

**PROJECT TITLE**

SNPs in multistep phosphorelay as a tool for enhanced drought tolerance in plants

**CONSORTIUM**

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## SUMMARY OF THE REPORT

Cytokinins (CKs) as a plant hormone regulates a broad spectrum of developmental responses from cell division to the onset of seed formation. In *Arabidopsis thaliana*, CK signaling is mediated via multistep phosphorelay (MSP) pathway through the concerted efforts of the different components including the sensor histidine kinases, ARABIDOPSIS HISTIDINE KINASE (AHK)2, AHK3, and AHK4 that recognized CK via its CHASE domain; ARABIDOPSIS PHOSPHOTRANSFER PROTEIN (AHP) that relays the signal from the AHKs in the cytoplasm to the response regulators in the nucleus; and the response regulators, ARR5-B and ARR5-A which regulate the transcription of many CK-responsive genes and serves as the feedback mechanism for the whole signaling pathway. Recently, many researches focused on the role of several MSP components on the regulation of plants' responses to abiotic stresses. Cytokinin receptors, AHK2, and AHK3 were reported to be upregulated by drought and osmotic stress and that *ahk2 ahk3* double mutant are drought tolerant (Tran et al., 2007; Nishiyama et al., 2011). Moreover, the stimulation of cytokinin biosynthesis, as well as the constitutional stimulation of AHKs improves plant growth, biomass production, and seed yield (Bartrina et al., 2017; Skalák et al., 2019). Furthermore, the MSP pathway integrates several external and internal signals into a single multistep pathway (Dobisova et al., 2017; Zdarska et al., 2019). All these suggest the multifaceted nature of CK (Pavlů et al., 2018). Because of these, we decided to identify naturally occurring genetic variability in the selected components of MSP, the sensor histidine kinases in *Arabidopsis thaliana* via the use of bioinformatics and experimental approaches, combining information from publicly available resources like the 1001 Genomes with PCR-based targeted sequencing. Ecotypes were pooled and selected by comparing the particular form of SNP they harbor with previous mutagenic studies (e.g., Bartrina et al., 2017). Potential candidate accessions containing SNP close to the CK-binding domain of AHKs were identified and investigated their CK responses using root-elongation assay and RT-qPCR analysis of CK reporter genes. The site-directed mutagenesis confirmed the altered binding capacity of identified AHK homologs providing direct evidence of naturally occurring variation in CK sensitivity among various *Arabidopsis* accessions. The genotypes revealing contrast phenotypes in cytokinin responsiveness were selected for the high-throughput phenotyping at VIB, UGent to determine the role of natural variability in terms of the drought response of the chosen ecotypes. Variation in terms of drought response was observed among the tested ecotypes. However, it was observed that ecotypes with less sensitivity to CK are more tolerant to drought. These results provide an initial confirmation that changes in the CK sensitivity can modulate stress response in plants particularly drought response.