

## **GUIGNARDIA CITRICARPA: AGROBACTERIUM-MEDIATED TRANSFORMATION AND FUNCTIONAL GFP EXPRESSION**

M.B.C. Rodrigues<sup>1</sup>, L.C.L. Fávaro<sup>1</sup>, A.P.S. Pallu<sup>1</sup>, A. Ferreira<sup>1</sup>, F.L.S. Sebastianes<sup>1</sup>, M.J.C. Rodrigues<sup>1</sup>, M.B. Spósito<sup>1</sup>, W.L. Araújo<sup>2</sup>, **A.C. Bogas**<sup>1</sup>, A.A. Pizzirani-Kleiner<sup>1</sup>

<sup>1</sup>*University of São Paulo, Piracicaba*, <sup>2</sup>*University of Mogi das Cruzes, Mogi das Cruzes, Brazil*

*Guignardia citricarpa* is the causal agent of Citrus Black Spot (CBS), an important disease in Citriculture. Due to the expressive value of this activity worldwide, especially in Brazil, understanding the functioning of this fungus is of utmost relevance, making possible to clarify its infection mechanisms and providing tools to control CBS. This work describes for the first time an efficient and successful methodology for genetic transformation of *G. citricarpa* mycelia which generated transformants expressing the gene encoding for the *gfp* (green fluorescent protein). Mycelia of *G. citricarpa* were transformed via *Agrobacterium tumefaciens*, which carried the plasmid pFAT-*gfp*, containing both genes encoding for hygromycin resistance (*hgr*) as well as *gfp*. The optimization of the agrotransformation protocol was performed testing different conditions (type of membrane; inductor agent concentration - AS - and coculture time). Results demonstrated that the best condition occurred with the utilization of Millipore membrane; 200 mg/mL of AS and 96 hours as coculture time. High mitotic stability (82%) displayed by the transformants along with the PCR technique confirmed the *hgr* gene insertion. In addition, it was observed the presence of *gfp* inside the mycelia by epifluorescence optical microscopy. The establishment of a transformation method for *G. citricarpa* opens a range of possibilities and facilitates the study of insertional mutagenesis and genetic knockouts, in order to identify important genes involved in the pathogenesis mechanisms and plant-pathogen interaction.

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