



ISBN 978-85-66836-16-5

HETEROLOGOUS EXPRESSION STUDIES IN *Nicotiana benthamiana* REVEAL CANDIDATE EFFECTOR PROTEINS FROM *Hemileia vastatrix* THAT ACCUMULATES INTO THE PLANT CELL NUCLEUS / Identificação de proteínas efetoras de *Hemileia vastatrix* que se localizam no núcleo da célula vegetal. <u>T. MAIA</u>¹, G. MARIN-RAMIREZ¹, S. H. BROMMONSCHENKEL¹. ¹Departamento de Fitopatologia/Instituto de Biotecnologia Aplicada a Agropecuária-BIOAGRO, Universidade Federal de Viçosa, MG, 36570-000, Brazil. Email: thiago.maia@ufv.br

Hemileia vastatrix secretes a variety of effectors that modify the function of the host cell allowing the biotrophic interaction. Some effectors are targeted to extracellular space (apoplastic effectors) whereas others can be translocated into the plant cells (cytoplasmic effectors). Heterologous expression in Nicotiana benthamiana cells is considered as an alternative approach to study effectors from H. vastatrix due to the limited availability of molecular genetic tools to perform functional studies in this fungus. In previous studies, we have selected small secreted proteins that suppress PAMP-triggered immunity in N. bethamiana. To determine the subcellular localization of these proteins, we cloned the coding sequence (without the signal peptide) to obtain candidate effector-GFP fusions under control of the 35S promoter and transferred the binary vector to Agrobacterium tumefaciens GV3101. We also cloned the gene that encodes one secreted protein that shows similarity to rust transferred protein referred to hereafter as HvRTP-01B. The Agro clones were infiltrated into leaves of 3-week-old N. benthamiana plants. Samples from the infiltrated leaves were examined 48h after infiltration using a Zeiss LSM510 META laser-scanning confocal microscope. Seven candidate effectors (HvEC-006, HvEC-016, HvEC-021, HvEC-036, HvEC-040, HvEC-064, and HvRTP-01B) accumulated in the nucleus. The candidate HvEC-056 accumulated into chloroplasts. The remaining HvEC-GFP fusion constructions did not showed informative subcellular. Studies are being carried out to identify the targets of the effectors localized in the nucleus.

Key words: Coffee rust, Effector biology, Subcellular compartments