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Differential expression of distinct soybean resistance genes interacting with Argentinean isolates of *Diaporthe phaseolorum* var. *meridionalis*

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Abstract Soybean Stem Canker (SSC), caused by *Diaporthe phaseolorum* var. *meridionalis* (*Dpm*), is an important disease of soybean in Argentina. There are five known dominant genes that confer resistance to SSC, *Rdm1* to *Rdm5*. Particularly, *Rdm2* was identified in cv. Tracy-M and then it was stabilized in the breeding line T2. The *Rdm4* gene was first identified in cv. Hutcheson. More recently it was found that this gene was linked to the *Rdm5* gene, defining the *Rdm4-5* resistance region in Hutcheson. The objective of this work was to analyze the behaviour of the dominant *Rdm2, Rdm4* and *Rdm5* genes interacting with the CE109 and CE112 local physiological races of *Dpm*, in different susceptible backgrounds (genotypes RA702 and J77-339). *Rdm4* and *Rdm5* segregated phenotypically as completely dominant genes in the specific interactions with the CE109 and CE112 isolates, respectively, in both susceptible backgrounds. Similarly, *Rdm2* segregated as expected for a complete dominant gene in the specific interaction with the CE109 isolate, in both susceptible backgrounds. However, when interacting with the CE112 isolate, the *Rdm2* gene did not segregate as expected for a completely dominant gene, neither in RA702 nor in J77-339 susceptible background. The distorted segregation of the *Rdm2* gene was due to incomplete penetrance. To the best of our knowledge this is the first report documenting changes in the degree of penetrance of a soybean resistance gene (*Rdm2*) depending upon the physiological race of *Dpm* which interacts with and the genetic background in which the *Rdm* gene is being expressed.

Keywords *Glycine max* · Incomplete penetrance · *Rdm* resistance genes

Abbreviations

cv	Cultivar
DNA	Deoxyribonucleic acid
DP	Dead plants
Dpc	*Diaporthe phaseolorum* var. *caulivora*
dpi	Days post inoculation
Dpm	*Diaporthe phaseolorum* var. *meridionalis*
DPR	Disease progress rate
IP	Incubation period
R	Resistant
S	Susceptible
SSC	Soybean stem canker
SSR	Single sequence repeats

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Introduction

Soybean Stem Canker (SSC) is a very important disease of soybean and the characterization of genotypes as susceptible or resistant to SSC is mandatory to fulfil the requirements for registration of a new soybean cultivar in Argentina (www.inase.gov.ar).

The causal agent of SSC is the fungus *Diaporthe phaseolorum* (Cooke & Ellis) Sacc, anamorph *Phomopsis phaseoli* (Desmaz) Sacc. The *Diaporthe/Phomopsis* complex constitutes an important pathogenic group for soybean with ample genetic diversity (Morgan-Jones 1985). This complex is composed by three varieties of *Diaporthe phaseolorum*: *D. phaseolorum* var. *meridionalis* Fernández (*Dpm*), and *D. phaseolorum* var. *caulivora* K. L. Athow and R. M. Caldwell (*Dpc*), both causal agents of SSC, and *D. phaseolorum* var. *sojae* (S. G. Lehman) Wehmeyer, causal agent of pod and stem blight; and by *Phomopsis longicolla* T. W. Hobbs (teleomorph unknown), primary agent of seed decay (Sinclair 1999).

The two varieties of *D. phaseolorum* causing SSC (i.e. *Dpm* and *Dpc*) were established based on morphological, biological, and ecological differences, as well as physiological capacity to induce stem canker (Keeling 1988; Fernández and Hanlin 1996).

Pathogenic variability among isolates causing SSC was reported in USA (Keeling 1985, 1988) and also in the main soybean producing area of Argentina (Pioli et al. 1999, 2003). The inter- and intra-varietal genetic variability of *D. phaseolorum* may be explained by the fact that the natural infective propagule of this fungus is the sexual form, allowing genetic recombination (Fernández and Hanlin 1996; Sinclair and Hartman 1999). In Argentina, the SSC caused by *Dpm* was first detected in southern Santa Fe Province, during the 1992–93 growing season and then an outbreak of the disease was reported in this area and in the northwest of Argentina in 1996–97 (Pioli et al. 1997; Ploper et al. 1999).

Resistance to SSC is controlled by four major, dominant, non-allelic genes, formerly named *Rdc1*, *Rdc2*, *Rdc3* and *Rdc4*, after *Dpc* (Kilen and Hartwig 1987; Bowers et al. 1993). However, the identification of these resistance genes was done with southern U.S. isolates of *D. phaseolorum*, later named var. *meridionalis.* Moreover, results in the literature demonstrated that genes for resistance to *Dpm* do not confer resistance to *Dpc* (Keeling 1985, 1988; Higley and Tachibana 1987; Kulik 1989; Pioli et al. 2003). Thus, to avoid misleading information in breeding for SSC resistance, it was proposed to rename these four major genes, conferring resistance to *Dpm*, as *Rdm1*, *Rdm2*, *Rdm3*, and *Rdm4* (Pioli et al. 2003).

Particularly, the *Rdm2* gene was identified and characterized, with the *Rdm1* gene, in the cultivar Tracy-M and then it was separated and stabilized in an experimental line (Kilen et al. 1985). The *Rdm4* gene was first identified in the cv. Dowling (Bowers et al. 1993) and an allele of this gene was characterized in the cv. Hutcheson (Tyler 1996).

In a recent report, using classical genetics and phenotypic analysis with two local physiological races of *Dpm*, a new *Rdm* gene, linked to *Rdm4,* and tentatively named *Rdm5,* was identified in cv. Hutcheson. This finding revealed the existence of a complex genomic region in cv. Hutcheson containing at least two resistance genes: the previously characterized *Rdm4* and the novel *Rdm5* gene, conferring specific resistance to CE109 and CE112 physiological races of the *Dpm*, respectively (Chiesa et al. 2009). In addition, at least four different local physiological races of *Dpm* were differentiated in the core soybean-producing area of Argentina (Pioli et al. 2003). Among them, isolate CE109 was avirulent to *Rdm1-Rdm4* genes while isolate CE112 was avirulent to *Rdm3* and *Rdm5* genes but moderately virulent to *Rdm1* and *Rdm2* genes and virulent to the *Rdm4* gene. Both isolates were highly and moderately virulent to the susceptible genotypes RA702 and J77-339, respectively (Pioli et al. 2003; Chiesa et al. 2009).

The objectives of this work were to perform a comprehensive analysis of the resistance conferred by *Rdm2*, *Rdm4* and the recently reported *Rdm5* genes, in the specific interaction with two previously characterized local physiological races of *Dpm*, segregating in two different susceptible genetic backgrounds. Evidence will be presented for incomplete penetrance and expressivity of the *Rdm2* gene interacting specifically with the CE112 isolate of *Dpm*.

Material and methods

Plant material and growing conditions

Four soybean genotypes were used: the cv. RA702 (*rdm/rdm*) and the line J77-339 (*rdm/rdm*), both as

female susceptible parents; and the line T2 (*Rdm2/Rdm2*) and the cv. Hutcheson (*Rdm4/Rdm4*; *Rdm5/Rdm5*), both as male resistant parents. The hybrid origin of the F_1 plants, obtained from the crosses RA702 × T2 and J77-339 × T2, as well as from the crosses RA702 × Hutcheson and J77-339 × Hutcheson, was confirmed by assaying a group of Single Sequence Repeats (SSR) molecular markers (Cregan et al. 1999), polymorphic between the susceptible and resistant genotypes, in each particular cross (Chiesa 2009). The parental genotypes, the crosses, the number of F_2 plants inoculated and the local isolates of *Dpm* tested are detailed in Table 1. Plants were grown in 8-cm diameter plastic pots, filled with a sterilized mix of humus-rich soil/perlite (3:1 v/v). Pots were maintained at field capacity by sub-irrigation throughout the experiment. The experiment was done in an environmentally controlled greenhouse with mean temperatures of 28±3 °C (day) and 18±3 °C (night) and mean photosynthetic active photon flux density of 500 μE $m^{-2}s^{-1}$ (400–700 nm), measured with a LI-COR 185a radiometer and 190 s sensor (LI-COR Ltd, Lincoln, Nebraska, USA).

Fungal mycelium and inoculation procedure

Dpm isolates CE109 and CE112 were collected from symptomatic plants with SSC in two different localities of Santa Fe Province, Argentina, in 1997/98. The isolates were morphological and molecularly characterized according to Fernández and Hanlin (1996), and deposited in the Centro de Referencia en Micología (CEREMIC), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina (Pioli et al. 2003). For this study, monothallic mycelium of the CE109 and CE112 isolates were recovered by hyphal-tipping from their respective cultures deposited in CEREMIC. Mycelia were first inoculated to the highly susceptible soybean genotype RA702 to test their pathogenicity. Isolates were recovered from the first internode above the inoculation point of five randomly chosen symptomatic plants. The

Table 1 Parental genotypes, crosses, and F_2 populations segregating for distinct Soybean Stem Canker resistance genes (*Rdm*) in diverse genetic backgrounds and *Diaporthe phaseolorum* var. *meridionalis* (*Dpm*) isolates tested

Parental genotypes[a]	Population	Nº of F_2 plants	Block[b]	Nº of F_2 plants	*Dpm* isolate[c]
RA702 × T2	RA02a	184	RA02a-1	83	CE109
			RA02a-2	101	CE109
J77-339 × T2	J02a	168	J02a-1	84	CE109
			J02a-2	84	CE109
RA702 × Hut.	RA045-a[d]	143	RA045-a1	73	CE109
			RA045-a2	70	CE109
J77-339 × Hut.	J045-a[e]	120	J045-a1	60	CE109
			J045-a2	60	CE109
RA702 × T2	RA02b	189	RA02b-1	95	CE112
			RA02b-2	94	CE112
J77-339 × T2	J02b	165	J02b-1	89	CE112
			J02b-2	76	CE112
RA702 × Hut.	RA045-b[d]	157	RA045-b1	78	CE112
			RA045-b2	79	CE112
J77-339 × Hut.	J045-b [e]	151	J045-b1	74	CE112
			J045-b2	77	CE112

[a] RA702 and J77-339 are susceptible parents (*rdm/rdm*); T2 (*Rdm2/Rdm2*) and Hutcheson (Hut.) (*Rdm4/Rdm4*; *Rdm5/Rdm5*) are resistant parents

[b] F_2 populations were randomly divided into block -1 and -2

[c] The inoculations with *Dpm* isolates, CE109 or CE112, respectively, were performed independently and simultaneously

[d] RA045-a, and RA045-b, are two subgroups of the same population (RA045; n=300), randomly formed

[e] J045-a, and J045-b, are two subgroups of the same population (J045; n=271), randomly formed

infected portion of the stem was superficially disinfected with 10 g l^{-1} sodium hypochlorite for 1 min, rinsed with sterilized water, dried on blotting paper and placed in Petri dishes with potato glucose agar (20 g l^{-1}) medium, acidified with lactic acid 2 ml l^{-1} (Roy and Miller 1983; Pioli et al. 2003). Petri dishes were incubated for 7 days, at 27±1 °C, with a 12 h of near UV light and 12 h of darkness. Before utilizing mycelia in the inoculations, the identity of re-isolated pathogens was checked further by the induction of sexual and asexual fructifications (Pioli et al. 1997). At the fully expanded trifoliate leaf stage (12–15 days old) seedling hypocotyl was wounded by cutting a thin portion of the external cellular layer of the stem with a sterile scalpel. The cut was made parallel to the hypocotyls axis, from top to bottom, and the bottom part of the sliced portion remained attached to the stem. A fragment of approximately 1.5×1.5 mm of mycelium was inserted into the wound and immediately covered with vaseline to avoid dehydration. Control seedlings were similarly wounded and covered with vaseline, but no mycelium was applied. During the first 72 h after inoculation seedlings were kept in high relative humidity (90–100 %) by covering them with a transparent polyethylene tent (Pioli et al. 2003). The inoculations with *Dpm* isolates, CE109 or CE112, were performed independently.

Disease evaluation

Plant-pathogen interactions were evaluated weekly from 7 days post inoculation (dpi). The progress of the disease during the experimental period was assessed using a rating scale with four qualitative, arbitrary categories. A value of 0 was assigned to plants without SSC symptoms, 0.3 to plants presenting only foliar chlorosis symptoms or a small bright canker, 0.6 to plants displaying symptoms of foliar necrosis and dark stem canker and 1 to dead plants (DP) (Pioli et al. 2003). The total period of evaluation was 35–50 dpi, depending on the disease progress rate. At the end of the evaluation period, the alive and healthy F_2 individuals of each inoculated population were qualitatively classified as resistant (R) meanwhile the dead F_2 plants were classified as susceptible (S), in order to determine the phenotypic ratio of R or S plants, respectively. The parental genotypes were classified as resistant (≤20 % DP) or susceptible (≥70 % DP), according to the scale described in Chiesa et al. (2009). Hybrid (F_1) plants from the crosses J77-339 × T2 and J77-339 × Hutcheson were inoculated to corroborate the dominance of the resistance genes, the phenotypic response and the progress of the disease in heterozygous individuals.

Disease progress curve and disease progress rate

For each population, the averaged disease progress curve was obtained for the groups of R and S individuals. The disease progress curve was obtained as the cumulative percentage of DP (%DP) on time. The disease progress rate (DPR, %DP day^{-1}) was then estimated as the slope of the regression of %DP on time. The DPR was utilized to quantify the speed of the disease increase of different *Rdm-Dpm* interactions in different genetic backgrounds. The ratio between DPRs of groups of S and R individuals was used to compare differences in the magnitude of disease progress among S and R individuals. The duration of the incubation period (IP, days) was estimated, for the compatible interactions, dividing the origin ordinate of the regression function by the DPR (Agrios 2005).

Calculation of the *Rdm* genes penetrance and expressivity

The penetrance of the *Rdm* genes was calculated as the number of individuals phenotypically classified as resistant out of the total number of individuals carrying at least one dominant allele of the *Rdm* gene, when inoculated with each analyzed local isolate of *Dpm*. The expressivity of the resistance, for R individuals, was quantified from the DPR, with higher DPR meaning lower expressivity (Pierce 2010).

Statistical analysis

For the analysis of each *Rdm-Dpm* interaction, in both susceptible backgrounds, a randomized block design with two blocks was used for each F_2 population, to take into account for possible environmental variations within the greenhouse (Table 1). To test differences between blocks, the homogeneity test was performed (Snedecor and Cochran 1989). Chi-square, χ^2, goodness of fit test was performed to check the hypothesis of Mendelian inheritance of the resistance (Lacadena 1988). All statistical analysis was done by using InfoStat Student Version software.

Results

Analysis of the *Rdm* genes in the specific interaction with the CE109 isolate of *Dpm*

The disease reaction to the CE109 isolate showed that the susceptible (*rdm/rdm*) parental genotype RA702 presented 17 dead plants out of 17 inoculated plants (100 %DP) and the line J77-339 presented 12 dead plants out of 17 inoculated plants (71 %DP), whilst the resistant genotype T2 (*Rdm2/Rdm2*) showed three dead plants out of 17 inoculated plants (18 %DP) and the cv. Hutcheson (*Rdm4/Rdm4; Rdm5/Rdm5*) showed no dead plants (0 %DP) (Table 2). In addition, five hybrid plants from the cross J77-339 × T2 (F_1, *Rdm2/rdm2*), inoculated with the CE109 isolate, showed one dead plant out of five inoculated plants (20 %DP), matching the resistant T2 parent phenotype. Also, the five hybrid plants from the cross J77-339 × Hutcheson (F_1, *Rdm4/rdm4*) inoculated with the CE109 isolate remained symptomless (0 %DP), matching the resistant Hutcheson parent phenotype (Table 2). In all described interactions, the disease reaction of the parental genotypes was indicative of the isolate virulence and effectiveness of the inoculation technique.

Table 2 Phenotypic reaction of soybean parental lines and their F_1 and F_2 progenies in different segregating populations derived from the crosses RA702 × T2; J77-339 × T2; RA702 × Hutcheson and J77-339 × Hutcheson in the specific interaction with the CE109 isolate of *Diaporthe phaseolorum* var. *meridionalis*. Plant-pathogen interaction was measured 35–50 days post inoculation, and the disease reaction was scored as described in Materials and methods

Parental lines & populations	Nº of plants inoculated	Disease reaction (R: S)[a]		χ^2	P
		Expected[b]	Observed		
RA702 (*rdm/rdm*)	17		0: 17		
J77-339 (*rdm/rdm*)	17		5: 12		
T2 (*Rdm2/Rdm2*)	17		14: 3		
Hutcheson (*Rdm4/Rdm4, Rdm5/Rdm5*)	17		17: 0		
F_1 (*Rdm2/rdm2*) [c]	5		4: 1		
F_1 (*Rdm4/rdm4*) [d]	5		5: 0		
Rdm2 segregation					
RA702 × T2					
F_2 - RA02a-1	83	62: 21	55: 28	3.12 ns	0.08
F_2 - RA02a-2	101	76: 25	75: 26	0.05 ns	0.82
J77-339 × T2					
F_2 - J02a-1	84	63: 21	57: 27	2.28 ns	0.13
F_2 - J02a-2	84	63: 21	55: 29	4.07 ns	0.04
Rdm4 segregation					
RA702 × Hutcheson					
F_2 - RA045-a1	73	55: 18	61: 12	2.66 ns	0.10
F_2 - RA045-a2	70	52.5: 17.5	60: 10	4.27 ns	0.04
J77-339 × Hutcheson					
F_2 - J045-a1	60	45: 15	46: 14	0.09 ns	0.77
F_2 - J045-a2	60	45: 15	49: 11	1.46 ns	0.23

[a] Resistant (R) and susceptible (S) are healthy and dead plants, respectively, determined at the end of the inoculation experiment

[b] Expected phenotypic segregation in F_2 generation (3R: 1S)

[c] Hybrid plants from the cross J77-339 × T2

[d] Hybrid plants from the cross J77-339 × Hutcheson

P probability

ns not significant differences, $P \geq 0.01$

In the specific interaction T2 (*Rdm2/Rdm2*)-CE109, the *Rdm2* gene presented incomplete penetrance (80 %), since some individuals carrying this complete dominant gene were phenotypically susceptible. This degree of incomplete penetrance was also observed in the hybrid individuals (*Rdm2/rdm2*). Conversely, in the specific interaction Hutcheson-CE109, the *Rdm4* gene presented complete penetrance (100 %), both in the parental genotype (*Rdm4/Rdm4*) and in the hybrid individuals (*Rdm4/rdm4*) (calculated from data of Table 2).

Rdm2 segregation in the specific interaction with the CE109 isolate in different susceptible backgrounds

In the RA702 susceptible background, the 83 F_2 plants of the RA02a-1 block segregated 55R: 28S (χ^2=3.12; P=0.08) and the 101 F_2 plants of the RA02a-2 block segregated 75R: 26S (χ^2=0.05; P=0.82) (Table 2). The χ^2 value for the homogeneity test between blocks was 1.37; P=0.25, indicating that the frequency counts were distributed identically across the different blocks. Therefore, the data from both blocks was pooled and the obtained χ^2 for the segregation of the whole population was 1.86 (P=0.17). Thus, the F_2 analysis showed that the *Rdm2* gene in the RA702 genetic background, in the specific interaction with the isolate CE109 of *Dpm*, segregated in a 3R: 1S phenotypic ratio characteristic of a single gene with complete dominance.

In the J77-339 susceptible background, the 84 F_2 plants of the J02a-1 block segregated 57R: 27S (χ^2=2.28; P=0.13) and the 84 F_2 plants of J02a-2 block segregated 55R: 29S (χ^2=4.07; P=0.04) (Table 2). The χ^2 value for the homogeneity test between both blocks was 0.11; P=0.75, indicating that the frequency counts were distributed identically across the different blocks. Thus, the data from both blocks was pooled and the obtained χ^2 for the segregation of the whole population was 6.23 (P=0.02). As a result, the F_2 analysis showed that the *Rdm2* gene in the J77-339 genetic background, in the specific interaction with the isolate CE109 of *Dpm*, segregated in a 3R: 1S phenotypic ratio characteristic of a single gene with complete dominance.

Consequently, the observed phenotypic segregation of the *Rdm2* gene, in the specific interaction with the CE109 isolate of *Dpm*, fitted to the expected segregation of a simple inherited, complete dominant gene in both susceptible genetic backgrounds (cv. RA702 and line J77-339).

Rdm4 segregation in the specific interaction with the CE109 isolate in different susceptible backgrounds

In the RA702 susceptible background, the 73 F_2 plants of the RA045-a1 block segregated 61R: 12S (χ^2=2.66; P=0.10) and the 70 F_2 plants of the RA045-a2 block segregated 60R: 10S (χ^2=4.27; P=0.04) (Table 2). The χ^2 value for the homogeneity test between blocks was 0.13; P=0.78, indicating that the frequency counts are distributed identically across the different blocks. Therefore, the data from both blocks was pooled and the obtained χ^2 for the segregation of the whole population was 7.27 (P=0.01). The results of the F_2 analysis showed that the *Rdm4* gene in the RA702 genetic background, in the specific interaction with the isolate CE109 of *Dpm*, segregated in a 3R: 1S phenotypic ratio characteristic of a single gene with complete dominance.

In the J77-339 susceptible background, the 60 F_2 plants of the J045-a1 block segregated 46R: 14S (χ^2=0.09; P=0.77) and the 60 F_2 plants of the J045-a2 block segregated 49R: 11S (χ^2=1.46; P=0.23) (Table 2). The χ^2 value for the homogeneity test between blocks was 0.45; P=0.50, indicating that the frequency counts were distributed identically across the different blocks. Thus, the data from both blocks was pooled and the obtained χ^2 for the segregation of the whole population was 1.11 (P=0.30), indicating that the *Rdm4* gene in the J77-339 genetic background, in the specific interaction with the isolate CE109 of *Dpm*, segregated in a 3R: 1S phenotypic ratio characteristic of a single gene with complete dominance.

The observed phenotypic segregation of the *Rdm4* resistance gene from cv. Hutcheson, in the specific interaction with the CE109 isolate of *Dpm*, was in agreement with the expected segregation of a simple inherited and complete dominant gene in the two susceptible genetic backgrounds, the cv. RA702 and the line J77-339.

Analysis of the *Rdm* genes in the specific interaction with the CE112 isolate of *Dpm*

The disease reactions to the CE112 isolate showed that the susceptible parental genotype RA702 presented 17

dead plants out of 18 inoculated plants (94 %DP) and J77-339 presented 13 dead plants out of 18 inoculated plants (72.2 %DP). However, when the resistant parents were evaluated, the disease reactions of the T2 line to the CE112 isolate showed 10 dead plants out of 18 inoculated plants (56 %DP) whilst cv. Hutcheson remained symptomless (0 %DP) (Table 3).

In the specific interaction T2-CE112, the *Rdm2* gene presented incomplete penetrance (44 %) since individuals carrying the *Rdm2/Rdm2* genotype were phenotypically susceptible. The reduced penetrance observed in the T2-CE112 interaction confirmed a previous report in which the T2 genotype was classified as moderately susceptible to the isolate CE112 of *Dpm* (Pioli et al. 2003). While, in the specific interaction Hutcheson-CE112, the *Rdm5* gene presented complete penetrance (100 %) against the CE112 isolate of *Dpm*.

Rdm2 segregation in the specific interaction with the CE112 isolate in different susceptible backgrounds

In the RA702 susceptible background, the 95 F_2 plants of the RA02b-1 block segregated 20R: 75S (χ^2=144.98; P<0.01) and the 94 F_2 plants of the RA02b-2 block segregated 17R: 77S (χ^2=162.40; P<0.01) (Table 3). The χ^2 value for the homogeneity test between blocks was 0.26; P=0.65, indicating that the frequency counts were distributed identically across the two blocks. When the F_2 data from both blocks was pooled the obtained χ^2 for the segregation of the whole population was χ^2= 359.02 (P<0.01). The segregation ratio of F_2 individuals of population RA02b (RA702 × T2) inoculated with the isolate CE112 of *Dpm*, did not fit to the 3R: 1S ratio expected for a complete dominant gene. Moreover, in the J77-339 susceptible background, the 89 F_2 plants of the J02b-1 block segregated 25R: 64S (χ^2=106.50; P<0.01) and the 76 F_2 plants of J02b-2 block segregated 19R: 57S (χ^2=101.33; P<0.01) (Table 3). The χ^2 value for the homogeneity test between blocks was 0.21; P=0.67, indicating that the frequency counts were distributed identically across the different blocks. As well, when the F_2 data from both blocks was pooled the obtained χ^2 for the segregation of the whole population was 207.71 (P<0.01). Consequently, the results indicated that in two different susceptible backgrounds, RA702 and J77-339, the segregation of F_2 individuals inoculated with the CE112 isolate

Table 3 Phenotypic reaction of soybean parental lines and their F_2 progenies in different segregating populations derived from the crosses RA702 × T2; J77-339 × T2; RA702 × Hutcheson and J77-339 × Hutcheson in the specific interaction with the CE112 isolate of *Diaporthe phaseolorum* var. *meridionalis*. Plant-pathogen interaction was measured 35–50 days post inoculations, and the disease reaction was scored as described in Materials and methods

Parental lines & populations	Nº of plants inoculated	Disease reaction (R: S)[a]		χ^2	P
		Expected[b]	Observed		
RA702 (*rdm/rdm*)	18		1: 17		
J77-339 (*rdm/rdm*)	18		5: 13		
T2 (*Rdm2/Rdm2*)	18		8: 10		
Hutcheson (*Rdm4/Rdm4, Rdm5/Rdm5*)	18		18: 0		
Rdm2 segregation					
RA702 × T2					
F_2 -RA02b-1	95	71: 24	20: 75	144.98 sd	< 0.01
F_2 -RA02b-2	94	70.5: 23.5	17: 77	162.40 sd	< 0.01
J77-339 × T2					
F_2 -J02b-1	89	67: 22	25: 64	106.50 sd	< 0.01
F_2 -J02b-2	76	57: 19	19: 57	101.33 sd	< 0.01
Rdm5 segregation					
RA702 × Hutcheson					
F_2 - RA045-b1	78	58.5: 19.5	59: 19	0.02 ns	0.90
F_2 - RA045-b2	79	59: 20	61: 18	0.27 ns	0.61
J77-339 × Hutcheson					
F_2 - J045-b1	74	55.5: 18.5	57: 17	0.16 ns	0.69
F_2 - J045-b2	77	58: 19	60: 17	0.28 ns	0.60

[a]Resistant (R) and susceptible (S) are healthy and dead plants, respectively, determined at the end of the inoculation experiment

[b]Expected phenotypic segregation for a dominant gene in F_2 generation (3R: 1S)

P probability

sd and ns significant and not significant differences, P<0.01 and P≥0.01, respectively

of *Dpm*, did not fit to the 3R: 1S ratio expected for a complete dominant gene.

Rdm5 segregation in the specific interaction with the CE112 isolate in different susceptible backgrounds

In the RA702 susceptible background, the 78 F_2 plants of the RA045-b1 block segregated 59R: 19S (χ^2= 0.02; P=0.90) and the 79 F_2 plants of the RA045-b2 block segregated 61R: 18S (χ^2=0.27; P=0.61) (Table 3). The χ^2 value for the homogeneity test between blocks was 0.05; P=0.80, indicating that the frequency counts were distributed identically across the different blocks. Therefore, when the data from both blocks was pooled the obtained χ^2 for the segregation of the whole population was 0.17 (P=0.71). Thus, the results of the F_2 analysis showed that the *Rdm5* gene in the RA702 genetic background, in the specific interaction with the isolate CE112 of *Dpm*, segregated in a 3R: 1S phenotypic ratio characteristic of a single gene with complete dominance.

In the J77-339 susceptible background, the 74 F_2 plants of the J045-b1 block segregated 57R: 17S (χ^2= 0.16; P=0.69) and the 77 F_2 plants of the J045-b2 block segregated 60R: 17S (χ^2=0.28; P=0.60) (Table 3). The χ^2 value for the homogeneity test between blocks was 0.01; P=0.90, indicating that the frequency counts were distributed identically across the two blocks. Thus, when the data from both blocks was pooled the obtained χ^2 for the segregation of the whole population was 0.56 (P=0.45), indicating that the *Rdm5* gene from cv. Hutcheson, in the J77-339 genetic background and in the specific interaction with the isolate CE112 of *Dpm*, segregated in a 3R: 1S phenotypic ratio characteristic of a single gene with complete dominance.

Consequently, it was verified that the phenotypic segregation of the *Rdm5* gene from cv. Hutcheson, in the specific interaction with the CE112 isolate of *Dpm*, fitted the expected segregation for a complete dominant gene in the two susceptible backgrounds, the cv. RA702 and the line J77-339 (Table 3).

Disease progress rate for interactions with the CE109 isolate of *Dpm*

When the parental genotypes were evaluated in the specific interaction with CE109 isolate, the DPR for the susceptible parents (*rdm/rdm*) RA702 and J77-339, was 2.9 and 2.1 %DP day^{-1}, respectively; whilst the DPR for the resistant parent T2 (*Rdm2/Rdm2*) as well as

Table 4 Disease Progress Rate (DPR) obtained from the disease progress curves of each *Rdm-Dpm* specific interaction for the parental genotypes, the F_1 and the R and S groups of F_2 individuals of each population

Parental genotypes & hybrids (F_1)	DPR (%DP.d^{-1})[a]	
	Isolate of *Dpm* tested	
	CE109	CE112
RA702	2.9	3.1
J77-339	2.1	2.1
T2	0.5	1.6
F_1 (*Rdm2*/*rdm2*)	0.5	na
Hutcheson	0.0	0.2
F_1 (*Rdm4*/*rdm4;Rdm5*/*rdm5*)	0.04	na
Rdm2 segregant populations (F_2)		
RA02a (S)[b]	3.3	–
RA02a (R)[c]	0.1	–
J02a (S)	2.7	–
J02a (R)	0.1	–
RA02b (S)	–	2.9
RA02b (R)	–	0.5
J02b (S)	–	2.6
J02b (R)	–	0.5
Rdm4 segregant populations (F_2)		
RA045-a (S)	2.3	–
RA045-a (R)	0.1	–
J045-a (S)	2.3	–
J045-a (R)	0.1	–
Rdm5 segregant populations (F_2)		
RA045-b (S)	–	2.8
RA045-b (R)	–	0.1
J045-b (S)	–	2.6
J045-b (R)	–	0.1

[a] The DPR was estimated as the slope of the regression of cumulative percentage of dead plants (%DP) on time, during the analyzed period. The R^2 value of regressions ranged from 0.80 to 0.99

[b] (S): Susceptible individuals from the crosses described in Table 1, grouped at the end of the inoculation assay based on the 3R: 1S or 1R: 3S segregation ratios

[c] (R): Resistant individuals from the crosses described in Table 1, grouped at the end of the inoculation assay based on the 3R: 1S or 1R: 3S segregation ratios

na: F_1 not available

for the hybrids (*Rdm2/rdm2*) was 0.5 %DP day^{-1} (Table 4). Thus, the progress of the disease was 5.8 and 4.2 times faster in the susceptible RA702 and J77-339 genotypes, respectively, than in the resistant T2 and hybrid genotypes. On the other side, the DPR for the resistant parent Hutcheson (*Rdm4/Rdm4*) and for the hybrid individuals (*Rdm4/rdm4*) was 0.0 %DP day^{-1} and 0.04 %DP day^{-1}, respectively (Table 4). Accordingly, the progress of the disease was 72.5 and 52.5 times faster in susceptible RA702 and J77-339 genotypes, respectively, than in the hybrids individuals from the cross J77-339 × Hutcheson (Table 4).

For the F_2 individuals of the *Rdm2*-segregant population RA02a, the DPR of the individuals phenotypically characterized as S and R was 3.3 and 0.1 %DP day^{-1}, respectively (Table 4). Therefore, for this specific interaction, the progress of the disease was 33 times faster in S than in R individuals. For the F_2 individuals of the *Rdm2*-segregant population J02a, the DPR of the individuals phenotypically characterized as S and R was 2.7 and 0.1 %DP day^{-1}, respectively (Table 4). As a result, in this specific interaction, the progress of the disease was 27 times faster in S than in R plants.

For the F_2 individuals of the *Rdm4*-segregant population RA045-a, the DPR of the individuals phenotypically characterized as S and R was 2.3 and 0.1 %DP day^{-1}, respectively (Table 4). Also, for the F_2 individuals of the *Rdm4*-segregant population J045-a, the DPR of the individuals phenotypically characterized as S and R was 2.3 and 0.1 %DP day^{-1}, respectively (Table 4). Therefore, the progress of the disease in *Rdm4*-segregant populations RA045-a and J045-a, in the specific interaction with CE109 isolate of *Dpm*, was 23 times faster in S than in R plants.

Disease progress rate for interactions with the CE112 isolate of *Dpm*

When the parental genotypes were evaluated in the specific interaction with CE112 isolate, the DPR was 3.1 and 2.1 %DP day^{-1} for the susceptible parents RA702 and J77-339, respectively; whilst the DPR was 1.6 and 0.2 %DP day^{-1}, for the T2 and Hutcheson parents, respectively (Table 4). Thus, the progress of the disease was 16 and 11 times faster in RA702 and J77-339 susceptible genotypes, respectively, than in cv. Hutcheson. However, the progress of the disease was only 1.9 and 1.3 times faster in RA702 and J77-339, respectively, than in the T2 genotype. In addition, the progress of the disease was 8 times faster in the moderately susceptible parent T2 compared to the resistant parent Hutcheson, confirming the incomplete penetrance of the *Rdm2* gene when interacting with the CE112 isolate.

For the F_2 individuals of the *Rdm2*-segregant population RA02b, the DPR of the individuals phenotypically characterized as S and R was 2.9 and 0.5 %DP day^{-1}, respectively (Table 4). Therefore, for this specific interaction, the progress of the disease was 5.8 times faster in S than in R plants. In the *Rdm2*-segregant population J02b, the DPR of the F_2 individuals phenotypically characterized as S and R was 2.6 and 0.5 %DP day^{-1}, respectively (Table 4). For this specific interaction the progress of the disease was 5.2 times faster in S than in R individuals. When comparing the DPR among groups of R individuals from population RA02a and J02a interacting with the CE109 isolate (0.1 %DP day^{-1}) and from population RA02b and J02b interacting with the CE112 isolate (0.5 %DP day^{-1}), it became evident a five times lower expressivity of the *Rdm2* gene when interacting with the CE112 isolate, in both susceptible backgrounds (calculated from data in Table 4).

For the F_2 individuals of the *Rdm5*-segregant population RA045-b, the DPR of the individuals phenotypically characterized as S and R was 2.8 and 0.1 %DP day^{-1}, respectively (Table 4). Thus, for this specific interaction, the progress of the disease was 28 times faster in S than in R plants. For the F_2 individuals of the *Rdm5*-segregant population J045-b, the DPR was 2.6 and 0.1 %DP day^{-1} for the individuals phenotypically characterized as S and R, respectively (Table 4). As result, for this specific interaction, the progress of the disease was 26 times faster in S than in R plants.

Additionally, the incubation period (IP) of susceptible genotypes RA702 and J77-339, calculated from the disease progress curves (data not shown), were 8 and 10 days, respectively, when interacting with the CE112 isolate, and 9 and 12 days, respectively, when interacting with the CE109 isolate.

Discussion

Regarding the *Rdm4* and *Rdm5* genes from cv. Hutcheson, the phenotypic segregations observed in this

work were consistent with the genotypic segregations of these resistance genes in the specific interaction with CE109 and CE112 isolates of *Dpm*, respectively, in the J77-339 genetic background (Chiesa et al. 2009). In addition, the results obtained with cv. RA702 extended the complete dominant behavior of these resistance genes to another highly susceptible genetic background (Tables 2 and 3). Remarkably, cv. Hutcheson behaved as completely resistant when it interacted with the CE109 and CE112 isolates (Tables 2 and 3).

Conversely, the observed phenotypic segregation of the *Rdm2* gene (from the T2 line) interacting with the CE112 isolate, fitted to the 1R: 3S ratio in both susceptible backgrounds (populations RA02b and J02b, Table 3), suggesting at first instance, that a single recessive resistance gene could be involved in this specific interaction. However, if a recessive gene would be involved, the T2 line should have displayed a complete resistant phenotype in the specific interaction with the CE112 isolate. That was not the case, since the T2 line showed 56 %DP (Table 3). In addition, the T2 genotype was previously characterized as moderately susceptible in the specific interaction with CE112 (Pioli et al. 2003). Thus, the hypothesis of a recessive resistance gene was not supported.

An alternative explanation for the unexpected phenotypic segregation of the *Rdm2* gene in the RA02b and J02b populations (Table 3) could be found considering incomplete penetrance of this gene when interacting with the CE112 isolate. Penetrance of a resistance gene is said to be reduced or incomplete when some individuals fail to express the trait, even though they carry the resistance allele (Pierce 2010). The T2 (*Rdm2*/*Rdm2*) parent showed 56 % of individuals susceptible to the CE112 isolate, indicating a penetrance of the resistance of 44 %. Considering the phenotypic reaction of the T2 parent, the same or even lower degree of penetrance should be expected in the *Rdm2/Rdm2* and *Rdm2/rdm2* F_2 individuals from the populations RA02b and J02b, when interacting with the CE112 isolate. The percentage of susceptible individuals was 74 and 64 % in RA02b and J02b segregant populations, respectively, indicating a penetrance of the resistance conferred by the *Rdm2* gene of 26 and 36 %, in RA02b and J02b populations, respectively (calculated from data in Table 3). Additionally, the susceptible background in which the *Rdm2* gene was segregating influenced also the degree of penetrance in this particular interaction (cf. penetrance in RA02b and J02b populations).

Interestingly, the penetrance of *Rdm2* gene in the specific interaction with the CE109 isolate, in the T2 parent and the hybrids individuals, was 80 % (calculated from data in Table 2). Although in the interaction with the CE109 isolate the *Rdm2* gene had not full penetrance, the phenotypic reaction allowed the characterization of the T2 line as resistant to this isolate, according to the scale described by Chiesa et al. (2009). Also, the observed phenotypic segregation of the R and S individuals of RA02a and J02a populations fitted well to the expected segregation for *Rdm2* as a complete dominant gene (Table 2).

Taken as a whole, our results for the *Rdm2* gene were consistent with a monogenic model with different degrees of incomplete penetrance, depending firstly on the physiological race of the pathogen (e.g. CE112 or CE109 isolates) and secondly on the genetic background (e.g. RA702 or J77-339).

Research on other plant-pathogen systems had shown phenotypic variation in the response to different pathogens, consistent with the presence of resistance genes with incomplete penetrance and/or expression. Particularly, incomplete penetrance of the resistance was reported in the interactions of *Cucumis melo* with *Melon Yellows Virus* (Nuez et al. 1999), *Beta vulgaris* with *Beet Necrotic Yellow Vein Virus* (Gidner et al. 2005), *Zea mays* with *Sugarcane Mosaic Virus* (Ingvardsen et al. 2010), *Eucalyptus grandis* with *Puccinia psidii* (Junghans et al. 2003), *Lactuca sativa* with the nematode *Meloidogyne javanica* (Maluf et al. 2002) and *Triticum aestivum* with *Mayetiola destructor* (Liu et al. 2005). These examples of incomplete penetrance depended upon the pathogen strain, the genetic background, the environmental conditions and the allelic variations of the resistance gene(s).

It is worth noting that the genetic control of the resistance was determined by examining the final ratios of resistant/susceptible plants in the F_2 progenies. Since the outcome of a plant-pathogen interaction is a sequence of processes in time, the qualitative characterization at the end of the evaluation period does not explain how rapidly this final point is reached. This is of particular interest when incomplete penetrance of the resistance is evident, like in the *Rdm2*-CE112 interaction. In this case, the use of the disease progress curves could help to better understand the differences among distinct plant-pathogen interactions. Moreover,

the DPR quantify in a single value the daily increase of compatibility of the interaction (or the speed of the disease in each particular interaction) (Campbell and Madden 1990). Particularly, the fact that the DPR in the T2-CE112 interaction was three times higher than the DPR of the T2-CE109 interaction (Table 4) confirms the lower penetrance of the *Rdm2* gene in the interaction with the CE112 isolate. Also, the higher DPR of the R individuals in the interaction with the CE112 isolate (populations RA02b and J02b) than the DPR of the R individuals interacting with the CE109 isolate (populations RA02a and J02a) confirms a reduced expressivity of the *Rdm2* gene in the incompatible interactions with CE112 (Table 4). Variable expressivity occurs when a resistant phenotype is expressed to a different degree among individuals with the same *R* gene (Pierce 2010). In this case, the expression of the resistance conferred by the *Rdm2* gene was modified by the isolates of *Dpm*.

Thus, the incomplete penetrance and reduced expressivity of the *Rdm2* gene in the resistant individuals are in agreement with the characterization of the T2 line as moderately susceptible in the specific interaction with the CE112 isolate (Pioli et al. 2003). On the other hand, for the *Rdm4*-CE109 and *Rdm5*-CE112 interactions, the DPRs for the groups of R individuals among different F_2 populations was always the same, indicating complete penetrance and expressivity for the *Rdm4* and *Rdm5* genes, independently of the genetic background (Table 4).

The approach using F_2 populations to evaluate penetrance and expressivity may be ideal for this kind of study, especially if the parents are widely divergent, as were the case in this study, since the background genotypes among F_2 plants were randomly distributed. The conclusions of this study will be reinforced when DNA markers linked to the different *Rdm* genes became available to label and follow these genes in segregant populations.

On the other hand, the DPR for each specific incompatible interaction allowed the verification, except for the *Rdm2*-CE112 interaction, that the analyzed resistance loci, in double or single genetic doses, restricted quickly the advance of the pathogen. The incompatible plant-pathogen interactions are highly dependent on the ability of the plant to recognize the pathogen early and fast in the first instants of the infection process and to respond against it properly (Chisholm et al. 2006; Jones and Dangl 2006; de Wit 2007). However, in the compatible interactions, S plants were incapable of avoiding the development of the fungus following the first stages of the infection. This behaviour was clearly demonstrated and quantified through the DPR (Table 4). The averaged progress of the disease was 27 times faster in S than in the R plants, when interacting with the two local races of *Dpm*. In the S plants, the defence responses potentially induced against each particular race of the pathogen were not activated, or activated too late, upon recognition of effectors or effector-mediated perturbations of host targets and they were not effective (Chisholm et al. 2006; Jones and Dangl 2006; van der Hoorn and Kamoun 2008).

Besides, the shorter averaged IP of RA702 (8.5 d) compared to J77-339 (11 d), together with the higher DPR of the former (Table 4), indicated a higher degree of susceptibility of cv. RA702 to the analyzed local isolates. Also the shorter averaged IP observed for CE112 (9 d) compared to CE109 (11 d) would suggest higher level of aggressiveness of CE112 isolate. Therefore, for breeding purposes, it must be taken into account that the genetic background of the parental genotypes and the local physiological races of the pathogen strongly influence the expression of some *Rdm* genes. In addition, the knowledge of the degree of penetrance and expression of a given *R* gene, interacting with different physiological races of the fungus, would be relevant for the selection of parental genotypes in programs aims to incorporate high level, long-lasting resistance to SSC. As far as we know, this is the first report documenting changes in the penetrance and expressivity of a soybean resistance gene (*Rdm2*) depending upon the physiological race of the pathogen which interacts with and the genetic background in which the *R* gene is being expressed. Nevertheless, continual surveillance of pathogenic variability of the fungus, as well as searching for new resistance genes, will be required to preserve SSC resistance over time.

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References

Agrios, G. N. (2005). *Plant pathology* (5th ed.). New York: Academic Press.

Bowers, G. R., Ngeleka, K., & Smith, O. D. (1993). Inheritance of stem canker resistance in soybean cultivars Crockett and Dowling. *Crop Science, 33*, 67–70.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology.* USA and Canada: J. Wiley & Sons.

Chiesa, M. A. (2009). Aspectos patogénicos y moleculares de la resistencia a la Cancrosis del Tallo de la Soja. Doctoral Thesis. University of Rosario, Santa Fe, Argentina. 184 pp.

Chiesa, M. A., Pioli, R. N., & Morandi, E. N. (2009). Specific resistance to soybean stem canker conferred by the *Rdm4* locus. *Plant Pathology, 58*, 1032–1038.

Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. *Cell, 124*, 803–814.

Cregan, P. B., Jarvik, T., Bush, A. L., Shoemaker, R. C., Lark, K. G., Kahler, A. L., Kaya, N., VanToai, T. T., Lohnes, D. G., Chung, J., & Specht, J. E. (1999). An integrated genetic linkage map of the soybean genome. *Crop Science, 39*, 1464–1490.

de Wit, P. J. G. M. (2007). How plants recognize pathogens and defend themselves. *Celular & Molecular Life Sciences, 64*, 2726–2732.

Fernández, F. A., & Hanlin, R. T. (1996). Morphological and RAPD analyses of *Diaporthe phaseolorum* from soybean. *Mycologia, 88*, 425–440.

Gidner, S., Lennefors, B. L., Nilsson, N. O., Bensefelt, J., Johansson, E., Gyllenspetz, U., & Kraft, T. (2005). QTL mapping of BNYVV resistance from the WB41 source in sugar beet. *Genome, 48*, 279–285.

Higley, P. M., & Tachibana, H. (1987). Physiologic specialization of *Diaporthe phaseolorum* var. *caulivora* in soybean. *Plant Disease, 71*, 815–817.

Ingvardsen, C. R., Xing, Y., Frei, U. K., & Lübberstedt, T. (2010). Genetic and physical fine mapping of *Scmv2*, a potyvirus resistance gene in maize. *Theoretical and Applied Genetics, 120*, 1621–1634.

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature, 444*, 323–329.

Junghans, D. T., Alfenas, A. C., Brommonschenkel, S. H., Oda, S., Mello, E. J., & Grattapaglia, D. (2003). Resistance to rust (*Puccinia psidii Winter*) in Eucalyptus: mode of inheritance and mapping of a major gene with RAPD markers. *Theoretical and Applied Genetics, 108*, 175–180.

Keeling, B. L. (1985). Soybean cultivar reactions to soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora* and pathogenic variation among isolates. *Plant Disease, 59*, 132–133.

Keeling, B. L. (1988). Influence of temperature on growth and pathogenicity of geographic isolates of *Diaporthe phaseolorum* var. *caulivora*. *Plant Disease, 72*, 220–222.

Kilen, T. C., & Hartwig, E. E. (1987). Identification of single genes controlling resistance to stem canker in soybean. *Crop Science, 27*, 863–864.

Kilen, T. C., Keeling, B. L., & Hartwig, E. E. (1985). Inheritance of reaction to stem canker in soybean. *Crop Science, 25*, 50–51.

Kulik, M. M. (1989). Variation in pathogenicity among isolates of *Diaporthe phaseolorum* f. sp. *caulivora*. *Mycologia, 81*, 549–553.

Lacadena, J. R. (1988). *Genética* (4th ed.). Madrid: AGESA.

Liu, X. M., Brown-Guedira, G. L., Hatchett, J. H., Owuoche, J. O., & Chen, M. S. (2005). Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from *T. turgidum* ssp. dicoccum to common wheat. *Theoretical and Applied Genetics, 111*, 1308–1315.

Maluf, W. R., Azevedo, S. M., Gomes, L. A. A., & Barneche de Oliveira, A. C. (2002). Inheritance of resistance to the root-knot nematode *Meloidogyne javanica* in lettuce. *Genetics and Molecular Research, 1*(1), 64–71.

Morgan-Jones, G. (1985). The *Diaporthe–Phomopsis* complex on soybean: Morphology. In: M.M. Kulik (Ed.), *Proc. Conf. Diaporthe–Phomopsis Disease Complex of Soybean* (pp. 1–7). Fort Walton Beach, FL: USDA-ARS, Springfield, VA.

Nuez, F., Picó, B., Iglesias, A., Esteva, J., & Juarez, M. (1999). Genetics of Melon yellows virus resistance derived from *Cucumis melo* ssp. *agrestis*. *European Journal of Plant Pathology, 105*, 453–464.

Pierce, B. A. (2010). *Genética: un enfoque conceptual* (3rd ed., p. 730). Buenos Aires: Editorial Médica Panamericana.

Pioli, R. N., Gattuso, S., Prado, D., & Borghi, A. (1997). Recent outbreak of stem canker (*Diaporthe phaseolorum* var. *meridionalis*) of soybean in Santa Fe, Argentina. *Plant Disease, 81*, 1215.

Pioli, R. N., Morandi, E. N., Gosparini, C. O., & Borghi, A. L. (1999). First report on pathogenic variability of different isolates of *D. phaseolorum* var. *meridionalis* on soybean in Argentina. *Plant Disease, 83*, 1071.

Pioli, R. N., Morandi, E. N., Martínez, M. C., Lucca, F., Tozzini, A., Bizaro, V., & Hopp, E. (2003). Morphologic, molecular and pathogenic characterization of *Diaporthe phaseolorum* variability in the core soybean producing area of Argentina. *Phytopathology, 93*, 136–145.

Ploper, L. D., Galvez, R., Gonzalez, V., Jaldo, H., Devani, M., & Salas, G. (1999). Effects of sowing date on the incidence of stem canker in soybeans. *Avance Agroindustrial, 19*, 33–38.

Roy, K. W., & Miller, W. A. (1983). Soybean stem canker incited by isolates of *Diaporthe* and *Phomopsis* spp. from cotton in Mississippi. *Plant Disease, 67*, 135–137.

Sinclair, J. B. (1999). *Diaporthe/Phomopsis* complex. In *Compendium of soybean diseases* (4th ed., p. 31). St. Paul: The American Phytopathological Society.

Sinclair, J. B., & Hartman, G. L. (1999). Soybean diseases caused by fungi. In *Compendium of soybean diseases* (4th ed., p. 11). St Paul: The American Phytopathological Society.

Snedecor, G. W., & Cochran, W. G. (1989). *Statistical methods* (8th ed.). Ames: Iowa State University Press.

Tyler, J. M. (1996). Characterization of stem canker resistance in "Hutcheson" soybean. *Crop Science, 36*, 591–593.

van der Hoorn, R. A. L., & Kamoun, S. (2008). From guard to decoy: a new model for perception of plant pathogen effectors. *The Plant Cell, 20*, 2009–2017.