	0.0026*	0.25
2	3.7799	0.0005**	12.29	0.0011*	0.23
3	3.3090	0.0030*	10.15	0.0035*	0.27
4	4.2899	0.0005**	15.79	0.0010**	0.48
5	3.9965	0.0005**	9.89	0.0026*	0.14
6	3.3652	0.0018*	8.39	0.0062	0.18
7	3.4217	0.0018*	9.43	0.0039*	0.19
8	3.4796	0.0012*	8.40	0.0061	0.17
9	4.2430	0.0002**	11.97	0.0016*	0.27
10	3.4956	0.0016*	13.19	0.0009**	0.27
11	3.4823	0.0021*	8.78	0.0060	0.23
12	2.8317	0.0130	5.88	0.0268	0.26
13	3.0178	0.0171	7.55	0.0205	0.43
14	3.0351	0.0063	7.70	0.0111	0.26
15	3.8376	0.0016*	12.58	0.0029*	0.46
16	4.5404	0.0015*	20.42	0.0014*	0.69
17	2.5937	0.0206	4.62	0.0407	0.15
18	2.9659	0.0112	5.62	0.0261	0.19
19	3.1973	0.0064	7.42	0.0139	0.29
20	3.2729	0.0052	9.04	0.0089	0.38
21	2.2775	0.0451	3.97	0.0648	0.21
22	2.6943	0.0270	6.08	0.0358	0.40
23	1.9637	0.2607	6.02	0.0576	0.55
24	4.0035	0.0041*	16.03	0.0039*	0.67
25	1.6382	0.1754	2.23	0.1661	0.18
26	1.8154	0.2581	4.27	0.1076	0.51

[1)]corresponds to set number in Table 6
** significant at $P \leq 0.001$ lsve.
* significant at $P \leq 0.005$ lsve.

sponse among these selected 19 individuals (Table 7). Set 16, comprised of three markers, pA136, G15d, and E01c, selected 5 individuals as Peking type and 2 of these had FI values <0.10. Of the 6 individuals selected as Essex type, 4 had FI values of >1.00. These 3 markers explained 69 % of the variation in the nematode response among these 11 selected individuals (Table 7). Set 24 (pA136, E01c, G15d, and S07a) selected 5 individuals as Peking type and 5 individuals as Essex type. These 4 markers explained 67 % of the total variation in the SCN response among these selected 10 individuals (Table 7). When 5 markers (set 26, Table 6 and 7) were used, two individuals were recognized as the Peking molecular type. FI value for these individuals were 0.02 and 0.62. Four individuals recognized as Essex type had FI values of 0.65, 0.73, 1.04, and 1.33. Since only 6 individuals were selected by this combination, even one offtype in the selected group offsets the significance of F-value dramatically. Thus, the marker combinations allowed selection of F_2 progeny with lower FI values; however, within the class of F_2 segregants representing Peking molecular type, variation in FI values was still observed. Until very close linkages with QTLs for SCN resistance are detected, compromise has to be made with respect to number of markers employed and the efficiency of the marker combination in regard to the number of selected genotypes for further breeder's manipulations.

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