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## GENETIC VARIABILITY OF *Colletotrichum lindemuthianum* BY SEQUENCING ITS REGIONS

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### INTRODUCTION

Anthracoze, caused by *Colletotrichum lindemuthianum*, is one of the most important diseases of the common bean (*Phaseolus vulgaris* L.) in Brazil and in other regions of the world (Pastor-Corrales et al. 1994). Anthracnose occurs more severely in places where relative humidity conditions above 91% and temperatures ranging from 18° and 22°C predominate (Kelly et al. 1994). High genetic variability of *C. lindemuthianum* has been described worldwide, and more than 247 different races of the pathogen have been identified, which 35 occur exclusively in Brazil (Nunes et al. 2013). The high number of physiological races and the complexity in the use of genetic resistance of the *C. lindemuthianum* fungus are evidence of wide virulence diversity (Pastor-Corrales et al. 1994). Therefore, the objective of this work was to characterize isolates of *C. lindemuthianum* from Pernambuco state of Brazil through sequencing of ITS regions.

### MATERIAL AND METHODS

These researches were conducted at Laboratório de Melhoramento do Feijoeiro Comum e Biologia Molecular do Núcleo de Pesquisa Aplicada a Agricultura (Nupagri), Universidade Estadual de Maringá and at the Centro de Estudos do Genoma Humano, Universidade de São Paulo. Seventeen isolates of *C. lindemuthianum* from Pernambuco state were used for ITS regions analyses. Genomic DNA extraction from mycelia mass was performed according to Cárdenas et al. (2012). PCR were carried out according to (Gardes and Bruns 1993) for the primer ITS 1F (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS 4 (5'TCCTCCGCTTATTGATATGC 3') (White et al. 1990). The PCR products were analyzed on 1.2% agarose gels stained with SYBR Safe (0.02%). The purification of the PCR products were performed utilizing the Kit PureLink PCR Purification Kit (Invitrogen®) and the sequencing was conducted in the ABI 3730 DNA Analyser with BigDye® Terminator v 3.1 Cycle Sequencing Kit. The analyses sequence were made by the BioEdit (version 7.0) and MEGA 5.2 software.

### RESULTS AND DISCUSSION

As illustrated in Table 1, the DNA sequences of 12 out of the 17 *C. lindemuthianum* isolates were compared with the sequences obtained from GenBank. The sequence analysis revealed that the race 2047 from the database showed 100% similarity with these isolates analyzed in the present study. The greatest genetic divergence was observed among the isolates CLPE 53 with the CLPE 87 and CLPE 55, which magnitude was 0.050. Conversely, the most of isolates were similar with genetic distance value of 0.000. The greater genetic variability presented by the 12 isolates was observed in the ITS 2 region, which are positioned between 391 a 463. Similar results were obtained by Balardin et al. (1999) who identified variability at ITS 2 region of *C. lindemuthianum*. The isolate CLPE 53 was the most divergent among all the isolates, revealing different SNPs at the ITS 2 region. Likewise, at ITS 2 region, the sequences of

CLPE 37, 38, 53, 56, 57 and 63 isolates showed the substitution of **A** by **C**, at position 455. Meanwhile, the isolate CLPE 87 which presented six SNPs, it were observed the following substitutions: **A** by **C**, at position 72, **A** by **G** at position 74, **A** by **G** at position 391, **C** by **A** at position 424, **C** by **A** at position 430 and **A** by **G** at position 434. It is emphasized that the region ITS 1 presented SNP at position 73, taking place **T** by **G** in the sequence of the isolates CLPE 29, 37, 38, 43, 49, 55, 56, 57 and 63 of *C. lindemuthianum*. These same isolates also showed the substitution of **A** for **G** at position 153. Based on sequencing the results presented here, revealed that the isolates from the state of Pernambuco presented reduced genetic variability.

**Table 1.** Single nucleotide polymorphisms (SNP) on sequences of the *Colletotrichum lindemuthianum* isolates at the ITS 1 and ITS 2 regions

Isolates	Positions																									
	72	73	74	153	391	424	430	431	434	435	436	437	440	441	442	443	444	446	447	448	455	461	462	463		
Race 2047	C	G	G	G	G	A	A	C	G	C	A	G	C	A	C	T	C	T	G	C	C	C	A	A		
CLPE 29	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
CLPE 37	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-		
CLPE 38	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-		
CLPE 43	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
CLPE 49	-	T	-	A	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
CLPE 53	-	-	-	-	-	-	T	T	C	G	C	A	G	C	A	C	T	C	T	G	A	-	C	-		
CLPE 55	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	T		
CLPE 56	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-		
CLPE 57	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-		
CLPE 63	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-		
CLPE 87	A	-	A	-	A	C	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
SNP	A/C	T/G	A/G	A/G	A/G	C/A	T/C/A	T/C	C/A/G	G/C	C/A	A/G	G/C	C/A	A/C	C/T	T/C	C/T	T/G	G/C	A/C	A/C	C/A	T/A		

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