MOLECULAR ANALYSES OF *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola* FOR PATHOTYPES CHARACTERIZATION

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Anthracnose and angular leaf spot caused by *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola*, respectively, are among the major bean (*Phaseolus vulgaris* L.) diseases in Brazil. Differential cultivars have been currently used for classification of these pathotypes. However, the differential sets of cultivars for these pathogens have only twelve cultivars and some carry the same genes (1,4,6). Therefore, they hardly represent all the host genes. So, it is not expected that the resistant genes present in these cultivars can classify precisely all pathotypes. Molecular markers have been used to help the classification of *C. lindemuthianum* pathotypes. DNA from pathotypes 64, 65, 73 and 89 of *C. lindemuthianum* has been amplified by RAPD technique. Specific RAPD markers have been identified using bulks of isolates of each pathotype. *Primers* OPAR09, OPAT18, OPAT09 and OPAO07 showed characteristic bands for such pathotype (2,3).

The objective of this research was to (i) amplify DNA from 41 isolates of 20 *C. lindemuthianum* pathotypes already identified (5) with *primers* OPAR09, OPAT18, OPAT09 and OPAO07 with the objective of verify the presence or absence of bands previously described as specific for pathotypes 64, 65, 73 and 89 and (ii) amplify DNA from 27 isolates of *P. griseola* pathotypes 63.23, 63.31, 63.39 and 63.55 with *primers* OPA07, OPG05 and OPA11 that could show specific DNA bands for these pathotypes.

Different from Mesquita et al. (1997), our results did not demonstrate the existence of specific RAPD markers for *C. lindemuthianum* and *P. griseola* pathotypes (Figures 1-a and 1-b). These can be explained based on the assumption that molecular studies may show different results when one use few isolates and pathotypes compared to a large number due to pathogen variability.

Nevertheless, the 20 *C. lindemuthianum* pathotypes analyzed with *primers* OPAR09, OPAT18 and OPAT09 could be defined in two groups: one being formed from isolates of pathotypes 23, 69, 64, 95, 65, 89, 87, 55, 81, 117, 339, 83, 102 and the other by isolates of the remaining pathotypes (Figure 2a). In the first group, four isolates of pathotype 64 showed genetic distance of zero. In the second group six isolates of pathotype 73 showed genetic distance of zero.

RAPD analysis of four *P. griseola* pathotypes with *primers* OPA07, OPG05 and OPA11 defined two groups: one being formed by four isolates of pathotype 63.23 and the second by isolates of the remaining pathotypes (Figure 2b). The second group could be subdivided in three subgroups: one formed by 5, 3, 2 and 4 isolates from pathotypes 63.23, 63.39, 63.31 and 63.55, respectively. The second, being formed by 1, 2 and 5 isolates from pathotypes 63.55, 63.39 and 63.31, respectively. The third formed by one isolate of pathotype 63.55. The pairs of pathotype-isolated 63.55/29-3 vs. 63.55/58-1 showed genetic distance zero.

According to our data, the DNA bulk technique was efficient to identify polymorphism among isolates of *C. lindemuthianum* and *P. griseola*, which may be used as a tool for genetic diversity studies among pathotypes. It is possible that using bulk techniques the polymorphic regions linked to virulence gene(s) may be reached and that such regions are similar among different pathotypes. The data generated by genetic distances classify the pathotypes into different groups and could help to identify genetic similarity among isolates.

More genetic and physiology studies of *C. lindemuthianum* and *P. griseola* will be necessary to find the appropriated molecular technique to reach the virulence genomic regions of the different pathotypes from these pathogens. Such basic knowledge would provide a system to better characterize pathotypes based on molecular markers.

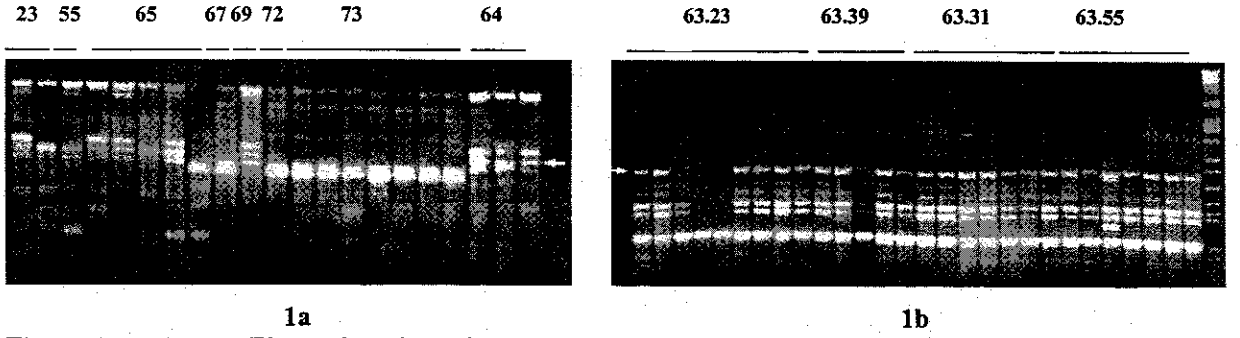
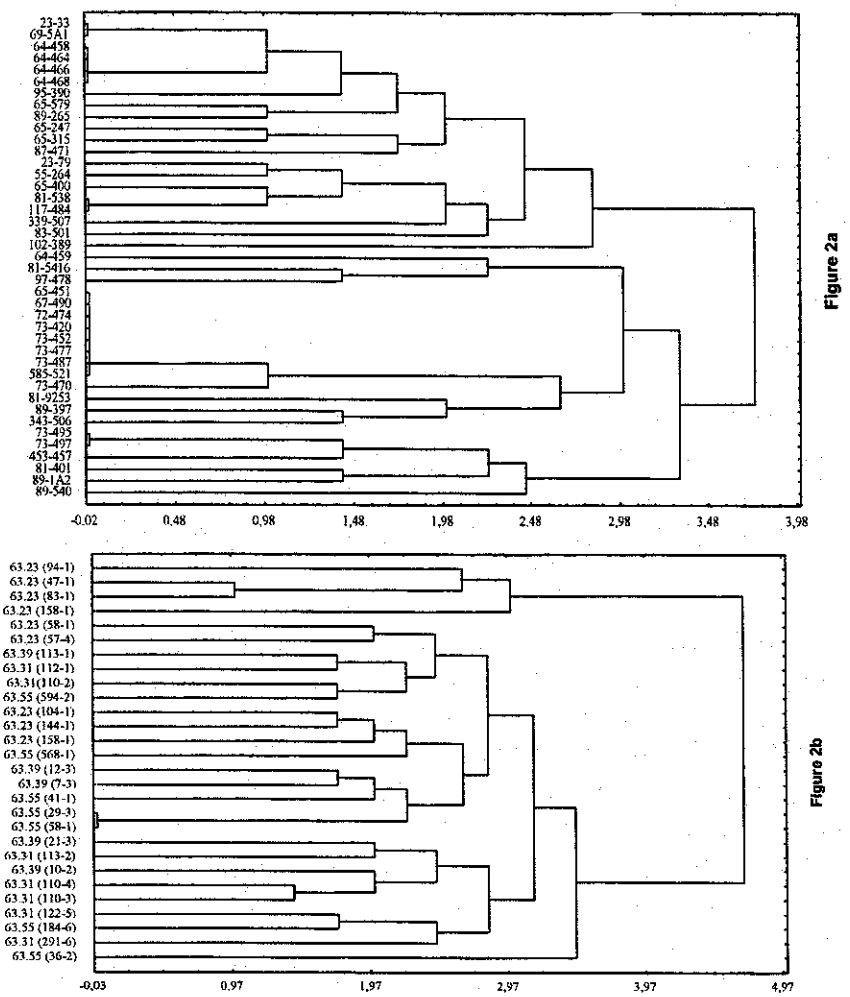


Figure 1a and 1b – Electrophoretic analyses of amplification products obtained with *primers* OPAR09 (1a) and OPG05 (1b) of DNA of isolates of *C. lindemuthianum* and *P. griseola* pathotypes. The arrows indicate the bands supposedly linked to isolates of *C. lindemuthianum* and *P. griseola* pathotypes 64 (1a) and 63.31(1b), respectively. The pathotype classification of each isolate is shown directly in the figure.



Figures 2a e 2b – Dendrograms obtained of genetic analysis of isolates of *C. lindemuthianum* (2a) and *P. griseola* pathotypes (2b).

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