The uniqueness of plants as self-sufficient and sessile organisms results from the evolution of complex systems in response to changing environmental conditions. Low temperatures, drought, high salinity or high metal content in the soil are common stress conditions that negatively affect plant growth and development, and crop productivity. Compared to other eukaryotic organisms, plant genomes have the ability to evolve rapidly, which increases their plasticity in response to stress and adaptation to new conditions. The processes of whole genome duplication (WGD) and intensive diploidization, which led to the appearance of larger number of gene variants (the so-called paralogs), played an important role in the evolution of plant. Despite high sequence homology, these genes exhibit differences in expression levels (sub- and neofunctionalization models) that may influence the functional changes of their direct protein partners. Despite the evidence for a relationship between novel adaptive traits and WGD, the mechanisms of their origin are still poorly understood.

Oilseed rape (*Brassica napus* L.) as a winter crop is particularly susceptible to drought during two periods. The first period is seed germination and root growth in early autumn. In the second period, the snowless winter is responsible for soil drought in early spring, which contributes to a shortening of the flowering period of rapeseed, less development of the plants themselves and less ability to regenerate damage caused by pathogens. Therefore, it is a challenge for modern biology and breeding to understand the changes in the transcriptome and proteome at different stages of plant development when plants are exposed to stress. Oilseed rape is an allopolyploid species that arose by via hybridization between diploid species of *B. rapa* (donor A genome) and *B. oleracea* (donor C genome), whose genomes had previously undergone three rounds of duplication. Because of its origin, *B. napus* is an excellent model for studying the mechanisms of functional divergence of duplicates leading to a plastic response to stress.

The goal of this project is to characterize the dynamics of proteome changes and differences in protein networks associated with two ABI1 (protein phosphatase 2C) bait proteins under drought and salt stresses in oilseed rape. These studies should allow us to learn about new elements that regulate plant responses to stress and increase their tolerance to adverse environmental conditions. It is well known that the functions of many proteins depend on specific physical interactions with other proteins that together control cellular processes and genotype-phenotype relationships. Therefore, it is important to determine the interactions among many proteins with the central role of the BnaABI1 paralogue protein to understand the changing physiological state of plants in response to different environmental stimuli. In this project we will compare the protein complex composition of two BnaABI1 isoforms under drought and salinity. Comparison of the two protein complexes will allow us to observe changes depending on the isoform and the state of the cells induced by stress, as well as reveal common and specific elements for each of them. The protein phosphatase ABI1 is a conserved regulatory hub protein that controls many stress-induced signaling pathways in plants. In our studies, we showed that despite the conserved basic phosphatase activity, 6 paralogs of the *BnaABI1* gene in oilseed rape underwent a functional divergence toward a specific activity under a specific dehydration stress. Comparison of the two protein complexes controlled by BnaABI1 will provide the first interactome map of ABI1 in *B. napus*. Knowledge of the total number of protein interactions dependent on extracellular stimuli is essential for a better understanding of signaling networks and cell state changes under stress in organisms.

To achieve the goals of the project, we will first conduct a global analysis of changes in the proteome of *B. napus* under drought and salinity stresses. We will use the differential proteomics approach based on the iTRAQ method. We will then define changes in the protein-protein interaction network associated with the BnaA01ABI1 and BnaC07ABI1 isoforms as the hubs. We will use an approach based on affinity chromatography coupled with mass spectrometry (AP-MS) technology. It comprises 5 steps: preparation of expression cassettes for both His-tagged isoforms, transient expression of recombinant proteins in *B. napus* protoplasts or plants, purification, identification of proteins by MS and analysis of protein complexes. The selected interactions between the BnaABI1 isoform and the target protein will be verified using Y2H, BiFC and pull down methods. We will also define the role of BnaABI1 in regulating the functions of its protein partners.