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Full Length Article

High Genetic Diversity and Aggressiveness of *Sclerotinia sclerotiorum* in Soybean (*Glycine max*) in Central Brazil Region

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Abstract

The diversity and aggressiveness inter- and intrapopulation of *Sclerotinia sclerotiorum* through mycelial compatibility groups and the evaluation of representative isolates of these groups were investigated for the disease severity in soybean plants. The isolates were derived from sclerotia collected in different soybean-producing regions of Central Brazil. For each location, mycelial compatibility analyses were conducted in a completely randomized design with two replications and 25 isolates. After the intrapopulation, interpopulation analyses were carried out, with a total of 31 isolates, using one or two representative isolates of each mycelial compatibility group of the intrapopulation analysis. For assessing isolates aggressiveness, the petioles of soybean cultivars M-SOY 7908 RR and BRSGO 7760 RR were inoculated with 21 representative isolates of mycelial compatibility. The length of lesions on the stem was measured three and seven days after inoculation. The experiment was arranged in a completely randomized factorial design, testing 21 (isolates) × 2 (cultivars), with 3 replications. In intrapopulation analyses, the genetic diversity of *S. sclerotiorum* was low, with at most three compatibility groups per population. In the interpopulation analyses, eight compatibility groups were detected, of which one with 19 isolates was predominant. The isolates differed significantly inaggressiveness on both the cultivars. M-SOY 7908 RR was found to be the most susceptible cultivar and SSSM25 the most aggressive isolate.

Keywords: *Glycine max*; Phytopathogenic diversity; Population genetic; Resistance; White mold

Introduction

Sclerotinia sclerotiorum, is one of the most important pathogens of soybean worldwide. This pathogen causes the white mold disease in a broad host range. Disease management is complicated by the long-term survival of sclerotia in the soil and the absence of resistance in elite, commercial cultivars (Willbur *et al.* 2019).

It is known that *S. sclerotiorum* isolates originating from different geographical areas and hosts may vary in morphological, physiological and genetic characteristics (Kull *et al.* 2004; Durman *et al.* 2005; Li *et al.* 2008; Garg *et al.* 2009). The expansion of the genetic diversity of most living organisms occurs by means of sexual reproduction (which is meiosporic in the case of fungi), but to date this type of reproduction has not been described for certain fungal species (Carlile *et al.* 2001). In contact areas of colonies of a same isolate or between different isolates of the same fungal species, the occurrence of sexual compatibility (sexual reproduction) and vegetative compatibility or incompatibility can be observed (Glass *et al.* 2000). Hyphal anastomosis allows the exchange of cell contents between different individuals in compatibility reactions, the onset of sexual reproduction, heterokaryon formation, occurrence of a parasexual cycle and nutrient distribution in the mycelium/colony (Glass *et al.* 2000; Hickey *et al.* 2002).

Based on the genetic description, fungi are called antagonistic when clearly distinguishable sexual and vegetative heterokaryons occur at the interface between hyphae of different isolates (Alexopoulos *et al.* 1996). Sexual reproduction can occur among both heterothallic and homothallic isolates (Robertson *et al.* 1998). The test of mycelial compatibility groups has been used to evaluate the genetic diversity in several plant-pathogenic fungi (Katan *et al.* 1991; Leslie 1993), including *S. sclerotiorum* (Kull *et al.* 2004; Li *et al.* 2008).

Population studies of *S. sclerotiorum* demonstrated the genetic diversity and provided evidence for clonal and/or sexual reproduction. Generally, clonal reproduction

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predominates in temperate regions (Hambleton *et al.* 2002), while sexual recombination occurs in warmer climate regions (Malvárez *et al.* 2007). Kohn *et al.* (1990) studied mycelial compatibility among 35 *S. sclerotiorum* isolates from different regions of Canada, and clustered the isolates in 28 groups of mycelial compatibility, 23 isolates were incompatible with all and 12 isolates formed compatibility groups with 2–3 isolates, indicating high genetic heterogeneity within the species. The reported degree of mycelial incompatibility suggests that genetic exchange occurs eventually in fungal populations. However, this exchange is more likely to occur through the ascus in sexual reproduction, since the high mycelial incompatibility between *S. sclerotiorum* isolates probably decreases the possibility of somatic heterokaryosis through hyphal fusion.

In Brazil, the formation of five mycelial compatibility groups and three clusters was detected by RAPD, suggesting moderate genetic diversity and sexual recombination among isolates from the tropics (Júnior *et al.* 2011). Meinhardt *et al.* (2002) reported two compatibility groups among 23 isolates. Differences between groups of mycelial compatibility as the aggressiveness of soybean plants were observed by Kull *et al.* (2004).

Despite the decades-old presence of *S. sclerotiorum* in Brazil, information about the population biology of this pathogen as well as on the aggressiveness of isolates of this fungus on soybean plants are still scarce, especially with regard to the Midwest region. The objectives of the present study were to investigate the genetic diversity in *S. sclerotiorum* populations on soybean in Central Brazil by the analysis of mycelial compatibility, and to assesse the aggressiveness of isolates in mycelial compatibility groups of soybean plants.

Materials and Methods

Population of *S. sclerotiorum*

Nine populations of *S. sclerotiorum* isolates were derived from sclerotia collected from eight soybean-producing regions in the Central Brazil. Sclerotia were collected from each location at approximately 50 points, then selecting 25 isolates per sampled field. The distance between the sampling points in each field was at least 10 m; only in the municipality of Silvânia, Goiás, two samples were collected from two fields on the same farm.

To obtain the isolates in the mycelial form, the sclerotia were sterilized in 96% alcohol and 2% sodium hypochlorite for 60 s in each solution and then placed on Petri dishes containing agar-water culture medium and incubated in a chamber, in constant darkness at 20 ± 1°C. After initiation of sclerotia germination, 6-mm diameter discs containing only tips of the fungal hyphae were removed from the edges of the colony and placed on Petri dishes containing PDA (potato dextrose agar) culture medium and incubated in a chamber under the above conditions.

Intrapopulation analysis of mycelial compatibility of *S. sclerotiorum* isolates

The experiments were conducted in a completely randomized design with two replications and 25 isolates per population, with one replication cultured in MPM (modified Patterson's medium) and the other in PDA (potato dextrose agar) culture medium. Two culture media were used, as in a previous study it was found that, in some cases, the MPM medium delays the growth of fungi, making evaluation difficult and inducing errors. The inhibition of mycelial growth was observed by Schafer and Kohn (2006) in the presence of MPM medium. Both culture media were used for greater insight and reliability in the assessments of mycelial reactions.

The MPM culture medium was prepared as proposed by Schafer and Kohn (2006), containing:0.68 g L^{-1} KH_2PO_4; 0.50 g L^{-1} $MgSO_4.7H_2O$; 0.15 g L^{-1} KCl, 1.0 g L^{-1} NH_4NO_3; 18.4 g L^{-1} D-glucose; 0.50 g L^{-1} yeast extract; 15.0 g L^{-1} agar; 200 μL solution of trace elements containing 95 mL distilled water; 5.0 g monohydrate citric acid; 5.0 g $ZnSO_4.7H_2O$; 1.0 g $Fe(NH_4)_2(SO_4)_2.6H_2O$; 0.25 g $CuSO_4.5H_2O$; 0.05 g $MnSO_4.1H_2O$; 0.05 g H_3BO_4; 0.05 g $Na_2MoO_4.2H_2O$; and 1 mL $CHCl_3$ as a preservative solution stored at 20 ± 2°C. The PDA culture medium was prepared as described by Zauza *et al.* (2007), containing 20 g L^{-1} D-glucose, 17 g L^{-1} agar and 200 g potato. Before pouring the culture media into Petri dishes, 1.000 μL of strawberry red dye (Mix Coralim®) were added to facilitate the visualization of incompatibility reactions.

The isolates were cultured in PDA for 5 d in the dark at 25 ± 1°C. After this period, 6-mm diameter mycelial discs were removed from the edge of the *S. sclerotiorum* colony and three discs were placed equidistantly in each Petri dish (diameter 9 cm), containing culture medium. After this, the Petri dishes were incubated in a BOD chamber in the dark at 25 ±1°C. The mycelial interaction reactions were evaluated 7 d after incubation, considering a reaction incompatible when a red line was detected and formation of aerial mycelium at the interface line. Reactions were considered compatible when the colonies grew freely in the culture medium and were fused.

Interpopulation analysis of mycelial compatibility of *S. sclerotiorum* isolates

Two isolates from each group of mycelial compatibility of each location were paired with each other, amounted to 31 isolates for interpopulation analysis. Some mycelial compatibility groups were represented by a single isolate since they were comprised of a solitary isolate. This experiment was conducted similarly to that described above. The results showed that the compatibility groups were distinct or similar to the groups already determined within each population/location.

Aggressiveness of *S. sclerotiorum* isolates in soybean

In the study of aggressiveness of *S. sclerotiorum* isolates on soybean, a single isolate from each compatibility group detected for each location was randomly chosen, totaling 21 isolates. These isolates (SSMO01, SSMO23, SSUB01, SSUB18, SSAF11, SSSI37, SSSI21, SSSI16, SSSI10, SSPA11, SSPA03, SSPA23, SSCS24, SSCS05, SSAN02, SSAN11, SSAN20, SSSM12, SSSM25, SSSM03 and SSSM10) were evaluated for aggressiveness on the cultivars M-SOY 7908 RR and BRSGO 7760 RR, since these two cultivars have been reported as highest and lowest disease incidence, respectively, among commercial varieties under field conditions in central region of Brazil. The cultivars were sown in 5 L pots filled with Oxisol soil under greenhouse conditions. The experiment was repeated once to verify the consistency of results.

The experiment was conducted in a completely randomized, factorial design with 21 (isolates) × 2 (cultivars), with 3 replications. Each replication consisted of five plants, i.e., a total of 15 plants per treatment. When the plants reached stage V3/V4, the third trifoliate leaf was cut with scissors, leaving about 3 cm of the stem for the insertion of the inoculum. For the stem inoculation, the entire length of 1000 μL plastic tips was filled with mycelial discs taken from the edge of inverted *S. sclerotiorum* colonies such that the upside down mycelium came to direct contact with the plant at the inoculation site.

The 21 isolates used in inoculations were grown in PDA culture medium for 3 d at 25 ± 1°C and a photoperiod of 12 h. Inoculated plants were maintained in a greenhouse for 7 d, at a mean temperature of 22°C and relative humidity of 90%. Evaluations consisted of measuring the lesion length on the stems, 3 and 7 d after inoculation. Subsequently, the average lesion length of five plants per replication was calculated.

Data from both experiments were subjected to normality and homogeneity tests of variance, using the SAS package (Statistical Analysis System) (SAS Institute 1999). Statistical tests were performed using Box-Cox power transformation (Box and Cox 1964). Once the statistical assumptions were met, analysis of variance was performed with the F test at 5% probability. The isolate means were compared by the Scott-Knott test and for cultivars; the Tukey test was applied at 5% probability, using the statistical program SISVAR (Ferreira 2000).

Results

Mycelial compatibility of *S. sclerotiorum* isolates

The pairing of the isolates in all possible combinations for each population produced incompatible and compatible reactions. The incompatible reaction was characterized by the presence of a red line, which were recorded from the top or the bottom of the colonies (Fig. 1A and B) and formation of aerial mycelium along the contact line of the isolates (Fig. 1C and D). The compatible response was defined as the absence of a line in the contact zone of the isolates (Fig. 1E and F). The results of the isolate compatibility analysis for each population are shown in Table 1.

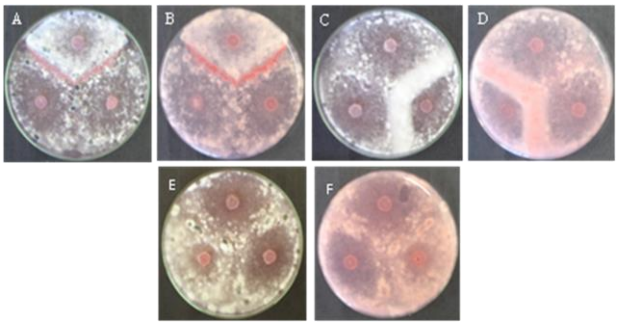

Fig. 1: Paired *S. sclerotiorum* isolates reacting with incompatibility (**A**, **B**, **C** and **D**), compatibility (**E** and **F**)

A single mycelial compatibility group (MGC1) was detected in both Água Fria-GO population and Silvânia-GO "B" population, denoting the existence of one clone in each sampled field (Table 1). In contrast, in the populations of Uberlândia-MG and Montividiu-GO, two compatibility groups per population were found. Group MCG1 was also present at these two locations, comprising 96% of the isolates, while the groups MCG7 in the population of Montividiu and MCG8 in the population of Uberlândia were represented by a single isolate (Table 1).

In soybean fields sampled in Patrocínio-MG and Silvânia-GO "A", three compatibility groups per population were detected. In both populations of Patrocínio and Silvânia "A", group MCG1 was represented by 23 isolates (92%), while MCG2 and MCG3, in the population of Silvânia, and MCG4 and MCG5 in the population of Patrocínio were constituted of a only one isolate (4%) (Table 1). In the population of Anápolis, group MCG1 comprised 88% of the isolates, while MCG2 contained only 12% (Table 1).

In the population of Chapadão do Sul, two compatibility groups, MCG1 and MCG3, were identified, consisting of 64 and 26% of the isolates, respectively (Table 1). In the population of São Miguel do Passa Quatro-GO, there were three mycelial compatibility groups: MCG1, consisting of 23 isolates (92%), and MCG4 and MCG6, represented by a single isolate (4%).

The results of the mycelial compatibility tests revealed low genetic diversity in *S. sclerotiorum* populations within each sampled field (intrapopulation) at the eight sampled municipalities, except for São Miguel do Passa Quatro-GO, Silvânia-GO, "A" and Patrocínio-MG, where three compatibility groups were found, although two were represented by a only one isolate. This indicates that the frequency of sexual recombination in these populations, if existent, is very low.

Table 1: Mycelial compatibility groups of *S. sclerotiorum* isolates detected in different municipalities

MCG	Isolates								
	Água Fria	Silvânia "A"	Silvânia "B"	Montividiu	Uberlândia	Patrocícnio	Anápolis	Chapadão do Sul	São Miguel do Passa Quatro
	SSAF01	SSSI01	SSSI26	SSMO01	SSUB01	SSPA01	SSAN01	SSCS02	SSSM01
	SSAF02	SSSI02	SSSI27	SSMO02	SSUB02	SSPA02	SSAN03	SSCS03	SSSM02
	SSAF03	SSSI03	SSSI28	SSMO03	SSUB03	SSPA03	SSAN04	SSCS07	SSSM03
	SSAF04	SSSI04	SSSI29	SSMO04	SSUB04	SSPA04	SSAN05	SSCS09	SSSM04
	SSAF05	SSSI05	SSSI30	SSMO05	SSUB05	SSPA05	SSAN06	SSCS13	SSSM05
	SSAF06	SSSI06	SSSI31	SSMO06	SSUB06	SSPA06	SSAN07	SSCS14	SSSM06
	SSAF07	SSSI07	SSSI32	SSMO07	SSUB07	SSPA07	SSAN08	SSCS17	SSSM07
	SSAF08	SSSI08	SSSI33	SSMO08	SSUB08	SSPA08	SSAN09	SSCS24	SSSM08
	SSAF09	SSSI09	SSSI34	SSMO09	SSUB09	SSPA09	SSAN10	SSCS25	SSSM09
	SSAF10	SSSI10	SSSI35	SSMO10	SSUB10	SSPA10	SSAN12	-	SSSM11
1	SSAF11	SSSI11	SSSI36	SSMO11	SSUB11	SSPA12	SSAN13	-	SSSM12
	SSAF12	SSSI12	SSSI37	SSMO12	SSUB12	SSPA13	SSAN14	-	SSSM13
	SSAF13	SSSI13	SSSI38	SSMO13	SSUB13	SSPA14	SSAN15	-	SSSM14
	SSAF14	SSSI14	SSSI39	SSMO14	SSUB14	SSPA15	SSAN16	-	SSSM15
	SSAF15	SSSI15	SSSI40	SSMO15	SSUB15	SSPA16	SSAN17	-	SSSM16
	SSAF16	SSSI17	SSSI41	SSMO16	SSUB16	SSPA17	SSAN18	-	SSSM17
	SSAF17	SSSI18	SSSI42	SSMO17	SSUB17	SSPA18	SSAN19	-	SSSM18
	SSAF18	SSSI19	SSSI43	SSMO18	SSUB19	SSPA19	SSAN20	-	SSSM19
	SSAF19	SSSI20	SSSI44	SSMO19	SSUB20	SSPA20	SSAN21	-	SSSM20
	SSAF20	SSSI22	SSSI45	SSMO20	SSUB21	SSPA21	SSAN22	-	SSSM21
	SSAF21	SSSI23	SSSI46	SSMO21	SSUB22	SSPA22	SSAN23	-	SSSM22
	SSAF22	SSSI24	SSSI47	SSMO22	SSUB23	SSPA24	SSAN24	-	SSSM23
	SSAF23	SSSI25	SSSI48	SSMO24	SSUB24	SSPA25	-	-	SSSM24
MCG	Água Fria	Silvânia "B"	Montividiu	Uberlândia	Silvânia "A"	Patrocínio	Anápolis	Chapadão do Sul	S. M. Passa Quatro
1	SSAF24	SSSI49	SSMO25	SSUB25	-	-	-	-	-
	SSAF25	SSSI50	-	-	-	-	-	-	-
2	-	-	-	-	SSSI21	-	SSAN02	-	-
	-	-	-	-	-	-	SSAN11	-	-
	-	-	-	-	-	-	SSAN25	-	-
3	-	-	-	-	SSSI16	-	-	SSCS01	-
	-	-	-	-	-	-	-	SSCS04	-
	-	-	-	-	-	-	-	SSCS05	-
	-	-	-	-	-	-	-	SSCS06	-
	-	-	-	-	-	-	-	SSCS08	-
	-	-	-	-	-	-	-	SSCS10	-
	-	-	-	-	-	-	-	SSCS11	-
	-	-	-	-	-	-	-	SSCS12	-
	-	-	-	-	-	-	-	SSCS15	-
	-	-	-	-	-	-	-	SSCS16	-
	-	-	-	-	-	-	-	SSCS18	-
	-	-	-	-	-	-	-	SSCS19	-
	-	-	-	-	-	-	-	SSCS20	-
	-	-	-	-	-	-	-	SSCS21	-
	-	-	-	-	-	-	-	SSCS22	-
	-	-	-	-	-	-	-	SSCS23	-
4	-	-	-	-	-	SSPA11	-	-	SSSM25
5	-	-	-	-	-	SSPA23	-	-	
6	-	-	-	-	-	-	-	-	SSSM10
7	-	-	SSMO23	-	-	-	-	-	-
8	-	-	-	SSUB18	-	-	-	-	-

The results of the compatibility test among the sclerotia sampling locations are shown in Table 2. When one or two isolates representing each compatibility group of all locations were paired in all possible combinations, eight compatibility groups were detected (MCG1, MCG2, MCG3, MCG4, MCG5, MCG6, MCG7, and MCG8, represented by, respectively, 19 (61.6%), 3 (9.6%), 3 (9.6%), 2 (6.4%), 1 (3.2%), 1 (3.2%), 1 (3.2%), and 1 isolates (3.2%) (Table 2).

In Silvânia-GO, isolates from different plots on a same farm behaved differently in mycelial compatibility analyses. While three groups of mycelial compatibility were detected in the field "A", the 25 isolates in field "B" were all compatible with each other, indicating the existence of a single clone in this field. The existence of more than one compatibility group in one field can be explained by the topographic position of this field on the property. It is located in a low land area, which contributes to a transfer of sclerotia from higher areas by rainwater, favoring the gene flow from the population of field "B".

Aggressiveness of *S. sclerotiorum* isolates

The results of the aggressiveness test of *S. sclerotiorum* isolates are presented in Table 3. The interaction cultivar-isolate was significant after 3 d of inoculation ($P \leq 0.05$), and after 7 d of inoculation ($P \leq 0.01$) (Table 3). All 21 isolates used in this study could induce disease in the

Table 2: Isolates and mycelial compatibility groups identified by interpopulation analysis of 31 *S. sclerotiorum* isolates collected on soybean fields in the Central region of Brazil

Isolates	Location/Population	MCG
SSSI37	Silvânia "B"	1
SSSI48		1
SSUB01	Uberlândia	1
SSUB18		8
SSUB21		1
SSAF03	Água Fria	1
SSAF11		1
SSMO23	Montividiu	7
SSMO01		1
SSMO20		1
SSSI21	Silvânia "A"	2
SSSI16		3
SSSI10		1
SSSI02		1
SSPA11	Patrocínio	4
SSPA23		5
SSPA17		1
SSPA03		1
SSAN02	Anápolis	2
SSAN11		2
SSAN21		1
SSAN07		1
SSCS24	Chapadão do Sul	1
SSCS14		1
SSCS05		3
SSCS01		3
SSSM10	São Miguel do Passa Quatro	6
SSSM12		1
SSSM15		1
SSSM03		1
SSSM25		4

Table 3: Lesion size (cm) caused by different *S. sclerotiorum* isolates, representing eight groups of mycelial compatibility on the soybean cultivars BRSGO 7760 RR and M-SOY 7908 RR, three and seven days after inoculation (d.a.i.)[2]

Isolates	MCG	Cultivars (3 d.a.i.)		Cultivars (7 d.a.i.)	
		BRSGO 7760 RR	M-SOY 7908 RR	BRSGO 7760 RR	M-SOY 7908 RR
SSMO01	1	0.30 d A	0.02 d A	0.37 d A	0.02 c A
SSUB01	1	0.77 c A	2.12 c A	1.53 d B	5.07 b A
SSSI16	3	0.28 d B	0.92 c A	1.63 d B	5.17 b A
SSSI21	2	0.42 d A	0.73 c A	2.67 c B	4.95 b A
SSAN11	2	0.38 d B	1.35 c A	3.12 c B	7.32 a A
SSAN02	2	1.05 c B	2.42 b A	3.63 b B	8.27 a A
SSPA11	4	1.03 c B	2.05 b A	4.02 b B	6.78 a A
SSPA23	5	1.82 b A	1.56 b A	4.05 b A	5.42 b A
SSCS05	3	1.87 b A	2.72 b A	4.73 b B	7.75 a A
SSPA03	1	1.42 b A	1.38 c A	5.38 a A	3.33 b B
SSSI10	1	1.63 b A	1.98 b A	5.40 a A	7.18 a A
SSAN20	1	1.08 c B	2.76 b A	5.65 a B	9.37 a A
SSMO23	7	2.55 b A	2.73 b A	5.68 a A	7.53 a A
SSSM10	6	1.00 c B	2.07 b A	5.77 a A	8.12 a A
SSSI37	1	1.95 b A	2.07 b A	5.83 a A	6.90 a A
SSCS24	1	2.93 a A	3.60 a A	5.97 a A	7.57 a A
SSAF11	1	2.92 a A	4.11 a A	6.22 a A	7.73 a A
SSSM03	1	3.23 a A	2.32 b A	6.30 a A	5.30 b A
SSSM12	1	3.25 a A	4.10 a A	7.23 a A	8.53 a A
SSUB18	8	3.63 a A	3.93 a A	7.70 a A	7.82 a A
SSSM25	4	1.85 b A	3.18 a A	8.02 a A	10.58 a A
Mean		1.68	2.29	4.80	6.70
CV (%)		31.19		22.63	

[1]Means in the original scale, but with statistical tests resulting from the Box-Cox Power Transformation (Box and Cox 1964)
Means followed by the same lower-case letters in the column and capital letter in the row, did not differ from each other by the Scott-Knott test, for the factor isolate, and Tukey test, for the factor cultivar, at 5% significance
[2]Combined analysis of two experiments

cultivars BRSGO 7760 RR and M-SOY 7908 RR, with the exception of isolate SSMO01, from Montividiu-GO, with low aggressiveness. There was disease evolution between the first assessment, 3 d after inoculation, and the second evaluation, 7 d after inoculation, showing that the conditions remained favorable to the pathogen development and

expression of its aggressiveness. Regardless of the isolate, cultivar M-SOY 7908 RR was more susceptible than cultivar BRSGO 7760 RR in both evaluations, confirming the observations under commercial field conditions.

Three days after inoculation, the isolates SSUB18, SSSM12, SSSM03, SSCS24, and SSAF11 were the most aggressive to cultivar BRSGO 7760 RR, causing lesions with a length range of 3.63 to 2.92 cm. The most aggressive isolates to cultivar M-SOY 7908 RR were SSAF11, SSSM12, SSUB18, SSSM25, and SSCS24, although with no statistical difference, causing lesion lengths of 4.11 to 3.18 cm (Table 3). Among the five most aggressive isolates to each cultivar, four isolates (SSAF11, SSSM12, SSUB18, and SSCS24) were common to both soybean cultivars used in this aggressiveness analysis. Three days after inoculation, the lesion length differed significantly between the cultivars for the isolates SSAN20, SSAN02, SSPA11, SSM10, SSAN11, and SSSI16 and the lesion length on BRSGO 7760 RR was shorter.

For cultivar M-SOY 7908 RR, seven days after inoculation, the most aggressive isolates were SSSM25, SSAN20, SSSM12, SSAN02, SSSM10, SSUB18, SSCS05, SSAF11, SSCS24, SSMO23, SSAN11, SSSI10, SSSI37, and SSPA11. To cultivar BRSGO 7760 RR, the most aggressive isolates were SSSM25, SSUB18, SSSM12, SSSM03, SSAF11, SSCS24, SSSI37, SSSM10, SSMO23, SSAN20, SSSI10, and SSPA23 (Table 3). Seven days after inoculation, the number of most aggressive isolates increased from 5 to 14 isolates; this shows that some isolates have a longer incubation period and therefore need more time to express their aggressiveness in soybean plants.

Among the most aggressive 14 isolates to cultivar M-SOY 7908 RR, 10 were common to cultivar BRSGO 7760 RR. Seven days after inoculation, the difference between cultivars in lesion length was significant for the isolates SSPA03, SSCS05, SSPA11, SSAN 02, SSAN11, SSSI21, SSSI16, and SSUB01.Cultivar M-SOY 7908 RR proved to be the most sensitive, except for isolate SSPA03, which induced the greatest lesion length in cultivar BRSGO 7760 RR (Table 3).

Discussion

The results of the mycelial compatibility tests revealed low genetic variability in *S. sclerotiorum* populations within each sampled field (intrapopulation) at the eight sampled municipalities, except for São Miguel do Passa Quatro-GO, Silvânia-GO, "A" and Patrocínio-MG, where three compatibility groups were found, although two were represented by a only one isolate. This indicates that the frequency of sexual recombination in these populations, if existent, is very low. The population of Chapadão do Sul was well-distributed in the two mycelial compatibility groups, unlike the other populations studied, in which the groups are represented by at most three isolates. Kolhi *et al*. (1992) also concluded that there is more than one *S. sclerotiorum* clone in a single field, in an analysis of 290 isolates collected in western Canada.

High genetic diversity was found in the interpopulation analysis, *i.e*., analyzing the populations resulting from the mixture of the mycelial compatibility groups of the eight municipalities These results agree from Mahalingam *et al*. (2020) based on MCG and microsatellite data which founded relatively a high level of gene and genotypic diversity in Sri Lanka (also a tropical country). Some researchers assert that sexual recombination occurs in regions with a warmer climate (Atallah *et al*. 2004; Malvárez *et al*. 2007).

In Silvânia-GO, isolates from different plots on a same farm behaved differently in mycelial compatibility analyses. While three groups of mycelial compatibility were detected in field "A", the 25 isolates in field "B" were all compatible with each other, indicating the existence of a single clone in this field. The existence of more than one compatibility group in one field can be explained by the topographic position of this field on the property. It is located in a lowland area, which contributes to a transfer of sclerotia from higher areas by rainwater, favoring the gene flow from the population of field “B”.

Among 40 isolates collected in different locations and different hosts, five groups of mycelial compatibility and three clusters were identified by RAPD, suggesting high genetic variability and sexual recombination among the studied isolates (Júnior *et al*. 2011). In another study, low genetic variability was observed in *S. sclerotiorum* populations. Among 23 isolates (21 isolates from common bean, 1 from potato and 1 from pepper), two compatibility groups were observed, aside from polymorphism among isolates of a same mycelial compatibility group (Meinhardt *et al*. 2002).

The intra-population genetic variability analysis was low because few compatibility groups were detected within the sampled fields, aside from the fact that these groups are represented by a maximum of three isolates. The only exception was the population of Chapadão do Sul, where the two existing compatibility groups are distributed more homogeneously in the sampled area. A different behavior was observed when the isolates from the different locations were paired with each other, resulting in eight compatibility groups. In this interpopulation analysis, group MCG1 comprised 62% of the isolates and present in all sampled fields. The other groups (MCG2, MCG3, MCG4, MCG5, MCG6, MCG7, and MCG8) contained at most three isolates, *i.e*., 9.6%. This information suggests that the gene flow of sclerotia transferred from one area to another through cultural practices, be it within a property or in relatively distant areas, as well as the use of contaminated seed, contribute little to the genetic variability of *S. sclerotiorum* populations. The variability could possibly be associated with mutations, nevertheless most clones have a low frequency, except in the population of Chapadão do Sul, where the frequency of the two clones was similar.

Despite a few earlier studies of genetic variability in *S. sclerotiorum* populations in Brazil, the number of isolates analyzed in this paper is higher than the number of isolates reported in the literature (Gomes *et al*. 2011; Júnior *et al*. 2011); in addition, the pathogen populations in this study were analyzed within each sampled soybean field and between fields in different Brazilian municipalities of Central Brazil, where white mold has become a major soybean disease, arousing concerns of producers as well as of researchers interested in the pathosystem *S. sclerotiorum* × *Glycine max* L.

No relationship was observed between mycelial compatibility groups and aggressiveness of isolates to soybean plants, since in a same mycelial compatibility group the isolates differed in lesion length on the stem. These results are in agreement with those of Atallah *et al*. (2004), Auclair *et al.* (2004) and Kull *et al.* (2004), who found that the MCG or microsatellite markers were not associated with specific characteristics of aggressiveness or ecological adaptations of the pathogen. Not all isolates of group MCG1 were equally aggressive to soybean plants. These results disagree with those of Otto-Hanson *et al*. (2011), who reported the existence of 64 mycelial compatibility groups in a population of 156 isolates collected in different regions of North America and France, where isolates of the same compatibility group did not differ in aggressiveness in common bean plants Kull *et al.* (2004) found 42 mycelial compatibility groups among 299 isolates from Diverse, DeKalb, Watseka Sets of Canada and Argentina. These compatibility groups differed in aggressiveness to soybean plants. The most aggressive were the groups with clustered isolates from different locations, e.g., Diverse, DeKalb and Watseka. These results are in agreement with our study, since the most aggressive isolates belonging to MCG1 are from different locations, such as São Miguel do Passa Quatro, Água Fria, Chapadão do Sul, Silvânia, Montividiu, Anápolis, and Patrocínio. It was also found that isolates from the same location differed in aggressiveness, as in the case of the isolates SSUB 18 and SSUB 01, from Uberlândia and SSMO 01 and SSMO 23, from Montividiu, differing from the findings of Kull *et al*. (2004) and Durman *et al*. (2003). Isolates that were incompatible with most isolates within their respective populations (SSSM 25, SSUB 18, SSSM 10, SSMO 23, SSCS 05, SSPA 11, and SSAN 02) were included in the group of 14 most aggressive isolates to cultivar M-SOY 7908 RR seven days after inoculation.

The reason for the variation in aggressiveness observed in this study may be related to the range of different municipalities in the Central region of Brazil where sclerotia were collected, therefore the existence of several compatibility groups would be more likely than of a clonal population (Kolhi *et al.* 1992; Durman *et al.* 2003). Aggressiveness variation of *S. sclerotiorum* has been a subject of many studies in differents crops (Ekins *et al.* 2007; Otto-Hanson *et al.* 2011; Attanayake *et al.* 2013) also in different geographic areas.

In soybean, white mold is a severely yield-limiting disease when favorable environmental conditions are met. Yield reductions are caused by reduced seed number and weight (Hoffman *et al*. 1998; Danielson *et al*. 2004) resulting from the girdling of stems and disruption of xylem and phloem (Willbur *et al*. 2019). Thus, understanding the diversity and aggressiveness of the pathogen is valuable to improve the effectiveness of control practices, particularly the strategic use of cultivar resistance. When analyzing the diversity and aggressiveness of this fungus, we found 19 different behaviors in two soybean cultivars, thus for the selection of resistant varieties more than one isolate should be used due to the genetic behave in *S. sclerotiorum* populations.

Conclusion

Interpopulation analyses detected high genetic diversity and eight groups of mycelial compatibility in Central Brazil region. The *S. sclerotiorum* isolates differ in terms of aggressiveness to soybean plants. This study has found different aggressiveness levels among the 14 *S. sclerotiorum* isolates from soybean collected in Central Brazil. The aggressiveness and diversity was not associated with mycelial compatibility groups.

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Author Contributions

RAG sample collection, analysis and made the write up, VDD and VPA interpreted the results and made the write up, RMO performed the experiments, KAGBA made the write up, MCM sample collection and financial support, MGC sample collection, made the write up and advisor

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