PHAEOISARIOPSIS GRISEOLA VIRULENCE PATTERN AND RAPD DIVERSITY

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Angular leaf spot (ALS) caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, is one of the most damaging diseases of common bean around the world. The fungus infects most plant aerial parts, especially leaves and pods, causing premature defoliation that culminate in poorly filled pods and reduced seed quality. In Brazil, the world's largest common bean producer and consumer, ALS is both prevalent and economically important. Without adequate disease control, yield reductions of up to 80% have been reported. In addition to yield losses, bean market quality may be affected. In this country, a major part of bean production is by medium/small or subsistence farmers who do not apply fungicides to their crops due to its high cost. As a result, the development of resistant cultivars would provide a more environmentally and friendly control alternative that could be used in integrated crop protection strategies. Breeding beans for ALS resistance is, however, complicated by the wide pathogenic variability that *P. griseola* presents, rendering a resistant variety to be susceptible in a different year or locality. In Brazil, all isolates that have been identified belong to the Mesoamerican gene pool which are capable of inducing disease in cultivars of both bean gene pools. This work presented the objectives (i) to study the pathogenic variability of *P. griseola* using the international differential cultivars set, (ii) to study the genetic diversity of this fungus by the RAPD technique and (iii) to investigate the hypothesis of the presence of multiple infections in angular leaf spot lesions, in leaves and pods, caused by this pathogen.

The 96 *P. griseola* isolates used in the present study, were obtained from naturally-infected bean leaves and pods of the genotypes FEB 209, FEB 200, FEB 170 and A 805, collected in the Embrapa Rice and Beans Experimental Station, in the county of Santo Antonio de Goias, Goias, Brazil. To verify the multiple infection hypothesis, one leaf and one pod were harvested from each of the above mentioned genotype. From each of these leaves and pods it was selected four lesions and from each lesion it was made three isolates, totaling 24 isolates (12 from leaf and 12 from pod) per genotype. For virulence analysis, the international set of 12 common bean differential cultivars were sown in aluminum pots containing 2,0 kg of soil at the rate of five seeds per pot. Conidia suspensions, for all inoculations, were obtained by culturing the fungus in bean-leaf-dextrose-agar medium. Inoculum was adjusted to 2×10^4 conidia mL^{-1}. The bean plants were then inoculated at the V_3 development stage by spraying the conidial suspension onto the upper and lower leaves surfaces. The inoculated plants were incubated in a moist chamber (> 95% RH) for 36 h. After this period of time, plants were transferred to greenhouse benches for another 14-18 days and evaluated for symptoms according to the 1-9 descriptive scale developed at CIAT. Plants rating from 1 to 3 were considered as resistant and from 4 to 9 as susceptible. For the molecular study *P. griseola* was grown in liquid medium (200 g potato and 10 g glucose/L of water) for 12-14 days. The RAPD reactions were carried out with primers OPA18, OPD06, OPD07, OPE09, OPJ10, OPK09, OPL12, OPL14 and OPL17 and performed in a final volume of 25 µL containing 25 ng of template DNA, 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.4 µM of one primer decamer, and 1

unit of *Taq* DNA polymerase. Amplification reactions were performed in a thermocycler model PTC – 100 as follows: a preliminary denaturation step (3 min at 94°C), followed by 45 cycles of denaturation (1 min at 94°C), primer annealing (1 min at 35°C) and prime extension (2 min at 72°C), and a final extension step of 10 min at 72°C. Amplified fragments were separated by electrophoresis on 1.5% agarose gel immersed in TBE (89 mM Tris-borate, 2 mM EDTA(pH8.0)) at 3 V cm^{-1}. DNA bands were visualized under UV light after staining the gels with ethidium bromide and photographed with the Eagle Eye photosystem.

Up to five different pathotypes were identified in either leaves or pods of each cultivar. In the present study, from the same lesion more than one pathotype was identified. The existence of more than one pathotype of the fungus in a single lesion is recognized as a multiple infection process. Pathotypes 63-63, 63-55, 63-31, 31-55, 63-47, 63-39 and 63-23 were identified (Table 1). All this variability had previously been described by other authors in Brazil. The fact that most of these pathotypes caused disease in all Andean cultivars suggest that only the incorporation of resistance genes from this gene pool may not be effective to control angular leaf spot under Brazilian conditions. As a result, the best way of controlling bean angular leaf spot could be pyramiding resistance genes from both Andean and Mesoamerican genes pools what could lead to effective and durable disease management.

Table 1. Pathotypes identification based on the reaction of 12 differential cultivars inoculated with 96 isolates of *Phaeoisariopsis griseola* collected in the S[to] Antonio de Goiás, GO

Pathotype	Differential Cultivars												Number of isolates
	Andean						Mesoamerican						
	1[a]	2	3	4	5	6	7	8	9	10	11	12	
31-55	+[b]	+	+	+	+	-[b]	+	+	+	-	+	+	7
63-23	+	+	+	+	+	+	+	+	+	-	+	-	5
63-31	+	+	+	+	+	+	+	+	+	+	+	-	9
63-39	+	+	+	+	+	+	+	+	+	-	-	+	5
63-47	+	+	+	+	+	+	+	+	+	+	-	+	6
63-55	+	+	+	+	+	+	+	+	+	-	+	+	25
63-63	+	+	+	+	+	+	+	+	+	+	+	+	39

[a]: 1-Don Timóteo; 2-G 11796; 3-Bolón Bayo; 4-Montcalm; 5-Amendoin; 6-G 5686; 7-PAN 72; 8-G 2858; 9-Flor de Mayo; 10-México 54; 11-BAT 332; 12-Cornell 49-242. [b]: +/- = Compatible/Incompatible reaction.

Based on nine RAPD primers, a total of 57 fragments were generated. Out of these 35 were polymorphic in at least one of the 96 *P. griseola* isolates under study. As a mean 6.33 bands/primer and 3.88 polymorphic bands/primer were produced. RAPD analysis performed on the 96 isolates revealed great genetic variability clustering them into six groups at a distance of 64%. No association was observed between results from the molecular analysis and the inoculation of the isolates in the international differentials.

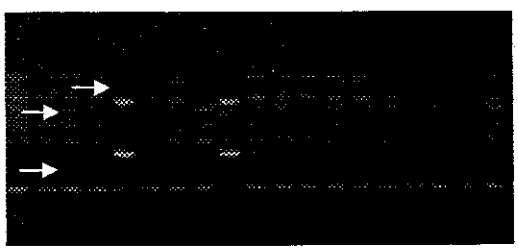

Figure 1. Electrophoretic analysis of amplification products obtained with primer OP L14. Arrows indicate polymorphic bands.

CIAT's *Phaseolus vulgaris* core collection for resistance to