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Review

"Determining the role of the MAPKKK17/18-ABI1 PP2C signalling module in regulating the cellular response to abscisic acid"

by

Sivakumar Krishnamoorthy

Plants are excellent in surviving changing environments, even under hostile conditions. The reason for this hardness is a powerful flexibility enabling the plants to change functionality and thereby to adapt to the new conditions. Central players in this adaptive process are differential gene expression and upstream signaling pathways including action of phytohormones. This work is about one of these phytohormones, the abscisic acid (ABA). Though this little molecule is known since long, we are still far away from understanding the complex regulatory pathways of ABA-signalling.

The thesis by Krishnamoorthy focuses on two aspects of ABA signaling: the role of i) MAP kinase kinase kinases and ii) of the ubiquitin E3 ligases. Recent publications show that both are involved in ABA signaling, but the exact mechanisms are still unknown. In detail, Krishnamoorthy analyzed the function of MAPKKK17 and MAPKKK18 in ABA signaling, investigated the cross-talk between ABA and auxin signaling using *ABI1*and *MAPKKK18* mutants and evaluated contribution of ubiquitin

E3 ligases *UPL4* and *UPL6* in ABA signaling. This is a rather novel and interesting approach.

In general, the work by Krishnamoorthy was very successful. To detect early signaling-events, he set-up a system to record responses to ABA application after 4 hours. The central methodological approach was RNAseq, allowing to investigate fast reprogramming of gene expression. To analyze the role of MAPKKK17 and 18 and their interaction with ABI1 and E3 ubiquitin ligases UPL4 and 6 in ABA signaling, gain- and loss-of-function mutants were used. This straight-forward approach resulted in highly interesting novel results. In detail, the results show that both MAPKKKs are involved in ABA signaling. On one hand, they seem to have overlapping roles in ABA signaling, e.g. in stomata regulation or ABA-jasmonic acid signaling. However, there are also distinct functions of these two MAPKKKs, e.g., a function of MAPKKK17 in RNA processing and a function of MAPKKK18 in developmental processes. Another interesting outcome is that in co-operation with ABI1, MAPKKK18 is involved in crosstalk between ABA and auxin signaling. In addition, it could be shown that UPL4 somehow regulates ABA sensitivity acting on root elongation and stomatal development. Both, UPL4 and UPL6 seem to be integrated in ABA signalling modules as potential fine-tuners. These results give valuable new insight into the complex, interactive ABA-signaling pathways. They will be of great value for future work.

The technical performance was excellent and the thesis is clearly and understandable written, with all information needed. Looking at transcriptomic data reveals a lot of information and the excellent approach used here, with transcriptomics of different and overlapping ABA responses of mutants of the key players, is highly important. The next steps would now be to focus on specific modules which are indicated by differential gene expression. Based on these results, it would be nice to create models, illustrating the functional performance of such hubs with the MAPKKKs and the E3 ubiquitin ligases. Besides all the tables with top regulated genes, that would have been a great addition. Another additional approach could be to investigate the role of the MAPKKKs on differential gene expression in more detail, concentrating for example on specific transcription factors. This could be done by qRT-PCR which would also validate the RNAseq data, and would allow a deeper time-scale of ABA response, checking response not only at one time-point (4h), but also at earlier and later stages.

The doctoral dissertation demonstrates the candidate`s general theoretical knowledge in the discipline and the ability to conduct independent scientific work.

The subject of the doctoral dissertation is an original solution to a scientific problem.

I highly recommend the thesis of Sivakumar Krishnamoorthy to be accepted and rate it with

1,3.

(magna cum laude)

Prof. Dr. Klaus Humbeck