

Recent climate changes caused increased occurrence of water shortage during vegetative and reproductive stages of cereal development. This stress has detrimental effect on plant physiology and yield. Thus, currently a priority should be given to developing new stress-tolerant cereal cultivars. This urgent need may be fulfilled by semi-dwarf mutants of cereals which are defective in biosynthesis of brassinosteroids (BRs) or reaction (known as signaling) to these phytohormones. BRs regulate various developmental and physiological processes and play a crucial role as modulators of plant architecture (height and stature). Plant architecture is one of the major determinants of plant reaction to environmental stresses, including drought. Importantly, recent reports (including publications from our group) indicated that mutants defective in the BR biosynthesis or signaling show reduced height and enhanced tolerance to drought at the stage of vegetative development. However, most studies in model and crop species were focused on the effects of drought only on the vegetative development. Importantly, transition from the vegetative to reproductive development is the most sensitive stage, and any stress encountered during this period severely affects plant physiology and yield. Currently, mechanisms of plant adaptations to drought exerted during this transition phase and role of BRs in regulation of the reproductive development under drought conditions remain largely unknown, even in model species, and require further research.

Thus, in order to fill such a significant gap in our knowledge of plant biology, in this project multidirectional approaches will be applied to study genetic, biochemical, and physiological adaptations of barley plants to drought exerted during the phase of reproductive development, and to analyze the effect of drought on yield parameters and chemical composition of grains. Application of semi-dwarf, barley mutants defective in the BR biosynthesis or signaling, along with reference genotypes, will enable determining the role of BRs in regulation of these processes. The following analyses illustrate the major objectives and expected outputs of this project:

- 1) phenotypic description of BR mutants and reference genotypes under optimal conditions and two-week drought stress (exerted at the transition to the phase of reproductive development) through observations of plant growth dynamics, relative water content in leaves, time of heading, total and productive tillering, grain weight per plant, and weight of 1,000 grains;
- 2) characterization of response to drought with physiological and biochemical approaches through measurement of chlorophyll accumulation, photosynthesis efficiency, gas exchange, and Rubisco enzyme activity, as well as analysis of carbohydrate (sugar) metabolism, and accumulation of proline which belongs to components of the cellular system of protection against the stress conditions;
- 3) elucidation of mechanisms regulating spike development under the control and drought conditions through transcriptome profiling (RNA-seq and smallRNAseq) and quantification of various phytohormones (BRs, gibberellins, auxins, and abscisic acid) in spikes using the HPLC-MS/MS method. Additionally, chemical composition of grains will be characterized using near-infrared spectroscopy;
- 4) characterization of genes and proteins differentially expressed in spikes of the BR mutants and reference cultivars developing under the control and drought conditions through determining spatial expression patterns of differentially expressed genes (DEGs), comparative analysis of the DEGs' expression in various organs and at different developmental stages, identification of transcription factor-binding sites and cis-acting elements within promoters of DEGs, and verification whether the identified DEGs are regulated by microRNAs. Interactions between proteins encoded by DEGs will be also predicted.

Plant material will include six genotypes: previously characterized semi-dwarf barley mutants, including two mutants deficient in the BR biosynthesis, two mutants defective in the BR signaling, and the 'Bowman' cultivar as a reference. In addition to the 'Bowman' cultivar, the 'Cam/B1' breeding line, adapted to the semi-arid environment, will be included as secondary reference during interpretation of physiological response to drought. The multidirectional analyses will be performed on vegetative (leaves) and reproductive (spikes) organs of barley plants, and at the subcellular, tissue and organ level to characterize the complex adaptational processes that occur during plant reaction to drought. The project will be implemented in close collaboration with Università degli Studi di Milano (Italy) and the Laboratory of Growth Regulators, Palacky University (Czech Republic).