

Towards understanding the suppression of host xylogenesis by the plant pathogen *Plasmodiophora brassicae*

Plasmodiophora brassicae is a biotrophic protist that attacks plants of the brassicaceae family. Progression of the disease in plant hosts leads to the formation of characteristic galls on the roots – hence the disease name “clubroot”. One of the characteristic symptoms of the disease is the inhibition of the maturation of xylem - the tissue involved in the distribution of water along with nutrients from the roots to the above-ground parts of the plant. Without a well-functioning xylem, plants wilt and die. The fight against this plant disease, which is the cause of economic losses in widely cultivated rapeseed crops, is carried out on many levels. The major method is to search for genetic sources of resistance. Unfortunately, after some time, the pathogen often acquires the ability to infect resistant varieties. A kind of reinforcement in the fight against this pathogen is the search for tolerant forms that, despite infection, can endure the disease for a long time, which also results in a reduction in crop losses caused by clubroot. However, the search for tolerant forms requires an understanding of the biological basis of the disease progression in the plant. Our research team works on this topic for many years and we have already described many aspects related to the reprogramming of plant development by *P. brassicae*. Unfortunately, the issue regarding xylem development is quite difficult to decipher. This is due, among other aspects, to the fact that this process is coordinated through many parallel pathways, and additionally, biochemical and metabolic changes in the vicinity of xylem precursor cells may result in a blockage of differentiation. It should be emphasized here that during the formation of the xylem, cells lose their contents and go through a stage of programmed cell death; this results in xylem being, to put it simply, lignified tubes through which transport takes place. Due to the above-mentioned issues, we need to use a method that can provide information to understand the cellular context of the changes that occur in the formation of galls.

In recent years, the single cell sequencing method has been successfully used for transcriptomic studies. It enables researchers to obtain the unique expression profile of each cell and this way to understand its developmental and physiological state. The whole method consists of isolating single cells from plant organs and then RNA, which contains information about which proteins will be synthesized by each cell, is extracted. This information allows the prediction of cell fate and if supported by research on biochemical and metabolic context, makes it possible to obtain a full appraisal of the changes occurring in the underground parts of plants infected by the *P. brassicae* pathogen. Together with scientists from Finland and Belgium, we will conduct the single cell sequencing experiment and data analysis. In addition, some of the work will be carried out together with scientists from the Czech Republic possessing automated phenomics chamber in which changes in plant growth and their life condition, including water status, can be automatically recorded. The latter point is closely related to the development of the xylem. After the initial interpretation and synthesis of the obtained data, we will proceed to the second phase of the project, the essence of which will be further functional verification. Based on the above-mentioned observations, we will select which regulatory pathways and which processes are disturbed by pathogen attack. Then, through direct visualization of changes in activity and accumulation of gene transcripts, and through the generation of mutations leading to the dysfunction, or precise manipulation of the operation of these genes, we will fully describe the mechanism underlying the vascular tissue development induced by the *P. brassicae* pathogen. Finally, we will use this information to obtain plants that, in the face of infection by *P. brassicae* and further development of the disease, retain the ability to form xylem to a high degree. We will assess the tolerance of these plants, and thus the practical possibility of using the knowledge obtained to create tolerant plants in the future.