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A common QTL for resistance to races 3 and 9 of soybean cyst nematode

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The aim of this work was to study the association between the *rhg1* gene and the soybean response to races 3 and 9 of *Heterodera glycines* (Soybean Cyst Nematode - SCN). The resistant cv. Hartwig was crossed with the susceptible line Y23 and the response to races 3 and 9 was evaluated in 135 and 128 Recombinant Inbred Lines (RIL), respectively. Nematode assays were performed in the greenhouse using a completely randomized design. Eight SSR markers covered a genomic region of 57 cM. Estimated heritabilities of resistance to race 3 and 9 were 80.97 and 80.39%, respectively, showing that a few major genes are segregating in the population. Applying the Composite Interval Mapping (CIM) method, the *rhg1* resistance gene was mapped between the SSR markers Satt275 and Satt038 at 2.0 and 3.0 cM from marker Satt038, explaining 29.11 and 20.01% of phenotypic variance in resistance to races 3 and 9, respectively. These SSR markers would be useful tools for assisting in the selection of SCN-resistant genotypes and for expediting the introgression of SCN resistance loci from cv. Hartwig to soybean elite cultivars.
Keywords: *Heterodera glycines*, mapping, molecular markers, resistance, soybean

Cervigni, G.D.L.; I. Schuster; M.F. Silva; C. S. Sediyama; E. G. de Barros & M. A. Moreira. 2007. Un QTL común para la resistencia a las razas 3 y 9 del nematode del quiste de la soja. *Rev. Fac. Agron. Vol 106 (**2**): 137-144.*

El objetivo de este trabajo fue estudiar la asociación entre el gen *rhg1* y la resistencia a las razas 3 y 9 de *Heterodera glycines* (Nematode del Quiste de la Soja - NQS). El cultivar resistente Hartwig fue cruzado con la línea susceptible Y23 y la respuesta a las razas 3 y 9 fue evaluada en 135 y 128 Líneas Endogámicas Recombinantes (LER), respectivamente. Los ensayos de respuesta al NQS fueron realizados en invernáculo bajo un diseño completamente aleatorizado. Ocho marcadores SSR fueron ordenados en una región genómica de 57 cM. La heredabilidad de la resistencia a las razas 3 y 9 fue 80,97 y 80,39%, respectivamente, indicando que pocos genes mayores estaban segregando en la población. Aplicando Mapeo por Intervalo Compuesto (MIC), el gen de resistencia *rhg1* fue mapeado entre los marcadores SSR Satt275 y Satt038, a 2,0 y 3,0 cM del marcador Satt038, explicando el 29,11 y 20,01% de la varianza fenotípica de la resistencia a la raza 3 y 9, respectivamente. Estos marcadores serían herramientas útiles para auxiliar en la selección de genotipos resistentes al NQS y acelerar la introgresión de *loci* de resistencia al NQS a cultivares élite de soja.

Palabras clave: *Heterodera glycines*, mapeo, marcadores moleculares, resistencia, soja

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INTRODUCTION

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinhoe, is a serious pest of soybean. This pest can cause yield losses of up to 100% (Agrios, 1998). In Brazil, the SCN was reported for the first time in the 1991/1992 growing season. It is currently distributed in nine Brazilian states, infecting 2,500,000 ha (Silva *et al*., 2002).

The most practical and economical method of controlling this parasite is the use of resistant cultivars in the rotation scheme, with no host or susceptible cultivars. In this context, creation of new soybean cultivars with resistance to SCN is constantly required. Breeding SCN resistant cultivars is a complex process and the great SCN genetic variability (Ross, 1962; Riggs *et al.*, 1988) usually results in short-lived cultivar resistance.

The genetic control of resistance to SCN in soybean is complex and not well understood. Several studies have been suggested that resistance to SCN includes recessive and dominant genes (Caldwell *et al*., 1960; Matson & Williams, 1965; Rao-Arelli & Anand, 1988; Rao-Arelli *et al.*, 1992). In addition, resistance genes can be linked or independent, and multiallelic (Anand & Rao-Arelli, 1989; Hancock *et al*., 1987). Genetic analysis also suggests that resistant genes may be shared among different resistant sources (Rao-Arelli & Anand, 1988) and the resistance is a quantitative character since it shows a continuous distribution of phenotypes as was observed by Mansur *et al*. (1993).

Molecular markers have provided information on the position and location of many SCN resistance quantitative trait loci (QTL) (Concibido *et al*. 2004). A QTL, mapped on the top of the molecular linkage group (MLG) G (Webb *et al.*, 1995; Concibido *et al*. 1997; Mudge *et al*., 1997), named *rgh1*, is considered the most important SCN resistance locus.

In Brazil, genetic variability of SCN is greater than in other soybean producing areas of the world; races 1, 2, 3, 4, 4$^+$, 5, 6, 9, 10, 14 and 14$^+$ have all been found in Brazil (Dias *et al.*, 1999). However, the most widely grown Brazilian soybean cultivars are only resistant to races 1 and 3 (Dias *et al*., 2005). Taking into account the great number of SCN races identified in Brazil, there is a great interest in conducting Marker-Assisted Selection (MAS) for SCN resistance in Brazilian breeding programs. MAS is less time-consuming that phenotypic selection, and a greater number of genotypes could be selected for, that carry resistance genes to several SCN races.

There are several reports associating *rhg1,* the most important QTL for resistance to SCN, and the resistance to race 3 (Webb *et al.*, 1995; Concibido *et al*. 1997; Mudge *et al*., 1997, Cervigni *et al.*, 2004), but until now, there are no studies involving race 9. This work was carried out in order to investigate the association between *rhg1* and the resistance to races 3 and 9 of the parasite. Race 3 was included to analyze, in the same population, the magnitude of the effect of this QTL on both races. The association between *rhg1* and the resistance to race 9 was analyzed by amplifying SSR markers linked to *rhg1* in a Recombinant Inbred Lines (RIL) population derived from a single cross between the resistant cultivar (cv) Hartwig and the Brazilian susceptible line Y23.

MATERIALS AND METHODS

Plant material and SCN assay

One hundred and eighty RILs segregating for SCN resistance to races 3 and 9 were obtained from a single cross between the resistant cv. Hartwig and the susceptible line Y23. F_2 plants obtained from 5 F_1 plants were selfed until F_6 generation using the Single Seed Descent (SSD) method (Brim, 1966). The response to SCN races 3 and 9 was evaluated on 135 and 128 RIL, respectively, in two subsequent experiments.

The SCN races 3 and 9 were believed to be nearly genetically homogeneous due to re-

production in small population size for more than 40 generations. Inocula of both races were maintained on the roots of the susceptible cv. Embrapa 20 growing in the greenhouse at 25 30ºC. At the inoculation time, the amount of cysts was increased in order to provide enough inoculum. Thirty days after inoculation, females and cysts were recovered on a 60 mesh sieve and disrupted in a tissue grinder to release the eggs. Six plants per RIL, 6 of the susceptible (Y23) and 6 of the resistant (Hartwig) cross parents were inoculated in each experiment. Additionally, six plants of each soybean differential lines: Peking, Pickett, PI 90763 and PI 88788, and the susceptible control to all SCN races, cv. Lee 68, were included in each experiment. In order to confirm the identity of the inoculated races, the female index (FI) was calculated according to Riggs *&* Schmidt (1988) as follows:

FI = (mean number of females present on each differential line) / (mean number of females present on cv. Lee 68) x 100

If the FI < 10% the differential soybean line is considered resistant, (R), if the FI e» 10% susceptible, (S). For race 3, Peking, Pickett, PI 90763 and PI 88788 are R, for race 9 only the PI 88788 and PI 90763 are R.

Seeds were germinated in sand at 25°C and each seedling was transferred to a 500-mL pot containing a mixture of sand and soil (1:2). After three days, each seedling was inoculated with approximately 1,000 eggs and juveniles of SCN, race 3 or 9. Plants were grown in a greenhouse at 25–30°C with a 16-h photoperiod.

On the 30th day after inoculation, plants were uprooted and their roots were washed with tap water over 60 mesh sieves to collect the cysts. At this time, the FI from differential soybean lines was estimated as mentioned above. The FI for each RIL was estimated in a similar manner replacing the mean number of females present on each differential line by the mean number of females of each RIL in the numerator, and replacing the average cv. Lee 68 by Y23 in the denominator.

Each plant was grown in individual pots corresponding to each plot (experimental unit). Pots were arranged in a completely randomized design with six replications (6 plants per RIL) for each race. Heritability of resistance to races 3 and 9 was estimated in a broad-sense from the expectances of the mean squares. Genetic correlation between resistance to SCN, race 3 and race 9, was estimated considering the response of 102 RILs inoculated with both SCN races. The analysis of variance (ANOVA) and parameter estimation were performed using the Genes program (Cruz, 1999) available at: http://www.ufv.br/dbg/genes/genes.htm.

SSR marker analysis

DNA samples were extracted from soybean leaves by the CTAB method (Keim *et al*., 1988), quantified in a spectrophotometer, and stored at 4ºC until use. Nine Simple Sequence Repeat (SSR) markers closely linked to the resistance gene *rhg1*, located on the top of the MLG G (Webb *et al*., 1995; Concibido *et al*. 1997; Mudge *et al*., 1997), were initially tested in both parents and those that gave clear polymorphic bands were amplified in the F_1 plants and in each of the 180 RILs. DNA amplification was performed in 25 ìL reactions containing 30 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.4 mM $MgCl_2$, 0.1 mM of each dNTP, 0.6 ìM of forward and reverse primers and 1 unit of *Taq* DNA polymerase. Amplifications were performed in 30 cycles, each consisting of one denaturation step at 94ºC for 1 min, one annealing step at 50ºC for 1 min, and one extension step at 72ºC for 2 min. The final cycle was followed by a 7 min extension step at 72ºC. The SSR products were resolved in a 3% agarose gel immersed in TBE (90 mM Tris-borate buffer; 1 mM EDTA, pH 8.0), or in a vertical, nondenaturing, 10% polyacrylamide gel using TAE buffer (40 mM Tris- Acetate buffer, 1 mM EDTA). Gels were stained with ethidium bromide (10 mg/ml) and photodigitalized using an Eagle Eye II system (Stratagene, La Jolla, CA).

Marker linkage and QTL analyses

To identify genomic regions associated with SCN resistance, the co-segregation between

DNA markers and SCN response was analyzed. Association between SSR marker and phenotypic values for races 3 and 9 was determined by multiple regression using the stepwise elimination method (SAS, 1990). Genetic distances between markers were estimated using a minimum LOD score of 3.0, maximum recombination frequency of 30% and the mapping function of Kosambi (1944). The goodness of fit to expected molecular marker segregation ratios was checked by the chi-square test.

QTLs were mapped applying the Composite Interval Mapping (CIM) method (Lander & Botstein, 1989) with the GQMol program (Schuster & Cruz, 2004) available at: http://www.ufv.br/dbg/gqmol/gqmol.htm. The position of putative resistance loci was inferred whenever the Likelihood Ratio (LR) score exceeded 15.88 and 10.67 (a = 0.05) for races 3 and 9 respectively, and after performing 1,000 permutations (Churchill & Doerge, 1994).

RESULTS

Phenotype data analysis

In both experiments, the inoculation results showed absence of 'race shift' for both SCN races (Table 1). FI values ranged from 0 to 450 for race 3 and from 0 to 255 for race 9, showing the presence of transgressive segregation in both experiments. Normal distribution for FI values was rejected for both races according to the Shapiro-Wilk test, with W = 0.9477 (P = 0.0000) and W = 0.8367 (P = 0.0000) for races 3 and 9, respectively. On the other hand, the phenotypic data sets for both races showed a continuous distribution (Figure 1). Heritabilities of 80.97 and 80.39% were estimated for races 3 and 9, respectively. The coefficient of genetic correlation (r_g = 0.17), calculated on the response to both races of SCN in 102 RILs, was statistically significant (P ≤ 0.01).

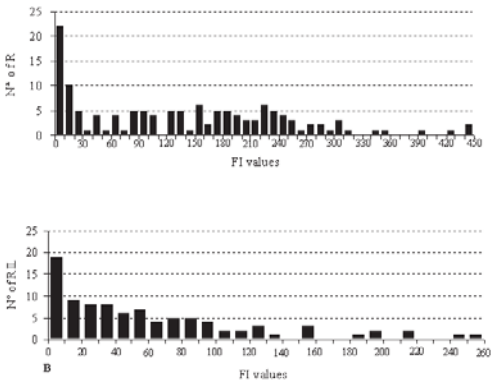

***Figure 1**. Frequency distribution of female index (FI) in a RIL population inoculated with SCN race 3 (A) and race 9 (B). The FI values were calculated according to Riggs & Schmidt (1988) using the susceptible parent Y23 in the denominator.*

Distribución del índice de hembras (FI) en una población de LER inoculada con la raza 3 (A) y 9 (B) del NQS. El FI fue calculado de acuerdo a Riggs & Schmidt (1988) usando el progenitor susceptible Y23 en el denominador.

***Table 1.** Mean number of females (MNF), female index (FI) and reaction of soybean race differential lines for **H. glycines**.*

*Número medio de hembras (MNF), índice de hembras (FI) y reacción de líneas de soja diferenciales de razas de **H. glycines**.*

Race of *H. glycines*	MNF[1] Lee 68	FI (%)[2] Hartwig	Y23	Peking	Pickett	PI 88788	PI 90763
3	182.0	2.5	39.7	3.9	0.8	4.4	1.4
		R	S	R	R	R	R
9	146.0	5.7	59.0	12.4	11.5	0.1	5.0
		R	S	S	S	R	R

[1]Mean of six replications; [2]FI (%) = (MNF on differential/MNF on Lee 68) x 100; S = susceptible (FI ≥10%) and R = resistant (FI < 10%).

[1]Media de seis repeticiones; [2]FI (%) = (MNF desarrolladas sobre genotipos diferenciadores/MNF desarrolladas sobre Lee 68) x 100; S = susceptible (FI ≥ 10%) y R = resistente (FI < 10%).

Molecular marker associated with SCN resistance

All nine SSR markers tested were polymorphic between the cross parents, cv. Hartwig and line Y23, and the alleles of all SSR from each parent were present in all five F_1. Eight SSR showed the expected segregation ratio for a RIL population (1:1), being arranged into a molecular linkage group (MLG) of 57 cM (Figure 2). The SSR marker Satt309 was discarded because of a severe deviation from the expected segregation ratio. Only one SSR marker, Satt038, was retained into the final multiple regression model. Its coefficient of determination (R^2) was 14.5% (F = 22.90; P < 0.0000) for SCN race 3 and 9.36% (F = 8.83; P < 0.0350) for SCN race 9. Using the CIM method, a genomic region associated with the resistance to both races was identified between markers Satt275 and Satt038, at 2.0 and 3.0 cM from marker Satt038, explaining 29.11% and 20.01% of the phenotypic variance for resistance to races 3 and 9, respectively (Figure 2).

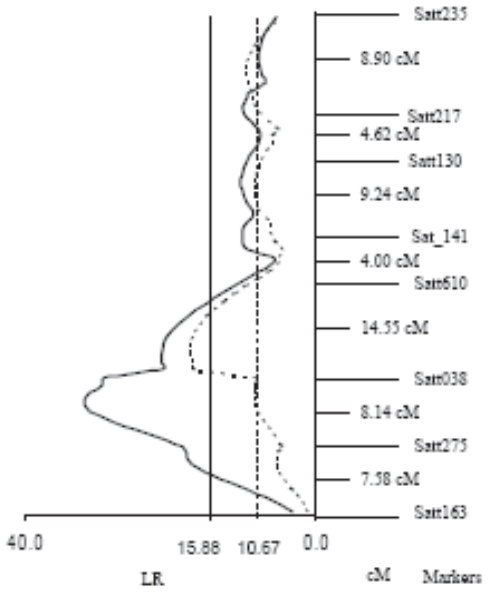

Figure 2. *Molecular linkage group (MLG) G; marker names and genetic distances in centiMorgans (cM) are shown on the right. Position of the resistance locus on the MLG G is indicated by plotting the Likelihood Ratio (LR). The black line (–) and dotted line (---) represent, respectively, the LR values obtained for races 3 and 9. The threshold LR values for race 3 (15.88) and race 9 (10.67) were established using 1,000 permutations.*

Grupo de ligamiento molecular (MLG) G; a la derecha se muestran los nombres de los marcadores moleculares y su distancia genética en centiMorgans (cM). La posición del locus de resistencia sobre el MLG G está indicada graficando la razón de probabilidad (LR). La línea negra entera (–) y la punteada (---) representan, respectivamente, los valores de LR obtenidos para la raza 3 y 9. El valor de LR umbral para las razas 3 (15,88) y 9 (10,67) fueron estimados realizando 1000 permutaciones.

DISCUSSION

Phenotype evaluation

Transgressive segregation is interesting in plant breeding because it allows the selection of genotypes superior to the parents. Genetic studies indicate that transgressive segregation typically results from recombination between parents that possess QTLs with antagonistic effects (Rieseberg *et al*., 2003).

The continuous distribution of FI values from both races indicates that the response to SCN is a quantitative trait, as it was reported by Mansur *et al*. (1993) and Webb *et al*. (1995). Normal data distribution would be expected when many genes of minor effects are controlling the trait. Conversely, the lack of normality in the data distribution detected by the Shapiro-Wilk test performed in our study, suggests the resistance to SCN is an oligogenic trait, which is in accordance to Caldwell *et al*. (1960), Matson & Williams (1965) and Mansur *et al*. (1993). The high values of broad-sense heritabilities obtained (80.97% for race 3 and 80.39% for race 9) also support this idea. Even though the estimation of the heritability depends on the genetic material and the method used (Falconer & Mackay, 1996), there is a good coincidence between the heritability for race 3 obtained here and the ones reported in the literature (Mansur *et al*., 1993; Webb *et al*., 1995). However, to our knowledge, this is the first report on the heritability of the resistance to SCN race 9. The significant genetic cor-

relation between the responses to both races of the parasite estimated here would indicate that some of the genes involved in the resistance to both races could be inherited together. The low magnitude of this correlation indicates, on other side, that resistance to both races is conditioned by different genes.

DNA marker analysis and mapping

The SSR marker Satt309 was mapped at 0.4 cM distal to *rhg1* (Cregan *et al.*, 1999). This marker was discarded because it showed distorted segregation. Segregation distortion for genetic markers closely linked to *rhg1* on MLG G was largely reported in several sources of resistance to SCN. This includes PI 437654 (Webb *et al*., 1995), PI 209332 (Mudge *et al*., 1997), Hartwig, used in this study as the resistant parent, Peking (Prabhu *et al*., 1999), and PI 88788 (Glover *et al.*, 2004).

The order of the 8 SSR arranged in a MLG of 57 cM differed from that obtained by Song *et al*. (2004). This could be explained by the fact that we used a population with a different genetic background from those used by Song *et al*. (2004). In addition, the inversions that we observed could have already been present in Hartwig.

The MLG G was previously associated to the resistance of SCN by Boutin *et al.* (1992). The *rgh1* resistance QTL has been mapped on top of the MLG G using RFLP markers (Webb *et al*., 1995; Concibido *et al*., 1997), flanked by the SSR Satt038 and Satt130 (Mudge *et al*., 1997) and Satt038 and Satt163 (Cervigni *et al*., 2004). This is a recessive gene first reported by Caldwell *et al*. (1960) and it is, together with *rhg2, rhg3* and *Rhg4*, involved in SCN resistance. We used the same population as Cervigni *et al,* (2004), in a different assay, to confirm the effect of *rgh1* against race 3, and to associate this effect with the one against race 9.

In the present study, we confirmed that *rhg1* explains a high proportion of the total phenotypic variation to race 3 in the cv. Hartwig (29.11%). High determination coefficients, ranging from 26.2 - 50.9% have been previously reported (Webb *et al*., 1995; Concibido *et al*., 1997; Mudge *et al*., 1997; Cervigni *et al*., 2004), demonstrating, one more time, that *rhg1* is a major gene that confers resistance to race 3 of SCN.

This gene has also been associated to the resistance to other races of SCN, such as races 1 and 6 (Concibido *et al*., 1997), 1, 2 and 5 (Yue *et al*., 2001). However, there are no reports correlating the *rhg1* gene with resistance to race 9. In our study, the *rhg1* gene explained 20.01% of the resistance to race 9 in cv. Hartwig, indicating that it is also involved in conferring resistance to this race. Our data demonstrate that *rhg1* is, at least, one of the genes that cause the significant genetic correlation obtained between both races of SCN. In an inheritance study, we found that resistance to race 3 and 9 was determined by four and two genes, respectively, one of them being common to both races (Cervigni *et al*., 2007). We demonstrate here that this common gene is *rhg1*.

The main goal of using molecular markers in crop improvement is to select indirectly desirable genotypes with maximum efficiency and speed, at a low cost. The use of SSR markers previously associated to the *rhg1* gene, allowed us to associate this gene with the resistance to race 9. The use of molecular markers closely linked to this gene would be an important tool for selection, in a simple way, of soybean genotypes carrying resistance to races 3 and 9 of the parasite, speeding up the obtention of SCN resistant cultivars in soybean breeding programs.

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