MOLECULAR CHARACTERIZATION OF *COLLETOTRICHUM LINDEMUTHIANUM* HAPLOIDS AND DIPLOIDS

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INTRODUCTION

The *Colletotrichum lindemuthianum* (Sacc. et Magnus) Lams.-Scrib., causal agent of anthracnose in common bean (*Phaseolus vulgaris* L) presents a wide genetic variability and it has motivated researchers to develop studies directed to the comprehension of the molecular interactions emphasizing this pathological system. The formation of conidial anastomosis tubes between conidia within acervuli has been observed during *C. lindemuthianum* conidiogenesis and described as a mechanism that favors exchange of nuclear material and organelles between incompatible strains. Conidial anastomosis tubes were detected in pairs or in multiple associations of conidia (Roca et al., 2003). Therefore, the present work had as objective to study the parasexual recombination in *Colletotrichum lindemuthianum* through analysis of auxotrophic mutants of races CL2047 and CL23 (haploids) using RAPD molecular marker.

MATERIAL AND METHODS

The experiments were carried out to obtain heterokaryons among the genetic complementary mutants. Mycelium plugs (5 mm) of each *nit* mutant were paired equidistantly apart (approximately 1.0 cm) on Petri dishes containing BM + NaNO3 for vegetative complementation tests. Those dishes were incubated at 22°C for 12 to 21 days and then examined for prototrophic heterokaryotic growth. The diploids CL2047-4//CL23-19 and CL23-14//CL23-15 were haploidized, which permitted the isolation of two recombinant parasexuals, haploids, CLrec6 and CLrec4. The DNA extraction was conducted according to the methodology proposed by Raeder and Broda (1987). The extracted DNA was submitted to PCR reactions using the primers RAPD OPC8 and OPF5.

RESULTS AND DISCUSSION

The recombinant CLrec4 presented an 800bp band which CL2047-4, when analyzed with the primer OPF5 (Figure 1). On the other hand, the analyses conducted with the primer OPC8 showed the presence of a 1,300bp band in the diploid CL23-14//CL23-15, which is not found in race CL23 (Figure 2). However, the recombinant CLrec6 presented two bands with 350bp and 800bp, being polymorphic in relation to the diploid CL2047-4//CL23-19. The recombinant CLrec4 demonstrated to be polymorphic in relation to the diploid CL23-14//CL23-15 by presenting a band with 1,000bp. The results showed the occurrence of a parasexual cycle in *C. lindemuthianum*, confirming the importance of this process in the variability generation in this pathogen.

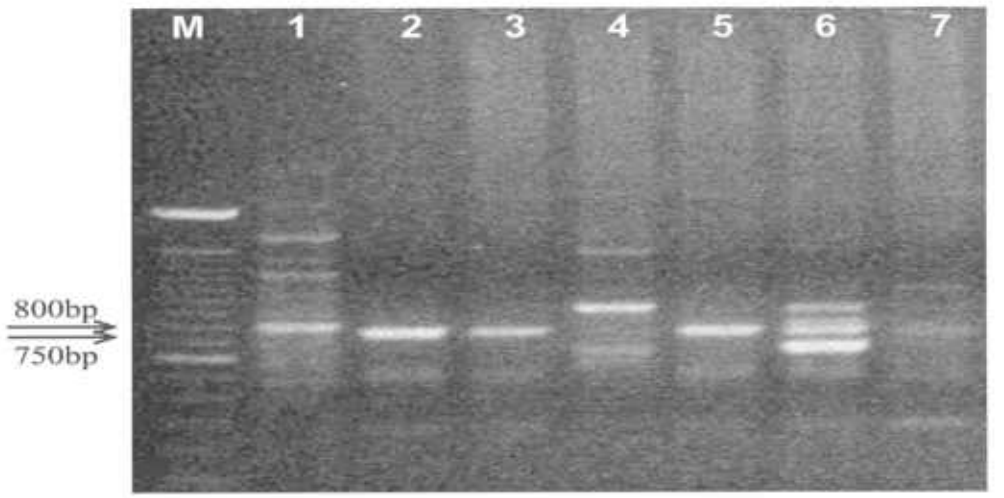

Figure 1 - Amplification of genomic DNA using OPF5 RAPD marker. Lanes are as follows: M, molecular weight marker (100bp ladder); 1, CL23; 2, CL2047; 3, CL2047; 4, CL23-14//CL23-15; 5, CL2047-4//CL23-19; 6, CLrec4; 7, CLrec6.

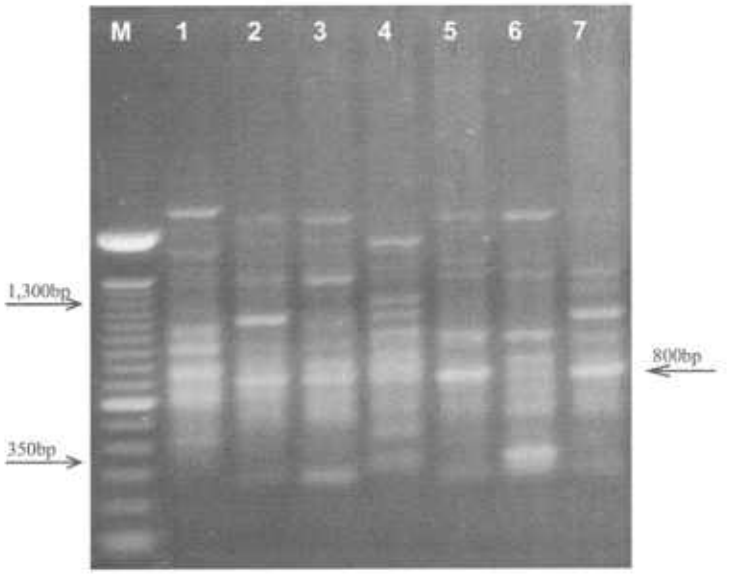

Figure 2 - Amplification of genomic DNA using OPC8 RAPD marker. Lanes are as follows: M, molecular weight marker (100bp ladder); 1, CL23; 2, CL2047; 3, CL2047; 4, CL23-14//CLR23-15; 5, CL2047-4//CL23-19; 6, CLrec4; 7, CLrec6.

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