ABSTRACT - APPLICATIONS OF BIOTECHNOLOGY IN ENVIRONMENTAL AND AGRICULTURAL AREAS;

MOLECULAR EVIDENCE OF PHYLLOSTICTA CITRICARPA AND OTHER SPECIES IN CITRUS BLACK SPOT IN THE AMAZON REGION

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Citrus black spot (CBS), caused by Phyllosticta citricarpa, represents one of the most destructive diseases in Brazilian citriculture, leading to yield losses of up to 85%. The disease is characterized by necrotic lesions on the fruit exocarp, depreciating commercial value and, in severe infections, inducing premature fruit abscission. Beyond its economic impact in domestic markets, CBS is categorized as an A1 quarantine pest by the European Union, restricting Brazilian citrus exports and reinforcing its phytosanitary relevance. While CBS has been extensively documented in southeastern Brazil, little scientific evidence exists on its occurrence and epidemiology in the northern region, particularly in the state of Amazonas. In 2021, CBS outbreaks were recorded in

a commercial sweet orange orchard in Manaus, comprising the cultivars ‘Pêra’ and ‘Valência.’ Given the absence of prior records for Amazonas, this study aimed to identify Phyllosticta species associated with symptomatic tissues through molecular phylogenetic analyses. Fruit and leaf samples exhibiting typical CBS lesions were collected, surface-sterilized, and subjected to fungal isolation. A total of 76 isolates with morphology consistent with Phyllosticta were obtained. Representative isolates were selected for DNA extraction, and the internal transcribed spacer (ITS) region of ribosomal DNA was amplified using primers V9G and ITS4. Amplicons were sequenced via the Sanger platform, and consensus sequences were assembled and aligned with reference Phyllosticta taxa. Phylogenetic reconstructions were performed using CIPRES computational resources. The phylogeny revealed three distinct clades: P. citricarpa (30 isolates), P. capitalensis (11 isolates), and P. bifrenariae (2 isolates). Among them, only P. citricarpa has been unequivocally recognized as the causal agent of CBS. The presence of P. capitalensis and P. bifrenariae in symptomatic tissues indicates possible secondary colonization, but their etiological role remains unresolved. Confirmation of pathogenicity, therefore, requires fulfillment of Koch’s postulates. These results represent the first molecular evidence of P. citricarpa in Amazonas and document, for the first time, the association of other Phyllosticta species with symptomatic citrus tissues in the state. The data expand current knowledge of pathogen diversity in Amazonian citriculture and emphasize the utility of molecular tools for accurate fungal identification in underexplored regions. Importantly, the confirmation of CBS in Manaus highlights a phytosanitary risk for local production, given the susceptibility of the cultivars evaluated and the favorable climatic conditions for disease development. This study contributes to the foundation for epidemiological monitoring and integrated management of CBS in Amazonas. Moreover, it provides relevant information for designing locally adapted control strategies and for supporting phytosanitary measures essential to preserve both regional productivity and international citrus trade.

Palavras-chave: fungus; manaus; citrus black spot ; phyllosticta spp.