A DREAM of NAC – molecular mechanism of transcriptional adaptation to DNA stress

Plants are repeatedly subjected to various environmental stresses including factors causing DNA damage. Most of arable lands in Europe and North America are acidic, similarly to soils in many developing countries in South America, Central Africa and Southwest Asia. Below a pH of 5.5, Aluminium (Al), the most abundant metal in the crust of the earth, becomes mobile in the form of phytotoxic Al^{3+} ions that cause inhibition of cell division and cell elongation in the root tip. An example for a crop that is highly sensitive to Al is barley. Previous work, including studies carried out by both partners in the frame of an European ERA-CAPS consortium, has revealed that aluminum is genotoxic agent that induces DNA damage. Here, we want to explore a novel transcriptional mechanism of tolerance of plants to genotosic agents.

The previous studies of German partner performed on Arabidopsis have revealed a potential involvement of one of NAC transcription factors and DREAM complex in DNA damage response (DDR). The newly identified NAC is a target of SOG1 transcription factor that is a master regulator engaged in DDR pathway in plants, whereas DREAM complex is best characterized in humans, where it acts as a repressor of cell cycle genes when a cell enters quiescence (G_0) phase. Our research hypothesis assumes that in plants this newly identified NAC together with DREAM components build up a complex, activated by DNA damage, to down-regulate the expression of cell cycle and growth promoting genes.

Under the presented project, the research work will be performed for two plants species: *Arabidopsis thaliana* that is a model dicot, and *Hordeum vulgare* (barley) that is a monocot crop with recently assembled genome sequence. The general plan of work consists of four main work packages. In the first one, WP1, we plan to identify barley ortologue of newly identified Arabidopsis NAC gene, based on transcriptome analysis of *hvsog1* mutant (that was developed by us in the previous project) and its parent variety, after treatment with genotoxic agents – aluminum and cisplatin. We will use our barley TILLING population to identify mutants carrying mutations in this orthologue and, eventually, in its paralogs. These mutants will be characterized for their response to different genotoxic agents using root growth assays and DNA damage analysis. Under the second work package, WP2, we will try to evaluate if there are specific components of DREAM complex that take part in DNA damage response. To assess this we will develop Arabidopsis and barley mutants in genes encoding selected components of DREAM complex. The analysis of mutants response to genotoxic agents will help to choose DREAM factors that are DNA-damage response specific. The goal of WP3 is to assign the composition and targets of an inhibitory transcriptional complex upon DNA damage. Under this task we will try to answer the question if analyzed NAC is a part of DREAM-containing complex or if it acts independently of DREAM complex under DNA stress. We also plan to identify its target genes in Arabidopsis by ChiP-seq and in barley by RNA-seq. The comparative analysis of obtained results for this two species will help to evaluate how conserved are this regulator elements between monocots and dicots. The last work package, WP4, includes analysis of the spatial and temporal response to DNA damage in root. If our hypothesis (that assume the influence of analyzed factors on repression of cell cycle/growth promoting genes upon DNA stress) holds true, mutations in analyzed NAC and DREAM complex components should lead to enhanced growth on genotoxic agents, what additionally make this work applicable. Potentially, barley lines carrying mutations in analyzed genes can be more tolerant to genotoxic Al^{3+} ions present in acidic soils that constitute an increasing area of arable soils in the world.