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Poznań 2026-05-28

Doctoral dissertation review of Mr Serhii Kozin
dissertation
"Engineering of tryptophan specialized metabolism in Arabidopsis thaliana for plant immunity"

Legal requirements justifying the review process

On 21st of April 2026, I was appointed as a reviewer of the abovementioned doctoral dissertation by the Scientific Council of IBCH PAS. The legal basis for this review is Article 29 sec. 2, point 14 of the Act of 30 April 2010 on research institutes (Journal of Laws of 2022, item 498), in accordance with Article 179 of the Act of 3 July 2018 — Provisions introducing the Law on Higher Education and Science (Journal of Laws of 2018, item 1669) and the Act of 14 March 2003 on academic degrees and titles and on degrees and titles in the field of arts (Journal of Laws of 2017, item 1789).

The evaluation of the dissertation was conducted in accordance with the criteria outlined in the Law on Higher Education and Science. This assessment focuses on the originality of Mr Serhii Kozin approach to the scientific problem, his demonstration of thorough theoretical knowledge, and his capability to carry out independent research.

I declare no conflict of interest; no common publications or projects with either the student or his supervisor – Professor dr hab. Paweł Bednarek.

General characteristics of the dissertation

Dissertation is shaped in classical style; it contains list of abbreviations, followed by introduction, objectives, materials and methods, results, discussion, conclusions, abstract (both Polish and English), and a list of literature references. Thesis is well-balanced and clearly written in appropriate scientific language. The data is well-presented with the highest scientific standards, ensuring good flow and aiding in following the scientific rationale behind the chosen experimental steps.

Based on the scientific problem being in the center of this research, as well as experimental design and presented results, proposed title is accurate and properly reflects the scientific merit of the work.

Justification for the accuracy of the dissertation topic

Plants have evolved a range of mechanisms to defend themselves against pathogen attacks. Some of them are very efficient, however if we take a closer look we can see that the actual choice of appropriate defence strategies presents considerable level of complexity. Biotic

interactions are amongst the most dynamically evolving scenarios in nature and, due to the constant battle between pathogens and their host, they continuously evolve. The first layer of defence is undoubtedly the development of mechanical barriers that impede pathogen entry. The next is pathogen detection, followed by the initiation of signaling cascades resulting in physiological and chemical reactions leading to various effects, from slowing the pathogen's growth, or restricting their spread, to undermining key aspects essential to its survival, sometimes even leading to the death of the host cell containing the intruder. Each of these aspects requires a certain amount of energy investment from the plant and the adoption of specific developmental strategies (e.g., limiting organ growth, photosynthesis, etc.). On the other hand, depending on how pathogens enter the plant, how they spread, and how they build their physiological niche will influence the effectiveness and appropriate selection of strategies. As you can see, the matter is quite complex.
In the doctoral thesis assessed here, the author sought to elucidate the temporal and spatial specificity of glucosinolate metabolites. The research also examined the potential for generating new alternative scenarios for their production by placing of their synthesis (with the help of genetic engineering) into distinct cellular and tissue environments. Furthermore, it explored the possibility of enabling the synthesis of defensive metabolites that are not naturally present in a particular species (Arabidopsis) but can typically emerge in response to pathogen attacks in related species (*Brassica rapa*).
Essentially, the entire work is closely linked to the research conducted for many years in the laboratory of Professor Bednarek, who is the supervisor of this thesis. The work concerns plant chemical defence. The primary research subject here is Arabidopsis thaliana, which, like other plants in the Brassicaceae family, uses glucosinolate synthesis as its chemical defence strategy, as well as the initiation of GLS catabolism at sites of damage/pathogen attack to produce toxic compounds. It should be noted, however, that although the group of compounds used is similar in Brassicaceae, individual species have their own unique ability or preference for the synthesis of specific secondary metabolites. This aspect is the main focus of this work in which attempts to engineer de novo particular metabolic pathways or gain optimal defence allocation strategy are taken. The author investigates also whether it is possible to produce defensive secondary metabolites characteristic of Brassica rapa by transferring the genes necessary for their production into the Arabidopsis genome. This thesis addresses fundamental aspects of chemical defence in plants, in particular it helps to understand which tryptophan-derived metabolites works as a defence component in pre-invasive or post-invasive situations.

Evaluation of the scientific value of the doctoral dissertation

The author clearly defines the research objectives, and the introduction effectively outlines the molecular basis of defence responses in plants. It discusses the current state of knowledge regarding chemical defence and provides a description of Arabidopsis as a model organism for these studies (critically pointing out interspecies differences in chemical defence). This well-structured introduction covers the subject comprehensively and offers a solid foundation for understanding the presented work. It also demonstrates the candidate's strong grasp of the topic.
Materials and methods part is well written and allows clear understanding of experimental design as well as technical repetitions of conducted experiment. I greatly appreciate that both information on the pathogen strain as well as the use of particular Arabidopsis multiple

mutant backgrounds and appropriate control genotypes is explained. Here I have just a single question; why the AAP1 (Amino Acid Permease 1) gene was used as a proxy for pathogen/host ratio accumulation studies? Is that gene constitutively expressed in the host, or it changes upon infection, and what is typical expression pattern?

As a biotic factor author uses *Plectosphaerella cucumerina* Strain BMM (PcBMM), a highly virulent, necrotrophic fungal pathogen. it has been found that the biosynthesis of tryptophan (trp)-derived metabolites (depleted in *cyp79B2cyp79B3* and *pen2* mutants) and their targeted delivery at pathogen contact sites are required for Arabidopsis basal resistance to *P.cucumerina, the combination of this patotype strain and appropriate mutants that are unable to produce GLS-related defence components is a perfect choice for this study. PcBMM* does not form an appressorium or penetrate into plant cells, instead it gets inside the plant thanks to successive degradation of host leaf cell layers.
To test the possibility that camalexin may also be involved in pre-invasive defence thanks to processing indolic glucosinolates within the external cellular layers upon pathogen attack, the author decided to drive the expression of *CYP71A13* gene that encodes cytochrome P450 enzyme, catalysing the conversion of indole-3-acetaldoxime into indole-3-acetonitrile (key step in camalexin biosynthesis), with the help of PEN2 (PENETRATION 2) atypical myrosinase, that is known from its function in hydrolysing GLS in external cell layers upon pathogen attack. Construct was introduced into *pen2 cyp71a12 cyp71a13* triple mutant background compromised in preinvasive GLS-based defence. This resulted however on in a mild increase of camalexin levels; therefore, as a second attempt both CYP71A13 and PAD3 (PHYTOALEXIN DEFICIENT) genes were expressed under PEN2 promoter. This resulted in appropriate re-construction of metabolic path, and was also reflected by increased resistance to PcBMM. Result shows that preinvasive, camalexin-based defence can be engineered in Arabidopsis.

Next part of the work presents an attempt to introduce the path necessary for brassinin production from the hydrolysis of indolic glucosinolates into *pen2 cyp71a12/13* mutant plants. This turned up to be very successful since the introduction of a single BABG gene, that encodes myrosinase involved in convertion of I3G isothiocyanate intermediate in brassinin pathway, is enough to “install” new chemical pathway of defence and increase plant resistance against the PcBMM. This points out that Arabidopsis myrosinases are less effective in processing that leads to brassinin production, however remaining components necessary for the whole process exist in this genotype. Here, however various degrees of resistance were observed in independent transgenic lines. An attempt to synthesise cyclobrassinin or spirobrassinin required additional expression of CYP71CR1 and CYP71CR2 respectively.
These results are interpreted by the author that it is chemical property of GLS metabolites that shapes their efficacy in PcBMM growth restriction, rather than the cell or tissue specific location of their synthesis.
Overall, I value this work very highly, and I appreciate its multidisciplinary nature. I believe these results provide a strong foundation for future engineering of chemical defence in Brassicaceae. Great research always leads to new questions, inspiring us to seek deeper understanding, therefore I decide to address few aspects below:

- Why only pathogen DNA score was used, and no visual inspection data or Trypan Blue staining was performed?

- Would that be worth to check host plant growth reduction upon PcBMM inoculation in all experimental variants?

- Do we get a definite answer that the cellular location and appropriate compartmentalisation of particular myrosinases have no importance?

- Would it be worth testing effects with different pathogens, having different ways of entering the plant to get the true appraisal of differences between native and engineered pathways?

- Is PEN2 expression in Arabidopsis always epidermal? Did anyone use PEN2 promoter-reporter lines to check PEN2 activation by pathogens that attack deeper cell layers or spread via vascular tissues?

- Would that be worth engineering chemical defence pathways exclusively in stomatal guard cells?

Final statement:

This doctoral dissertation submitted meets the requirements specified in the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended), as well as in the Procedure for Awarding the Doctoral Degree at the Institute of Bioorganic Chemistry PAS No. 40/2025/Internet/RN_140 of 18 March 2025). I therefore request that the Scientific Council of the Institute of Bioorganic Chemistry PAS admit M. Sc. Serhii Kozin to the further stages of the proceedings for the award of the doctoral degree.
In light of the essential significance of this research and its contributions to the field of applied sciences, along with the high quality of the research data presentation, I respectfully request that the IBCH PAS consider awarding a distinction for this dissertation.

Prof. Robert Malinowski

Signed by /
Podpisano przez:

Robert Malinowski

Date / Data:
2026-05-29 13:16

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