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Phytopathology/ Original Article

Resistance of common bean lines to *Sclerotinia sclerotiorum* isolates under different environmental conditions

Abstract – The objective of this work was to evaluate the reaction of common bean (*Phaseolus vulgaris*) lines to *Sclerotinia sclerotiorum*, the aggressiveness of the isolates, and the inheritance of resistance to white mold, under two environmental conditions. The experiments were carried out under field and greenhouse conditions. In each one, four isolates were inoculated in 14 common bean lines using the straw-test inoculation method and severity on plants. No common bean line with a high level of resistance to white mold was identified, and most of the lines were moderately resistant to the pathogen. In general, the BRS Campeiro, BRS Radiante, BRSMG Talismã, and Ouro Negro cultivars stand out for resistance. Under field conditions, the UFLA 3 isolate discriminates well the lines, while, under greenhouse conditions, UFLA 26 and UFLA 92 are the most aggressive isolates. Furthermore, according to the genetic study, most of the observed variation can be attributed to horizontal resistance, although a small part is associated with vertical resistance.

Index terms: *Phaseolus vulgaris*, diallel analysis, white mold.

Resistência de linhagens de feijoeiro-comum a isolados de *Sclerotinia sclerotiorum* em diferentes condições ambientais

Resumo – O objetivo deste trabalho foi avaliar a reação de linhagens de feijoeiro (*Phaseolus vulgaris*) ao fungo *Sclerotinia sclerotiorum*, a agressividade dos isolados, e a herança de resistência ao mofo-branco, em duas condições ambientais. Os experimentos foram realizados em condições de campo e casa de vegetação. Em cada um deles, quatro isolados foram inoculados em 14 linhagens de feijoeiro-comum pelo método de inoculação “straw test” e a severidade nas plantas foi avaliada. Não se identificaram linhagens de feijão-comum com alto nível de resistência ao mofo-branco, e a maioria delas mostrou-se moderadamente resistente ao patógeno. Em geral, as cultivares BRS Campeiro, BRS Radiante, BRSMG Talismã e Ouro Negro destacam-se quanto à resistência. Na condição de campo, o isolado UFLA 3 discrimina bem as linhagens, enquanto, na casa de vegetação, UFLA 26 e UFLA 92 são os isolados mais agressivos. Além disso, de acordo com o estudo genético, a maior parte da variação encontrada pode ser atribuída à resistência horizontal, embora uma pequena parte esteja associada à resistência vertical.

Termos para indexação: *Phaseolus vulgaris*, análise dialélica, mofo-branco.

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Introduction

White mold is one of the most important diseases of common bean (*Phaseolus vulgaris* L.) (Schwartz & Singh, 2013; Lehner & Mizubuti, 2017). This disease is caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, which has a high genetic variability, even in clonal populations (Abreu et al., 2019; Attanayake et al., 2019; Abán et al., 2021). Sclerotia are the central component of epidemics as they are long-term survival structures, and as a resistant structure, they can survive for a long time in the soil. They are formed in the presence of a host susceptible to *S. sclerotiorum* and are observed internally and externally in association with the stems and pods, which hinders the management of white mold, especially with fungicides. The use of resistant cultivars is an efficient alternative in an integrated disease management to control white mold (Roese et al., 2018).

The choice of plant breeding strategy for resistance to pathogens depends on such knowledge and the type of inheritance (Nelson et al., 2018). The genetics of resistance to white mold is considered physiological and quantitative in nature. In the field, the resistance reaction of plants has been associated with physiological resistance and escape mechanisms, which involve plant architecture and flowering season. The most efficient strategy for controlling the disease is combining these two aspects. Moreover, the physiological resistance is highly complex, typically involving various polymorphisms with small effects, which makes resistance breeding a major challenge (Derbyshire et al., 2022). So far, efforts and progress of breeding programs to develop efficient cultivars have not been fully successful in Brazil and worldwide (Schwartz & Singh, 2013; Ferreira et al., 2018).

Results obtained in inoculation assays may not be always associated with results from the field (Ferreira et al., 2019). Therefore, the artificial inoculation of plants under greenhouse and field conditions should be an integral part of an accurate assessment of common bean resistance to this pathogen, especially because the variability of the plant reaction has been associated with evaluation problems (Kim et al., 2000; Kull et al., 2004). The selection of resistant genotypes both in the field and greenhouse can be a strategy to obtain genotypes with complete resistance.

The objective of this work was to evaluate the reaction of common bean lines to *S. sclerotiorum*, the aggressiveness of the isolates, and the inheritance of resistance to white mold, under two environmental conditions.

Materials and Methods

The reaction of thirteen cultivars (BRSMG Majestoso, BRSMG Uai, BRS Radiante, BRS Esplendor, BRS VALENTE, Ouro Negro, BRS Campeiro, BRSMG Tesouro, BRSMG Talismã, BRSMG União, BRS 9435 Cometa, Pérola, and BRS Estilo), and a common bean line from a recurrent selection program (RP-2) to four isolates of *Sclerotinia sclerotiorum* were assessed. The isolates were collected from a common bean production field, in the cities from the state of Minas Gerais, Brazil, as follows: UFLA 3, in Ijaci; UFLA 26, in Lambari; UFLA 54, in Viçosa; UFLA 92, in Patos de Minas.

To assess the reaction of the lines to *S. sclerotiorum* and aggressiveness of the fungal isolates, the straw-test inoculation method (Petzoldt & Dickson, 1996) was used with modifications. Initially, the inoculum preparation for field and greenhouse experiments consisted of the growth of each *S. sclerotiorum* isolate in PDA (potato dextrose agar) medium. After four days, the discs with mycelium were transferred to petri dishes (90 mm inner diameter) containing PDA medium and then kept, at 22±1°C, in a BOD (biochemical oxygen demand) for four days in the dark.

A field experiment was carried out in an experimental area of the Biology Department, Institute of Natural Sciences at the Universidade Federal de Lavras, in the municipality of Lavras, in the state of Minas Gerais (MG), Brazil (21°13'36.47"S, 44°57'40.35"W, at 975 m altitude). According to Köppen-Geiger's classification, the region climate is a Cwa type, mesothermic with mild summers and drought during winter. The soil of the experimental area is classified as Oxisol, with a clayey texture (Santos et al., 2018). In the field, a randomized complete block experimental design was carried out with three replicates. The plot consisted of 1 m row containing 15 common bean plants. Mycelium discs were removed from the edges of the 4-day colonies, using 200 μL pipette tips (Axygen, Glendale, USA), and put at the apex of the main stem of 42 day-plants (Leite et al., 2016). Seven days after the inoculation, the disease severity was visually assessed, using a diagrammatic scale from 1 to 9 (Terán et al., 2006), where: 1 represents plants without symptoms, and 9 represents plant death.

A greenhouse experiment was conducted in a randomized complete block design with three replicates. The plot consisted of three plants, in a 3 L plastic pot containing a mixture (2:1) of sifted soil and the commercial substrate Tropstrato HT (Vida verde, Mogi Mirim, SP, Brazil). Plants were kept in a greenhouse with 25±2°C and 75 % moisture. Mycelium discs were removed from the edges of the 4-day colonies, using 200 µL pipette tips, and put at the apex of the main stem of 28-day-old plants (Abreu et al., 2019). Seven days after inoculation, the reaction of all common bean plants to white mold was assessed on a diagrammatic 1-9 scale (Singh et al., 2014); where: 1 represents no sign of inoculated internode infection, immune or completely healthy; and 9 represents the disease infection >1 inch after the second inoculation node, leading to plant death.

For statistical analysis, the severity scores were subjected to individual and joint analysis of variance for each isolate, for both environmental conditions (greenhouse and field), and the means were compared by Scott-Knott's test, at 5% probability, with the aid of the SAS software (SAS Institute, Cary, EUA). For the genetic analyses, a diallel analysis was performed according to Melo & Santos (1999), following model: $Y_{ij} = \mu + r_i + a_j + S_{ij} + \varepsilon_{ij}$, where: Y_{ij} is the severity of the disease exhibited by cultivar i, when it was subjected to the inoculation of isolate j; µ is the overall mean; r_i is the fixed effect of horizontal resistance of cultivar i; a_j is the fixed effect of the aggressiveness of isolate j; s_{ij} is the fixed effect of vertical resistance of cultivar i inoculated with the isolate j; and ε_{ij} is the experimental error associated with the observation Y_{ij}.

The percentage of variation (v) observed for the sources of variation of the diallel analysis was calculated by the expression:

$$v(\%) = \frac{SS_{sv}}{SS_{total} - SS_{error}}$$

where: SS_{SV} is the sum of squares of the sources of variation (for which GAA is the general aggressiveness ability, GRA is the general reaction ability, and SIA is the specific interaction ability); SS_{total} is the sum of squares of the total variation; SS_{error} is the sum of squares of the error.

Results and Discussion

The response of genotypes to white mold varies depending on the isolate used and the assessment environment (Silva et al., 2014; Porto et al., 2019). The estimates of the coefficient of variation from all analyses of variance were of similar magnitudes to those reported in the literature (Silva et al., 2014). The results of the joint analyses of variance indicated that the sources of variation environment, isolate, and environment × isolate interaction were significant (Table 1). The breakdown of the environment × isolate interaction shows that the interaction was complex, mainly due to the behavior of the UFLA 92 isolate (Figure 1). In a breeding program aiming at resistant lines, the artificial inoculation in plants, in greenhouse and field, should be part of the evaluation of the common bean plant resistance to *S. sclerotiorum*. In general, the UFLA 3 and UFLA 26 isolates showed a similar behavior in both experiments (field and greenhouse). Thus, the reactions of common bean lines to *S. sclerotiorum* indicate that the assessment environment, that is, field and greenhouse, influenced the severity of white mold. Some authors have associated this fact with the variability of isolate aggressiveness (Kull et al., 2004). Willbur et al. (2019) highlighted the importance of using a panel representative of mildly to strongly aggressive isolates for the screening of host genotypes. Moreover,

Table 1. Summary of the joint analyses of variance for reaction of common bean (*Phaseolus vulgaris*) lines to the inoculation of four *Sclerotinia sclerotiorum* isolates, under two environment conditions (field and greenhouse).

Source of variation	Severity of white mold	
	Degree of freedom	Mean square
Common bean lines (L)	13	2.09^{ns}
Environments (E)	1	40.82**
Isolates (I)	3	64.41**
L x E	13	2.22^{ns}
L x I	39	1.35^{ns}
E x I	3	77.88**
L x E x I	39	0.96^{ns}
Error	110	0.5
Severity mean (± SD)[1]		4.39 (±0.54)
Coefficient of variation (%)		16.12

**Significant by the F-test, at 1% probability. ^{ns}Nonsignificant. [1]Determined on the basis of a diagrammatic scale from 1 to 9 (Terán et al., 2006). SD, standard deviation.

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information on the isolate aggressiveness is important, not only for the knowledge of the pathogen variability, but also for the selection of common bean plants in different assessment environments.

For the field experiment, the interaction lines × isolates were significant, which indicates that there is difference between the isolate aggressiveness levels, and that the reaction to the pathogens was not coincident in all assessed lines. In general, the common bean lines showed moderate resistance to all isolates except for UFLA 92. This result indicates that, in the field, the environmental conditions did not allow of the expression of the aggressiveness of the UFLA 92 isolate. In addition, the different behavior of this isolate between the two environments exemplifies a complex interaction between pathogen and host (Figure 1). This interaction has also been observed in other studies with this pathogen (Silva et al., 2014; Leite et al., 2016) and other important pathogens from common bean crop (Pereira et al., 2015), confirming the potential of phytopathogenic fungi to adapt to environmental conditions and produce different physiological responses.

Only the UFLA 3 isolate discriminated the lines (Table 2); for this isolate, the severity means ranged from 2.5 to 5.6, and lines were separated in three groups. Group showed the lowest scores (mean 3.47), it contains ten lines – 'BRSMG Majestoso', 'BRS Esplendor', 'BRS VALENTE', 'Ouro Negro', 'BRSMG

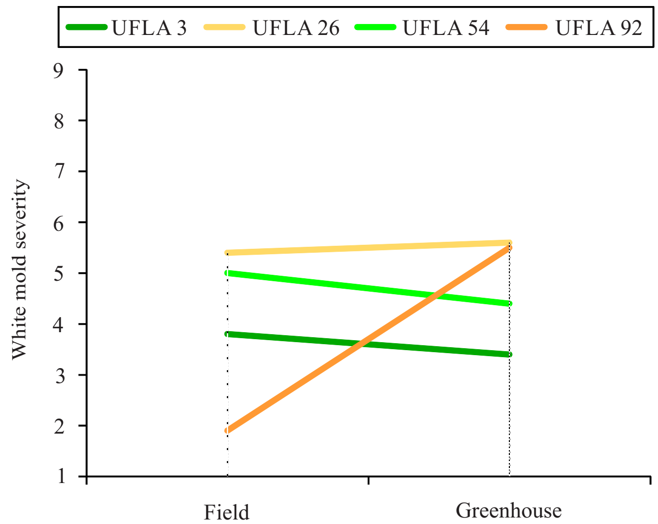

Figure 1. Means of white mold severity [diagrammatic scale from 1 to 9 (Terán et al., 2006)] in common bean (*Phaseolus vulgaris*) lines subjected to the inoculation of four *Sclerotinia sclerotiorum* isolates (UFLA 3, UFLA 26, UFLA 54, and UFLA 92), under field and greenhouse conditions.

Table 2. Means of white mold severity in common bean (*Phaseolus vulgaris*) lines caused by four *Sclerotinia sclerotiorum* isolates assessed under field (F) and greenhouse (GH) conditions. Colors indicate the reaction of lines to severity means according to Paula Júnior et al. (2012): green (means < 3) are indicative of resistant plants; yellow (means from 3.1 to 6) show moderately resistant plants; and orange (means > 6) show susceptible plants[(1)].

Common bean line	UFLA 3 isolate		UFLA 26 isolate		UFLA 54 isolate		UFLA 92 isolate		Means of lines[(2)]	
	Field	GH	Field	GH	Field	GH	Field	GH	Field	GH
BRSMG Majestoso	3.6 C	3.4 A	5.9 A	6.8 A	4.8 A	4.9 A	2.1 A	6.5 A	4.1	5.4
BRSMG Uai	5.6 A	4.1 A	5.4 A	6.3 A	4.9 A	3.7 B	2.3 A	5.8 A	4.6	5.0
BRS Radiante	4.3 B	3.0 A	5.4 A	4.7 B	4.5 A	4.7 A	1.9 A	4.3 B	4.0	4.2
BRS Esplendor	3.8 C	3.4 A	5.1 A	6.3 A	4.5 A	4.4 A	1.7 A	5.6 A	3.8	4.9
BRS VALENTE	3.6 C	3.6 A	4.9 A	5.8 B	5.6 A	3.8 B	1.9 A	5.7 A	4.0	4.7
Ouro Negro	3.9 C	3.4 A	5.7 A	5.0 B	5.6 A	4.8 A	1.9 A	3.3 B	4.3	4.1
BRS Campeiro	4.4 B	3.5 A	5.4 A	3.3 C	5.0 A	3.6 B	1.9 A	3.3 B	4.2	3.4
BRSMG Tesouro	3.8 C	3.5 A	4.9 A	5.1 B	4.8 A	3.8 B	2.1 A	6.3 A	3.9	4.7
BRSMG Talismã	2.5 C	3.1 A	5.2 A	5.3 B	5.2 A	3.8 B	1.9 A	4.9 A	3.7	4.3
BRSMG União	3.4 C	3.2 A	5.1 A	6.7 A	4.7 A	3.3 B	2.2 A	6.2 A	3.9	4.9
BRS 9435 Cometa	3.2 C	3.4 A	6.1 A	6.0 A	5.2 A	5.0 A	1.9 A	5.9 A	4.1	5.1
Pérola	3.5 C	3.6 A	6.4 A	5.3 B	4.8 A	4.9 A	1.9 A	6.8 A	4.2	5.2
BRS Estilo	4.2 B	3.5 A	4.8 A	5.0 B	4.9 A	5.4 A	1.8 A	6.7 A	3.9	5.2
RP2	3.4 C	3.4 A	4.9 A	7.0 A	5.4 A	5.2 A	2.3 A	6.0 A	4.0	5.4
Coefficient of variation (%)	14.7	14.6	16.1	16.0	12.6	17.1	21.7	15.6		
Means of isolates[(1)]	3.8	3.4	5.4	5.6	5	4.4	1.9	5.5		
Standard deviation	0.71	0.26	0.49	1.00	0.36	0.69	0.18	1.15		

[(1)]Means followed by equal letters in the columns do not differ, by Scott-Knott's test, at 5% probability. Severity determined on the basis of diagrammatic scales from 1 to 9 (Terán et al., 2006; Singh et al., 2014), assessed under field (F) and greenhouse (GH) conditions.

Tesouro', 'BRSMG Talismã', 'BRSMG União', 'BRS 9435 Cometa', 'Pérola', and RP2. According to the classification scheme of Paula Júnior et al. (2012), only 'BRSMG Talismã' was resistant to this isolate in the field, and the others were moderately resistant to it. In this classification, plants with scores from 1 to 3 were considered resistant, those classified from 3.1 to 6 were moderately resistant, and those with scores above 6 were considered susceptible to *S. sclerotiorum*.

For the greenhouse experiments, the sources of variation lines, isolates, and lines × isolates interaction were significant in the analysis of variance. The isolate UFLA 3 did not discriminate common bean lines, in contrast to what was observed in the field (Table 2). BRS Campeiro was the only cultivar classified in the groups with the lowest severity means: 3.3, 3.6, and 3.3, for UFLA 26, UFLA 54, and UFLA 92 isolates, respectively. For UFLA 26, BRS Campeiro cultivar was significantly different from the other common bean lines.

UFLA 26 and UFLA 92 were the most aggressive isolates in greenhouse, as they showed 5.6 and 5.5 severity means, respectively. Due to its aggressiveness, the UFLA 26 isolate can be recommended in experiments seeking to identify common bean genotypes resistant to *S. sclerotiorum*; moreover, this isolate maintains its high level of aggressiveness under different environmental conditions.

Positive correlations between the severity assessments performed in the field and greenhouse have been observed in some studies (Soule et al., 2011; Leite et al., 2016; Chauhan et al., 2020). Breeders could take advantage of this correlation to obtain high levels of resistance to white mold (Schwartz & Singh, 2013), since the results obtained in the field derive from the combination of physiological resistance and escape mechanisms (Abreu et al., 2019).

In the genetic study, the general aggressive ability (GAA) was highly significant, at 1% probability in both environments, but the specific interaction ability (SIA) was significant at 5% probability in the field. Eighty-nine percent of the variation observed were due to the GAA, which is indicative of quantitative resistance (Melo & Santos, 1999). The general reaction ability (GRA) was significant only in greenhouse experiment. The significant specific intereaction ability (SIA) is, initially, an indication of vertical resistance. However, the low magnitude may also represent differences of variability of common bean reactions to the isolates, as well as the difference of variability of the isolate aggressiveness (Table 3).

According to the diallel analysis, the source of variation GAA explained 89% and 55% of the variation in the field and greenhouse experiments, respectively (Table 3). All GAA estimates were significant, UFLA 92 isolate had a GAA estimate of 0.79 (Figure 2), which indicates a higher level of aggressiveness than the value obtained in the field. In both experiments, UFLA 26 isolate showed positive GAA estimates, therefore, it shows a higher level of aggressiveness.

In the greenhouse experiment, the GRA estimates of the cultivars showed that six (42%) of the 14

Table 3. Summary of the Anova of the partial diallel for reaction of common bean (*Phaseolus vulgaris*) lines inoculated with the four *Sclerotinia sclerotiorum* isolates under field and greenhouse conditions.

Source of variance	Degree of freedom	Mean squares	
		Field	Greenhouse
GAA	3	97.1**	45.2**
GRA	13	0.59	3.71**
SIA	39	0.71*	1.58**
Error	110	0.41	1.05
Severity means[(1)]		4.04	4.74

**, *Significant by the F-test, at 1% and 5% probability, respectively. Source of variance: GAA, general aggressiveness ability; GRA, general reaction ability; SIA, specific interaction ability. [(1)]Determined on the basis of a diagrammatic scale from 1 to 9 (Terán et al., 2006).

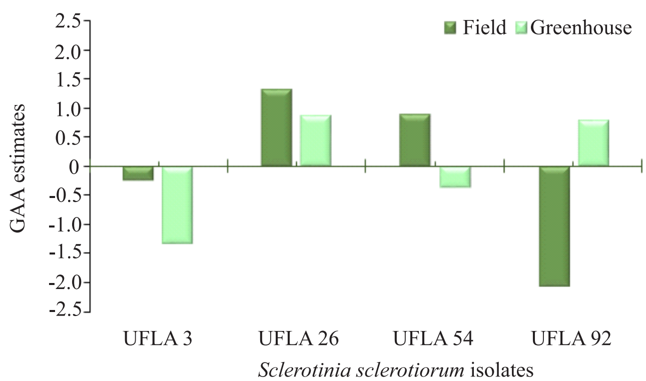

Figure 2. Estimates of general aggressiveness ability (GAA) of four *Sclerotinia sclerotiorum* isolates in experiments performed with common bean (*Phaseolus vulgaris*) lines under field and greenhouse conditions.

genotypes had significant values, two of them with negative estimates (Figure 3). Twelve (21.43%) and six (10.71%) of the 56 SIA estimates were significant in the greenhouse and field experiments.

In the present study, the occurrence of a weak host × pathogen interaction in the two assessment environments was compatible with the predominance of quantitative resistance (Niks et al., 2015). Moreover, the horizontal resistance was confirmed as well. In a literature survey, at least 40 genomic regions (quantitative trait loci) of common bean were found to be involved in resistance to white mold, confirming the quantitative nature of this resistance (Soule et al., 2011; Campa et al., 2020).

Common bean Ouro Negro, BRS Campeiro, and BRSMG União cultivars showed significant SIA estimates for, at least, two isolates in the greenhouse experiment. And in the field, for two isolates, the SIA estimates were significant in the BRS 9435 Cometa cultivar (Table 4). A significant SIA value allows of the estimation of specific effects for each isolate/line combination, albeit at a lower magnitude. Silva et al. (2014) also found a small significant difference for SIA in the reaction of common bean cultivars to *S. sclerotiorum* isolates (Silva et al., 2014). The significant effects provide information on the cultivars and isolates that contributed most to interaction.

Continuous search for resistance sources is therefore a priority in common bean breeding, and studies in different environments will be critical to achieve a

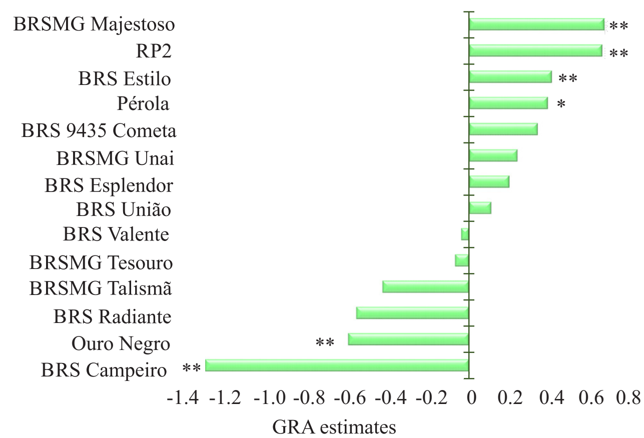

Figure 3. Estimates of the general reaction ability (GRA) of 14 common bean lines to four *Sclerotinia sclerotiorum* isolates in the experiments conducted under greenhouse conditions. Bars with **, * indicate means significant at 1% and 5% probability, respectively, by the t-student test.

Table 4. Estimates of the specific interaction ability (SIA) for the reaction of 14 common bean (*Phaseolus vulgaris*) lines to four *Sclerotinia sclerotiorum* isolates under field (F) and greenhouse (GH) conditions.

Common bean line	*Sclerotinia sclerotiorum* isolate							
	UFLA 3		UFLA 26		UFLA 54		UFLA 92	
	Field	GH	Field	GH	Field	GH	Field	GH
BRSMG Majestoso	-0.27	-0.71	0.48	0.49	-0.24	-0.08	0.03	0.29
BRSMG Uai	1.30**	0.41	-0.48	0.48	-0.59	-0.95*	-0.22	0.06
BRS Radiante	0.53	0.13	0.04	-0.33	-0.48	0.85*	-0.08	-0.64
BRS Esplendor	0.27	-0.19	-0.03	0.51	-0.2	-0.14	-0.04	-0.17
BRS VALENTE	-0.13	0.17	-0.44	0.21	0.62*	-0.56	-0.05	0.18
Ouro Negro	-0.08	0.61	0.06	-0.01	0.38	1.00**	-0.35	-1.59**
BRS Campeiro	0.44	1.37**	-0.16	-0.97*	-0.12	0.48	-0.16	-0.89*
BRSMG Tesouro	0.08	0.09	-0.26	-0.44	-0.1	-0.53	0.28	0.88*
BRSMG Talismã	-0.94**	0.1	0.12	0.06	0.54	-0.06	0.29	-0.1
BRSMG União	-0.24	-0.37	-0.09	0.95*	-0.07	-1.16**	0.41	0.58
BRS 9435 Cometa	-0.68*	-0.34	0.66*	0.04	0.16	0.28	-0.14	0.02
Pérola	-0.43	-0.27	0.89**	-0.69	-0.29	0.11	-0.18	0.85*
BRS Estilo	0.53	-0.35	-0.44	-1.03**	-0.02	0.65	-0.07	0.73
RP2	-0.36	-0.66	-0.35	0.72	0.41	0.13	0.29	-0.19

**, * Significant at 1% and 5% probability by the t-student test.

better understanding for the *S. sclerotiorum* – common bean interaction.

Conclusions

1. Environmental conditions (in the field and in greenhouse) positively influence the general aggressiveness ability of *Sclerotinia sclerotiorum* isolates artificially inoculated in common bean (*Phaseolus vulgaris*) plants.
2. No common bean lines with a high level of resistance to white mold are identified, most of which are moderately resistant to the pathogen; in general, the BRS Campeiro, BRS Radiante, BRSMG Talismã, and Ouro Negro common bean cultivars stand out for resistance.
3. Under field conditions, the isolate UFLA 3 well discriminate the lines, while under greenhouse conditions, UFLA 26 and UFLA 92 are the most aggressive isolates.
4. A predominance of horizontal resistance is observed in the *S. sclerotiorum*/common bean pathosystem.

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