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Review of the Ph.D dissertation entitled:

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

by Msc Sivakumar Krishnamoorthy

Formal basis for the issuance of the review

This review of the doctoral thesis by Msc Sivakumar Krishnamoorthy was prepared on the basis of the resolution of the Scientific Council of the Biological Sciences Discipline at Adam Mickiewicz University in Poznan, dated 23 January 2026, and the letter from the Dean of the Faculty of Biology, Prof. Beata Messyasz, dated 16 February 2026.

Mr Sivakumar Krishnamoorthy, MSc, completed his doctoral thesis at the Department of Biotechnology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan. His work was supervised by Dr hab. Agnieszka Ludwików, associate professor at the Adam Mickiewicz University.

The subject of the doctoral thesis and the rationale for choosing the research topic

The subject of the doctoral thesis by Mr. Sivakumar Krishnamoorthy, MSc, was to investigate the role of MAPKKK17 and MAPKKK18 kinases and ABI1 phosphatase in *Arabidopsis thaliana* in response to abscisic acid (ABA), using differential transcriptome analysis of the *mkkk17-1*, *mkkk18-1*, and *abi1td* mutants compared to the wild-type (WT Col-0). Additionally, the PhD student explored the role of two HECT-type ubiquitin E3 ligases, UPL4 and UPL6, in ABA signalling and their interaction with upstream components in the signalling pathway, employing phenotypic tests on knockout mutant lines and plants overexpressing *UPL4* and *UPL6*. ABA is a primary stress phytohormone, regulating plant responses to adverse abiotic environmental

conditions. The interaction of ABA with MAP kinase cascades – key signalling modules and with the proteasome regulation system highlights the network complexity. This network ensures the specificity of the response, enabling plant organisms to adapt to changes in their external environment. Although the main components of the ABA signalling pathway are already well characterized, the mechanism of ABA integration with additional regulatory levels, such as MAPKKKs and E3 ubiquitin ligases, remains largely unknown.

The PhD student undertook ambitious tasks: (1) characterizing the role of MAPKKK18, MAPKKK17 kinases, and ABI1 phosphatase in ABA signalling in *Arabidopsis thaliana*, including identifying ABA-responsive genes regulated by these proteins; (2) determining the physiological role of the ubiquitin-proteasome system components, UPL4 and UPL6 ligases, in ABA-dependent development of roots and stomatal apparatus, and their significance in transcription regulation. The multilayer model proposed by the PhD student shows that MAPKKK17/18 and ABI1 determine transcriptional responses to ABA, while UPL4/UPL6 regulate proteasomal processes. This model will contribute to a better understanding of the molecular basis for balancing plant growth and adaptation to stress. The subject of this dissertation is important and aligns with current research trends.

Formal Evaluation of the Doctoral Dissertation

The doctoral dissertation is a 157-page document written in English, featuring 32 figures and 16 tables. The bibliography is extensive, comprising 370 references. The structure follows the standard format for doctoral dissertations. The dissertation includes an Abstract (both in Polish and English), a list of abbreviations, an Introduction, Hypotheses and Objectives, Materials and Methods, Results, and Discussion. The work concludes with a concise Summary, in which the PhD student presents the most significant findings from the research, as well as two Final Conclusions based on these results. Additionally, the dissertation contains lists of figures and tables. The title of the dissertation is correctly formulated, and the research topic presented in the thesis is consistent with its title.

Although the thesis has been written correctly, and the careful editing and layout of the text deserve praise, the PhD student has not managed to avoid a few minor shortcomings. A common error noted in many parts of the reviewed thesis is the use of italics for protein names, whilst gene names and Latin species names are not italicized. In section 3.2. (p. 46) a list of molecular markers is missing. On pp. 48 and 55, in the sections referring to the growth of mutant and Col-0 seeds, there should be a reference to Chapter 3.9. instead of Chapter 1.1. or 3.4. Similarly, on p. 57, when referring to plant cultivation conditions and the application of exogenous ABA, a reference to Chapter 3.10. should have been included, rather than to Sections 3.4. and 3.5. The description of sample preparation for RNA-seq is found in Sections 3.11.-3.13., not as indicated in Sections 3.6. and 3.7. (p. 57), whilst the description of how the GO enrichment analysis was conducted is not included in Section 3.14. but in Section 3.19. The reference to subsections 5.1.3. and 5.3.2. (pp. 89-90) as well as to section 3.1.7 (p. 95) and sections 3.1., 3.3., 3.5. (p. 97) is also incorrect.

I would like to emphasise that these errors do not affect my positive overall assessment of the thesis.

Substantive Evaluation of the Doctoral Dissertation

Upon evaluating the dissertation substantively, I can confidently state that the research design, selection of research methods, conduct of the study, and interpretation of the results all meet the required standards.

In the 'Introduction' chapter, the PhD student reviewed the latest literature and provided a clear and comprehensive description of the canonical ABA signalling pathway – PYR/PYL/RCAR-ABI1-SnRK2. He then characterized in detail the MAP kinases in *Arabidopsis thaliana* and explained the mechanisms regulating signal transduction via MAP kinase cascades, highlighting their role in intracellular signalling in plants. The PhD candidate devoted particular attention to the fundamental role of the MAPKKK18/MAPKKK17 cascade in ABA signalling. In the following subchapters, Mr Sivakumar Krishnamoorthy discussed issues related to the selective degradation of proteins by the cellular 'clean-up system', known as the ubiquitin-26S proteasome. He described E3 ubiquitin ligases as key enzymes involved in the ubiquitination process. Above all, the PhD student focused on HECT-type E3 ubiquitin ligases, known as UPL ligases, which are the subject of his research. He discussed their role in regulating components of the ABA signalling pathway. The final subsections of the Introduction are devoted to the interactions between ABA and auxins in plant developmental processes. These include seed germination, root elongation, and lateral root formation. Mr Sivakumar Krishnamoorthy also discussed MAP kinase signalling pathways, focused on the activation of the common MKK3-MPK1-RBK1 module. Although antagonistic and/or synergistic interactions between phytohormones are not the primary focus of this study, they are undoubtedly of great interest. Given the novelty of this topic, I would ask the PhD student to explain the mechanism by which the MKK3-MPK1-RBK1 module is activated upon auxin perception. In addition, is this mechanism similar to that involved in the activation of this module in the presence of ABA?

This section has only one shortcoming:

1. Figure 3 (p. 36) shows that ABA-dependent inhibition of PP2C phosphatases blocks SnRK2 kinase activity, which is incorrect and conflicts with the accepted mechanism and previous description.

However, reading the Introduction was a pleasure.

The Introduction clearly highlights the main findings that justify the research significance. It demonstrates the PhD candidate extensive understanding of the topic and his ability to select pertinent academic literature that supports the study.

The PhD thesis clearly defines five objectives suitable for doctoral research and presents two research hypotheses, which are supported by the findings.

The 'Materials and Methods' chapter outlines the study methodology, research material and cultivation conditions, the kits and reagents used, and the bioinformatics tools, following typical conventions. It may be beneficial to include a description or reference to the *upl4.1*, *upl4.2*, and *upl6* mutant lines, as well as information on the repository (SRA or GEO) where the raw RNA-seq data are deposited.

The chapter, 'Results', is divided into six subsections, in which the PhD student presents in detail the results from the experiments conducted during the various research tasks. Each stage of the research discussed begins with a statement of its objective, supporting understanding of the experimental approach chosen by the PhD candidate, and concludes with a careful summary of the results obtained. The thorough description of the experiments, accompanied by 27 figures and 14 tables, is comprehensive and raises no concerns.

Questions and comments regarding the results of the thesis:

1. The PhD student states that GO enrichment analysis of 164 down-regulated genes in the *abi1td* mutant revealed a significant overrepresentation of auxin-related processes (p. 89). He concludes that GO enrichment analyses of both *abi1td* (Fig. 28) and *mapkkk18* (Fig. 22) mutants suggest that ABI1 and MAPKKK18 inhibit the expression of auxin-related genes as part of the ABA response (p. 90). I can't agree with this conclusion. If auxin-related gene expression is down-regulated in *abi1td* and *mapkkk18* mutants, ABI1 and MAPKK18 are likely activators rather than inhibitors.
2. Mr Sivakumar Krishnamoorthy confirmed by RT-qPCR that *UPL4* and *UPL6* genes were not transcribed in *upl4.1*, *upl4.2*, and *upl6* mutants. Were the analyzed knockout lines also tested for the presence of the UPL4 or UPL6 protein? On page 96, the statement: "RT-qPCR analysis confirmed the disruption of gene expression in these knockout lines, as evidenced by the absence of 18S rRNA transcript" is unclear. Please explain.
3. The results description did not provide detailed documentation of the verification of lines overexpressing the *UPL4* and *UPL6* genes. Could you please clarify the criteria used to select lines for functional studies? In addition, the PhD student generated two complementation lines for the *UPL4* and *UPL6* genes: 35S: *UPL4-GFP-HIS/upl4.1* and 35S: *UPL6-GFP-HIS/upl6.1*, the characteristics of which he also omitted from the results description. Could you please share information about the expression of the *UPL4* and *UPL6* genes in these lines compared to the wild-type and the overexpression lines (35S: *UPL4-GFP-HIS/WT-4* and 35S: *UPL6-GFP-HIS/WT-3*)?

I would like to ask the PhD candidate to address the above comments and answer the questions posed during the public defence.

The most significant achievements of the PhD candidate's research:

1. Demonstrating that MAPKKK17 and MAPKKK18 kinases perform distinct, though partially overlapping, functions. MAPKKK17 primarily regulates RNA metabolism and post-transcriptional processes in response to ABA, while MAPKKK18 links ABA signalling to developmental plasticity, particularly in the development of organs and regulation of stomatal density.
2. Demonstrating that ABI1 phosphatase and MAPKKK18 kinase modulate auxin-responsive genes, indicating ABA and auxin interaction through shared transcriptional networks.
3. Furthermore, demonstrating the roles of HECT-type ubiquitin E3 ligases UPL4 and UPL6 in ABA-dependent plant development reveals their distinct functions: UPL4 acts as a positive regulator of root growth and a negative regulator of stomatal development, thereby controlling

stomatal patterning, whereas UPL6 inhibits epidermal cell proliferation and affects stomatal density.

The "Discussion" chapter presents a comprehensive analysis in which the PhD student thoughtfully compares his own findings with the literature on the functional similarities and differences between MAPKKK17 and MAPKKK18 kinases in ABA signalling and in the regulation of developmental processes. The discussion also considers the interaction between the "growth hormone" auxin and the "stress hormone" ABA, both of which balance plant development in response to environmental conditions, and explores the roles of the HECT-type E3 ubiquitin ligases UPL4 and UPL6 in ABA-dependent regulation of root and stomatal development. I found these sections especially engaging.

In terms of content, I hold the thesis submitted for review in very high regard. The structure of the dissertation appears well considered and effectively organized.

Final conclusion

In summary, I conclude that the doctoral thesis submitted for review by Mr Sivakumar Krishnamoorthy, MSc, demonstrates a sound theoretical grounding in the discipline of Biological Sciences and the ability to conduct independent research.

The subject of the doctoral thesis is an original solution to a significant scientific problem. The doctoral thesis submitted for review meets the conditions set out in Article 187(1)-(2) of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2025, item 1691, as amended).

In view of the above, I hereby request the Scientific Council for Biological Sciences at Adam Mickiewicz University in Poznan to admit the PhD candidate to the subsequent stages of the doctoral procedure.

Agnieszka Kiełbowicz-Matuk

dr hab. Agnieszka Kiełbowicz-Matuk