GENETIC DIVERSITY OF *Phaeoisariopsis griseola* BY THE RAPD METHOD

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Angular leaf spot, caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferr., has been recognized as one of the most important air borne disease of common beans (*Phaseolus vulgaris* L.) in Brazil. Control methods for this disease includes cultural practices, chemical control and genetic resistance.

The development of new bean cultivars resistant to angular leaf spot needs the previous acknowledgment of the pathogen variability. It has been demonstrated that *P. griseola* presents great variability. The method universally accepted to determine such variability is through the inoculation of a bean differential set. The advent of the DNA recombinant technique offers new methodologies for a more complete and secure investigation of the genetic of plants and phytopathogenic fungi. Diversity and characterization studies of several plant species and fungi have been realized by the Random Amplified Polymorphic DNA (RAPD). The major advantages of this approach are its simplicity, the universality of used primers, tolerance to a wider range of DNA concentration and the lack of environment influences in the results.

Erlenmeyer flasks of 250 mL of liquid medium (200 g Potato and 10 g glucose per liter of water) were inoculated with 5-6 agar disks of 0,7 cm in diameter from each isolate. The cultures were placed in a rotary shaker (110-120 rpm) and incubated at room temperature for 12-15days. Mycelia were harvested by filtration through filter paper and placed in liquid nitrogen and then transferred to -80 °C refrigerator until DNA extraction. DNA extraction was performed according to the SDS procedure using TE buffer for final dilution of the samples. RAPD reactions were carried out with the primers OP K 07, K 09, L 12, L 14, L 17, R 03, R 04 e R 17. Amplification reactions were performed in thermocycler model PTC-100™. Each reaction of 25 µL contained 25 ng of DNA, 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 0,4 uM of one primer decamer, and one unit of Taq DNA polymerase. Each amplification cycle consisted of one initial DNA denaturation step of 3 minutes at 94 °C followed by 45 cycles of 1 minute at 94 °C, 1 minute at 35 °C and 2 minutes at 72 °C and a final extension step of 10 minutes at 72 °C. Amplification products were separated by electrophoresis in a 1.5% agarose gel and visualized under UV light and photographed with the Eagle Eye II photosystem (Stratagene Inc, La Jolla, CA). The DNA bands obtained for each individual were scored based on their presence (1) or absence (0). Only the most intense bands were considered. Cluster analysis was done by the Unweighted pair-group average and Euclidean distances. All calculations were done with the program Statistica, version 5.0.

The analyze of 98 *P. griseola* isolates collected at Damolandia and Inhumas county, State o Goiás, Brazil, revealed great genetic diversity. According to the method used and at a distance level of 62,5%, isolates were clustered in 4 groups (Figure 1.). Groups 1 and 4 were formed by isolates originated only from Damolandia (4 isolates) and Inhumas (2 isolates) counties, respectively, while groups 2 and 3 were formed by isolates from both counties. Although the obtained dendrogram did not show any clustering in these groups according to the isolate origin, it was possible to observe a tendency of the isolates in these two groups be from Inhumas and Damolandia counties, respectively. A non linkage among RAPD markers and the pathotypes

used in this study was observed. No pathotype specific band were observed in the present study. By the distance matrix it was possible to observe that the distance among isolates varied from 0,00 to 5,39, indicating great variability among the isolates of the fungus *P. griseola* what is in accordance with the variability found for this pathogen determined by inoculating the differential set. Although the RAPD allows a more complete study of the *P. griseola* genome, when compared to the virulence test, unhappily this technique does not permit the identification of which amplified locus is linked to virulence in the fungus. This fact makes this technique not appropriated for the identification of *P. griseola* pathogenic variability.

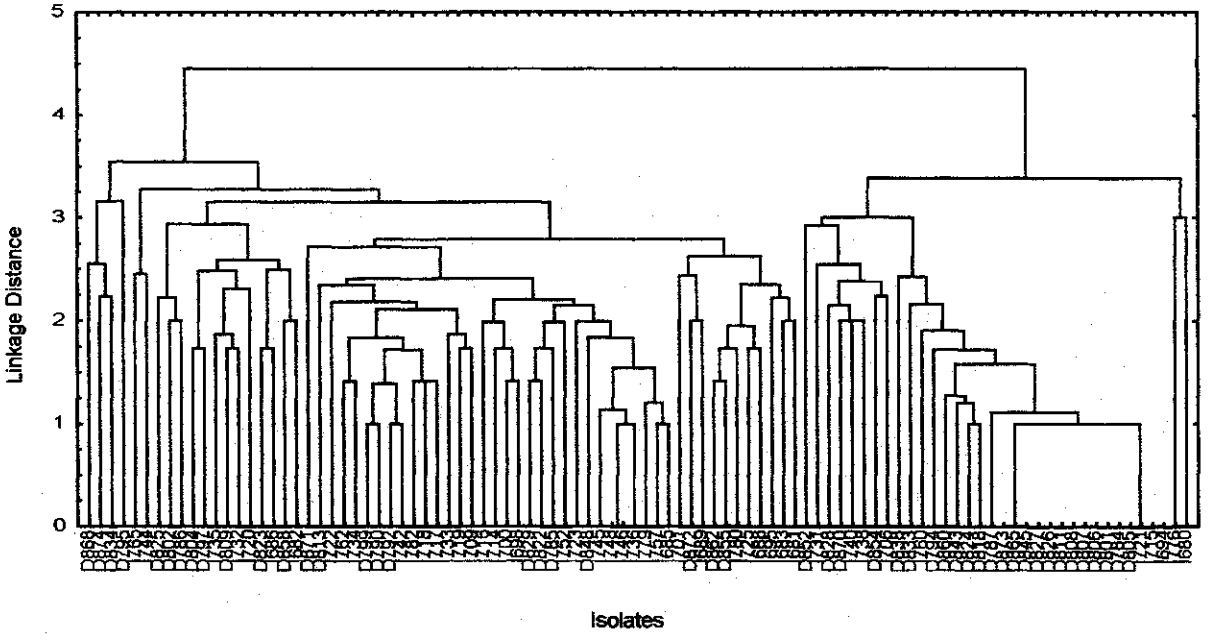

Figure 1. Dendogram of 96 isolates of *Phaeoisariopsis griseola* based on the RAPD method using 8 primers (OP K 07, K 09, L 12, L 14, L 17, R 03, R 04 e R 17). Embrapa Rice & Beans, 2002. (D = Damolandia; I = Inhumas).