Institut de recherche
en biologie végétale

Montréal, April 15th, 2026

Doctoral Dissertation Review of Deeksha Singh:

"The Role of PHLOEM EARLY DOF transcription factors (PEARs) in *Plasmodiophora brassicae* - driven phloem reprogramming in *Arabidopsis thaliana* (L.) Heynh."

1. Justification for conducting the review

By Resolution No. 4/K/2026 of the Scientific Council of IGR PAN, adopted on 6 March 2026, concerning the appointment of reviewers of the doctoral dissertation, pursuant to Art. 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 Art. 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) and the Regulations of the Scientific Council, I was appointed as reviewer of the doctoral dissertation of Deeksha Singh. This dissertation was carried out under the supervision of Prof. dr hab. Robert Malinowski from the Institute of Plant Genetics of the Polish Academy of Sciences in Poznań, Poland. I declare that I have no common publications or projects with either the student or her supervisor.

2. Justification of the choice of dissertation topic

Plants of the *Brassicaceae* family constitute a valuable and economically significant group of crop species, including Canola (*Brassica napus*), cabbage (*Brassica oleracea*), and many others that are widely cultivated worldwide for food, oil production, and animal feed. These crops are, however, highly susceptible to clubroot disease, caused by the soil-borne protist *Plasmodiophora brassicae*, which represents one of the most devastating and economically damaging diseases affecting Brassicaceae globally. Understanding the molecular and cellular mechanisms underlying this plant-pathogen interaction is therefore of great scientific and practical importance.

Arabidopsis thaliana, a member of the Brassicaceae family and closely related to many cultivated Brassica species, is one of the most powerful model organisms in plant biology. Its well-annotated genome, extensive mutant collections, and broad range of molecular tools make it an ideal system for investigating fundamental biological mechanisms. Crucially, knowledge generated in *Arabidopsis* can be effectively translated to crop species, offering direct perspectives for breeding programmes aimed at developing disease-resistant varieties.

In this context, I conclude that the Doctoral Candidate has undertaken research that is both scientifically important and practically relevant, with clear implications for the understanding of one of the most economically significant diseases affecting Brassicaceae crops.

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3. Formal assessment of the dissertation

The doctoral dissertation of Deeksha Singh submitted for evaluation is written in English and comprises a summary in both English and Polish, a list of abbreviations, an introduction, main hypotheses and aims, materials and methods, results and discussion, conclusions, future perspectives, references, co-author statements, and the full text of two peer-reviewed publications. The results presented in the dissertation are based on the following two publications:

1. Singh D., Blicharz S., Stefanowicz K., Ragni L., Michalak K., Bagniewska-Zadworna A., and Malinowski R. (2022). Combining Clearing and Fluorescence Microscopy for Visualising Changes in Gene Expression and Physiological Responses to Plasmodiophora brassicae. Journal of Visualized Experiments 186. DOI: 10.3791/64297 (IF: 1.2)
2. Singh D., Blicharz S., Roszak P., Helariutta Y., and Malinowski R. (2025). PHLOEM EARLY DOF genes are induced within clubroot galls as a consequence of cambial stimulation and vascular reprogramming during Plasmodiophora brassicae infection in Arabidopsis thaliana. Journal of Experimental Botany 76(16):4521–4537. DOI: 10.1093/jxb/eraf225 (IF: 5.7)

The two articles were published in reputable international scientific journals. The second publication in particular, appearing in the Journal of Experimental Botany, represents a high-quality contribution to the field with a strong impact factor. In both publications, Deeksha Singh appears as the first author, demonstrating her leading role in conducting the research, analysing the data, and preparing the manuscripts. According to the co-author statements included in the dissertation, her contributions encompassed experimental design and execution, data analysis and interpretation, and writing of the manuscripts, which clearly attests to her significant and central involvement in this work.

The dissertation is structured in a logical manner. The introduction provides an overview of the literature, the hypotheses and aims are well formulated, and the materials and methods are described with sufficient detail. The results and discussion section coherently presents the main findings of both publications, and the conclusions are supported by the data. There are also some future perspectives provided. The title of the dissertation accurately reflects the subject matter of the publications on which it is based. Overall, the dissertation meets the formal requirements for doctoral dissertations.

4. Substantive assessment of the dissertation

Summary

The summary is clearly written and provides a concise and informative overview of the research conducted, the methods employed, and the main findings.

Introduction

The introduction provides a general description of clubroot disease and its biological significance. However, in my assessment, it is relatively short and does not sufficiently contextualise the topic. Several important elements are missing. At the beginning I am missing a broader context of the economic impact of clubroot disease on Brassicaceae crops that would be a valuable to establish the agricultural relevance of this work and a broad context of this study (please contextualize your study). Second, a general description of the genetic network governing phloem development is broadly absent, which makes it difficult for the reader to fully appreciate the significance of PEAR genes in this context (please provide). Third, the role of plant hormones

— particularly auxin and cytokinin — in phloem development and in the clubroot disease process is not introduced in the introduction, even though these hormones are central to the results presented later. On page 30, it is only briefly mentioned that "auxins play a pivotal role in defining and shaping nearly all facets of plant development", without any supporting citations (please discuss it). Finally, a comparison between *Arabidopsis thaliana* and economically important Brassicaceae crops in the context of clubroot disease susceptibility and response would have strengthened the justification for using *Arabidopsis* as the model system.

Main hypotheses and aims of the study

The hypotheses are clearly stated and scientifically sound. However, the aims could be more precisely formulated. Aim 1 states the objective as using promoter-GUS fusions, whereas the true scientific objective is to understand changes in the expression of key PEAR genes during gall formation — the GUS approach is merely the tool. Similarly, Aim 3 is framed around the use of methods rather than the scientific question being addressed, namely the role of auxin and cytokinin in *Plasmodiophora brassicae* infection. It is also notable that while auxin and cytokinin are central to Aim 3 and the results, they do not appear in the hypotheses, which represent a missed opportunity to articulate a more specific and testable prediction regarding hormonal regulation of phloem reprogramming during infection.

Materials and methods

The materials and methods are described in sufficient detail, largely through reference to the two publications included in the dissertation. The experimental pipeline is well illustrated, and the method description seems sufficient to reproduce the data. The choice of genetic tools, reporter lines, and chemical inhibitors is appropriate.

Results, discussion and conclusions

The results are clearly presented and coherently discussed in relation to the published literature. The most important findings of this dissertation can be summarised as follows: (i) the development of an optimised fixation and clearing pipeline enabling high-quality fluorescence microscopy of complex clubroot galls; (ii) the demonstration that PEAR genes, including PEAR1, DOF6, and HCA2, are transcriptionally upregulated within galls during Pb infection; (iii) the surprising finding that even in higher-order pear mutants with severe phloem developmental defects, Pb infection still strongly induced ectopic phloem proliferation, suggesting that PEAR upregulation is a downstream consequence of broader vascular reprogramming rather than a direct driver of phloem overproduction; and (iv) evidence that auxin signalling responses are strongly induced across host vascular tissues during gall formation, while cytokinin signalling appears to be dampened.

Regarding the observation that higher-order pear mutants still exhibited strong ectopic phloem proliferation upon Pb infection, the author concludes that PEAR induction is a downstream effect of altered vascular cell fate. While this interpretation is plausible, the Doctoral Candidate could have elaborated further on what upstream mechanisms the pathogen may be exploiting — for example, whether direct manipulation of cambial activity, hormonal crosstalk, or other regulatory factors could explain this result independently of PEARs.

Regarding the choice of 16 DAI as the sampling time point for hormonal reporter experiments, a more explicit justification would have been helpful.

The conclusions are appropriate and well supported by the data.

Future perspectives

This section is rather general and would benefit from greater specificity. Several points deserve elaboration. When stating that "there always remains a scope for further improvements" in terms of the imaging method, the author should specify which concrete improvements are envisioned. Similarly, the claim that "this approach has greatly expanded the existing histological toolkit" should be substantiated by explicitly stating what was previously impossible or limited and how this method overcomes those constraints. When mentioning "future developments and inclusion of more advanced imaging methods", the author should name specific techniques and future directions of improvements.

The suggestion that "the pathogen may either accomplish this by targeting molecular pathways or indirectly via physiological means by tweaking the hormonal crosstalk between auxin and cytokinin" lacks precision — it should be clarified which specific molecular pathways are being referred to, and why hormonal crosstalk should not itself be considered a molecular mechanism. Furthermore, the possible involvement of other phytohormones in gall formation is raised but not explored — the author should indicate which hormones they consider most likely to be involved, on what basis, and how their involvement could be experimentally tested in future work.

Literature

In my assessment, the selection of cited works for the discussed substantive aspects of the work is in general appropriate. The Author used the most significant publications related to the topic of the dissertation, consisting of articles published in reputable international scientific journals.

5. Final conclusion

This dissertation addresses important scientific questions at the interface of plant developmental biology and plant-pathogen interactions. It constitutes a coherent research study, combining methodological innovation with fundamental biological discoveries. The Author demonstrated a clear ability to integrate diverse experimental approaches (fluorescence microscopy, genetic tools, and pharmacological treatments), and to interpret the obtained results. In my opinion, the work makes a meaningful contribution to science. The main areas where improvement would be beneficial concern the contextualisation of the study in the introduction, which would benefit from providing broader context and a more thorough presentation of the hormonal and genetic networks underlying studied mechanism, as well as the future perspectives section, which would gain significantly from greater specificity regarding future experimental directions and methodological developments.

In summary, I conclude that the doctoral dissertation of Deeksha Singh entitled "The Role of PHLOEM EARLY DOF transcription factors (PEARs) in *Plasmodiophora brassicae* - driven phloem reprogramming in *Arabidopsis thaliana* (L.) Heynh." meets the requirements set out in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science (consolidated text: Journal of Laws of 2024, item 1571, as amended). In view of the above, I hereby request that Ms. Deeksha Singh, M.Sc., be admitted to the further stages of the proceedings for the conferral of the degree of Doctor. At the same time, I recommend to the Scientific Council of the Institute of Plant Genetics PAN in Poznań the acceptance of the dissertation and the admission of Deeksha Singh, seeking the academic degree of doctor, to further stages of the doctoral procedure.

Best wishes,



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