VIRULENCE AND GENETIC DIVERSITY OF *PSEUDOCERCOSPORA GRISEOLA* ISOLATES FROM PARANÁ STATE, BRAZIL

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INTRODUCTION

The angular leaf spot, caused by the pathogenic fungus *Pseudocercospora griseola,* is one of the most widespread diseases in common bean (*Phaseolus vulgaris* L.) producing areas. The use of resistant cultivars is indicated as an effective strategy to control the ALS pathogen (Miklas et al. 2006). Various resistance genes, named *Phg*-, conferring race specific resistance to different races of *P. griseola* have been identified. However, the strength of the resistance is complex, and a pathogen such *P. griseola* shows high diversity in its virulence (Pastor-Corrales and Tu 1989). In this work, the differential cultivars of angular leaf spot were used to characterize *P. griseola* isolates from infected common bean plants from Ponta Grossa, Paraná state, Brazil. In addition, these isolates were characterized through sequencing of the internal transcribed spacer (ITS) region.

MATERIAL AND METHODS

This work was conducted at the Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) at the Universidade Estadual de Maringá (UEM), Paraná state, Brazil. Leaves with angular leaf spot symptoms from common bean infected plants were collected in Ponta Grossa, located in the center of Paraná state. Monosporic cultures of isolates of the fungus *P. griseola* were performed as described by Sanglard et al. (2009). A total of five isolates were obtained and the cultures were replicated in Petri dishes containing green bean medium (200 g of green bean, 17 g of agar, 3 g of $CaCo_3$ and distilled water to complete 1000 mL) and kept in BOD in absence of light at 25°C for 12 days. The race identification was performed using the set of 12 ALS differential cultivars proposed by Pastor-Corrales and Jara (1995). Ten seeds of each differential cultivar were sown in trays containing soil in trays and kept in greenhouse condition for 17 days. The first trifoliolate leaf of each plant was inoculated with a spore suspension adjusted to 2×10^4 spores.mL^{-1} and transferred to a chamber for 72 h at 22°C and humidity >95%. The evaluation of the symptoms was performed according the scale proposed by Inglis et al. (1988). The genomic DNA extraction was performed from the mycelial mass previously stored at -20°C, using a CTAB protocol. The ITS1-5.8S-ITS2 regions of the rDNA were amplified by PCR using the primers ITS1F (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') and the PCR products were visualized in a 0.8% agarose gel. The PCR product was purified using the PureLink PCR Purification Kit (Invitrogen) and the sample were sequenced at the Centro de Estudos do Genoma Humano e Células-Tronco CEHG-CEL of the Universidade de São Paulo (USP), São Paulo state, Brazil. The analysis of the DNA sequences and construction of the phylogenetic tree were performed using BioEdit 7.2.5 and MEGA 5.2.2 software. For the phylogenetic tree construction, nine sequences of the ITS1 and ITS2 regions of *Pseudocercospora* spp. from the GenBank database were selected, based on their similarity to the isolates from Paraná state (Figure 1).

RESULTS AND DISCUSSION

All *P. griseola* isolates using the ALS differential cultivars allowed the identification only the race 63-63. This is the first report of the occurrence of 63-63 race of *P. griseola* in Paraná state. Race 63-63 showed high virulence, overcoming the resistance of all differential cultivars. This race is broadly distributed through the Brazilian producing areas and its presence was confirmed in Santa Catarina, Minas Gerais and Goiás states (Nietsche et al. 2001, Sartorato 2002). Phenotypic and molecular data on pathogen variability have supported the separation of *P. griseola* into two major groups: Andean (*P. griseola f. griseola*) and Mesoamerican (*P. griseola f. mesoamericana*) (Guzmán et al., 1999). Therefore, the compatibility of race 63-63 to the Andean and Mesoamerican cultivars Don Timóteo, G 11796, Bolón Bayo, Montcalm, Amendoin, G 5686, PAN 72, G 2858, Flor de Mayo, Mexico 54, BAT 332, Cornell 49-242, revealed a tendency of the isolates belong to the Mesoamerican gene *pool*. The phylogenetic tree based on the DNA sequences of the ITS-rDNA regions evidenced the presence of two major groups (Figure 1). A group of isolates recovered from species *P. vitis, P. eucalyptorum* and *P. paraguayensis*, and a group including the *P. griseola* isolates. The five isolates from Ponta Grossa (PG001-PG005) show high similarity to the *P. griseola* f. *mesoamericana* isolates retrieved from the GenBank database, confirming the Mesoamerican origin of the isolates collected in Paraná. On the other hand, the Mesoamerican group was clearly divergent from the Andean isolates previously described in the literature.

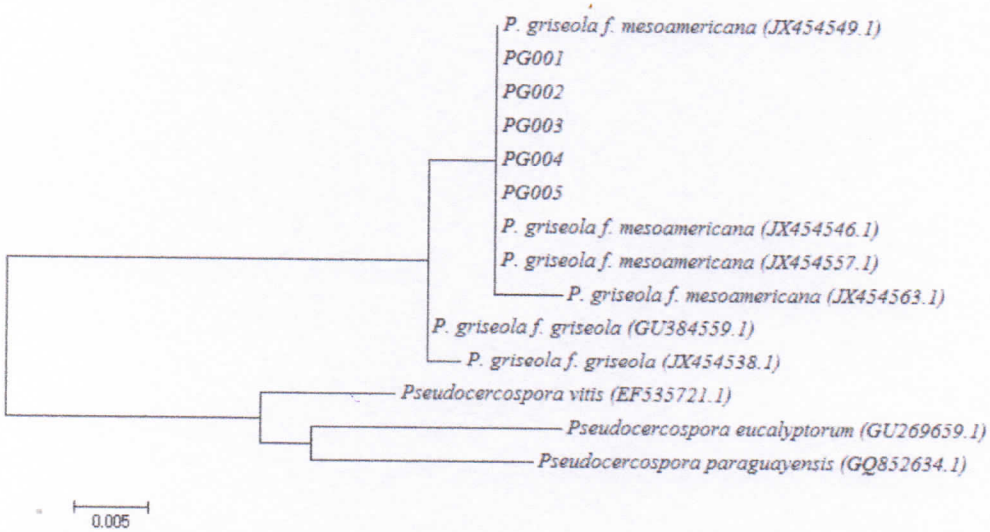

Figure 1. Phylogenetic tree of 14 isolates of *Pseudocercospora spp.*, based on DNA sequences of ITS1-5.8S-ITS2 genomic region.

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