ORIGIN OF SPORES TO START AN ANGULAR LEAF SPOT EPIDEMIC

Sartorato, A.[1], Mesquita, G.M.[1], Boogaerts, J.[1], Garcia, R.A.V.G.[2]

[1]Embrapa Arroz e Feijão, C. Posta 179, CEP 75375-000, Santo Antônio de Goiás - GO,
[2]Universidade Federal de Goiás, Goiânia - GO - sartorat@cnpaf.embrapa.br

Common bean is one of the most popular leguminous crop cultivated in Brazil. It is the host of innumerous diseases including angular leaf spot, caused by the fungus *Phaeoisariopsis griseola*, which is responsible for up to 70% yield losses in the field. The objective of this work was to study the origin of *P. griseola* spores to start an angular leaf spot epidemic.

A randomized complete block design with 3 treatments (control, resistant and susceptible cultivar) and 4 replications were used. To provide a source of inoculum 3 bean plants was transferred to the center of each plot (except in the control plot) 44 days after sowing. The inoculum source plants stayed in the center of each plot for 7 consecutive days. Before been transferred to the field, these plants were inoculated in the greenhouse, in the V3 development stage, with a inoculum concentration of 2×10^4 spores ml^{-1} of the isolate Ig 746,. Three leaflets showing angular leaf spot symptoms were collected from each plot 57, 64 and 75 days after sowing. From each leaflet it was prepared a monosporic isolate. DNA from each monosporic isolate was extracted and amplified using the RAPD technique and the primers OPK 10, OPL 14, OPL 17, OPL 18, OPR 03 e OPR 13. Amplification reactions were performed in a thermocycler model PTC-100™ in a reaction volume of 25 µL containing 25 ng of DNA, 0.1 mM of each dNTP, 2.0 mM of $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.4 µM of one primer decamer and 1 unit of *Taq* DNA polymerase. Each amplification cycle consisted of 15 seconds at 94°C, 30 seconds at 35 °C and 60 seconds at 72 °C. After 40 cycles, a final extension step of 10 minutes at 72 °C was accomplished. Amplification products were separated by electrophoresis in a 1.5% agarose gel and photographed with the Eagle Eye II photosystem. The DNA bands amplified in the RAPD reactions were then scored according to their presence (1) or absence (0) for each pathogen isolate. The matrix so generated was submitted to cluster analysis, which was performed by the unweighted pair-group average and the Squared Euclidean distance methods.

The fungus *P. griseola* presented great genetic diversity (Figure 1). At a genetic distance of 15% arbitrary limit it was possible to divide the isolates in three groups. The first two groups are formed by one isolate each. The third group presented a total of 106 isolates. It is also possible to observe that the subgroup that contains the isolate used as a control (Ig 746) presented six other isolates very similar to those from the control, probably, indicating that these isolates had their origin from the isolate Ig 746, or that in the field there were some isolates very similar to the control isolate. The fact that angular leaf spot is seed transmitted is not relevant in this pathosystem since the percentage of disease transmission is no higher that 2,5%. All other isolates of the third group were divided in other subgroups, indicating that they are different from each other and from those belonging to the control isolate subgroup. As a conclusion, one could say that the isolates of *P. griseola* that reached the experimental area are genetically different from the control isolate (Ig 746) suggesting that an angular leaf spot epidemic starts from spores that comes from the outside of a bean field.

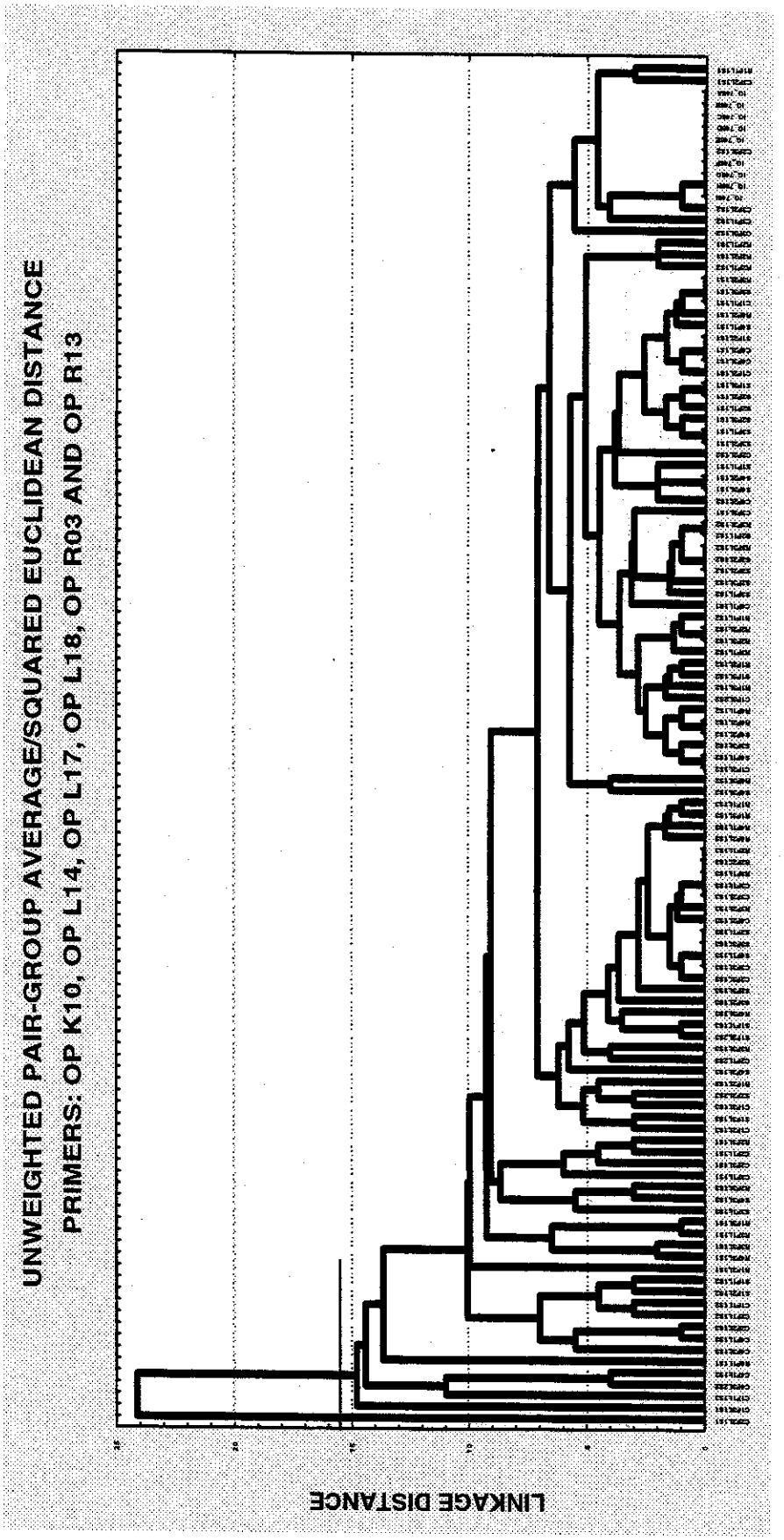

Figure 1. Dendogram of 108 isolates of *Phaeoisariopsis griseola* based on the random amplified plymorphic DNA (RAPD) method using six primers (OPK 10, OPL 14, OPL 17, OPL 18, OPR 03 and OPR 13).