In response to stress stimuli, plants must reallocate internal resources from growth and development to adaptation processes that enable their survival under adverse conditions. Common abiotic stresses in the natural environment include drought and fluctuations in soil nitrogen levels. The mechanisms regulating adaptive strategies to these stressors are not fully understood, particularly those controlling cross-responses, which remain elusive. Given the multifunctional nature of brassinosteroids (BRs)—growth-promoting steroid hormones—their signaling pathways are likely to be crucial for plant development and responses to both biotic and abiotic stresses. Recent studies have highlighted the increasingly recognized multi-regulatory role of long non-coding RNAs (lncRNAs) in key molecular and biological processes; however, our understanding of lncRNA functionality is still in its infancy. Collectively, these insights form the foundation of the proposal.

The aim of this project is to elucidate the molecular mechanisms underlying the cross-response of spring barley to drought and extremes in nitrogen concentration, as well as to explore its interconnection with brassinosteroid signaling. We will investigate how stress-induced alterations in lncRNAs contribute to transcriptomic, metabolic, and phenotypic adjustments. In particular, we will focus on the role of lncRNAs in coordinating the actions and interactions of phytohormones in barley leaves. Furthermore, we will elucidate the mechanisms through which brassinosteroid-mediated regulation affects barley's response to combined drought and varying nitrogen concentrations. Our research hypothesis posits that brassinosteroid signaling is a critical component of the plant's cross-response to drought and nitrogen extremes. Moreover, both nitrogen deficiency and excess in the soil significantly influence the molecular, metabolic, and phenotypic responses of barley to drought; however, the effects of low and high nitrogen levels differ substantially. Additionally, we will examine whether plant adaptation to stress stimuli, as well as the cross-regulation of brassinosteroids and other phytohormones, is regulated by lncRNAs and their interactions with specific microRNAs (miRNAs) and mRNAs.

The selection of appropriate research methodologies and specific plant materials is essential for achieving the project's objectives and validating its hypotheses. We will conduct a series of complementary and coordinated experiments to investigate barley varieties that differ in their brassinosteroid signaling efficiency. Specifically, we will analyze the wild type cv. Bowman alongside its near-isogenic line, BW312 (*uzu 1.b*), which exhibits a dwarf phenotype due to brassinosteroid insensitivity caused by a missense mutation in the *HvBRI1* gene encoding the brassinosteroid receptor. Modifications of coding and non-coding transcripts induced by varying water regimes and nitrogen levels will be assessed through next-generation sequencing. Additionally, we will examine metabolic remodeling using an untargeted HPLC/MS system, alongside changes in phytohormone accumulation resulting from combined drought and low/high nitrogen conditions, analyzed via a targeted UPLC/MS system. Non-destructive digital assessments (using an innovative multispectral imaging platform, the PlantEye system), post-harvest phenotyping, and physiological measurements will be conducted to evaluate the phenotypic plasticity of aerial plant parts exposed to drought and varying levels of nitrogen deficiency or excess in the soil. Data integration concerning various parameters will be achieved using multivariate analysis methods. Special emphasis will be placed on the network analysis of brassinosteroid signaling-related genes, as well as the co-expression and co-reaction modules to which they belong. Additionally, the role of lncRNAs in regulating metabolic adjustments will be elucidated in barley leaves exhibiting disrupted brassinosteroid perception, in comparison to leaves with unaltered brassinosteroid signaling. In summary, it is crucial to highlight that this study will employ **2** barley genotypes subjected to multi-omic analyses across **4** different treatments and **3** time points. This approach will result in **24** research variants, enabling a comprehensive assessment of the dynamic processes occurring in barley across both temporal and environmental dimensions under abiotic stresses. Importantly, a follow-up omics study over the next **2** years will further enhance the statistical power of the findings.

The integrated research and knowledge gathered throughout this project may serve as a foundation for biological advancements and methodologies aimed at improving barley and other crop plants, particularly as barley increasingly emerges as a model organism. The synthesis of data from various experimental approaches will significantly enhance our understanding of molecular adaptation in plants to abiotic stresses, as well as elucidate how regulatory sequences within the barley genome influence its development. Our research findings, along with existing literature, indicate that there are promising new avenues for investigating the molecular mechanisms underlying barley's response to abiotic stress, which are highly desirable from both scientific and breeding perspective. This exploration should undoubtedly encompass the study of lncRNAs, and the implementation of the project aligns perfectly with this area.