Euphytica (2009) 165:1–4
DOI 10.1007/s10681-008-9760-z

REVIEW

Inheritance of resistance in soybean PI 567516C to LY1 nematode population infecting cv. Hartwig

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Received: 11 January 2008 / Accepted: 27 June 2008 / Published online: 12 July 2008

Abstract Worldwide, cyst nematode (SCN) *Heterodera glycines* is the most destructive pathogen on cultivated soybean (*Glycine max* (L.) Merr.). In the USA yield losses in 2001 were estimated to be nearly 60 million dollars. Crop losses are primarily reduced by the use of resistant cultivars. Nematode populations are variable and have adapted to reproduce on resistant cultivars overtime because resistance primarily traces to two soybean accessions. Recently cv. Hartwig was released which has comprehensive resistance to most SCN populations. A virulent nematode population LY1 was recently selected for its reproduction on Hartwig. LY1 population originated from a mass mating of Race 2 (HG Type 1.2.5-) females with Race 5 (HG Type 1.2-) males. LY1 nematode population infects currently known sources of resistance except PI 567516C. The female indices obtained on PI 567516C and Hartwig were 7% (resistant) and 155% (susceptible), respectively. However, the genetic basis of LY1 resistance in soybean PI 567516C is not known. Resistant PI line 567516C was crossed to susceptible cultivar Hartwig to generate 105 $F_{2:5}$ families. These families together with parents, seven indicator lines and a susceptible control cv. Lee-74 were evaluated for response to LY1 nematode population in the greenhouse. Chi-square analysis showed resistance in PI567516C to LY1 was conditioned by one dominant and two recessive genes (*Rhg*, *rhg*, *rhg*). Chi-square value was 0.15 and $P = 0.70$. This information will be useful to soybean researchers for developing resistant cultivars to nematode population that infects Hartwig.

Keywords Chi-square analysis · cv. Hartwig · *Heterodera glycines* · LY1 · PI 567516C · Resistance · Soybean (*Glycine max* (L.) Merr.) · Soybean cyst nematode

Introduction

Worldwide, soybean (*Glycine max* (L.) Merr.), grown for its edible protein and oil, is a very important agronomic crop. Soybean yields are reduced each year by nematodes and diseases. Soybean cyst nematode, *Heterodera glycines* Ichinohe (SCN) reduces yield more than any other soybean pest in the world. SCN causes yield reductions by feeding on plant nutrients, retarding root growth, and inhibiting Bradyrhizobium nodulation. In the USA yield losses in 2001 were estimated to be nearly 60 million dollars (Wrather et al. 2001). Resistant cultivars have

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been an effective means of control. Soybean breeders have been evaluating the soybean germplasm collection for sources of resistance. Most evaluations for nematode resistance included Races 3, 5, and 14 corresponding to HG Types 0-, 2.5.-, 1.3.-, respectively (Niblack et al. 2002). A total of 118 resistant PI (Plant Introduction) lines were identified with resistance to one or more of these Races (Arelli et al. 1997). They also bio-assayed these 118 accessions for resistance to Races 1 and 2 corresponding to HG Types 2.5.- and 1.2.5-, respectively (Arelli et al. 1997, 2000). Although, more than 100 SCN resistance sources have been identified, the genetic base of host resistance is very narrow. Many resistant cultivars have been developed in the USA but, nearly all have resistance genes from Peking and PI 88788 (Diers and Arelli 1999). Additionally, both soybean lines Peking and PI 88788 were reported to have major resistance genes in common (Arelli and Anand 1998). Widespread use of these resistance sources has caused major shifts in nematode populations and nematodes have adapted to reproduce on resistant cultivars overtime.

Recently, soybean cultivar Hartwig was released which has comprehensive resistance to most nematode populations (Anand 1992). Hartwig is infected by a few isolates of Race 4 population of SCN (corresponding to HG Type 1.2.3.5.6.7). Additionally, a virulent nematode population LY1 also reproduces on Hartwig including its primary source of resistance, PI437654 (Young 1998). Among known sources of resistance to nematode populations including Peking, and PI 88788 soybean PI 567516C was the only available source resistant to LY1 nematode population (Young 1999). Recently, Arelli and Young (2005) confirmed its resistance to LY1 nematodes. Chen et al. (2006) determined that PI567516C and Hartwig are genetically unique. The same study also concluded that PI567516C is genetically unique from most other sources including Peking and PI88788. It is highly desirable to introgress resistance genes from PI 567516C into Hartwig for developing germplasm with more comprehensive resistance to known nematode populations parasitizing PI437654-derived cultivars. However, resistance genes in PI567516C for LY1 nematode have not been characterized. The objective was to determine inheritance of resistance in soybean line PI 567516C to LY1 nematode population.

Materials and methods

Plant materials

Seeds of soybean PI 567516C were obtained from Soybean Germplasm Collection, USDA-ARS, Urbana-Champaign, IL (courtesy of Randall Nelson, Soybean Curator) for making crosses. PI 567516C is a maturity group IV soybean, with purple flowers, grey pubescence, greenish yellow seed and having resistance to nematode Races 1, 3 and moderate resistantance to Race 2, corresponding to HG Types 2.5.-, 0-, 1.2.5.-, respectively(Young 1995; Arelli et al. 1997). This line was crossed with Hartwig in 1999 to generate segregating progenies for greenhouse evaluations. A total of 105 F2:5 families from PI567516C × Hartwig were bioassayed for reaction to the LY1 nematode population. A modified method of greenhouse evaluation followed an established protocol (Arelli et al. 2000). A set of differentials or indicator lines were included in each evaluation to characterize the nematode population. These indicator lines were PIs 548402 (Peking), 88788, 90763, 437654, 209332, 89772, 548315 (Cloud) and a susceptible control 548658 (cv. Lee 74).

Nematode population

LY1 nematode population was developed from a mass mating of SCN Race 2 (HG Type 2.5-) females with SCN Race 5 (HG Type 1.3-) males. Eggs resulting from the cross were cultured on Hartwig and then on cultivar Hutcheson in alternate generations for 14 cycles, followed by 13 continuous generations of reproduction on Hartwig. Female Index after 26 generations of selection on Hartwig was 34% and continued selection for several more cycles has produced nematode population with a female index of nearly 100%. This population was then maintained on Hutcheson for ten generations.

Nematode bioassay

The plants of each F2:5 family and indicator lines were grown in clay pots (2–3 seeds per 7.5-cm-diam pot) filled with silt loam soil and the test was completely randomized. Both soil and pots were steam sterilized. Seven seedlings were grown from each F2:5 family and each seedling represented a single replication. After germination, the seedlings were thinned to one per pot and were grown for 4–5 days prior to their inoculation with LY1 nematode eggs. Each pot was inoculated with approximately 2,000 eggs of nematodes in 5 ml. suspension of water. Plants were grown for 30 days in a greenhouse with an evaporative cooling system and under-bench heating at 27 ± 1°C. At the end of 30 days, each root ball together with soil was separated individually from pots. The soil was removed gently from plant roots by softly crumbling the root ball. Plant roots were individually washed with a strong jet of water to dislodge the females and cysts that were counted under a stereomicroscope. A Female Index (FI%) was calculated to evaluate the nematode response to each individual seedling (Golden et al. 1970; Schmitt and Shannon 1992).

$$\mathrm{FI\%} = (\text{Number of females on a given individual plant/Average number of females on Lee 74}) \times 100.$$

The test was repeated three times.

Based on average reaction of seven plants in three separate bioassays, each F2:5 family was categorized as resistant (R) or susceptible (S). We defined resistance as <10% FI established for plant reaction to nematode (Niblack et al. 2002). The Chi-square (χ^2) test using Yates correction analysis to adjust for small population size was used to test the goodness of fit for appropriate genetic hypotheses.

Results and discussion

The reaction of LY1 nematode population (FI) on indicator lines and parental lines is included in Table 1. There was environmental variation (data not shown) in the greenhouse evaluations. Even the parental lines had variation for FI values, however, they were uniformly classified as resistant or susceptible (Table 2). The reaction was consistent for the families in three evaluations with the same families categorized as either resistant or susceptible in all evaluations. Range and means for FI are included in Table 2. Transgressive segregation for resistance was observed within F2:5 families.

Table 1 Reaction of the LY1 nematode population on indicator lines and parents (Average of three tests)

Soybean	Female Index (%)
PI 548402 (Peking)	83
PI 88788	45
PI 90763	92
PI 437654	260
PI 209332	172
PI 89772	55
PI 548316 (Cloud)	130
PI 548658 (cv. Lee 74)	100
cv. Hartwig	155
PI 567516C	7

One-hundred and five (F2:5) families from the cross PI567516C with Hartwig were evaluated against LY1 nematode population, and five families were categorized resistant and 100 susceptible. This was a good fit to an expected three-gene model, *Rhg*, *rhg*, *rhg* (one dominant and two recessive genes) with a segregation ratio of 3 (Resistant):61 (Susceptible) (Table 3). The Chi-square (χ^2) value was 0.15 with a *P* value of 0.70. We have not designated gene symbols for these genes due to the difficulties involved in tests for allelism.

The genetic data for PI567516C showing resistance involving three major genes are consistent with previous studies that have shown SCN resistance in general is a complex trait. Three and four gene models for HG Types 2.5.-; 0-; 2. -; and 1.3.6.- (corresponding to Races 1, 3, 5, and 14, respectively) were proposed in previous studies. It has been also postulated that several minor genes are involved in conditioning resistance to SCN populations in soybean. Caldwell et al. (1960) reported the

Table 2 LY1 nematode Female Index, means and ranges of parents and 105 F2:5 families from a cross between PI 567516C and Hartwig (Average of three bioassays)

Parents & families	Total number of plants/ families	FI% Means (Resistant)	FI% Range (Resistant)	FI% Means (Susceptible)	FI% Range (Susceptible)
PI 567516C (R)	7	7	0–11		
cv. Hartwig (S)	7			152	84–220
F2:5 Families	105	5.3	2.6–8.0	164	18–310

Table 3 Chi-square analysis for $F_{2:5}$ lines in PI 567516C × Hartwig population for LY1 nematodes. (Average of three tests)

Number of families							
Total	Observed		Expected		Hypothesized	χ^2	*P*-value
	Resistant	Susceptible	Resistant	Susceptible	Resistance genes		
105	5	100	4.9	100.1	*Rhg rhg rhg*	0.15	0.70

inheritance of resistance to SCN (probably using Race 1 population) in soybean Peking, proposing three recessive genes *(rhg1*, *rhg2*, *rhg3*). Later, an additional dominant gene (*Rhg4)* was reported in Peking by Matson and Williams (1965), using most likely a Race 3 population. Arelli et al. (1992) identified an additional dominant gene in soybean PI 88788 that was later designated *Rhg5* (Arelli 1994). Arelli et al. (1992) used a SCN Race 3 population for their research. We do not know how many of these reported genes for resistance are in common or different with soybean PI567516C for LY1 nematode population.

Generally, environmental variation is a common factor in nematode studies. Greenhouse bioassays for nematode reaction are highly labor-intensive, tedious and time consuming. We grew each experiment for bioassays at different times and hence in different environments. It is possible that the small population size of 105 F2:5 families has caused either an overestimation or underestimation of number of resistant and susceptible families. However, it appears that any biased estimations did not cause a serious problem because the data consistently fit the proposed genetic model of 3(R):61(S) in each of the three separate experiments.

Soybean line PI567516C is the only known source of resistance to the virulent nematode population LY1 which infects PI437654 and its derived cultivars including Hartwig. Hartwig is the only known soybean cultivar with broad resistance available to several nematode populations (Races or HG Types). We are developing germplasm with more comprehensive resistance to LY1 nematodes in Hartwig background. This germplasm should be useful to soybean breeders as a valuable source material for breeding resistant cultivars in different maturity groups. We await mapping data for confirming these resistance genes in soybean PI567516C to specific linkage groups. Mapping data will also determine which genes in PI567516C are common or different to genes mapped in other SCN resistance sources.

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