

The most dangerous flax pathogen is *Fusarium oxysporum* sp. *lini* (Foln), which causes fusarium wilt of flax. This dangerous disease of flax can cause up to 20% loss in its cultivation. The results of our preliminary studies show that the non-pathogenic strain of *F. oxysporum* (Fo47), which is a commonly known endophyte, colonizes flax without causing disease symptoms, and also limits the spread of the pathogenic strain of *F. oxysporum* in flax, thus reducing disease symptoms. Flax sensitized with a non-pathogenic strain is more resistant to infection with a pathogenic strain. Moreover, we have shown that a non-pathogenic strain of *F. oxysporum* colonizes not only flax roots, but also its shoots. This information is important because previous reports indicated that the Fo47 endophyte mainly colonizes plant roots and does not penetrate shoots. Previous studies on the interaction of flax and Fo47 have focused only on the molecular mechanisms of plant response in the studied system. However, we still know little about the molecular mechanisms underlying Fo47 endophytic function. We do not know what fungal proteins are key in the colonization of plants (in our case flax) by a non-pathogenic strain of *F. oxysporum*. We do not know why Fo47 colonizes flax shoots. We do not know which Fo47 proteins initiate flax sensitization and what is the mechanism of their action. We do not know how the expression of genes encoding proteins secreted by fungal strains of various pathogenicity and responsible for the colonization and sensitization of plants is regulated.

The aim of this project is to understand the molecular mechanisms of action of non-pathogenic *F. oxysporum* (in the interaction of flax and Fo47) in the processes of colonization and sensitization of flax. Due to the complexity of these processes, in the project we will focus on one group of proteins that may play a key role in them. These are enzymes that degrade plant cell wall polymers, the action of which facilitates the penetration of plant tissues, provides free glycans used by the fungus as an energy source or building blocks, and at the same time releases elicitors that activate plant defense response pathways. Both non-pathogenic and pathogenic fungal strains secrete enzymes that degrade plant cell wall polymers, but plant colonization by these two strains differs, which may be due to different gene expression of these proteins. Therefore, it is equally important to determine what mechanisms are responsible for regulating the expression of these genes. During the project, we will answer two important questions: Are and which fungal proteins that degrade plant cell wall polymers are responsible for the colonization of flax roots and shoots by a non-pathogenic strain of *F. oxysporum*, and do the same proteins activate the flax sensitization process? Are the mechanisms of epigenetic regulation of genes encoding proteins that degrade plant cell wall polymers responsible for differences in the colonization of flax roots and shoots by Fo47 and Foln?

The project implementation will include the following stages. The first stage will be the identification of molecular targets for Fo47 modification. A comparative analysis of the transcriptome of Fo47 and Foln fungi from flax treated with these fungi will be performed, on the basis of which differentiating transcripts will be selected. The promoters of these genes will then be compared to see whether their different expression may be due to different promoter sequences. In the second stage, gene constructs for silencing selected genes will be prepared, and then transgenic fungi Fo47 with silenced genes will be produced. Subsequently, in the third stage, transgenic fungi with changed properties will be used to determine the functions of selected proteins in the colonization and sensitization process. The flax plants will be treated with fungi and then we will monitor the progress of colonization at many different time points, including: by macroscopic and microscopic observations and the content of fungal genetic material. The last stage will be to check whether epigenetic mechanisms (DNA methylation, histone methylation and acetylation, miRNA) regulate the expression of selected genes in the non-pathogenic and pathogenic strain of *F. oxysporum*.

The project will result in understanding the role of fungal proteins that degrade flax cell wall polymers in the process of colonization and sensitization by a non-pathogenic strain of *F. oxysporum*. In the future, this comprehensive knowledge of the flax-Fo47 interaction will contribute to the conscious use of Fo47 as a potential biocontrol agent increasing the resistance of flax to infections caused by the pathogenic strain of *F. oxysporum* in field crops.