**Variability within race 65 of *Colletotrichum lindemuthianum* collected in different regions from Brazil through sequencing of ITS regions**

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**INTRODUCTION:** Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc & Magnus) Briosi and Cavara, is one of the most serious diseases of common bean in the worldwide (Pastor-Corrales and Tu 1994). The disease is managed through deployment of resistant cultivars, but new pathotypes present a challenge to the successful implementation of this strategy (Singh and Schwartz2010). Among the races of *C. lindemuthianum,* the pathotype 65 stands out for being confirmed its presence in various regions of Brazil and the world (Balardin et al. 1997; Gonçalves-Vidigal et al. 2008; Davide and Souza 2009). One way to detect genetic variability in plant pathogens is by amplifying the ITS region (Internal Transcribed Spacer) ribosomal DNA (rDNA) by PCR. Thus, the objective of this study was to characterize isolates of *C. lindemuthianum* race 65 from different regions of Brazil through sequencing of ITS regions.

**MATERIALS AND METHODS:** The experiments 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 the race 65 were used for ITS regions analyses. Twelve isolates were obtained from the mycology collection of NUPAGRI Laboratory and five were kindly provided by Dr. Tamires Ribeiro of the Instituto Agronômico de Campinas, Campinas, São Paulo state. The genomic DNA extraction from mycelial mass where performed according to Cárdenas et al. (2012). The PCR conditions for the primers iniciadores ITS1F (5' CTTGGTCATTTAGAGGAAGTAA 3') (Gardes and Bruns, 1993) and ITS4 (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 where made using the Kit PureLink PCR Purification Kit (Invitrogen®) and the sequencing was performed using the ABI 3730 DNA Analyser with BigDye® Terminator v 3.1 Cycle Sequencing Kit). For the sequence analyses BioEdit (version 7.0) and MEGA 5.2 softwares where used.

**RESULTS AND DISCUSSION:** DNA sequences of 17 analyzed isolates in this study were compared with the sequence of race 23 (Table 1). Variability of *C. lindemuthianum* isolates was inferred from the sequence comprising ITS 1 and ITS 2 regions and the 5±8S rRNA gene. Differences among sequences of isolates due to single nucleotide substitutions were observed in the ITS 1 and ITS 2 regions. The results revealed the presence of a SNP at position 79 of ITS 1 region, occurring substitution of **C** by **T** in the sequence of the isolate 3, from Mato Grosso. In turn, the isolate 4 from Parana State presented a SNP at position 120, where there was an exchange of **G** by **C**. It was observed wide variability in the sequences from the isolate 8 collected in Santa Catarina, which presented three SNPs, verifying the following substitutions: **C** by **A** at position 83, **G** by **T** at position 119 and **G** by **A** at position 199. Interesting results were observed in isolates 9 and 12 from Santa Catarina; 14 and 15 from São Paulo which showed similar SNPs at positions 119 and 199 where there was an exchange of **G** by **T** and **G** by **A**, respectively. Additionally, isolate 14 also presented a SNP at position 156 enabling the substitution of **A** by **G**. The sequences of the isolates 13 and 17 from São Paulo, resulted in the

following substitutions: **G** by **A** at position 199 and **G** by **T** at position 119, respectively. Davide and Souza (2009) obtained similar results of the existence of pathogenic variation within the race 65. Furthermore, at ITS 2 region, the sequences of 8, 9, 12, 14, 15 and 17 isolates showed the substitution of **C** by **A** at position 501. The sequence of the isolate 4 at position 480 presented a substitution of **G** by **A.** Moreover, the sequence of the isolate 16 revealed the presence of three SNPs at positions 435, 436 and 470 with the following substitutions, respectively: **C** by **G**, **G** by **A** and **A** by **C**. Similar results were obtained by Balardin et al. (1999) who found variability at ITS 2 region of *C. lindemuthianum*. The greatest genetic divergence was observed among isolates 10 (Santa Catarina) and 3 (Mato Grosso), which magnitude was 0.772. However, the most similar isolates were 2 and 7, with genetic distance value of 0.002; these ones are from Mato Grosso and Santa Catarina, respectively. Most of the variability observed in the sequence analysis of our 17 isolates from race 65 of *C. lindemuthianum* was in the ITS 1 region. The results obtained in this study revealed the existence of high genetic variability among and within the race 65 through analysis of ITS regions.

**Table 1.** Single nucleotide polymorphisms (SNP) on sequences of the isolates from race 65 of *Colletotrichum lindemuthianum* at the ITS 1 and ITS 2 regions

| Isolates | Position | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **79** | **83** | **119** | **120** | **156** | **199** | **435** | **436** | **470** | **480** | **501** |
| **Race 23** | T | C | G | G | A | G | C | G | A | G | C |
| **3** | C | - | - | - | - | - | - | - | - | - | - |
| **4** | - | - | - | C | - | - | - | - | - | A | - |
| **8** | - | A | T | - | - | A | - | - | - | - | A |
| **9** | - | - | T | - | - | A | - | - | - | - | A |
| **12** | - | - | T | - | - | A | - | - | - | - | A |
| **13** | - | - | - | - | - | A | - | - | - | - | - |
| **14** | - | - | T | - | G | A | - | - | - | - | A |
| **15** | - | - | T | - | - | A | - | - | - | - | A |
| **16** | - | - | - | - | - | - | G | A | C | - | - |
| **17** | - | - | T | - | - | - | - | - | - | - | A |
| **SNP** | T/C | C/A | G/T | G/C | A/G | G/A | C/G | G/A | A/C | G/A | C/A |


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