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Efficiency of microsatellite markers in assisted selection for resistance to soybean cyst nematode (race 3)

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ABSTRACT - *Four microsatellite sequences were tested in soybean DNA from cultivars and segregating genotypes. Three of them were close to the resistance locus rhg1 on molecular linkage group G (Satt309, Sat_168, Sat_163) and one was close to Rhg4 locus on group A2 (Sat_162). Progenies previously classified as cyst nematode (SCN) resistant and others with unknown reaction were tested, using the resistant cultivars Liderança, and Renasscença and the susceptibles 'Cristalina' and 'OCEPAR-4' as control. The best primer for resistance to SCN was Sat_162. Twenty segregant progenies tested with Sat_162 presented a 150 bp band for homozygous resistant genotypes and 200 bp for susceptible ones, and both for the heterozigous genotypes. Previous studies have shown that this microsatellite marker is efficient to select genotypes carrying Peking-derivative resistance. 'Peking' takes part in the in the genealogy of all segregant progenies evaluated in this study. Thus, the obtained results showed that Sat_162 distinguished resistant and susceptible soybean genotypes to SCN, race 3.*

Key words: SCN, race 3, marker assisted selection, microsatellites, soybean.

INTRODUCTION

The soybean cyst nematode (SCN) (*Heterodera glycines* Ichnohe) counts among the most important problems for soybean cultivation since losses due to this pathogen can be total, depending on its soil density (Noel 1992). First detected in 1992, in Brazil, specifically in Nova Ponte, Iraí de Minas and Romaria, Minas Gerais State, this nematode has nowadays reached most of the soybean production areas in Brazil. Mauro et al. (1999) emphasize that the use of resistant cultivars in rotation with susceptible ones and non-host species is the most economic and reliable method to control SCN.

Thus, the development of resistant cultivars is an important step in direction of disease control and reduced imposed pathogen-caused losses. Identifying resistant segregant lines to SCN in any breeding program is a difficult and expensive process. Firstly, the pathogen must be collected from infested areas, then transport, and multiplied. It is also necessary to inoculate a large number of segregant progenies and evaluate them for resistance to SCN. Obviously all these steps involve contamination risks on SCN free areas and there may be problems regarding the evaluation of the segregant lines.

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The development of SCN resistant lines would be easier if an effective, rapid and reproducible system was available to identify inbred lines carrying the resistant alleles. Cregan et al. (1999a, b) has used some microsatellite markers to identify resistant and susceptible lines to SCN, race 3. Among the developed microsatellite markers some are close to the resistance locus rhg1, on linkage group G (Satt309, Sat_168, Sat_163 and Sat_141), while others are close to the resistance locus Rhg4, on linkage group A2 (Sat_162, Satt632 and Sat_157). According to the same author (Personal communication) the most polymorphic sequences are Satt309, Sat_168, Sat_163 and Sat_162.

Microsatellites are intensely used for assisted selection and correspond to short DNA sequences constituted by 1-4 tandem repeated nucleotides. They are frequent in eukaryote genome made up of highly polymorphic loci (Ferreira and Gratapaglia 1998). These markers are codominant, multialelic, and have an expected heterozigozity of around 0.7. Besides, microsatellites are abundant and disperse in the whole genome and the derived polymorphism can be analyzed by PCR. Furthermore, the marker information based on the primer sequence is easily exchangable among laboratories contributing to the cooperative efforts in research and development (Brondani et al. 1998).

Considering all the advantages of using microsatellites in assisted selection, this study's primary intent was to verify the efficiency of some of these microsatellite markers at distinguishing resistant and susceptible segregant Brazilian soybean progenies to SCN, race 3.

MATERIAL AND METHODS

Several crosses (Table 1) among resistant and susceptible soybean cultivars led to subsequently F_3 and F_4 generations, of which some progenies were cultivated in a SCN (race 3) infested field, in Iraí de Minas, Minas Gerais State, Brasil, aiming at the selection of resistant plants using the methodology proposed by Arantes et al. (1998). The selection was also performed in a SCN (race 3) infested area, cultivated with the susceptible cultivar Conquista, sown 45 days before. In this area, plants in the V_6 stage (Fehr and Caviness 1977), with SCN symptoms were uprooted and the genotypes to be evaluated were cultivated in the same rows, which were openned at 0.5 m apart, coinciding with the rows of Conquista. Each progeny to be evaluated was derived from a single plant selected in the previous generation. Thus, different numbers of progenies from each cross were evaluated (see Table 2). Resistant and susceptible cultivars to SCN were sown among the progenies. Thirty-four days later, ten plants were carefully removed from each plot and evaluated to obtain the number of females and cysts in the roots. All genotypes were evaluated according to the rating system used by Hartwig (1985), which is the most commonly used method for this purpose, as follows: 0 = no cysts on the roots; 1 = 1 to 5 cysts; 2 = 6 to 10 cysts; 3 = 11 to 20 cysts; 4 = more than 20 cysts. Results for the evaluations of the ten sampled plants are shown in Table 2.

Table 1. List of crosses used for field and laboratory tests aiming at SCN resistance (race 3)

Crosses	Genealogy (R x S)[1]	Generations
JAB 99-40	BR 92-15440 x Cristalina	F_3
JAB 99-41	BR 90-4617 x Cristalina	F_3
JAB 99-42	BR 91-10569 x Cristalina	F_3
JAB 99-43	Hartwig x Paraná	F_3
JAB 99-44	Delsoy x Cristalina	F_3
JAB 99-45	(BR 90-4722 x Cristalina) x Cristalina	F_3
JAB 99-10	BR 92-15440 x Cristalina	F_4
JAB 99-11	BR 91-10569 x Cristalina	F_4
JAB 99-12	BR 92-15440 x Cristalina	F_4
JAB 99-13	Delsoy x Cristalina	F_4
JAB 99-14	BR 90-4617 x Cristalina	F_4
JAB 99-17	Hartwig x Paraná	F_4
JAB 99-18	Hartwig x EMBRAPA 1	F_4
JAB 99-19	Hartwig x Paraná	F_4
JAB 99-21	Hartwig x Cristalina	F_4

[1] R: Resistant to SCN (race 3); S: Susceptible to SCN (race 3).

Plants classified as resistant were transported to Jaboticabal, State of São Paulo, and replanted in 5-L plastic pots, remaining in the soybean breeding program at UNESP. Samples of the first young leaves produced after replantation, as well as samples from resistant (Renascença and Liderança) and susceptible cultivars (Cristalina, OCEPAR-4, FT-Cometa, Conquista, IAS-5 and BR-16) and 20 progenies with unknown reaction to SCN (race 3) were collected for DNA extraction, using the CTAB protocol (Ferreira and Grattapaglia 1998). A molecular analysis was then performed in order to verify the efficiency of the microsatellite marker at distinguishing SCN (race 3) resistant and susceptible genotypes, and also to determine the correspondence between the results obtained in the field to those obtained in the laboratory.

Four evaluated microsatellite markers (Satt309, Sat_168, Sat_163 and Sat_162) showed polymorphism for the resistant and susceptible alleles of SCN (race 3). According to Cregan (1999a, b), two resistant (134 bp and 125 bp) and two susceptible alleles (131 bp and 149 bp) were observed for Satt309. For Sat_168, two resistant (157 bp and 171 bp) and one susceptible allele (179 bp) were detected. Regarding Sat_163, one resistant (242 bp) and at least two susceptible alleles (208 bp and 233 bp) were observed. For Sat_162, one allele for susceptiblity, close to 200 bp, and another for resistance, close to 150 bp, were detected.

The PCR reactions were carried out in a MJ Thermocycler, model PTC 100, using a program with an initial 30 seconds denaturation step at 95 °C, followed by 11 cycles of 30 seconds of denaturation (94 °C), annealing (57 °C) and extension (72 °C). The annealing temperature was reduced by 1 °C interval from 57 to 46 °C, from cycles 1 to 11. These steps were followed by 22 cycles of denaturation, annealing and extension at 94, 46 and 72 °C, respectively. The program ended with a 4 °C soak. The PCR reaction mixture was set up as follows: 1X PCR buffer (10 mM Tris-HCl pH 8.3 and 50 mM KCl); 1.5 mM $MgCl_2$;

Table 2. Evaluation of soybean cultivars and progenies for SCN resistance, according to Hartwig (1985), in field test carried out in Irai de Minas, MG, Brasil

Row	Genotype	Scores									
		1	2	3	4	5	6	7	8	9	10
1	Conquista	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
2	Renascença	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	JAB 99-40-2	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0
4	JAB 99-40-3	4.0	3.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0
5	JAB 99-40-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	JAB 99-40-5	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0
7	JAB 99-40-6	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0
8	JAB 99-40-8	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0	4.0	4.0
9	JAB 99-40-13	3.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0
10	JAB 99-40-14	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0
11	JAB 99-41-2	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0
12	JAB 99-41-4	3.0	3.0	3.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
13	JAB 99-41-8	4.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0	4.0	4.0
14	JAB 99-41-9	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
15	JAB 99-41-10	4.0	3.0	3.0	4.0	3.0	3.0	3.0	4.0	4.0	4.0
16	JAB 99-41-12	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0
17	Cristalina	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
18	Liderança	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	JAB 99-41-14	1.0	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
20	JAB 99-42-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	JAB 99-42-5	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0	4.0
22	JAB 99-42-6	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
23	JAB 99-42-7	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0
24	JAB 99-42-8	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
25	JAB 99-42-10	3.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0	4.0
26	JAB 99-42-11	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
27	JAB 99-42-12	3.0	3.0	3.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
28	JAB 99-43-2	4.0	3.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
29	JAB 99-43-3	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
30	JAB 99-43-4	3.0	3.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0
31	JAB 99-43-9	4.0	4.0	4.0	4.0	3.0	3.0	4.0	4.0	4.0	4.0
32	JAB 99-43-11	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
33	OCEPAR-4	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0
34	Renascença	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35	JAB 99-43-13	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	3.0	3.0
36	JAB 99-44-3	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
37	JAB 99-44-4	4.0	4.0	4.0	3.0	4.0	3.0	4.0	4.0	4.0	4.0
38	JAB 99-44-5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
39	JAB 99-44-6	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	3.0
40	JAB 99-44-8	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0
41	JAB 99-44-10	3.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0	3.0
42	JAB 99-44-12	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
43	JAB 99-44-13	4.0	3.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0	4.0
44	JAB 99-44-15	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
45	JAB 99-44-16	3.0	3.0	3.0	3.0	3.0	4.0	3.0	3.0	3.0	3.0
46	JAB 99-45-3	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
47	JAB 99-45-6	4.0	4.0	3.0	3.0	4.0	4.0	4.0	4.0	4.0	4.0
48	JAB 99-10-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
49	FT-Cometa	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
50	Liderança	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
51	JAB 99-10-4	2.0	2.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
52	JAB 99-10-5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
53	JAB 99-11-2	3.0	3.0	3.0	3.0	3.0	4.0	3.0	3.0	3.0	3.0
54	JAB 99-11-3	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0
55	JAB 99-11-5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
56	JAB 99-12-1	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0
57	JAB 99-12-2	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
58	JAB 99-12-4	2.0	2.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
59	JAB 99-12-5	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0
60	JAB 99-13-1	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0
61	JAB 99-13-2	3.0	1.0	0.0	3.0	3.0	3.0	0.0	1.0	1.0	3.0
62	JAB 99-13-3	3.0	3.0	3.0	3.0	3.0	4.0	3.0	3.0	3.0	3.0
63	JAB 99-14-1	3.0	3.0	3.0	3.0	4.0	3.0	3.0	3.0	3.0	3.0
64	JAB 99-14-2	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
65	IAS-5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

to be continued

Row	Genotype	Scores									
		1	2	3	4	5	6	7	8	9	10
66	Renascença	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
67	JAB 99-14-3	3.0	4.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
68	JAB 99-14-4	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
69	JAB 99-14-5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
70	JAB 99-17-1	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0
71	JAB 99-17-2	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
72	JAB 99-17-3	2.0	1.0	1.0	2.0	2.0	2.0	1.0	2.0	2.0	1.0
73	JAB 99-17-4	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
74	JAB 99-18-2	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0	4.0
75	JAB 99-18-3	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
76	JAB 99-18-4	3.0	4.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
77	JAB 99-19-2	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
78	JAB 99-19-4	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0
79	JAB 99-21-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
80	JAB 99-21-3	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
81	JAB 99-21-4	4.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0
82	JAB 99-21-5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
83	BR-16	4.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0	4.0
84	Liderança	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0

0.15 mM dNTP's; 0.15 µM for each primer (forward and reverse); 100 ng of genomic DNA, 1 Unit of Taq DNA Polimerase and Milli-Q water up to a final volume of 50 µL. After amplification the PCR products were separated on a 3.5% MetaPhor® Agarose gel under a 100 V for four hours, and visualized by staining with ethidium bromide (0.5 ug ml^{-1}). The results were visualized and documented in a Kodak-EDAS 290 Photodocumentation system.

RESULTS AND DISCUSSION

The four microsatellites were tested using Brazilian soybean cultivars (Renascença, Liderança, Cristalina, OCEPAR-4, FT-Cometa, Conquista, IAS-5 and BR-16) and segregant populations (Table 1) developed by the breeding program of the Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual de São Paulo "Júlio de Mesquita Filho" (UNESP), Jaboticabal Campus.

All the primers were tested and the results re-confirmed by several replications of the protocol. Primer Sat_163 produced bands close to 230 bp and 240 bp, which is very similar to the resistance band of 242 bp and to the susceptibility band of 232 bp. Furthermore, fragments with little base pair differences (10 bp) tend to make the discrimination more difficult when using agarose gel. On the other hand, Sat_168 produced just one band (150 bp) for both resistant and susceptible cultivars. This band is equivalent to the one of 157 bp for resistance being, however, common to some susceptible genotypes. Thus, this marker did not show efficiency for evaluating the crosses. Fragments of 131 and 134 bp were observed, respectively, for the susceptible and resistant cultivars when amplifying with primer Satt309. Moreover, the small differences between fragments (3 pb) disqualified the primer for a recommendation in assisted selection using agarose gel. With respect to markers Sat_168, Cregan (1999a) concluded that for a safer classification of the gene rhg1 the simultaneous use of this marker with the marker Satt309 would be necessary, since the allele of 157 bp often appears both in the resistant and susceptible cultivars. Resistant genotypes carrying the 157 bp allele in the Satt309 locus must carry the 134 bp allele in the same locus. On the other hand, susceptible genotypes carry the 131 bp in this locus, resulting in more laborious and more expensive selection process, due to the great number of reactions required to distinguish the 131 and 134 bp alleles.

Primer Sat_162 produced bands close to 150 bp in the resistant genotypes and close to 200 bp in the susceptibles ones. This was true both for progenies and cultivars (Renascença, Liderança, Cristalina, OCEPAR-4, FT-Cometa, Conquista, IAS-5 and BR-16), corresponding respectively to the resistant and susceptible alleles described by Cregan et al. (1999b). This distance between fragments allows an easy discrimination of resistant and susceptible genotypes in agarose gel. Figures 1 to 3 show the results derived from DNA samples amplified with microsatellite Sat_162.

For Figure 1, all the genotypes were previously evaluated in the field for resistance to SCN, race 3, according to methodology developed by Arantes et al. (1998). Each genotype was a plot in the field, and 10 plants per plot were evaluated (Table 2). Plants scored 0 or 1 were considered as resistant, according to criteria of Hartwig (1985). Thus, from a total of 720 evaluated plants, just 80 were selected as resistant (progenies JAB 99-40-4, JAB 99-40-5, JAB 99-40-14, JAB 99-41-14, JAB 99-42-1, JAB 99-10-1, JAB 99-10-5 and JAB 99-21-2). These plants were transported to Jaboticabal, SP. Only 31 survived, corresponding to the evaluated plants with primer Sat_162, as shown in Figure 1. All the 31 plants

classified as resistant in field tests and the resistant cultivars Renascença and Liderança produced a 150 bp band (Figure 1), while the susceptible controls (OCEPAR-4 and Cristalina) produced a 200 bp band, showing a high correspondence between field tests and the results of DNA amplification with the microsatellite Sat_162.

Results shown in Figures 2 and 3 are related to 20 segregant genotypes (F_3 and F_4) with unknown reaction to SCN. The same Figures show segregation for fragments 150 bp and 200 bp, corresponding to progenies with resistant and susceptible reaction to the pathogen. The resistant control (Renascença) showed the 150 bp fragment and the susceptible (Cristalina) the 200 bp fragment. As expected, due to the progeny segregation, some progenies showed both fragments, which may be ascribed to the heterozigozity. Owing to the high correspondence between the field evaluations and molecular results, for both susceptible and resistant genotypes, the marker Sat_162 was incorporated in the process of marker assisted selection (MAS) carried out in our laboratory. Thus, about 300 segregant genotypes were screened using the microsatellite Sat_162 as SCN (race 3) resistance marker, as part of the second cycle of MAS. Some of these results are displayed in Figure 4, where there is a sample of F_3 plants evaluated with marker Sat_162. We observe segregation for the 150 bp and 200 bp, indicating the possible presence of resistant and susceptible alleles, as previously mentioned.

Previous studies carried out by Cregan et al. (1999a) showed that the microsatellite Sat_162 is very efficient for SCN assisted selection of genotypes carrying Peking-derivative resistance, and Peking takes part in the genealogy of all SCN resistance sources used in this study. According to Young (1999), microsatellites can be 99% accurate in predicting lines that are susceptible in subsequent greenhouse assays. Thus, this microsatellite is particularly indicated and very effective in the assisted selection of resistant genotypes carrying the Peking resistance genes.

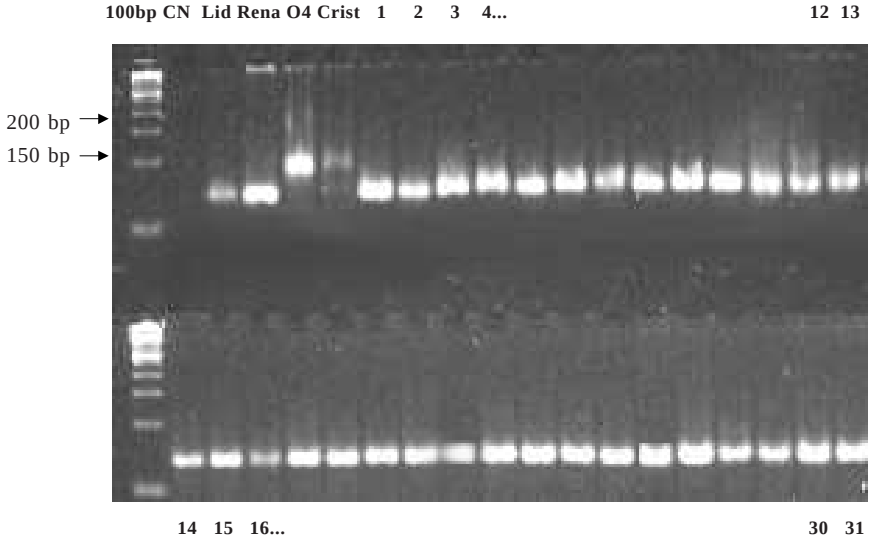

Figure 1. Amplification with microsatellite Sat_162 of DNA samples taken from the resistant cultivars Liderança and Renascença (Lid and Rena), the susceptibles Ocepar 4 and Cristalina (O4 and Crist) and thirty-one F_3 and F_4 genotypes previously classified as resistant in field tests (1 to 31).

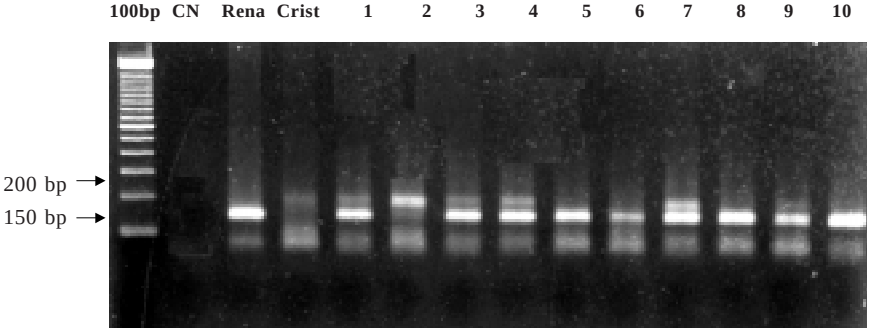

Figure 2. Amplification with microsatellite Sat_162 of DNA samples taken from the resistant cultivar Renascença (Rena), the susceptible Cristalina (Crist), and ten segregant genotypes (1 to 10).

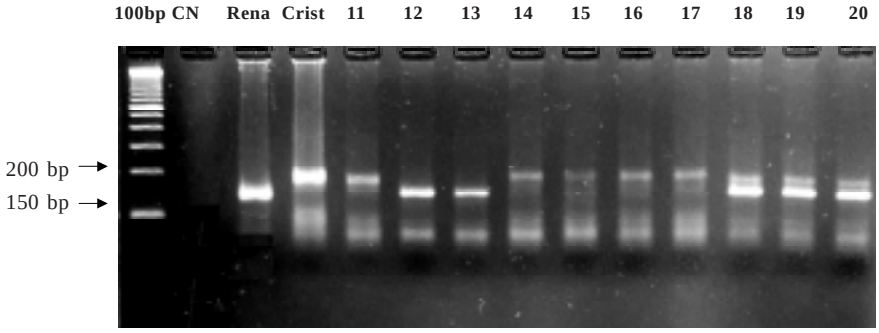

Figure 3. Amplification with microsatellite Sat_162 of DNA samples taken from the resistant cultivar Renascença (Rena), the susceptible Cristalina (Crist), and ten segregant genotypes (11 to 20).

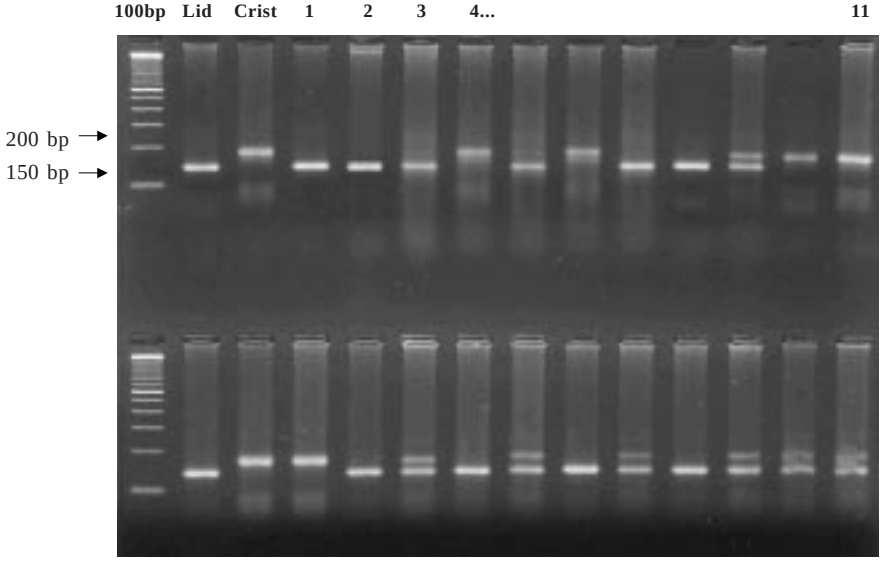

Figure 4. Amplification with microsatellite Sat_162 of DNA samples taken from 24 segregant progenies, using the resistant cultivar Liderança (Lid) and the susceptible Cristalina (Crist) as controls.

CONCLUSIONS

1. The best and most effective microsatellite for assisted selection aiming at SCN resistance, race 3, in Peking-derivative populations, was Sat_162 marker.

2. Results for assisted selection using the microsatellite marker Sat_162 were equivalent to the results obtained in the field evaluations for resistance to SCN (race 3).

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Eficiência de marcadores microssatélites na seleção assistida para resistência ao nematóide do cisto da soja (raça 3)

RESUMO - *Quatro seqüências de microssatélites foram testadas utilizando-se DNA de cultivares e populações segregantes de soja. Três delas localizam-se próximas ao locus de resistência rhg1, no grupo G de ligação (Satt309, Sat_168, Sat_163) e a outra próxima ao locus Rhg4 no grupo A2 (Sat_162). Progênies previamente classificadas como resistentes ao nematóide do cisto (SCN) e outras com reação desconhecida foram testadas, usando como controles os cultivares resistentes Renascença e os Liderança suscetíveis 'Cristalina' e 'OCEPAR-4'. O melhor microsatélite para seleção assistida objetivando resistência ao*

SCN, raça 3, foi o Sat_162. Vinte genótipos segregantes testados com Sat_162 apresentaram bandas de 150 pb (resistente) ou de 200 pb (suscetível), e ambas para os genótipos heterozigotos. Estudos prévios demonstraram que Sat_162 é eficiente para seleção de genótipos portadores de genes de resistência do cultivar Peking. Todas as progênies segregantes avaliadas possuem 'Peking' na sua genealogia. Os resultados com Sat_162 evidenciaram que o mesmo pode ser utilizado para a seleção assistida de genótipos resistentes a SCN, raça 3.

Palavras-chave: SCN, raça 3, seleção assistida por marcador, microsatelites, soja.

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