## Research project objectives

Viruses cause epidemics in all major cultures of agronomic importance, representing a serious threat to global food security. As strict intracellular pathogens, they cannot be controlled chemically therefore knowledge related to the molecular bases of viral infections and crop resistance is key to facing viral attacks in fields. In this project, we will analyze several factors related to the dynamics of virus variability, its interactions with the host, persistence in the environment, and re-emergence of viral disease. The project will focus on one tobamovirus, cucumber green mottle mosaic virus (CGMMV) which is responsible for substantial economic losses in cucurbit crops worldwide. Cucurbits are among the most cultivated vegetables and their global production is estimated at approx. 151 million tons. CGMMV is challenging to control due to its high transmissibility by contact, as it can readily survive in water and soil, and propagate through infected seeds and plants. Recently, increasing outbreaks of CGMMV have been noticed in Poland in cucumber crops leading to yield and quality losses. As traditional plant protection methods cannot mitigate CGMMV disease, understanding the molecular mechanisms of viral infections and crop resistance is crucial for addressing viral control of epidemics. The goal of this project is to perform comprehensive studies to analyze:(i) the occurrence and distribution of CGMMV in cucumber crops as well as irrigation water, which can be the source of infective virus in greenhouses (ii) the genetic variability of CGMMV, and its evolutionary dynamics, (iii) the impact of single mutations on symptom development on infected plants and virus accumulation, and (iv) the transcriptomic profile of cucumber plants infected by CGMMV to determine the molecular pathways involved in plant's response to infection This type of study has not been performed in Poland before.

## Research project methodology

The research planned in the project will be conducted using the most modern molecular biology techniques, including high-throughput sequencing (HTS). Surveys will be performed in commercial cucumber production (greenhouses) during 2025-2027. In the framework of the project, we will verify the presence of CGMMV in cucumber plants as well as in irrigation water coupling concentration, HTS-based metagenomics, and biological infectivity tests. The full-length genome sequences of the chosen CGMMV isolates (mild and severe) will be obtained and compared with others described to date. The sequences will be analyzed to estimate the conservative and variable regions and unique point mutations that could be responsible for symptom development. Moreover, infectious cDNA clones will be constructed which are required to analyze the impact of particular point mutations, identified in the framework of the project and from the literature data, on symptoms development in infected plants and virus accumulation. The detailed analysis of the CGMMV population structure will be executed using the isolates collected by our research team in previous years, sequences obtained during this project, and those available in GenBank. The evolutionary analysis will be performed using bioinformatic tools that allow for establishing the phylogenetic relationships among isolates, the occurrence of recombination events, and the correlation between the CGMMV genetic diversity, geographical distribution, and host species. With this data, we will be able to shed light on the epidemiological and evolutionary dynamics of CGMMV in Poland and worldwide, especially considering the growing distribution of plant material across the globe.

Finally, we also propose to use the RNA-Seq tool to generate the data that will allow predicting molecular networks of interactions between different CGMMV genotypes and economically important plant-cucumber, that is, how different genes and transcription factors (TF) change their expression in a coordinated manner in response to infection. This will illustrate how host-virus interplay and how the virus manipulates the host resources in its own benefit.

## Expected impact of the research project on the development of science

The realization of this project will be an important step in expanding knowledge about the epidemiology, CGMMV population diversity, and host-pathogen interactions to identify targets for breeding or engineering resistance against CGMMV. The results of the distribution of the virus will be posted on the Agrophage Signaling Platform of Institute Plant Protection-NRI (https://www.agrofagi.com.pl/), which allows for monitoring the occurrence of threats in crops. Systematic monitoring of pests is a very important element of Integrated Pest Management. The project will be performed in the framework of international cooperation with researchers from Consejo Superior de Investigaciones Científicas (Spain), the Netherlands Institute of Ecology, and the National Institute of Biology (Slovenia) who have vast experiences in the evolutionary and epidemics study of plant viruses. The results of this research will be disseminated through presentations at relevant scientific meetings and published in international journals within the fields of phytopathology, evolutionary biology, and plant virology. The participation and scholarship for the PhD student have been planned in the project, and research will be carried out as part of PhD dissertation.