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Inheritance of Cyst Nematode Resistance in a New Genetic Source, *Glycine max* PI 494182

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Abstract

Worldwide, cyst nematode (*Heterodera glycines* Ichinohe) is the most destructive pathogen of cultivated soybean. In the USA, current annual yield losses are estimated to be nearly a billion dollars. Crop losses are primarily reduced by the use of resistant cultivars. Nematode populations are variable and have adapted to reproduce on resistant cultivars over time because resistance primarily traces to two soybean accessions. It is important to use diverse resistance sources to develop new nematode resistant cultivars. Soybean PI 494182 is a recent introduction from Japan and found to be resistant to multiple nematode populations. It is yellow seeded and maturity group 0. We have determined inheritance of resistance in PI 494182 using $F_{2:3}$ families derived from cross PI 494182 X cv. Skylla. Skylla is a susceptible parent. Three nematode populations, races 1, 3, and 5, corresponding to HG types 2.5.7, 0, and 2.5.7 were used to bioassay 162 $F_{2:3}$ families in greenhouse experiments. Based on Chi-square tests, a two-gene model is proposed for resistance to race 1 and a three-gene model is proposed for conditioning resistance to both races 3 and 5. Correlation coefficient analysis indicated that some genes conditioning resistance to races 1, 3, and 5 are shared or closely linked with each other. These results will be useful to soybean breeders for developing soybean cultivars for broad resistance to nematodes.

Key words: Inheritance of resistance, *Heterodera glycines*, *Glycine max*, Chi-square analysis

Introduction

Soybean [*Glycine max* (L.) Merr] grown for its edible protein and oil, is a very important agronomic crop worldwide. Soybean yields are reduced each year by nematodes and other pests. Soybean cyst nematode, *Heterodera glycines* Ichinohe (SCN), reduces yield more than any other pest in the world. In the USA, estimated annual total yield reduction is valued at over a billion dollars and current losses in the South are valued at nearly US$60 million (Koenning 2006). Resistant cultivars have been an effective means of nematode control. Many resistant cultivars have been developed in the USA, but nearly all have resistance genes from Peking and/or PI 88788 (Diers and Arelli 1999). Widespread use of these resistance sources has caused major shifts in nematode populations. Over time, these variable populations have adapted to resistant cultivars. Genetically diverse sources of resistance may provide durable resistance.

Soybean PI 494182 (cv. Suzhime) is a recent introduction from Japan and is resistant to SCN races 1, 3, and 5 (Arelli et al. 2000; Young 1995). These races correspond to HG Types 2.5.7, 0, and 2.5.7, respectively (Niblack et al. 2002). PI 494182 is a yellow-seeded soybean which is commercially desirable trait in cultivar breeding. PI494182 was evaluated for genetic variation and its genetic relationship was determined with other SCN-resistant accessions. One hundred twenty-two genotypes were evaluated by 85 Simple Sequence Repeat markers from 20 linkage groups. Non-hierarchical (VARCLUS) and hierarchial (Ward's) clustering were combined with multidimensional scaling (MDS) to determine relationships among tested lines. The 122 lines were grouped into seven clusters by two different clustering methods and the MDS results consistently corresponded to the assigned clusters. Assigned clusters were dominated by genotypes that posses one or more unique SCN resistance genes and were associated with geographical origins. The results of analysis of molecular variance (AMOVA) showed that the variation differences among clusters and individual lines were signifi-

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cant. PI494182 was assigned to cluster VI and is genetically very distinct from other sources of SCN resistance including Peking, PI88788, and PI437654 (Chen et al. 2006) and potentially may have different gene/s for nematode resistance. Soybean line Peking is the predominant source of SCN resistance in the cultivars grown in the southern USA. Additionally, it is used in Argentina, Brazil, and China. Soybean PI 494182 can be an excellent alternate or additional source of SCN resistance in developing cultivars, especially to be grown in the fields infested with races 1, 3, and/or 5. However, resistance genes in PI 494182 for these nematode races have not been characterized. The objective was to determine inheritance of resistance in PI 494182 to SCN races 1, 3, and 5.

Table 1. Reaction of seven indicator lines and two parents for three nematode races.

Soybean line	Female Index(%) Race 1	Female Index (%) Race 3	Female Index (%) Race 5
PI 548658 (Lee-74)	100	100	100
PI 548402 (Peking)	1	2	3
PI 88788	57	1	68
PI 90763	0	1	0
PI 437654	2	0	0
PI 209332	70	1	143
PI 89772	2	0	0
PI 548316 (Cloud)	73	10	86
PI 494182	4	1	9
cv. Skylla	110	71	154

Materials and Methods

Plant Materials

Seeds of soybean PI 494182 were obtained from Soybean Germplasm Collection, USDA-ARS, Urbana-Champaign, IL (Courtesy of Randall Nelson, Curator). PI 494182 is maturity group 0 soybean. It is determinate in growth habit, with purple flowers, gray pubescence, brown pod color, and narrow leaflets. To produce $F_{2:3}$ families, soybean PI 494182 was crossed with cv. Skylla. Skylla is a SCN susceptible soybean that was developed at Michigan State University (Wang et al. 2006). It is maturity group II cultivar.

Nematode Cultures

Collection and culturing methods of three near-homogeneous populations of SCN races 1, 3, and 5 used in this research have been reported (Arelli et al., 2000; Qiu et al. 1999).

Briefly, a SCN field population was obtained from Cape Girardeau County, Missouri. The field was known to have planted with cultivars having resistance to races 3 and 14 for several years. The collected nematodes were cultured on the roots of susceptible cv. Hutcheson (Buss et al. 1988) in the greenhouse, adopting limited inbreeding for several generations. This population was categorized as race 1 (HG Type 2-) based on its reaction to standard differentials according to classification system of Golden et al. (1970) and Schmitt and Shannon (1992), and is being maintained on Hutcheson.

A race 3 nematode population (HG Type 0-) was obtained from a soybean field planted to Essex cultivar (Smith and Camper 1973) at Ames Foundation, located near Grand Junction, Tennessee (Courtesy of L. D. Young, USDA-ARS) in the summer of 1994. Essex is susceptible to nematode populations. The nematode population was increased on the roots of Essex in the greenhouse for several more generations (approximately 15 to 20). The population is currently maintained on a susceptible soybean Hutcheson.

A race 5 nematode population was collected at the Rhodes Farm of the University of Missouri, near Clarkton, Missouri in summer of 1993, where soybean cv. Bedford (Hartwig and Epps 1978) was grown for several years. The collected nematode population was cultured in the greenhouse by limited inbreeding on the roots of soybean PI 88788 for over forty generations. Soybean PI 88788 is susceptible to SCN race 5 population (HG Type 2-) and these cultured nematodes are currently maintained on susceptible Hutcheson. These near-homogeneous nematode populations provide consistent reactions on soybean plants.

Originally, these three nematode races 1, 3, and 5 were confirmed by race determination tests available at that time, using four standard soybean differentials, Pickett-71, Peking, PI 88788, PI 90763, and a susceptible control Hutcheson for their classification. Using this system both races 1 and 5 can be separated based on Pickett-71 reaction; Pickett-71 is resistant to race 1 but is susceptible to race 5. For our bioassays, we followed a revised classification scheme, HG Type (Niblack et al. 2002) which included seven indicator lines, [PI 548402 (Peking), PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, PI 548316 (Cloud), and a standard susceptible control Lee-74 (PI 548658)] for better characterization of nematode populations. However, HG Type of classification system will not distinguish races 1 and 5 for their reaction.

Bioassay

The bioassays were performed in the greenhouse at the USDA-ARS, Jackson, TN during the years 2004-2007. Methods for the SCN bioassays performed in the greenhouse followed established protocols (Arelli et al. 2000) with the exception of using water bath system. Each plant was grown in a 7-cm clay pot filled with steam-sterilized soil on a greenhouse bench top with an evaporative cooling and under bench heating system. A computerized system has regulated duration of light in the greenhouse during the bioassays for proper growth of soybean seedlings and nematode infestation. A female index (FI) was used to evaluate the nematode response of each seedling (Golden et al. 1970; Schmitt and Shannon 1992) and was expressed in percentage. It is calculated based on number of female nematodes/average number of females on Lee-74 X 100. A FI of < 10% was considered a resistant (R) reaction whereas, 10% was defined as susceptible (S) reaction on soybean plants.

Evaluations for SCN races 1, 3, and 5 were conducted separately and each test was repeated once. The nematode reaction was based on female development on the roots of seven plants

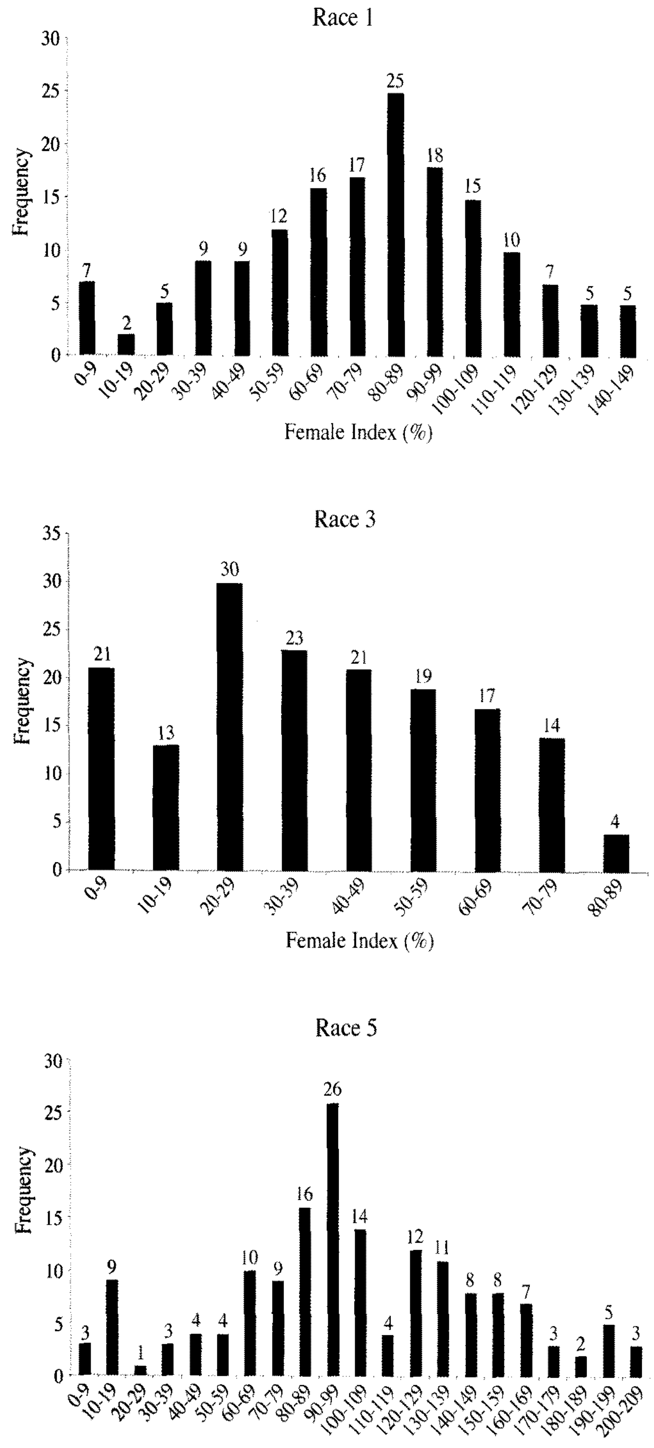

Fig. 1. Frequency distribution for female index (FI) to SCN races 1, 3, and 5 in 162 $F_{2:3}$ lines from PI494182 x cv. Skylla.

from each $F_{2:3}$ family. A female index was calculated and each family was classified either resistant or susceptible category. The Chi-square test was used to test the goodness of fit for the gene models we proposed. We used Pearson's correlation coefficient to relate resistance genes postulated for different races.

Table 2. Chi-square analyses for 162 $F_{2:3}$ families from cross PI 494182 x cv. Skylla for nematode races 1, 3, and 5.

Total	Segregation				Hypothesized Resistance Genes	Chi Square	P-Value %
	Observed		Expected				
	Reaction to Race 1						
	R	S	R	S			
162	7	155	10.125	151.875	*rhg,rhg*	*1.028*	*50<p>30*
	Reaction to Race 3						
	R	S	R	S			
162	20	142	22.7	139.3	*Rhg ,Rhg ,rhg*	*0.394*	*70<p>50*
	Reaction to Race 5						
	R	S	R	S			
162	3	159	2.53	159.47	*Rhg, rhg, rhg*	*0.085*	*50<p>30*

Results and Discussion

Data from the SCN bioassay indicated that the responses to SCN races 1, 3, and 5 were normally or near-normally distributed (Fig. 1). Therefore, the experimental design and data collection were appropriate for further genetic analysis.

The reaction of three nematode populations, races 1, 3, and 5 on indicator lines and the parents is included in Table 1. One hundred and sixty-two $F_{2:3}$ families were evaluated against each of three races. We have categorized seven uniformly resistant (non-segregating resistant) and 155 susceptible (including segregating) families for reaction to race 1 nematode population. The data for Chi-square analysis fit for a two recessive gene model (*rhg, rhg*) based on, 1 (resistant):15 (susceptible plus segregating) genetic ratio and included in Table 2. We have previously included segregating into susceptible category and used only two phenotypic classes (resistant and susceptible) in F_3 generation for Chi-square analysis (Yue et al. 2000).

The categorization of families for reaction to race 3 nematode population includes 20 uniformly resistant (non-segregating resistant) and 142 susceptible (including segregating). Based on the Chi-square analysis, a three-gene model (*Rhg, Rhg, rhg*) is proposed (Table 2) for a genetic ratio of 9 (resistant): 55 (susceptible plus segregating). Another three-gene model, *Rhg, rhg, rhg* (one dominant and two recessive genes) is proposed for reaction to nematode population race 5 based on a genetic ratio of 1 (resistant) : 63 (susceptible plus segregating) (Table 2).

The reaction to different races for the same $F_{2:3}$ families were determined using correlation analysis. The results (Table 3) show that resistance to race 1 is significantly correlated with resistance to race 3 and race 5, implying that some genes conditioning resistance to races 1, 3, and 5 are shared or closely linked with each other.

All HG Types (SCN races) used in this study were confirmed according to reaction on the standard indicator lines (differentials) and susceptible control (Lee 74). Parents and indicator lines included in all inoculations in this study showed reactions to the populations of HG Types consistent with the protocols

Table 3. Pearson's correlation coefficient of reaction of 162 $F_{2:3}$ plants to three different SCN HG Types (races).

	HG Type 2.5.7 (Race 1)	HG Type 0 (Race 3)	HG Type 2.5.7 (Race 5)
Race 1	1.000	0.539 <0.0001	0.488 <.0001
Race 3	0.539 <.0001	1.000	0.236 0.0025
Race 5	0.488 <.0001	0.236 0.0025	1.000

established by Schmitt and Shannon (1992) and Niblack et al. (2002). Significant variation was observed in the response of $F_{2:3}$ families, parents and indicator lines, and also between replications for all HG Types tested (data not included), suggesting that the reactions of individual plants to nematode populations are affected significantly by environment.

The genetic data for PI494182 showing resistance to SCN races 1, 3, and 5 involving two to three major genes are consistent with previous studies. All studies including our own have shown that SCN resistance in general is a complex trait. In soybean Peking, three recessive and one dominant gene were proposed that conditioned resistance to nematode populations (Caldwell et al. 1960; Matson and Williams 1965). Arelli et al. (1992) identified an additional dominant gene *Rhg5* in soybean PI 88788. It is also postulated that several minor resistance genes in soybean are involved in conditioning resistance to SCN populations. We have not done allelism tests to determine how many of these reported genes are in common or different with soybean PI 494182. We are developing germplasm with resistance to SCN races 1, 3, and 5 in maturity groups II and III. This germplasm should be useful to breeders as a valuable source material for breeding resistant cultivars for genetic diversity. We await mapping data for confirming and assigning these resistance genes to specific linkage groups in soybean PI 494182.

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