Resumos do VI SBGMP • 28 de maio a 2 de junho de 2017
Parque Metalúrgico Augusto Barbosa Centro de Artes e Convenções da UFOP • Ouro Preto • MG
www.sbg.org.br - ISBN978-85-89109-06-2

THE p24 PROTEIN OF *Citrus leprosis virus-C* (CiLV-C) IS A TRANSMEMBRANE PROTEIN WITH TYPE II TOPOLOGY

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Palavra-chave: *Citrus leprosis virus-C*, membrane fractionation, Bimolecular Fluorescence Complementation (BiFC), viral transmembrane protein, membrane protein topology

Citrus leprosis, caused by *Citrus leprosis virus-C* (CiLV-C), is considered one of the major viral diseases of citrus in Brazil. Currently, the Brazilian state of São Paulo is the largest producer of orange juice and an estimated annual cost around U$ 80 million is used to control this disease. Given the importance of the disease, many efforts have been made to unravel the pathosystem and to develop effective control methods of citrus leprosis. However, detailed understanding of the molecular processes involved in the manifestation of the citrus leprosis is still needed. For instance, until present there is no information about the functionality of the viral ORFs. In this context, the present work presents functional studies of the CiLV-C p24 protein through studies of the association of p24 with the plant membrane network. For this purpose, chemical treatment with Na_2CO_3 and Urea after membrane fractionation from *Nicotiana benthamiana* leaf cells agroinfiltrated with p24 construct revealed that the p24 protein is an integral membrane protein. Additionally, the Bimolecular Fluorescence Complementation (BiFC) assay was performed to understand into which membranous subcellular compartment the p24 N- or C-terminal is exposed. BiFC relies on the capacity of two non-fluorescent fragments of the Yellow Fluorescent Protein (YFP), i.e. its N-terminus (N-YFP) and C-terminus (C-YFP), to interact with each other when they are over expressed in the same subcellular compartment. The C-YFP fragment was targeted either to the cytosol (C-YFPcyt) or to the endoplasmic reticulum (ER) lumen (C-YFPer), and it was co-agroinfiltrated in tobacco leaves with the counterpart N-YFP fragment fused either to the N- or C-terminus of the p24 protein. Reconstitution of the fluorescence was observed when the p24, carrying N-YFP fused at the C-terminus was co-expressed with the C-YFPer and also when p24 N-terminus fused to the N-YFP was co-expressed with C-YFPcyt indicating that in plant cells, the N-terminal region of p24 is exposed to the cytoplasm whereas C-terminal domain faces the ER luminal side (type II topology) Taken together these results suggest that the p24 is a transmembrane protein with putative transmembrane domains and that p24 presents type II membrane protein topology.

Supported by FAPESP and Embrapa