

Viruses cause many economically important plant diseases often leading to yield quantity and quality losses. Due to the fact that no chemical treatment methods are available it is very important to gain the knowledge about virus biology and mechanisms which allow their adaptation to different environmental conditions and hosts. It allows to develop new plant protection strategies and phytosanitary measures. One of the viruses infecting a wide range of plants, including economically important plants (like tomato, potato, cucumber, strawberry), ornamentals (like marigold, narcissus) and woody plants (like black locus, grapes, currants) is *Tomato black ring virus* (TBRV). Since 1999, several surveys have been conducted in the fields and glasshouses which confirmed its constant presence in Poland. In the Department of Virology and Bacteriology of Institute of Plant Protection-National Research Institute, 22 TBRV isolates from different hosts which vary in pathogenicity and virulence, were collected. Moreover, the presence of defective RNAs (D-RNAs) which arose from the viral genome during prolonged passages in one host was observed. It has been shown that D-RNAs have influence on biological properties, replication and accumulation of the virus in plant tissues. Still, little is known about mechanisms determining disease development, adaptation to different host species or impact of D-RNAs on viral replication.

Currently, research on specific co-existence of virus and its host in the context of new crop protection strategies development are carried out using advanced molecular technologies. One of them are artificial constructs (called infectious clones) which might be introduced to plants by bacteria of *Agrobacterium* genus and allow to obtain infectious virus in plants. This type of constructs allows for genome manipulation, analysis of the molecular evolution of the virus, analysis of genetic determinants of variability or study the vector-pathogen-host interactions.

Therefore, the main aim of this project is the construction of TBRV infectious clones expressing green-fluorescence protein (GFP) encoding gene for three isolates (from tomato, zucchini and black locus) varied in host range and pathogenicity, which will allow to conduct more detailed studies on its biology, replication and virus-host interactions. By using them we will be able to perform fluorescence microscopy observation of the virus in plants (tomato, zucchini and tobacco), its location in plant cells and visualization of the infection processes day by day. Moreover, the constructs bearing D-RNA molecule will be constructed. It will allow for analysis of different plants hosts infected with three isolates with additional RNA particles versus original isolates on the cellular level. The experiments will bring the knowledge about role of D-RNA in infection process, genes expression and will allow to investigate the course of pathogenesis from local infection in inoculated leaves to virus systemic movement and disease development.

The results of this project will significantly expand the knowledge about TBRV biology, replication and mechanisms affecting on possibility to infect different plant species which is essential for development of new plant protection strategies. Moreover, the implementation of tasks in the following project will initiate the extensive research on the interactions between the virus and the host.