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ORIGINAL ARTICLE

Comparison of inoculation methods for selecting common bean genotypes with physiological resistance to white mold

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Abstract
Although different methods of inoculation have been proposed to assess the reaction of common bean to white mold (WM) caused by *Sclerotinia sclerotiorum*, a thorough comparison among them is lacking. In this study, six approaches were tested to identify the most reproducible and efficient method for discriminating six common bean genotypes of *carioca* market class based on their resistance to white mold. These included: modified straw test (ST), cotton pad (CP), infected flower on intact plant (IFIP) or on detached leaf (IFDL), and mycelium disc on intact plant (MDIP) or on detached leaf (MDDL). All experiments were conducted in a greenhouse or laboratory in a completely randomized design with four replicates. Several statistics including coefficient of variation (CV), standard deviation, intraclass correlation coefficient (ICC), *p* value for Bartlett's test for homoscedasticity and sensitivity ratio (SR) were used as criteria for discrimination. The Spearman's correlation coefficient was used to test the association between the methods. Results showed ST as the most suitable for selecting WM-resistant genotypes, followed by the IFIP method.

Keywords *Phaseolus vulgaris* L. · White mold · Disease resistance · Plant breeding

Introduction

Fungal diseases are among the main biotic constraints that limit yield and increased production costs in common bean (*Phaseolus vulgaris* L.) crops worldwide (Singh and Schwartz 2010). White mold, also known as Sclerotinia stem rot, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most destructive diseases of common bean. Under favorable conditions, white mold epidemics may lead to severe losses in both seed yield and quality (Schwartz and Steadman 1989; Schwartz and Singh 2013).

More than 400 plant species have been described as hosts for *S. sclerotiorum*, including cultivated and wild species (Boland and Hall 1994). Such wide range of hosts favors survival and persistence of the pathogen at different environments and production systems, making disease control more difficult. In addition, the fungus produces resistance structures (sclerotia) after colonizing different host tissues, such as stems, leaves, flowers and pods. Sclerotia detach easily from advanced lesions or during plant harvest, and may remain viable in the soil for periods as long as many years (Schwartz and Steadman 1989; Miklas et al. 2013).

In addition to chemical and cultural control practices, such as the use of a lower plant density and the rational use of irrigation, the use of common bean cultivars with upright growth habit, lodging resistance, early maturity and some level of physiological resistance has been recommended to improve disease control (Paula Júnior et al. 2009; Miklas et al. 2013). Therefore, common bean cultivars combining morphological and agronomic traits that help avoidance of the disease and physiological resistance have been highly demanded by growers. So far, efforts and progress of breeding programs to develop cultivars possessing these traits have not been fully successful in Brazil and worldwide (Miklas et al. 2013; Schwartz and Singh 2013; Lehner et al. 2015; Ferreira et al. 2018).

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The inheritance of resistance of common bean to white mold is complex, composed of genetic or physiological resistance and disease avoidance mechanisms (Miklas et al. 2013; Schwartz and Singh 2013). Although simple inheritance of resistance has been reported (Genchev and Kiryakov 2002; Schwartz et al. 2006; Antonio et al. 2008), partial or quantitative resistance with additive effects is predominant and with medium to high environmental effects (Carneiro et al. 2011; Schwartz and Singh 2013; Leite et al. 2016; Vasconcellos et al. 2017). Plants with upright growth and good architecture that are resistant to lodging and have a porous canopy usually contribute to disease avoidance (Paula Júnior et al. 2009; Vieira et al. 2010; Miklas et al. 2013).

Different artificial inoculation methods have been developed and used to evaluate the reaction of the common bean to white mold and select genotypes with physiological resistance (Steadman et al. 1997; Miklas et al. 1992; Schwartz et al. 2006; Tolêdo-Souza and Costa 2007). Studies that compared these methods with regards reproducibility and effectiveness in discriminating resistant genotypes are limited. Terán and Singh (2009) were the first to compare three methods to identify physiological resistance in dry bean genotypes with different evolutionary origins. In Brazil, carioca is the most consumed common bean in Brazil, with approximately 70% of the national preference, for which limited information on screening methods is available. Time and cost-effective inoculation methods, that also require less labor, are essential for identifying resistant genotypes and, consequently, the effective use and exploitation of these sources by breeding programs. The objective of this work was to evaluate a range of methods for inoculating *S. sclerotiorum* in common bean with regards reproducibility and effectiveness in discriminating common bean genotypes based on their physiological resistance.

Materials and methods

Genetic material, plant grown and experimental design

Six common bean genotypes of the carioca market class were used in the different artificial inoculations methods: five cultivars (BRS Requinte, BRSMG Madrepérola, BRS Cometa, Pérola and BRS Estilo) and an elite line (CNFC 9500). The main agronomic traits of these common bean genotypes are presented in Table 1. Based on their known reaction to white mold in the field, and a modified straw test as reported by Ferreira et al. (2014), BRS Requinte was used as a susceptible control and CNFC 9500 was used as a resistant control. All seed samples were obtained from the collection of the Brazilian Agricultural Research Corporation (Embrapa) common bean breeding program (Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil).

In all cases, plants were grown in 8.0 L pots with two plants per pot, which were placed in a greenhouse. For planting, pots were filled with commercial substrate (Plantmax®) and soil (red latosol) at an 1:1 mixture. Fertilization was performed according to the technical recommendations for the common bean crop (Barbosa and Gonzaga 2012). Whenever needed, plants were staked using wooden sticks and cotton ropes.

For each method, all six common bean genotypes were tested (Table 1). All experiments were conducted at Embrapa Arroz e Feijão in a greenhouse or laboratory using a completely randomized design with four replicates, *i.e.*, four pots each with two plants. Thus, eight plants per genotype were evaluated in each experiment. Mean disease score of two plants per pot were used in the statistical analysis.

Inoculation and disease assessments

S. sclerotiorum inoculum

In all experiments, inoculations were performed using BRM 29673 *S. sclerotiroum* isolate, obtained from common bean plants in a commercial growing area in Ponta Grossa, PR, Brazil. This isolate has been maintained in our collection at Embrapa and has used in all experiments given its known aggressiveness.

Inoculation methods

Straw test The straw test (ST) was modified from Petzoldt and Dickson (1996), who adapted from Terán et al. (2006). The fungal isolate was streaked on Petri dishes containing potato dextrose agar (PDA) supplemented with chloramphenicol (1:1000) and maintained for 72 h in a BOD incubator at 20 ± 1 °C and a 12 h photoperiod. Three days after the second streaking, inoculations were performed when the plants reached the V4 phenological stage (third trifoliate leaf unfolded). The stem of the second trifoliate of each plant was sectioned approximately 1.0 cm from the axillary bud using a sterilized scalpel. In the cross-section of the cut, a 200 μL micropipette filter tip was inserted to add a 5.0 mm diameter disc of PDA medium colonized by the fungus. One stem per plant was inoculated. Subsequently, the plants were kept in a greenhouse for 8 days at 25 ± 5 °C and at a relative humidity of more than 85%. The disease intensity were evaluated using an ordinal descriptive scale proposed by Terán et al. (2006): 1 = plants with lesion only at the inoculation site; 2 = lesion development beyond the inoculation site; 3 = lesion reaching the entire axillary bud and the opposite side of the inoculated stem; 4 = lesion occupying all sides of the inoculated stem or 10% of the plant infected; 5 = 30% of the plant infected; 6 = 50% of the plant infected; 7 = 70% of the plant infected; 8 = 90% of the plant infected; and 9 = dead plant or with generalized necrosis.

Table 1 Main agronomic traits of the six common bean genotypes of carioca market class used for artificial inoculation with the fungus *S. sclerotiorum*, the causal agent of white mold

Genotype	100-seed weight (g)	Maturity cycle[1]	Growth habit	Direct mechanical harvesting	Breeding institution	Year of registration[2]
Pérola	26.5	Normal	Indeterminate semi-upright	Not adapted	Embrapa	1994
BRS Requinte	24.5	Normal	Indeterminate semi-prostrate	Not adapted	Embrapa	2004
BRS Cometa	24.5	Medium-early	Indeterminate upright	Adapted	Embrapa	2007
BRS Estilo	26.0	Normal	Indeterminate upright	Adapted	Embrapa	2009
BRSMG Madrepérola	25.0	Medium-early	Indeterminate prostrate	Not adapted	UFV/UFLA/Epamig/Embrapa	2011
CNFC 9500	24.0	Normal	Indeterminate upright	Adapted	Embrapa	NA

[1] Maturity cycle: Normal (85–94 days) and medium-early (75–84 days)

[2] Official registration on MALFS – Ministry of Agriculture, Livestock and Food Supply (Brazil); NA: not applicable

Cotton pad The cotton pad (CP) was modified from Bastien et al. (2012). Isolate BRM 29673 was initially streaked on Petri dishes containing PDA medium supplemented with chloramphenicol (1:1000) and were maintained in a BOD incubator at 20 ± 1 °C and a 12 h photoperiod. After 72 h, two 5.0 mm diameter discs containing mycelia of the fungus were removed and then transferred to Erlenmeyer flasks where they were submerged in 600 mL of potato dextrose [PD, potato (200 g/L) and dextrose (20 g/L)] liquid medium. This medium was shaken for 96 h at 110 rpm. Subsequently, the medium containing the mycelium of the fungus was homogenized in a blender for 30 s, and the inoculum suspension was obtained. Sterilized cotton pads (approximately 2.0 × 2.0 cm) were immersed in the inoculum suspension and placed on the axial buds of common bean plants in the V4 phenological stage, with one bud inoculated per plant. The plants were kept in a greenhouse at 25 ± 5 °C and at a relative humidity of more than 85%. After 8 days of inoculation, the length of the lesions developed at the inoculation sites was measured, using a pachymeter (DGH Technology), along the branches of the plants covered by the cotton pad.

Infected flower on intact plant The infected flower on intact plant (IFIP) method was modified from Schwartz et al. (1978). Non-senescent flowers of bean plants grown and kept in a greenhouse were initially collected and disinfested with sodium hypochlorite and distilled water (0.5%). They were subsequently air dried on sterile paper, placed in Petri dishes containing PDA medium supplemented with chloramphenicol (1:1000) and *S. sclerotiorum* mycelium and cultured for 72 h as previously described for the CP method. After 48 h, the infected flowers were used in the inoculations, and a flower was transferred with sterilized forceps to each of the three leaflets of the same trifoliate leaf in each inoculated plant in the V4 stage. After that, the plants were kept in a greenhouse at 25 ± 5 °C and at a relative humidity of more than 85%. At 8 days after inoculation, the length of the lesions formed in each leaflet was measured using a pachymeter. The score attributed to each experimental plot was obtained by the mean length of the lesions observed in the three leaflets of each of the two inoculated plants.

Infected flower on a detached leaf The infected flower in a detached leaf (IFDL) inoculation method was modified from Leone and Tonneijck (1990). Inoculum was prepared as described for the IFIP method. Detached leaflets were inoculated and placed in sterile 9.0 cm Petri dishes containing two layers of autoclaved filter paper and moistened with 3.0 mL of distilled water. Three leaflets of the same trifoliate leaf were detached and used to inoculate plants in the V4 stage. Subsequently, the plates with the inoculated leaflets were kept in a BOD incubator at 20 ± 1 °C and a 12 h photoperiod. After 48 h and 72 h of inoculation, the length of the lesions formed at the inoculation sites was measured using a pachymeter. The score attributed to each experimental plot was obtained through the mean length of the lesions observed in the three leaflets of each of the two inoculated plants. The area under the disease progress curve (AUDPC) was calculated using the following estimator: $AUDPC = \Sigma[((y_1 + y_2) \div 2) \times (t_2 - t_1)]$; where y_1 and y_2 correspond to the successive evaluations performed at times t_1 (48 h) and t_2 (72 h), respectively.

Mycelium disc in an intact plant The inoculation of *S. sclerotiorum* by the mycelium disc in an intact plant (MDIP) method was performed as described for the ST method. Discs of PDA medium and fungal mycelium with a 2.0 mm diameter were placed on three leaflets of the same trifoliate leaf of each inoculated plant in the V4 stage. Subsequently, the plants were kept in a greenhouse for 8 days at 25 ± 5 °C and at a relative humidity of more than 85%. The inoculated plants were evaluated by measuring, using a pachymeter, the length of the lesions formed in each leaflet. The score attributed to each experimental plot was obtained by the mean length of the lesions observed in the three leaflets of each of the two inoculated plants.

Mycelium disc on detached leaf The mycelium disc in a detached leaf (MDDL) inoculation method was modified from Leone and Tonneijck (1990). Preparation of the inoculum followed the procedure described for the ST method. Inoculation using discs of PDA medium and fungal mycelium was performed as described for the MDIP method. However, detached leaflets were inoculated and placed in sterile 9.0 cm Petri dishes containing two layers of autoclaved filter paper moistened with 3.0 mL of distilled water. Three leaflets of the same leaf per plant in the V4 stage were detached and inoculated. Subsequently, the plates with the inoculated leaflets were maintained in a BOD incubator at 20 ± 1 °C and a 12 h photoperiod. After 48 h and 72 h of inoculation, the length of the lesions formed at the inoculation sites was measured using a pachymeter. The score attributed to each experimental plot was obtained by the mean length of the lesions observed in the three leaflets of each of the two inoculated plants. The AUDPC was estimated as described for the IFDL method.

Statistical analysis

For each inoculation method, several statistics were obtained as suggested by Otto-Hanson et al. (2009): coefficient of variation (CV), standard deviation, intraclass correlation coefficient (ICC), *p* value for Bartlett's test for homoscedasticity and sensitivity ratio (SR). The SR was given by $SR\ (M/N) = |dM/dN| / (\sigma_M/\sigma_N)$; where M and N are two methods of inoculation; |dM/dN| is the angular coefficient from regressing N against M; and σ_M and σ_N are the standard deviations associated with the M and N methods, respectively.

The straw test was used as standard for comparison, given it the most widely used method worldwide (Petzoldt and Dickson 1996; Terán et al. 2006; Schwartz and Singh 2013; Jhala et al. 2014; Ferreira et al. 2014). To estimate the SR, a linear relationship between M and N was assumed. The square root of the residual mean square (RMS) was used to obtain the standard deviation. It was also assumed that the SR distribution fits the Snedecor F distribution. Therefore, the following considerations were made: if SR (M/N) > 1, the M method is superior to N; if SR (M/N) < 1, the N method is superior; and if the SR (M/N) = 1, the methods are equivalent.

The intraclass correlation coefficient was given by ICC $=\sigma_t^2/\left(\sigma_t^2+\sigma_e^2\right)$; where σ_t^2 is the treatment variance; and σ_e^2 is the residual variance. The correlation between inoculation methods was also estimated using Spearman's correlation coefficient. For grouping the means related to the reactions of the genotypes to the white mold in each test or inoculation method, the Scott-Knott method was applied at 5% significance. Statistical analyses were performed using R software (R Core Team 2013).

Results

The mean reactions that defined the ranking of the genotypes varied among the methods (Table 2), due to interactions between genotypes and methods or even to the errors associated with each method. The discrimination ability among the common bean genotypes by each inoculation method was influenced by the magnitude of the error associated with each observation (experimental plot or replicate).

Using ST, cultivar BRS Cometa and the elite line CNFC 9500 were the only ones that differed from the others ($P > 0.05$). Using CP, BRS Estilo had the lowest lesion length compared to other genotypes. Using IFIP, both BRS Cometa and CNFC 9500 genotypes showed small lesion size followed by BRS Estilo and Pérola, which had intermediate lesion size, and BRS Requinte and BRSMG Madrepérola with large lesions. Using IFDL, BRS Cometa and CNFC 9500 showed the smallest mean lesion sizes; Pérola showed intermediate response, and BRSMG Madrepérola, BRS Estilo and BRS Requinte showed large lesions. Using MDIP, BRS Cometa, BRS Estilo and CNFC 9500 genotypes performed best, followed by Pérola and BRSMG Madrepérola, which had intermediate scores, and by BRS Requinte, which had the largest mean lesion size. Finally, when using MDDL, BRS Requinte was the only one that differed from the other genotypes, suggesting greater susceptibility (Table 2).

The lowest coefficients of variation (CV) were observed for IFDL (5.22%) and MDDL (5.69%) followed by IFIP (9.32%), ST (11.69%), MDIP (23.47%) and CP (52.65%) (Table 3). Regarding the magnitude of the standard deviations, the lowest scores were observed for the IFDL (0.11), MDDL (0.32) and ST (0.62). Standard deviations were 4.22, 4.36 and 30.45 for MDIP, IFIP and CP method, respectively (Table 3). The ST method had the highest ICC score (0.92) followed by the IFIP (0.89), MDIP (0.85) and IFDL (0.76) method (Table 3), suggesting moderate to good reproducibility. In contrast, CP (0.31) and MDDL (0.35), ICC scores were below 0.50, and thus less reproducible.

The *p* values of Bartlett's test of homogeneity of variance ranged from 0.03 to 0.84. The CP method showed the lowest p value (0.03) among all methods (Table 3). The angular

Table 2 Mean reaction of the five common bean genotypes to white mold when evaluated by different methods of artificial inoculation

Genotype	Inoculation method[1]					
	ST	CP	IFIP	IFDL	MDIP	MDDL
Pérola	6.50 b	78.47 b	48.84 b	6.88 b	22.54 b	5.59 a
BRS Requinte[2]	6.50 b	62.88 b	61.92 c	7.20 c	35.85 c	6.36 b
BRS Cometa	2.25 a	54.96 b	28.33 a	6.70 a	11.15 a	5.84 a
BRS Estilo	7.25 b	15.64 a	46.92 b	6.99 c	8.27 a	5.64 a
BRSMG Madrepérola	6.75 b	87.74 b	59.24 c	7.12 c	20.63 b	5.60 a
CNFC 9500[2]	2.75 a	47.38 b	35.39 a	6.69 a	9.45 a	5.70 a

[1] ST: modified straw test (scores from 1 to 9); CP: cotton pad (lesion length in mm); IFIP: infected flower in an intact plant (lesion length in mm); IFDL: infected flower in a detached leaf, considering the area under the disease progress curve – AUDPC (lesion length in mm); MDIP: mycelium disc in an intact plant (lesion length in mm); and MDDL: mycelium disc in a detached leaf, considering the area under the disease progression curve – AUDPC (lesion length in mm). Values followed by the same letter in each method (column) do not differ from each other at 5% significance by the Scott-Knott method

[2] Genotypes selected as controls based on their reaction to white mold in the field and in a screening using a modified straw test as reported by Ferreira et al. (2014); BRS Requinte was used as the susceptible control, and CNFC 9500 was used as the resistant control

Table 3 Experimental variation coefficients, standard deviations, intraclass correlation coefficients and *P* values associated with Bartlett's test for the different methods of artificial inoculation with the fungus *S. sclerotiorum*

Inoculation method[1]	Coefficient of variation (CV) (%)	Standard deviation[2]	Intraclass correlation coefficient (ICC)	Bartlett's test (*P* value)[3]
ST	11.69	0.62	0.92	0.84
CP	52.65	30.45	0.31	0.03
IFIP	9.32	4.36	0.89	0.41
IFDL	5.22	0.11	0.76	0.21
MDIP	23.47	4.22	0.85	0.25
MDDL	5.69	0.32	0.35	0.50

[1] ST: modified straw test; CP: cotton pad; IFIP: infected flower in an intact plant; IFDL: infected flower in a detached leaf; MDIP: mycelium disc in an intact plant; and MDDL: mycelium disc in a detached leaf

[2] Standard deviation values estimated by the square root of the RMS of each artificial inoculation method

[3] Null hypothesis: adjustment of the data to homoscedasticity

coefficients obtained by the regression between the ST method and the other methods were significant and positive for IFIP, IFDL and MDIP, but not significant for CP and MDDL (Table 4). This results for CP may be due to its high experimental error (CV = 52.65%) (Table 3).

Sensitivity ratio (SR) values showed that all of the methods were inferior to ST, given their they were significantly lower than ST (SR < 1) (Table 5). However, due to lack of significant relationship between ST and CP and ST and MDDL, these methods cannot be assumed to have poorer technical merit than the ST Table 6.

Finally, based on Spearman's correlation coefficients results by the ST method were highly associated with IFIP and IFDL, but not with CP, MDIP and MDDL. Results of the CP method were significantly, although weakly, associated only with MDIP. IFIP results were highly associated with IFDL and MDIP, and the same trend was observed for IFDL and MDIP. Results of the IFDL and MDDL were not significantly associated (Table 6).

Discussion

In our study, the use of six commercial cultivars, which are adapted to Brazilian environmental conditions, was prioritized due to the lack of information regarding white mold reaction under controlled environment conditions. We found that BRS Requinte cultivar did not show physiological resistance to the disease, corroborating the reaction to the white mold observed in the field and in the artificial inoculation (Ferreira et al. 2014). BRS Requinte can thus be considered a susceptible control suitable for artificial inoculation experiments based on the results of all tested methods. Also corroborating previous reports, the elite line CNFC 9500 performed well as a resistant control due to being ranked at the top for resistance in all but CP method. The same was observed for BRS Cometa, which together with CNFC 9500 were at the top for physiological resistance to white mold. These genotypes show an upright plant growth and adaptation to direct mechanical harvest, which favors the avoidance from white mold in the field (Paula Júnior et al. 2009; Vieira et al. 2010; Miklas et al. 2013). The combination of morphological and phenological traits with physiological resistance to white mold in the same genotype is the main goal of breeding programs (Miklas et al. 2013; Schwartz and Singh 2013; Lehner et al. 2015; Ferreira et al. 2018).

Previously, Otto-Hanson et al. (2009) reported lower CV score for the detached leaf method (25%) than for the straw test (35%), but both were higher in comparison to our results. In contrast, Kull et al. (2003) reported higher CV scores for the leaf detached method compared with the cut stem method, which was similar to the ST method tested in the present study. The CV and standard deviation are scale-dependent measurements and, therefore, are influenced by the magnitude

Table 4 Angular coefficients obtained by regression of N in M where M and N are two methods of artificial inoculation of the fungus *S. sclerotiorum*. All methods were compared to the straw test (N)

Inoculation method[1]	Angular coefficient[2]				
	CP	IFIP	IFDL	MDIP	MDDL
ST	0.3386^{ns}	4.8525**	0.0762**	2.0968*	0.0019^{ns}

[1] ST: modified straw test; CP: cotton pad; IFIP: infected flower in an intact plant; IFDL: infected flower in a detached leaf; MDIP: mycelium disc in an intact plant; and MDDL: mycelium disc in a detached leaf

[2] Significant coefficients based on the t-test. **1% significance, *5% significance and ^{ns} not significant

Table 5 Estimates of the sensitivity ratio between M and N where M and N are two methods of artificial inoculation of the fungus *S. sclerotiorum*. All methods were compared to the straw test (N)

Inoculation method[1]	Sensitivity ratio (SR)[2]				
	CP	IFIP	IFDL	MDIP	MDDL
ST	0.0693**	0.6937*	0.4156**	0.3098**	0.0036**

[1] ST: modified straw test; CP: cotton pad; IFIP: infected flower in an intact plant; IFDL: infected flower in a detached leaf; MDIP: mycelium disc in an intact plant; and MDDL: mycelium disc in a detached leaf

[2] SR values significantly lower than one (SR < 1) according to the F test. **1% significance and *5% significance

of the scores or measurements that are given to the experimental observations or plots. Although such measurements are important indicators of experimental quality, they do not allow an efficient comparison with respect to the quality and technical merit of each artificial inoculation method.

The intraclass correlation coefficient (ICC), a measure of reliability, is suitable for assessing the homogeneity of two or more measurements and interpreted as the proportion of the total variability attributed to the evaluated object (Shrout and Fleiss 1979). Thus, in the context of this study, ICC indicated the compliance degree between the repetitions inherent to each inoculation method tested. Thus, the higher the ICC the greater the reproducibility of the inoculation method. As to the sensitivity ratio (SR), our results agreed with Kull et al. (2003) who also found ST superior to other methods when inoculating *S. sclerotiorum* on cotyledons and on detached leaves using bean and soybean plants.

The positive associations between same of the methods may be due to the expression of common genes involved in the responses of each genotype for the different methods or different plant organs. Falconer & Mackay (1996) stated that the main genetic cause of the correlation is pleiotropy, which is the simultaneous effect of the same gene in two or more traits studied. The lack of association of the responses between some methods suggests that resistance may be associated with different genomic regions and, therefore, can be governed by different genes or even activate different response mechanisms. Thus, simultaneous or sequential inoculation of the same plant or genotype with different inoculation methods may provide greater genetic gains related to the physiological resistance to the disease by favoring the identification of different genes or response mechanisms.

Collectively, our analyses showed that the ST method is the most suitable selecting common bean genotypes for physiological resistance to white mold given its discrimination ability and reproducibility. Terán and Singh (2009) also compared the modified straw test or cut-stem, infected bean flower, and infected oat seed, to screen for resistance to white mold, and concluded that the straw test was the best method. In addition to being most used worldwide (Petzoldt and Dickson 1996; Terán et al. 2006; Schwartz and Singh 2013; Jhala et al. 2014; Ferreira et al. 2014), ST is already being used by the Embrapa common bean breeding program for the routine selection of genotypes with physiological resistance to white mold, and it can be adopted by others in Brazil and worldwide even by breeding programs for other host crops of the disease. It is important to highlight the low cost and technical simplicity of execution associated with the ST, thus making it an accessible method to many research groups worldwide. The IFIP method also performed well and could used as an alternative option.

The resistance of common bean to white mold is composed of physiological resistance and avoidance mechanisms in the field (Miklas et al. 2013; Schwartz and Singh 2013). The former is a quantitative trait with additive effects and, therefore, highly influenced by the environment (Carneiro et al. 2011; Schwartz and Singh 2013; Leite et al. 2016; Vasconcellos et al. 2017). Therefore, results obtained in inoculation assays may not be always associated with results from the field. Our common bean breeding program at Embrapa

Table 6 Spearman's correlation coefficients of the reaction of common bean genotypes to white mold when artificially inoculated with six different methods

Inoculation method	Correlation coefficients					
	ST	CP	IFIP	IFDL	MDIP	MDDL
ST	–					
CP	−0.1095^{ns}	–				
IFIP	0.6927**	0.1722^{ns}	–			
IFDL	0.6532**	0.1261^{ns}	0.8835**	–		
MDIP	0.2656^{ns}	0.4765*	0.6713**	0.5522**	–	
MDDL	-0.0978^{ns}	0.1809^{ns}	0.1383^{ns}	0.2496^{ns}	0.3357^{ns}	–

**1% significance, *5% significance and ^{ns} not significant

focuses on combining avoidance mechanisms and physiological resistance to white mold in a same elite genotype. Therefore, the availability of efficient, cost-effective and reproducible methods of *S. sclerotiroum* inoculation is highly demanded.

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