A NEW INOCULATION PROCEDURE TO EVALUATE ANGULAR LEAF SPOT DISEASE IN BEAN PLANTS (*Phaseolus vulgaris* L.) FOR BREEDING PURPOSES

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Introduction - Angular leaf spot incited by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris is a severe disease in common bean (*Phaseolus vulgaris* L.). This disease has been considered one of the main problems responsible for the low yield of this crop in Brazil. Pyramiding resistance genes to overcome diseases caused by pathogens has been proposed as an strategy to obtain durable and wide spectrum resistance. However, pyramiding can be a difficult task when only conventional inoculation techniques are used to evaluate the plant symptoms (Faleiro et al., 2003). The objective of this study was to develop an alternative method for inoculation of *P. griseola* using excised bean leaves.

Material and Methods - *P. griseola* race 63.23 was obtained from a monosporic culture. The fungus was grown in petri dishes containing tomato juice agar medium. The resulting spores and mycelia were scrapped smoothly with a spatula and filtered through gauze and the spore concentration was adjusted to 2.0 x 10^4 conidia/mL. For the excised leaf method, which was based on Tu (1986), 18 days after germination the middle follicle of the first trifoliate leaves of each plant was excised when they had reached approximately two-thirds of their full development. The detached leaves were inoculated by immersion into a spore suspension and placed in petri dishes (90 x 15 mm) on a filter paper moistened with 3.0 mL of fresh distilled water. The petri dishes were incubated for 18 days in a BOD kept at 19°C, under a 12 hour daily light regime (Phillips® TLT 20W/75RS) at about 28 µmoles $m^{-2}s^{-1}$. The two remaining follicles of the first trifoliate leaves were sprayed with a spore suspension on both leaf surfaces and incubated in a mist chamber (20-22°C; 100% relative humidity). To test the inoculation methods the 12 angular leaf spot differential cultivars (Table 1) and cultivars Vermelho (susceptible control) and AND 277 (resistant control, gene *Phg-1*) (Carvalho et al., 1998) were used. One hundred and fifty-six F_2 plants from a cross between cultivars AND 277 and Vermelho were inoculated with *P. griseola* race 63.23 by the two inoculation methods. The data obtained in the evaluations were submitted to the chi-square test to determine the efficiency of both methods to detect the segregation of one dominant resistance gene (*Phg-1*) in the F_2 population (Table 2).

Results and Discussion -The symptom evaluation showed that the proposed inoculation procedure was efficient to evaluate angular leaf spot resistance/susceptibility (Figure 1). The 12 common bean differential cultivars and the cultivars Vermelho and AND 277 presented the same scores when inoculated by both methods (Table 1). The F_2 plants used in this work also presented the same phenotypes when tested either with the proposed inoculation procedure or with the conventional inoculation method (Table 2). Out of 156 F_2 plants, 120 were resistant and 36 were susceptible to *P. griseola* race 63.23. The chi-square test support the hypothesis that a single dominant gene is responsible for resistance of AND 277 to the *P. griseola* race 63.23 (Table 2). The excised leaf inoculation procedure shows some advantages when compared with the conventional inoculation method. The alternative procedure is non-destructive. Susceptible plants can be kept alive even after their reaction to the pathogen is determined. In addition, different pathotypes can be simultaneously evaluated with leaves from the same plant. The proposed inoculation procedure is now being used in association with molecular markers to aid the selection of resistant plants in the bean breeding program of the Federal University of Viçosa. This program aims to pyramid anthracnose, angular leaf spot and rust resistance genes in common bean elite-cultivars.

Table 1. Reaction of the bean angular leaf spot differential series and of control cultivars inoculated by two different methods with *P. griseola* race 63.23. Each value represents the evaluation of four different plants.

Cultivars	Binary value	Reaction degree and phenotype		
		Conventional method	Excised leaf method	Phenotype[c]
Don Timoteo	1	9	9	S
G 11796	2	9	9	S
Bolon Bayo	4	9	9	S
Montcalm	8	9	9	S
Amendoin	16	9	9	S
G 5686	32	9	9	S
Pan 72	1	9	9	S
G 2858	2	9	9	S
Flor de Mayo	4	9	9	S
Mexico 54	8	1	1	R
BAT 332	16	9	9	S
Cornell 49-242	32	1	1	R
[a]AND 277	-	1	1	R
[b]Vermelho	-	9	9	S

[a]Resistant control; [s]Susceptible control; [c]Resistant (R) and Susceptible (S).

Table 2. Angular leaf spot resistance/susceptibility of F_2 plants derived from a cross between Vermelho and AND 277, inoculated by *P. griseola* race 63.23, using the excised leaf and conventional inoculation methods.

Inoculation method	Expected ratio[a]	Observed ratio	χ^2	P-value[b]
Excised leaf	3:1	120:36	0.307	57.91%
Conventional	3:1	120:36	0.307	57.91%

[a]3:1 (R_:rr), expected ratio for the segregation of one dominant resistance gene in the F_2 population; [b]Estimated probability value.

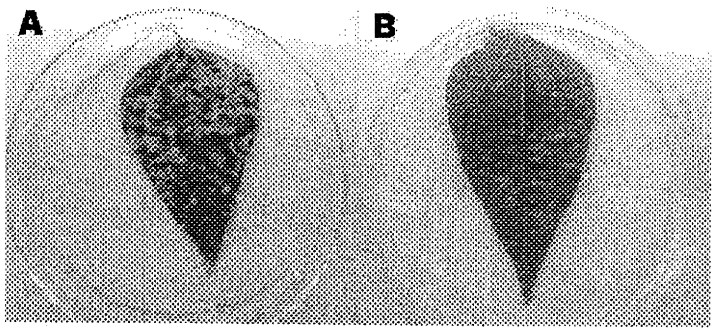

Figure 1. Angular leaf spot symptoms observed on leaves of cultivars (**A**) Vermelho (susceptible control) and (**B**) AND 277 (resistant control) inoculated by the excised leaf method.

Acknowledgement: This work was financed by grants from CNPq and FAPEMIG.

References

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