

MOLECULAR CHARACTERIZATION OF *Exobasidium vexans* USING ITS PRIMERS

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Blister blight caused by *Exobasidium vexans* is a devastating leaf disease in tea (*Camellia sinensis*) in almost all tea growing regions in Asia. This disease causes serious crop losses under inclement weather conditions besides affecting quality of made tea. Although tea cultivars show varying degrees of resistance/susceptibility to blister blight, a cultivar showing total resistance to blister blight has not yet been identified. Being an obligatory parasite, there is little information available on the variation of *E. vexans*, and the techniques needed for its molecular studies. The objective of this study was to test the feasibility of ITS 1-F and ITS4-B primers for studying molecular diversity of *E. vexans*. DNA was extracted using 'QIAGEN-Plantmini-DNA extraction kit'. Basidiospores of *E. vexans*, were collected through spore-fall technique from four locations/regions. Pure cultures of common phylloplane Ascomycete, *Pestolotiopsis* sp., *Cladosporium* sp., and *Poria hypolateritia*

(Basidiomycete) were used as comparisons. The quality and quantity of the genomic DNA were determined by comparing with DNA markers on agarose gel. PCR was carried out using ITS 1-F and ITS4-B primer combination. The primer pair amplified Internal Transcribed Spacer (ITS) regions of *E. vexans* and *P. hypolateritia*, but not the Ascomycete fungi tested. Banding pattern of ITS-PCR product was observed on polyacrylamide gel. A prominent band of approximately 700bp of *E. vexans* collected from St. Coombs Estate, Talawakelle and *P. hypolateritia* was re-PCR'd and

sequence-characterized. The DNA sequences of both species showed, high similarity (>80%) with the DNA sequences of Basidiomycete fungi in the Genbank. When subjected to BLAST analysis, ITS sequences of *E. vexans* showed 77-89% homology with other *Exobasidium* spp., thus confirming the accuracy of the amplified region.

This research suggests that ITS 1-F and ITS4-B primer pair can be successfully used to study the genetic diversity of *E. vexans* in Sri Lanka and to discriminate *E. vexans* from phylloplane Ascomycete contaminants.

Key words: Basidiospores, Blister blight, *Camellia sinensis*, *Exobasidium vexans*, ITS region