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**Evaluation of the doctoral dissertation of M.Sc. Muhammad Kamran entitled „CRK5 regulates senescence, photosynthesis, and dissipation of energy as heat in *Arabidopsis thaliana* by inhibitory effect on salicylic acid signaling"**

**General information about the dissertation**

Doctoral dissertation of Mr Muhammad Kamran submitted for review is composed of three scientific articles preceded by a brief summary in English and in Polish, list of abbreviations and a more extensive summary (30 pages) divided into chapters: Introduction, Research hypothesis and objectives, Materials and methods, Main results and general comments, Conclusions, and References.

First article is a review paper whereas the other two are original research articles. The impact factor of the three journals are high and amount to 5.2, 6.9 and 4.8, respectively. Full details about these articles are as follows:

Article 1: Kamran, M., Burdiak, P., & Karpiński, S. (2025). Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells, 14(3), 176. https://doi.org/10.3390/cells14030176. (Ministerial score: 140; IF: 5.2)

Article 2: Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, Plant Physiology, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046. (Ministerial score: 140; IF: 6.9)

Article 3: Kamran, M., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer review in BMC Plant Biology, Springer Nature. (Ministerial score: 140; IF: 4.8)

Pursuant to Article 187 paragraph 3 of the Act of 20 July 2018, the Law on Higher Education and Science, in my review I focus on the three points discussed below.

**Assessment with justification whether the doctoral dissertation presents the general theoretical knowledge of the person applying for a doctoral degree in a specific discipline**

The first article of the series describes a current state of knowledge on chosen aspects of plant stress defense, in particular, sensing of stress in chloroplasts, generation of stress-related signals and their propagation in and between plants. A crucial role in generation of these retrograde signals is attributed to the absorbed energy in excess (AEE) at photosystem II antenna and its harmless dissipation via the xantophyll cycle, envisaged by nonphotochemical quenching of chlorophyll a fluorescence (NPQ) and to reduction of plastoquinone (PQ) pool. Various signaling molecules are

described, among them proteins (kinases, transcription factors, and other regulators), stress hormones and reactive oxygen species (ROS) and their dual role in regulation of the acclimation to stress or damage (senescence and cell death) is enhanced. An attention is also put to the transmission of these signals in systemic way throughout the plant (systemic acquired acclimation, SAA) and to the surrounding plants (network acquired acclimation, NAA).

Some of the described stress responses are well known, such as signaling from the PQ pool, the action of stress hormones, and cross- tolerance, whereas others represent very recent discoveries. For example, the phenomenon of NAA involving a network of ROS, electric and NPQ signals is a very recent discovery of the research group headed by prof. Stanisław Karpiński. The description of specific actions of Receptor-like Kinases and transcription factors in SAA is a valuable update on this timely and intensively studied topic.

This review contains 2 figures and 2 tables, all of high quality. They present a current status of knowledge about signaling from chloroplasts under stress, systemic communication between leaves of the same plant and network communication between plants via changes in NPQ, ROS and electric signals. Whereas tables summarize the knowledge about various stress factors, phytohormones and chemical compounds associated with induction of cross-tolerance in plants and the dual role of transcription factors in regulation of cross-tolerance and cell death.

This review introduces a great number of references (218). Among them are older publications of fundamental importance for this topic as well as a very recent ones. Based on this thorough analysis of the literature, the objectives of the work were clearly defined and a novel hypothesis has been formulated that a specific protein CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 (CRK5) functions as a key upstream negative regulator of salicylic acid-dependent signaling pathway that coordinate dark-induced senescence, antioxidant homeostasis, and cell death in Arabidopsis. This hypothesis was further verified in the next two articles of the series.

Apart of a big portion of theoretical knowledge supplied by a review paper, a more-focused literature analysis is provided (as an Introduction chapters) in the next two research articles. Introduction chapter in article 2 guides the reader deeply into the topic of SA action and involvement of receptor-like kinases in stress signaling to develop a hypothesis that CRK5 is a negative regulator of senescence through SA-signaling pathway. Whereas, an Introduction chapter in article 3 makes an overview of literature concerning the involvement of SA and CRKs in regulation of senescence and cell death through modifications of ROS and antioxidant system.

To summarize this part of my review, Muhammad Kamran demonstrates a good knowledge of biology of plant stress responses, as well as presents an ability to carefully analyze literature data, recognize knowledge gaps and formulate a novel and timely hypothesis.

**Assessment with justification whether the doctoral dissertation demonstrates the ability of the person applying for a doctoral degree to independently conduct scientific or artistic work.**

In all articles included in the doctoral dissertation Muhammad Kamran is the first author and has a major contribution to them.

In the first article - he participated in the experimental design of the study and he collected resources for this study, prepared figures and tables, wrote a draft version, reviewed and edited the final version. Altogether, his contribution was estimated at 70 %.

In the second article - he participated in experimental design of the study, crossing and PCR validation, he cultivated plants and generated double mutants, performed stress treatment (dark-induced senescence) and collected plants for analysis, performed phenotypic analysis and measurements of ethylene content with gas chromatography, foliar temperature with thermal imaging camera, chlorophyll *a* fluorescence parameters and their heterogeneity (with imaging system and with Dual PAM-100) and gas exchange, as well as he evaluated protein amount by immunobloting. He participated in preparation of the samples and analysis concerning confocal microscopy, pigment content, salicylic acid content, and of RNA-seq library. He performed the statistical analysis of the data and resource collection for the article, prepared all figures and tables, as well as he wrote a draft version, then review and edited the final version. Altogether, his contribution was estimated at 60 %.

In the third article – he participated in conceptualization and experimental design of the study, he cultivated plants and generated the double mutants through genetic crossing and PCR validation, performed stress treatment (dark-induced senescence), collected the material for analysis, performed phenotypic analysis and measurements of relative ion leakage, blue staining for cell death and staining for ROS and prepared RNA-seq library and analysis. He participated in measurements of activities of antioxidative enzymes (APC, CAT, SOD), $H_2O_2$ accumulation, in preparation of samples and analysis concerning carotenes, xantophyll, phenolics, and in determination of radical scavenging activity through DPPH and ABTS measurements. In this article, his contribution was estimated at 67.5 %.

In addition to preparation of articles included in the doctoral dissertation, Muhammad Kamran is also a co-author of 3 research papers (1 published in Plants and 2 published in Cells) and is a co-author of 3 posters presented at International Conferences: The Society for Experimental Biology 2025 in Antwerp, (Belgium), Plant Biology Europe Congress 2023 in Marseille (France), and in The 33rd International Conference on Arabidopsis Research 2023 in Chiba (Japan). He also participated in Summer School at Warsaw University of Life Sciences and he was the executor of grant Opus 20 UMO-2020/39/B/NZ3/02103 of the National Science Centre.

Considering the scientific activity of Ph.D. student and his considerable participation at all stages of preparation of scientific articles (such as: literature mining and recognition of knowledge gaps, design of experiments and its performance with a plethora of variable and modern methodologies, analysis of results and their presentation, discussion of obtained results with literature, preparation of manuscript draft and of its final version) I am fully convinced that Muhammad Kamran is well prepared to conduct the independent scientific research at the excellent level of science.

**Assessment with justification whether the doctoral dissertation constitutes an original solution to a scientific problem, an original solution in the application of the results of one's own scientific research?**

All three articles are of high scientific standard. The articles are published in (article 1 and 2) or submitted to (article 3) a high ranked scientific journals. I also appreciate that these works were done as part of a grant Opus 20.

The most important achievements of this dissertation include:

1. A careful description of a current state of knowledge concerning plant defense mechanisms involving sensing of stress in chloroplasts, generation of stress-related signals and their propagation in and between plants.
2. Providing a genetic, molecular and physiological evidence to support the view that CRK5 negatively regulates dark-induced senescence (DIS) by inhibition of SA signaling. This has been demonstrated with the use of *crk5* mutant and with mutants with low and high SA content, such as: SA-induction-deficient-2 (sid2), a transgenic plant possessing a bacterial salicylate hydrolase gene (NahG), constitutive expresser of PR genes 1 ( *cpr1*) and with the use of double mutants on the *crk5* background, such as: *crk5*/NahG, *crk5*/*sid2* in which the phenotype of *crk5* was reversed.
3. Providing evidence that regulation of senescence governed by CRK5 does not involve ethylene signaling. This has been shown with the use of ethylene-insenitive-2 (*ein2*) mutant and double mutant *crk5*/*ein2*.
4. Discovery that CRK5 positively regulates photosynthesis. It promotes photosynthetic efficiency and photo-protective heat dissipation and this regulation acts by inhibition of SA-dependent signaling.
5. Description of transcriptomic changes which provides an evidence that CRK5 regulates gene expression by inhibiting SA and ROS signaling.
6. Identification of the action of CRK5 on redox homeostasis during senescence. It was documented that this modulation involves carotenes and xanthophylls, enzymatic and non-enzymatic (various phenolic compounds) antioxidants and $H_2O_2$ accumulation.

In general, these deliveries put a new light into the understanding of a molecular background of plant stress responses by revealing a new role for CRK5 protein. These achievements are novel and has a high scientific value.

**Critical notes**

I have found only a few minor inaccuracies or gaps in the dissertation:

a/ The scheme presented in Fig. 1 of article 1 is very informative, however in such a complex picture some connections or their lack might be questionable. For example, in the text (top of p.6) it is written that "MPK4 regulate stomatal closing within seconds, leading to enhanced photorespiration, triggering ROS generation, and ethylene synthesis" (referring to Fig.1) but on this figure such connections from MPK4 are not shown.

b/ Another doubt is related to the time range for chlorophyll *a* fluorescence and NPQ given in the article 1. On p.5 (at the beginning of 2nd paragraph) it is written that AEE dissipation as heat and fluorescence happens within pico- or nanoseconds. Similar information ("pico-, nano-, micro-sec") is given in Fig.1 (box on the top right) in relation to NPQ. I cannot agree with these because the fastest peak of chl fluorescence ("J" peak during transient) appears after 2 ms of excess light whereas the maximum of chl fluorescence (FM) is reached at about 1 s. The induction of NPQ is slower and demands 30-60 s.

c/ In my opinion, the title of the chapter no 3 in article 1 („cross-tolerance") is not fully representative for its content because this part mainly describes retrograde signaling.

d/ In the legend of Fig. 1 on p.6 in article 1 an error occurred: instead of "stormless" should rather be "stromules".

e/ In the discussion of results of heat emission from leaves in article 2 it might be worthy to mention that SA changes the pattern of heat emission from mitochondria by activation of the alternative respiratory flux.

f/ In article 3, the following two formulations wrongly descript the relationship between CRK5 and DIS, although, they may be considered as language mistakes:

l. 315-316: "CRK5, through the prevention of SA-signaling, accelerates dark-induced senescence and cell death"

l. 404-405: "Together, these results indicate that CRK5 inhibits the SA-signaling and foliar ROS levels, thus accelerating DIS, characterized by enhanced ROS production and CD."

g/ In the discussion of results in article 3 it might be worthy to address the point that elevated SA levels inhibit CAT activity.

However, these are only a minor issues and do not affect the high scientific value of this dissertation.

**Final grade**

I, hereby, declare that the reviewed Ph.D. thesis by **Muhammad Kamran** meets the criteria pursuant to the Act of 20 July 2018 on Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended) and Resolution No. 89-2022/2023 of the SGGW Senate dated June 26, 2023, regarding the adoption of the Regulations for conducting proceedings for awarding the doctoral degree at the Warsaw University of Life Sciences and request that the Research Discipline Council of Biological Sciences of the Warsaw University of Life Sciences accepts **Muhammad Kamran** for further stages of doctoral proceedings.

Due to the high scientific value of this work which discovers an important regulatory role of CRK5 protein in retrograde signaling and in plant stress acclimation I, hereby, request that the thesis is accepted with distinction.

Kraków, 12.06.2026

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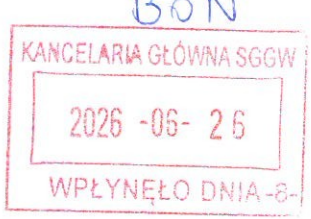



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