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Review of PhD thesis of Lakshmipriya Perincherry, M.Sc.
entitled "The role of *Fusarium* mycotoxins and lytic enzymes in fusariosis of pea (*Pisum sativum* L.)"

The doctoral dissertation of Lakshmipriya Perincherry presented for review was prepared under the supervision of prof. dr hab. Łukasz Stępień at the Institute of Plant Genetics - Polish Academy of Sciences in Poznań, Poland. At this point, it should be underlined that the team led by prof. Stępień has extensive experience among other things in research on the understanding of the molecular background for the ability of pathogenic *Fusarium* species to synthesize various groups of mycotoxins.

The reviewed dissertation consists of a series of four thematically related scientific articles published in 2019-2021, in journals included in the *Journal Citation Reports* (JCR) database. The series includes one review article and three original papers:

1. Perincherry, L., Lalak-Kańczugowska, J., & Stępień, Ł. (2019). *Fusarium* produced mycotoxins in plant-pathogen interactions. Toxins. 11 (11), 664, doi: https://www.mdpi.com/2072-6651/11/11/664.
2. Perincherry, L., Ajmi, C., Oueslati, S., Waśkiewicz, A., & Stępień, Ł. (2020). Induction of *Fusarium* lytic enzymes by extracts from resistant and susceptible

cultivars of pea (*Pisum sativum* L.). Pathogens, 9(11), 976. doi:10.3390/pathogens9110976.

3. Perincherry, L., Urbaniak, M., Pawłowicz, I., Kotowska, K., Waśkiewicz, A., & Stępień, Ł. (2021a). Dynamics of *Fusarium* mycotoxins and lytic enzymes during pea plants' infection. International Journal of Molecular Sciences, 22(18), 9888. doi: https://doi.org/10.3390/ijms22189888.
4. Perincherry, L., Witaszak, N., Urbaniak, M., Waśkiewicz, A., & Stępień, Ł. (2021b). Effects of secondary metabolites from pea on *Fusarium* growth and mycotoxin synthesis. Journal of Fungi (under communication at the time of submitting the doctoral dissertation) 7(12), 1004; doi:https://doi.org/10.3390/jof7121004.

All the papers are multi-authored, and Lakshmipriya Perincherry, M.Sc. is their first author. It should be underlined that in all these research papers the PhD student is additionally a corresponding author (in two with the supervisor). Although the percentage share of the PhD student's contribution to the preparation of individual papers was not given, the detailed description contained in the declarations and the *Author contributions* subsections embedded in each manuscript clearly indicate that her role in the preparation of the indicated papers was leading. It follows from the attached declaration statements that Ms. Perincherry, M.Sc. took part in planning experiments and their execution, interpretation of the results and preparation of manuscripts. She participated in a series of biochemical and molecular analyses including antifungal activity tests, enzyme activity assays and gene expression studies. As a corresponding author, she also led the manuscript revision processes. The total value of the indicated publications according to the current criteria of the Ministry of Science and Higher Education is 360 points and the value of the IF index according to the JCR list (according to the publication year) is 18.762. The research comprising the doctoral dissertation of L. Perincherry, M.Sc. was financed as part of the National Science Center project (NCN OPUS 2017/25/B/NZ9/01210).

The reviewed dissertation consists of the following elements: a summary in English and Polish; a commentary including the *Introduction*, *Research hypotheses and objectives*,

Materials and methods, *The most important results and discussion*, *Summary and conclusions*, and *References*; a set of four articles constituting the subject of the dissertation, and declaration statements of the co-authors of individual publications. It is a pity that the so-called supplementary information in the form of additional tables and figures, which in fact would give a more complete picture of the wide spectrum of analyses carried out by the PhD student was not included in the dissertation. To sum up, the dissertation meets the formal requirements for this type of PhD Thesis.

Substantive evaluation of the dissertation

In terms of merit content, I highly appreciate the original research works written by Lakshmipriya Perincherry. The attached commentary has been synthetically and transparently prepared, referring to subsequent experimental works. The information presented in the *Introduction* section is largely based on the review article included in the dissertation. In my opinion, this section could be enriched with a short description of the general offensive strategy of pathogenic *Fusarium*, since during the infection process *Fusarium* species employ not only mycotoxins and plant cell wall-degrading enzymes to subvert target host cells. The *Materials and methods* presented in the commentary could also be more detailed in relation to the description of the methodology of the statistical analysis indicating the number of technical and/or biological repetitions in particular experiments. It is worth noting that the commentary to the dissertation was supported by appropriately selected citations, which proves very good knowledge of the literature on the subject.

The overriding goal of the doctoral dissertation was to monitor the *in vitro* and *in vivo* effect of pea plant extracts on the lytic enzymes and mycotoxins produced by *Fusarium* representatives, as well as to provide understanding of the effect of pea plant-derived secondary metabolites on the growth and metabolism of the selected *Fusarium* strains. The specified goal clearly defines the scope of successively analyzed issues in the following research papers. The above-mentioned goal was realized through five specific research hypotheses. In line with the hypotheses adopted by the Author (1) pea plant extracts can alter the production of extracellular cell wall degrading enzymes and mycotoxin levels in

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Fusarium cultures; (2) expression of lytic enzyme-coding genes can change as the effect of secondary metabolites present in the plant extracts; (3) pea cultivars with different resistance levels display diverse metabolic profiles, which relates to their responses to infection by *Fusarium* strains; (4) accumulation of mycotoxins in pea plants may be dependent on their disease resistance characteristics; (5) *Fusarium* species show alteration in the growth and mycotoxin biosynthesis when exposed to individual secondary metabolites present pea extracts.

It is known that annually 25–50% of crops harvested worldwide are contaminated with mycotoxins, which may be passed from the contaminated plant-derived feed to animals and eventually to humans. Thus, knowledge on the biochemical and molecular characteristics of pathogens producing mycotoxins, including *Fusarium* representatives, is crucial for the development and implementation of effective protection systems for crops. Both the experimental model and the goal of the research are important from the theoretical and application point of view, therefore I consider the research topic undertaken by L. Perincherry, M.Sc. to be extremely important for plant pathology and plant disease management.

The four papers included in the dissertation have already been positively evaluated by reviewers and editors of recognized journals in the field of mycology and biochemistry. For this reason, I do not see the need for a substantive re-review of the already published articles. Considering the obligation of the reviewer, I will only mention the most important achievements obtained from the conducted research.

The first work of the cycle published in 2019 (publication 1.), which is a review article, provides an excellent introduction to the topic of the dissertation. The review presents not only the mechanisms of mycotoxin biosynthesis in the *Fusarium* genus under various environmental conditions, but also describes host responses to *Fusarium*-produced mycotoxins. I find this section particularly valuable giving the holistic view of the role of mycotoxins in plant-pathogen interactions.

Subsequent experimental papers included in the dissertation are based on a common research model, which consists of two different *Fusarium* species such as *F. proliferatum*

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(strains PEA1 and PEA2) and *F. oxysporum* (strains 34 OX and 1757 OX) that were treated (1) with extracts obtained from resistant (Sokolik) and susceptible (Santana) pea plants, (2) with pea plant-derived secondary metabolites or (3) used for pea plant inoculation. In the work published in 2020 (publication 2.), L. Perincherry, M.Sc. assessed the role of various substrates and pea plant extracts in inducing the cell wall-degrading enzymes and mycotoxin production in the pathogenic *Fusarium*. Although the addition of the extract derived from resistant pea plants to the fungal growing medium provoked only statistically non-significant growth inhibition of the selected strains, both extracts were effective in inducing the activity of lytic enzymes such as β-glucosidase, pectate lyase and xylanase. In addition, the extract from resistant pea provoked a slight reduction in fumonisin FB1 production in *F. proliferatum* PEA1 cultures.

As indicated in the next work published in 2021 (publication 3.), pea extracts induced *Fusarium* cell wall-degrading enzymes also at the transcript accumulation level. However, the pattern of gene expression induced by the plant extract was dependent on the pea cultivar, which means that the extracts from susceptible pea induced a sudden increase in gene expression, whereas the resistant one elicited expression at a lower level. The subsequent experiment related to plant infection studies revealed that β-glucosidase, xylanase, pectate lyase and lipase activities were differentially accelerated in the following days after pea inoculation with both pathogens. Additional mycotoxin quantification in infected pea roots showed that more beauvericin was accumulated in the susceptible cultivar, confirming that the toxin production determines the aggressiveness of the pathogen.

The last work of the cycle published also in 2021 (publication 4.) was aimed at identification of the differentially expressed secondary metabolites in resistant and susceptible pea cultivars. Based on metabolic profiling, the resistant cultivar accumulated a high level of phenylpropanoid compounds. Therefore to understand the role of these compounds on *in vitro* growth and mycotoxin biosynthesis, selected metabolites were added to the growing medium of the pathogens. Coumarin, spermidine, p-coumaric acid, isoorientin and quercetin were able to reduce the growth of the pathogen; however, a higher level of p-coumaric acid was found

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to enhance the growth of *F. proliferatum* strain PEA1. In addition, the metabolites were able to greatly inhibit the biosynthesis of fumonisin B1 and beauvericin.

Summarizing the obtained results, it should be emphasized again that the results of the conducted research, apart from the cognitive value in terms of biology and pathobiology of necrotrophs, are of great practical importance. This is due to the fact that understanding of the role of pathogen-derived factors such as lytic enzymes and mycotoxins in plant–pathogen interactions is necessary for the design a successful disease-management program. The adopted concept, including the research methods and data analysis used, made it possible to verify the research hypothesis and achieve the main goal of the dissertation. The four conclusions resulting from experimental works correspond to the assumptions adopted in the thesis. However, in my opinion, hypothesis no. 2. "Expression of lytic enzyme-coding genes can change as the effect of secondary metabolites present in the plant extracts" has not been fully verified, because only the effect of the extracts on the expression of genes, and not individual metabolites or the metabolite pool, was assessed by the Author.

After analyzing the whole dissertation I would ask the Author to comment on the following remarks, to which I did not find an answer in the content of the comments and in subsequent manuscripts:

- What is the genetic background of the resistance of the used pea cultivars to the selected *Fusarium* species/strains?
- The pea extracts were taken from 20-day-old Sokolik and Santana plants, but the metabolic profiling presented in publication no. 4. was performed for 1-month-old plants – has it been confirmed that the metabolic profile (quantitatively) does not change during pea development?
- Were the activities of selected lytic enzymes also determined in the pure pea extracts?

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- In the Commentary the Author stated that the pathogen produced more enzymes during infection in the resistant cultivar than in the susceptible one - was the protein accumulation also studied?
- Could the Author extend the statement contained in publication no. 1. that "there are some tolerant varieties with obvious yield decreases a 'cost' of tolerating the disease".
- According to the *Materials and Methods* sections in the experimental publications all the treatments were carried out in triplicates (no. 2. and 3.) or duplicates (no. 4.) - were they biological or technical repeats?
- Why was statistical analysis of the selected lytic enzyme activities in publication no. 2. presented only for the 8th day of plant extract supplementation to fungal strains?
- Was quantitative or molecular evaluation of the effectiveness of the pea plants infection carried out by the Author in addition to phenotype observations (nos. 3. and 4.)?
- Why were infection studies included in publication no. 4. carried out on 7-day-old plants, whereas metabolic profiling of the selected cultivars was prepared on 1-month-old plants?
- How can you explain the result included in publication no. 4. that a greater amount of p-coumaric acid (100ng/mL) promoted fungal growth?

In conclusion, the dissertation is written correctly and clearly with few exceptions. For example, in the Commentary L. Perincherry, M.SC. write "Similar to that of root β-glucosidase activity, the xylanse activity was also higher in PEA1- and 34 OX-infected Santana roots. However, no difference was observed for Santana roots." - is it really about the Santana cultivar? In general, I can state that the dissertation submitted for assessment provides valuable information and brings new knowledge concerning *Fusarium* patho-biology. The raised questions and comments contained in the review are intended to systematize the valuable results contained in this dissertation and do not diminish its value.

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Lakshmipriya Perincherry, M.SC. has demonstrated very good skills in the use of various research methods starting with classical methods used in phytopathology to molecular analyses. The way the dissertation was prepared and expertise in the discussed topic testify to the scientific maturity of the PhD student.

Final conclusion

In view of the positive evaluation of the doctoral dissertation of Lakshmipriya Perincherry, M.Sc. presented above I submit a motion to the Scientific Council of the Institute of Plant Genetics, Polish Academy of Sciences in Poznań to admit her to further stages of the PhD degree conferral procedure.

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