

Inheritance of resistance to soybean cyst nematode races 3 and 14 in soybean RIL and F₂ populations

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Abstract – The objective of this work was to evaluate the soybean inheritance of resistance to cyst nematode races 3 and 14. The following populations were evaluated: one population of recombinant inbred lines (RILs) [Hartwig (resistant) x Y23 (susceptible line)] for races 3, 14 and 9; one population of families F_{2,3} [M-SOY 8001 (resistant) x MB/BR 46 – Conquista (susceptible)] for race 3; and one population of families F_{2,3} [(S5995 (resistant) x BRSMG Renascença (susceptible)] for race 14. In RIL populations, four epistatic genes were identified which conditioned resistance to race 14, and three epistatic ones for resistance to races 3 and 9. The lack of one gene provided moderate resistance under all situations. The highest number of genes for resistance to race 14 points out that genes responsible for lower effects might be involved. In population F_{2,3} from M-SOY 8001 x MB/BR 46 – Conquista, one recessive gene for moderate resistance and two recessive genes complete resistance to race 3 were identified. Two recessive genes conditioning moderate resistance to race 14 were identified in population F_{2,3} from the crossing S5995 x BRSMG Renascença. These results will be useful in designing crossings, involving these parentals, with higher possibility to accumulating genes that provide resistance to several SCN races.

Index terms: *Glycine max*, *Heterodera glycines*, improvement, recombinant inbred lines, soybean cyst nematode.

Herança da resistência ao nematóide de cisto da soja, raças 3 e 14, em populações de linhagem endogâmica recombinante e F₂ de soja

Resumo – O objetivo deste trabalho foi avaliar, em soja, as heranças da resistência ao nematóide de cisto da soja, raças 3 e 14. As seguintes populações foram avaliadas: uma linhagem endogâmica recombinante (RILs) [Hartwig (resistente) x Y23 (suscetível)], para as raças 3, 14 e 9; uma população de famílias F_{2,3} [(M-SOY 8001 (resistente) x MB/BR 46 – Conquista (suscetível)], para a raça 3; e uma população de famílias F_{2,3} [S5995 (resistente) x BRSMG Renascença (suscetível)], para a raça 14. Foram identificados nas populações RILs quatro genes epistáticos que condicionaram resistência à raça 14 e três genes epistáticos para a resistência às raças 3 e 9. A falta de um dos genes proporcionou resistência moderada em todas as situações. Na população F_{2,3} de M-SOY 8001 x MB/BR 46 – Conquista, foi identificado um gene recessivo para resistência moderada à raça 3 e dois genes recessivos para a resistência completa. Na população F_{2,3} do cruzamento S5995 x BRSMG Renascença foram identificados dois genes recessivos que condicionaram resistência moderada à raça 14. Estes resultados visam contribuir no delineamento de cruzamentos, que envolvem estes parentais, com maior possibilidade de acumular genes que conferem resistência às diversas raças do nematóide de cisto da soja.

Termos para indexação: *Glycine max*, *Heterodera glycines*, melhoramento, linhagem endogâmica recombinante, nematóide do cisto da soja.

Introduction

Soybean cyst nematode (*Heterodera glycines* Ichinohe – SCN) is the pathogen that causes more damages to soybean [*Glycine max* (L.) Merrill] throughout the world (Wrather et al., 2003). The most secure and economical control of SCN is the use of

resistant cultivars associated to the rotation of non-host crops (Embrapa, 2006). The development of resistant cultivars involves the identification of genes in resistance sources and their transfer to susceptible cultivars.

Although the identification of new resistance sources and characterization of their genes have been accomplished by several studies (Diers et al., 1997;

Zhang et al., 1999), only genes from few sources were incorporated into commercial soybean cultivars, as those genes frequently come from PI 88788 and Peking, in USA, and from Hartwig in Brazil (Concibido et al., 2004; Embrapa, 2006). This fact associated to the wide diversity of the SCN physiologic races (Niblack et al., 2002) rather causes certain vulnerability in the cultivars available to producers (Embrapa, 2006). To avoid the broken resistance by SCN in the recommended cultivars, it is necessary to increase the diversity of resistance genes used in breeding programs.

Eleven SCN races (1, 2, 3, 4, 4⁺, 5, 6, 9, 10, 14 and 14⁺) were detected in Brazil, in ten states. However cultivars available for cropping in the country showed resistance only to races 1 and 3. Moderate resistance to other races was also found (Embrapa, 2006). In breeding programs, to obtain varieties resistant to several SCN races base-populations with genes are necessary for resistance to several SCN races, besides knowledge of the genetic relationship between sources of resistance and inheritance.

For resistance to SCN, the type of gene action and the number of resistance genes vary according to different sources. Recessive and dominant genes were reported such as *rhg1*, *rhg2*, *rhg3*, *Rhg4* and *Rhg5* (Caldwell et al., 1960; Rao-Arelli et al., 1992; Qiu et al., 1997; Concibido et al., 2004), besides different alleles for the same locus in different PI (Brucker et al., 2005). The *rhg1* gene has a great importance in the control of resistance to several races of SNC (Silva et al., 2007). The mostly studied resistance sources are Peking, PI88788, PI90763, PI437654, Hartwig, and PI438489B (Concibido et al., 2004). However, some resistance genes are common in PI and other are unique ones, therefore characterizing a restrict genetic base of soybean (Dong et al., 1997).

In Brazil, Hartwig cultivar is widely used in genetic breeding programs. It derived from PI 437654 and is resistant to most races of SCN, except for 4⁺ and 14⁺. Studies about the inheritance for resistance to race 3 in Hartwig cultivar, as well as to races 3 and 5 in PI 437654, are available (Faghihi et al., 1995). However, no studies are available in the literature about inheritance for resistance to race 14. Race 3 is found in eight Brazilian states, race 14 is found in four and race 9 in two (Embrapa, 2006).

The objective of this work was to study the inheritance for resistance to SCN, races 3, 9 and 14 in Hartwig cultivar, using a RIL population derived from the crossing of this resistance source with the susceptible line Y23, besides studying resistance to races 3 and 14 in improved materials, using two F_{2:3} breeding populations.

Material and Methods

The population of recombinant inbred lines (RILs) was obtained from Hartwig (resistant) x Y23 (susceptible line) in the experimental field of Universidade Federal de Viçosa, Viçosa, MG. F₂ plants obtained from five F₁ plants were advanced until generation F₆ by using the single seed descent method (SSD) under greenhouse conditions. The F_{6:7} RILs were evaluated for resistance to races 3 and 9 of SCN, whereas F_{6:9} RILs were evaluated for resistance to race 14. The numbers of evaluated RILs were 134, 110, and 128 for resistance to races 3, 9, and 14, respectively.

The two populations of F_{2:3} families were obtained from M-SOY 8001 (resistant to race 3) x MB/BR 46 – Conquista (susceptible) and S5995 line (moderately resistant to race 14) x BRSMG Renascença (susceptible) crosses, in Cooperativa Central de Pesquisa Agrícola – Coodetec, Cascavel, PR, Brazil. Sixty-five families from the first cross were phenotypically evaluated for resistance to SCN race 3. In the second cross, 66 families were evaluated for resistance to race 14. Resistance source in M-SOY 8001 is Coker 6738. The line S5995 was identified as moderately resistant to race 14 in a germplasm screening, but the source of resistance is not know.

The experiments were carried out in greenhouse conditions. A complete randomized design was used, and four to six plants were assessed per F_{2:3} family, and three to six plants per RIL, under 25 to 30°C and 16 hours of light.

In RIL populations, the evaluations for resistance to races 3 and 9 were performed in Universidade Federal de Viçosa, over the years 2001 and 2002, respectively, whereas the evaluation for resistance to race 14 was performed in 2004, in Embrapa Soja, Londrina, PR, Brazil. Both F_{2:3} populations were evaluated in Embrapa Soja over the year 2003.

The inocula for races 3, 9 and 14 were kept in a susceptible cultivar for all the races in the greenhouse. Populations, parents, soybean differential cultivars (Peking, Pickett, PI 90763 and PI 88788) and the lines susceptible to all races control (Lee) were included in the experiments. Seeds were placed to germinate in sand at 25°C. Each seedling (two to three days old) was transplanted individually to a 0.5 L clay pot containing a mixture of soil and sand at 1:2 ratio. Four thousand eggs per pot were inoculated simultaneously at transplant.

The data set were obtained thirty days after inoculation, when the individual plants were removed from the pots, and their roots were washed under a strong jet of water in a 20 mesh sieve attached to another

60 mesh one, and cysts were counted under a stereoscopic microscope. The mean cysts of each F_{2:3} family or RIL were transformed in female index (FI) estimated as follows: FI = 100 x cyst average numbers in one given family (F_{2:3} or RIL)/cyst average numbers in the susceptible family.

Data for the susceptible parents from each population were used in the denominator of the expression above to calculate the FI for each family, and assess the genetic differences among the parents of each cross. To confirm SCN race, FI was calculated by substituting the denominator of the expression above by the number of cysts and females in Lee (susceptible standard cultivar), as proposed by Riggs et al. (1988).

The RIL classification in relation to the different races was assessed according to Schmitt & Shannon (1992), as follows: resistant (R), with FI < 10; moderately resistant (MR) – with FI ≥ 10 and < 30; moderately susceptible (MS) – with FI ≥ 30 and < 60; and susceptible (S) – with FI ≥ 60.

The results obtained were analyzed using chi-square test as follow:

$$\chi^2 = \sum_{i=1}^r \frac{(o_i - e_i)^2}{e_i}$$

in which: r is a number of classes, and o_i and e_i are the observed and expected counts for class i.

The phenotypic correlation coefficient between resistance to SCN races 3, 9 and 14, was estimated according to Cruz (2006), considering the response of

67 RILs inoculated with the three SCN races. Evaluation of the significance was made through Mantel's test (Manly, 1997).

Results and Discussion

The genetic variability of each population for resistance to SCN was shown by the high variation in the mean number of cysts detected in the parents, and by the finding of a wide variation of resistance for the races observed within each population (Table 1). Intense transgressive segregation for reduction in the cyst numbers was observed in F_{2:3} families evaluated for resistance to race 14, therefore evidencing allelic and genic interactions present in the control of the trait. The transgressive segregation is interesting to the breeder, since it allows for selecting descendants with a number of genes that are favorable and superior to parents (Ramalho et al., 2000). In the S5995 line x BRSMG Renascença population, the mean number of cysts from race 14 in parent S5995 was high (Table 1), compared to resistant parents in the other crosses, so pointing out that this resistance source shows moderate resistance to race 14 of SCN. Thus, in this population, there would be segregating just genes that provide moderate resistance to race 14.

The distribution of the RIL and F_{2:3} families, according to the classification based on FI, are shown in Table 2. In both F₂ populations, most plants were classified in the intermediate classes (MR and MS). Probably, this is a reflex of the allelic interactions involved in the control of

Table 1. Mean cyst number in parental (R – resistant, and S – susceptible), populations, and the maximum and minimum values in different populations.

Variable	Hartwig x Y23 RIL/raça 3	Hartwig x Y23 RIL/raça 9	Hartwig x Y23 RIL/raça 14	M-SOY 8001 x Conquista F ₂ /raça 3	S5995 x Renascença F ₂ /raça 14
Parent R	4.6	8.4	0.0	1.9	76.3
Parent S	39.3	59.2	275.1	165.6	284.8
Population	90.5	35.8	174.2	71.23	98.6
Min -Max	0.4-278.5	0.6-150.6	0.0-351.6	0.5-179	35.63-203.4
N ⁽¹⁾	134	110	128	65	66

⁽¹⁾N = number of families F_{2:3} or RILs evaluated in each cross.

Table 2. Number of plants (NP) and mean female index (MFI), obtained for different classes of RIL and F_{2:3} families: resistant – R (FI < 10); moderately resistant – MR (FI ≥ 10 and < 30), moderately susceptible – MS (FI ≥ 30 and < 60); and susceptible – S (FI ≥ 60).

Population - race	R		MR		MS		S		Total of plants
	NP	MFI	NP	MFI	NP	MFI	NP	MFI	
RIL - 3	16	3.93	14	11.43	6	28.41	98	119.32	134
RIL - 9	11	2.69	29	12.56	19	37.17	43	66.02	110
RIL - 14	10	5.62	7	66.95	45	127.01	66	240.93	128
F _{2:3} - 3	7	5.21	16	34.05	25	72.87	17	124.33	65
F _{2:3} - 14	0	-	33	62.90	27	118.82	6	204.78	66

the character. It must be taken into account that the dominance effects cannot be detected in RIL populations, because of their genetic nature. In addition, a higher number of susceptible plants were detected in RIL populations.

In order to evaluate the inheritance of the soybean resistance to SCN, by chi-square test, two analyses for each race were accomplished in the different populations (RIL and $F_{2:3}$): one considering the moderate resistance (MR), with cutting point for FI<30; and other considering the complete resistance (R) with cutting point for FI<10 (Table 3).

The probabilities associated to the heterogeneity test among populations were 9.73% for populations that segregated 1:3, 74.65% for populations with segregation 1:7, and 42.26% for populations with segregation 1:15, which demonstrates the great consistence of the obtained data.

A higher gene number for resistance to SCN was detected for RIL populations, therefore indicating higher number of resistance genes in Hartwig cultivar than in the other resistance sources already improved, used in the other populations. Hartwig cultivar, has genes for resistance to most SCN races described, except for races 4⁺ and 14⁺. Thus, it is expected to present a higher number of resistance genes than the cultivars recently introduced by the breeding programs.

In all cases, one extra gene was necessary for the complete resistance in relation to the moderate one. In the cross S5995 x BRSMG Renasença, despite the detection of two recessive genes for moderate resistance, there was no possibility to know how many genes are necessary to condition complete resistance,

since no $F_{2:3}$ family with IF<10 was detected in this population (Table 3).

In the $F_{2:3}$ population M-SOY 8001 x MB/BR 46 (Conquista), evaluated for resistance to race 3, a recessive gene (segregation 1R:3S) conferring moderate resistance was detected, besides two recessive and epistatic genes (1R:15S) for complete resistance, that is, the simultaneous presence of both recessive genes are necessary for plants to have complete resistance. In the RIL population Hartwig x Y23, two epistatic genes (1R: 3S) were identified for moderate resistance to race 3, and three epistatic genes (1R:7S) for complete resistance (Table 3). The epistasis identified in RILs indicates the necessity of the presence of two genes for plants to show moderate resistance, and three genes for plants to be completely resistant. Because RIL populations are constituted by homozygous lines, it is not possible to know if involved genes are dominant or recessive. However, corroborating in number with the results of this study, Faghihi et al. (1995) found two genes for resistance to race 3 in a population derived from Hartwig cultivar, as being one recessive and another dominant.

The fact that two recessive genes confer resistance to race 3 in the population M-SOY 8001 x MB/BR 46 – Conquista, differing from Hartwig cultivar, where one recessive and one dominant gene are responsible for resistance (Faghihi et al., 1995), is an indication that genes from other resistance source may be found in cultivar M-SOY 8001, which transforms this cultivar a good source to be used in breeding programs for resistance to SCN.

In RILs assessed for resistance to race 9, two epistatic genes conditioning moderate resistance (1R:3S),

Table 3. Evaluation of the soybean resistance inheritance to SCN in RIL (Hartwig x Y23) populations evaluated for resistance to races 3, 9 and 14, and in $F_{2:3}$ populations (M-SOY 8001 x MB/BR46 – Conquista) evaluated for resistance to race 3, and S5995 x BRSMG Renasença for resistance to race 14⁽¹⁾.

Race	Evaluation	Population	R	S	Hypothesis		χ^2	P (%)	Total
					GN	Segr.			
3	FI<30 (MR)	M-SOY 8001 x Conquista	23	42	1	1:3	3.74	5.31	65
		Hartwig x Y23	30	104	2	1:3	0.49	48.51	134
	FI<10 (R)	M-SOY 8001 x Conquista	7	58	2	1:15	2.26	12.87	65
		Hartwig x Y23	16	118	3	1:7	0.04	84.47	134
9	FI<30 (MR)	Hartwig x Y23	40	90	2	1:3	2.31	12.87	110
	FI<10 (R)	Hartwig x Y23	11	99	3	1:7	0.06	81.36	110
14	FI<30 (MR)	S5995 x BRSMG Renasença	32	34	2	7:9	0.60	43.81	66
		Hartwig x Y23	17	111	3	1:7	0.00	94.80	128
	FI<10 (R)	S5995 x BRSMG Renasença	0	66	-	-	-	-	66
		Hartwig x Y23	10	118	4	1:15	0.05	81.58	128

⁽¹⁾FI: female index; R: number of resistant individuals; S: number of susceptible individuals; GN: gene number; segr.: segregation; P: probability.

and three epistatic genes (1R:7S) for complete resistance were identified. Although it is not possible to know if involved genes are dominant or recessive, these results are also relevant, since no reports about qualitative inheritance for resistance to this race are available in the literature.

For race 14, in the population of families $F_{2,3}$, two recessive genes were identified (7R:9S). This type of inheritance is characterized by the action of gene duplication, since only one recessive gene is necessary to condition the moderate resistance. As the resistant parent of this crossing (S5995) was classified as MR, and two genes were identified for moderate resistance, it is evident that more than two genes are necessary to condition complete resistance to this race, but S5995 line does not have this (or these) additional gene(s). In the RIL populations, three epistatic genes (segregation 1R:7S) were necessary to confer moderate resistance to this race, and four epistatic genes (segregation 1R:15S) for complete resistance.

For complete resistance, four genes for race 14 and three for races 3 and 9 were found in the RIL populations. For moderate resistance, three resistance genes were necessary for race 14, and two for race 3. The highest number of genes needed for resistance to race 14 is an indication that these additional genes have lower effects.

Based on the 67 RILs evaluated simultaneously for resistance to the three races (3, 9 and 14), the phenotypic correlation coefficient was estimated. Only for the races 3 and 14, the correlation obtained (0.20) was statistically significant by Mantel's test at 5% probability. The data pointed out that the genetic components associated to the resistance for both SCN races are at least partly jointly inherited.

According to the results obtained in the present study, it is not possible to say there are common genes segregating for those different races. However, the lack of a higher correlation for resistance between races rather suggests that different breeding strategies should be adopted for each race.

The development of cultivars resistant to one specific race of SCN is a tough and slow procedure. But, eight SCN races were grouped into four groups, according to their diversity, based on evaluations performed in 524 lines and cultivars of soybean. The groups formed were: races 2, 4 and 14; 6 and 9; 1 and 3; and race 5 (Kim et al., 1998). This suggests that for those different races of each group, some resistance genes probably

exist in common in soybean genotypes, which can explain the fact that though most Brazilian cultivars have been selected for resistance to race 3, they are also resistant to race 1. Based on the cited work, it is presumed the improvement for resistance to race 14 would also raise resistance level for races 2 and 4.

In Brazil, however, most cultivars available for cropping show resistance only to races 1 and 3, and moderate resistance to the other races derived from Hartwig cultivar (Embrapa, 2006). Therefore, it is necessary to increase the diversity of the resistance genes used in breeding programs. So, new genes for resistance to different races must be identified in PIs and transferred to the commercial cultivars. In addition, other resistance sources should also be used in breeding programs, in order to allow resistance genes to be rotated by the farmers. In this sense, M-SOY 8001 cultivar, which probably shows genes for resistance to race 3, different from those found in the Hartwig cultivar, might be of great use in breeding programs, since it is already an adapted material.

The results of the present study may contribute to the experimental design for crosses with higher possibility of accumulating genes for resistance to several SCN races.

Conclusions

1. Different number of genes are necessary to confer resistance to races 3, 9, and 14 of the soybean cyst nematode.
2. The higher is the number of resistance genes to soybean cyst nematode races 3, 9, and 14 present in a specific background, the higher is the resistance level obtained to this pathogen.
3. The lack of higher correlation for resistance between races suggests that different breeding strategies must be adopted for each race.

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