Vol. 17(35), pp. 1076-1081, 29 August, 2018
DOI: 10.5897/AJB2018.16584
Article Number: 13B31C058389
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Simple and rapid detached leaf technique for screening common beans (*Phaseolus vulgarise* L.) *in vitro* against angular leaf spot (*Pseudocercospora griseola*) disease

Yayis Rezene[1,3*], Kassahun Tesfaye[2,3], Clare Mukankusi[4], Esther Arunga[5] and Paul Gepts[6]

[1]Southern Agricultural Research Institute, Molecular Biotech Lab., P. O. Box 06 Awassa, Ethiopia.
[2]Ethiopian Biotechnology Institute, P. O. Box 32853, Addis Ababa, Ethiopia.
[3]Department of Microbial Cellular and Molecular Biology, Addis Ababa University, Ethiopia.
[4]International Centre for Tropical Agriculture (CIAT), P. O. Box 6247, Kampala, CIAT-Uganda.
[5]Department of Agricultural Resource Management, Embu University College, P.O. Box 6-60100, Embu, Kenya.
[6]University of California, Davis Department of Plant Sciences, MS 11 Shields Avenue Davis, California 95616-8780, United States of America.

Received 4 July 2018, Accepted 31 July 2018

A rapid and reliable detached-leaf technique was developed for screening common bean (*Phaseolus vulgaris*) genotypes against angular leaf spot (ALS) disease caused by *Pseudocercospora griseola.* It is helpful to differentiate between susceptible and resistance common bean genotypes. Detached leaves of common bean tested genotypes were inoculated by immersing them into a spore suspension followed by placing into petri dishes at room temperature on cotton moistened with tap water. After 10-day incubation, the leaves were monitored for symptom development. This screening method was effective to determine the pathotype of *P. griseola* isolates on a set of ALS differential common bean genotypes. With this specific screening technique, all known sources of ALS resistance genes, including MEX-54, BAT332, and AND277 exhibited resistant reactions to ALS and showed no reaction and symptom development following inoculation with *P. griseola* isolates collected from Ethiopia. However, all other common bean genotypes tested together with genotypes known for their susceptibility with reaction scores 4 to 9 developed lesions in the interveinal regions of the leaves. Hence, the proposed simple and rapid screening technique was efficient, low-cost, and able to differentiate between resistant and susceptible common bean genotypes. It can be used for screening in marker-assisted gene pyramiding and backcrossing programs to facilitate early selection of seedlings in segregating progenies.

Key words: Detached leaf, *Pseudocercospora griseola*, genotype screening.

INTRODUCTION

The angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola*, infects common bean (*Phaseolus vulgaris* L.) throughout its centre of origin and domestication range in the Americas, which extends from the Northwest Argentina to Northern Mexico (Pastor-Corrales et al., 1998). Moreover, this specific disease is

*Corresponding author. E-mail: rezene77@gmail.com.

economically important in Africa (Ddamulira et al., 2015; Olango et al., 2017). It is also a major constraint to common bean production in Ethiopia where this is an important food and economically foreign exchange crop (Lemessa et al., 2011). The ALS disease is favoured by intermittent cool and warm and wet and dry weather (Correa-Victoria et al., 1989).

A precise and reproducible disease screening protocol is important in common bean disease resistance breeding programs to differentiate susceptible and resistant lines in fixed lines and segregating progenies. Screening for ALS resistance may be conducted either in the field or in a screen house; however, both involve tedious procedures and are faced with challenges (Schwartz et al., 1982; Pastor-Corrales et al., 1998; Mahuku et al., 2003; Olango et al., 2017). Field screening is dependent on the natural occurring environmental conditions which are highly unpredictable to cause the right disease pressure in addition to being compromised by the simultaneous occurrence of other bean pathogens. In addition, knowledge of the causative *P. griseola* pathotype is usually lacking. On the other hand, resistance evaluation under screen house conditions helps to control the unpredictable disease conducive environment and a number of studies have used this method to evaluate common bean genotypes for *P. griseola* resistance using specific pathogen isolates (Ddamulira et al., 2014; Ddamulira et al., 2015; Olango et al., 2017). However, the achievements of greenhouse screening depend on seedling age, inoculum quality and quantity, inoculation technique, and pre- and post-inoculation favourable environmental conditions (Twizeyimana et al., 2007) that are tedious and are prone to human error. The detached leaf disease screening method has been reported as an alternative and rapid technique for screening common bean germplasm for resistance against anthracnose disease (Tu, 1986; Pereira et al., 2013) and whit mold (Teran and Singh, 2009). It was also reported in soybean by Twizeyimana et al. (2007) to screen soybean genotypes for resistance to white-mold disease; the results from the detached leaf assay were reported by the same author and were significantly correlated and comparable with the results of screening house and field screening results. The method has also been reported for screening wheat for fusarium head blight resistance as an alternative disease screening technique (Browne and Cooke, 2004).

Conventional screening methods for common bean (involving attached leaves) against angular leaf spot disease [CIAT screening protocol (Pastor-Carlos et al., 1998)] have been commonly used as an evaluation technique. However, conventional screening techniques have their own drawback for screening common bean for ALS resistance.

The objective of this study was to evaluate an alternative, simple, low cost and high efficiency *in vitro* screening technique for common bean genotypes against angular leaf spot caused by *P. griseola* that could be used in common bean breeding programmes.

MATERIALS AND METHODS

Description of common bean genotypes

This study was conducted at the Molecular Biotech Lab of the Southern Agriculture Research Institute (SARI), Awassa, Ethiopia. Nine common bean genotypes (Table 1) were selected from a field screening trial during the main growing seasons of 2017 based on their resistance reactions to ALS under natural infection. They included 'kirmson' and G4564 that showed susceptible reaction both in field screening conditions and during the evaluation with the proposed detached-leaf technique. The HSPS10, HSPS11, and HSPS12-genotypes from the present breeding program have a resistant reaction under natural infestation and *in vitro* with the proposed method. The MEX-54, BAT233 and AND277 are three ALS differentials with known sources of resistance, whereas Redwolaita (RW) is a common bean cultivar known for its susceptibility.

Inoculum preparation and pathogenicity test

Inoculations were conducted with three strains of *P. griseola*: (1) KA060, received from CIAT-Uganda (Kawanda, Uganda); (2) two pure isolates *Pg 001* and *Pg 002* that had been previously collected from Wondogenet and Areka bean growing areas of Ethiopia. These isolates were previously characterized for their virulence. Tested strains were grown onto V8 agar medium in the Petri dishes. The resulting spores and mycelia were scrapped smoothly with a spatula and filtered through gauze; the spore concentration was adjusted to 2.0×10^4 conidia/mL using a hemocytometer.

Common bean plants were grown in the screening house. After 18 days of germination, the middle leaflet of the first trifoliate leaf of each plant was detached from each test plant genotype. At this stage, leaves were approximately two-thirds of their full development. The detached leaves set from each genotype were inoculated separately by immersion into the spore suspension and placed in Petri dishes (90 × 15 mm) onto the cotton moistened with 3.0 mL of tap water (Figure 1). The humidity of the Petri dishes was monitored and maintained using applications of watering every three days throughout the incubation period. Dew formed inside the Petri dish allowed the development of the pathogen. The petri dishes were kept at 24°C and incubated for 18 days under a 12-h daily light regime at room temperature.

To determine the pathotype of *P. griseola*, a set of 12 ALS differential and their binary numbers includes, Don Timoteo (1), G1179 (2), Bolon Bayo (4), Montcalm (8), Amendoin (16), G5686 (32), PAN 72 (1), G2858 (2) Flor de Mayo (4), Mexico 54 (8), BAT 332 (16), and Cornell 49242 (32). In addition, backcross (BC) progenies from the cross MEX-54, VAX-6 and Redwolaita from a gene pyramiding program were also used in this experiment to differentiate susceptible and resistant plants (Table 2). The popular common bean cultivar 'Redwolaita', which is susceptible, but widely grown by farmers and the resistant parents carrying known resistance genes, including MEX-54, BAT233 and AND277, were used as control in this study.

Determining virulence phenotypes

The virulence analysis was made for each monosporic *P. griseola*

Table 1. Reaction of common bean genotypes under naturally existing angular leaf spot pathogen (*Pseudocercospora griseola*) at Wondogenet and Areka during the main cropping seasons of 2017 and validation with the proposed method of screening using two identified pathotypes in both location.

Genotype	Genepool[2]	Wondogenet17	Areka17	Detached leaf method[1]	
		ALS (1-9)	ALS (1-9)	*Pg 001*	*Pg 002*
kirmson	A	8.0	8.3	+	+
G4564	A	8.5	8.3	+	+
HSPS10	M	2.0	2.3	-	-
HSPS11	M	2.0	1.8	-	-
HSPS12	M	1.0	1.3	-	-
MEX54	M	4.5	2.3	-	-
BAT332	M	1.0	3.3	-	-
AND277	A	3.0	4.8	-	+
RW[3]	M	8.0	9.3	+	+
LSD		2.3	2.4		
CV		22.7	22.8		

[1]+, Compatible or susceptible; -, incompatible or resistant. [2a], Andean; M, Mesoamerican. [3]Redwolaita.

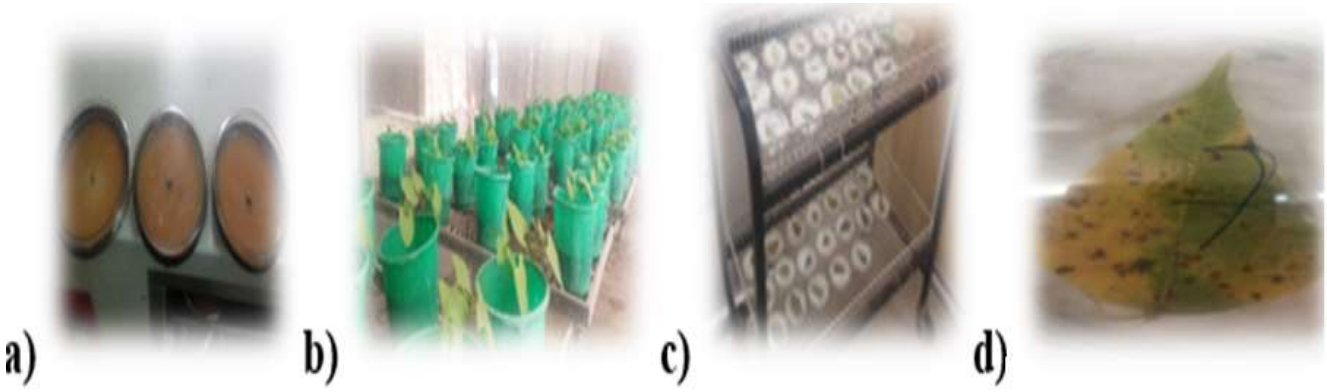

Figure 1. The proposed rapid screening technique for common bean genotypes against *Pseudocercospora griseola*, the causal agent of angular leaf spot. **a)** Single spore isolates. **b)** Common bean plants growing in the screening house. **c)** Detached leaf in Petri dish inoculated with single spore isolates and maintained until symptom development. **d)** Detached leaf *in vitro* showing ALS symptom development after inoculation.

isolate by inoculating a set of 12 common bean differentials as proposed by Pastor-Corrales et al. (1999). The differentials include six large-seeded Andean origin and six small to medium seed sized Middle American origin (Table 3). Three seeds from each differential were grown in plastic pots containing a mixture of soil, sand and organic manure in three replications in the screen house. The plants were inoculated as mentioned earlier.

Disease reaction assessment and data analysis

The treatments were arranged in a randomized complete block design with three replications. Three common bean seedlings per genotype per replication were inoculated as described. The inoculated detached leaves with spore suspension were monitored every day for disease symptom development and the CIAT disease 1 to 9 scoring protocol (Pastor-Carlos et al.,1998) was utilized in which plants that scored 1 to 3 were considered incompatible (-) or resistant and plants scored 4 to 9 were considered as compatible (+) or susceptible. Disease evaluations on individuals on individual detached leaf were made at 10, 12, 14 and 17 days after inoculations, using the 1 to 9 rating scale, where 1=immunity (no visible symptoms) and 9=very severe symptoms (van Schoonhoven and Pastor-Carlos, 1987).

RESULTS AND DISCUSSION

Evaluation of pathogenicity of *P. griseola* isolates

In vitro (Figure 1) pathogenicity of specific *P. griseola* isolates, collected from the diverse common bean growing regions of Ethiopia was confirmed by using sets of common bean differentials (Table 3). Hence, the screening technique allowed differentiating between the resistance and susceptible common bean genotypes

Table 2. Reaction of back cross(BC) segregating progenies for the angular leaf spot resistance.

Code#	Pedigree	Reaction to Pg 001 isolate	Reaction to Pg 002 isolate
KT 001	BC4[RW/MEX(+g796)] F4	-	-
KT 002	BC4[RW/MEX(+g796)] F4	-	-
KT 003	BC4[RW/MEX(+g796)] F4	-	-
KT 004	BC3[RW/MEX(+g796)] F4	-	-
KT 005	BC3[RW/MEX(+g796)] F4	-	-
KT 006	BC4[RW/VAX(+SAP6)]	+	+
KT 007	BC4[RW/VAX(+SU91)]	+	+
KT 008	BC4[RW/MEX(+g796)]	-	-
KT 009	BC4[RW/MEX/VAX(+SAP6/g796)]	-	-
KT 011	RW(REDWOLAITA)	+	+
KT 012	BC4[IB/VAX6(+SAP6)]	-	-
KT 013	BC4[IB/VAX6(+SAP6/+SU91)]	-	-
KT 014	BC4[IB/MEX(+g796)]	-	+
KT 015	IBADO(IB)	-	+
KT 016	BC4[IB/VAX/MEX(+SU91/g796)]	-	+
KT 017	BC4[IB/VAX+SU91)]	+	+
KT 018	BC4[(IB/MEX(+g796)]	-	-
	VAX6	+	+
	REDWOLAITA(RW)	+	+
	MEX54	-	-

Table 3. Common bean ALS differential cultivar and binary system for pathotype determination study using the proposed rapid technique to confirm predetermined pathotype.

Code	Cultivar ID	Seed size	Binary value	R Gene present	KA060	Pg 001	Pg 002
A	Don Timoteo	Medium	1	1 dominant	+	+	+
B	G 11796	Large	2		-	+	-
C	Bolón Bayo	Large	4		+	+	+
D	Montcalm	Large	8	2 recessives	+	+	+
E	Amendoim	Large	16	2 recessives	+	+	+
F	G 5686	Large	32	1 dominant	+	-	-
G	Pan 72	Small	1	1 dominant	+	+	+
H	G 2858	Medium	2	1 dominant	+	+	-
I	Flore De Mayo	Small	4	2 duplicates	+	+	+
J	Mexico 54	Medium	8	*Ph-2, Ph5, ph-6*	+	-	-
K	BAT332	Small	16	*ph-6*^{2}	+	-	-
L	Cornell 49242	Small	32	*Ph-3*	+	-	-
					61:63	31:7	29:5

Source: Caixeta et al. (2002) and Mahuku et al. (2004).

(Table 3 and Figure 2). The results from this specific screening technique are comparable and can be used for screening of common bean as resistance/susceptible against leaf spot disease.

The backcross progenies with known sources of *Phg-2* gene showed higher level of resistance than those without this specific locus (Table 2). All of the susceptible common bean genotypes under the natural infestation during field screening were similarly susceptible and those with high level of resistance were also resistance to the angular leaf spot caused by *P. griseola* under the rapid screening technique which was used in this specific study.

The procedure is currently part of the common bean

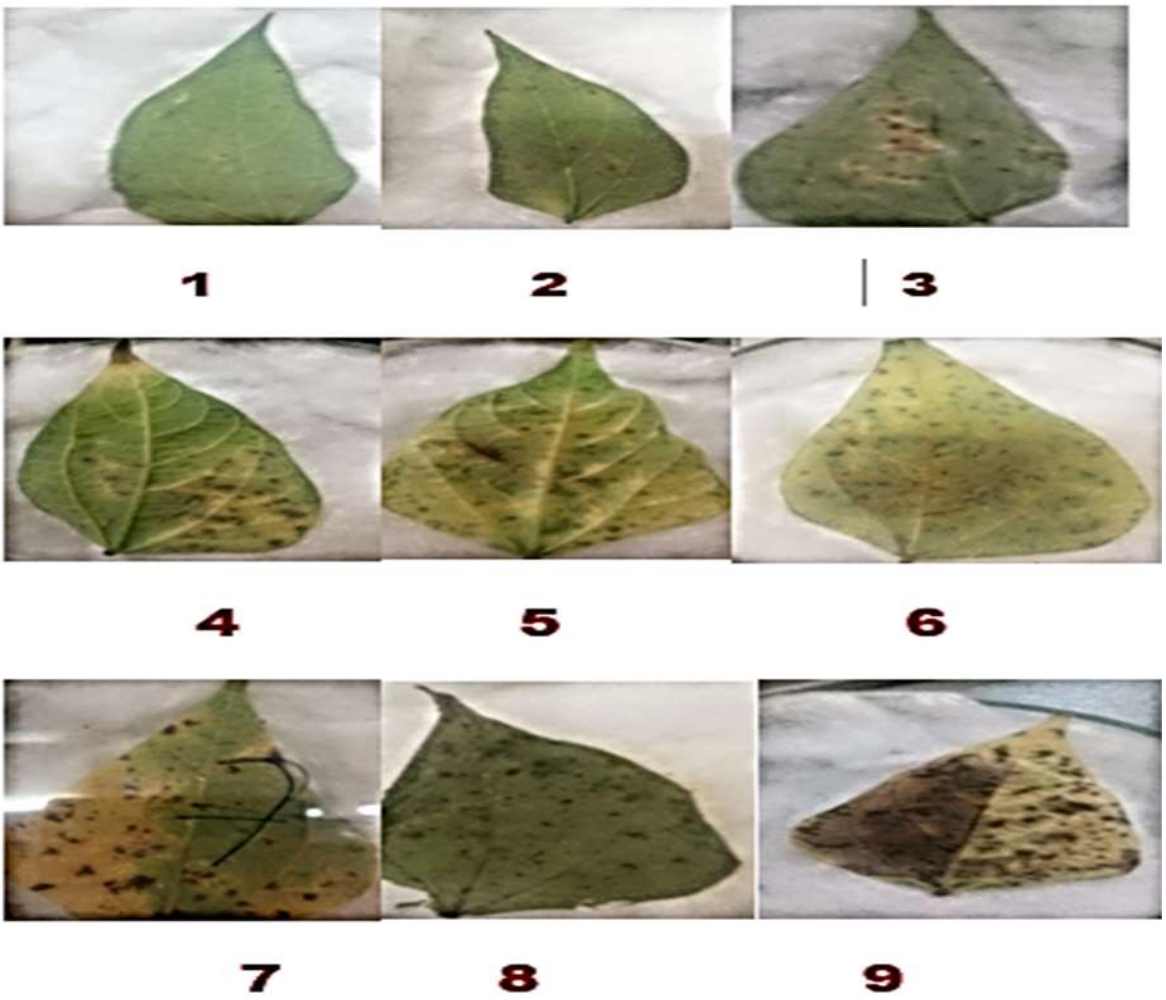

Figure 2. Different disease scoring of leaves inoculated with *Pseudocercospora griseola*, the causal agents of angular leaf spot disease, using the proposed detached-leaf inoculation method. 1 = no visible symptoms; 3 = 10 to 20% of plants infected and/or about 5% of the total plant area affected by the pathogen; 5 = 40 to 50% of plants infected and/or about 20% of plant area affected; 7 = 60 to 70% of plants infected and/or about 40% of plant area affected; and 9 = >80%. Scores 1 - 3 considered as a resistance; 4 to 6, moderately resistance; 7 to 9, susceptible.

breeding program at SARI, Ethiopia and is used in combination with molecular markers to facilitate selection of resistant progenies in each backcross generation. The program adopted this specific technique for screening pyramided back cross progenies with multiple resistance which would have been difficult with the conventional screening technique. Tu (1986) demonstrated many advantages of the detached-leaf screening method of common bean against anthracnose disease as compared to the attached-leaf conventional method of screening. This method can prevent unnecessary interactions between pathogens during bean evaluation for resistance to different pathogens or different races of the same pathogen. With this method of screening, a bean plant can be screened repeatedly because inoculations can be done at any growth stage without affecting the growth and seed production of the rest of the plant. This method also saves time in breeding for resistance and works very well on newly expanding leaves because they appear to have less contamination. This method can speed up selection of progenies during the marker-assisted backcross gene pyramiding and can be used with molecular markers to select progenies with disease resistance traits during each subsequent backcross generations. From the evaluation of a large number of common bean genotypes, four lines of common beans were identified as resistant under field screening at the Wondogenet and Areka research farms during the 2017 cropping season under naturally occurring epidemics. Contrasting pathogen population were also exposed with the proposed screening technique and inoculated with individual pathotype of *P. griseola*. The genotypes showed comparable reaction with both methods. Hence, the proposed screening technique was found to reliably

assess the disease reaction.

In breeding programs and during gene pyramiding, it will also be challenging when using only conventional method of screening to evaluate plant symptoms but this alternative rapid detached leave method is proposed for marker-assisted gene pyramiding in combination with molecular markers to aid the selection of resistant progenies during each backcross generation. In addition, the proposed method can help during evaluation of progenies for different pathotypes using leaves from the same plant simultaneous evaluation from the same plant and this could be difficult with conventional evaluation method (Table 3 and Figure 2).

Finally, the result determined from this specific *in vitro* screening technique through predetermined pathotype (Ddamulira et al., 2014) which was received from CIAT-Uganda (Table 1) revealed that the proposed procedure and the screening technique was comparable and efficient for pathotype determination using sets of common bean differentials.

Conclusion and implications for common bean breeding

The proposed rapid and efficient *in vitro* screening technique can be used as an alternative method in common bean breeding programs for screening genotypes for ALS resistance caused by *P. griseola*. This screening technique can be used in combination with molecular makers to assist and facilitate progeny selection during a marker-assisted gene pyramiding and backcrossing program. The methods can be used during simultaneous evaluation of progenies for multiple resistance as a single plant can be evaluated several times at any growth stage without affecting the growth of the plant, thus, facilitating a selection program at a reduced cost. This alternative screening technique will provide great opportunity for breeders working in gene pyramiding and marker-assisted back-crossing programs to evaluate common bean genotypes and progenies for multiple disease resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the funding and support from the Kirkhouse Trust. They also extend their thanks to the South Agricultural Research Institute (SARI) for support during the experiment and the CIAT & ABC partner countries for sharing the germplasm and *P. griseola* isolates.

REFERENCES

Browne RA, Cooke BM (2004). Development and evaluation of an in vitro detached leaf assay for pre-screening resistance to Fusarium head blight in wheat. European Journal of Plant Pathology 110(1):91-102.

Correa-Victoria FJ, Pastor-Corrales MA, Saettler AW (1989). Angular leaf spot. 2. ed . In: Schwartz HF, Pastor-Corrales MA (eds.). Bean production problems in the tropics. Centro Internacional de Agricultura Tropical (CIAT), Cali, CO.pp. 59-75.

Ddamulira G, Mukankusi C, Ochwo-Ssemakula M, Edema R, Sseruwagi P, Gepts P (2015). Gene Pyramiding Improved Resistance to Angular Leaf Spot in Common Bean. American Journal of Experimental Agriculture 9(2).

Ddamulira G, Mukankusi C, Ochwo-Ssemakula M, Edema R, Sseruwagi P, Gepts P (2014). Identification of new sources of resistance to angular leaf spot among Uganda common bean landraces. Canadian Journal of Plant Breeding 2(2):55-65.

Lemessa F, Waktole S, Mulatu W (2011). Association between angular leaf spot (Phaeoisariopsis griseola (Sacc.) Ferraris) and common bean (*Phaseolus vulgaris* L.) yield loss at Jimma, Southwestern Ethiopia. Plant Pathology Journal 10(2):57-65.

Mahuku GS, Jara C, Cajiao C, Beebe S. (2003). Sources of resistance to angular leaf spot (Phaeoisariopsis griseola) in common bean core collection, wild Phaseolus vulgaris and secondary gene pool. Euphytica 130(3):303-313.

Olango N, Tusiime G, Mulumba JW, Nankya R, Fadda C, Jarvis DI, Paparu P (2017). Response of Ugandan common bean varieties to *Pseudocercospora griseola* and angular leaf spot disease development in varietal mixtures. International Journal of Pest Management 63(2):119-127.

Pastor-Corrales MA, Jara C, Singh SP (1998). Pathogenic variation in, sources of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. Euphytica 103(2): 161-171.

Pereira AC, Celin EF, Rezende LL, Carneiro VQ, Lehner MDS, Paula Júnior TJ, Carneiro JES (2013). Alternative method for inoculation of *Colletotrichum Lindemuthianum* on common beans. Annual Report of the Bean Improvement Cooperative 55:139-140.

Schwartz HF, Corrales MP, Singh SP (1982). New sources of resistance to anthracnose and angular leaf spot of beans (*Phaseolus vulgaris* L.). Euphytica 31(3):741-754.

Teran H, Singh SP (2009). Efficacy of three greenhouse screening methods for the identification of physiological resistance to white mold in dry bean. Canadian Journal of Plant Science 89(4):755-762.

Twizeyimana M, Ojiambo PS, Ikotun T, Paul C, Hartman GL, Bandyopadhyay R (2007). Comparison of field, greenhouse, and detached-leaf evaluations of soybean germplasm for resistance to *Phakopsorapachyrhizi*. Plant Disease 91(9):1161-1169.

Tu JC (1986). A detached leaf technique for screening beans (*Phaseolus vulgaris* L.) *in vitro* against anthracnose (*Colletotrichum lindemuthianum*). Canadian Journal of Plant Science 66(3):805-809.

Van Schoonhoven A, Pastor-Corrales MA (comps.) (1987). Standard system for the evaluation of bean germplasm. Centro Internacional de Agricultura Tropical (CIAT), Cali, CO. 56 p.