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Review of the doctoral dissertation of Muhammad Kamran, MSc.

entitled "CRK5 regulates senescence, photosynthesis, and dissipation of energy as heat in Arabidopsis thaliana by inhibitory effect on salicylic acid signaling" supervised by
***professor Stanisław Karpiński, PhD, DSc Eng.**, Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology, Warsaw University of Life Sciences*
and co-supervisor
***Paweł Burdiak , PhD, DSc Eng.**, Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology, Warsaw University of Life Sciences*

Legal and formal basis of the review

This review was prepared on the basis of the resolution of the Warsaw University of Life Sciences dated April 23, 2026 (Resolution No. 30/NB-2025/2026), appointing me as a reviewer in the procedure for conferring the doctoral degree upon Muhammad Kamran MSc. and, requesting the preparation of this doctoral dissertation review and a determination of whether it meets the requirements specified for this type of work.

The doctoral dissertation submitted by Muhammad Kamran, MSc, incorporates data previously partially published by the Author across three journal articles. The comprehensive summaries in both Polish and English encompass 9 pages (pp. 12–21), whereas the full English description of the dissertation's scope—including conclusions and literature—spans 30 pages (pp. 22–52). The cumulative dissertation consists of three manuscripts: one published original research article, one published review paper, and one original research article currently under peer review. All three are associated with highly reputable journals of international standing, specifically:

1. **Muhammad Kamran, Paweł Burdiak** and **Stanisław M. Karpiński** , Crosstalk between abiotic and biotic stresses responses and the role of chloroplast retrograde signaling in the cross-tolerance, *Cells* 2025, 14, 176, (ministerial score 140, IF:5,2)
2. **Muhammad Kamran, Paweł Burdiak**, Roshanak Zarrin Ghalami, Anna Rusaczonek, Maria Duszyn, Kinga Gołębiewska, Piotr Gawroński and **Stanisław M. Karpiński**, The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic and signaling, *Plant Physiology* 2025, 200, 1-12 (ministerial score 140, IF:6,9)
3. **Muhammad Kamran, Paweł Burdiak**, Anna Rusaczonek, Roshanak Zarrin Ghalami, and **Stanisław M. Karpiński** CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid and signaling pathway, submitted to *BMC Plant Biology* (ministerial score 140, IF:4,8)

In all of these papers, prof. Karpiński is the senior and corresponding author. Based on the signed co-author statements, the PhD candidate's contribution to all three publications is unquestionably significant.

Comparing the sizes of the smallest known plant and animal genomes reveals that they are remarkably similar. This is intriguing, as plants—which might appear structurally simpler—would intuitively be expected to require fewer genes. This phenomenon warrants closer examination. Aside from the fundamental distinction between autotrophy and heterotrophy, the primary difference between plants and animals lies in their response to stress factors. Because the vast majority of plants are sessile, they are highly vulnerable to environmental changes and cannot physically escape stressors; consequently, they spend their entire lifespan, sometimes spanning thousands of years, in a single location. Throughout the growing season, plants require relatively stable access to light while simultaneously enduring numerous environmental challenges. Their survival and response optimization depend heavily on a complex genetic system. This requires a vast amount of genetic information to effectively mitigate diverse environmental pressures, typically classified as biotic and abiotic stresses.

Recent studies demonstrate that responses to various types of stress share identical or highly similar elements. Until recently, salicylic acid was considered a key and defining element of the response to biotic stress; however, this view is now outdated. The PhD candidate focused precisely on this aspect of cellular signaling in response to stress factors and the optimization of light intensity. The Author presented arguments that facilitate a better understanding of the signal transduction mechanism. This phenomenon is directly related to the optimal activity of numerous enzymatic proteins, as well as the flux of metabolites through the membranes of specific cellular compartments. A detailed analysis of these issues also indicates that plants can utilize incoming radiation only within a strictly limited range. One mechanism enabling plants to thrive in a changing environment is the optimization of the levels and mutual proportions of photosynthetic pigments (chlorophylls and carotenoids). Fluctuations in the intensity and spectral composition of incoming light are often rapid, requiring an immediate response and adaptation. Excess excitation energy (AEE) can also be dissipated through biophysical processes, namely by being converted into thermal energy. The generated thermal energy can significantly influence the course and efficiency of physiological processes. Fluorimetric analysis is a rapid and unambiguous method for determining photochemical activity within the photosynthetic apparatus, enabling the assessment of specific segments of the electron transport chain (ETC). Certain parameters, such as the Non-Photochemical Quenching (NPQ) value, prove particularly useful for the objectives specified in this doctoral dissertation.

Understanding how plants respond to stressors and the subsequent sequence of metabolic events—particularly when facing a combination of biotic and abiotic stresses—occupies much of plant physiologists' attention. Specifically, research focuses on how plants react when exposed to intense versus low light intensity, and how these responses vary depending on the developmental stage. The presented studies examined the role of cysteine-rich receptor-like kinase 5 (CRK5) as a negative regulator of salicylic acid-dependent pathways, which regulate dark-induced senescence (DIS), ROS homeostasis, and induced cell death (CD). A key question raised was how signaling coordinated by CRK5 and salicylic acid modifies the management of excess excitation energy (AEE), which can either dissipate as heat or lead to elevated ROS levels and CD induction. The analysis primarily focused on the role of kinases and transcription factors (TFs) in retrograde signaling from chloroplasts, the transcriptional response, and stress-induced CD. Plant reactions were investigated regarding the initiation of cross-tolerance responses and the integration of SA signaling into cell senescence, photosynthesis, and AEE dissipation, as well as the signaling coordination among SA, ROS, and CD pathways.

Detailed evaluation of the dissertation with justification

The research presented in this dissertation clearly posed a challenge for the author at every stage – from the initial concept, through methodology development, analysis, and interpretation of results, to the final visual presentation. Completing this work required the candidate to master numerous advanced techniques, including RNA sequencing and bioinformatics analysis. I believe that interpreting such a massive dataset must have been the greatest challenge. Overall, the dissertation deserves a positive evaluation due to its multifaceted approach to the research problem, advanced methodology, and meticulous preparation. Nevertheless, I have the impression that during the initial planning phase, the experimental design became overly complicated.

I highly value the choice of topic and the core subject of the manuscript. Climate change is one of the most critical issues facing the world today. Rising temperatures, combined with prolonged periods of drought, accelerate evaporation, ultimately leading to increased soil salinity.

Arabidopsis thaliana is a highly fascinating species for plant biologists. A particularly interesting property is its flexible metabolic regulation system, which enables the plant to complete its life cycle in different environments. Additionally its genome is sequenced and we know many well described mutants. It is therefore no surprise that this species has been used for many years by researchers worldwide—including the team of the Doctoral Candidate's Supervisor—as an excellent model for studying plant homeostasis mechanisms in the presence of abiotic and biotic stress.

The first of the presented articles covers a very broad subject, focusing on how plants react to diverse stresses. This publication provides experimental evidence of retrograde signaling from chloroplasts that triggers a nuclear defense response. The cited measurements show changes in the level of non-photochemical quenching (NPQ), which simultaneously serves as a component inducing signals for chloroplast retrograde signaling. Furthermore, the article highlights the role of NPQ in initiating responses that stem from the reduction state of plastoquinone, redox state modifications, the ROS pool, and hormonal signals. Coordinated reactions in both systemic responses and acquired acclimation ultimately lead to the resistance of plant tissues. **The central role of CRK5 in plant metabolism is also briefly mentioned,** including its involvement in the induction of cell death (CD) and the coordinated response to both biotic and abiotic stresses.

In the conclusions of this paper, following a highly substantive argumentation, the authors state that plants are "...environmentally smart and intelligent, they communicate...." While I understand the authors' intentions behind these words, their further dissemination may lead to misunderstandings, especially regarding living plants. **What is Candidate's opinion in this matter?**

Regarding this article, I have also one reservation: namely, **what does the PhD student's involvement in "planning experimental work" mean in the context of a review paper?**

In the second article, which constitutes the core of this dissertation, the Author presents experimental work aiming to precisely determine the role of CRK5 in integrating DIS signaling and modulating the impact of AEE linked to the SA signaling pathway. For this study, crk5 and cpr1 mutant lines with impaired SA and ethylene synthesis were utilized. It was successfully demonstrated that CRK5 acts as a regulator of photosynthesis, promoting plant growth and AEE energy dissipation, which results in lowered leaf temperature and accelerated DIS, while simultaneously serving as a negative regulator of the SA pathway. This response is accompanied by transcriptional changes in an entire suite of transcription

factors related to WRKY, SA-pathway-regulated senescence, photosynthesis, and NPQ under prolonged darkness.
A crucial element of this research involved analyzing the phenotypes obtained by crossing these mutant lines. These observations led to the conclusion that CRK5 specifically modulates the SA-dependent pathway. Furthermore, SA accumulation was found to induce a phenotype similar to that of the crk5 mutant, and exogenously applied SA decreased NPQ and leaf temperature regardless of the genotype. This provides additional evidence that SA acts as a negative regulator of non-photochemical quenching (NPQ)—the thermal dissipation of excess light energy. Consequently, it was concluded that CRK5 maintains high photosynthetic activity by inhibiting SA-driven responses.

The third article demonstrates a consistent continuation of the previous research line, specifically tracking the links between salicylic acid (SA) signaling, the cellular redox state, and the initiation of cell death (CD). In the crk5 mutant, an accumulation of reactive oxygen species (ROS) and accelerated CD were observed, accompanied by increased cellular electrolyte leakage. A depleted pool of low-molecular-weight antioxidant compounds (xanthophylls and carotenoids) along with an enriched pool of phenolics reflect a high ROS-scavenging demand, which is further supported by enhanced antioxidant enzyme activity. These correlations are corroborated by transcriptomic analyses revealing the activation of key pathways involved in CD, ROS scavenging, and redox state alterations. These shifts point to a critical role for CRK5 in SA-dependent cellular reprogramming. Thus, it was demonstrated (consistent with the findings in the second paper) that SA signaling is negatively regulated by CRK5, which consequently explains the observed gene expression patterns under DIS exposure.
Regarding the co-author contribution statements, **specifying individual percentages with a precision of 0.5% is unjustified**, as it is highly improbable that such contributions can be quantified with this level of accuracy.

In summary, the three articles collectively demonstrate that plants exposed to a multitude of environmental stresses utilize the CRK5 protein to modulate SA signaling, adjust antioxidant capacity, and regulate cell death (CD) to maintain stable growth and ensure optimal development, particularly under excessively intense light conditions. Given the long-established regulatory role of chloroplasts in cellular metabolism, alongside the pivotal function of CRK5 in basic plant cell physiology, investigating the interdependencies among these specific processes is highly justified.
However, when evaluating the Author's conclusions, **it is questionable whether the data support such a far-reaching statement regarding a uniquely crucial role for this protein in plant acclimation to ongoing global climate change.** While CRK5 is undoubtedly important for general acclimation processes—which inherently encompass responses to global warming—the same logic could apply to virtually any physiological study addressing the regulation of pathways directly or indirectly influenced by temperature. Elevated temperatures naturally modify the vast majority of physiological processes, including light energy absorption and dissipation. Nevertheless, such broad physiological interconnectedness does not justify asserting that this specific work is directly linked to global warming. The diagrams selected by the author to summarize the three articles (Figs. 2, 5 and 8) show similar relationships and are, to some extent, repetitive. However, they are useful for understanding the significant progress that has been made.
Chronologically, from the very beginning of the work on this topic, the starting point of the entire doctoral dissertation was a mature concept that had already been repeatedly verified by Prof. Karpiński's research group. This concept, presented in the first article of this PhD thesis,

was supported by very strong arguments in favor of conducting the experimental verification described in the subsequent two papers. Evaluating the thesis as a whole, one must appreciate the consistency, the mutual integration of the three texts, and the development of the research concept, which, in my assessment, is exemplary. The multifaceted and mutually reinforcing confirmations at the physiological, molecular, and biochemical levels provide a strong conviction that the presented concepts will be difficult to refute. Without questioning either the maturity of the concept or its positive experimental verification, I would like to ask about the basis for the conviction expressed in the final sentence of the abstract (first paper) that there is a common molecular system regulating the cell death (CD) process, acclimation, and cross-tolerance; however, whether this constitutes a single system remains unknown. **How would the Doctoral Candidate comment on such a view?**
The comparison of plant responses to biotic and abiotic stress is not a new topic; nevertheless, global literature continuously produces numerous papers indicating that certain responses are specific to both types of stress. The dissertation cites an extensive body of literature highlighting the limitations of such a perspective. However, the physical presence of potential pathogens within a plant likely poses an additional challenge to its development. The organism must cope with this factor as well, which will inevitably trigger a specific response. **Could you comment on this aspect?**

Concluding Remarks
The doctoral dissertation submitted by Muhammad Kamran, MSc, presents substantial experimental data, parts of which have been published in the three peer-reviewed articles detailed above. Furthermore, the accompanying co-author contribution statements leave no doubt regarding the dominant and leading role of Mr. Kamran in the conception, execution, and preparation of these publications. Mr. Muhammad Kamran, MSc, demonstrated extensive theoretical knowledge and familiarity with modern techniques necessary for conducting research work. In addition, the PhD candidate performed a vast amount of research, which constitutes a significant contribution to the progress of studies on the CRK5 protein and will undoubtedly be utilized in the future by researchers working with other plants as well. Beyond the core chapters of this thesis, the PhD candidate has co-authored three experimental papers (one in *Plants* and two in *Cells*) and presented three posters at international scientific conferences. This entire body of work consistently focuses on plant stress physiology, directly or indirectly reinforcing the core subject of his doctoral dissertation.

Conclusion
I, the undersigned, hereby state that the reviewed doctoral dissertation of Mr. Muhammad Kamran meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (consolidated text: Journal of Laws of 2024, item 1571, as amended). Specifically, it demonstrates the Doctoral Candidate's general theoretical knowledge in the field of exact and natural sciences within the discipline of biological sciences, proves his ability to independently conduct scientific work, and constitutes an original solution to a scientific problem. Therefore, I request that Mr. Muhammad Kamran, MSc, be admitted to the subsequent stages of the procedure for the award of the doctoral degree in the field of exact and natural sciences in the discipline of biological sciences. Taking in account the huge number of experiments and their relevance and also the quality of presented publications I propose considering the dissertation for distinction.

/-/ Zbigniew Miszalski

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