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Review of the Ph.D dissertation entitled: Evaluating modes of resistance to *Plasmidiophora brassicae* in *Arabidopsis thaliana* by MSci. Soham Mukhopadhyay, under the supervision of dr hab. Robert Malinowski and dr William Truman

MSci. Soham Mukhopadhyay carried out his experimental work in the Department of Integrative Plant Biology, Institute of Plant Genetics of the Polish Academy of Science in Poznań under the supervision of dr hab. Robert Malinowski and dr William Truman

The dissertation consists of nine chapters including the Abstract, Introduction, Objectives and Hypothesis, Materials and Methods, Discussion, Conclusions List of figures, List of Tables and Bibliography.

Plasmodiophora brassicae is a well known pathogen of important crops including rape and different species and cultivars of the *Brassica* genus that causes clubroot disease. This disease has been shown to cause significant losses in plant productivity worldwide, predominantly in the temperate climate. Significant progress has been made in elucidating the mechanisms of infection, plant resistance and the evolutionary arms race between the parasitic protist and its host plants, however, the current state of knowledge of plant – pathogen interactions (and plant – microorganisms in general) is still scarce. The development of efficient plant protection strategies that take into account the necessity to maximize the efficiency of available resource utilization and to limit energy consumption is a pending need. This

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development requires broadening our understanding of plant biotic and abiotic interactions. The work described in this dissertation puts another piece in the puzzle of understanding the plant – pathogen interaction and provides the reader with new insight into the mechanisms of resistance of the Brassicaceae against *Plasmodiophora brassicae*.

The Introduction of the dissertation is a comprehensive overview of the biology of the pathogenic protist, economic importance of the disease, disease management strategies and a thorough description of the known mechanisms of plant resistance against *Plasmodiophora brassicae.* This part of the Introduction makes up for the majority of the content of the Introduction and provides the reader with an up to date review of the available reports concerning the wide spectrum of plant immunity in responses to the pathogen including pathogen recognition, signaling leading to the plant response and a description of the response itself. The Author emphasis the role of phytohormones in plant tolerance against the protist and distinguishes between salicylic acid and jasmonic acid dependent mechanisms in plant tolerance/susceptibility. An important aspect of the interaction are mutual adaptations of the microorganism to the plant and *vice versa* that determine pathogenicity and resistance of *Plasmodiphora* and the plant respectively. This evolutionary arms race was comprehensively described in this section of the dissertation. The description provides the reader with all the insight necessary to understand the scope of the investigations and accordingly justifies the approach, rationale and methodology used.

The Introduction is followed by a description of the objective of the study and the hypothesis. The objectives are clearly written, however, there is too much focus placed on the methodology in the description. I my opinion the aim of the study was to: "…identify the genetic factors underpinning resistance…" not to: "…screen the natural germoplasm diversity of Arabidopsis accessions …". The screening was merely the method used to identify factors determining plant resistance against the pathogen. Similarly with the hypothesis. The hypothesis in this case should refer to plant resistance against clubroot disease. The investigations presented in the dissertation had a very clear purpose, but it was not to verify whether

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transcriptomics or GWAS can be used to identify the mechanisms of plant resistance against clubroot disease. The mentioned above issues were not the subject of the study.

The materials and methods used in the study are well justified and seem optimally selected to answer the questions raised in the dissertation. There are however a few issues that require clarification:

1. P 55 only 71 *Arabidopsis thaliana* accessions are listed in table 3, even though the Materials and Methods (and figure caption) section states that 142 were used in the study. It seems to me that the table is incomplete
2. P 58 Pathogen assay – *q*PCR and Disease Index Scoring. I did not find any literature reference to the pathogen load quantification method used in the study. If this is a standard method used for *P. brassicae* quantification *in planta* than an appropriate reference should be included. If not, more methodological details should be included (reaction optimalization, specificity of the primers, etc.)
3. P 59 More technical details about qPCR should be provided (see above). No information on how expression was calculated
4. P 62 Transcriptomics. I understand that the RNAseq was performed by Genomed, but more technological details about the analysis should be provided. The description of library preparation was limited to information about poly-A selection and the length of pair-end reads
5. P 62 RNAseq data analysis pipeline. I would like to know what was actually done by the Author of the dissertation.
6. P 63 Statistical analysis and other bioinformatics software. There is practically no information about the statistical analysis performed. Some of this information was provided while describing the results (I fully support this mode of description), but basic information should be provided in the materials and methods.

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The results are very well described and illustrated. Most importantly though, the purpose of performing every single analysis was clearly and convincingly justified. The description of the results is sound and coherent with the aims; the reader does not have wonder what was the purpose of performing a particular experiment in respect of the primary aim of the study. The results also show the amount of work performed by the Author. The data set used in the GWA study is a good example of the hard work done. Showing the combined pathogen load and gall formation criteria was a very nice way to describe pathogen progression and plant resistance. A few issues require clarification:

1. P 69 what was actually used for GWAS? Was it relative pathogen load as stated on page 69 or the DI?
2. P 74 the Author mentioned the expression of *RPB1-like 4*, but did not show an illustration of the results (graph)
3. P 75, 77 (and on a few more occasions) the Author mentions that *ccd*B was replaced with the sequence being cloned into the vector. This is a feature characteristic for Gateway cloning (negative selection) and I completely do not understand why it was emphasized (on several occasions, including the figures) so much. This info could have been included in the materials and methods, but why in the results?
4. P 84 Figure 22 I am not convinced that used Student's t-testing is the optimal approach to test statistical significance. I think that ANOVA with the Dunnet's post hoc would be more appropriate
5. P 85 Fig 23 no indication of what gene is represented on a particular graph. Where three reference genes used here?
6. In the transcriptomic analysis, I would consider comparing two sets of data: the DEG of Col-0 mock vs Col-0 *P b*. with DEG Est-1 mock vs Est-1 *P. b.*
7. P 115 Was chitosanase activity (or gene expression) measured in plants ectopically expressing *hypocrea lixii* chitosanase?

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Additional remarks:

The ambiguity of the immune marker gene expression in Est-1 and Col-0 transformants (*pRPB1::RPB1*) provides evidence of the multigene origin of the plants response to *P. brassicae*. I very much liked the nice experimental approach (even though more markers genes should be used) and the way it was discussed in the results section and then latter in the discussion.

Precise language should be used eg. P 73 "…Tsu-0 carries 2 copies of *RPB1* identical at the aa level…" this implies that the gene has an identical aa sequence. Colloquialism, such as "reps" and a few others should also be avoided.

The discussion is well written. It is straight to the point, coherent and concise. I found very little speculation, the vast majority of the conclusions were well justified in the results. I was surprised by the fact of such a variability in the presence of the functional *RPB1* locus in different *Arabidopsis thaliana* accessions. Can the Author shed some more light on this fact in regard to adaptation to different climatic conditions? Is there a correlation between this locus and the occurrence of clubroot disease?

In conclusion, the doctoral thesis of MSci. Soham Mukhopadhyay undoubtedly provides significant input into our understanding of the mechanism of plant resistance against *Plasmodiophora brassicae* and the plant – microorganism interaction in general. Overall, I consider the research performed by the Author of the dissertation as highly valuable, novel and of high scientific level.

In my opinion, the thesis of MSci. Soham Mukhopadhyay fulfils all the requirements of a PhD thesis and thus can be considered for a doctorate. I fully support the acceptance of the dissertation in its current form and I would strongly support further steps towards doctoral thesis acceptance. I hearby declare that the PhD. Thesis entitled **Evaluating modes of resistance to *Plasmidiophora brassicae* in *Arabidopsis thaliana*** by MSci. Soham Mukhopadhyay meets all criteria pursuant to art. 187 of the Act of 20 July 2018 The Law on Higher Education and Science (Journal of laws 2018, item 1668, as amended).

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