SSR markers linked to stem canker resistance in soybean *Glycine max*

Marcadores SSRs ligados a la resistencia al cancro del tallo en soja *Glycine max*

Javier Ramón Gilli [1*], Gabriel Ricardo Vellicce [2], Clarisa Noelia Bernardi [1]

Abstract

In this work, we studied 40 samples of *Diaporthe phaseolorum* var. *meridionalis* (Dpm), causal agent of stem canker in soybeans (SCS). In the susceptible genotype Golondrina65 the isolate RSF12 showed the highest percentage of the dead plant index (DP = 85.7 %) and was used to characterize all known sources of resistance to Dpm. The soybean MJ19RR experimental line showed the best behaviour against this isolate with a DP = 2.4 % and was used to develop a segregating population with the susceptible cultivar FT-2001. In the F_2 generation, the chi-square test determined a 3:1 ratio of resistant plants against susceptible plants, as expected for a dominant gene. In order to advance in our study we propose, as objective, to locate in the soybean genetic map the resistance to *Diaporthe phaseolorum* var. *meridionalis*. The Bulked Segregant Analyses and the genetic linkage study identified a region of chromosome 6 of the genetic map of soybean, located at 13.3 cM from the Satt433 *locus* associated with resistance to SCS. The soybean experimental line MJ19RR was selected as the best source of resistance, and it is available in the active bank of soybean germplasm of INTA, for the genetic control of this disease. The results obtained in this work represent a first approximation for the understanding of the genetic basis of resistance to SCS.

Keywords

Diaporthe phaseolorum var. *meridionalis* • molecular markers • fungal resistance • *Glycine max*

---

1   EEA INTA Marcos Juárez. Laboratorio de Biotecnología. Ruta provincial N° 12. km 2. C.P. 2580. Marcos Juárez. Córdoba. Argentina. * gilli.javier@inta.gob.ar

2   Estación Experimental Agroindustrial Obispo Colombres (EEAOC). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA). Av. William Cross 3150. C. P. T4101XAC. Las Talitas. Tucumán. Argentina.

Resumen

En este trabajo estudiamos 40 muestras de *Diaporthe phaseolorum* var. *meridionalis* (Dpm), agente causal del cancro del tallo en soja (CTS). En el control susceptible Golondrina65, el aislado RSF12 presentó el mayor porcentaje del índice de plantas muertas (DP = 85,7 %) y fue utilizado para caracterizar las fuentes de resistencia conocidas al Dpm. La línea experimental de soja MJ19RR mostró el mejor comportamiento frente a este aislado, con un valor de DP= 2,4 %, y fue utilizada para desarrollar una población segregante con el cultivar susceptible FT-2001. En la generación F_2 la prueba de chi-cuadrado determinó una proporción 3:1 de plantas resistentes *versus* plantas susceptibles, como se espera para un gen dominante. Para avanzar en nuestro estudio, proponemos como objetivo localizar en el mapa genético de soja la resistencia a *Diaporthe phaseolorum* var. *meridionalis*. El Bulked Segregant Analyses y el estudio de ligamiento genético identificaron una región del cromosoma 6 del mapa genético de soja, a 13,3 cM del *locus* Satt433, asociada a la resistencia al CTS. Además la línea experimental de soja MJ19RR fue seleccionada como la mejor fuente de resistencia disponible en el banco activo de germoplasma de soja de INTA para el control genético de esta enfermedad. Los resultados obtenidos en este trabajo representan una primera aproximación para la comprensión de las bases genéticas de la resistencia al CTS.

Palabras claves

Diaporthe phaseolorum var. *meridionalis* • marcadores moleculares • resistencia a hongos • *Glycine max*

Introduction

Soybean stem canker (SSC) is caused by *Diaporthe phaseolorum*, was first reported in USA in 1940s and was one of the pathogens with major impact on soybean yield. A variant named meridionalis was identified in 1973 in southern USA with two different stages: the asexual one as *Phomopsis phaseoli* var. *meridionalis* in infected plant tissue, and the sexual phase, as *Diaporthe phaseolorum* var. *meridionalis* (Dpm) on plant detritus (14). In Argentina, *D. phaseolorum* var. *meridionalis* was first reported in 1992 (7). It is currently distributed all over the soybean production areas with four different physiological breeds identified according to response to inoculation of different resistant cultivars (10).

SSC resistance is controlled by five major, dominant, nonallelic genes: *Rdm1* and *Rdm2* in cv. Tracy-M (11); *Rdm3* in cv. Crockett, *Rdm4* in cv. Dowling and cv. Hutcheson (2, 3) and *Rdm5* in cv. Hutcheson (20). The pyramiding of these resistance genes could be the better strategy for control all physiological breeds causing SSC. In this sense, marker assisted selection is a tool that is currently available in most breeding programs, however there is a lack of information about the mapping of marker associated with resistance to SSC.

With the objective of locating the genetic resistance to *Diaporthe phaseolorum* var. *meridionalis* in the genetic soybean map, it was used a Bulked

Segregant Analysis (BSA) strategy (13) in a F2 population derived from a simple cross between the MJ19RR x FT-2001 genotypes.

The BSA is a simple strategy that is used for a first approach to locate genomic regions associated with important agronomic traits. It is based on the disequilibrium of the segregation due to genetic linkage and consists in of comparing two DNA bulks from plants of a segregating population (generation F2) derived from a simple cross or a backcross (generation BC1F2).

MATERIALS AND METHODS

Fungal isolation

D. phaseolorum var. *meridionalis* were obtained from infected plants showing typical SSC symptoms from soybean production fields located in Córdoba and Santa Fe regions of Argentina, during the 2013/2014 harvest season. Isolation was conducted by the method of Keeling (9). *D. phaseolorum* var. *meridionalis* were cultivated on potato dextrose agar (PDA) plates at 27 ± 2°C for 5 days, and then maintained at room temperature for 45 days in order to induce perithecium fructification; afterwards, the cultures were maintained at 4°C. Morphologic characterization considered the aspect of the colony, the perithecium, the pycnidium and whether there were α or β conidia present (5). Isolates fitting to *D. phaseolorum* var. *meridionalis* description were subcultured on new PDA media, finally virulence studies of each isolate were performed by inoculation of the susceptible control Golondrina65 under greenhouse conditions.

Plant Materials

The genotypes used included: the susceptible control Golondrina65 and the cultivars Tracy-M, Crockett, Dowling, Hutcheson, MJ19RR, Hartwig, Pickett71, FT-2001, Peking. In addition 147 F_2 plants were obtained after crossing the contrasting parents FT-2001 (susceptible) and MJ19RR (resistant). The cross was performed in INTA Marcos Juarez in January 2015, 147 F_2 seeds were obtained from a single plant F_1.

Phenotypic screening

The toothpick method (9) was used to screen response to SSC under green-house conditions. For rating resistance of cultivars against SSC, a random blocks design was used with three replications of 15 plants each. In order to rate the 147 F_2 plants a complete randomized design was performed, while three replicates with 15 plants randomly distributed of Golondrina65 were included as positive control to the inoculation. Seven days after emergence, plants were inoculated with Dpm mycelium and kept at a 25-30°C temperature with 100% of relative humidity (RH) for 48 h. Subsequently, the plants were maintained in a greenhouse for 25 days before rate disease severity was recorded. A longitudinal section of the stem was taken to measure pathogen penetration into plant tissue (photo 1, page XXX). Genotype resistance was rated as the average value of the percentage of dead plants index (% DP) in three repli-cates using the Equation 1:

$$\%DP = \frac{(DP+\frac{IP}{2})100}{TP}*100 \quad (1)$$

where:
DP = number of dead plants
IP = number of infected plants
TP = total number of plants

Four levels of disease severity were established to rank cultivars response to Dpm, according to percentage of death plant index (% DP): resistant R = 0 to 14.9%, moderately susceptible MS = 15 to 49.9 %, susceptible S = 50 to 84.9% and highly susceptible HS = 85 to 100% (11), while the individuals of F_2 mapping populations were scored in two levels: as resistant when they did not developed disease symptoms, and susceptible when they showed disease symptoms (photo 1).

DNA extraction

DNA was purified from leaf tissue (15) and suspended in TE buffer (Tris-HCl 10 mM, EDTA 1 mM pH = 8), the concentration was determined by means of electrophoresis on 0.8% agarose gel and comparison with standard samples. For bulk segregant analysis, equimolar solutions were obtained from 15 resistant plants (resistant bulk: RB), and the 15 susceptible plants (susceptible bulk: SB).

PCR amplification

Genetic analysis was performed by PCR amplification of 84 SSR markers covering the 20 chromosome of the soybean genome (table 1, page XXX). The selection of the SSR was based on the location of the clusters of disease resistance genes previously reported (SoyBase, available in: http://www.soybase.com, accessed, September 2015).

Photo 1. Reaction of soybean genotypes 25 days after inoculation with *D. phaseolorum* var *meridionalis*. A and B, resistant reaction in MJ19RR; C and D, susceptible reaction in FT-2001, showing necrosis caused by fungal growth on hypocotyl plant.

Foto 1. Reacción de genotipos de soja luego de 25 días de la inoculación con *D. phaseolorum* var *meridionalis*. A y B, reacción de resistencia en MJ19RR. C y D, reacción susceptible en FT-2001, se observa necrosis causada por el hongo en el hipocotilo de las plántulas.

Table 1. Chromosome (Chr) and SSR markers used in the genetic characterization of the parents MJ19RR and FT-2001.

Tabla 1. Cromosomas (Chr) y marcadores SSRs usados en la caracterización de los parentales MJ19RR y FT-2001.

Chr	SSR markers
1	Sat_036; Satt184.
2	Sat_069; Satt005; Sat_135.
3	**Sat_125**; **Satt154**; Satt584; Satt393.
4	Sat_042; **Satt139.**
5	**Satt382**; Satt545; Satt174.
6	**Satt281**; **Satt079**; Satt202; Satt316; Satt371; **Satt307**; **Satt433**; S**att460**; Sct028; **Satt277**; Satt286; Satt319; Satt357.
7	Satt245; Satt590.
8	Satt409; Sat_162; Sat_157; Satt632.
9	Sat_020; Sat_119; **Satt518**; Satt337.
10	Sat_108; Satt445.
11	**Satt509**: **Satt415**; Satt332.
12	Sat_118; Satt541.
13	Satt030; **Satt516**; Satt334; Satt657.
14	Satt063; Satt168; Satt416.
15	Sat_107; Sat_112; **Satt384**; Satt602; Satt369.
16	Satt596; Satt244; Sat_396; Satt285; **Satt547**.
17	Sat_001; Satt458; Satt301; Satt574.
18	Satt131; **Satt309**; Satt288; Sat_141; Sat_163; Satt505; Satt012; Satt472; **Satt191**; Satt517; **Satt038**; **Satt130**; Satt610; Satt503.
19	**Satt182**; Satt462; Satt652.
20	**Satt440**; Satt127.

Bold letters indicate polymorphic markers among both parents.

Letras en negrita indican los marcadores polimórficos entre parentales.

SSR amplification was conducted with a GeneAmp PCR System 9700 (Applied Biosystems, Framingham, MA, USA), using a final volume of 15μl containing 50 ng of DNA of the resistant bulk, the susceptible bulk or the population parents, 1x GoTaq Buffer Green (1.5mM of Cl_2Mg), 0.2 mM of each dNTPs, 1U of GoTaq polymerase (PROMEGA, Madison, US), and 0.5 μm of each primer. Amplification conditions were as follows: 94°C for 120 s; 35 cycles of 92°C for 45 s; 47°C for 45 s; 68°C for 45 s; and 68°C for 60 s. PCR products were separated by electrophoresis on 12% polyacrylamide gels, stained with ethidium bromide solution (10 mg/ml) and visualized under UV light. The correct size of amplicons was analyzed by comparison with the reference genotype Williams82 (SoyBase, available in: http://www.soybase.com, accessed June 2016).

Linkage analysis

Linkage analysis was performed with 147 F_2 plants derived from the MJ19RR x FT-2001 cross and map construction were accomplished with GQMol software (4), using distance unit of Kosambi with 3.0 LOD score and maximum recombination distance of 50 cM. Graphics were obtained with GGT 2.0 software (21).

RESULTS

Fungal isolation

Plants from soybean fields putatively infected with Dpm were collected; out of 40 samples, 37 produced isolates fitting Dpm description. These were used to inoculate the susceptible control Golondrina65 in order to confirm their identity and measure their virulence on soybean. The isolates that produced % DP values from 80% to 100% were considered highly virulent. The isolate RSF12 obtained in Roman (29°30'49" S, 59°46'40" W), Santa Fe, Argentina, produced the highest % DP values and was selected for further analysis.

Reaction of MJ19RR to RSF12 isolate

The responses of susceptible control Golondrina65 and nine soybean cultivars (Tracy-M, Crockett, Dowling, Hutcheson, MJ19RR, Hartwig, Pickett71, FT-2001, Peking) to inoculation with RSF12 are presented in table 2 (page XXX). Eighty five plants of Golondrina65 in six replicates were inoculated, all of them presented typical SSC symptoms corresponding to a highly susceptible reaction (HS) with values % DP of 85.7 ± 4.5. A sub-set of four genotypes produced a susceptible reaction (S) in terms of % DP: Hartwig (69.3 ± 3.4%), Pickett71 (67.7 ± 4.2%), FT-2001 (67.3 ± 2.1%) and Peking (53.1 ± 1.6%), while Dowling, Crockett and Hutcheson produced a moderately susceptible reaction (MS) with % DP values of 23.4 ± 0.8%, 20.5 ± 1.1% and 15.6 ± 2.9%, respectively. On the other hand, Tracy-M and MJ19RR had a resistant reaction (R) with % DP values of 12.5 ± 2.5% and 2.4 ± 1.6%, respectively. It is noteworthy that the MJ19RR was the only genotype that presented no dead plants by inoculation with SSC.

Inheritance of the resistance

The phenotypic analysis of 147 F_2 plants by inoculation with Dpm isolate RSF12 produced 113 and 34 plants showing resistant and susceptible reactions, respectively. The chi-square value $\chi2 = 0.28 < 3.86$ at a $p \leq 0.05$ confirmed a 3:1 mendelian segregation that fitted in with a frequency of a single dominant gene (table 3, page XXX).

Table 2. Percentage of dead plant index (% DP) of soybean genotypes inoculated with isolate RSF12 of *Diaporthe phaseolorum* var. *meridionalis.*

Tabla 2. Porcentaje del índice de plantas muertas (% DP) de genotipos de soja inoculados con el aislado RS12 de *Diaporthe phaseolorum* var. *meridionalis.*

Cultivars	Number of Plants	% DP	Reaction (11)
Golondrina65[a]	85	85.7 ± 4.5	Highly Susceptible
Hartwig	41	69.3 ± 3.4	Susceptible
Pickett71	41	67.7 ± 4.2	Susceptible
FT-2001	45	67.3 ± 2.1	Susceptible
Peking	38	53.1 ± 1.6	Susceptible
Dowling	41	23.4 ± 0.8	Moderately Susceptible
Crockett	40	20.5 ± 1.1	Moderately Susceptible
Hutchenson	45	15.6 ± 2.9	Moderately Susceptible
Tracy–M	42	12.5 ± 2.5	Resistant
MJ19RR	40	2.4 ± 1.6	Resistant

[a] The susceptible control Golondrina65 was tested in six repetitions randomly distributed among the soybean cultivars and the 147 plants of the mapping population.

[a] El control susceptible Golondrina65 fue incluido en seis repeticiones distribuidas al azar entre los cultivares y las 147 plantas de la población de mapeo.

Table 3. Chi-square for the resistance *locus* Rdm_{MJ19RR} and the SSR markers in F_2 generation of MJ19RR x FT-2001 and its positions (MP) in the soybean molecular map (19).

Tabla 3. Chi-cuadrado para el *locus* de resistente Rdm_{MJ19RR} y los marcadores SSRs en la generación F_2 de MJ19RR x FT-2001 y sus posiciones (MP) en el mapa molecular de soja (19).

Locus	MP (cM)	Hypothesis	Expected	Observed	X^2
Satt079	117.8	1:2:1	36.75:73.50:36.75	39:66:42	1.66
Satt307	121.30	1:2:1	36.75:73.50:36.75	35:61:44	3.64
Satt433	128.30	1:2:1	36.75:73.50:36.75	39:82:26	4.26
Rdm_{MJ19RR}		3:1	110.25:36.75	113:34	0.28

Polymorphism detection

All the amplified fragments showed the expected size as reported for the reference genotype Williams 82. Out of all 84 SSR, 22 were polymorphic between the parent cultivars MJ19RR and FT-2001 (table 1, page XXX).

In order to detect the SSR which were near the region of resistance gene, we considered PCR sensitivity as reported for bulk segregant analysis (13). The markers Satt382 from Chr 5, Satt433 from Chr 6, Satt182 from Chr 19, and Satt152 from Chr 3 were selected as candidates considering low intensity amplification the resistant

allele in susceptible bulk (SB) as a sign of low recombination between the markers and the resistance gene.

Mapping SSC resistance in MJ19RR

Out of the four markers (Satt382, Satt433, Satt182 and Satt152) analyzed in the 34 susceptible F_2 plants, only in Satt433, the susceptible allele (a band of 200 bp), was observed in 26 of the 34 plants; whereas in the remaining eight plants, the resistant allele (290 bp) was amplified. These latter plants represented recombination events between the marker and the resistance gene (photo 2 and figure 2, page XXX).

Eleven additional SSR were selected from the Satt433 genomic region covering about 40 cM, five of which produced polymorphic bands in the parent genotypes (table 1, page XXX). Satt433, Satt079 and Satt307 were included in the analysis and the chi-square test confirmed the segregation of theses markers with mendelian ratio (table 3, page XXX). Using GQMOL the Satt433 marker was positioned at 25.1 cM from Satt307 and at 34.2 cM from Satt079, whereas resistance to SSC (Rdm_{MJ19RR}) was located at 13.3 cM from Satt433 (figure 1, page XXX). This region was not previously reported to be associated with SSC resistance.
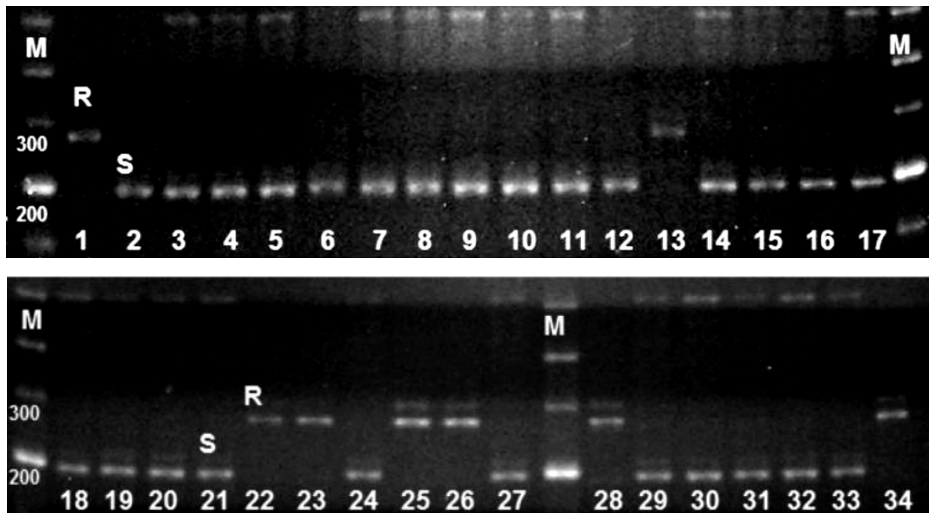
Photo 2. Satt433 amplification in 34 susceptible plants of mapping population. R, resistant allele of MJ19RR, S. susceptible allele of FT-2001. M, molecular weight marker.

Foto 2. Amplificación de Satt433 en las 34 plantas susceptibles de la población de mapeo. R, alelo resistente de MJ19RR; S, alelo susceptible de FT-2001. M, marcador de peso molecular.
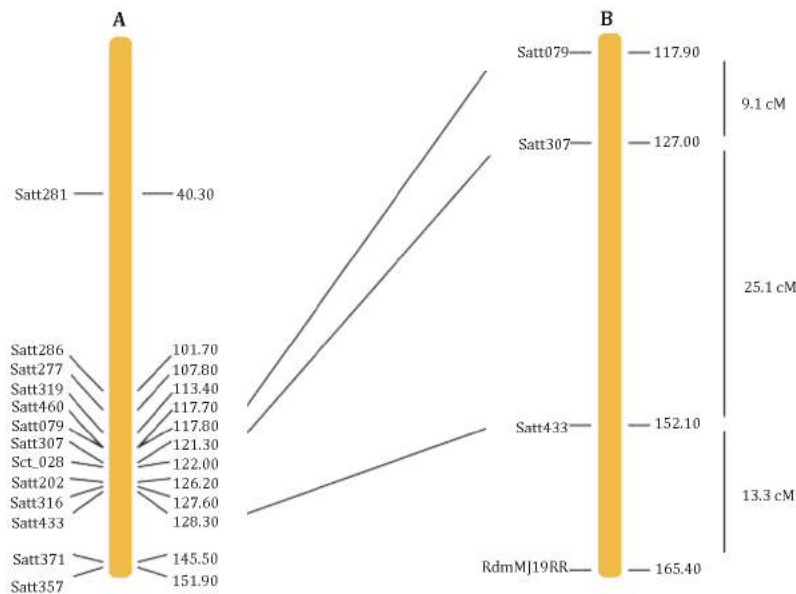
Figure 1. Section of chromosome 6 showing position of Rdm_{MJ19RR} locus. A, reference map (19). B, genetic map obtained in the present study.

Figura 1. Sección del cromosoma 6 donde se localiza el *locus Rdm$_{MJ19RR}$*. A, mapa de referencia (19). B, mapa genético obtenido en el presente trabajo.

The recombination in the 34 susceptible plants was evaluated with the Satt079, Satt307 and Satt433 markers. As shown in figure 2 (page XXX), recombination in all analyzed *locus* were observed. Satt371 and Satt357, located in the distal region of Chr 6, were monomorphic for MJ19RR and FT-2001 and it was not possible to analyze recombination at the distal end of this chromosome.

Resistance sources characterization

Nine soybean genotypes, among which are all known sources of SSC resistance, were analyzed with seven SSR markers of the Chr 6 region where Rdm_{MJ19RR} was mapped (figure 3, page XXX). The resistant allele from Satt433 was amplified in MJ19RR, Tracy M, Crockett and Dowling. The only resistant genotype that did not showed the resistance allele was Hutcheson. Amplifications of the *loci* Satt316 and Satt202 produced the same alleles in MJ19RR, Tracy-M, Crocket, Peking, FT-2001, Pickett71 and Hartwig, except Tracy-M and FT-2001 that showed different alleles for Satt316. With the Satt307 and Satt079 markers all genotypes amplified different alleles than MJ19RR, except Hutcheson and Dowling for the Satt079 *locus*. Analysis with Satt371 marker showed the same allele in MJ19RR, FT-2001, Pickett71 and Hartwig, while Satt357 amplified the same allele in all genotypes except in Hutcheson. Overall, these results indicate that there was no a clear relationship between the haplotypes studied.
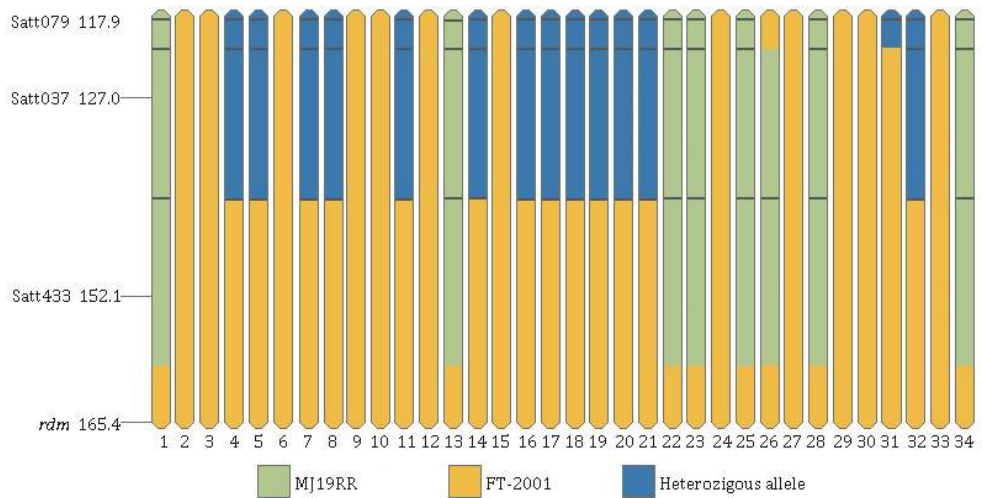
Figure 2. Recombination in distal region of chromosome 6 in 34 susceptible plants of mapping population. Orange fragments denote susceptible allele of FT-2001, green fragments denote the resistant allele of MJ19RR blue fragments, denote heterozygous regions.

Figura 2. Recombinación de la región distal del cromosoma 6 en las 34 plantas susceptibles de la población de mapeo. Los fragmentos naranjas indican el alelo susceptible de FT-2001, fragmentos verdes indican el alelo resistente de MJ19RR mientras que fragmentos azules indican regiones heterocigotas.
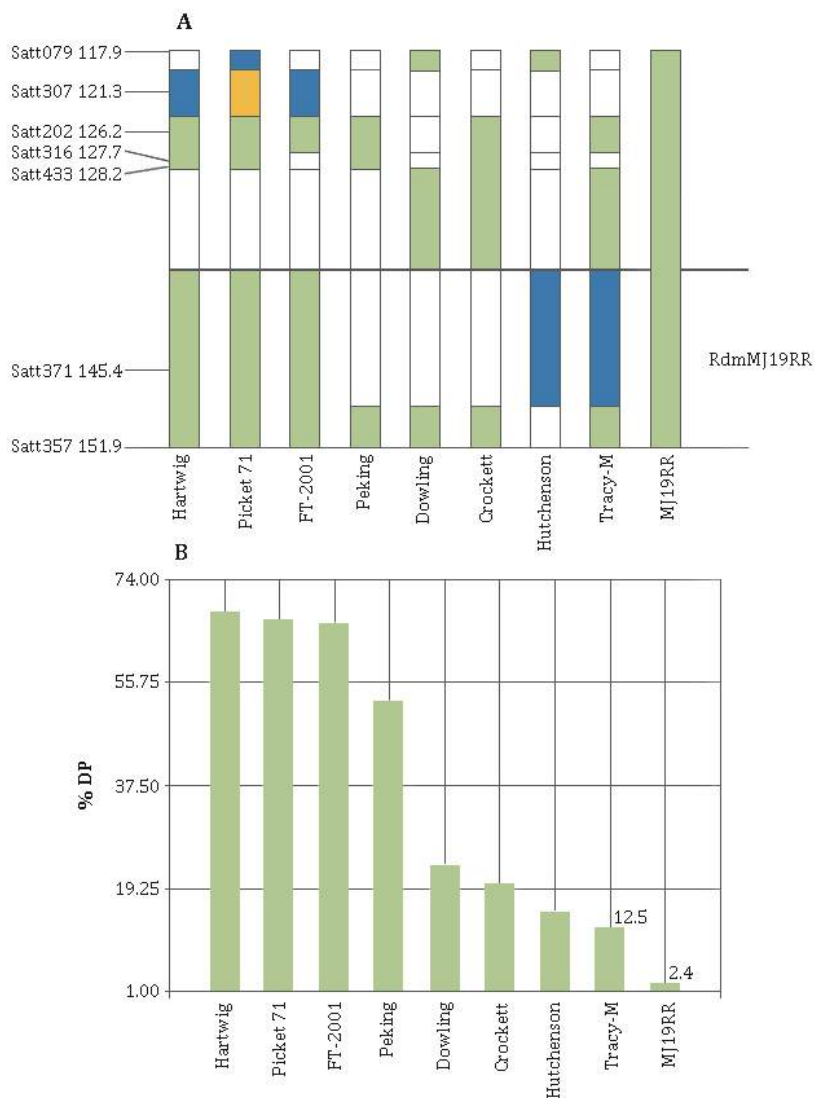
Figure 3. A: allelic combination of the distal end of chromosome 6 in all known Dpm resistance sources; green fragments denote presence of alleles resistant of MJ19RR. **B:** percentage of dead plant index (% DP) with response to Dpm (RS12) inoculation.

Figura 3. A: combinación alélica del extremo distal del cromosoma 6 en todas las fuentes de resistencia al Dpm conocidas; fragmento verde representa los alelos del genotipo resistente MJ19RR. **B:** Porcentaje del índice de plantas muertas (% DP) como respuesta a la inoculación con Dpm (RS12).

Discussion

In this paper we present results the first genomic approximation to stem canker resistance using the bulk segregant analysis. The selection of SSR candidates according to the intensity of the resistant allele amplification in the susceptible bulk was efficient to localize the genomic region associated with the resistance. This strategy allowed the identification of genomic region of interest, using few polymorphic molecular markers, a common situation when we used domestic parental for develop mapping population. When analyzing generation F_2 of the mapping population, we showed that the genetic resistance of MJ19RR was the result of a single dominant gene. In this case, the hypothesis was confirmed considering 113 F_2 plants as resistant, which did not show symptoms (immune), while the remaining 34 susceptible plants showed clearly identifiable symptoms throughout the hypocotyls (photo 1, page XXX). This stark contrast between resistant and susceptible plants in the mapping population leaves no doubt of the inheritance of this gene and demonstrates the consistency of the resistance reaction of MJ19RR against an aggressive isolate of Dpm.

The genotypic analysis located the resistance at Chr 6, linked to Satt433 marker at 13.3 cM (figure 1, page XXX). The recombination between Satt433 and Rdm_{MJ19RR} in eight susceptible plants suggested that resistance to SSC could be located in the distal region of Chr 6 (figure 2, page XXX). The lack of polymorphism between MJ19RR and FT-2001 at Satt371 and Satt357 prevented the recombination study in the distal end of chromosome.

This region on Chr 6 of the soybean map was previously reported as responsible for resistance to sudden-death syndrome (6, 8, 16), to *Phytophthora sojae* (12), to Asian soybean rust in cv. FT-2001 (18). Also, resistance to *Heterodera glycines* was mapped by (1, 22). These findings support the idea of a clustered location of resistance genes related to biotic stress, which is very valuable for breeding programs.

The presence of physiological breeds of Dpm that show a differential response to different *Rdm* genes was reported (17), who observed that there are very aggressive breeds in Argentina which are controlled only by the *Rdm1* gene, however lower % DP values were observed when this gene is accompanied by *Rdm2* in the Tracy-M genotype. In our research, the Tracy-M showed % DP values corresponding to the resistance reaction (R), but Dowling, Crocket and Hutchenson showed moderately susceptible (MS) responses (table 2, page XXX). This result suggests that RSF12 is a very aggressive isolate, because only is controlled by Tracy-M (*Rdm1/Rdm2*).

Although we are not aware of the resistance source from where MJ19RR originated, reaction similarities between MJ19RR and Tracy-M could indicate that both genotypes share a genetic base for Dpm resistance. Using SSR marker, we aligned the haplotypes of our parental genotypes with all the possible Dpm resistance sources known and their respective reactions to RSF12 isolation (figure 3, page XXX). The comparison of the genomic region from distal end of Chr 6 for genotypes MJ19RR and Tracy-M showed similarities for Satt357 *locus* amongst both genotypes. In this sense, Tracy-M could be the sole possible responsible for MJ19RR resistance, considering that all the sources of resistance was the tested in this experiment.

The *Rdm2* gene controls less aggressive breeds which are also controlled by other known genes (*Rdm1,3,4*) (17), while in our research the RSF12 variant was controlled only by Tracy-M genotype, a combination of *Rdm1* and *Rdm2* genes. This way if the resistance of MJ19RR derived of Tracy-M, then it should be through the *Rdm1* gene. Nevertheless, the agronomic difference of the % DP values between MJ19RR (2.4%) and Tracy-M (12.5 %), the lack of dead plants and the sole resistance gene in MJ19RR support the idea that these genotypes have different genetic basis for Dpm resistance.

Anyway our experiments were unable to determine if the MJ19RR response is product of *Rdm1* an allelic variation of this or a new gene of other locus.

Finally, in this paper we showed that resistance of MJ19RR control a very aggressive Dpm variant through a single gene, indicating that these genotype is a very important tool for genetics breeding programs.

Conclusions

The RSF12 was the most aggressive isolate while MJ19RR was the only genotype where no dead plant resulted from inoculations, this fact revealed to MJ19RR as a promising source of resistance to SSC.

The genetic resistance of MJ19RR to SSC is determined by a single gene, located at the distal end of chromosome 6 at 13.3 cM of the Satt433 marker.

This study determined that the agronomic reaction of the soybean genotype MJ19RR against the RSF12 isolate is different from the rest of the known resistance sources, this is a strong indicator of the presence of a new gene.

References

1. Arriagada, O.; Mora, F.; Dellarossa, J. C.; Ferreira, M. F.; Cervigni, G. D.; Schuster, I. 2012. Bayesian mapping of quantitative trait loci (QTL) controlling soybean cyst nematode resistant. Euphytica. 186: 907-917. DOI: 10.1007/s10681-012-0696-y.
2. Bowers, G. R.; Ngeleka, K.; Smith, O. D. 1993. Inheritance of stem canker resistance in soybean cultivars Crockett and Dowling. Crop Sci. 33: 67-70. DOI: 10.2135/cropsci1993.0011183X003300010010x.
3. Chiesa, M. A; Pioli, R. N.; Morandi, E. N. 2009. Specific resistance to soybean stem canker conferred by the Rdm4 *locus.* Plant Pathol. 58: 1032-1039. DOI: 10.1111/j.1365-3059.2009.02145.x.
4. Cruz, C. D.; Schuster, I. 2004. GQMOL-Aplicativo computacional para análise de dados moleculares e de suas associações com caracteres quantitativos. Universidad Federal do Viçosa. Viçosa. Brazil.
5. Fernández, A. F.; Hanlin, R. T. 1996. Morphological and RAPD analyses of *Diaporthe phaseolorum* form soybean. Micologia. 88: 425-440. DOI: 10.2307/3760884.
6. Iqbal, M. J.; Meksem, K.; Njiti, V. N.; Kassem, M. A.; Lightfoot, D. A. 2001. Microsatellite markers identify three additional quantitative trait loci for resistance to soybean sudden-death syndrome (SDS) in Essex × Forrest RILs. Theor. Appl. Genet. 102: 187-192. DOI: 10.1007/s001220051634.
7. Ivancovich, A. J.; Botta, G. L.; Annone, J. 1992. Aparición del cancro del tallo en cultivos de soja del área de la E.E.A. Pergamino. Información N°95. EEA INTA Pergamino.
8. Kazi, S.; Shultz, J.; Afzal, J.; Johnson, J.; Njiti, V. N.; Lightfoot, D. A. 2008. Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome. Theor. Appl. Genet. 116: 967-977. DOI: 10.1007/s00122-008-0728-0.

9. Keeling, B. L. 1982. A seedling test for resistance to soybean stem canker caused by *Diaporthe phaseolorum* var. caulivora. Phytopathology. 72: 807-809. DOI: 10.1094/Phyto-72-807.

10. Keeling, B. L. 1988. Influence of temperature on growth and pathogenicity of geographic isolates of *Diaporthe phaseolorum* var. caulivora. Plant Dis. 72: 220-222. DOI 10.1094/PD-72-0220.

11. Kilen, T. C.; Hartwig, E. E. 1987. Identification of single genes controlling resistance to stem canker in soybean. Crop Sci. 27: 863-864. DOI: 10.2135/cropsci1987.0011183X002700050005x.

12. Li, X.; Han, Y.; Teng, W.; Zhang, S.; Yu, K.; Poysa, V.; Li, W. 2010. Pyramided QTL underlying tolerance to Phytophthora root rot in mega-environments from soybean cultivars 'Conrad'and 'Hefeng 25'. Theor. Appl. Genet. 121: 651-658. DOI: 10.1007/s00122-010-1337-2.

13. Michelmore, R. W.; Paran, I.; Kesseli, R. V. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic region by using segregation population. P. Ntl. Acad. Sci. USA. 88: 9828-9832. DOI: 10.1073/pnas.88.21.9828.

14. Morgan-Jones, G. 1989. The *Diaporthe/Phomopsis* complex: Taxonomic considerations. Proceeding of the World Soybean Research Conference IV. Buenos Aires. Argentina. 1699-1706.

15. Murray, M. G.; Thompson, W. F. 1980. Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res. 8: 4321-4325. DOI: 10.1093/nar/8.19.4321.

16. Njiti, V. N.; Meksem, K.; Iqbal, M. J.; Johnnson, J. E.; Kassem, M. A.; Zobrist, K. F.; Kilo, V. Y.; Lightfoot, D. A. 2002. Common loci underlie field resistance to soybean sudden death syndrome in Forrest, Pyramid, Essex, and Douglas. Theor. Appl. Genet. 104: 294-300. DOI: 10.1007/s001220100682.

17. Pioli, N. R.; Morandi, E. N.; Martínez, M. C.; Lucca, F.; Tozzini, A.; Bizarro, V.; Hopp, H. E. 2003. Morphologic, molecular, and pathogenic characterization of *Diaporthe phaseolorum* variability in the core soybean-producing area of Argentina. Phytopathology. 93: 136-146. Available in: http://dx.doi.org/10.1094/PHYTO.2003.93.2.136.

18. Silva, D. G. C.; Yamanaka, N.; Polizel, A. M.; Brogin, R. L.; Pereira, S. S.; Nogueira, L. M.; Passianoto, A. L. L.; Catelli, L. L.; Arias, C. A. A.; Nepomuceno, A. L. 2006. Mapeo de genes de resistencia a roya asiática en soja. Resúmenes 3° Congreso de Soja del MERCOSUR. Rosario. Argentina.

19. Song, Q. J.; Marek, L. F.; Shoemaker, R. C.; Lark, K. G.; Concibido; V. C.; Delannay, X.; Specht, J. E.; Cregan, P. B. 2004. A new integrated genetic linkage map of the soybean. Theor. Appl. Genet. 109: 122-128. DOI: 10.1007/s00122-004-1602-3.

20. Tyler, J. M. 1996. Characterization of stem canker resistance in ¨Hutcheson¨ soybean. Crop Sci. 36: 591-593. DOI: 10.2135/cropsci1996.0011183X003600030011x.

21. Van Berloo, R. 2008. GGT2.0: versatile software for visualization and analysis of genetic data. J. Hered. 99: 232–236. DOI: 10.1093/jhered/esm109.

22. Yue, P.; Arelli, P. R.; Sleper, D. A. 2001. Molecular characterization of resistance to *Heterodera glycines* in soybean PI 438489B. Theor. Appl. Genet. 102: 921-928. DOI: 10.1007/s001220000453.