Theor Appl Genet (2005) 111: 44–49
DOI 10.1007/s00122-005-1970-3

ORIGINAL ARTICLE

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Rhg1 alleles from soybean PI 437654 and PI 88788 respond differentially to isolates of *Heterodera glycines* in the greenhouse

Received: 22 November 2004 / Accepted: 17 February 2005 / Published online: 10 May 2005

Abstract The production of resistant soybean [*Glycine max* (L.) Merr.] cultivars is the most effective means for controlling losses from soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe). The major resistance gene in most SCN resistance sources is *rhg1*, which has been mapped as a quantitative trait locus onto linkage group G. Our objective was to determine whether the SCN resistance sources PI 437654 and PI 88788 have different functional alleles at *rhg1* based on resistance phenotypes. Populations segregating for resistance alleles at *rhg1* from both PI 88788 and PI 437654 and at *Rhg4*, a second SCN resistance gene from PI 437654, were developed. These populations were screened for resistance to the *H. glycines* inbred isolates PA3 (HG type 7) and TN14 (HG type 1.2.5.7) in the greenhouse and evaluated with molecular markers linked to both *rhg1* and *Rhg4*. Each isolate test was repeated, and the evaluations were done on a single-plant and a line-mean basis in Test 1, and solely on a single-plant basis in Test 2. Across two tests with the TN14 isolate, plants with the PI 437654 allele for a marker linked to *rhg1* had significantly ($P < 0.0001$) less SCN reproduction than plants carrying the PI 88788 allele. A marker linked to *Rhg4*, however, was not significantly associated with resistance to TN14. Across two tests with the PA3 isolate, alleles of *rhg1* from both sources gave a resistant reaction, although plants homozygous for the PI 88788 allele had significantly ($P < 0.05$) greater resistance than plants with the PI 437654 allele. The marker allele from PI 437654 linked to *Rhg4* was significantly ($P < 0.0005$) associated with greater resistance than the PI 88788 allele in both PA3 tests, and resistance was dominant. There was a significant interaction between alleles at *rhg1* and *Rhg4* in both PA3 tests. These results suggest that PI 437654 and PI 88788 each have a different functional SCN resistance allele at or close to *rhg1*. These allelic differences have implications that breeders should consider before incorporation into cultivars.

Communicated by F.J. Muehlbauer

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Introduction

Genetic mapping studies over the last decade have provided information on the locations and effects of many soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe; HG) resistance quantitative trait loci (QTLs; Concibido et al. 2004). A QTL mapping to linkage group (LG) G, designated the *rhg1* gene (also designated cqSCN-001), is considered to be the most important SCN resistance gene in soybean cultivars. This gene has been mapped from PI 437654 (Webb et al. 1995), PI 209332 (Concibido et al. 1996), Peking, PI 88788, PI 90763 (Concibido et al. 1997), and PI 89772 (Yue et al. 2001). In most of these sources, *rhg1* was found to provide the greatest SCN resistance among all mapped resistance QTLs.

Although resistance at *rhg1* has been mapped from several SCN resistance sources, there have been no studies to determine whether different functional alleles exist at this locus. The screening of these sources with a range of SCN isolates has shown that sources have different resistance profiles (Diers et al. 1997). These differences could result from these sources having different alleles at *rhg1* or because they have unique sets of additional SCN resistance QTLs. For example, PI 437654 has been shown to carry *rhg1*, *Rhg4*, and at least four additional resistance QTLs (Concibido et al. 2004). This may at least partially explain its resistance to a broad range of SCN isolates. In contrast, PI 88788, which has resistance to a narrower range of SCN isolates, has been shown to have only one QTL in addition to *rhg1* (Concibido et al. 2004; Glover et al. 2004).

It has been documented that a series of alleles exists for some qualitative resistance genes in soybean. The genes *Rps1* and *Rps3*, which give resistance to *Phytophthora sojae* Kaufmann and Gerdemann, each have multiple alleles that are defined on the basis of their responses to different races of *Phytophthora sojae* (Grau et al. 2004). Unfortunately, it is not possible to test for the presence of different alleles at a SCN resistance locus using traditional allelism tests due to the quantitative nature of SCN resistance and confounding effects from other resistance loci. However, it should be possible to test for different functional alleles at SCN resistance genes using linked markers in populations segregating for alleles from different sources. Information on whether the *rhg1* alleles differ among resistance sources and whether some alleles provide greater resistance than others would be helpful to breeders as they develop SCN-resistant cultivars. The objective of this study was to determine if different functional alleles of *rhg1* could be detected from PI 437654 and PI 88788.

Materials and methods

Development of plant material

Populations of soybean [*Glycine max* (L.) Merr.] lines segregating at *rhg1* were developed from a cross of Bell (Nickell et al. 1990), which carries SCN resistance from PI 88788, with PI 437654 (Anand et al. 1988). Previous studies have confirmed that Bell has resistance at *rhg1* (Glover et al. 2004) and that the sizes of the simple sequence repeat (SSR) fragments for markers closely linked to *rhg1* in Bell are consistent with those from PI 88788 (Fig. 1). Populations were developed from F_2 plants that were heterozygous for Satt309, a SSR marker located 0.4 cM from *rhg1* (Cregan et al. 1999), and Satt424, a SSR marker located approximately 16.5 cM from *Rhg4* (Soybase 2004). A population of F_3 plants derived from a selected F_2 plant was grown in the field in 2003, and approximately 80 F_3 plants were individually threshed to form $F_{3:4}$ lines. Some lines were discarded due to insufficient seed production, and the remaining lines were used in the Test 1 evaluations. In Test 2, a F_3 plant grown in the field in 2003 that was heterozygous for both *rhg1* and *Rhg4* and produced over 500 F_4 seed was selected. Each SCN isolate was tested on F_4 plants derived from this selected plant. By testing populations that were selected from heterozygous F_2 and F_3 plants, we were able to reduce the effect of background segregation of other SCN resistance genes in our population.

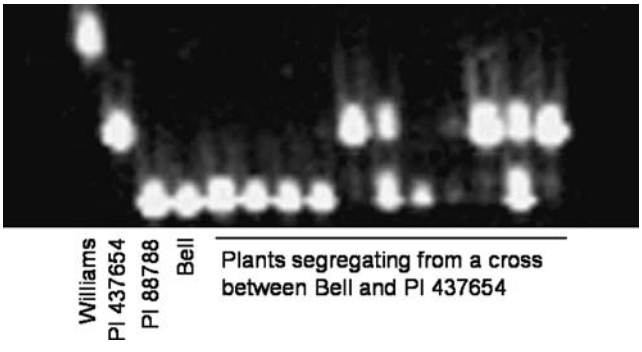

Fig. 1 Segregation of the SSR marker Satt309, which is linked to *rhg1*. *Glycine max* (L.) Merr. cv. Williams is susceptible to SCN. PI 437654 and PI 88788 are sources of resistance, and Bell is a SCN-resistant cultivar that has resistance from PI 88788

Genetic marker evaluation

To increase the efficiency of Test 1, four plants from each $F_{3:4}$ line were genotyped with SSR markers to identify those segregating for *rhg1*. Those lines identified as segregating at this locus were not used in SCN screens because they provide less power to detect QTLs than homozygous lines. SSR marker evaluation was carried out on tissue obtained from seedlings germinated on germination paper in a growth chamber for 72 h. DNA was extracted from the unifoliate leaves or cotyledons using a slightly modified version of the quick extraction method described by Bell-Johnson et al. (1998). PCR was carried out according to Cregan and Quigley (1997) with the SSR marker Satt309, and the products were separated by electrophoresis on 6% non-denaturing polyacrylamide gels (Wang et al. 2003), and visualized under UV light. Low DNA quantity allowed only a few lines to be identified as being derived from heterozygous plants, and only 11 lines were discarded prior to the evaluation of lines with the SCN isolate PA3 and eight lines prior to evaluation with the TN14 isolate. For both Tests 1 and 2, each plant in the SCN greenhouse test was genotyped with fluorescently tagged versions of Satt309 and Satt424 using the previously described PCR conditions. These PCR products were analyzed on an ABI Prism 377 DNA Sequencer (Applied Biosystems-Perkin Elmer, Foster City, Calif., USA). Gel images were viewed with GENESCAN V3.1 analysis software (Applied Biosystems) and manually scored.

Greenhouse assay

Plant evaluations for SCN resistance were done in a thermo-regulated water bath system according to Niblack et al. (2002). Resistance tests were initiated by germinating seed on germination paper for 48 h at 24°C. On the day of transplanting, cysts were crushed to release the eggs (Riggs and Schmitt 1991), which were counted along with J2s (second-stage juveniles) under a microscope in five 25-µl samples to quantify the inoculum. Seedlings of each entry were selected on viability and uniformity and planted individually into a 2.5 (diameter) × 20-cm-long PVC tube filled with steam-sterilized sandy soil that had been infested with approximately 3,000 eggs + J2s. Prior to planting, 24

tubes were packed with soil into large plastic crocks. Each crock was suspended in the water bath, and the infected plants were grown under a 16/8-h (light/dark) photoperiod and watered as needed for 30 days. The water bath system maintains a constant soil temperature of 27 ± 1°C, thereby providing optimal conditions for SCN growth. Fertilizer was applied bi-weekly to aid in plant growth. Approximately 30 days after soil infestation, the cysts and females were washed from the roots of each plant, collected on a 250-μm-aperture wire mesh sieve, and counted under a stereo microscope. A female index (FI) was calculated for each entry using the following formula (Golden et al. 1970): $(N_1/N_2)\times100$, where N_1 is the average number of females per entry and N_2 is the average number of females on the susceptible check Lee 74. Lines with FI of less than ten were classified as resistant (Golden et al. 1970).

The soybean populations were evaluated with SCN isolates TN14 and PA3. TN14 reproduces well on PI 88788 but not on PI 437654, whereas both parents are resistant to isolate PA3 (Table 1). In all tests, a single plant in a tube was an experimental unit. Both Tests 1 and 2 and the evaluations with TN14 and PA3 were separated by time. In each test, the test populations, Bell, the susceptible check Lee 74, and the HG-type differentials were arranged in a completely randomized design. In the Test 1 evaluation of TN14, 56 lines were replicated four times and Lee 74 was replicated six times; Bell and the HG-type differentials were left out of the test. In the Test 1 evaluation with PA3, 48 lines were replicated three times, the differentials and Bell four times, and Lee 74 eight times. In Test 2, 250 F_4 plants tracing to a single F_3 plant were tested on an individual plant basis for each SCN isolate. The HG-type differentials and Bell were replicated five times for both isolates, and Lee 74 was replicated ten times in the PA3 evaluation and seven times in the TN14 evaluation.

Data analysis

Data from the two tests and two isolates were analyzed separately using analysis of variance with PROC GLM from SAS (SAS Institute 1988). Associations between markers and FI were tested in a one-factor analysis of variance. For Test 1, the data were analyzed on a line mean basis and an individual plant basis. For Test 2, the data were analyzed only on an individual plant basis because the population had no line structure. Dominant genetic effects were detected with a single degree of freedom contrast that tested for the difference between the mid-value between the two homozygous classes and the heterozygous class. Two-factor analysis of variance was employed to test for significant interactions between Satt309 and Satt424. Chi-square analysis was done to determine if each marker fit segregation expectations. The Test 1 results on a progeny-mean basis and the Test 2 results on a single-plant basis were tested for fit to 1/4 homozygous for alleles from PI 437654, 1/2 heterozygous or heterogeneous, and 1/4 homozygous for alleles from PI 88788 (1:2:1). The Test 1 results on a single-plant basis were tested for fit to 3/8 homozygous PI 437654, 1/4 heterozygous, 3/8 homozygous for PI 88788 (3:2:3).

Results

In both tests, SCN reproduction on the HG-type differentials, the parent Bell, and the susceptible check Lee 74 was as expected (Table 1). The mean number of cysts and females on Lee 74 ranged from 102 for PA3 in Test 1 to 844 for TN14 in Test 2. The HG type of PA3 was 0 in Test 1 and 7 in Test 2. Although this isolate is inbred, we have previously found that it will alternate between these two types in greenhouse tests. In Test 2, TN14 was a HG-type 1.2.5.7, which is also consistent with observations from previous studies. Both PI 437654 and Bell, the parents of the population, were resistant to PA3. In contrast, PI 437654 was resistant to TN14 while both Bell and its resistance source PI 88788 were susceptible (Table 1). This is consistent with the mean FI of the population of 1.7 for PA3 in Test 1 and 3.0 in Test 2 compared with a mean FI of the population of 76.8 for TN14 in Test 1 and 64.4 in Test 2. There was significant genetic variability among lines for FI after inoculation with TN14 and PA3 in Test 1 ($P < 0.0001$).

The populations were tested for association between resistance and Satt309, which is closely linked to *rhg1* (Cregan et al. 1999). A significant association ($P < 0.05$) was found between Satt309 and resistance to both SCN isolates on a single-plant and progeny-mean basis in

Table 1 *Heterodera glycines* (HG)-type test results for nematode isolates used to inoculate soybean populations

Isolate	Test	Number on Lee 74	Female Index[a]							Bell	HG-type
			1 Peking	2 PI 88788	3 PI 90763	4 PI 437654	5 PI 209332	6 PI 89772	7 PI 548316		
PA3	1	102.0	0.0	1.5	0.0	0.0	0.0	0.0	1.5	0.0	0
PA3	2	119.3	3.0	3.5	1.0	0.2	6.3	0.8	15.8	3.7	7
TN14	1	133.6	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND
TN14	2	844.0	19.9	79.5	0.8	0.6	85.7	0.5	82.1	84.6	1.2.5.7

[a]Female index = (average number of females on indicator line)/(average number of females on Lee 74) × 100
[b]ND, Not determined because the HG-type test was not done for TN14 in Test 1

Table 2 Female index values, probabilities for marker association, R^2 values of markers, and probabilities for dominance deviation identified in populations segregating for *rhg1* and *Rhg4*. Plants or lines in the populations were placed into the homozygous PI 437654, PI 88788, and heterozygous classes based on the segregation of Satt309 and Satt424

Test	Isolate	Marker	Linked gene	Genotypic classes			Marker ($P > F$)	R^2	Dominance ($P > F$)
				PI 437654 Female Index:	Heterozygous	PI 88788			
Test 1:									
Single plant[a]	TN14	Satt309	*rhg1*	21 (69)[b]	106 (36)	101(109)	0.0001	0.4	0.0001
Progeny mean	TN14	Satt309	*rhg1*	24 (6)	76 (34)	113 (13)	0.0001	0.32	NS[c]
Single plant	TN14	Satt424	*Rhg4*	76 (68)	80 (67)	74 (79)	NS	0.00	NS
Progeny mean	TN14	Satt424	*Rhg4*	72 (11)	80 (35)	63 (10)	NS	0.00	NS
Single plant	PA3	Satt309	*rhg1*	3 (57)	0.7 (42)	0.3 (41)	0.0001	0.12	NS
Progeny mean	PA3	Satt309	*rhg1*	3 (12)	1 (25)	0.3 (11)	0.04	0.14	NS
Single plant	PA3	Satt424	*Rhg4*	0.6 (46)	0.5 (49)	3 (46)	0.0001	0.15	0.006
Progeny mean	PA3	Satt424	*Rhg4*	0.5 (8)	0.8 (31)	4 (9)	0.0005	0.29	0.02
Test 2:									
Single plant	TN14	Satt309	*rhg1*	0.7 (40)	78 (119)	84 (48)	0.0001	0.64	0.0001
Single plant	TN14	Satt424	*Rhg4*	65 (55)	62 (103)	65 (49)	NS	0.00	NS
Single plant	PA3	Satt309	*rhg1*	3 (35)	4 (134)	2 (71)	0.01	0.04	0.05
Single plant	PA3	Satt424	*Rhg4*	1 (61)	2 (123)	7 (56)	0.0001	0.17	0.004

[a]Means of genotypic classes are determined on a single-plant or progeny-mean basis
[b]The number of plants or lines are in parenthesis
[c]Not significant at $P < 0.05$

Test 1 and on a single-plant basis for Test 2 (Table 2). For TN14, lines or individual plants homozygous for the PI 437654 allele had significantly ($P < 0.0001$) greater resistance than those homozygous for the PI 88788 allele. The difference between the homozygous classes was most dramatic in Test 2, ranging from a mean FI of 0.7 for homozygous PI 437654 plants to 84 for homozygous PI 88788 plants. This response is consistent with the greater resistance of PI 437654 to TN14 than PI 88788. Significant dominant effects were observed on an individual plant basis in both tests, with susceptibility to TN14 being dominant over resistance. In neither test was Satt424 significantly associated with resistance to TN14.

Both Satt309 and Satt424 were significantly ($P < 0.05$) associated with resistance to PA3 on a progeny-mean basis in Test 1 and on a single-plant basis in both tests. This significance occurred despite all of the marker class means having a FI below 10 and classified as resistant (Golden et al. 1970). Plants or lines homozygous for the PI 88788 allele of Satt309 showed greater resistance than plants homozygous for the PI 437654 allele, which is opposite the trend observed for TN14. A significant dominance deviation was observed for Satt309 in Test 2 only. Consistent with the response to TN14, resistance was recessive to susceptibility. For Satt424, which is linked to *Rhg4*, greater resistance to PA3 was associated with the allele from PI 437654. Significant dominance deviations were associated with Satt424 in both tests. In contrast to *rhg1*, resistance at *Rhg4* was dominant to susceptibility. Two-way analysis of variance revealed a significant ($P < 0.05$) interaction between Satt309 and Satt424 on a single-plant basis in both PA3 tests, indicating that an epistatic interaction exists between *rhg1* and *Rhg4*. This interaction shows that the PI 437654 allele for *rhg1* interacts with the PI 437654 allele for *Rhg4* to achieve full resistance to PA3 (Fig. 2). In contrast, full resistance occurs with the PI 88788 allele at *rhg1* regardless of the presence of the *Rhg4* allele. This

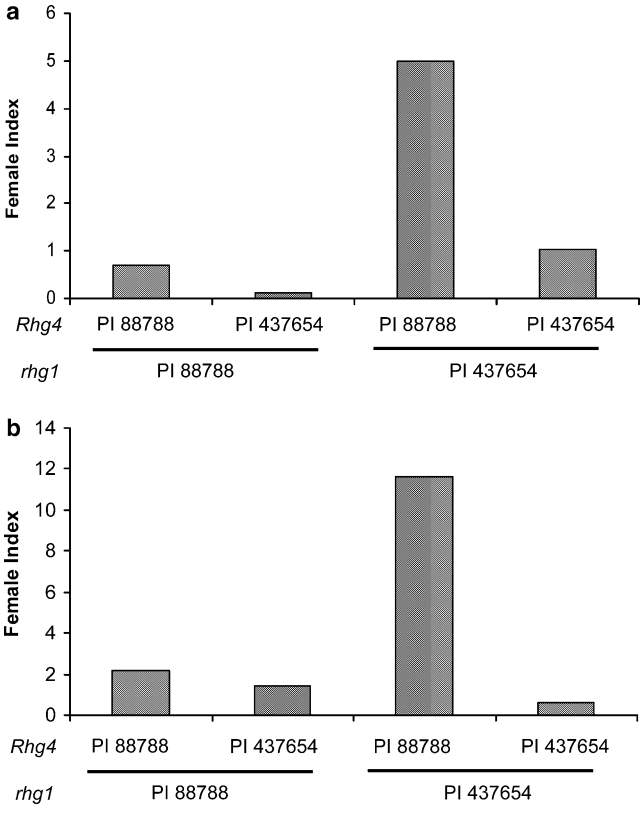

Fig. 2 Mean female index values of plants inoculated with *H. glycines* isolate PA3. The means are of plants predicted to be homozygous for a combination of *rhg1*, based on Satt309, and *Rhg4*, based on Satt424. **a** Results from Test 1 on a single-plant basis, **b** results from Test 2

interaction was not significant on a progeny-mean basis in Test 1, likely because of an insufficient number of lines in each genotypic class.

Chi-square analysis revealed significant ($P < 0.05$) segregation distortion for Satt309 on both a single-plant and progeny-mean basis for the TN14 evaluation in Test 1 and for the PA3 evaluation in Test 2. In these distorted populations, fewer plants or lines homozygous for PI 437654 alleles were observed than expected (Table 2). Satt309 is linked to *rhg1*, and both Webb et al. (1995) and Glover et al. (2004) observed segregation distortion associated with *rhg1* in populations developed from crosses between a susceptible cultivar and PI 437654 or Bell. In both studies, they observed fewer lines than expected that were homozygous for the *rhg1* resistance allele. Our results show that when the PI 437654 and PI 88788 *rhg1* alleles both segregate in a population, there is selection against PI 437654 homozygotes. For the distortion observed for Satt309 in Test 2, the selection against the PI 437654 homozygotes must have occurred during seed formation or during seed germination and plant development, because the seed used in the test were harvested directly from a heterozygous plant. Further research is needed to uncover the mechanism of segregation distortion at *rhg1*. Significant segregation distortion was also detected for Satt424 in Test 1 on a single-plant basis with PA3. This distortion resulted from an over-representation of heterozygotes. We are not aware of other published reports of segregation distortion at *Rhg4*.

Discussion

We have shown that PI 437654 and PI 88788 have different functional alleles at *rhg1* based on resistance phenotype. The *rhg1* allele from PI 437654 provides resistance to TN14, whereas the PI 88788 allele gives a susceptible reaction. In contrast, both parents provide a resistance reaction to PA3, although the PI 88788 allele confers greater resistance than the PI 437654 allele. In addition to demonstrating that different functional alleles exist, these results clearly show that the *rhg1* allele from PI 88788 gives isolate specific resistance to SCN. Although it has been previously shown that SCN resistance QTLs provide varying levels of resistance to SCN isolates (Concibido et al. 2004), we have more definitively demonstrated this isolate specificity.

There is a possibility that we are not testing different alleles at the same locus from these sources of resistance, but instead are testing resistance alleles at closely linked loci. Genetic tests or the cloning and validation of different alleles from each source are needed to resolve whether these are the same or different genes. Although *rhg1* candidate genes have been cloned (Hauge et al. 2001; Meksem and Lightfoot 2001), complementation studies to confirm this cloning have not been reported in the literature.

Rhg4 was significantly associated with resistance to PA3. The resistance allele for *Rhg4* was from PI 437654, which is consistent with previous results indicating that PI 437654 has resistance at this locus (Webb et al. 1995). There is no evidence that Bell or PI 88788, the latter being the source of SCN resistance for Bell, has a resistance allele at *Rhg4* (Concibido et al. 1997; Glover et al. 2004). This suggests that the populations were segregating for alleles that confer resistance and susceptibility at *Rhg4*. In addition, *Rhg4* conferred resistance to PA3 only, similar to the PI 88788 allele from *rhg1*.

The interaction we observed between *rhg1* and *Rhg4* is consistent with results observed by Meksem et al. (2001) who studied a population developed from crossing Forrest, which has SCN resistance from Peking, and the susceptible cultivar Essex. In this population, they reported an epistatic interaction between these genes and found that only when the resistance alleles from both genes were combined was full resistance achieved. This is similar to the interaction we observed between the *rhg1* and *Rhg4* alleles from PI 437654 for PA3. Meksem et al. (2001) also used the isolate PA3 to test their population (P. Arelli, personal communication). The consistency of this interaction in both Peking and PI 437654 combined with their similar resistance profile and close genetic distance (Diers et al. 1997) support the idea that these two resistance sources have similar genes for SCN resistance.

Our results also agree with previous reports that resistance at *rhg1* is recessive. The *rhg1* gene symbol was designated in a genetic study in which resistance in Peking was explained by three recessive genes (Caldwell et al. 1960). After SCN resistance QTLs were mapped, the major SCN resistance gene on LG G was defined as *rhg1* because this placement was most consistent with results from classical genetic studies (Cregan et al. 1999). Concibido et al. (1997) described the resistance at *rhg1* to be partially recessive in a mapping study, although their ability to show recessiveness was hampered by their use of partially inbred $F_{2:3}$ lines. In most other soybean mapping studies, inbred lines are used because of the self-pollinating nature of the crop and the increased ability to detect QTLs in inbred populations (Cowen 1988). The recessive resistance of *rhg1* is unusual because resistance genes are typically dominant (Agrios 1997). One example of a recessive resistance gene is the *mlo* gene that confers resistance to powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal) in barley (*Hordeum vulgare* L.). This gene has been cloned, and it does not fit any of the major classes of disease resistance genes (Buschges et al. 1997) and may be a negative regulator of defense responses.

rhg1 has been classified as both a qualitative gene and a QTL. The original genetics work done on SCN treated the trait as qualitatively inherited, which allowed researchers to name the resistance genes. However, when resistant and susceptible genotypes are crossed, resistance reactions typically segregate continuously in pop-

ulations. In this study and in a previous study by Brucker et al. (2005), we successfully evaluated *rhg1* as essentially a qualitative disease resistance gene. This was done through the use of populations derived from partially inbred plants, inoculations with inbred nematode isolates, and careful greenhouse testing.

To our knowledge, this is the first report of allelic diversity for nematode resistance genes. Our finding that the widely deployed SCN resistance sources PI 88788 and PI 437654 have different functional alleles at *rhg1* has implications for soybean breeders. In populations developed from crosses between these sources of resistance, breeders should be aware of which allele they are selecting. If they are breeding cultivars that will be grown in fields infested with SCN populations that have a pathogenicity similar to the PA3, either *rhg1* allele could be selected, however the *rhg1* allele from PI 437654 would be preferable if fields are infested with SCN populations similar to TN14. Breeders can select for these alleles through markers-assisted selection or by phenotyping plants for resistance. The SSR marker Satt309 can differentiate between the *rhg1* alleles and could be used to select either allele (Fig. 1). Alternatively, evaluation of plants that have been inoculated with TN14 would discriminate between these alleles.

Further work is needed to more fully describe the resistance profiles of the *rhg1* alleles from PI 437654 and PI 88788. For example, lines with SCN resistance from PI 88788 are more resistant to a HG-type 1.2.3.6.7 isolate than lines with the PI 437654 source of resistance (Cary 2003). This difference could be the result of the PI 88788 allele at *rhg1* providing greater resistance than the PI 437654 allele to this isolate. In addition, more work is needed to determine if other SCN resistance sources have different functional *rhg1* alleles.

References

Agrios GN (1997) Plant pathology, 4th edn. Academic, San Diego

Anand S, Gallo KM, Baker IA, Hartwig EE (1988) Soybean plant introductions with resistance to Races 4 or 5 of soybean cyst nematode. Crop Sci 28:563–564

Bell-Johnson B, Garvey G, Johnson J, Lightfoot D, Meksem K (1998) Biotechnology approaches to improving resistance to SCN and SDS: methods for high throughout marker assisted selection. Soybean Genet Newsl 25:115–117

Brucker E, Niblack T, Kopisch-Obuch FJ, Diers B (2005) The effect of *rhg1* on reproduction of *Heterodera glycines* in the field and greenhouse and associated effects on agronomic traits. Crop Sci (in press)

Buschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van der Lee T, Diergaarde P, Groenendijk J, Topsch S, Vos P, Salamini F, Schulze-Lefert P (1997) The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Caldwell BE, Brim CA, Ross JP (1960) Inheritance of resistance of soybeans to the cyst nematode, *Heterodera Glycines*. Agron J 52:635–636

Cary TR (2003) Northern regional soybean cyst nematode test report. University of Illlinois, Urbana

Concibido VC, Denny RL, Lange DA, Orf JH, Young ND (1996) RFLP mapping and marker-assisted selection of soybean cyst nematode resistance in PI 209332. Crop Sci 36:1643–1650

Concibido VC, Lange DA, Denny RL, Orf JH, Young ND (1997) Genome mapping of soybean cyst nematode resistance genes in 'Peking', PI 90763 and PI 88788 using DNA markers. Crop Sci 37:258–264

Concibido VC, Diers BW, Arelli PR (2004) A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci 44:1121–1131

Cowen NM (1988) The use of replicated progenies in marker-based mapping of QTLs. Theor Appl Genet 75:857–862

Cregan PB, Quigley CV (1997) Simple sequence repeat DNA marker analysis. In: Caetano-Anolles G, Gresshoff PM (eds) DNA markers: protocols, applications and overviews. Wiley, New York, pp 173–185

Cregan PB, Mudge J, Fickus EW, Danesh D, Denny R, Young ND (1999) Two simple sequence repeat markers to select for soybean cyst nematode resistance conditioned by the *rhg1* locus. Theor Appl Genet 99:811–818

Diers BW, Skorupska HT, Rao-Arelli AP, Cianzio SR (1997) Genetic relationships among soybean plant introductions with resistance to soybean cyst nematodes. Crop Sci 37:1966–1972

Glover KD, Wang D, Arelli PR, Carlson SR, Cianzio SR, Diers BW (2004) Near isogenic lines confirm a soybean cyst nematode resistance gene from PI 88788 on linkage group J. Crop Sci 44:936–941

Golden AM, Epps JM, Riggs RD, Duclos LA, Fox JA, Bernard RL (1970) Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis Rep 54:544–546

Grau CR, Dorrance AE, Bond J, Russin JS (2004) Fungal diseases. In: Boerma HR, Specht JE (eds) Soybeans: improvement, production, and uses, 3rd edn, vol 16. American Society of Agronomy, Madison, pp 679–763

Hauge BM, Wang ML, Parsons JD, Parnell LD (2001) Nucleic acid molecules and other molecules associated with soybean cyst nematode resistance. US Patent No. 2003005491

Meksem K, Lightfoot D (2001) Novel polynucleotides and polypeptides relating to loci underlying resistance to soybean cyst nematode and methods of use thereof. US Patent Application No. 09772134

Meksem K, Pangiotis P, Hyten D, Njiti V, Lightfoot DA, Arelli P (2001) Forrest resistance to soybean cyst nematode is bigenic: saturation mapping of the *rhg1* and *Rhg4* loci. Theor Appl Genet 103:710–717

Niblack TL, Arelli PR, Noel GR, Opperman CH, Orf JH, Schmitt DP, Shannon JG, Tylka GL (2002) A revised classification scheme for genetically diverse populations of *Heterodera glycines*. J Nematol 34:279–288

Nickell CD, Noel GR, Thomas DJ, Waller R (1990) Registration of 'Bell' soybean. Crop Sci 30:1364–1365

Riggs RD, Schmitt DP (1991) Optimization of the *Heterodera glycines* race test procedure. J Nematol 23:149–154

SAS Institute (1988) SAS/STAT user's guide, version 6.03. SAS Institute Cary, N.C.

Soybase (2004) Soybase class browser [Online]. Iowa State University. Available at http://soybase.ncgr.org/cgi-bin/ace/generic/search/soybase (verified 21 Oct., 2004)

Wang D, Shi J, Carlson SR, Cregan PB, Ward RW, Diers BW (2003) A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop Sci 43:1828–1832

Webb DM, Baltazar BM, Rao-Arell AP, Schupp J, Clayton K, Keim P, Beavis WD (1995) Genetic mapping of soybean cyst nematode Race-3 resistance loci in the soybean PI 437.654. Theor Appl Genet 91:574–581

Yue P, Sleper DA, Arelli PR (2001) Mapping resistance to multiple races of *Heterodera glycines* in soybean PI 89772. Crop Sci 41:1589–1595