UNIVERSITY OF WARSAW

Faculty of Biology
Institute of Experimental Plant Biology and Biotechnology
Department of Plant Anatomy and Cytology, Plastid Nanomorphology Group
dr hab. Łucja Kowalewska, prof. UW

Warsaw, 6.07.2026

Review of the doctoral thesis 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"

prepared under the supervision of Prof. dr hab. inż. Stanisław Karpiński

(assistant supervisor: Dr inż. Paweł Burdiak)

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology,

Warsaw University of Life Sciences

Formal assessment

The doctoral thesis of M.Sc. Muhammad Kamran is a collection-type dissertation comprising three thematically coherent, peer-reviewed articles published or submitted in international journals between 2025 and 2026. The thesis includes Polish and English summaries, a list of abbreviations, an introduction, a statement of research hypotheses and objectives, a materials and methods section, a presentation of main results with general commentary, conclusions, a reference list, and the full texts of the constituent articles.

The articles comprising the thesis are:

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 (**review article**; IF: 5.2; Ministerial score: 140 pts)

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

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*

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 502 133 959
e-mail: lucja.kowalewska@uw.edu.pl
http://www.png.biol.uw.edu.pl

salicylic acid signaling pathway. **BMC Plant Biology**, Springer Nature (under peer review). (IF: 4.8; Ministerial score: 140 pts)

The combined Impact Factor amounts to **16.9** and the total Ministerial score to **420 points**. M.Sc. Muhammad Kamran is the first author on all three publications; the role of corresponding author is held by Prof. Karpiński in all three cases.

The individual contribution of M.Sc. Muhammad Kamran to each publication, as declared in the co-authorship statements submitted to the Biological Sciences Discipline Council of the Warsaw University of Life Sciences, is summarized below.

In **Article 1** (review article, *Cells*, **70%**): the candidate's contribution encompassed resource collection, preparation of all figures and tables, drafting of the original manuscript, and participation in its final editing. It should be noted that the co-authorship statement also lists "participation in the experimental design of the study" among the declared contributions. However, Articlehow 1 is a review article containing no original experimental work, as confirmed both by its content and by the Author Contributions statement published therein, which attributes to M.Sc. Kamran the roles of *resources*, *writing – original draft preparation*, and *visualization* only. It is therefore not clear to the reviewer how the candidate interprets the term "experimental design" in the context of a review paper. This discrepancy does not affect the assessment of the candidate's dominant contribution to this publication, but it is noted as a formal inconsistency in the co-authorship declaration.

In **Article 2** (experimental, *Plant Physiology*, **60%**): the candidate's contribution encompassed participation in the experimental design; generation of mutant lines, plant cultivation, and experimental execution across a broad range of physiological, biochemical, and molecular methods (including chlorophyll fluorescence, thermal imaging, gas exchange, western blot, pigment analysis, SA quantification, and RNA-seq library preparation); statistical analysis of the majority of datasets; and preparation of all figures and tables, drafting, and editing of the manuscript.

In **Article 3** (experimental, *BMC Plant Biology*, **67.5%**): the candidate's contribution encompassed participation in the conceptualization and experimental design; generation of mutant lines, plant cultivation, and experimental execution (including cell death assays, ROS measurements, antioxidant enzyme activities, pigment and phenolic content, radical scavenging activity, and RNA-seq library preparation and analysis); statistical analysis of all key parameters; and preparation of all figures and tables, drafting, and editing of the manuscript.

The declared contributions (60–70%) reflect a substantial and dominant individual engagement in all three publications and leave no doubt as to the leading role of M.Sc. Muhammad Kamran in the research constituting this dissertation. The thesis fulfills the formal requirements set out in the Act of 20 July 2018 Law on Higher Education and Science (Journal of Laws 2024, item 1571, as amended).

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 22 55 42 103,
e-mail: lucja.kowalewska@uw.edu.pl
http://www.biol.uw.edu.pl

Scientific assessment

General remarks

The doctoral thesis of M.Sc. Muhammad Kamran addresses a timely and important question in plant stress biology – how plants coordinate phytohormonal signaling, photo-protective energy management, and cell fate decisions under conditions of energy imbalance. The central hypothesis is clearly formulated, testable, and convincingly supported by the experimental data presented. The thesis is logically structured: the review article (Article 1) establishes a broad conceptual framework, while Articles 2 and 3 progressively dissect the molecular and physiological mechanisms through which CRK5 governs SA-mediated senescence, photo-protection, and redox homeostasis.

Article 1 – Review (Cells, 2025)

The review published in *Cells* provides a broad synthesis of current knowledge on chloroplast retrograde signaling, cross-tolerance, systemic and network-acquired acclimation (SAA and NAA), and their regulation by ROS, phytohormones, and transcription factors. The article is comprehensive and well written. It demonstrates the candidate's capability to synthesize a complex research field. It provides a theoretical framework for the subsequent experimental work, positioning CRK5 at the interface of extracellular stress perception, SA signaling, cellular redox homeostasis, and cell death control.

It should be noted that the thematic scope of the review extends considerably beyond the thesis's core subject. Topics such as cross-tolerance in crops, epigenetic priming, biotic stress interactions, and mitochondrial retrograde signaling receive extensive coverage yet are largely disconnected from the experimental work on CRK5 and DIS presented in Articles 2 and 3. However, this does not affect the scientific validity of the review.

Article 2 – Plant Physiology (2026)

The study published in *Plant Physiology* represents the most substantial and original contribution of the thesis. The work convincingly demonstrates that CRK5 is a central regulatory hub linking SA signaling with DIS and photo-protective energy dissipation. Several findings deserve recognition.

The demonstration that loss of CRK5 leads to reduced non-photochemical quenching (NPQ) and lower foliar temperatures (ΔT_{max}) under conditions of absorbed energy in excess, and that these phenotypes are fully reversed in SA-deficient double mutants, establishes a previously unrecognized connection between SA signaling and thermal energy dissipation. This is a novel observation with broad implications for understanding how phytohormonal status influences chloroplast photoprotection. The use of thermal infrared imaging (FLIR) to quantify ΔT_{max} as a proxy for NPQ-dependent heat dissipation is an innovative methodological contribution that complements classical chlorophyll fluorescence measurements. The transcriptomic analysis further provides molecular-level support for the physiological observations, revealing extensive SA-dependent reprogramming of WRKY transcription factors, senescence-associated genes, and NPQ-related genes in *crk5* under prolonged darkness.

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 22 55 42 103,
e-mail: lucja.kowalewska@uw.edu.pl
http://www.biol.uw.edu.pl

An additional observation is that *crk5* and *cpr1* mutants display similar physiological phenotypes but markedly different transcriptomic profiles. *crk5* exhibits more extensive transcriptional reprogramming than *cpr1* in darkness. The authors acknowledge this discrepancy and suggest that CRK5 functions upstream of CPR1 in the regulatory hierarchy, but the molecular basis for the quantitative difference in transcriptional response between the two SA-accumulating lines remains unresolved.

Article 3 – BMC Plant Biology (under peer review)

The manuscript submitted to *BMC Plant Biology* extends the findings of Article 2 by demonstrating that CRK5 preserves antioxidant homeostasis and prevents CD during DIS. The data showing elevated ROS accumulation, increased electrolyte leakage, reduced carotenoid and xanthophyll pools, and enhanced enzymatic antioxidant activities in *crk5* and *cpr1* mutants, together with the full phenotypic rescue in *crk5/sid2* and *crk5/NahG* backgrounds, build a coherent and internally consistent picture of CRK5 as a guardian of cellular redox homeostasis through SA suppression. The transcriptomic data reveal strong induction of senescence-associated genes, metacaspases, and autophagy components in *crk5* under darkness. The comparison between *crk5* and *cpr1* transcriptomes again reinforces CRK5's proposed upstream position.

Finally, please note an editorial error in this manuscript. The heading of the first Results section reads "CRK5, through the prevention of SA-signaling, **accelerates** dark-induced senescence and cell death," which is the opposite of what the paper demonstrates. CRK5 **prevents** DIS and CD by inhibiting SA signaling; the intended heading evidently referred to the consequence of CRK5 loss-of-function, not to CRK5 itself. This error should be corrected before publication.

Methodological remarks

The multi-level experimental approach integrating physiological, biochemical, cellular, and transcriptomic methods is comprehensive and appropriate for the biological questions addressed. The thermal imaging protocol represents a significant methodological contribution. Several observations are nonetheless worth raising.

Terminology: cell death vs. programmed cell death. Throughout the thesis, the term "cell death" (CD) is used as the primary abbreviation, defined broadly as "a genetically controlled process." However, the relationship between CD and "programmed cell death" (PCD) – a term used extensively in Article 1 and occasionally in Articles 2 and 3 – is never formally clarified. The distinction matters: the experimental assays employed to quantify CD (trypan blue staining and electrolyte leakage) detect membrane integrity loss generally and do not specifically distinguish programmed from non-programmed cell death. The thesis would benefit from a clearer terminological framework specifying whether the CD measured here should be classified as PCD *sensu stricto*, senescence-associated cell death, or a broader category encompassing both.

DIS as an experimental model. The thesis cites Buchanan-Wollaston et al. (2005), which demonstrates significant differences in gene expression and signaling pathways between developmental and dark/starvation-

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 22 55 42 103,
e-mail: lucja.kowalewska@uw.edu.pl
http://www.biol.uw.edu.pl

induced senescence in *Arabidopsis*. However, the work does not discuss the implications of this finding for the transferability of its conclusions to natural developmental senescence. Given that the thesis's broader framing invokes the physiological importance of leaf aging in relation to plant productivity, a brief critical assessment of how well the DIS model recapitulates natural senescence would be appropriate.

Kinase activity of CRK5. Throughout the thesis, CRK5 is described as a functional receptor-like kinase, but no biochemical kinase activity assay is presented and no direct phosphorylation substrate is identified in the context of SA regulation. The mechanism by which CRK5 kinase activity suppresses SA signaling remains inferential.

Temporal dynamics of DIS. All experiments compare control plants with plants after 4 days of continuous darkness. No time-course data are presented covering intermediate time points (days 1, 2, or 3). The absence of kinetic information limits the ability to determine when *crk5* and *cpr1* begin diverging from Col-0 during DIS, and at which stage CRK5 function is most critical. As a minor point of language, the thesis states on page 27 that the central hypothesis "has been proven". In an empirical context, "supported" or "confirmed" would be more appropriate, as hypotheses are corroborated by evidence rather than proven.

Main achievements of the dissertation

In my assessment, the dissertation's principal achievements are the following:

1. **A novel link between SA signaling and thermal energy dissipation, connecting phytohormonal status to chloroplast photo-protection in a previously unrecognized way.**
2. **A genetic dissection of SA-dependence, through a well-designed panel of single and double mutants.**
3. **A comprehensive, multi-level experimental integration. The candidate integrates physiological, biochemical, cellular, and whole-transcriptome analyses into a coherent mechanistic model of CRK5 as a hub coordinating hormonal signaling, redox homeostasis, and cell-fate decisions during dark-induced senescence.**
4. **Development of a new methodological tool - the FLIR-based thermal imaging protocol for quantifying ΔT_{max} under variable light conditions.**

Questions for the doctoral defense

Question 1. The thesis demonstrates that loss of CRK5 function leads to elevated SA accumulation and its downstream consequences. However, the direct molecular mechanism by which CRK5 kinase activity suppresses SA biosynthesis or signaling is not established. Can CRK5 kinase activity be confirmed biochemically *in vitro*? What is the proposed direct molecular target of CRK5 in the SA pathway under conditions of dark-induced senescence, and how does this relate to the MPK3/MPK6–WRKY70 cascade described in the context of biotic stress by Zhao et al. (2022)?

Question 2. Both *crk5* and *cpr1* mutants display elevated SA and similar physiological phenotypes (reduced NPQ, lower ΔT_{max}, accelerated DIS), yet their transcriptomic profiles differ substantially: *crk5* exhibits far more extensive transcriptional reprogramming than *cpr1*. How does the author interpret this discrepancy?

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 22 55 42 103,
e-mail: lucja.kowalewska@uw.edu.pl
http://www.biol.uw.edu.pl

Question 3. The DIS model used throughout the thesis involves transferring plants to continuous darkness for four days. The thesis cites Buchanan-Wollaston et al. (2005), which reports significant differences in gene expression between dark/starvation-induced and developmental senescence in *Arabidopsis*. How does the author assess the physiological relevance of the DIS model for conclusions about CRK5's role in natural leaf aging? Have any of the key phenotypic findings been verified under conditions of natural developmental senescence?

Question 4. Throughout the thesis, the terms "cell death" (CD) and "programmed cell death" (PCD) are used, but their relationship is never formally defined. The experimental readouts used to quantify CD – trypan blue staining and electrolyte leakage – detect membrane damage non-specifically and do not distinguish PCD from other forms of cell death. How does the author define CD in the context of this thesis? Should the cell death observed in *crk5* and *cpr1* during DIS be considered PCD *sensu stricto*, senescence-associated cell death, or stress-induced necrosis? What additional experimental evidence would be required to make this classification?

Question 5. Statistical comparisons among the genotypes in Articles 2 and 3 are reported as pairwise t-tests, with letter-based homogeneous groups shown above the bar charts. Could the author describe the statistical workflow in more detail – in particular, whether a one-way ANOVA or a non-parametric equivalent was applied before the pairwise comparisons, and how the homogeneous groups and any correction for multiple comparisons were determined?

Final conclusion

Having reviewed the doctoral thesis of M.Sc. Muhammad Kamran in its entirety, I conclude that it constitutes an original contribution to the scientific problem addressed and fulfills all statutory requirements for a doctoral dissertation as defined by the Act of 20 July 2018 — Law on Higher Education and Science (Journal of Laws 2024, item 1571, as amended), in the manner specified by Resolution No. 89-2022/2023 of the Senate of the Warsaw University of Life Sciences of 26 June 2023 on the adoption of regulations governing doctoral degree award procedures at the Warsaw University of Life Sciences. Therefore, I submit to the Academic Council of the Biological Sciences Discipline of the Warsaw University of Life Sciences a motion to accept the presented doctoral thesis and to admit M.Sc. Muhammad Kamran to the subsequent stages of the doctoral procedure, including the public defense.

Sincerely,

Łucja Kowalewska

Podpisany elektronicznie przez
Łucja Kowalewska; Uniwersytet Warszawski
06.07.2026
10:30:28 +02'00'

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 22 55 42 103,
e-mail: lucja.kowalewska@uw.edu.pl
http://www.biol.uw.edu.pl