ONGOING MOLECULAR STUDIES OF EUCALYPTUS POWDERY MILDEW IN BRAZIL

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INTRODUCTION

Powdery mildew diseases are caused by biotrophic fungi in the Erysiphales. These fungal pathogens are easily observed by the whitish powdery appearance caused by their colonization of the aerial surfaces on living plants (Stadnik & Rivera, 2001) (Figure 1). In Brazil, powdery mildew of *Eucalyptus* spp is increasing under the current nursery production systems, and the causal agents are generally recognized as the anamorphic stage, such as *Oidium* spp or *Oidium eucalypti* Rostrup. (Mucci *et al.*, 1980). Unfortunately, pathogen identification is hampered by the absence of sexual reproductive structures in Eucalyptus powdery mildew pathogens of Brazil (Bedendo, 2011). Accurate identification of these powdery mildew fungi is essential for developing disease management practices, such as resistance-breeding and screening programs, which are dependent on the pathogen species or race. In addition, accurate identification of powdery mildew pathogens will enhance chemical control methods, because different species may respond differently to various fungicides that have specific modes of action.

OBJECTIVES

The main objectives of this project are to: i) Identify the powdery mildew pathogens that infect *Eucalyptus* in different regions of Brazil by molecular and morphological characterization; ii) Generate transcriptome sequencing data of Eucalyptus powdery mildew; and iii) Identify genetic markers from transcriptome sequences that can be used to analyze the genetic diversity and population structure among isolates of Eucalyptus powdery mildew pathogens.

MATERIAL AND METHODS

Eucalyptus powdery mildew specimens were collected from mini-clonal hedges in greenhouses from several clonal *Eucalyptus* nurseries in five states of Brazil (Figure 2). For DNA-based identification, total cellular DNA was extracted from mycelia and conidia using a Chelex® method (Walsh *et al.* 1991). ITS and 28S rDNA (LSU) regions were amplified by PCR and sequenced using the primers ITS5/ITS4 and PM3/TW14, respectively. Resulting sequences were aligned with homologous sequences available on GenBank. Phylogenetic trees were obtained using Bayesian analysis.

For morphology-based identification, mycelia and conidia from infected leaf surfaces were scraped into a drop of lactic acid on a glass slide for light microscopy. Morphological characters, such as size and shape of conidia; presence or absence of fibrosin bodies; nature of conidiogenesis; characteristics of the conidiophore (e.g., size and shape of the foot cell); and hyphal morphology were observed and recorded. Transcriptome studies are in progress to develop genetic markers for use in population genetic analyses or identifying genes involved in host-plant infection and environmental interactions.

PRELIMINARY RESULTS

Of 82 samples of powdery mildew pathogens collected from *Eucalyptus* plants, 48 samples yielded sufficient DNA for PCR, which resulted in 40 sequences of ITS rDNA and 48 sequences of 28S rDNA. Searches on Blastn

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(http://blast.ncbi.nlm.nih.gov) revealed that all isolate sequences were very similar to species within the genus *Podosphaera* for both genetic regions. For phylogenetic analysis, the sequences were aligned with 33 ITS rDNA sequences of *Podosphaera* spp available on GenBank.

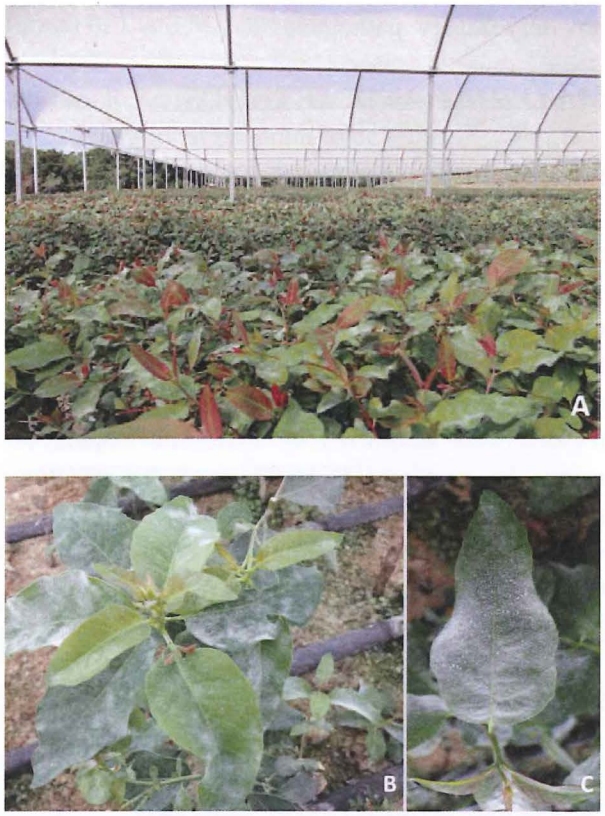

Figure 1. A) *Eucalyptus nursery in State of Minas Gerais, Brazil, with plants showings powdery mildew signs and symptoms;* ***B)*** *Plants highly infected with powdery mildew pathogen; and* ***C)*** *Eucalyptus leaf with myce*lia and conidia of powdery mildew pathogen.

Results of phylogenetic analyses for both DNA regions showed that all sequences generated in this study were comprised within a single clade of *Podosphaera pannosa*, supported by high posterior probability (97%) (28S rDNA results not shown) (Figure 3). Phylogenetic and sequence analysis revealed that all pathogen isolates of Eucalyptus powdery mildew possessed identical (or nearly identical) sequences for the ITS and 28S rDNA regions. Identical rDNA sequences could be indicative of a clonal population structure, perhaps attributable to lacking sexual reproduction in tropical regions or perhaps reflecting a recent introduction of this pathogen to Brazil. Thus, continued studies using other genetic markers are needed to confirm the population structure of Eucalyptus powdery mildew pathogens in Brazil

Figure 2. *Collection locations in Brazil (Font: Google Maps).*

REFERENCES

Alfenas, A.C., Zauza, E.A.V., Mafia, R.G., and Assis, T.F. de. 2009. Clonagem e Doenças do Eucalipto. Viçosa: Editora UFV.

Bedendo, I.P. 1995. Oídios. Pages 27-38 In: Bergamin Filho, A.; Kimati, H.; Amorim, L., eds. Manual de fitopatologia, 3ed. v.1.

Mucci, E.S.F., Pitta, G.P.B., and Yokomizo, N.K.S. 1980. O oídio em mudas de eucalipto. Boletim Técnico Instituto Florestal, 34, n.1, p.27-38.

Silva, M.D.D., Alfenas, A.C., Maffia, L.A., and Zauza, E.A.V. 2001. Pages 201-205 In: Etiologia do oídio do eucalipto. Fitopatologia Brasileira.

Stadnik, M.J. and Rivera, M.C. 2001. Oídios. Jaguariúna: Embrapa Meio Ambiente.

Walsh, P.S., Metzger, D.A., and Higuchi, R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506-513.

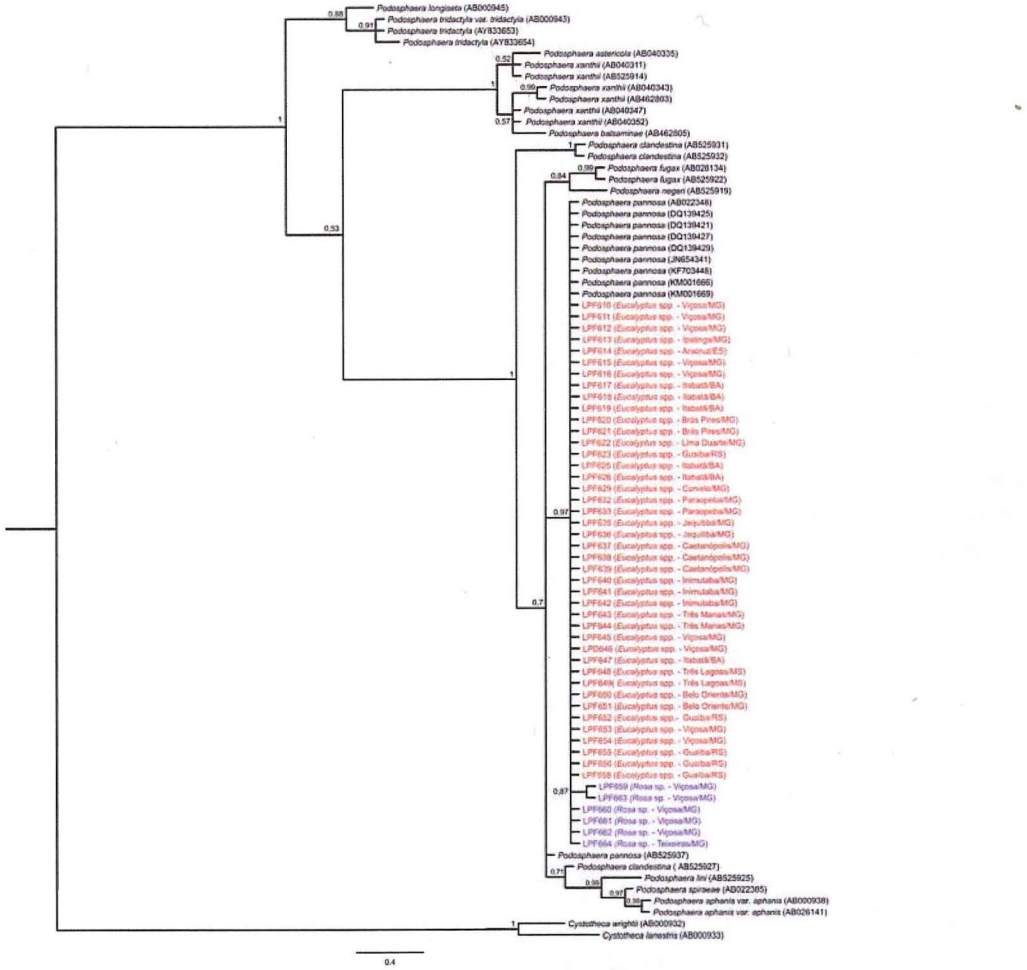

Figure 3. *ITS sequence-based Bayesian phylogenetic analysis of powdery mildew pathogens of Eucalyptus spp (depicted in red). Posterior probability support percentages are indicated at the branch nodes.*