

SUMMARY

Biochemical and physiological characteristics of *mkk2* insertion mutant of *Arabidopsis thaliana* (L.) Heynh.

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The mitogen-activated protein kinases cascade (MAPKs cascade) is a universal pathway found in all known eukaryotic cells. It plays a central role in the perception and transduction of growth, developmental and stress signals. The MAPK cascade has a hierarchical structure and consists of three levels: MAPKKK-MAPKK-MAPK. Activation of MAPKKKs by upstream signals results in sequential phosphorylation of their downstream MAPKKs and MAPKs and resultant/subsequent changes in the activity of other enzymes or selected transcription factors. MKK2 (mitogen-activated protein kinase kinase 2) and its role in plant metabolism has not been thoroughly studied so far. The aim of this thesis was to characterize the phenotype of *A. thaliana* with inhibited expression of *MKK2* and to determine the function of *MKK2* in *A. thaliana* growth and development as well as stress responses.

The conducted research has attempted to verify the following hypotheses:

1. *MKK2* participates in the processes of growth and development in *A. thaliana*.
2. *MKK2* affects the response of plants treated with selected, abiotic stress factors: excess light and salinity.

Plant genotyping confirmed the presence of an insert in the *MKK2* gene and homozygosity of the *mkk2* mutant's *A. thaliana* SAIL line. In addition, *MKK2* gene expression in *mkk2* insertional mutants was completely inhibited, what allowed to draw conclusions about the function of this gene. *mkk2* mutant plants yielded significantly more biomass and its rosette area was larger compared to wild type (WT) *A. thaliana*. In order to explain these differences, measurements of photosystem activity, content of photosynthetic pigments, selected photosystem proteins and RubisCO were performed. In addition, gas exchange, stomatal density, stomatal index, the water content, the level of selected phytohormones in *A. thaliana* leaves were determined. Based on the obtained results, it was found that inhibition of *MKK2* expression in *A. thaliana* was associated with increased: quantum yield of both photosystem I (PSI) and photosystem II (PSII), structural protein content of the PSII light-harvesting antenna complex Lhcb2, chlorophyll and carotenoids per area of the leaf, and a more efficient CO₂ assimilation. In addition, an increase in the concentration of indolilo-3-acetic acid (IAA) and salicylic acid (SA) was observed as well as a reduction in the content of abscisic acid glucose ester (ABA-GE) and jasmonic acid (JA) in *mkk2* mutants. The results indicate a small relationship between *MKK2* inhibition and the activity of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and non-specific peroxidase (POX). On the other hand, differences in H₂O₂ concentration between *mkk2* mutants and WT plants suggest an involvement of *MKK2* in ROS metabolism.

The presented data indicate that the phenotypic effect of accelerated growth, biomass production and leaf area was due to the more efficient photosynthetic activity of *mkk2* mutants. In addition, the size of the parenchyma cells and the increased water content that probably determined plant biomass most likely resulted from IAA production activated in *mkk2* mutants. These results indicate a significant contribution of *MKK2* as a negative regulator in the processes of growth and development in *A. thaliana*. *MKK2* participates in plant metabolism through changes in the activity of photosynthesis, phytohormonal content and antioxidative system. *MKK2* kinase also mediates the response to stress factors: salinity

(NaCl) and the excess light stress (EL), which indicate a role of *MKK2* in plant acclimatization mechanisms. The diagram summarizes the conclusions of the conducted research (Fig. 1).

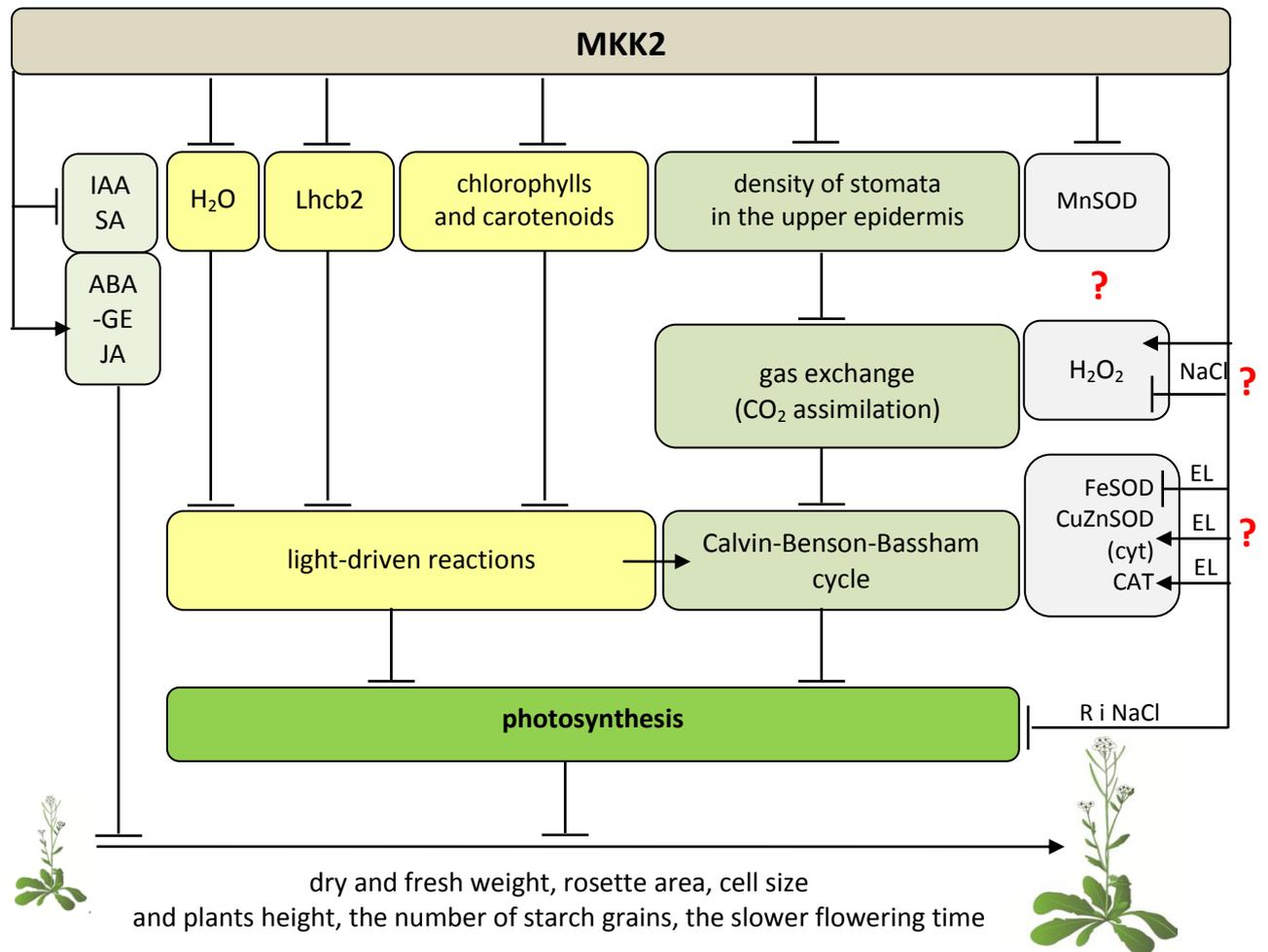


Fig. 1. Scheme presented *MKK2* kinase regulation of growth-development and stress processes in *A. thaliana*. CAT - catalase; cyt - cytosolic; EL - excess light stress; H₂O₂ - hydrogen peroxide; IAA - indolyl-3-acetic acid; Lhcb2 - structural protein of the photo-light complex of PSII; MnSOD / FeSOD / CuZnSOD - manganese / ferric / copper-zinc superoxide dismutase; NaCl - sodium chloride; R - recovery conditions; SA - salicylic acid. Stress conditions in which changes were observed were given over the lines. The remaining lines indicate control conditions. ? - indicates the need for further research due to the more sophisticated regulatory mechanisms in antioxidative processes. Mark "⊥" indicates inhibition and "↓" stimulation of the process.