SOYBEAN CYST NEMATODE RESISTANCE DERIVED FROM *GLYCINE TOMENTELLA* IN AMPHIPLOID (*G. MAX* × *G. TOMENTELLA*) HYBRID LINES

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ABSTRACT

Bauer, S., T. Hymowitz, and G. R. Noel. 2007. Soybean cyst nematode resistance derived from *Glycine tomentella* in amphiploid (*G. max* × *G. tomentella*) hybrid lines. Nematropica 37:277-285.

Soybean cyst nematode, *Heterodera glycines*, is an important yield limiting pest in major soybean production areas throughout the world. The primary component of *H. glycines* management is the planting of resistant cultivars in rotation with nonhost crops. Several genes that confer resistance to *H. glycines* have been identified, but populations of *H. glycines* that can overcome all of the genes are known. In order to find additional resistance genes, 491 accessions of the perennial soybeans *G. argyrea, G. canescens, G. clandestina, G. curvata, G. cyrtoloba, G. dolichocarpa, G. falcata, G. latifolia, G. microphylla, G. pindanica, G. tabicina*, and *G. tomentella* were evaluated for resistance to *H. glycines* HG Type 0. All species, except *G. curvata* and *G. pindanica,* had at least one accession that was immune to *H. glycines. Glycine tomentella* (accession PI 483218, 2n = 78), which was immune to *H. glycines,* was used to introgress resistance into intersubgeneric hybrids of *G. max* (cv. Altona) and this *G. tomentella* accession. Amphiploid hybrid lines ($2n = 118$) resulting from this hybridization were backcrossed to *G. max* (cv. Clark 63) to develop fertile lines ($2n = 40$). Fifty clones were developed, and when challenged with *H. glycines* HG Type 0, the clones also were immune to the nematode.

Key words: Glycine argyrea, G. canescens, G. clandestina, G. curvata, G. cyrtoloba, G. dolichocarpa, G. falcata, G. latifolia, G. max, G. microphylla, G. pindanica, G. tabicina, G. tomentella, Heterodera glycines, resistance, perennial soybean, soybean, soybean cyst nematode.

RESUMEN

Bauer, S., T. Hymowitz, y G. R. Noel. 2007. Resistencia al nematodo quiste de la soya derivada de *Glycine tomentella* en líneas híbridas anfiploides (*G. max* × *G. tomentella*). Nematropica 37:277-285.

El nematodo quiste de la soya, *Heterodera glycines*, es una plaga importante en la producción de soya a nivel mundial. El componente más importante para el manejo de *H. glycines* es la siembra de variedades resistentes en rotación con cultivos no hospedantes. Se han identificado varios genes que confieren resistencia a *H. glycines*, pero también se conocen poblaciones que pueden reproducirse en presencia de todos estos genes. Con el fin de hallar genes de resistencia adicionales, se evaluó la resistencia a *H. glycines* Tipo HG 0 de 491 accesiones de soya perenne *G. argyrea, G. canescens, G. clandestina, G. curvata, G. cyrtoloba, G. dolichocarpa, G. falcata, G. latifolia, G. microphylla, G. pindanica, G. tabicina* y *G. tomentella.* En todas las especies, excepto en *G. curvata* y *G. pindanica*, se encontró por lo menos una accesión inmune a *H. glycines.* Se utilizó *Glycine tomentella* (accesión PI 483218, 2n = 78), la cual es inmune a *H. glycines,* para incorporar resistencia en híbridos intersubgenéricos de *G. max* (cv. Altona) y esta accesión de *G. tomentella.* Se retrocruzaron las líneas híbridas anfiploides ($2n = 118$) resultantes de esta hibridización con *G. max* (cv. Clark 63) para desarrollar líneas fértiles ($2n = 40$). Se desarrollaron 50 clones, que al evaluarse con *H. glycines* Tipo HG 0, también fueron inmunes al nematodo.

Palabras clave: Glycine argyrea, G. canescens, G. clandestina, G. curvata, G. cyrtoloba, G. dolichocarpa, G. falcata, G. latifolia, G. max, G. microphylla, G. pindanica, G. tabicina, G. tomentella, Heterodera glycines, nematodo quiste de la soya, resistencia, soya, soya perenne.

INTRODUCTION

Soybean cyst nematode, *Heterodera glycines* Ichinohe, causes crop loss in all major soybean, *Glycine max* (L.) Merrill, production areas throughout the world (Riggs, 2004). In the United States from 1996 to 2004, nation-wide crop loss due to *H. glycines* has ranged from 28% to 54%, which is more than any other pathogen (Wrather and Koenning, 2004). Management of *H. glycines* relies primarily on crop rotation and planting of resistant cultivars. However, resistance may not be durable due to apparent loss of resistance genes in proprietary cultivars and populations of *H. glycines* that can defeat all resistance genes identified in *G. max* (Bond *et al.*, 2006; Dias *et al.*, 1998). Riggs *et al.* (1998) evaluated six accessions of five perennial species for host suitability to *H. glycines* and also evaluated *G. max* × *G. tomentella* hybrids. Resistance was identified in the perennial species. However, of 65-70 *G. max* × *G. tomentella* (PI483218) hybrids tested against races 3, 5, and 14 none were resistant (FI < 10). In order to identify other sources of resistance and perhaps additional mechanisms of resistance, we evaluated 491 accessions of the perennial soybean species *G. argyrea* (9), *G. canescens* (36), *G. clandestina* (46), *G. curvata* (4), *G. cyrtoloba* (14), *G. dolichocarpa* (3), *G. falcata* (6), *G. latifolia* (17), *G. microphylla* (24), *G. pindanica* (1), *G. tabicina* (114), and *G. tomentella* (217) for resistance to *H. glycines*. Our objectives were to evaluate accessions of several species of perennial soybean for resistance to *H. glycines* and to determine whether resistance could be introgressed into clones of *G. tomentella* × *G. max* hybrids to provide potential sources of resistance.

MATERIALS AND METHODS

Seeds of the perennial accessions were placed on moist filter paper in Petri plates and germinated. If available, five seedlings of each accession were transplanted into 7-cm diameter clay pots containing 180 cm^3 of sand and placed on a greenhouse bench. Depending on the accession, plants were grown for 3-12 months. Supplemental HID lighting was provided to maintain a 14-hour day length. One week before inoculation, seedlings of *H. glycines*-susceptible cv. Lee 74 soybean were transplanted into pots as described above. Plants were inoculated with 3,000 eggs and J2 obtained from females and cysts of Hg Type 0 raised on Lee 74 soybean (Assunção *et al.*, 2004) and maintained on a greenhouse bench for approximately 1 month. Supplemental heating and cooling were provided to maintain soil temperatures of 24-30°C.

When mature females appeared on roots of Lee 74 soybean (1 month), the root system of each plant was dipped vigorously several times in a beaker containing 3 L of water to remove soil and females from the roots. Roots were placed on nested 850- and 250-µm-pore sieves and sprayed with a strong stream of water to remove adhering females. The soil was suspended in the water and gravity sieving using the nested sieves was used to extract females from the soil suspension (Cobb, 1918). Females were collected from the 250-µm-pore sieve and counted with the aid of a dissecting microscope. The thoroughly cleaned roots were placed in a drying oven at 70°C for 48 hours. Roots were removed, allowed to equilibrate for 24 hours at room temperature and humidity, and weighed.

A female index (FI) ((no. females per 0.1 g dry root of accession/no. females per 0.1 g dry root of Lee 74) × 100) was calculated for each accession and a rating of resistance classified as: resistant (R): $0 \le FI < 10$; moderately resistant (MR): $10 \le FI < 30$; moderately susceptible (MS): $30 \le FI < 60$; and susceptible (S): $FI \ge 60$ (Assunção *et al.*, 2004).

Development of hybrids was described previously (Singh *et al.*, 1993). Briefly, a hybrid of *G. tomentella* (accession PI 483218, $2n$ = 78) and *G. max* (cv. Altona, $2n$ = 40) was developed by treating progeny of the cross with colchicine to develop amphiploids ($2n$ = 118). Progeny were back crossed six times to cv. Clark 63 (*G. max*) to produce monosomic alien addition lines. These were selfed to produce disomic alien addition lines, thus proving these lines were fertile. Clones (female sterile, male fertile) from the amphiploids were derived from 50 plants and propagated from cuttings of the mother plant as described previously (Patzoldt *et al.*, 2007). Briefly, stems with three nodes were treated with the root promoting hormone powder Rhizopon AA#2 (Phytotronics, Earth City, MO) and placed under supplemental lighting at 25°C in tubes containing sterile deionized water. After 2-3 weeks, plants with well developed roots were transplanted into 7-cm diameter pots containing 180 cm^3 of sand. Each clone was replicated three times and inoculated with 3,000 eggs/pot with the Hg Type 0 population used in the evaluation of perennial soybean. Growing conditions and evaluations were as described above for the perennial species. The experiment was repeated for 22 of the clones evaluated for resistance to a population of *H. glycines* developed by repeated selection on cv. Hartwig.

In order to confirm that the lack of *H. glycines* development was not do to some aspect of the production of clones, clones derived from *G. tomentella* accessions and identified as resistant, moderately resistant, or susceptible to *H. glycines* were evaluated. Those accessions were PI 446949 (R), PI 446952 (MR), PI 446953 (MR), and PI 505214 (S). PI 483218 was used as the resistant control. The protocol and experimental conditions described above for evaluations of clones of PI 483218 were followed except that plants were inoculated with 4,000 eggs.

RESULTS

Among the 491 accessions evaluated, resistance to *H. glycines* was identified in each of the 12 species, *G. argyrea, G. canescens, G. clandestina, G. curvata, G. cyrtoloba, G. dolichocarpa, G. falcata, G. latifolia, G. microphylla, G. pindanica, G. tabicina,* and *G. tomentella* (Table 1). The reaction of representative accessions of each species is provided in Table 1. Information on the 491 accessions evaluated is available on the Germplasm Resources Information Network (GRIN), which may be accessed at http://www.ars-grin.gov/. All of the species except *C. curvata* and *G. pindanica* had at least one accession that was immune to *H. glycines.* However, only four accessions of *C. curvata* and one of *G. pindanica* were evaluated. Approximately 56% of the 491 accessions were immune or expressed a high level of resistance. However, *G. canescens* accessions PI440940 and PI505157, *G. clandestina* accession PI440952, *G. cyrtoloba* accession PI 505171 and PI505169 were classified as MS ($30 \leq IP < 60$). Accessions classified as susceptible ($FI \geq 60$) were *G. cyrtoloba* accession PI505171, *G. microphylla* accession PI 509489, and *G. tomentella* accession PI505214. Additionally, *G. tomentella* accessions PI 446949, PI 446952, PI446953, and PI 505214 used subsequently to evaluate levels of resistance in clones were classified as MR (FI = 13%), MR (FI = 17%), MR (FI = 20%) and MR (FI = 17%), respectively. The clone developed from immune PI483218 was immune.

All 50 of the amphiploid hybrid clones were immune to *H. glycines* HG Type 0 (Race 3) and the 22 clones evaluated with the *H. glycines* population selected on Hartwig were considered immune (Table

Table 1. Greenhouse evaluation of some accessions of *Glycine* species for resistance to *Heterodera glycines* Hg Type 0.

Species	PI[x]	FI[y]	Resistance rating[z]
argyrea	595793	2.9	R
argyrea	595794	0.0	R
argyrea	595795	0.0	R
argyrea	595796	0.0	R
argyrea	599400	0.0	R
canescens	440927	0.0	R
canescens	440928	0.0	R
canescens	440935	24.0	MR
canescens	440940	49.0	MS
canescens	505157	32.8	MS
canescens	509454	0.0	R
canescens	509455	0.0	R
clandestina	440952	55.0	MS
clandestina	440954	0.6	R
clandestina	440969	0.0	R
clandestina	446943	0.0	R
clandestina	509470	0.0	R
clandestina	546961	0.0	R
clandestina	546968	13.8	MR
curvata	499931	2.3	R
curvata	505164	1.7	R
curvata	505167	3.5	R
cyrtoloba	440962	0.3	R
cyrtoloba	440963	50.5	MS
cyrtoloba	440964	5.9	R
cyrtoloba	499923	0.0	R
cyrtoloba	505171	60.1	S
cyrtoloba	505172	0.0	R
cyrtoloba	505177	0.0	R
dolichocarpa	IL 1370	1.6	R
dolichocarpa	IL 1371	0.0	R
dolichocarpa	IL 1372	0.0	R
falcata	233139	0.0	R
falcata	505180	2.3	R

[x]Plant Introductions are permanent USDA numbers; IL are temporary accession numbers.
[y]Female Index of parasitism where FI = ((no. females per 0.1 g dry root of accession/no. females per 0.1 g dry root of Lee 74) × 100).
[z]Resistance classified as: resistant (R): $0 \le IP < 10$; moderately resistant (MR): $10 \le IP < 30$; moderately susceptible (MS): $30 \le IP < 60$; and susceptible (S): $IP \ge 60$.

Table 1. (Continued) Greenhouse evaluation of some accessions of *Glycine* species for resistance to *Heterodera glycines* Hg Type 0.

Species	PI[x]	FI[y]	Resistance rating[z]
falcata	509473	0.1	R
falcata	509474	0.0	R
falcata	509475	0.0	R
falcata	595817	0.0	R
latifolia	319696	0.0	R
latifolia	321394	0.0	R
latifolia	378709	0.0	R
latifolia	440978	0.0	R
latifolia	440980	0.0	R
latifolia	509480	27.4	MR
latifolia	559294	0.0	R
microphylla	339664	0.0	R
microphylla	339665	1.8	R
microphylla	446941	6.6	R
microphylla	509485	0.0	R
microphylla	509486	0.0	R
microphylla	509487	0.0	R
microphylla	509489	95.9	S
pindanica	595818	12.3	MR
tabacina	193232	0.0	R
tabacina	237286	0.0	R
tabacina	248253	0.3	R
tabacina	373986	17.3	MR
tabacina	446999	5.1	R
tabacina	447000	0.0	R
tabacina	483198	0.0	R
tomentella	233501	0.0	R
tomentella	320547	0.2	R
tomentella	441000	8.6	R
tomentella	446948	22.5	MR
tomentella	483218	0.0	R
tomentella	505214	129.7	S
tomentella	505215	0.0	R

[x]Plant Introductions are permanent USDA numbers; IL are temporary accession numbers.
[y]Female Index of parasitism where FI = ((no. females per 0.1 g dry root of accession/no. females per 0.1 g dry root of Lee 74) × 100).
[z]Resistance classified as: resistant (R): 0 ≤ IP < 10; moderately resistant (MR): 10 ≤ IP < 30; moderately susceptible (MS): 30 ≤ IP < 60; and susceptible (S): IP ≥ 60.

Table 2. Number of *Heterodera glycines* females on roots of amphiploid clones of *Glycine max* × *G. tomentella* hybrids.

Clone	Hg Type 0 No. females per plant	Clone	Hartwig selection No. females per plant
Lee 74	176	Lee 74	244
		Hartwig	109
1	0	1	0
3	0	3	0
3C	0	—	—
4	0	4	0
4A	0	—	—
4B	0	—	—
5	0	5	0
6	0	—	—
8	0	8	0
10	0	10	0
10A	0	—	—
10B	0	—	—
11	0	—	—
11d	0	—	—
13	0	13	0
15	0	15	0
15b	0	—	—
16	0	—	—
17	0	17	0
18	0	—	—
20	0	20	0
21	0	21	0
22	0	22	0
23	0	23	0
28	0	—	—
29	—	29	0
30	0	—	—
31	—	31	0
33	0	33	0.3[z]
34	0	—	—
35	0	35	0
36	0	36	0

[y]Only one plant survived to the time of inoculation.
[z]Presence of females in these samples is believed due to contamination of screens as clone 44 was the first processed after Lee 74 and was followed by clones 33, 41, and 42.

Table 2. (Continued) Number of *Heterodera glycines* females on roots of amphiploid clones of *Glycine max* × *G. tomentella* hybrids.

Clone	Hg Type 0 No. females per plant	Clone	Hartwig selection No. females per plant
36D	0	—	—
38	0	—	—
39	0	—	—
40	—	40	0[y]
40B	0	—	—
41	0	41	0.3[yz]
42	0	42	1[yz]
43	0	—	—
44	0	44	9[yz]
44A	0	—	—
46-1	0	—	—
47	0	—	—
48	0	—	—
51	0	—	—
51a	0	—	—
51b	0	—	—
52a	0	—	—
52b	0	—	—
53	0	—	—
55	0	—	—
56	0	—	—

[y]Only one plant survived to the time of inoculation.
[z]Presence of females in these samples is believed due to contamination of screens as clone 44 was the first processed after Lee 74 and was followed by clones 33, 41, and 42.

2). The few females recovered from the sieve are believed due to contamination with females from the susceptible control. The population selected on cv. Hartwig had an FI of 45 when compared to the number of females that developed on Lee 74. *Glycine tomentella* × *G. max* clones developed from *H. glycines*-susceptible PI 446949, PI 446952, and PI446953 expressed levels of resistance to *H. glycines* similar to those observed in the evaluation of the PIs. However, PI 505214 was not susceptible, since the observed FI was 17, indicating a MR response. Thus, the FIs for clones of PI 446949, PI 446952, PI446953, and PI 505214 all indicated a MR reaction.

DISCUSSION

Perennial soybean species constitute new sources of resistance to *H. glycines*. Exploitation of these new genes will depend on the ability to develop hybrids between the perennial *Glycines* species and *G. max* that may be used to make crosses with *G. max* leading to the development of agronomically acceptable cultivars. The immune response of the clones indicates that an additional resistance gene or genes exist. Immune responses to sources of resistance in *G. max* to *H. glycines* are not common. When first evaluated, PI437654 was reported as immune to all races of *H. glycines* (Anand *et al.*, 1988). Anecdotal reports have since indicated a few females from some populations of *H. glycines* will develop on PI437654. In Brazil a field population that had never been challenged with the PI437654 source of resistance reproduced on cv. Hartwig (PI437654 source of resistance) (Dias *et al.*, 1998). Cultivars derived from this source of resistance do no have adequate yield in the absence of, or in the presence of low numbers of *H. glycines.*

The MR response (FI = 13%) of clones of PI446949, which expressed an R reaction (FI = 3.4%) in the evaluation of plants grown from seeds was not surprising. However, the MR response PI505214, which was susceptible in the evaluation of plants grown from seeds, was unexpected. It is possible that the adventitious roots that develop from cuttings affect penetration of J2 or the establishment of syncytia for feeding. Transformed roots were not as effective in providing numbers of females equal to those that developed on normal roots of seedlings (Cho *et al.*, 2000).

The lack of durability of *G. max* resistance to *H. glycines* continues to be problematic. Exploitation of the resistance of perennial soybeans may provide new genes and/or mechanisms of resistance that will be durable. Of special interest to soybean breeders and to soybean producers would be the incorporation of resistance in cultivars of both *H. glycines* and Asian soybean rust (causal organism *Phakopsora pachyrhizi*) obtained from the same *G. tomentella* × *G. max* clones (Patzoldt *et al.*, 2007).

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