PROJECT TITLE

Phenotyping of BPM1-mediated heat stress response in Arabidopsis thaliana

CONSORTIUM

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SUMMARY OF THE REPORT

In *Arabidopsis thaliana*, BPM proteins from the MATH-BTB family participate in the ubiquitin-proteasomal protein degradation pathway (UPS) as Cullin3-dependent E3 ligase substrates. By mediating protein degradation, BPMs are involved in the regulation of various plant processes that enhance phenotypic and physiological adaptability and the ability of plants to survive in a changing environment. Recent research has revealed a novel role for BPM1 protein in RNA-directed DNA methylation (RdDM) through direct interaction with DMS3 and RDM1, known components of the RdDM machinery. Through *de novo* DNA methylation, RdDM has a role in controlling genome integrity and gene expression, particularly during plant acclimation to fluctuating environmental conditions. Given the current and expected climate change, increased temperature has become one of the most important parameters affecting all living organisms, including plants. Therefore, it is invaluable to identify and characterize the components of signaling network, as well as influence of heat stress on biochemical, physiological and morphological changes. The main purpose of our project was to further elucidate the contribution of BPM1 to heat stress response in *Arabidopsis* using high-throughput plant phenotyping. We designed an experiment in which 23 different *Arabidopsis* genotypes with modified UPS and RdDM mechanisms were exposed to 40 °C for six hours. A total of 80 plants of each genotype were divided into four groups – a control group and three treatment groups. The control group remained under growth conditions throughout the experiment, while the treatment groups were exposed to 40 °C at different stages of development. The first treatment group was exposed to 40 °C when plants had two rosette leaves, while the second group was exposed when plants had eight rosette leaves. The third group was exposed to 40 °C at both two and eight rosette leaves stages. RGB images were taken daily, while chlorophyll fluorescence was measured immediately after the heat treatment, and 24 and 48 hours after the stress. For further gene expression and biochemical analyses, ten replicates per genotype and treatment were collected 48 hours after the last treatment. For fresh and dry weight analysis, seven replicates were collected one week after the last treatment, while three replicates will be collected after seed maturation for germination tests. The data obtained so far revealed differences in growth and chlorophyll fluorescence parameters between the different genotypes and treatment groups. Through further analysis, we expect to identify *Arabidopsis* lines which are more tolerant to heat stress based on phenotypic, physiological, and biochemical profiles. We also plan to correlate the heat-tolerant *Arabidopsis* lines and their gene expression to determine whether the response to heat stress is mediated by the ubiquitin-proteasome pathway or by *de novo* DNA methylation through the RdDM machinery.