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Screening common bean germplasm for resistance to genetically diverse *Sclerotinia sclerotiorum* isolates from Argentina

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ABSTRACT. White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a devastating disease that affects the common bean (*Phaseolus vulgaris*. L) crop worldwide. In Argentina, white mold has been detected in all bean production areas, reaching seed yield and quality losses up to 100% on susceptible common bean cultivars under favorable weather conditions. The aim of this study was to screen the physiological resistance of 20 common bean accessions to five genetically distinct isolates of *S. sclerotiorum* collected from the main common bean growing area of Argentina, using the greenhouse straw test. The white mold reaction was scored at 7, 14, and 21 days post-inoculation using a 1 (no disease symptoms) to 9 (severely diseased or dead plants) scale and the area under the disease progress curve (AUDPC) was determined. Highly significant differences ($p < 0.001$) were observed between isolates, accessions and genotype x isolate interaction at the three evaluations dates. All cultivars and lines were susceptible at the end of the assessment, except line A 195 which was resistant to white mold against the five isolates tested and was significantly different from all accessions. This work represents a valuable contribution to regional breeding programmes aimed to obtain cultivars with durable resistance.

Keywords: *Phaseolus vulgaris*; white mold; disease resistance; straw test.

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Introduction

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a devastating disease that affects the common bean (*Phaseolus vulgaris*. L) crop worldwide, especially in humid temperate regions (Singh & Schwartz, 2010). *S. sclerotiorum* is a homothallic fungus, which reproduces sexually by self-fertilization, resulting in the formation of apothecia that produce clonal ascospores, and asexually by mycelial germination of sclerotia (Merriman, 1976; Schwartz, Steadman, & Coyne, 1978). Under favorable weather conditions, white mold disease affects all aerial part of plants, both in vegetative and reproductive growth stages. Lesions are initially small, circular, dark green, and water soaked. White fungal mycelium growth followed by hard black sclerotia is observed in internal and external tissues of the plant, causing plant wither and death eventually (Steadman & Boland, 2005). Sclerotia can survive in soil for five or more years making this disease difficult to control. Infected seeds, sclerotia mixed with seed, infested soil, irrigation water and wind-blown ascospores, can spread the disease (Steadman & Boland, 2005).

Common bean commercial cultivars with white mold resistance are not available, but accessions with partial resistance have been identified in controlled environmental studies and field trials in wild beans (Terpstra & Kelly, 2008; Mkwaila, Terpstra, Ender, & Kelly, 2011), Middle-American (Ender & Kelly, 2005; Mkwaila et al., 2011) and Andean beans (Singh, Terán, Lema, Schwartz, & Miklas, 2007; Mkwaila et al., 2011; McCoy, Higgens, & Steadman, 2012). Moreover, introgression of higher levels of white mold resistance from *Phaseolus* species of the secondary gene pool into common bean has been performed (Schwartz, Otto, Terán, Lema, & Singh, 2006; Singh, Terán, Schwartz, Otto, & Lema, 2009, Singh et al., 2012, Singh, Schwartz, Viteri, Terán, & Otto, 2014a).

Physiological resistance and plant architectural avoidance traits, such as upright growth habit and open canopy, condition white mold reduction and are used for cultivar development in breeding programs (Kolkman & Kelly, 2003; Miklas, Porter, Kelly, & Myers, 2013). Both characteristics are quantitatively inherited and avoidance and resistance QTLs have been identified (Mkwaila et al., 2011; Pérez-Vega et al., 2012; Miklas et al., 2013, Hoyos-Villegas, Mkwaila, Cregan, & Kelly, 2015; Vasconcellos et al., 2017).

White mold physiological resistance detection is commonly performed in the greenhouse and different screening methods have been reported (Schwartz & Singh, 2013). However the straw test or cut-stem method (Petzoldt & Dickson, 1996) using one or more pathogen isolates, and multiple inoculations on the same plant have been widely applied for the identification of common bean accessions with high levels of broad-spectrum resistance (Pascual et al., 2010; Singh et al., 2014a; Viteri, Otto, Terán, Schwartz, & Singh, 2015).

In Argentina, which is among of the five major exporters of common bean worldwide, white mold has been detected in all bean production areas, reaching seed yield and quality losses up to 100% on susceptible common bean cultivars under favorable weather conditions (Singh & Schwartz, 2010; Ploper, González, Díaz, & Vizgarra, 2016). The disease is mainly managed with fungicides during flowering, combined in some cases with increased-row spacing, deep plowing and the use of upright cultivars, and often has been difficult and costly. For a successful integrated management of the disease, the knowledge about pathogenic variation in a particular geographical region is essential to minimize yield losses and reduce production costs in a context of sustainable cropping. In this sense, recent studies concerning the population variability of the white mold pathogen in the northwestern of Argentina, which is the main bean production area of the country, have been conducted (Abán et al., 2018). Molecular markers and mycelial compatibility groupings (MCG) revealed a high variability, suggesting the occurrence of both, clonal and sexual reproduction in *S. sclerotiorum* populations from common bean fields in northwestern of Argentina. Moreover, most of the isolates analyzed resulted highly aggressive towards bean plants in the greenhouse straw test. The study generated valuable information for regional common bean breeding programs aimed to obtain broadly adapted cultivars with durable resistance. Thus, the objective of this study was to assess the physiological resistance of 20 common bean accessions to five genetically distinct isolates of *S. sclerotiorum* collected from the main common bean growing area of Argentina.

Material and methods

Plant materials

A total of 20 common bean accessions were evaluated for white mold resistance under greenhouse conditions. The evaluated materials included eight cultivars, ten lines and two checks, with different seed type and growth habit (Table 1). The resistant check used was line A 195, a registered white mold resistant germplasm (Singh et al., 2007). On the other hand, the cultivar Leales 24 INTA, an Argentinian cultivar susceptible to white mold in the field and with low physiological resistance (Abán et al., 2018), was used as a susceptible check. Among the lines evaluated, four (Cachucheño, MSZ, PF1 and PP) had shown intermediate levels of resistance to white mold in naturally infested fields in the main production areas in northwestern Argentina. The cultivars Leales B30 INTA, Leales CR5 INTA, NAG 12 INTA, and Perla INTA, which are commonly grown in the northwestern of Argentina, were incorporated in the present study due to their desirable agronomic traits (Table 1). Some lines and varieties introduced from CIAT (Centro Internacional de Agricultura Tropical), IAPAR (Instituto Agronômico do Paraná, Brasil), USDA-ARS (United States Department of Agriculture-Agricultural Research Service), and UFLA (Universidade Federal de Lavras, Brasil) with no previous evaluation for white mold resistance were also included in the analysis. The evaluated germplasm is part of the work collection of the INTA breeding program of Argentina.

Table 1. Seed type, growth habit, origin and response to different common bean diseases for 20 accessions tested for white mold resistance.

	Seed				
Accesions	Type	Size[1]	Growth habit[2]	Origin	Resistance (R)/Tolerance (T)[3]
Checks					
A 195	Cream	Large	I	CIAT	R: WM, ALS; T: H, D
Leales 24 INTA	Black	Small	II	INTA	T: ALS
Cultivars					
Alubia Sel. Cerrillos INTA	White	Large	I	INTA	T: D
Leales B30 INTA	White	Large	I	INTA	R: WB; T: CBB
Leales CR5 INTA	Cranberry	Large	I	INTA	T: ALS
NAG 12 INTA	Black	Small	II	INTA	T: D, ALS
Perla INTA	White	Large	I	INTA	-
IPR Garça	White	Medium	I	IAPAR	T: D
IPR Uirapuru	Black	Small	IIa	IAPAR	T: D
Sea 5	Carioca	Small	IIb	UFLa	T: D
Lines					
Cachucheño	White	Large	I	-	-
C-01-25	Black	Small	IIa	CIAT	-
MSZ	White	Large	I	-	-
PF1	White	Large	I	-	T: ALS
PP	White	Large	I	-	-
Otto 45-79	Red	Medium	I	USDA/ARS	T: CBB
Otto 50-2	Light red	Large	I	USDA/ARS	T: CBB
Vax 1	Cream striped	Small	IIa	CIAT	R: CBB
Vax 3	Red	Small	IIa	CIAT	R: CBB
Vax 6	Red	Small	IIa	CIAT	R: CBB

[1]Size: Medium = 25 to < 40 g 100^{-1} seeds; small = < 25 g 100^{-1} seeds; large = > 40–60 g 100^{-1} seeds. [2]Growth habit: I = determinate upright, II = indeterminate upright (IIa: no climbing ability; IIb some climbing ability). [3]WM = White Mold; WB = Web Blight; CBB = Common bacterial blight; ALS = Angular Leaf Spot; D = Drought; H = Heat.

Sclerotinia sclerotiorum isolates

Five *S. sclerotiorum* isolates (SS8, SS87, SS154, SS22, and SS54) were used to evaluate physiological resistance to white mold. Isolates with different characteristics were selected based on previous studies (Abán et al., 2018) in order to identify common bean accessions with different levels of resistance. Isolates showed differences in aggressiveness and represented a distinct mycelial compatibility group, URP haplotype and location (Table 2). Isolates with different levels of aggressiveness were included in the analysis to detect potential cross-over interactions.

Table 2. Origin and features of the five *S. sclerotiorum* isolates used in this study.

Isolate	MCG[1]	URP Haplotype[2]	Aggressiveness[3]	Locality
SS8	3	29	HA	Orán, Salta, Argentina (-23° 18' 28,4''; -64° 11' 98,1'')
SS154	42	43	HA	Tartagal, Salta, Argentina (-22° 39' 45,7''; -63° 33' 45,9'')
SS87	21	33	HA	Gral. Ballivián, Salta, Argentina (-22° 59' 23,6''; -63° 52' 57,9'')
SS22	4	20	HA	Campichuelo, Salta, Argentina (-23° 06' 83,0''; -64° 01' 16,9'')
SS54	16	52	WA	Palma Sola, Jujuy, Argentina (-23° 47' 47,3''; -64° 26' 48,9'')

[1]MCG = Mycelial compatibility group. [2]URP Haplotype = Haplotype determined by URP (Universal Rice Primers) markers. [3]Aggressiveness: HA = Highly aggressive; WA = Weakly aggressive. Isolates were characterized in a previous work (Abán et al., 2018).

Inoculum preparation and greenhouse evaluations

Original isolates were maintained in the refrigerator at 4°C as sclerotia. A single sclerotium was surface-sterilized by immersion (70% ethanol for 2 min., 3% sodium hypochlorite solution for 1 min.), rinsed three times with sterile distilled water and dried in sterilized paper towels. Sclerotia were then bisected and transferred aseptically to potato dextrose agar (PDA) plates. After 72h of incubation at 20-22°C, each isolate was purified by hyphal tip isolation and placed aseptically onto new PDA plates to initiate each greenhouse experiment.

The tests under greenhouse conditions were conducted using the modified straw method (Terán et al., 2006). Five seeds of each accession were sown in 3 L-pots placed in a greenhouse at 25 ± 2°C with a 12h photoperiod. After emergence, seedlings were thinned to three plants per pot and allowed to develop to V5 growth stage. A randomized complete block design with 5 replicates was performed. Each replication consisted of three plants grown in a 3 L-pot, and 5 pots per genotype.

For the first inoculation, the main stem was cut below the fifth node leaving a 3-cm internode intact (Kull et al., 2003; Terán et al., 2006). A 200 µL Eppendorf tip stacked with two plugs of fresh mycelial from a 48-h-old PDA culture was carefully placed mycelial-side down over the cut-stem internode. In plants with a resistant white mold score a second and a third inoculation with the same *S. sclerotiorum* isolate was made 7 and 14 days after the first inoculation, respectively. Inoculated plants were incubated in a growth chamber at 22 ± 2°C with 12-h photoperiod under high humidity (80%) using humidifiers. Plants inoculated with the pure PDA plug without pathogen served as controls.

Reaction to white mold was scored on a single plant seven days after inoculation (Short-term evaluation), using the modified 1 to 9 scale according to Terán et al. (2006).To confirm the resistance, an intermediate-term evaluation and a long-term evaluation were made measuring the severity of the progression of the disease at 14 DPI and 21 DPI, respectively. Based on the disease severity scores (DS) plants were classified as resistant (1 ⩽ DS ⩽ 4), intermediate (4 < DS < 7), and susceptible (7 ⩽ DS ⩽ 9). Using these data, the area under the disease progress curve (AUDPC) (Cooke, 2006) was calculated with the following formula (Shaner & Finney, 1977):

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+n1} + Y_i)/2][X_{i+1} + X_i]$$

where: Y_i is the white mold disease severity at the *ith* observation, X_i is the time (days) at the *ith* observation, and *n* is the total number of observations. Disease severity score data and AUDPC values for each evaluation date were analyzed by analysis of variance (ANOVA), and means were compared using Fisher's least significant difference (LSD) at p = 0.05 using INFOSTAT statistical software (Di Rienzo et al., 2014). Correlation coefficients were calculated between mean white mold scores for 20 bean accessions and five *S. sclerotiorum* isolates for the three evaluations dates.

Results and Discussion

In the present study, the physiological resistance of 20 common bean accessions was assessed at 7, 14, and 21 days post-inoculation with five genetically distinct isolates of *S. sclerotiorum* collected from the main common bean growing area of Argentina. Mean squares were highly significant (p < 0.001) for isolates, accessions and genotype x isolate interaction in the three evaluation dates (7, 14, and 21 DPI) (Table 3), suggesting that the response to physiological resistance to white mold may vary according to the *S. sclerotiorum* isolate. Pascual et al. (2010), Viteri et al. (2015), and Lehner et al. (2016) also found a significant interaction between common bean accessions and *S. sclerotiorum* isolates. In contrast, non-significant interaction was reported by Otto-Hanson, Steadman, Higgins, and Eskridge (2011). These differences could be attributed to the inoculation method, the variability of isolates, the variability of the germplasm used, and the environmental conditions under which the experiments were performed.

Table 3. Mean squares from the analysis of variance for white mold scores for 20 accessions of common bean evaluated at 7, 14 and 21 days post-inoculation (DPI) with five isolates of *S. sclerotiorum*.

		Mean Squares		
Source	df	7 DPI[1]	14 DPI	21 DPI
Model	99	18.94***b	34.13***	22.56***
Accession	19	90.15***	165.10***	102.70***
Isolate	4	8.18***	5.13***	15.52***
Accession x Isolate	76	1.71***	2.92***	2.89***
Error	1,400	0.24	0.26	0.14
Total	1,499			

[1]7 DPI (Short-term evaluation); 14 DPI (Intermediate-term evaluation); 21 DPI (Long-term evaluation). *** Significance at p < 0.001.

White mold disease scores and the AUDPC values increased across the three evaluations (Table 4). The isolate SS54 characterized as weakly aggressive in previous reports (Abán et al., 2018), was the least aggressive in the three evaluation dates (Table 5) with a mean disease score of 6.39 and AUDPC value of 110.63. In contrast, significant differences (p < 0.05) were observed between SS8, SS154, SS87, and SS22, characterized as highly aggressive in previous studies (Abán et al., 2018). At 7 DPI, the isolate SS8 was the most aggressive with a mean disease score of 5.12. However, at 14 and 21 DPI the isolate SS87 was the most aggressive with mean disease scores of 7.01 and 8.34, respectively (Table 5).

Table 4. Mean white mold scores and area under the disease progress curve (AUDPC) for 20 bean accessions in the greenhouse at 7, 14, and 21 days post- inoculation (DPI) with *S. sclerotiorum* isolates.

	7 DPI		14 DPI		21 DPI	
Accessions	Mean	AUDPC	Mean	AUDPC	Mean	AUDPC
Checks						
A 195	2.40	11.30	3.12	30.13	3.96	56.14
Leales 24	6.01	23.84	8.60	77.57	9.00	142.37
Cultivars						
Leales CR5 INTA	4.04	16.29	5.64	51.20	7.29	97.81
IPR Garça	4.16	16.35	5.65	51.02	7.51	98.24
Alubia Sel. Cerrillos INTA	4.49	17.18	6.08	54.50	7.97	106.84
Perla INTA	4.73	18.83	7.15	61.95	8.61	118.16
Leales B30 INTA	5.15	19.88	7.25	65.07	8.61	122.70
NAG 12 INTA	6.28	24.57	8.97	79.28	9.00	144.27
IPR Uirapuru	6.40	23.25	9.00	81.00	9.00	145.22
SEA 5	7.21	27.33	9.00	90.77	9.00	152.07
Lines						
OTTO 45-79	3.85	15.99	5.52	49.36	7.32	95.66
OTTO 50-2	3.87	15.72	6.25	51.49	8.04	103.59
MSZ	4.20	16.57	5.75	52.30	7.15	97.63
PF1	4.52	18.20	6.48	56.35	8.29	110.32
Cachucheño	4.67	18.04	6.50	57.43	7.91	110.07
PP	4.81	18.12	6.85	59.99	8.73	116.42
VAX 1	4.63	17.99	6.99	60.04	8.20	114.20
VAX 3	4.75	18.83	7.61	63.94	8.79	123.19
VAX 6	4.95	20.01	7.29	62.21	8.73	121.32
C-01-25	6.05	23.32	8.53	76.68	9.00	140.78
Mean	4.86	19.74	6.91	61.93	8.11	117.16
LSD[1] ($p \leqslant 0.05$)	0.16	2.57	0.16	4.80	0.12	7.34

[1]LSD = Least significant difference to compare white mold score.

Table 5. Mean white mold disease score at 7, 14, and 21 days post-inoculation in response to *S. sclerotiorum* isolates SS8, SS154, SS87, SS22, and SS54.

	Number of days post-inoculation					
Pathogen isolate	7	14	21	Mean	AUDPC[1]	LSD[2] ($p \leqslant 0.05$)
SS8	5.12	6.91	8.15	6.73	116.28	0.10
SS154	4.81	6.99	8.06	6.62	118.28	0.12
SS87	4.88	7.07	8.40	6.78	118.03	0.09
SS22	4.85	6.93	8.20	6.66	116.04	0.11
SS54	4.67	6.72	7.78	6.39	110.63	0.09
Mean	4.86	6.91	8.11	6.63	115.85	0.10
LSD[3] ($p \leqslant 0.05$)	0.08	0.08	0.06	0.06	3.67	-

[1]AUDPC = area under the disease progress curve. [2]LSD = Least significant difference to compare means between number of days post-inoculation within isolate. [3]LSD = Least significant difference to compare means between isolates within an evaluation date, and between AUDPC values.

In the short-term evaluation**,** bean accessions differed significantly ($p < 0.001$) based on mean white mold scores and AUDPC values (Table 4). Mean white mold scores varied from 2.40 (A 195) to 7.21 (Sea 5) and mean AUDPC values varied from 11.3 (A 195) to 27.33 (Sea 5). Among the accessions evaluated the check A 195 was resistant to the five pathogen isolates with the lowest mean white mold scores ($\leqslant 2.73$) (Table 6). Similarly, lines Otto 45-79 and Otto 50-2 exhibited resistant mean white mold scores (< 4). However, both accessions were intermediate to pathogen isolate SS8 and Otto 50-2 was intermediate to SS87 (Table 6). Most of the cultivars had intermediate reactions with mean white mold disease scores > 4 and < 7. The accessions Leales CR5 INTA (4.04), IPR Garça (4.16), and MSZ (4.20) had significantly lower mean white mold scores than the other cultivars evaluated. However, Leales CR5 INTA was resistant to pathogen isolates SS22 and SS54 (Table 6). On the other hand, the cultivar Sea 5 was the most susceptible genotype with a mean white mold disease score of 7.21 and AUDPC value of 27.33. Sea 5 was the only genotype susceptible to all the pathogen isolates with only one inoculation (Table 6).

In the intermediate-term evaluation, the mean white mold reaction score varied from 3.12 (A 195) to 9.00 (IPR Uirapuru and Sea 5) and the mean AUDPC varied from 30.13 (A 195) to 90.77 (Sea 5) (Table 4). All accessions that exhibited resistant mean white mold scores in the short-term evaluation decreased to intermediate mean white mold scores after two inoculations, except for A 195 which continued showing a

resistance response to all isolates tested (Table 6). The mean white mold score of the lines Otto 45-79 and Otto 50-2, decreased to intermediate white mold scores of 5.52 and 6.25, respectively. Among the accessions with intermediate white mold scores, Leales CR5 INTA (5.64), IPR Garça (5.65), and MSZ (5.75) had scores significantly ($p < 0.05$) lower than PF1, Alubia Sel. Cerrillos INTA and Cachucheño. Accessions Perla INTA and Leales B30 INTA had intermediate mean white mold scores, but with disease scores < 6 (Table 6). Genotype Leales B30 INTA was susceptible to all isolates, except for SS54 with an intermediate mean white mold score of 6.27. Lines Vax 1 decreased to susceptible mean white mold scores except for isolate SS8 (5.67) and SS87 (6.67) (Table 6). Similarly, Line Vax 3 was susceptible to all isolates, except for isolate SS8 (6.13). The accessions C-01-25, Nag 12 INTA, IPR Uirapuru, and Vax 6 decreased to susceptible scores to all isolates (Table 6).

Table 6. Mean white mold scores of 20 bean accessions in the greenhouse at 7, 14, and 21 days post-inoculation with five *S. sclerotiorum* isolates (SS8, SS154, SS87, SS22, and SS54) from common bean fields of Argentina.

	Sclerotinia sclerotiorum														
	SS8			SS154			SS87			SS22			SS54		
Accessions	7	14	21	7	14	21	7	14	21	7	14	21	7	14	21
Checks															
A 195	2.7	3.4	4.0	2.7	3.4	4.0	2.2	2.9	4.0	2.2	2.9	3.9	2.1	2.9	3.9
Leales 24	6.7	9.0	9.0	6.1	9.0	9.0	6.5	9.0	9.0	5.3	8.0	9.0	5.4	8.0	9.0
Cultivars															
Leales CR5 INTA	4.2	5.5	7.2	4.1	5.6	6.7	4.2	6.5	8.0	3.8	5.4	8.0	3.9	5.2	6.5
IPR Garça	4.2	5.8	7.1	4.1	5.3	7.0	4.2	5.7	9.0	4.1	5.7	8.0	4.1	5.8	6.5
Alubia Sel. Cerrillos INTA	4.6	6.4	8.5	4.3	5.7	7.5	4.3	6.3	8.9	4.6	6.0	7.5	4.6	6.0	7.5
Perla INTA	4.8	7.3	8.9	4.3	6.8	8.5	4.9	7.7	9.0	5.1	7.1	8.6	4.5	6.9	8.0
Leales B30 INTA	5.5	8.0	9.0	5.1	7.3	8.5	5.2	7.6	9.0	5.1	7.1	8.5	4.8	6.3	8.0
NAG 12 INTA	6.1	9.0	9.0	6.3	9.0	9.0	6.0	8.9	9.0	6.8	9.0	9.0	6.1	9.0	9.0
IPR Uirapuru	7.0	9.0	9.0	6.5	9.0	9.0	5.9	9.0	9.0	6.4	9.0	9.0	6.2	9.0	9.0
SEA 5	7.9	9.0	9.0	7.6	9.0	9.0	6.7	9.0	9.0	7.7	9.0	9.0	6.2	9.0	9.0
Lines															
Otto 45-79	4.1	5.8	7.5	3.7	5.4	7.1	3.9	5.8	8.0	3.7	5.4	8.0	3.7	5.2	6.0
Otto 50-2	4.1	5.5	7.5	3.8	6.8	8.2	4.2	6.5	8.0	3.6	6.4	8.5	3.6	6.1	8.0
MSZ	4.3	5.9	7.3	4.2	5.7	7.0	4.3	5.9	8.0	4.1	5.7	6.7	4.1	5.7	6.7
PF1	4.4	6.8	9.0	4.3	6.3	8.3	4.2	6.8	8.0	4.9	6.3	8.1	4.8	6.2	8.0
Cachucheño	4.5	6.5	8.7	4.3	6.2	7.7	5.0	6.7	8.1	4.8	6.5	7.4	4.7	6.5	7.6
PP	5.0	7.0	9.0	4.5	7.3	8.9	4.8	6.3	9.0	5.0	7.2	8.8	4.8	6.4	8.0
VAX 1	4.3	5.7	7.3	4.7	7.7	8.8	4.8	6.7	8.0	4.9	7.9	8.9	4.5	7.0	8.0
VAX 3	4.8	6.1	8.0	4.5	7.7	8.9	4.9	7.4	9.0	4.8	8.8	9.0	4.7	8.0	9.0
VAX 6	6.0	7.5	9.0	5.0	7.9	9.0	4.5	6.6	7.7	4.5	7.3	9.0	4.8	7.2	9.0
C-01-25	7.1	9.0	9.0	6.1	8.7	9.0	6.0	9.0	9.0	5.6	8.0	9.0	5.5	8.0	9.0
Mean	5.1	6.9	8.2	4.8	7.0	8.1	4.8	7.0	8.3	4.9	6.9	8.2	4.7	6.7	7.8
LSD[1] ($p \leq 0.05$)	0.3	0.3	0.2	0.5	0.5	0.4	0.3	0.3	0.1	0.3	0.4	0.3	0.3	0.4	0.3

[1]LSD = Least significant difference to compare white mold score.

In the long-term evaluation, all common bean accessions were susceptible to white mold, except for the check A 195 that maintained resistant white mold scores to all isolates even after three inoculations (Table 6). The average AUDPC values varied from 56.14 (A 195) to 152.07 (Sea 5) across the isolates (Table 4). The accessions Leales CR5 INTA, IPR Garça, MSZ, and Otto 45-79 were intermediate resistance (mean white mold score 6.00 – 6.67) to white mold to isolate SS54. Also, Leales CR5 INTA and IPR Garça were intermediate to SS154 and SS22, respectively.

No significant crossover interactions between accessions and pathogen isolates were observed. This was confirmed by the significant positive correlation values obtained between the mean white mold disease scores of the 20 bean accessions and the five pathogen isolates at the three evaluation dates (7, 14, and 21 DPI) (Table 7). These results are in agreement with Pascual et al. (2010) who used four *S. sclerotiorum* isolates of different aggressiveness to screen 19 common bean accessions of which five showed intermediate resistance to all isolates 21 days post inoculation, and no crossover interactions were reported. Similarly, Viteri et al. (2015) reported no crossover interactions while screening 31 bean accessions using four isolates of different geographical origin.

Our results confirm the success of performing three inoculations per plant for the identification of bean accessions highly resistant to *S. sclerotiorum* isolates. Terán and Singh (2009) previously reported multiple

inoculations and disease assessment at different times in order to select breeding lines with improved physiological resistance to white mold. According to Viteri et al. (2015), the screening methodology using multiple pathogen isolates of different aggressiveness and inoculations per plant, and evaluations that cover both vegetative and reproductive stages is the severest test ever applied in common bean to assess the response against white mold disease. Long-term evaluations of white mold allow discarding presumed resistant accessions evaluated in earlier stages (7-14 DPI) (Viteri et al., 2015). When accessions were maintained for 21 days, the advance of the disease was generally observed and accessions that were intermediate resistant to white mold ended susceptible. For instance, lines Otto 45-79 and Otto 50-2 were resistant and had lower AUDPC values in response to three isolates (SS154, SS22, and SS54) at 7 days post-inoculation. If we only used the short-term evaluation, we would probably select these accessions; however, they were intermediate after two inoculations per plant at 14 DPI and susceptible at 21 DPI. Therefore, evaluations should be delayed at least at 21 days post-inoculation or longer, in order to cover both vegetative and most of the reproductive growth stages, as previously reported (Singh et al., 2014a; Singh, Schwartz, Terán, Viteri, & Otto, 2014b; Terán & Singh, 2009). Although the straw test is an aggressive method to evaluate physiological resistance, the reaction of these lines should be evaluated in the field since greenhouse evaluations favor the growth of the fungus in optimal conditions, which is less likely to find in the field.

Table 7. Correlation coefficient between 20 common bean accessions and five *S. sclerotiorum* isolates at 7, 14 and 21 days post-inoculation.

Number of days post-inoculation	Pathogen isolate				
		SS8	SS154	SS22	SS87
7	SS154	0,80*			
	SS22	0,77*	0,82*		
	SS87	0,83*	0,78*	0,78*	
	SS54	0,76*	0,73*	0,82*	0,80*
14	SS154	0,79*			
	SS22	0,74*	0,88*		
	SS87	0,88*	0,83*	0,82*	
	SS54	0,83*	0,87*	0,91*	0,88*
21	SS154	0,76*			
	SS22	0,74*	0,89*		
	SS87	0,83*	0,76*	0,83*	
	SS54	0,81*	0,89*	0,85*	0,77*

*Significant at $p \leq 0,001$.

The physiological resistance of A 195 is effective against genetically diverse local accessions of the pathogen. Among the common bean accessions evaluated A 195 was the most resistant. Genotype A 195 was highly resistant to the five *S. sclerotiorum* isolates even after three inoculations confirming its physiological resistance to white mold as previously reported for Brazil, Spain and the United States (Pascual et al., 2010; Pérez-Vega et al., 2012; Schwartz & Singh, 2013; Viteri & Singh, 2015; Lehner et al., 2015; Lehner et al., 2016). A 195 is a common bean line with large opaque beige seed, derived from the cross of two Andean lines (Red Kloud and ICA 10009), and developed by Singh et al. (2007) at CIAT. In previous studies, this line showed partial levels of resistance (21 DPI mean white mold score of 4.77) to different highly and weakly aggressive *S. sclerotiorum* isolates (Viteri et al., 2015) including one pathogen isolate (ARS12D) collected in Tartagal, Salta, Argentina in 2012. In the present study, the mean white mold score observed for A 195 at 21 DPI was 3.96. These higher values may be due to the variability in aggressiveness of the isolates used in both studies.

Two independent complementary dominant genes were reported to conferring resistance in A 195 in response to either highly or weakly aggressive isolates (Viteri & Singh, 2015). Furthermore, the resistance QTLs WM2.2, WM7.1, and WM8.3 have been identified as conferring white mold physiological resistance in this line (Viteri et al., 2015). Moreover, line A 195 has a determinate growth habit Type I, which is an important avoidance mechanism since it creates a less conducive microclimate for *S. sclerotiorum* to colonize blossoms and stems and also facilitates mechanical harvesting (Schwartz, Casciano, Asenga, & Wood, 1987; Kolkman & Kelly, 2002). This line also exhibits resistance to Bean common mosaic virus (BCMV) and angular leaf spot and has a moderate level of resistance to drought and heat stresses (Singh et

al., 2007). All these characteristics make A 195 a good candidate genotype to be used by breeders to improve Argentinean common bean lines.

Regarding the cultivars analyzed Leales CR5 INTA, IPR Garça, and MSZ, with growth habit type I, exhibited high levels of intermediate resistance to white mold after one and two inoculations to all isolates. These accessions deserve attention in future studies because they have high levels of resistance to other diseases.

The results generated in the present study provides, for the first time, information on the physiological resistance of 20 common bean accessions against representative isolates of the main common bean production area of Argentina using the greenhouse straw test. These results are valuable for regional common bean breeding programs aimed to obtain broadly adapted cultivars with durable resistance, contributing to the development of sustainable management strategies to minimize yield losses due to white mold in bean production.

Conclusion

Line A 195 can be used as a donor parent in recurrent and gamete selection methods for improving white mold resistance in regional common bean breeding programs.

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