VARIABILITY AMONG *COLLETOTRICHUM LINDEMUTHIANUM* ISOLATES BY RAPD MARKERS

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner is one of the most important diseases in common bean. Control strategies include, mainly, the development of resistant cultivars. However, the major limitation for developing durable resistance in common bean cultivars is the magnitude of variability in *C. lindemuthianum* which has been reported worldwide (Mahuku & Riascos, 2004; Talamini et al., 2004). Understanding the pathogenic variability is a fundamental point in breeding program. Combining virulence and molecular analysis will lead to a better understanding of the variability present in *C. lindemuthianum*. Therefore, the objective of this study was to analyze the pathogenic and genetic diversity of *C. lindemuthianum* isolates collected in Minas Gerais State, to generate data to be used in breeding programs for resistance to common bean anthracnose.

MATERIAL AND METHODS

48 isolates of *C. lindemuthianum* obtained from naturally-infected common bean cultivars were used in this study. Isolates were collected in four regions from Minas Gerais State, Brazil: Buriti, Coromandel, Monte Carmelo and Patos de Minas (Alto Paranaíba Region); Januária and Unaí (North of Minas Region); Lambari, Lavras and Luminárias (South of Minas Region); and Viçosa (Forest Zone Region), as shown in Table 1.

Table 1. *Colletotrichum lindemuthianum* isolates, pathotypes (P) and counties (C) of Minas Gerais State, Brazil.

Isolate	P	C[1/]	Isolate	P	C	Isolate	P	C	Isolate	P	C
1	65	BU	13	65	LM	25	81	VI	37	65	VI
2	65	BU	14	65	LM	26	81	VI	38	65	VI
3	69	BU	15	8	LV	27	81	VI	39	81	VI
4	81	BU	16	64	LV	28	81	VI	40	81	VI
5	65	CO	17	65	LV	29	81	VI	41	81	VI
6	65	MC	18	65	LV	30	89	VI	42	83	VI
7	65	MC	19	65	LV	31	89	VI	43	87	VI
8	81	PM	20	73	LV	32	65	VI	44	87	VI
9	81	PM	21	73	LV	33	65	VI	45	337	VI
10	87	PM	22[2/]	73	LV	34	65	VI	46	337	VI
11	65	JÁ	23[2/]	73	LV	35	65	VI	47	337	VI
12	81	UN	24[2/]	73	LV	36	65	VI	48	337	VI

[1/] County: BU: Buriti; CO: Coromandel; MC: Monte Carmelo; PM: Patos de Minas; JA: Januária; UN: Unaí; LM: Lambari; LV: Lavras; LU: Luminárias; VI: Viçosa.
[2/] Isolates of sexual stage (*Glomerella cingulata* f.sp. *phaseoli*)

C. lindemuthianum isolates were grown in liquid medium for 7 days (110 rpm at 20°C). The RAPD reactions were carried out with the *primers* OP A13, OP AQ11, OP AS19, OP BA03, OP BA06, OP BA08, OP BB01, OP BB03, OP BB05, OP BB08, OP BB12, OP BB13, OP BB15 and OP BB19 and

performed in a final volume of 14 µl containing 4 µl water, 35 ng of genomic DNA, 50 µM of each dNTP and 0.4 µM oligonucleotide *primer*, 50 mM Tris-HCl, pH 8.0, 2.0 mM $MgCl_2$, 20 mM KCl, and 0.6 units Taq DNA polymerase. Amplification was programmed for 1 initial desnaturation cycle (94°C for 2 minutes), followed by 38 cycles of 2 minutes at 94°C, 15 seconds at 37°C and 1 minute at 72°C and a final extension step of 2 minutes. Amplification products were separated by electrophoresis and visualized under UV light before to be photographed with the Kodak EDA – 290 photographic camera. The genetic similarities and clustering analysis were performed by using the Nei and Li coefficient and UPGMA, respectively. The analysis of molecular variance (AMOVA) was performed.

RESULTS AND DISCUSSION

A total of 64 polymorphic bands were used to analyze the 48 *C. lindemuthianum* isolates. An average of 4.57 bands was generated per *primer*. The genetic similarity among the isolates ranged from 0.65 to 0.99. The clustering showed the occurrence of five groups. For the AMOVA (Table 2), each region, except the North of Minas Region (only two isolates), was considered as a population. The AMOVA showed that the genetic differentiation among regions is significant (Φ_{ST} = 0.0394, p < 0.016), with 3.94% and 96.06% of the genetic variability being among regions and within regions (populations), respectively. The free exchange of seeds among regions may have contributed substantially to the increased variability within regions instead of among regions variance. Further clustering at pathotype level was performed, where each pathotype (except the 8, 64, 69, 83 and 89 pathotypes) was considered a population, and an analysis of molecular variance was carried out (Table 2). The greater part of the variability was detected within pathotypes (75.24%). It was also observed that the genetic variability among pathotypes was highly significant (Φ_{ST} = 0.248, p < 0,000).

Table 2. Summary of AMOVA of three regions (AP, SM and ZM), from Minas Gerais State and for seven pathotypes of fungus (*C. lindemuthianum*) evaluated by RAPD markers.

S.V.	DF	SS	Variance components	% Total	Φ_{ST}	P
Regions						
Among regions	2	23.592	0.3014	3.94	0.039	0.016
Within regions	43	315.625	7.3401	96.06		
Total	45	339.217	7.6415	100.00		
Pathotypes						
Among pathotypes	4	86.678	2.0727	24.76	0.248	0.000
Within pathotypes	37	232.989	6.2970	75.24		
Total	41	319.667	8.3696	100.00		

CONCLUSIONS

The existence of high variability, has been demonstrated which validated studies emphasizing the great potential of this fungus to generate variability, and the need to assess the mechanisms involved in obtaining this genetic variability.

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