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Association of RFLP markers with loci conferring broad-based resistance to the soybean cyst nematode (*Heterodera glycines*)

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Abstract Soybean cyst nematode (SCN) is a major soybean yield-limiting pest. The present study was conducted to map broad-based SCN resistance loci from the cultivar 'Hartwig'. Two-hundred $F_{2:3}$ lines derived from the cross 'Williams 82' × 'Hartwig' were screened with a fourth-generation SCN inbred and 56 polymorphic molecular markers. Allele states and phenotypes were analyzed using stepwise regression and the model selection was made at $P \leqslant 0.01$. Four unlinked RFLP markers (A006, A567, A487, A112) were associated with SCN resistance and the partial coefficient of determinations (R^2) were 91%, 1%, 1%, and 1%. We have mapped a new, major SCN resistance locus (A006) and three minor loci (A567, A487, A112). This complete mapping will accelerate the transfer of broad-based resistance without linkage drag and aid in the determination of relationships among various SCN-resistant germplasm sources.

Key words Resistance genes · Soybean cyst nematode · Hartwig · RFLP mapping · PI 437654

Introduction

In the north central region of the United States, the soybean cyst nematode (SCN) (*Heterodera glycines*) is the most yield-suppressing disease of soybean (*Glycine max* (L.) Merr.). The yearly average production loss is 49 million bushels (Doupnik 1993). Once a field is infested, the number of nematodes may be reduced but they cannot be eliminated. The most economically viable management solution to SCN infestation is the use of SCN-resistant soybean cultivars. The genetic complexity and heterogeneity of SCN field populations have been obstacles in understanding the nature of soybean resistance to SCN (Faghihi et al. 1986a, b). Genetic studies have shown that SCN resistance is conferred by multiple genes (Caldwell et al. 1960; Matson and Williams 1965; Myers and Anand 1991; Rao-Arelli et al. 1992; Rao-Arelli 1994; Faghihi et al. 1995). The use of molecular markers offers an efficient alternative to the tedious task of screening germplasm with heterogeneous SCN field populations and permits the efficient selection of polygenic forms of SCN resistance.

Molecular markers have been used to map resistance genes to several species of nematodes. Markers have been linked to several loci conferring resistance to the potato cyst nematode (*Globodera rostochiensis*). Both Barone et al. (1990) and Kreike et al. (1993) mapped loci (*Gro*) conferring resistance to potato cyst nematode in *Solanum spegazzinii*. In addition, the *H1* locus in *S. tuberosum* has also been mapped with molecular markers (Pineda et al. 1993). Markers have been linked to the *Cre* locus in *Triticum aestivum*, conferring resistance to the cereal cyst nematode (*H. avenae*) (Williams et al. 1994) and to the *Hs1* locus conferring resistance to the beet cyst nematode (*H. schachtii*) in *Beta* (Salentijn et al. 1994). Besides cyst nematodes, the root knot nematode (*Meloidogyne incognita*) resistance gene *Mi* has been fine mapped in tomato (*Lycopersicon esculentum*) (Messeguer et al. 1991).

RFLPs have been used to map SCN resistance genes in soybean. Weisemann et al. (1992) found two markers proximal to *Rhg4* based solely on the tight linkage of *Rhg4* to the *i* locus. However, no genotypes were screened with SCN. Concibido et al. (1994) attempted to map SCN resistance from PI 209332. Screening with a SCN field population, RFLP markers were linked with SCN resistance loci on linkage groups A and K. Based on known linkages, it is possible that the putative locus on group A is also *Rhg4*.

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Our objective was to associate molecular markers with SCN resistance from the soybean cultivar Hartwig. Hartwig is unique in that it confers resistance to all known races of SCN (Anand 1992).

Materials and methods

Plant material

Two-hundred $F_{2:3}$ lines were derived from the cross 'Williams 82' (susceptible) × 'Hartwig' (resistant). Hartwig was derived from the cross 'Forrest' × PI 437654 and appears to have retained most of the resistance of PI 437654.

Nematode assay

F_3 seedlings and parents were tested for SCN resistance using a SCN inbred developed from a race-three wild-type population as previously described (Faghihi et al. 1995). The number of females developed on each root system was recorded. For each $F_{2:3}$ line, phenotypes were determined from the transformed mean ($^{10}\log_{x+1}$) of four replications (Faghihi et al. 1995).

Molecular markers

A total of 211 RFLP markers (Keim and Shoemaker 1988) and ten simple sequence repeats (SSR) (Akkaya et al. 1992) were screened for polymorphism in the parental materials.

Data collection and analysis

DNA extraction, restriction digestion, blotting, and hybridizations were the same as previously described (Vierling et al. 1994). In brief, DNA preparations from bulked $F_{2:3}$ seedlings were digested with either *Eco*RI, *Eco*RV, *Hin*dIII, or *Xba*I, separated on a 0.7% agarose gel, and probed with ^{32}P-labeled insert DNA. SSR data were amplified according to Akkaya et al. (1992) and separated on 4% acrylamide gels. Combined RFLP and SSR data were analyzed using Mapmaker (Lander et al. 1987). Allele states and phenotypes were analyzed using stepwise regression and the model selection was made at $P \leqslant 0.01$.

Results

Nematode screening

For parental material, the average number of females developed was 0 on Hartwig and 197 for Williams 82. Female counts on individual F_3 plants ranged from 0 to 544 and average counts for $F_{2:3}$ lines ranged from 0 to 345 (Faghihi et al. 1995) (Fig. 1). $F_{2:3}$ lines were divided into resistant, segregating, and susceptible cells using Ward's minimum-variance analysis (Faghihi et al. 1995). The mean number of females developed on resistant $F_{2:3}$lines was 0 and the mean on susceptible $F_{2:3}$ lines was 163.

Molecular-marker analysis

Fifty-three of the two-hundred-and eleven RFLP markers and three of the ten SSRs were polymorphic between the parental material. These 56 molecular markers were screened using 200 $F_{2:3}$ lines and used to construct a molecular-marker linkage map. The linkage map consisted of 18 groups and 725 map units. The placement of markers to linkage groups was consistent with previously published probe locations (Shoemaker and Olson 1993).

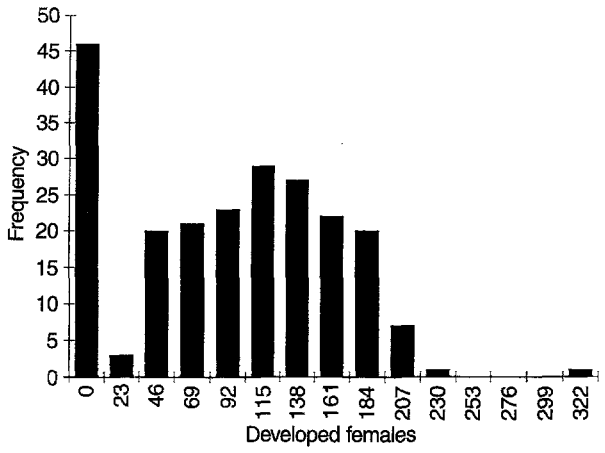

Fig. 1 Mean frequencies of developed females per root system of $F_{2:3}$ lines

Markers associated with SCN resistance

Four unlinked RFLP markers were statistically significant and associated with SCN resistance (Table 1). The model coefficient of determination (R^2) was 94% and the partial R^2 of marker A006 (91%) accounted for most of the model R^2. Markers A567, A487, and A112 each contributed an additional 1% to the model R^2.

In Table 2 are shown the means of females developed for the genotypic classes of each individual marker, regardless of the genotype of the other significant markers. For each marker, the average number of developed females was lowest in the homozygous Hartwig (HH) genotypic class. A006 was the only marker that showed an appreciable decrease of females when in a heterozygous state.

Examination of the genotypic combinations of all four markers showed that $F_{2:3}$ lines that were HH at all loci averaged 0 developed females, which is identical to the parental line Hartwig (Table 3). $F_{2:3}$ lines that were WW at all four loci averaged 181 developed females,

Table 1 Markers significantly ($P < 0.01$) associated with SCN resistance

Marker	Linkage group	R^2	F	Prob > F
A006	B	0.91	1293.3	0.0001
A567	S	0.01	17.5	0.0001
A487	A	0.01	11.7	0.0008
A112	F	0.01	10.2	0.0018

Table 2 Average number of developed females for $F_{2:3}$ genotypes of the four significant RFLP markers

Markers	HH[a]	HW	WW
	Average number of developed females		
A006	5	69	162
A567	39	131	144
A487	46	152	133
A112	54	127	148

[a] HH = Hartwig/Hartwig
HW = Hartwig/Williams 82
WW = Williams 82/Williams 82

Table 3 Average number of developed females for combinations of $F_{2:3}$ genotypes of the four significant RFLP markers

Markers				Developed females
A006	A567	A487	A112	
HH[a]	HH	HH	HH	0
HH	WW	WW	WW or HW	36
WW	HH	HH	HH	72
WW	WW	WW	WW	181

[a] HH = Hartwig/Hartwig
HW = Hartwig/Williams 82
WW = Williams 82/Williams 82

which is similar to Williams 82. Averages of developed females on $F_{2:3}$ lines with various combinations of HH and WW allele states ranged between the parental average.

Discussion

The histogram (Fig. 1) shows a bimodal distribution, with a peak at 0 and with the remainder of the $F_{2:3}$ lines having a normal distribution. Previous genetic analyses of SCN resistance in several crosses using Hartwig and one of its parent PI 437654 suggested resistance was conferred by 1–4 genes depending on the other parent (Myers and Anand 1991; Faghihi et al. 1995).

Our approach for locating SCN resistance loci was to detect statistically significant associations between $F_{2:3}$ lines and the transformed mean of the number of females found on the root systems. $F_{2:3}$ lines were used because they can be replicated. Since SCN resistance is conferred by more than one gene, using a replicated mapping population will allow for the detection of both major and minor effects (Cowen 1988; Carbonell et al. 1993). Using the SCN inbred should increase the accuracy of our $F_{2:3}$ line phenotypic screening by decreasing the variability associated with SCN field populations (Faghihi et al. 1995). $F_{2:3}$ lines were separated into resistant, segregating, and susceptible cells using Ward's minimum-variance cluster analysis (Faghihi et al. 1995). Minimum-variance cluster analysis was used to produce an unbiased separation of phenotypic cells, eliminating an arbitrary designation of phenotypes. Stepwise regression, which is an improved version of the forward-selection procedure, was recommended by Draper and Smith (1966) as the best variable-selection procedure. Stepwise regression re-examines variables at every stage of regression using a partial F criterion compared with a pre-selected ($P < 0.01$) percentage point of the appropriate F distribution. This allows for a judgement of the contribution of each variable as though it had been the most recent entry into the model, despite its actual point of entry. Therefore, any variable that proves non-significant can be removed from the model though it may have been the best single variable at an earlier stage.

Four unlinked RFLP markers showed significant effects on SCN resistance. Marker A006 explained 91% of the total variation and probes A567, A487, and A112 each explained an additional 1% of the total variation (Table 1). Comparison of the three genotypes of each individual marker showed that all markers had their lowest average of developed females in the HH class, with A006 having the lowest. Additionally, A006 was the only marker that showed a large decrease in females between the HW and WW genotypes. This was expected, considering the large R^2 associated with marker A006.

When analyzing genotypic combinations, HH at all loci averaged 0 females, which was identical to Hartwig. Lines with all WW genotypes averaged 181 females, which was very similar to Williams 82, which averaged 197 females. Lines that were HH at just the major locus (A006) and WW or HW at the other loci averaged 36 females, whereas WW at A006 and HH at the other loci averaged 72 females. The fact that only lines with HH genotypes at all four loci showed complete resistance, indicated that all four loci are needed for complete resistance. Comparing the number of developed females with the individual locus or loci combinations showed there was an additive effect on the number of developing females.

We have mapped a new, major SCN resistance locus (A006) and three minor loci (A567, A487, A112) with a model R^2 of 94%. We believe the high R^2 was due to several factors. As stated by Cowen (1988), the use of replicated phenotypic data allowed the detection of minor associations (A567, A487, A112). Using an inbred SCN line decreased the variability associated with screening germplasm with SCN field populations (Faghihi et al. 1995). Genotype-by-phenotype analysis showed that all four loci were required for complete resistance. Our complete mapping of SCN resistance will accelerate the production of SCN-resistant cultivars. Thus it may be possible to introgress resistance loci without linkage drag. Molecular mapping of SCN resistance loci will also elucidate the relationships between different sources of SCN resistance.

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