

Filling in the missing pieces of the brassinosteroids puzzle: uncovering yield potential, mechanisms of stomatal differentiation and genetic regulation of spike development in semi-dwarf, brassinosteroid mutants of barley

The limited area of available arable lands has been a challenge to agriculture, especially given that the major part of the cultivable area is already in use. Taking into account the constantly growing world population, there is a pressing need to meet the global food demand. Therefore, it is necessary to obtain higher yield per unit area of arable land which is already problematic to expand. Additionally, one of the most challenging factors for crop productivity is an ongoing climate change manifested by several environmental stresses, including more frequent episodes of prolonged and severe drought. For that reason, it is extremely important to develop new high-yielding cereal cultivars which at the same time would display increased tolerance to drought stress.

Brassinosteroids (BRs), a group of steroid phytohormones, regulate various aspects of growth and development throughout plant life cycle, including reproduction which affects yield potential in cereals. However, most of the studies have been conducted in *Arabidopsis thaliana* and several crucial aspects regarding the role of BRs in plant development in other species, such as barley representing the fourth most significant cereal crop worldwide, remains unclear until now. It is known that defects in the BR biosynthesis or signaling result in plant growth reduction (semi-dwarfism) and erect plant stature which are considered as favorable traits in cereal breeding. Moreover, our preliminary research revealed that one of the two barley BR-mutants which will be used in the project, does not exhibit negative side-effects on the grain weight, contrary to the majority of cereal BR-related mutants. These mutants display also enhanced tolerance to drought stress during the vegetative phase of development. Nevertheless, the role of BRs in regulation of inflorescence development is unresolved, especially in cereals exposed to drought stress during phase of reproductive development. Additionally, the involvement of BRs in stomatal differentiation has yet to be determined in monocots, even in the model species – rice.

In light of the above considerations, the presented project aims to:

- 1) Characterize yield parameters in the BR mutants and reference barley cultivars grown under normal and dense planting field conditions, taking advantage of semi-dwarf and erect phenotype of the BR mutant lines used in the project
- 2) Elucidate mechanisms regulating stomatal differentiation under optimal and drought conditions in the BR mutants of barley and reference genotypes
- 3) Clarify the BR-dependent regulation of spike development under optimal watering (control) and drought stress conditions through transcriptome profiling in the BR mutants and reference barley cultivars

Each of the planned research tasks in this project is preceded by already performed preliminary research and uses versatile and multidirectional approaches to clarify the above-mentioned aspects. We plan to answer the question whether the analyzed BR mutants will outperform the reference genotypes as regards yield-related parameters in the field trial, taking into account the similar weight of grains produced by these mutants when compared with the reference cultivars and their unique phenotype. If our hypothesis proves correct and the mutant lines exhibit higher yield, our research may have practical implications in the future. Next, with the use of microscopic, molecular and bioinformatic approaches, the linkage between BR-related pathways and stomatal differentiation will be determined. Additionally, with the use of data from transcriptome sequencing the connection between BR-related pathways and biological processes underlying spike development under drought stress will also be examined. **We expect that the results of this project will significantly contribute to the advancement of knowledge regarding BRs and their involvement in mechanisms that remained elusive until now. The outcome of these studies will be unique and novel not only for barley biology, but also for cereals as a group of crops of significant importance for global agriculture.**