

Brassinosteroids (BRs) are steroidal plant hormones. Studies on mutants defective in the BR metabolism, conducted in the model species *Arabidopsis thaliana*, indicated that BRs regulate a broad range of processes during plant life cycle. Analyses of the mutants implied that the abnormalities in the BR metabolism result in reduced plant height (dwarfism or semi-dwarfism) and changes in plant architecture. Reason for commencement of the project stems from the following aspects. The processes of BR biosynthesis and response were characterized to the highest degree in *A. thaliana*, however, they are far less elucidated in crop species, including cereals. In barley (*Hordeum vulgare*) only recently we have identified few genes, which encode enzymes involved in the BR biosynthesis, however, the process remains not fully understood. In a recent research conducted in our laboratory, physiological reactions of semi-dwarf, BR-deficient barley mutants to water scarcity were characterized. Interestingly, the mutants showed an enhanced tolerance to the stress. However, the genetic mechanisms mediating the regulation of plant reaction to drought and a role of BRs in these processes remain largely unknown (even in *Arabidopsis*). Taking into account the above aspects, **the aims of the project include:**

- 1) Identification of genes responsible for semi-dwarf, BR-dependent phenotype of barley mutants, which were identified and selected during our previous experiments
- 2) Functional analysis of the genes *HvCPII*, *HvSTE1*, and *HvDWF5*, which have been identified in our laboratory and encode enzymes potentially involved in the early stages of the BR biosynthesis in barley
- 3) Analysis of the global gene expression profile (transcriptome profiling) in the identified semi-dwarf barley mutants, which are defective in the BR metabolism, under optimal-watering conditions and during drought stress
- 4) Determining metabolite accumulation profile in the semi-dwarf barley mutants which are defective in the BR metabolism, under the optimal-watering and drought stress conditions.

The project will be implemented with application of the state-of-the-art methods and in the international cooperation with scientists representing the Lund University (Sweden), the Palacký University (Czech Republic), and the Institut de Biologie Moléculaire des Plantes (France). The identification of genes responsible for phenotype of the semi-dwarf barley mutants will be performed with application of the Next Generation DNA Sequencing (NGS) technique. In this approach exomes, which constitute the coding part of the genome, will be sequenced (Exome capture). This methodological approach is particularly useful in determining a genetic basis of traits in species with large genomes (as it is in barley), because it enables specific analysis of the coding sequences, which usually make up only a small part of the genome. The mutants which constitute a material of the study have been selected by us during a large-scale phenotyping experiment, characterized physiologically, and divided into allelic groups (in each group mutations are located in the same gene). It provides a significant input into the further research, which will be conducted in cooperation with the Lund University.

The functional analysis of the *HvCPII*, *HvSTE1*, and *HvDWF5* genes will be performed with the TILLING (*Targeting Induced Local Lesions IN Genomes*) technique. The plant population HorTILLUS will constitute a material of this research. The HorTILLUS population was established in the Department of Genetics, University of Silesia, and constitutes a significant one on a European scale platform for the functional analysis of barley genes. The research is aimed at identifying a series of alleles (versions) of the analyzed genes. In order to improve effectiveness of the research, the most suitable fragments of the genes for the functional analysis have already been determined with the bioinformatics tools. Identified mutants will constitute a material for quantifications of the endogenous BRs in order to validate the role of the analyzed genes in the BR biosynthesis. The endogenous BR quantifications will be performed with the ultra-high performance liquid chromatography (UHPLC) and mass spectrometry (MS/MS) in cooperation with the Palacký University.

Plant material for the analysis of global gene expression profile (transcriptome profiling) and the metabolite accumulation profiling will be the semi-dwarf barley mutant lines, which are deficient in the BR metabolism, and a reference cultivar. In these experiments plants of these genotypes will be grown under optimal-watering (control) and drought conditions. The transcriptome profiling analysis will be conducted with the state-of-the-art, high throughput RNA sequencing (RNA-Seq) method. The metabolite accumulation profile will be determined with the ultra-high performance liquid chromatography (UHPLC) and the mass spectrometry (MS/MS) in cooperation with the Institut de Biologie Moléculaire des Plantes. Results of the transcriptome profiling experiment and determining the metabolite accumulation profile will provide insights into the mechanisms of genetic and biochemical regulation of reaction to drought, what is particularly important for understanding of this crucial physiological process in plants, especially in cereals. Moreover, a comparison of the transcriptomic data and the metabolite accumulation profile between the analyzed mutants and the reference cultivar will also allow a role of BRs in the regulation of this mechanism to be determined.