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Review of the Doctoral Thesis of Ms. Yufeng Guan "The effect of nitric oxide on histone protein acetylation status in *Phytophthora infestans* (Mont.) de Bary"

The review has been done based on the agreement WB/DZ/125/2025 signed by prof. dr hab. Piotr Pawluć, the Deputy Rector of AMU in the name of its Collegium Biologicum.

The Doctoral Thesis of Ms. Yufeng Guan, conducted at the Institute of Experimental Biology, Department of Plant Ecophysiology of the University of Adam Mickiewicz (AMU) in Poznan under the supervision of Prof. dr hab. Magdalena Arasimowicz-Jelonek is dedicated to the mechanisms of genetic plasticity and adaptation to the environmental stresses by the economically significant pathogen *P. infestans* (Mont.) de Bary.

The pathogen is the oomycete causing root rot and foliar blights in plants. It attacks potatoes (Solanum sp.), including *S. tuberosum*, the source of staple food worldwide. The disease caused by *P. infestans* is known as potato blight or late blight. The pathogen is occupying cool and moist niches of the environment, where potatoes are usually vastly cultivated.

In general oomycetes including *P. infestans* do not survive in nature so well, except from its plant hosts, including plant debris, such as potato tubers collected or left in the soil after the harvest. The infested symptomless tubers and tubers infected but overlooked and then used as planting material transfer the disease onwards. Tubers containing hyphae and oospores form the inoculum which can cause devastating effects in the following season.

It has been demonstrated that the pathogen is very versatile and can survive, sporulate and undergo the whole disease cycle in many environmental conditions far beyond its optimal temperature and moisture. This rapid adaptation to the environment as well as the internal signals from the plant are the main topics of this PhD Dissertation.

STRUCTURE

The entire monograph encompasses as much as 179 pages. The PhD Dissertation is written in English, except the abstract in Polish. The structure of the Thesis results from the fact that two publications (including the semi-review and the results of the doctoral study) have been published already and another manuscript has been submitted to a scientific journal of JCR list.

The Thesis start with the Abstract in English, followed by Streszczenie in Polish. Then the above mentioned publications are enumerated and followed by the list of abbreviations.

The Introduction occupies 5 pages and it is followed by the Discussion of results presented on 13 pages. The results are summarized on 3 pages containing 11 Conclusions. Then the dissertation contains the Summary presented on 2 pages, including the illustration showing the sequence of events found by the PhD Candidate that led to changes in histone (de)acetylation status, which may result of nitric oxide production induced by the host and /or the environment. The Literature contains 62 positions. Then the Dissertation contains two publications and one manuscript submitted for the review, namely:

Publication 1

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S., Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle. Plant Physiology and Biochemistry, 216, 109129. (IF_{2025}=5.7; 70 MNiSW points)

Publication 2

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U. K., Sobieszczuk-Nowicka, E., Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology. Molecular Plant Pathology, 25(7), e13497. (IF_{2025}=4.9; 100 MNiSW points)

Manuscript 3

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. PLOS Pathogens (under review).

After the publications the PhD Dissertation contains the list of statements of co-authors showing their roles in the presented publications and the manuscript. The PhD Dissertation is finalized by the list of other achievements of Ms. Yufeng Guan.

In spite of the unusual order of this PhD Dissertation I confirm that such structure is logically guiding the reader through subsequent stages of the conducted research, presenting their objectives and hypotheses that were then verified during the study. In the case of both publications and the manuscript it was generally clear what was done by the PhD Candidate. It is also possible to compare it to the input of the other co-authors.

The acceptance of a large portion of the research work by reputable scientific journals indicate the interest of the scientific community in the knowledge and results of the conducted research. It also confirms the suitability of the adopted methodologies.

The research presented in the Thesis was done as part of the Project funded by the National Science Center OPUS 2018/31/B/NZ9/00355, entitled: “The effect of nitric oxide on histone protein acetylation status in *Phytophthora infestans* (Mont.) de Bary”. It is also declared that the funding was partly obtained through the National Science Center project Sonata BIS 2017/26/E/NZ4/00226, entitled “Nitroxyl as an alternative or competitive to nitric oxide metabolic link in plants in the model *Arabidopsis thaliana*”. However, in all three papers the acknowledgement is only mentioning the OPUS project and never mentions Sonata BIS so this information is inconsistent between the PhD Thesis and the publications/ manuscript.

METHODOLOGY

The methodology used in this work was fully appropriate to obtain the objectives of the experiments. The materials and methods were described with great accuracy and the descriptions were sufficient to repeat them in the other research laboratories, provided the materials available to the Candidate were accessible to the others (eg. the virulent and avirulent isolate of *P. infestans* towards potato cv. Sarpo Mira). The main research methods involved studies conducted in laboratory conditions.

The work presented in Publication 1 ("The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle") declared by the Candidate contained the following activities:

1) culturing, propagation and preparation of inoculum of the avirulent and virulent isolates;
2) preparation of reactive nitrogen modulators;
3) collection of materials for analyses
4) preparation and implementation of experiments involving:
    a) measurements of nitric oxide emission using NO chemiluminescence analyses,
    b) detection of peroxynitrite formation,
    c) RNA isolation and gene expression measurements,
    d) chromatin immunoprecipitation and the ChiP-qPCR analyses,
    e) immunoblot analysis of histone global and site-specific acetylation studies,
    f) phylogenetic analyses and conserved domain distribution of PifHAM1 and its ortholgs,
5) analyses concerning the isolation of histone-enriched proteins for immunoassays and determination of histone H3 and H4 total acetylation levels.

Moreover in Publication 1 the declaration says that the Candidate performed statistical analyses and participated in the preparation of the first version of the manuscript. However *CRediT authorship contribution statement* of this article says that "M. Arasimowicz-Jelonek (MAJ) and J. Floryszak-Wieczorek (JFW) designed the research, Y. Guan, J. Gajewska, and E. Sobieszczuk-Nowicka (ESN) performed the experiments; MAS, JFW and ESN analyzed the data and supervised the individual stages of the study. MAJ and JFW wrote the article. MAJ, JFW, ESN and S. Hartman reviewed and edited the manuscript." There is some discrepancy between the statement in the PhD Thesis saying that Y. Guan prepared the first version of the manuscript and the statement in the publication. I guess it results from necessary simplifications and Y. Guan in fact participated in the preparation of the first version of the manuscript by preparing the tables and figures (excluding Fig. 9). She has also formatted the manuscript, but this job, although very tiring – is rather technical. The same discrepancy concerns the role of statistical analyses in the process of analysing the data. Was it purely technically done by Y. Guan or by this she participated in data analysis? Please comment on this during the public defense of the PhD as the authorship of different activities in the papers subjected to achieving a doctor's degree must be fully clear. In this case the question is whether Ms. Guan performed technically the experiments without the input in data summary and drawing conclusions or she also participated in creative stages of the work.

The work presented in Publication 2 ("Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology") performed by Ms Yufeng Guan contained conducting *in silico* analysis of gene structure, protein sequence and protein-protein interaction network of HDACs and HATs in *P. infestans.* According to the declaration, the Candidate participated in forming the concept of this work and the preparation of the first version of the manuscript (by delivering Figures 1a-h and 2 ab, as well as Supplementary Figure S1 and two supplementary tables S1 and S2). Moreover, Ms. Guan participated in preparing the corrected version of the manuscript following the reviewers' remarks, including the point-by-point responses to their comments.. In this case the publisher does not list the engagement of the co-authors in the publication but the statement in the Thesis suggest that the main role of the Candidate started from the conceptualization, it has gone through the performance of great parts of experiments and was finalized by preparing the draft and final version of the manuscript. This by no doubt counts as the fully professional scientific input in the published research article, which is a combination of a review and the new knowledge. Both the Publication 1 and Publication 2 are in a very mature final form.

In the case of Manuscript 3 ("Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress") the Candidate has done the research steps 1-3 and 4c like in the Publication 1 and also:

a) studied the recombinant expression of PifHDAC3 in *Escherichia coli,*
b) quantified the activity of HDACs,
c) constructed of the 3D modelling of PifHDAC3,
d) performed in silico analyses of PifHDAC3 model potential in S-nitrosative sites,
e) done the phylogenetic analysis
f) studied the conserved domain distribution of PifHDAC3 and its orthologs,
g) participated in the analysis concerning chromatin immunoprecipitation assay and ChiPseq data analysis as well as the validation of ChiPseq results using ChiP-qPCR.

Moreover in Manuscript 3 the Candidate performed statistical analyses. The work of the Candidate did not include conceptualization. However it was connected with the preparation of the first version of the manuscript, which this time included writing of the first version of the manuscript, preparation of graphs and tables as well as formatting the article. Based on the statement in the submitted manuscript Ms Guan has not been involved in the final review and editing. Please be specific and explain your role in this manuscript.

The Thesis of Ms. Yufeng Guan described in detail the contributions of the other researchers received during her studies. The thorough description of the involvement of the other contributors reflect the proper ethical approach of the Candidate. Achievements in science usually result from the collective effort but everybody should get the credit from his/her own work. In spite of some discrepancies it may be concluded that the involvement of the Candidate spreads from forming the hypothesis via the preparation of plant and pathogen materials, followed by the performance of complex experiments at the molecular level and they are finalized by preparing graphs and tables and participation in writing of the draft manuscript, its polishing to the form required for article submission and correcting the manuscript until it is accepted by the reviewers.

This involvement is different depending on the article and not all elements of the above mentioned work concern the involvement of Ms Guan in each article submitted for the evaluation for doctoral degree.

ARTICLE METRICS

The share of the Candidate in each publication shows her important role in the presented studies. The article data and metrics of the Publications 1 and 2 are as listed below:

Publication 1 "The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle": the first authorship, share 60%, the other co-authors: 12,5%; 7,5%; 5%; 5% and 10% (the share of the corresponding author and the Supervisor Prof. M. Arasimowicz-Jelonek). The article was received on 14 May 2024, revised on 11 September 2024, accepted on 13 September 2024. It has been available online since 14 September 2024 and the Version of Record (that is the date the finalized version of the article was made available in the journal) has been available since 16 September 2024. The article is already cited 3 times. The publisher: Elsevier, the article has been published in Open Access.

Publication 2 "Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology": the first authorship, share 55%, the shares of the other co-authors: 12,5%; 7,5%; 7,5%; 7,5% and 10% (the share of the corresponding author and the Supervisor Prof. M. Arasimowicz-Jelonek). The article was first published on 21 July 2024. The article has been cited 2 times. The publisher is Wiley, the article has been published in Open Access.

Manuscript 3 Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress". The journal *PLOS Pathogens* began operation in September 2005 as the 5th journal of the Public Library of Science (PLOS), a non-profit open-access publisher.

SIGNIFICANCE OF FINDINGS

The aim of the research was to determine the effect of nitric oxide (NO) on expression of genes connected with the pathogenicity of *Phytophthora infestans* via histone (de)acetylation. The Candidate assumed that these changes, if found, enhance the adaptability and plasticity of the pathogen. The hypothesis was based on knowledge that the pathogen lacks 5-methylcytosine DNA modifications, so reversible histone acetylation and deacetylation may regulate its gene expression. It was explained that in *P. infestans* these processes are mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). In the presented studies, the stress caused by NO (nitrosative stress) was mimicked by the use of modulators of the reactive nitrogen species (RNS), which were applied to the cultures of *P. infestans*.

The material for the study was the virulent and avirulent isolate of *P. infestans* against the potato (*Solanum tuberosum*) cv. Sarpo Mira with the defined resistance gene. I do not know why the symbol of the virulent strain was *vr* and not *avr*, as opposed to *Avr* symbol for the gene in avirulent strain. Is there any reason for this? The opposite gene variants are usually marked with *avr/Avr* symbols.

The studies proved that indeed nitrosative stress resulted in significant changes in the global acetylation of some histones, which correlated with the induced expression of *HAT* genes. The up-regulation of the pathogen's biotrophic phase-related and pathogenicity-related genes was observed as a result of RNS-mediated acetylation in histones.

The histone deacetylase PifHDAC3 was associated with colonization of potato by *P. infestans* and it was abundant under NO-mediated stress both in vitro and in plants. Its highest levels were observed in response to nitrosative stress triggered by RNS. Chromatin immunoprecipitation sequencing (ChIP-seq) profiling after nitrosative stress showed that PifHDAC3-associated genes include those related to the propagation of *P. infestans*.

The studies proved the expression of numerous pathogenesis-related and life-cycle related genes of *P. infestans* was triggered by NO or RNS via histone (de)acetylation. NO-signaling and nitrosative stress were termed as crucial in the operation of *P. infestans* under changing environmental conditions. The work clarified molecular mechanisms of epigenetic changes in *P. infestans* and possibly also in many (or all?) oomycetes. The studies helped in understanding the role of epigenetic mechanisms of (de)acetylation in reprogramming of transcripts connected with the pathogenicity of *P. infestans.* The main achievement of this study is the demonstration of relationships between the acetylation status and the pathogen metabolism and its ability to attack the host-plant.

SIGNIFICANCE

Some years ago the research on plant pathogens was usually performed by a defined small group of plant pathologists. In recent years phytopathological research is also conducted by scientists from other complementary specialties, including plant molecular physiologists. This mostly result in a more in-depth understanding of the mechanisms related to plant-pathogen interactions. This topic is very important due to great economic impact of many pathogens on crop plants. However, only few crop failures have been as devastating as the potato late blight. In the 1840s the disease spread from North America to Europe and already in mid-1845s it led to the severe crop losses in Europe having its highest impact on Ireland. It caused the Great Famine resulting in one million of deaths and one million of emigrations, causing the reduction of the population by nearly 25% due to potato late blight only. The Great Famine lasted till 1852 and it is worth remembering the great role of the Polish traveler Paweł Edmund Strzelecki in decreasing the effects of the disease. His humanitarian help was extended to over 200 thousand children on the Green Island.

Like many microbes, *P. infestans* still attacks plants and is hard to combat; still the best and simplest way to control the disease is its prevention by avoiding the use of contaminated tubers. There are also programmes for the use of fungicide (chromisticide? SARocide? Stramenopilacide?) protection when rows of potatoes start to touch each other in the field.

It is very important to consider the weather and the area where potatoes are grown, including nearby plantations of early varieties that could be a source of infection. For the first treatment, a strong, comprehensive solution that protects the plantation from primary infections may be done by the mixture containing systemic propamocarb hydrochloride, cymoxanil, and fluazinam. The latter has the ability to kill oospores of the pathogen.

This treatment ensures thorough protection of the entire plant, preventing early disease outbreaks. There are several other chemical compounds used throughout the growing season. However, *P. infestans* is still seriously damaging potatoes and tomatoes; the relative species from the same genus are pathogenic to several other crops.

The total area under potato cultivation worldwide is currently approximately 20 million hectares, and annual harvests exceed 300 million tons. In the past, potatoes saved many societies from famine, and today they constitute a staple food and animal feed in many countries around the world. According to FAOSTAT data China is the world's leading potato producer in the global potato industry. In 2023, India, Ukraine, Russia, and the United States, followed by Germany, Bangladesh, France, Pakistan and Egypt contributed to the potato market as 'Top 10' country-producers worldwide. This ranking shows that the leading continent in potato production is Asia, with Europe in second place, followed by North America and Africa. Poland ranks fourteenth among the world's largest potato producers. According to FAO, Polish producers obtained nearly 5.6 million tons of potato tubers from the area of a bit less than 190 thousand hectares.

Due to all these facts the studies devoted to understanding of the mechanisms of plant pathogen interactions using the model *Solanum* and *Phytophthora* are of great value and importance.

POINTS FOR DISCUSSION

I would like to raise the following points during the discussion with the Candidate:

1) NO-signaling and nitrosative stress was termed as crucial trigger of the activity of *P. infestans* under changing environmental conditions; how important is this pathway in epigenetic changes found in *P. infestans*? The PifHDAC3 histone deacetylase was abundant under NO-mediated stress. Does it necessarily mean nitric oxide is the main cause of epigenetic mechanisms in *P. infestans*?

2) What is the contribution of NO-signaling and nitrosative stress in the other plant-pathogen interactions? Are there any known examples of other similar interactions in oomycetes or the other plant pathogens described in the scientific literature?

3) What is the role of NO-signaling and nitrosative stress in nature in its broad sense? How universal is this mechanism/phenomenon in biology?

4) Why is the transcriptional reprogramming mediated by RNS, leading to stronger pathogenicity of *P. infestans*, associated with the higher adaptability of the pathogen? Pathogens killing the plants/animals are usually regarded as more severe but less adapted.

5) Which stage of pathogen development/life cycle is more affected the NO-mediated signaling pathway? Biotrophic or saprotrophic phase?

6) In the publications you indicated that the increased knowledge connected with your studies on the mechanisms of plant-pathogen interactions may improve disease control strategies. Can you give an example of a possible strategy based on the results of your study? How could your method complement the use of chemicals and contribute to the Integrated Pest Management practices? Potato producers seek for new smart solutions.

CONCLUSION

I have no doubt that in respect to the structure, methodology, quality of research and importance of findings the PhD Dissertation "The effect of nitric oxide on histone protein acetylation status in *Phytophthora infestans* (Mont.) de Bary" is a significant and valuable step in science. The Doctoral Thesis of Ms Yufeng Guan meets the requirements of the Act on academic degrees and titles. With full confidence I recommend Ms Yufeng Guan for the next steps of the doctoral procedure, leading to the degree of philosophy doctor in natural sciences in the discipline of biology.

My opinion about the quality of experimental work as well as the preparation of every aspect of the PhD thesis is highly positive. I value the hard work behind these complicated and complex studies. The research techniques used were advanced, the studied problem was of great importance, scientific value of obtained results was high. Moreover, the Candidate was very proficient in statistics, showed computational skills, deep data analyses as well as writing and illustrating the results. Due to high quality of the research done and important findings obtained by the Candidate I am convinced that Ms Yufeng Guan deserves distinction for her work. The aim of her work was very ambitious and the research needed numerous high quality and complex experiments on a very precisely prepared infected plant material. Due to all these arguments I am appealing to the Disciplinary Council and the Faculty Council of Collegium Biologicum of Adam Mickiewicz University to award Ms Yufeng Guan with a special distinction or prize.

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