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ARTICLE

Resistance of soybean genotypes to the reniform nematode in a controlled environment

Carlos Lasaro Pereira de Melo[1*], Wanderléia Rodrigues dos Santos[2] and Guilherme Lafourcade Asmus[1]

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Abstract - *The aim of this study was to characterize the resistance to the reniform nematode of soybean genotypes derived from crosses with at least one parent resistant to* Rotylenchulus reniformis *or* Heterodera glicynes*, or to both. Two experiments in a greenhouse of Embrapa Western Region Agriculture, arranged in a completely randomized design, evaluated 199 genotypes with five replications. Sixty days after artificial inoculation (1000 eggs and larval forms), the nematodes were extracted from the roots and the genotypes evaluated for the number of eggs and larval forms per gram of root (NGR) and for the reproduction factor (RF). Sixty-five genotypes were resistant (RF <1.0), with mean RF significantly equal to M-SOY 8001. The highest number of lines resistant to reniform nematodes, in the different study combinations of crosses, were derived from the genotypes Custer, PI 437654, Fayette, BRSGO Ipameri, BRSMT Pintado, and BRS 262.*

Key words: Glycine max, Rotylenchulus reniformis, *plant breeding, phenotypic characterization.*

INTRODUCTION

Brazil is the world's second largest producer of soybean, planted on about 24.1 million ha and yielded about 75.3 million tons in the 2010/11 growing season. In the same period, the state of Mato Grosso do Sul grew 5.1 million tons in an area of 1.8 million ha (Conab 2011).

The soybean breeding programs contributed essentially to the advancement of agricultural production by the development of cultivars adapted to the varied environmental conditions. The selection of soybean varieties resistant to diseases, pests and nematodes has been a key target of breeding programs. The nematode species *Rotylenchulus reniformis* is one of major threats to plant health of soybean crops in production areas in the southern region of the United States (Robbins et al. 1994a). This nematode is widely spread in Brazil, parasitizing roots of annual and perennial species. It infects mainly roots of pineapple, banana, coffee, castor bean, passion fruit, tomato, but causes greatest damage to cotton and soybean (Robbins et al. 2002, Asmus and Ishimi 2009).

The presence of nematode *R. reniformis* was reported in 2002 in Mato Grosso do Sul, in the main soybean producing areas (Asmus 2004). In cotton, high population densities of this plant nematode can cause losses of over 60% (Robinson 2002). Damages of up to 32% have been reported in soybean crops with high densities of reniform nematodes.

The use of cultivars resistant to reniform nematode is one of the most efficient and inexpensive control strategies, indirectly providing higher stability and increased durability of these cultivars (Harville et al. 1985). Furthermore, the method is believed to be easily assimilated by producers, leading to a reduction of environmental impacts by a reduced use of agrochemicals. However, the lack of cultivars with confirmed genetic resistance prevents a wider use of this strategy.

Studies have shown that genes that confer resistance to reniform nematodes are close to those that confer resistance to soybean cyst nematode (SCN), especially in genotypes derived from Forrest, Custer, Pickett, Hartwig, PI 437654, Peking, Centennial, PI 90763, and Cordell (Rebois et al. 1970, Robbins et al. 1994b, Robbins and Rakes 1996, Ha et al. 2007). Most authors suggest a genetic linkage between the locus of resistance to *Heterodera glycines* and *R. reniformis*.

The characterization of soybean lines and cultivars for resistance to reniform nematodes in Brazil confirmed the resistance of the genotypes Forrest and Custer, aside from

[1] Embrapa Agropecuária Oeste, BR 163, km 253,6, CP 449, 79.804-970, Dourados, MS, Brazil. *E-mail: carlos.lasaro@embrapa.br
[2] Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Av. Brasil, Centro, 15.385-000, Ilha Solteira, SP, Brazil

shedding light on new sources of nematode resistance in the commercial cultivars M-SOY 8001 and CD 201 (Asmus and Schirmann 2004). The objective of this study was to characterize soybean genotypes of crosses with at least one parent resistant to *R. reniformis*, *H. glycines* or to both, for resistance to reniform nematode, in a controlled environment.

MATERIAL AND METHODS

The experiments were conducted in a greenhouse and in the nematology laboratory of Embrapa Western Region Agriculture, in Dourados, Mato Grosso do Sul, from August 2009 to January 2010. Lines derived from segregating populations conducted by the pedigree method were evaluated in the F_6, F_7, F_8 and F_9 generations of 48 crosses (Table 1), in which at least one parent was resistant to the nematode *R. reniformis* or *H. glycines* (races 1 and 3), or to both.

These genotypes were tested for reaction to *R. reniformis* in two experiments: the first evaluated 86 and the second 113 genotypes, all from the soybean breeding program of Embrapa (Table 1), of which 159 were conventional and 40 transgenic (glyphosate-resistant). The commercial cultivars M-SOY 8001 and BRS 239 were included as resistance and susceptibility standards, respectively, in both experiments. The experiments were arranged in a completely randomized design with five replications.

Soybean was sown in 500- ml polyethylene cups containing 400 ml of a soil - sand substrate (1:1) previously sterilized by solarization (Ghini et al. 2002). A standardized fertilization of 0.4 g of 0-20-20 (NPK), i.e., 300 kg ha^{-1}, was applied per unit. Four seeds were sown per cup, inoculated as described below with 2 ml of Nitragin Cell Tech at a concentration of $3x10^9$ viable cells of *Bradyrhizobium* sp. per ml, and thinned to one seedling per cup seven days after emergence.

The inoculum was obtained from sour passion fruit plants grown in 2000 ml pots for about six months after inoculation with a *R. reniformis* population from an infested area in the surroundings of Maracaju, MS, where the presence of this nematode had first been detected, causing damage to soybean in the state (Asmus et al. 2003). The passion fruit roots were ground as described by Coolen and D'Herde (1972).

About 10 days after sowing, the seedlings were individually inoculated with 5 mL of aqueous suspension containing 1,000 *R. reniformis* eggs and juveniles. The inoculum was divided in two 2.5 ml portions and filled into two holes, 2" deep, both 1 cm away from the root collar of the soybean seedlings, in September and November 2009, in the experiments I and II, respectively.

Sixty days after inoculating the plant nematode, a sufficiently long period for two full nematode generations, the genotypes were evaluated in their respective experiments. The roots of each plant were carefully washed in tap water and stored in plastic bags in the refrigerator until the beginning of the extraction process. The root mass was weighed on an electronic precision scale and nematodes extracted by the method described by Coolen and D'Herde (1972).

After extraction, the nematodes were inactivated in a 55 ºC water bath for 5 min and fixed in formalin (2%). The suspension was used to estimate the number of eggs, juveniles, young males and females and adults under a binocular optical microscope (magnification 40 X), by a slide count of 1mL aliquots.

From the estimated number of eggs, juveniles and/or adults in the roots and the fresh root weight, the reproduction factor (RF) and the average number of nematodes per gram of root (NGR) were calculated. The ratio between the final population in the root (Pf) and the initially inoculated population (Pi) was defined as RF. The NGR was defined as the ratio of the total number of nematodes in the roots by the root mass in grams. According to Oostenbrink (1966), genotypes with RF below or equal to one (1.0) are considered resistant.

Table 1. Crosses, number of lines and generations of tested soybean genotypes for resistance to *R. reniformis* in a greenhouse - Experiments I and II

No.	Crosses	Lines	Generation
	Experiment I		
1.	Forrest3 x BRS 2312	6	F7
2.	BRSGO 204 x (BRSGO Chapadões2) x BRSMG 250 (Nobreza3)	3	F6
3.	BRS Invernada3 x PI 561356	2	F6
4.	Custer3 x BR96-25619	31	F7
5.	BR96-25619 x Custer3	31	F7
6.	Forrest3 x BR96-25619	3	F7
7.	BR96-25619 x Forrest3	2	F7
8.	PI4376543 x Hartwig3	3	F8
9.	BRS 2622 x {BRS 244 RR x Shiranui-1}	5	F7
	Subtotal	**86**	

To be continued...

No.	Crosses	Lines	Generation
	Experiment II		
10	PI4376543 x Hartwig3	7	F8
11	Ipameri2 x (Fayette1 x PI 4376543)	13	F7
12	(BRSMT Pintado2 x MGBR 46 Conquista) x (Fayette1 x PI 4376543)	8	F7
13	(Fayette1 x PI 4376543) x (BRSMT Pintado2 x MGBR 46 Conquista)	11	F7
14	BRI01-1038 x M-SOY 80013	1	F8
15	BRI01-11556 x BRS Jiripoca CRNC3	1	F7
16	MGBR00-506163* x BR02-65900	2	F6
17	MGBR00-506163* x BR03-75127	8	F6
18	BRS 2622 x BR04-203094	2	F6
19	A 9000 RG x NK 4121132	1	F6
20	GOBR99-7010063* x BR03-81463 RR	4	F6
21	BRAS99-105533* x BRB02-2293-L1=BR 28*6RR	4	F6
22	Anta 82 RG2 x BR02-65840	9	F8
23	Anta 82 RG2 x BR02-64151	7	F8
24	BR02-78000 x Anta 82 RG2	3	F8
25	BRS 246RR x {Bedford2 x {Forrest*2z x {(UFV 1*2 x Davis-1) x [(Bossier x Paraná) x FT Estrela]}}}	1	F8
26	[(BRS133*2 x Ga93-92932) F4 Res. SCN and MRNG] x [CD205 x (BRS 134*5 x E96-246)] Res. Fe 1	1	F8
27	CD 202 x (CD 2011 x BRS 133)	1	F9
28	(CD-2021 x (GOBR83-600402 x (HIll x PI 227687))	2	F9
29	BRS 2312 x BRSGO Luziânia	1	F9
30	BR 98-17205 x BRS 2312	1	F9
31	Embrapa 48 x NK 4121132	2	F9
32	NK 4121132x BRS 232	1	F9
33	BRS 239 x [BRS 2312 x (PI 200487 x BRS 133)]	1	F9
34	BRS 184 x BRS 2312	1	F9
35	BRS 2312x BRS Sambaíba	1	F9
36	BRS 262 x (M-SOY 5826 x CD 206)	2	F8
37	BRS 2312 x (BRSGO Chapadões2 x BRI98-641)	2	F8
38	BRS 2312 x (BRSGO Chapadões2 x BRI98-641)	4	F9
39	{Sharkey2 x {Hartwig3 x {FT 5 x {[FT 10*2 x (FT 6*2 x SS 1-A)] x TGX 297-192-C}}}} x {(Santa Rosa x Tracy) x [(BR 16*3 x BRM92-6600) x (BR 16*5 x IAC 12)]}	1	F8
40	BRS 2622 x M-SOY 5942	1	F8
41	[(BR96-25917 x Foster IAC2) 2] x {BRS 66*2 x [BRS 134*2 x (Embrapa 59*2 x E96-246)]}	1	F9
42	CD2011 x BRS 134RR	1	F9
43	CD 201*21 x (E96-246 x BRS 133)	2	F9
44	[BR93-11995 x (E99-99 x OC95-3455)] x CD 2011	1	F9
45	BRS 2622 x BRS 255 RR	1	F8
46	{Cordell*43 x {Hartwig3 x [(UFV 1*2 x Davis-1)*3 x FT Estrela]}} x BRS 245 RR	1	F8
47	{Cordell3 x [Hartwig3 x (Embrapa 4*4 x Tracy M)]} x {(Santa Rosa x Tracy) x [(BR 16*3 x BRM92-6600) x (BR 16*5 x IAC 12)]}	1	F9
48	{BRS 133 x (Embrapa 63 RR x PI 230971)(RR??)]} x {[(BRS 133*2 x Ga93-9293(2)) F4 Res. SCN and Res. NG] x [Embrapa 134*5 x E96-246) x Embrapa 61] Res. Fe 1}	1	F8
	Subtotal	113	
	Total	199	

[1] Resistant parent to *R. reniformis,* [2] Resistant parent to *H. glycines* (SCN races 1 and 3, principalmente),[3] Resistant parent to *R. reniformis* and *H. glycines*. *Pedigree: MGBR00-50616=Sharkey x {[Forrest*4 x (Bossier x Paraná)] x [(UFV 1*2 x Davis-1) x FT Estrela]}; GOBR99-701006= {Hartwig*4 x [OC 8 x (TGX 342-351-D x Paranagoiana*4)]} x [Sharkey*2 x (Dourados*4 x SS1)]; BRAS99-10553=Sharkey x {Bedford x {Hartwig x {[(Cristalina CARDF-56 x IAC 7R) x BR 11] x Tracy M}}}

The homogeneity of variance was assessed by the Liliefors test, which indicated that the variances of the data set were not homogenous. Due to the presence of correlation between the mean and variance of each genotype evaluated, the NGR data were transformed to Log_{10} (x+1) and the RF data transformed to square root (x +1), where *x* is the original value of the variable. The purpose of this transformation was the stabilization of the variances. Subsequently, the NGR and RF data were subjected to analysis of variance using the statistical software SAS 9.1 (SAS Institute 2003). The cluster test of Scott-Knott and the Pearson correlation analysis were run on software Genes (Cruz 2006).

RESULTS AND DISCUSSION

The analysis of variance showed a significant difference ($p < 0.01$) among genotypes, in both experiments, for the variables reproduction factor (RF) and number of nematodes per gram of root (NGR). These results indicated the presence of phenotypic variation for resistance to reniform nematodes, indicating different resistance responses of the genotypes to this plant nematode. The coefficients of variation ranged from 18.7 to 25.5% (Tables 2 and 3) and were considered appropriate for experiments on the interaction of soybean and plant nematodes (Asmus 2004, Torres et al. 2006, Asmus 2008).

There was little variation in the mean RF and NGR between experiments I and II, and the highest values were observed in experiment II (Table 3). A likely explanation for this variation lies in the fact that the mean temperature inside the greenhouse was higher in experiment II (27.2 ºC) than in experiment I (22.6 ºC), which is closer to the optimal growth and reproduction temperature of *R. reniformis* (Rebois 1973).

Figure 1 (A and B) illustrate the variability among the soybean genotypes in experiments (I and II), compared with the mean of trait RF. The frequency distribution showed 10 classes of frequencies in experiments I and II. Importantly, the susceptibility (BRS 239) and resistance control (M-SOY 8001) were in different phenotypic classes, as expected. In the first experiment, 12 genotypes had a higher mean RF than the susceptible cultivar control BRS 239. On the other hand, 30 genotypes were allocated in the same class as the resistant cultivar, M-SOY 8001, and 12 other genotypes had a mean RF of up to 1.0 (Figure 1A). In experiment II, 20 genotypes had a greater resistance in the phenotypic class of the resistant cultivar M-SOY 8001. Of the 115 genotypes tested in this experiment, 69 were nematode-susceptible, since the mean RF was higher than 2.1, i.e., higher than of the susceptible cultivar BRS 239 (Figure 1B).

In both experiments, the mean RF of the resistance standard cultivar M-SOY 8001 was close to zero (0.17 in experiment I and 0.15 in experiment II), representing a reduction of at least 83% compared to the initial nematode population. The RF values of BRS 239 were 1.95 and 1.44 in experiments I and II, respectively (Tables 2 and 3). These results were similar to those reported by Asmus (2004), who concluded that the RF of cultivar M-SOY 8001 indicated resistance, while BRS 239 was susceptible to reniform nematodes.

The mean cluster analysis of experiment I formed two statistical groups for the variables RF and NGR. With regard to RF, the performance of 42 genotypes indicated resistance (RF <1.0), with means statistically equal to M-SOY 8001. Of these, NGR of 31 genotypes was low, with no difference from M-SOY 8001 (Table 2). The RR and NGR of the other

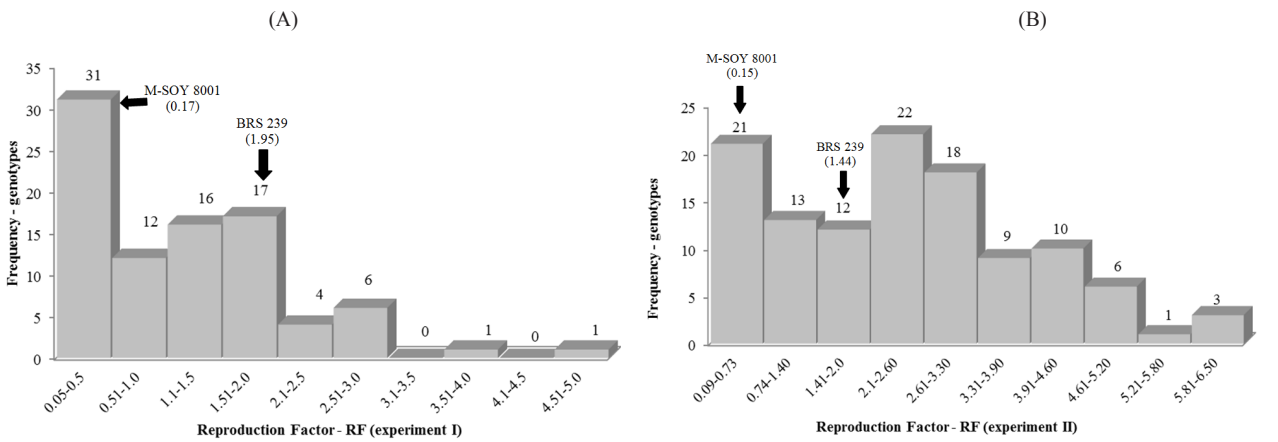

Figure 1. Frequency distribution of the reproduction factor (RF) of reniform nematodes. (A) 88 soybean genotypes in experiment I and (B) 115 soybean genotypes in experiment II.

Table 2. Comparison of means of reproduction factor (RF) and number of nematodes per gram of root (NGR) of *Rotylenchulus reniformis* in 88 soybean genotypes, evaluated in a greenhouse (Experiment I)

GENOTYPE[1]	RF*	NGR*	GENOTYPE	RF*	NGR*	GENOTYPE	RF*	NGR*
BRMS08-1720	0.05 a[2]	3.73 a	BRMS08-1817	0.53 a	32.14 a	BRMS08-1619	1.53 b	71.23 b
BRMS08-1797	0.07 a	3.93 a	BRMS08-1639	0.57 a	33.85 b	BRMS08-1811	1.66 b	97.10 b
BRMS08-1626	0.09 a	4.52 a	BRMS08-1280	0.61 a	30.28 a	BRMS08-1512	1.72 b	139.77 b
BRMS08-5808	0.11 a	10.86 a	BRMS08-1598	0.63 a	31.24 a	BRMS08-1726	1.72 b	70.05 b
BRMS08-2109	0.11 a	6.75 a	BRMS08-1305	0.64 a	38.04 b	BRMS08-1732	1.75 b	111.44 b
BRMS08-5766	0.12 a	14.94 a	BRMS08-1421	0.70 a	56.59 b	BRMS08-1635	1.80 b	114.05 b
BRMS08-2107	0.13 a	8.89 a	BRMS08-1805	0.75 a	49.48 b	BRMS08-1669	1.84 b	82.91 b
BRMS08-1785	0.13 a	8.66 a	BRMS08-1416	0.79 a	85.39 b	BRMS08-1646	1.85 b	89.45 b
BRMS08-1612	0.14 a	6.45 a	BRMS08-1772	0.83 a	48.96 b	BRMS08-1511	1.88 b	103.48 b
BRMS08-1746	0.14 a	7.71 a	BRMS08-1699	0.83 a	41.08 b	BRMS08-1890	1.91 b	106.76 b
BRMS08-1715	0.15 a	7.27 a	BRMS08-2037	0.88 a	54.74 b	BRMS08-1614	1.93 b	84.46 b
BRMS08-1869	0.15 a	6.00 a	BRMS08-1680	0.94 a	44.71 b	**BRS 239**	**1.95 b**	**104.17 b**
BRMS08-1804	0.16 a	7.48 a	BRMS08-5762	0.97 a	52.14 b	BRMS08-1284	2.04 b	119.40 b
M-SOY 8001	**0.17 a**	**9.34 a**	BRMS08-1895	1.03 b	63.21 b	BRMS08-1787	2.05 b	79.05 b
BRMS08-5776	0.18 a	11.73 a	BRMS08-1426	1.09 b	131.73 b	BRMS08-2121	2.06 b	96.68 b
BRMS08-1661	0.18 a	11.04 a	BRMS08-1290	1.11 b	57.12 b	BRMS08-1769	2.07 b	81.45 b
BRMS08-1935	0.18 a	11.67 a	BRMS08-1711	1.13 b	54.25 b	BRMS08-1676	2.16 b	113.89 b
BRMS08-1842	0.18 a	12.16 a	BRMS08-1725	1.15 b	61.72 b	BRMS08-1881	2.19 b	105.09 b
BRMS08-1634	0.19 a	9.67 a	BRMS08-1911	1.19 b	122.63 b	BRMS08-1816	2.21 b	157.97 b
BRMS08-1795	0.20 a	11.58 a	BRMS08-1719	1.24 b	90.39 b	BRMS08-1781	2.22 b	130.58 b
BRMS08-1729	0.23 a	12.00 a	BRMS08-1829	1.25 b	79.00 b	BRMS08-1607	2.56 b	138.84 b
BRMS08-5764	0.23 a	15.47 a	BRMS08-1600	1.37 b	115.22 b	BRMS08-1918	2.56 b	167.79 b
BRMS08-1586	0.26 a	33.42 a	BRMS08-1802	1.38 b	62.47 b	BRMS08-1870	2.61 b	116.90 b
BRMS08-1784	0.27 a	20.66 a	BRMS08-1289	1.40 b	88.77 b	BRMS08-1913	2.62 b	126.02 b
BRMS08-1898	0.29 a	12.58 a	BRMS08-1659	1.42 b	84.24 b	BRMS08-1304	2.78 b	201.52 b
BRMS08-2132	0.33 a	20.09 a	BRMS08-1799	1.42 b	77.24 b	BRMS08-1604	2.93 b	147.83 b
BRMS08-1788	0.34 a	18.62 a	BRMS08-1694	1.48 b	93.73 b	BRMS08-1972	3.66 b	197.14 b
BRMS08-1766	0.40 a	14.79 a	BRMS08-1807	1.48 b	73.41 b	BRMS08-1949	4.53 b	230.65 b
BRMS08-1888	0.44 a	80.09 b	BRMS08-1601	1.48 b	66.06 b			
BRMS08-1679	0.48 a	27.36 a	BRMS08-1642	1.51 b	70.35 b			
Mean square	0.378**	0.874**						
Pearson correlation	0.995**							
Mean	1.14	65.97						
Maximum	4.53	230.65						
Minimum	0.05	3.73						
CV (%)	24.57	25.49						

[1] Mean value of five replications per genotype.
*Original data.
[2] Means followed by the same letter, in the column, belong to the same group by the Scott-Knott test ($p<0.05$).
**Significant ($p<0.01$).
For analysis of variance the values of reproduction factor (RF) were transformed in square root (x+1) and number of nematode per gram of root (NGR) in Log10 (x+1).

44 genotypes were higher, statistically equal to BRS 239, expressing susceptibility to *R. reniformis*. In experiment II, five groups were formed based on the means, and resistance to *R. reniformis* (RF <1.0) was observed in 23 of the 115 genotypes, statistically equal to M-SOY 8001. For the variable NGR, only 9 of the 23 genotypes with RF<1.0 were considered statistically equal to cultivar M-SOY 8001 (Table 3). Twenty genotypes did not differ from BRS 239 (mean RF 0.94 - 1.92). In addition, the mean RF of 70 genotypes was above 2, statistically different from BRS 239, ranking them as better multipliers of reniform nematodes than the standard susceptibility cultivar. For 31 of these 70 genotypes, the mean NGR was high and statistically different from BRS 239 (Table 3).

The results of correlation analysis indicated that RF and NGR were significantly correlated, i.e., the lower the mean RF, the lower the mean NGR and vice versa. These analyses showed a significant correlation of RF with high NGR in experiment I (r = 0.995) and in experiment II (r = 0.988) (Tables 2 and 3).

Of the 199 genotypes evaluated in both experiments, 65 genotypes were resistant to *R. reniformis*, with RF below 1.0 and statistically equal to M-SOY 8001, and possibly contain more traits of adaptation for Brazilian conditions (Tables 2 and 3), especially in the state of Mato Grosso do Sul.

Of the 48 tested crosses (segregating populations), 17 resulted in at least one reniform- nematode resistant line, with RF < 1.0 (Table 4). Of the resistant lines, 48 were derived from the crosses labeled 4, 5, 9, 11, and 13 (Table 4). Studies showed that genotypes with cyst- nematode resistance derived from PI 437654, Custer, Forrest, Hartwig, PI 90763, and Peking are potentially resistant to reniform nematodes as well (Robbins et al. 1994a, Robbins et al. 1994b, Robbins et al. 2002, Asmus 2008).

Among the crosses of which over 10 lines were evaluated, the one from which the highest proportion of resistant progenies was derived (in relation to the number of lines evaluated per cross) from *BRSGO Ipameri x (Fayette x PI 437654)*. This result was expected because the three parents were resistant to *H. glycines* with possible resistance to *R.*

Table 3. Comparison of means of reproduction factor (RF) and number of nematodes per gram of root (NGR) of *Rotylenchulus reniformis* in 115 soybean genotypes, evaluated in a greenhouse (Experiment II)

GENOTYPE[1]	RF*	NGR*	GENOTYPE	RF*	NGR*	GENOTYPE	RF*	NGR*
BRMS08-2348	0.09 a	6.79 a	BMR-7743	1.74 b	148.74 c	BMR-87459RR	2.94 c	289.07 d
M-SOY 8001	**0.15 a**	**12.55 a**	BMR-1872	1.87 b	115.28 c	BRMS08-2143	2.95 c	206.28 c
BRMS08-2214	0.18 a	79.2 b	BRMS08-11980	1.88 b	213.86 c	BRMS08-2303	2.98 c	427.39 e
BRMS08-2465	0.19 a	11.34 a	BR07-34298	1.90 b	140.92 c	BRMS08-10972	3.01 c	255.65 c
BRMS08-2474	0.25 a	17.74 a	BRMS08-12271	1.92 b	204.82 c	BMR-10558	3.12 c	153.86 c
BRMS08-2210	0.26 a	22.44 a	BRMS08-10953	1.97 b	94.87 c	BRMS08-2323	3.13 c	433.39 e
BRMS08-2481	0.32 a	18.75 a	BRMS08-10904	2.01 c	109.58 c	BRN06-11457	3.13 c	494.69 e
BRMS08-2512	0.33 a	9.68 a	BRMS08-2458	2.07 c	212.88 c	BRMS08-10949	3.19 c	252.41 c
BRMS08-2480	0.33 a	34.76 a	BMR-7780	2.07 c	165.21 c	BRMS08-10957	3.36 c	207.77 c
BRMS08-2244	0.34 a	37.16 b	BRMS08-2140	2.08 c	162.94 c	BRMS08-12045	3.37 d	259.43 d
BRMS08-2507	0.35 a	16.70 a	BRI07-0644	2.10 c	182.22 c	BRMS08-2136	3.46 d	245.79 c
BRN06-16845	0.41 a	22.29 a	BRMS08-11981	2.12 c	172.52 c	BRMS08-12300	3.51 d	240.13 c
BMR-8204	0.45 a	46.53 b	BRMS08-10692	2.12 c	214.18 c	BRMS08-2133	3.53 d	369.56 d
BRMS08-10882	0.48 a	48.20 b	BRMS08-10971	2.15 c	195.46 c	BR07-26590	3.59 d	351.24 d
BRMS08-10887	0.49 a	46.73 b	BRMS08-2311	2.17 c	159.35 c	BRMS08-11915	3.63 d	271.17 d
BMR-5583	0.52 a	45.11 b	BMR-10419	2.17 c	89.07 b	BR06-75833	3.70 d	313.50 d
BRMS08-2171	0.57 a	46.43 b	BRMS08-2416	2.24 c	218.14 c	BRMS06-13911248	3.77 d	376.39 d
BR07-34344	0.60 a	46.42 b	BRMS08-11913	2.24 c	182.36 c	BRMS08-2496	4.05 d	240.51 c
BRMS08-2189	0.64 a	48.78 b	BRMS08-2184	2.28 c	131.54 c	BRMS08-2138	4.08 d	283.98 d
BMR-9563	0.65 a	72.64 b	BRMS08-12031	2.35 c	268.36 d	BRMS08-10960	4.12 d	283.07 d
BRMS08-2174	0.69 a	69.61 b	BRMS08-10912	2.35 c	204.79 c	BRMS08-11752	4.15 d	359.27 d
BRMS08-2255	0.74 a	77.79 b	BRMS08-11786	2.40 c	235.79 c	BRMS08-2329	4.24 d	491.91 d
BRMS08-2203	0.81 a	64.10 b	BR06-62539	2.43 c	209.44 c	BRMS08-11793	4.32 d	490.28 d
BRMS08-11801	0.84 a	179.41 c	BMR-84190RR	2.43 c	152.96 c	BRMS08-10892	4.48 d	567.66 e

To be continued...

BRMS08-2462	0.94 b	70.95 b	BRMS08-10961	2.46 c	137.44 c	BRMS08-11781	4.50 e	356.71 d
BRMS08-2506	1.02 b	71.78 b	BRI07-0319	2.47 c	156.82 c	BRMS08-10974	4.52 e	232.22 c
BRN06-12048	1.05 b	99.21 c	BRMS08-10886	2.49 c	229.65 c	BRMS08-5504	4.53 e	406.68 e
BRMS08-10414	1.08 b	118.35 c	BRMS08-11838	2.54 c	229.87 c	BR06-75792	4.64 e	263.03 d
BRMS08-2187	1.12 b	95.35 c	BR06-77758	2.59 c	183.83 c	BR06-63905	4.83 e	322.46 d
BRMS08-12295	1.20 b	163.47 c	BR07-31387	2.66 c	217.32 c	BRMS08-11976	4.88 e	515.63 e
BRMS08-2208	1.24 b	85.02 b	BMR-86253RR	2.76 c	239.38 c	BRQ06-2539	4.90 e	507.28 e
BRMS06-603089	1.39 b	165.19 c	BMR-10463	2.80 c	185.51 c	BRMS08-10893	4.96 e	629.64 e
BRMS08-12042	1.39 b	214.71 c	BRMS08-2248	2.82 c	201.35 c	BRMS08-11784	5.01 e	404.97 d
BRMS08-2468	1.41 b	81.18 b	BRMS08-2144	2.83 c	333.49 d	BMR-87069RR	5.29 e	368.25 d
BRS 239	**1.45 b**	**194 c**	BRMS08-11805	2.84 c	211.27 c	BRMS08-2307	5.83 e	602.46 e
BRMS08-2360	1.53 b	95.59 c	BRMS08-10897	2.84 c	219.96 c	BRMS08-10876	6.45 e	675.85 e
BRMS08-2222	1.54 b	149.75 c	BRMS08-10409	2.86 c	223.15 c	BRMS08-10910	6.47 e	306.82 d
BRMS08-4576	1.62 b	148.83 c	BMR-87695RR	2.91 c	410.00 d			
BMR-10557	1.64 b	145.80 c	BRMS08-2135	2.93 c	246.83 c			
Mean square	0.754**	0.865**						
Pearson correlation	0.988**							
Mean	2.39	209.11						
Maximum	6.47	675.85						
Minimum	0.09	6.79						
CV(%)	24.20	18.75						

[1] Mean value of five replications per genotype.
*Original data.
[2] Means followed by the same letter, in the column, belong to the same group by the Scott-Knott test ($p<0.05$).
**Significant ($p<0.01$).
For analysis of variance the values of reproduction factor (RF) were transformed in square root (x+1) and number of nematode per gram root (NGR) in Log10 (x+1).

reniformis, as of parent PI 437654. Asmus (2008) evaluated 31 soybean genotypes for resistance to *R. reniformis*. The results showed that most genotypes considered resistant to *R. reniformis* had previously been described as resistant to races 3 and/or 1 of soybean cyst nematode (SCN), as reported by Robbins and Rakes (1996).

Of the 65 lines considered resistant, the cultivars Custer, PI 437654, Fayette, BRSGO Ipameri, BRSMT Pintado, and BRS 262, in different cross combinations, generated a significant number of resistant progenies (> 73% resistant lines) (Table 4). Other cultivars such as BRS 231, Forrest, Hartwig, BRSGO Chapadões, BRSMG 250 (Nobreza), Anta 82 RG, Sharkey, and NK 412131 and the line GOBR99-701006 also originated resistant lines, though less frequently.

In the USA, there was a significant reduction in the rate of *R. reniformis* resistance of the genotypes used, due to the almost exclusive use of PI 88788 as resistance source to *H. glycines,* which is susceptible to *R. reniformis* (Robbins et al. 2002). On the other hand, most of the genotypes in this study were derived from parents with SCN resistance, mainly to the races 1 and 3. The cultivars BRSGO Ipameri, BRSMT Pintado and BRS 262, recommended for Brazil, performed well as resistance sources to reniform nematodes (Table 4). These cultivars were described as SCN-resistant by their breeders, for being progenies of at least one of the main sources of resistance to this plant nematode (Embrapa 2010).

Dias et al. (2009) mentioned the complexity in understanding the genetic basis of *H. glycines* resistance in soybean, due to the possibility of involving gene blocks or few genes with multiple alleles. This genetic behavior was also reported in studies on the genetic inheritance of *R. reniformis* resistance in soybean (Williams et al. 1981**,** Harville et al. 1985). Ha et al. (2007) evaluated genotype PI 437654 with molecular markers and proved that the genes of *R. reniformis* resistance are close to those that control *H. glycines* resistance. Furthermore, these authors showed that resistance is controlled quantitatively by genes with unequal effects. This hypothesis of quantitative inheritance of resistance may explain the low proportion of resistant lines (around 33% of all tested lines) detected in this study (Table 4).

The results of this study show that the variability for resistance to reniform nematodes in the studied genotypes was high, allowing the selection of resistant lines. Under controlled conditions, the variable RF is sufficient to determine the

resistance to *R. reniformis*. The cultivars BRSGO Ipameri, BRSMT Pintado, and BRS 262, suitable for nation-wide planting and described in this study as promising resistance sources to reniform nematodes, could be used soon in breeding programs. Therefore, to increase the chances of success in the development of reniform-nematode resistant cultivars in soybean breeding programs, in view of the lack of information on resistance to *R. reniformis*, it is recommended that effort should be invested in the evaluation and selection of segregating populations derived from parental genotypes with resistance to *H. glycines,* at least to the races 1 and 3.

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Table 4. Crosses that originated resistant lines (RF<1.0) to *R. reniformis* in a greenhouse - Experiments I and II

No.[1]	Crosses	Tested	Lines RF<1	% resistant lines
1	Forrest[3] x BRS 231[2]	6	2	3.1
2	(BRSGO 204 x BRSGO Chapadões[2]) x BRSMG 250 (Nobreza[3])	3	2	3.1
4	Custer[3] x BR96-25619	31	13	20.0
5	BR96-25619 x Custer[3]	31	15	23.1
6	Forrest[3] x BR96-25619	3	1	1.5
7	BR96-25619 x Forrest[3]	2	2	3.1
8	PI 437654[3] x Hartwig[3]	10	2	3.1
9	BRS 262[2] x {BRS 244 RR x Shiranui-1}	5	5	7.7
11	BRSGO Ipameri[2] x (Fayette[2] x PI 437654[3])	13	8	12.4
12	(BRSMT Pintado[2] x MGBR 46 Conquista) x (Fayette[2] x PI 437654[3])	8	1	1.5
13	(Fayette[2] x PI 437654[3]) x (BRSMT Pintado[2] x MGBR 46 Conquista)	11	6	9.2
20	GOBR99-701006[3*] x BR03-81463 RR	4	2	3.1
22	Anta 82 RG[2] x BR02-65840	10	1	1.5
31	Embrapa 48 x NK 412113[2]	2	1	1.5
35	BRS 231[2] x BRS Sambaíba	1	1	1.5
36	BRS 262[2] x (M-SOY 5826 x CD 206)	2	2	3.1
39	{Sharkey[2] x {Hartwig[3] x {FT 5 x {[FT 10*2 x (FT 6*2 x SS 1-A)] x TGX 297-192-C}}}} x {(Santa Rosa x Tracy) x [(BR 16*3 x BRM92-6600) x (BR 16*5 x IAC 12)]}	1	1	1.5
	Total		65	100%

[1] Number of cross in Table 1. [2] Parent resistant to H. glycines (race 3), [3] Parent resistant to R. reniformis and H. glycines (races 1 and 3).
*Pedigree: GOBR99-701006= {Hartwig*4 x [OC 8 x (TGX 342-351-D x Paranagoiana*4)]} x [Sharkey*2 x (Dourados*4 x SS1)]

Resistência de genótipos de soja ao nematoide reniforme em ambiente controlado

Resumo - *O objetivo do trabalho foi caracterizar genótipos de soja oriundos de cruzamentos com pelo menos um genitor resistente a* Rotylenchulus reniformis *ou* Heterodera glicynes*, ou ambos, quanto à resistência ao nematoide reniforme. Dois experimentos foram conduzidos em casa de vegetação na Embrapa Agropecuária Oeste, em delineamento inteiramente casualizado, totalizando 199 genótipos com cinco repetições. Após 60 dias da inoculação artificial (1000 ovos e formas larvais), os nematoides foram extraídos das raízes e os genótipos avaliados quanto ao número de ovos e formas larvais por grama de raiz (NGR) e ao fator de reprodução (FR). Sessenta e cinco genótipos foram considerados resistentes (FR<1,0), com valores médios de FR significativamente iguais à M-SOY 8001. Os genótipos Custer, PI 437654, Fayette, BRSGO Ipameri, BRSMT Pintado e BRS 262, nas diferentes combinações de cruzamentos avaliadas, foram os que geraram o maior número de linhagens resistentes ao nematoide reniforme*.

Palavras-chave: Glycine max, Rotylenchulus reniformis, *melhoramento de plantas, caracterização fenotípica.*

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