RESUMO - VEGETAL E INVERTEBRADOS

P61 PROTEIN OF CITRUS LEPROSIS VIRUS C ELICITS AN HYPERSENSITIVE-LIKE RESPONSE IN NICOTIANA BENTHAMIANA

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Citrus leprosis virus C (CiLV-C, genus Cilevirus, family Kitaviridae) is the prevalent causal agent of citrus leprosis, the main viral disease affecting citrus groves in Brazil. Differently from other plant viruses, CiLV-C does not systemically invade any of its known plant hosts, remaining restricted to cells around the inoculation site by its vector, mites of the species Brevipalpus yothersi. Phenotypically, local necrotic and chlorotic lesions of citrus leprosis resemble the outcome of a hypersensitivity response (HR) as a consequence of an incompatible plant-pathogen interaction. Previous transcriptomic and histochemical studies indicated that the plant response to CiLV-C involves the activation of the plant immune system, including the induction of the salicylic acid (SA) pathway, suppression of jasmonic acid (JA) pathway, ROS burst, upregulation of HR-related genes, and cell death, which in sum may lead to the

HR-like phenotype. However, the nature of the viral factor inducing such a response remains to be determined. To address this, ORFs from CiLV-C were individually expressed in Nicotiana benthamiana plants by Agrobacterium-mediated infiltration. After three days, visual evaluation of the treated leaves detected patches of dead tissues where the construction expressing the ORF p61 was infiltrated. Leaves treated with other ORFs remained phenotypically unaffected. Histochemical test using DAB reagent on p61-infiltrated leaves showed ROS burst, whereas RT-qPCR revealed the up-regulation of SA- and HR-related genes, and LC-MS/MS analyses detected an increased SA level and the reduction of the JA accumulation. In parallel, the infiltration with a p61 frameshift mutant that blocks the P61 production did not induce cell death, confirming that P61, but not its coding sequence, triggers the HR-like response. Altogether, our results revealed that the expression of CiLV-C P61 protein mimicries the responses observed during the plant-CiLV-C interaction, suggesting this protein as an elicitor of the plant immune system and the citrus leprosis symptoms. Moreover, recent reports revealed the accumulation of P61 protein in the endoplasmic reticulum (ER) and the further disruption of this membrane system. Since severe ER stress might lead to cell death, new experiments aiming to reveal the role of the ER stress in the development of P61-triggered cell death are in progress.

Financial support: FAPESP (2014/50880-0, 2014/00366-8, 2016/23870-9, 2017/50222-0, and 2019/02137-0).