Adam Mickiewicz University in Poznan

Faculty of Biology

Institute of Experimental Biology

Department of Plant Ecophysiology

Yufeng Guan

DOCTORAL DISSERTATION

The effect of nitric oxide on histone protein acetylation status in *Phytophthora infestans* (Mont.) de Bary

Supervisor: Prof. dr hab. Magdalena Arasimowicz-Jelonek

Poznan 2025

Uniwersytet im. Adama Mickiewicza w Poznaniu

Wydział Biologii

Instytut Biologii Eksperymentalnej

Zakład Ekofizjologii Roślin

Yufeng Guan

ROZPRAWA DOKTORSKA

Rola tlenku azotu w regulacji stanu acetylacji białek histonowych u *Phytophthora infestans* (Mont.) de Bary

Promotor: Prof. dr hab. Magdalena Arasimowicz-Jelonek

Poznań 2025

Acknowledgement

Completing this research journey would not have been possible without the unwavering support, guidance, and encouragement of numerous individuals to whom I owe my deepest gratitude.

First and foremost, I extend my sincerest appreciation to my supervisor, ***Prof. dr hab. Magdalena Arasimowicz-Jelonek****, whose expertise, patience, and insightful feedback were indispensable to this work. Your rigorous academic standards, willingness to engage in inquisitive scientific discussions, and unwavering belief in my potential pushed me to strive for excellence even during moments of doubt. Thank you for being not only a mentor but also a source of inspiration.*

I am also deeply grateful to my master's supervisor, ***Prof. dr hab. Jolanta Floryszak-Wieczorek****, for first introducing me to the fascinating world of nitric oxide and sparking my curiosity about its mysterious effects. Her extraordinary kindness, patience, and encouragement not only guided me through the early stages of my scientific journey but also laid a solid foundation for my passion for studying nitric oxide signaling. I will always cherish the knowledge, inspiration, and confidence I gained under her guidance.*

To my colleagues at ***Department of Plant Ecophysiology and Department of Plant Physiology****, thank you for fostering a collaborative and intellectually stimulating environment. Your camaraderie made this journey both productive and enjoyable.*

I sincerely thank ***all the co-authors*** *of the publications for the fruitful collaboration.*

I am equally indebted to ***my friends****, who provided emotional sustenance throughout this demanding process.*

Finally, and most profoundly, I thank ***my family****. To my parents, thank you for your sacrifices, unconditional love, and the values of perseverance you instilled in me. This achievement is as much yours as it is mine.*

Financing

This research was performed as part of:

Project funded by the National Science Center OPUS 2018/31/B/NZ9/00355, project title: **“The effect of nitric oxide on histone protein acetylation status in *Phytophthora infestans* (Mont.) de Bary”**.

And partly through the funding by the National Science Center:

Sonata BIS 2017/26/E/NZ4/00226, project title **“Nitroxyl as an alternative or competitive to nitric oxide metabolic link in plants in the model *Arabidopsis thaliana*”**

NARODOWE CENTRUM NAUKI

TABLE OF CONTENTS

ABSTRACT

Emerging evidence suggests that the high phenotypic plasticity of one of the world's most destructive phytopathogens, *Phytophthora infestans* (Mont.) de Bary, is driven by epigenetic mechanisms that enable its rapid adaptation to internal signals and environmental stressors, including the host-plant. Notably, *Phytophthora* lacks 5-methylcytosine DNA modifications, suggesting that reversible histone modifications—particularly acetylation and deacetylation—play a central role in gene regulation in these microorganisms. In *P. infestans,* these processes are mediated by 33 histone acetyltransferases (HATs) and 11 histone deacetylases (HDACs). Recent studies have shown that a potent signaling molecule, nitric oxide (NO), beyond its diverse regulatory roles, may also function as an epigenetic modulator of gene expression in both animals and plants. Although NO role in microbial epigenetics remains underexplored, it may accumulate in pathogen structures during critical developmental transitions and under stress.

Based on the above, the primary aim of the research was to determine whether and to what extent NO and the following nitrosative stress to which *P. infestans* is exposed during its lifecycle affect the histone (de)acetylation patterns, thereby modulating gene expression to enhance adaptability and/or pathogenicity. The research was based on a comparative analysis between the virulent (vr) MP977 and avirulent (Avr) MP946 isolates of *P. infestans* against the potato (*Solanum tuberosum* L.) 'Sarpo Mira', genotype with the *R3a* resistance gene, to identify changes that may affect the pathogen's virulence. The experiments included the saprophytic phase (*in vitro*) and the microorganism's parasitic phase (*in planta*). To mimic nitrosative stress, specific reactive nitrogen species (RNS) modulators were applied to the pathogen culture.

Firstly, a significant increase in the formation of NO and its derivative, peroxynitrite, was documented in *P. infestans*' structures during both the sporulation phase and *in planta* growth. It has been shown that similarly to *in planta* conditions, pharmacologically induced nitrosative stress results in significant changes in the global acetylation of histones H3 and H4. The observed hyperacetylation of histone H3 lysine 56 (H3K56ac) and histone H4 lysine 16 (H4K16ac) correlated with the induction of the expression of *HAT* genes, *i.e.*, *PifHAM1* and *PifHAC3*, which may catalyze the formation of H4K16ac and H3K56ac, respectively. The RNS-mediated changes in histone architecture in the form of enriched H3K56ac and H4K16ac mark accumulation in the promoter regions of the molecular markers of the

pathogen's biotrophic phase (*i.e.*, *Avr3a* and *Hmp1*) and other critical pathogenicity-related genes (*CesA1*, *CesA2*, *CesA3*, *sPLD-like1*) up-regulated their expression.

Subsequent *in silico* characterization and identification of RNS-responsive nuclear HDACs in *P. infestans* revealed that PifHDAC3 potentially catalyzes H3K56ac deacetylation and shows the highest level of transcript accumulation in response to NO. Notably, PifHDAC3 showed high abundance under nitrosative environments (*in vitro* and *in planta*); however, RNS did not provoke S-nitrosation and inhibition of recombinant PifHDAC3.

As PifHDAC3 was associated with the host colonization by *P. infestans*, the final stage of the study evaluated whether NO and the subsequent shift in the redox environment could affect the HDAC's recruitment to chromatin. Thus, chromatin immunoprecipitation sequencing (ChIP-seq) profiling provided insight into the key pathways regulated by PifHDAC3 in Avr/vr *P. infestans* exposed to a nitrosative environment, and revealed PifHDAC3-targeted genes involved, including those related to the pathogen's offensive strategies in a genotype-dependent manner. Notably, the NO availability led to the displacement of PifHDAC3 from the *Avr3a* promoter, and the loss of repressive chromatin structure enabled the transcriptional activation of *Avr3a*.

Summarizing, the dynamic interplay between RNS and HATs/HDACs is vital in influencing the expression of diverse *P. infestans* genes and documents NO as an essential epigenetic signal in the pathogen biology. By altering the histone (de)acetylation status, NO/RNS trigger the transcriptional reprogramming of genes related to metabolic, developmental, and offensive strategies, which may promote high adaptability to new (micro)environments. Thus, NO signaling and nitrosative stress play a crucial role in the operation of *P. infestans*' under environmental pressure.

Key words: nitric oxide, reactive nitrogen species, nitrosative stress, epigenetic modifications, histone (de)acetylation, *Phytophthora infestans,* late blight.

STRESZCZENIE

Coraz więcej dowodów wskazuje na to, że duża plastyczność fenotypowa jednego z najbardziej niszczycielskich fitopatogenów na świecie, *Phytophthora infestans* (Mont.) de Bary, jest efektem mechanizmów epigenetycznych, które umożliwiają mu szybką adaptację do zmiennych sygnałów wewnętrznych i stresorów środowiskowych, w tym również rośliny-gospodarza. Co istotne, 5-metylocytozyna w DNA nie została wykryta u *Phytophthora*, stąd odwracalne modyfikacje białek histonowych, w szczególności acetylacja i deacetylacja, mogą odgrywać kluczową rolę w epigenetycznej kontroli ekspresji genów. U *P. infestans* procesy te są kontrolowane przez 33 acetylotransferazy histonowe (HATs) i 11 deacetylaz histonowych (HDACs). Zarówno u roślin, jak i u zwierząt, cząsteczka sygnałowa - tlenek azotu (NO) może również funkcjonować jako epigenetyczny modulator ekspresji genów. Chociaż rola NO w epigenetyce mikroorganizmów pozostaje niezbadana, cząsteczka ta może być formowana w strukturach patogenów podczas krytycznych przemian rozwojowych i ekspozycji na stresy.

Wobec powyższego, nadrzędnym celem badań było wyjaśnienie, czy i w jakim stopniu, NO oraz następujący stres nitrozacyjny, na który patogen jest narażony podczas swojego cyklu życiowego, wpływa na wzorce (de)acetylacji histonów i zmiany ekspresji genów, prowadzące do efektywnej adaptacji i/lub patogeniczności *P. infestans*. Badania oparto na analizie porównawczej pomiędzy izolatem awirulentnym (Avr) MP946 i wirulentnym (vr) MP977 *P. infestans* względem odmiany ziemniaka (*Solanum tuberosum* L.) Sarpo Mira z genem odporności *R3a*, co pozwoliło na śledzenie zmian, potencjalnie związanych z wirulencją patogenu. Eksperymenty obejmowały fazę saprofityczną (*in vitro*) i pasożytniczą mikroorganizmu (*in planta*). W celu stworzenia warunków stresu nitrozacyjnego patogen był eksponowany na specyficzne modulatory reaktywnych form azotu (RNS).

W pierwszym etapie badań wykazano, że wzmożone formowanie NO i jego pochodnej, nadtlenoazotynu, towarzyszyło fazie sporulacji oraz wzrostowi patogenu *in planta*. Zarówno w warunkach *in planta*, jak i *in vitro*, stres nitrozacyjny prowadził do istotnych zmian w globalnej acetylacji histonów H3 i H4. Zaobserwowana hiperacetylacja lizyny 56 histonu H3 (H3K56ac) i lizyny 16 histonu H4 (H4K16ac) korelowała z indukcją ekspresji genów *HATs*, tj., *PifHAM1* i *PifHAC3*, które odpowiedzialne są za formowanie H4K16ac i H3K56ac. Zależne od RNS zmiany w architekturze histonów, w postaci wzbogaconej akumulacji znaczników H3K56ac i H4K16ac, w regionach promotorowych

molekularnych markerów fazy biotroficznej patogenu (*Avr3a* i *Hmp1*) oraz innych krytycznych genów związanych z patogenicznością (*CesA1*, *CesA2*, *CesA3*, *sPLD*-like1), wpływały na wzrost ekspresji tych genów.

W kontynuacji badań, przeprowadzona charakterystyka *in silico* i identyfikacja jądrowych HDACs wrażliwych na RNS wykazały, że PifHDAC3 potencjalnie katalizuje deacetylację H3K56ac i reprezentuje najwyższy poziom akumulacji transkryptu w odpowiedzi na NO u Avr/vr *P. infestans*. Ponadto immunoanaliza wykazała, że w środowisku nitrozacyjnym *in vitro* oraz *in planta*, białko PifHDAC3 występuje powszechnie. Nie stwierdzono jednak aby RNS wywołały S-nitrozację i inhibicję PifHDAC3.

Z uwagi na stwierdzony związek PifHDAC3 z kolonizacją ziemniaka przez *P. infestans*, ostatni etap badań wyjaśniał, czy NO i zależne od niego komórkowe zmiany redoks mogą wpływać na rekrutację PifHDAC3 do chromatyny. Analiza immunoprecypitacji chromatyny (ChIP-seq) umożliwiła identyfikację kluczowych procesów bezpośrednio regulowanych przez PifHDAC3 u *P. infestans* w warunkach środowiska nitrozacyjnego. Wykazano, że *loci* genów docelowych dla PifHDAC3 obejmują, m.in., geny zaangażowane w strategie ofensywne *P. infestans*. Stwierdzony efekt był zależny od genotypu patogenu. Dowiedziono, że dostępność NO prowadzi do przemieszczenia PifHDAC3 z promotora *Avr3a*, a zniesienie represyjnej struktury chromatyny umożliwia aktywację transkrypcyjną *Avr3a*.

W podsumowaniu należy podkreślić kluczową rolę NO jako ważnego sygnału epigenetycznego w biologii *P. infestans* oraz dynamiczną interakcję między RNS a HATs/HDACs w regulacji ekspresji genów. Wykazano, że NO/RNS, poprzez zmianę statusu (de)acetylacji histonów, stymulują proces przeprogramowania transkrypcyjnego genów związanych ze strategiami metabolicznymi, rozwojowymi i ofensywnymi patogenu i jego szybką adaptację do nowych środowisk. Wykazano, że sygnalizacja zależna od NO oraz związany z tą cząsteczką stres nitrozacyjny, odgrywają strategiczną rolę u *P. infestans* egzystującego w warunkach presji środowiska.

Słowa kluczowe: tlenek azotu, reaktywne formy azotu, stres nitrozacyjny, modyfikacje epigenetyczne, (de)acetylacja histonów, *Phytophthora infestans*, zaraza ziemniaka.

LIST OF PUBLICATIONS INCLUDED IN THE DISSERTATION

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*=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*=4.9; 100 MNiSW points)

Publication 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).

*IF given according to the latest list for 2025.

LIST OF ABBREVIATIONS

5mC	5-methylcytosine
Avr3a	Effector Avr3a
CesA1	Cellulose synthase 1
CesA2	Cellulose synthase 2
CesA3	Cellulose synthase 3
ChIP	Chromatin immunoprecipitation
cPTIO	2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide
GSNO	S-nitrosoglutathione
HACs	Histone acetyltransferases CBP family
HAFs	Histone acetyltransferases TAFII250 family
HAGs	Histone acetyltransferases GNAT family
HAMs	Histone acetyltransferases MYST family
Hat1s	Histone acetyltransferases Hat1 family
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
Hmp1	Haustorium-specific gene 1
NO	Nitric oxide
Npp1	Necrosis-inducing *Phytophthora* protein 1
$ONOO^-$	Peroxynitrite
PiCAT2	*Phytophthora infestans* catalase 2
PPI	Protein-protein interaction
PTMs	Post-translational modifications
RNS	Reactive nitrogen species
SIN-1	3-morpholinosydnonimine
SPLD-like1	Small phospholipase D-like 1
TSA	Trichostatin A
TSS	Transcription Start Site
TTS	Transcription Termination Site

INTRODUCTION

Phytophthora infestans (Mont.) de Bary is regarded as one of the most devastating phytopathogens worldwide (Fry, 2008; Fry *et al.*, 2015). The pathogen is a hemibiotrophic oomycete that causes potato late blight disease, generating substantial economic losses worldwide, accounting for approximately ten billion dollars annually (Dong and Zhou, 2022). Under optimal environmental conditions such as high air humidity (> 90%) and low temperatures (16 °C) accompanied by rains*, P. infestans* can devastate potato crops even within a week (Janiszewska *et al.*, 2021). As a heterothallic fungal-like microorganism, two mating types (A1 and A2) are necessary for *P. infestans* to complete the sexual cycle (Fry, 2008). The coexistence of both mating types is strongly linked to the increased genetic variation of *P. infestans*, which has been observed in certain emerging geographic regions in recent years (Babarinde *et al.*, 2024). Consequently, the increased infectious potential of *P. infestans*, driven by shifts in its population structure, is a critical factor underlying its variability in pathogenicity and virulence toward new cultivars and species, as well as its growing resistance to fungicides (Forbes, 2012; Michalska *et al.*, 2016). Additionally, continuous climate change influences plant disease epidemiology, and *P. infestans* can swiftly adapt to shifts in temperature (Wu *et al.*, 2019). Thus, the climate crisis can also accelerate the evolution of microorganisms and alter the plant-microbe relationship, enabling the emergence of new pathogenic strains. The molecular mechanisms underlying *P. infestans* colonization and adaptation to new or adverse environments remain poorly understood. Nevertheless, there is proof that the high pathogen plasticity derives from the epigenetic regulation of gene expression, which contributes to *Phytophthora's* rapid adaptation to various stresses and endogenous stimuli. Because 5-methylcytosine (5mC) - the predominant DNA modification in mammals and plants - has not been detected in *Phytophthora* species (Chen *et al.*, 2018), reversible acetylation of histone proteins may play a pivotal role in the epigenetic control in gene expression of these fungal-like microorganisms.

Epigenetics refers to non-genotoxic, reversible, and temporary genetic processes that regulate gene expression without changing DNA sequence or genotype (Dupont *et al.*, 2009; Watson and Riccio, 2009; Tirado, 2014). Histone proteins play a fundamental role in regulating chromatin structure and gene expression. Histones undergo post-translational

modifications (PTMs), which physically restrict the accessibility of the transcriptional machinery to specific genomic regions, making *loci* more or less available for transcription. These epigenetic modifications on histones may include methylation, acetylation, phosphorylation, ubiquitylation, and SUMOylation (Weinhold, 2006). Among these, histone acetylation, a process occurring at the ε-amino groups on the N-terminal of histone tails, is particularly critical for transcriptional regulation. This modification is dynamically controlled by two types of enzymes: histone acetyltransferases (HATs), which catalyze the addition of an acetyl group from acetyl-CoA, and histone deacetylases (HDACs), which are responsible for the removal of these groups (Wang *et al.*, 2016; Narita *et al.*, 2019). Acetylation neutralizes the positive charge of lysine residues, weakening histone–DNA interactions and promoting a more open chromatin structure, which facilitates transcriptional activation (Sterner and Berger, 2000; Nitsch *et al.*, 2021). Consequently, histone acetylation leads to gene upregulation, while deacetylation inhibits gene expression (Verdone, 2006). In relation to oomycetes, five families of HATs have been identified, including histone acetyltransferases GNAT family (HAGs), histone acetyltransferases TAFII250 family (HAFs), histone acetyltransferases CBP family (HACs), histone acetyltransferases MYST family (HAMs), and histone acetyltransferases Hat1 family (Hat1s). Moreover, there are only three classes of HDACs in oomycetes: classes I, II, and III, while class IV is absent. In *P. infestans*, 33 HATs have been identified and distributed into these five families (Wang *et al.*, 2016). In turn, the identified 11 HDACs are grouped into three distinct classes (Wang *et al.*, 2016).

The specific role of histone acetylation in pathogenic microorganisms is currently not well understood. However, research on *Phytophthora* species has shown that histone acetylation is crucial for their growth, reproduction, pathogenicity, and adaptation to environmental conditions (Zhao *et al.*, 2015; Wang *et al.*, 2016, 2020*a*). In *P. infestans*, all 11 HDACs and 33 HATs were found to be differently expressed throughout ten stages of development during the infection process and under various stress conditions. This indicates that HDACs and HATs are involved in many key biological processes in this pathogenic microorganism (Wang *et al.*, 2016). Additionally, transformants of *P. infestans* in which HDACs were silenced exhibited changes in sex hormone production and produced defective asexual and sexual structures, highlighting the essential role of HDACs in pathogen reproduction (Wang *et al.*, 2020*a*). Several studies have also demonstrated the role of HDACs in gene silencing in *P. infestans* (van West *et al.*, 2008; Vetukuri *et al.*, 2011). In *P.*

sojae, the HAT, Gcn5 has been identified as essential for the pathogen's adaptation to oxidative stress. As documented by Zhao *et al.*, (2015), the application of hydrogen peroxide led to reduced growth in Gcn5-silenced mutants, emphasizing the importance of Gcn5 in the tolerance of *P. sojae* to oxidative stress conditions.

Over the past two decades, NO has been recognized as a key signaling molecule in humans, animals, plants, and microorganisms. In pathogenic microorganisms, NO can act as a sophisticated regulator involved in developmental processes, virulence, host survival, and responses to the changing environments (Arasimowicz-Jelonek and Floryszak-Wieczorek, 2013; Arasimowicz-Jelonek and Floryszak-Wieczorek, 2016). In *P. infestans*, both saprophytic and parasitic stages are associated with the production of NO and other RNS (Izbiańska *et al.*, 2019). Overproduction of RNS may lead to nitrosative stress and induce alterations at the cellular level through the modifications of nucleic acids, proteins, and lipids (Ischiropoulos, 2003; Wang *et al.*, 2021). Nevertheless, a recent study has shown that *P. infestans* possesses a multifaceted system of metabolic sensors that control RNS balance *via* its enzymatic detoxification. This system enables the pathogen to thrive in various microenvironments characterized by nitrosative stress, thereby preventing redox misbalance and reducing NO-dependent modifications of biomolecules (Gajewska *et al.*, 2023). Research studies conducted on animal and plant systems have implicated that RNS may influence the alterations in chromatin (Nott *et al.*, 2008; Okuda *et al.*, 2015; Mengel *et al.*, 2016; Ageeva-Kieferle *et al.*, 2021; Drozda *et al.*, 2022*a*). There is experimental evidence that NO directly influences the expression and activity of HDACs. For example, in human lung cancer cells, treatment with NO donors significantly increased the expression of S-nitrosated HDAC6, which was associated with reduced enzymatic activity (Okuda *et al.*, 2015). In contrast, NO-mediated S-nitrosation of HDAC2 in mouse and rat spinal neuron cells exhibited no detectable impact on its activity (Nott *et al.*, 2008). Further studies on Arabidopsis have demonstrated that exogenous NO reduces HDAC activity in protoplasts and nuclear extracts (Mengel *et al.*, 2016). Additionally, light intensity-dependent fluctuations in NO levels have been shown to affect global histone acetylation dynamics (H3, H3K9, and H3K9/K14), thereby influencing HDA6 activity (Ageeva-Kieferle *et al.*, 2021). These studies suggest that HDACs in animals and plants play central roles in redox signaling pathways, translating NO production into epigenetic responses.

The epigenetic landscape of oomycetes is still largely unexplored, particularly regarding the potential role of nitric oxide and its derivatives in epigenetic regulation. In view of the above, it can be hypothesized that NO and the resulting nitrosative stress to which *P. infestans* is exposed throughout its lifecycle may influence histone (de)acetylation patterns, thereby modulating gene expression to enhance the pathogen's adaptability and/or pathogenicity. Therefore, the primary aim of the presented dissertation was to determine whether and to what extent NO and the following nitrosative stress influence the (de)acetylation patterns of histone proteins in pathogenic oomycete *P. infestans*. The research specifically addressed (1) the characterization of RNS-dependent acetylation profiles in Avirulent/virulent (Avr/vr) *P. infestans* and (2) the identification of RNS-dependent histone acetylation patterns that contribute to transcriptional reprogramming crucial for the pathogen's offensive strategy.

Those mentioned above (primary) aim was achieved by implementing the following research tasks:

1. Determination of reactive nitrogen species (RNS) formation in *P. infestans* at different developmental stages and during *in planta* growth– ***Publication 1***.

2. Analysis of histone H3/H4 acetylation patterns under nitrosative stress at the selected developmental stages of *P. infestans* – ***Publication 1***.

3. Verification of the transcriptional status regarding the RNS-dependent relative abundance of H3/H4 acetylation marks (H3K56ac and H4K16ac) on developmental and pathogenicity-related marker genes – ***Publication 1***.

4. *In silico* characterization of *P. infestans* HATs/HDACs, including the identification of RNS-sensitive HATs/HDACs – ***Publications 1, 2 and 3***.

5. Analysis of the effect of nitrosative stress on the functionality of the selected NO-sensitive HDAC(s) complexes (ChIP-sequencing of PifHDAC3 complexes) – ***Publication 3***.

The avirulent (Avr) MP946 (race 1.3.4.7.10.11) and the virulent (vr) MP977 (race 1.2.3.4.6.7.10) isolates of *Phytophthora infestans* (Mont.) de Bary in reference to the potato cv. Sarpo Mira (carrying the *R* genes *R3a*, *R3b*, *R4*, *Rpi-Smira1*, and *Rpi-Smira2*) were used in the experiments to elucidate the influence of NO and the following nitrosative stress on the (de)acetylation patterns of histone proteins in *P. infestans*. Additionally, both *in vitro* and *in planta* phases of *P. infestans* were analyzed in the experiments, reflecting the saprophytic and parasitic phases of the pathogen.

According to Gajewska *et al.*, (2023), selected concentrations of RNS modulators were applied to the pathogen culture to induce nitrosative stress conditions, *i.e.*: (i) donors - S-nitrosoglutathione (GSNO) at a concentration of 400 μM and 3-morpholinosydnonimine (SIN-1) at a concentration of 5 mM; (ii) scavengers –2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide (cPTIO) at a concentration of 500 μM and ebselen at a concentration of 200 μM. Control cultures were treated with sterile water. Hyphae were harvested at 0, 2, 24, 48, and 72 h post-treatment and either processed immediately or snap-frozen in liquid nitrogen, followed by storage at −80 °C for subsequent analyses.

DISCUSSION OF RESULTS

Current research collectively indicates that the nitro-oxidative environment, dynamically regulated by internal and external stimuli, may influence chromatin structure and epigenetic modifications. Although NO production in the oomycete pathogen *P. infestans* has been observed during both saprophytic and parasitic phases (Izbiańska *et al.*, 2019), its precise role and timing throughout the pathogen's life cycle remained unknown.

NO production is accelerated during sporulation and plant colonization

The first phase of the dissertation research aimed to elucidate the role of NO and its derivatives in coordinating the lifestyle and life cycle of *P. infestans*. Chemiluminescence-based quantification of NO emission was performed to show the RNS dynamics in *P. infestans* across different developmental stages (non-sporulating hyphae – 5-day-old culture, sporulating hyphae – 12-day-old culture, and zoospores) and during *in planta* growth (hyphae of vr *P. infestans* collected from potato tubers collected at 3 and 6 days post-inoculation (dpi)). NO levels varied significantly across the analyzed stages, with the lowest level detected in non-sporulating hyphae, followed by zoospores (generated from sporulating cultures), while sporulating hyphae produced the highest NO levels (*ca*. 5-fold rise in the comparison to non-sporulating hyphae) (*Publication 1, Fig.1A*). Nitric oxide production during host colonization was more abundant (*ca*. 15-fold rise in the comparison to sporulating hyphae of vr *P. infestans*) and showed dynamic changes associated with the biotrophy-necrotrophy switch (*Publication 1, Fig.S1*). The highest NO generation was detected in hyphae growing *in planta* during the necrotrophic phase (6 dpi). The NO level even exceeded the signal production observed in the earlier, biotrophic phase (3 dpi) (*ca*. 1.25-fold rise in the comparison to the biotrophic phase) (*Publication 1, Fig.1B*). Additionally, the formation of NO derivative, peroxynitrite ($ONOO^-$), was also determined across the developmental stages of *P. infestans*. The results indicated that the formation of $ONOO^-$ in *P. infestans* structures was strictly related to the NO levels when growing *in vitro*. Specifically, sporulating hyphae generated the highest level of $ONOO^-$, followed by zoospores; in turn, non-sporulating hyphae exhibited the lowest level of $ONOO^-$. However, no statistically significant difference in $ONOO^-$ levels was observed among the biotrophic and necrotrophic phases (*Publication 1, Fig.1C*). The detection of both RNS across distinct *in vitro* developmental stages of the Avr MP946 isolate revealed formation patterns

comparable to those observed in the vr MP977 (presented for sporulating hyphae; *Publication 1, Fig.1B, C*). These findings indicate the acceleration of NO and $ONOO^-$ formation during the sporulation phase and *in planta* growth of *P. infestans*, underscoring their potential importance in regulating key stages of the oomycete's life cycle.

NO enhances global histone acetylation in *P. infestans*

NO has been established as a key regulator of epigenetic transcriptional reprogramming in both animals (Watson and Riccio, 2009) and plants (Mengel *et al.*, 2016). Moreover, NO production and biological activity have been well documented across diverse plant pathogens (Wang and Higgins, 2005; Floryszak-Wieczorek *et al.*, 2007; Prats *et al.*, 2008; Turrion-Gomez and Benito, 2011; Samalova *et al.*, 2013; Izbiańska *et al.*, 2019); however, direct evidence linking NO to epigenetic regulation in *P. infestans* remains elusive. Our previous identification of core histones H3 and H4 as potential RNS sensors undergoing nitration in *P. infestans* (Izbiańska *et al.*, 2019) suggested a possible mechanism for NO-mediated epigenetic modifications in this pathogen. Thus, to address the gap, the following step of the study aimed to investigate the effect of NO and $ONOO^-$ on global histone H3 and H4 acetylation levels. The application of RNS modulators to Avr/vr pathogen cultures revealed that GSNO as NO donor, significantly enhanced the total H3 acetylation status in all analyzed developmental stages *i.e.*, the non-sporulating, sporulating hyphae, and zoospores of the vr *P. infestans (Publication 1, Fig.2A)*. In turn, both RNS donors promoted the total histone H4 acetylation in sporulating hyphae and zoospores of vr *P. infestans* (*Publication 1, Fig.2C*). Notably, developmental stage-dependent analysis of total H3 and H4 histone acetylation in Avr *P. infestans* showed that RNS also accelerated the modification level, particularly in sporulating hyphae and zoospores. This effect was most pronounced in response to SIN-1 treatment, an $ONOO^-$ donor (*Publication 1, Fig.2B, D*). Given that the most pronounced changes in global histone acetylation levels induced by NO/RNS were observed in sporulating hyphae, this developmental phase was selected for further analyses, including the detection of histone acetylation marks by western blot. The obtained results indicated that RNS differentially modulated histone acetylation marks, specifically, global acetylation marks *i.e.*, H3ac and H4ac, and site-specific acetylation marks including, H3K36ac, H3K56ac, H4K5ac, and H4K16ac (*Publication 1, Fig.3*). Notably, H3 acetylation and hyperacetylation of H3K56ac mediated by RNS were detected in Avr and vr isolates of *P. infestans* (*Publication 1, Fig.3A-D*). In addition, GSNO enhanced the acetylation level of H3K36, H4, and H4K16 in vr isolate *P. infestans* (*Publication 1, Fig.3A, B*). In turn, the

accumulation levels of H4ac and H4K16ac were promoted by SIN-1 in both isolates of *P. infestans* (*Publication 1, Fig.3A-D*). During *in planta* growth, the pathogen exhibited a 2-fold increase in H3K56ac abundance compared to *in vitro* conditions, indicating a strong epigenetic response to the host environment. Moreover, direct contact with host tissues further enhanced the enrichment of other analyzed histone acetylation marks, including the H4K16ac mark (*Publication 1, Fig.3E, F*), suggesting that host-derived signals may actively modulate histone acetylation to influence pathogen gene expression.

RNS modify the distribution status of H4K16ac and H3K56ac marks on developmental and pathogenicity-related genes

Recent studies in redox epigenetics highlight the role of RNS in modulating chromatin dynamics to regulate gene expression (Mengel *et al.*, 2017; Ageeva-Kieferle *et al.*, 2021). Thus, to determine whether RNS-dependent histone acetylation contributes to transcriptional reprogramming critical for the development and virulence of *P. infestans*, the study next examined the correlation between transcriptional status and the relative enrichment of H3K56ac and H4K16ac marks on selected genes. These genes were associated with key biological processes, including essential life cycle genes (*CesA1*, *CesA2*, *CesA3*) and pathogenicity-related genes (*sPLD-like1* and *PiCAT2*). To this end, a chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) assay was performed using antibodies specific to H3K56ac and H4K16ac, along with primers targeting the promoter regions of the selected genes (*Publication 1, Fig.5, 6*). Following GSNO treatment, an elevated level of the active mark accumulation - H3K56ac was observed at the promoter regions of genes encoding cellulose synthase 1, 2, and 3 (*CesA1*, *CesA2*, *CesA3*) in both *P. infestans* isolates (*Publication 1, Fig.5A-C*). In turn, SIN-1 treatment promoted H3K56ac accumulation in the promoter region of small phospholipase D-like 1 (*sPLD-like1*) and also *CesA3* in both genotypes (*Publication 1, Fig.5C-D*). No significant RNS-mediated changes in H3K56 acetylation levels were detected at the *Phytophthora infestans* catalase 2 (*PiCAT2*) promoter region in Avr/vr *P. infestans* (*Publication 1, Fig.5E*). The donors of RNS also did not induce any significant changes in the distribution levels of the other active mark - H4K16ac on the promoter of *CesA1*, *CesA2*, and *CesA3* in both isolates (*Publication 1, Fig.6A-C*); nevertheless, increased H4K16ac levels at *sPLD-like1* and *PiCAT2* promoters under nitrosative conditions were detected, with significant higher mark accumulation in Avr isolate (*Publication 1, Fig.6D, E*).

Next, to determine whether overproduction of RNS affects *P. infestans* lifestyle, the relative abundance of H3K56ac and H4K16ac active marks on critical (marker) genes of the biotrophic (*Avr3a* and *Hmp1*) and necrotrophic (*Npp1*) phases of the pathogen was monitored during *in planta* growth. To this aim, vr *P. infestans* cultures were pretreated for 5 hours with RNS modulators, and zoospore suspensions were subsequently obtained to perform plant inoculation. Significant enrichment of H3K56ac and H4K16ac was detected at the *Hmp1* promoter (4-fold and 3-fold increases, respectively) and at the *Avr3a* promoter (2-fold increase) at 24 hours post-inoculation (hpi) with GSNO-pretreated *P. infestans*; however, it declined at later hpi (48 and 72 hpi) but remained elevated compared to controls, which constituted water-pretreated *P. infestans* (*Publication 1, Fig.7A, B*, *and Fig.8A, B*). In contrast, the necrotrophic marker *Npp1* exhibited no significant changes in response to RNS pretreatment compared to control at the post-infection stage (*Publication 1, Fig.7C and Fig.8C*). Importantly, an enrichment of the H3K56ac and H4K16ac marks on the promoter region of *Avr3a* and *Hmp1* after plant inoculation, positively correlated in timing to these genes expression (*Publication 1, Fig.S3*). These results demonstrate that RNS-mediated histone acetylation preferentially primes the transcriptional activation of biotrophic-phase genes, implicating NO in redox-sensitive chromatin remodeling as a mechanism driving the biotrophic lifestyle of *P. infestans*.

In silico characterization of *P. infestans* HATs/HDACs identifies RNS-sensitive HATs/HDACs potentially engaged in the observed hyperacetylation of H3K56 and H4K16

The second phase of the research within the framework of the presented dissertation focused on the characterization of *P. infestans* HATs/HDACs, including the identification of RNS-sensitive HATs/HDACs potentially engaged in the observed RNS-mediated hyperacetylation of H3K56 and H4K16 in Avr/vr *P. infestans*. First, the determination of phylogenetic relationships and the distribution of conserved domains and motifs in PifHATs/PifHDACs was performed, providing new insights into the characteristics of these genes in *P. infestans*. Thus, *in silico* analysis included the most recent database of *P. infestans* to refine the classification and functional annotation of PifHATs and PifHDACs. Phylogenetic analysis classified 33 PifHATs into five families, including HAGs (23), HAFs (1), HACs (4), HAMs (1), Hat1s (1), as well as three putative novel HATs (HAT1, 2, and 3). Among the five PifHAT families identified in *P. infestans*, the HAG family contains the most

significant number of members, totaling 23. In turn, HAMs, Hat, and HAFs contain only one member (*Publication 2, Fig.1E and S1*). The study also analyzed the exon-intron structures, which are crucial for alternative splicing, regulation of gene expression, and evolutionary conservation. Among the *PifHATs*, *PifHAM1* contains the highest number of exons (8), while several other histone acetyltransferases have only one exon. Notably, the 5′-UTR and 3′-UTR are present in only three *HATs*: *PifHAG10*, *PifHAG6*, and *PifHAG5* (*Publication 2, Fig.1F*). Each of the five PifHAT families exhibits distinct conserved domains and motifs that are critical to their acetyltransferase activity (*Publication 2, Fig.1G, H*). All members of the 23 HAG family possess the Acetyltransferase_1 domain (PF00583), which is essential for catalyzing histone acetylation. The sole member of the HAF family, PifHAF1, contains both AA_kinase and AT_1 domains, suggesting potential dual roles in acetylation and kinase functions. PifHat1, belonging to the Hat1 family, has the Hat1_N domain (PF10394), which is specific to this acetyltransferase group (*Publication 2, Fig.1G, H*). Furthermore, all four members of the HAC family share the KAT11 (SM001250), Bromodomain (PF00439), and PHD (SM000249) domains. PifHAC3 uniquely features the ZnF-TAZ (SM000551) and Znf-ZZ (SM00291) domains, which likely enhance protein-protein interactions (*Publication 2, Fig.1G, H*). These domains facilitate diverse epigenetic functions: Bromodomains recognize acetylated lysine residues to modulate chromatin structure (Marmorstein and Berger, 2001); CHROMO domains aid in chromatin condensation; ZnF-TAZ domains mediate protein interactions (Bienz, 2006); and PHD domains contribute to chromatin remodeling. The single HAM member, PifHAM1, contains the MOZ_SAS (PF01853), Tudor-knot (PF11717), and zf-MYST (PF17772) domains. Importantly, the KAT11 domain has been identified as an ortholog of Rtt109 (the regulator of Ty1 transposition gene product 109), which primarily catalyzes acetylation at H3K56 (Han *et al.*, 2007; Tang *et al.*, 2008). Additionally, the zf-MYST domain is recognized as a significant acetyltransferase that catalyzes the formation of H4K16ac (Wang *et al.*, 2020*b*). Thus, *in silico* analysis of the phylogenetic and conserved core domains enabled the selection of nuclear PifHATs potentially catalyzing the formation of H3K56ac and H4K16ac in *P. infestans* (*Publication 1, Table S3; Fig.S2*). Four *HATs*, *i.e.*, *PifHAC1, PifHAC2, PifHAC3*, and *PifHAC4* containing the KAT11 domain, were identified as potentially responsible for H3K56 acetylation (*Publication 1, Table S3*). PifHAM1 containing the zf_MYST domain was indicated as a major HAT catalyzing the formation of H4K16ac (*Publication 1, Table S3; Fig.S2*).

To further verify whether the observed RNS-mediated hyperacetylation of H3K56 and H4K16 was linked to transcriptional upregulation of the above-identified *PifHATs* candidates, changes in their expression profiles in Avr/vr *P. infestans* growing *in vitro* at the sporulating stage were determined. The results indicated that, among all candidate *PifHATs*, only *PifHAC3* and *PifHAM1* were upregulated by RNS donors (GSNO/SIN-1) in both isolates of *P. infestans*, correlating with increased H3K56ac and H4K16ac levels, respectively (*Publication 1, Fig.4A-D*). Specifically, *PifHAC3* showed an early upregulation after RNS donors' treatment in both isolates of *P. infestans* (*Publication 1, Fig.4A, B*). However, the highest *PifHAC3* expression (*ca*. 2-fold) induced by GSNO was recorded in vr isolate at 48h after treatment (*Publication 1, Fig.4A*). In the case of *PifHAM1*, GSNO induced long-period expression in the vr isolates (*Publication 1, Fig.4C*), while SIN-1 triggered a slightly delayed response, upregulating *PifHAM1* starting from 2 and 24 h after the modulator treatment in vr and Avr isolates, respectively (*Publication 1, Fig.4C, D*). These results link RNS signaling and HATs by influencing histone architecture through acetylation mediated by PifHAC3 and PifHAM1, which leads to the deposition of acetylation marks H3K56ac and H4K16ac on the promoter regions of key life cycle and pathogenicity-related genes.

Regarding PifHDACs, 11 members were classified into three classes (I, II, and III), with classes I and II each containing four HDACs and class III containing three (*Publication 2, Fig.1A and S1*). Among *PifHDACs*, exon numbers varied widely: *PifHDAC4* and *PifHDAC2* had the fewest number of exons (one each), whereas *PifHDAC6*, *PifHDAC8*, *PifHDAC1*, and *PifHDAC5* contained the maximum number of exons (seven each) (*Publication 2, Fig.1B*). Notably, 5′ untranslated region (UTR) was only presented in *PifSir2*.2 in *PifHDACs*. Among the three classes of PifHDACs, classes I and II share a common conserved domain and motifs, the Hist_deacetyl domain (PF00850), which catalyzes the removal of the acetyl group by cleaving an amide bond (*Publication 1, Fig.1C, D*). (Leipe and Landsman, 1997). While class III featured the Sir2 domain (PF02146). Hist_deacetyl and SIR2 family domains predominated in *P. infestans* HDACs are critical for recognizing acetylated amino alkyl groups. In contrast, the SIR2 family domain shared by PifSir2.1, PifSir2.2, and PifSir2.3 is implicated in transcriptional silencing, cell cycle progression, and chromosome stability in *P. infestans* (Brachmann *et al.*, 1995).

To further investigate the role of NO/RNS in the epigenetic regulation of transcriptional reprogramming in the Avr/vr *P. infestans*, the expression patterns of the genes encoding nuclear PifHDACs were evaluated. As previously, Avr/vr *P. infestans* isolates at the sporulating stage were pretreated with RNS modulators, and the transcript levels of *PifHDAC1, PifHDAC2, PifHDAC3, PifHDAC5,* and *PifHDAC7* were determined (*Publication 3, Fig.1A-F; Fig.S1A-D*). Among these, *PifHDAC3* was identified as the most sensitive to RNS, reaching the highest transcript accumulation following NO donor treatment in both Avr and vr *P. infestans*. In the Avr MP946 isolate, a significant upregulation of *PifHDAC3* expression was observed starting from 48h after RNS donors treatment. It peaked at 72h after GSNO application, reaching a *ca*. 6-fold increase in transcript accumulation (*Publication 3, Fig.1C*). In the vr MP977 isolate, an approximately 3-fold increase in *PifHDAC3* transcript accumulation was observed only at 2nd h after GSNO treatment (*Publication 3, Fig.1D*). Thus, *PifHDAC3* displayed a genotype-dependent temporal pattern of expression. Notably, phylogenetic analysis indicated that PifHDAC3 clusters closely to human HDAC1 and HDAC2. Domain conservation analysis further showed that PifHDAC3 harbors key conserved domains, including Hist_deacetyl (PF00850) and Acuc domains (IPR003085), which are shared with its human homologs (*Publication 3, Fig.S2*). These findings imply that PifHDAC3 may regulate the deacetylation of lysine 56 on histone H3 in *P. infestans*, similar to the functions of HDAC1 and HDAC2 in other eukaryotes. The nitrosative and host environments promote hyperacetylation of H3K56 in *P. infestans* (*Publication 1, Fig. 3*), indicating that PifHDAC3 may play a vital role in regulating the transcriptional status of key developmental and pathogenicity-related genes. Previous studies have documented that HDAC3 is implicated in oxidative stress responses in pathogenic fungi. For instance, in the entomopathogenic and endophytic fungus *Metarhizium robertsii* (Metchnikoff) Sorokin, HDAC3 has been shown to regulate ergosterol biosynthesis and tolerance to oxidative stress. Deletion of *HDAC3* in *M. robertsii* significantly reduces its resilience to oxidative stress induced by insect and plant cellular environments (Liu *et al.*, 2024). As nitrosative stress co-occurs with oxidative stress, referred to as nitro-oxidative stress, this modification highlights the pivotal role of HDAC3 in transcriptional reprogramming in response to such nitro-oxidative conditions across diverse pathogens, including *P. infestans*.

PifHDAC3 is highly abundant in environments with nitrosative stress, yet it does not undergo S-nitrosation

Next, to determine whether the protein encoded by *PifHDAC3* contributes to the previously observed H3K56 hyperacetylation in *P. infestans* structures following inhibition of NO-dependent activity, a specific antibody against the HDAC3- fungal-like protein (anti-PifHDAC3) was constructed, along with a recombinant protein. First, the approach enabled the characterization of PifHDAC3 protein as highly abundant under both artificially created and naturally occurring nitrosative stress conditions within the host environment. Treatment with GSNO resulted in an approximately 40% increase in PifHDAC3 protein accumulation in both isolates of *P. infestans* (*Publication 3, Fig.2A*). Consequently, the host-derived nitrosative environment accelerated PifHDAC3 accumulation during the later stages of disease progression, specifically from 72 hpi. The protein abundance continued to rise over time, reaching approximately a 12-fold increase by 120 hpi (*Publication 3, Fig.2C*). Importantly, PifHDAC3 protein accumulation was preceded by a substantial increase in transcript levels, which revealed a 40-fold increase at 48 hpi (in relation to 0 hpi), and exceeded 300-fold increase in the following hpi (*Publication 3, Fig.2E*). Comparing PifHDAC3 protein levels in the vr MP977 isolate grown *in vitro* versus *in planta* revealed a substantially higher accumulation, approximately 3-fold during interaction with the host (*Publication 3, Fig.2D*). Several studies have demonstrated that HDACs play functional roles in various phytopathogens during host invasion (Elías-Villalobos *et al.*, 2015; Lee *et al.*, 2019; Lin *et al.*, 2021; Villota-Salazar *et al.*, 2023). In *P. infestans*, all *HDACs* have been previously found to be expressed throughout various stages of infection (Wang *et al.*, 2016). Notably, *PifHDAC3* was strongly upregulated starting from 48 hpi, suggesting its potential role in the pathogen's offensive strategy, *in planta* development and/or transition from biotrophy to a necrotrophic phase.

Subsequently, the relationship between RNS-mediated S-nitrosation and PifHDAC3 enzymatic activity was assessed. The recombinant PifHDAC3 protein, expressed and purified from *E. coli*, was subjected to an *in vitro* HDAC activity assay in the presence of RNS modulators and trichostatin A (TSA, an HDAC inhibitor). Notably, neither RNS modulators nor TSA could significantly affect PifHDAC3 activity (*Publication 3, Fig.2B*). Although *in-silico* analyses identified two putative S-nitrosation sites within PifHDAC3 (*Publication 3, Fig.S3*), suggesting it might be a potential target of the NO-dependent PTM, no measurable signal was found after detection of biotinylated proteins, coupled with

western blot analysis, using an anti-PifHDAC3 antibody (*Publication 3, Fig.S4*). These findings indicated that PifHDAC3 is unlikely to undergo NO-mediated S-nitrosation. Feng *et al.*, (2011) had previously revealed that human HDAC8 catalytic activity is also unaffected by exogenous NO in the form of sodium nitroprusside. Additionally, recombinant HDAC1, HDAC2, and HDAC3 expressed in *E. coli* displayed varying sensitivities to NO donors. Unlike HDAC2, which was highly sensitive to NO donors, the reduced activity of HDAC1 was not attributed to S-nitrosation, and HDAC3 activity remained unaffected (Colussi *et al.*, 2008). Interestingly, although S-nitrosated HDAC2 in neurons retained its catalytic activity, this modification was crucial for its release from DNA, and resulting in an elevation in histone acetylation levels (Nott *et al.*, 2008). Collectively, these studies indicate that animal HDACs act as key components in redox-signaling pathways, mediating the conversion of NO signals into epigenetic modifications.

Nitrosative stress leads to the recruitment of PifHDAC3 to transcriptionally and metabolically active regions in a genotype-specific manner

Based on previous findings that identified PifHDAC3 as a gene responsive to NO and the subsequent shift in the redox environment, and linked its expression to potato colonization by *P. infestans*, the third phase of this study focused on assessing whether NO influences PifHDAC3 recruitment to chromatin. To investigate this, chromatin immunoprecipitation sequencing (ChIP-seq) profiling was conducted. Due to the complexity of the analysis, only GSNO, as a physiological NO donor, and cPTIO, as an NO scavenger, were selected to modulate the nitrosative environment in the pathogen structures.

First, to identify genotype-specific patterns of PifHDAC3 chromatin recruitment, a comparative analysis of PifHDAC3-bound genes under control conditions in Avr MP946 and vr MP977 *P. infestans* were performed (*Publication 3, Fig.3*). A total of 1562 PifHDAC3-bound *loci* were identified in Avr MP946; in contrast, only 545 *loci* associated with PifHDAC3 were identified in vr MP977 (*Publication 3, Fig.3*). Overall, PifHDAC3 was found to bind 427 genes shared across both genotypes, with targets enriched in processes such as proteasome activity, cofactor biosynthesis, tyrosine metabolism, and secondary metabolite production, highlighting its role in metabolic regulation and protein turnover. This observation aligns with Haas *et al.*, (2009), who identified core metabolic genes as essential for the survival of *P. infestans* during host interaction. Additionally, the Avr MP946-specific *loci* (1135) were associated with RNA processing, nucleotide metabolism, and the

metabolism of fatty acids and carbohydrates. In turn, the identified 118 specific targets of vr MP977 were linked to peroxisome activity and nucleotide sugar metabolism pathways. These differences suggest that the vr MP977 isolate may employ epigenetic mechanisms to enhance virulence by modulating responses to nitro-oxidative stress. Peroxisomes, which play a key role in managing RNS and ROS, were among the enriched pathways. Additionally, nucleotide sugar metabolism, essential for glycoconjugate synthesis and polysaccharide biosynthesis, is vital for pathogenicity (Hardham and Suzaki, 1990; Gerardy-Schahn *et al.*, 2001).

Next, PifHDAC3 occupancy under nitrosative stress in Avr/vr *P. infestans* was assessed. In the Avr genotype, GSNO treatment resulted in 531 PifHDAC3-bound *loci*, with 446 shared with the control and 85 unique to GSNO exposure (*Publication 3, Fig.4A*). Although NO donor reduced the overall number of binding events, it redirected PifHDAC3 to *loci* enriched in fatty acid degradation, inositol phosphate metabolism, and amino sugar/nucleotide sugar metabolism, indicating transcriptional reprogramming toward lipid and carbohydrate metabolism. When the co-application of cPTIO and GSNO scavenged NO, PifHDAC3 binding was reduced to 430 genomic *loci*. Among these, 373 peaks were shared with the control. At the same time, 57 were unique to cPTIO-co-treated hyphae (*Publication 3, Fig.4B*). The cPTIO-specific targets were enriched in proteasome function, phagosome formation, and cyanoamino acid metabolism, suggesting a redirection of PifHDAC3 toward protein turnover and stress response pathways. Following GSNO treatment of vr MP977 genotype, PifHDAC3 was found to bind to 492 *loci*, with 274 of these overlapping with targets identified in control conditions (*Publication 3, Fig.5A*). The targets unique to GSNO treatment were associated with DNA replication, 2-oxocarboxylic acid metabolism, nucleocytoplasmic transport, and mismatch repair. These observations align with trends noted in the Avr *P. infestans* and suggest that PifHDAC3 may play a role in stabilizing the genome under nitrosative stress. In turn, treatment of vr MP977 genotype with NO scavenger increased PifHDAC3 binding to 709 *loci*, including 379 unique peaks and 330 shared with control (*Publication 3, Fig.5B*). The cPTIO-specific targets were enriched in glycolysis/gluconeogenesis, amino acid biosynthesis, and nucleocytoplasmic transport, highlighting an increased involvement of PifHDAC3 in energy production and biosynthesis under NO depletion in cellular environment.

The genome-wide PifHDAC3 occupancy analysis demonstrated that NO signaling modulates PifHDAC3 chromatin occupancy in a genotype-dependent and position-specific

manner. The Avr MP946 genotype was found to be more sensitive to NO, exhibiting a marked global loss of PifHDAC3 binding upon GSNO treatment and only partial recovery in response to cPTIO. In contrast, vr MP977 maintained robust PifHDAC3 occupancy under nitrosative conditions, with redistribution toward the transcription start site (TSS), and further shifts binding to the transcription termination site (TTS) upon NO scavenging (*Publication 3, Fig.6*). These dynamic and localized alterations in PifHDAC3 binding underscore the flexible and resilient nature of chromatin regulation in the virulent genotype of *P. infestans*. Notably, the earlier stage of this dissertation research determined that the elevation of acetyltransferase expression (*e.g.*, *PifHAC3*; *Publication 1, Fig. 4A, B*) under nitrosative stress may neutralize PifHDAC3 activity and promote this plasticity. Interestingly, cPTIO treatment in the vr MP977 genotype enhances PifHDAC3 binding near the TTS rather than the gene body or TSS, implying a function in post-transcriptional regulation or mRNA processing. This distinct response may contribute to the pathogen's adaptability under low RNS conditions, consistent with previous studies that demonstrate *P. infestans* utilizes numerous NO-detoxifying systems to maintain homeostasis during infection (Gajewska *et al.*, 2023). The vr MP977, in comparison to the Avr MP946, possesses a more complex virulence factor set (1, 2, 3, 4, 6, 7, and 10) for *P. infestans*. Consequently, its capacity to retain PifHDAC3 occupancy in the face of nitrosative stress might be attributed to an epigenetic resilience mechanism that contributes to its pathogenic potential. The observed NO-induced redistribution of PifHDAC3, especially at the TSS, may increase pathogenicity-associated gene expression through facilitating promoter-specific chromatin remodeling. These findings lend support to the hypothesis that NO positively modulates the pathogen's offensive strategy, at least in part by altering the positioning and function of PifHDAC3, and that genotype-specific epigenetic responses to NO may help clarify various virulence patterns.

NO-dependent redox changes modulate PifHDAC3 binding at the *Avr3a* effector *locus*

P. infestans genome possesses diverse potential effector genes; nevertheless, the *Avr3a* effector gene is crucial to the pathogen’s virulence, as silencing *Avr3a* substantially reduces the pathogen's capacity to cause disease (Bos *et al.*, 2010). Moreover, RNS overproduction elevated the levels of H3K56ac and H4K16ac marks in the promoter region of *Avr3* during the post-infection phase (*Publication 1, Fig.7A and Fig.8A*). This augmentation was positively correlated with *Avr3a* expression throughout time (*Publication 1, Fig.S3A*) (Guan *et al.*, 2024). Thus, in the final stage of the study, ChIP analysis was

employed to assess the PifHDAC3 occupancy upstream of the *Avr3a* transcription start site (TSS) in Avr MP946 and vr MP977 *P. infestans* genotypes exposed to nitrosative conditions (*Publication 3, Fig.7A*). In Avr MP946, PifHDAC3 was strongly enriched at −1586 bp upstream of the *Avr3a* TSS under control conditions, suggesting the gene is transcriptionally suppressed in optimal, non-stressed circumstances. Following GSNO treatment, PifHDAC3 binding shifted slightly to −1736 bp, accompanied by a reduction in overall signal intensity, indicating that NO signaling promotes PifHDAC3 displacement from the *Avr3a* promoter. This disruption of repressive chromatin architecture may enable transcriptional activation of *Avr3a*, reinforcing the link between NO-dependent redox changes and pathogen virulence. The observed results were consistent with the previously obtained results showing, that NO alters histone acetylation profiles in *P. infestans*, increasing H3/H4 acetylation under nitrosative stress and potentially derepressing virulence genes (*Publication 1, Fig.7A and Fig.8A*). The diminished PifHDAC3 binding under nitrosative conditions suggests that NO disrupts its repressive function, thereby allowing the transcriptional activation of pathogenicity-related *loci*. Conversely, cPTIO treatment restored PifHDAC3 binding to the −1586 bp site, leading to a slight increase in signal intensity compared to the control, thereby enhancing chromatin repression. This suggests that NO scavenging elevates PifHDAC3 recruitment, possibly suppressing *Avr3a* expression. In the vr MP977 genotype, PifHDAC3 was undetectable at the *Avr3a* promoter region under control or GSNO-treated conditions. This is consistent with either gene activation or structural variation at this *locus*. However, cPTIO treatment resulted in detectable PifHDAC3 enrichment at −1586 bp upstream of the TSS, suggesting that NO depletion can trigger *de novo* recruitment of PifHDAC3, even at a *locus* typically inactive in this genotype. Quantification of ChIP-seq signals (*Publication 3, Fig.7B*) further highlighted the inverse relationship between NO availability and PifHDAC3 binding in Avr MP946.

The ChIP-qPCR validation of PifHDAC3 enrichment at the promoter region of the *Avr3a* effector gene in Avr/vr *P. infestans* was also performed. Under control conditions, PifHDAC3 was significantly enriched at the −1571 bp region upstream of the *Avr3a* TSS in the Avr MP946, indicating repressive chromatin configuration at this effector *locus* (*Publication 3, Fig.8A*). In contrast, the vr MP977 exhibited no significant PifHDAC3 occupancy at this *locus* (*Publication 3, Fig.8A*), as determined by both ChIP-qPCR and ChIP-seq analyses, suggesting a lack of transcriptional repression at *Avr3a*, possibly due to genotype-specific regulatory differences or promoter rearrangement. Following GSNO

treatment, a marked decrease in PifHDAC3 binding was detected at the −1571 bp site in the Avr *P. infestans*, with a slight shift in enrichment to −1740 bp (*Publication 3, Fig.8B*). This redistribution of PifHDAC3 correlated with the global decrease in PifHDAC3 chromatin occupancy observed in ChIP-seq analysis under nitrosative stress. These findings support the hypothesis that NO promotes the release of PifHDAC3 from the *Avr3a* promoter, potentially facilitating gene activation and contributing to increased pathogen virulence. Upon cPTIO treatment, PifHDAC3 enrichment at the −1571 bp site was enhanced in both Avr and vr genotypes (*Publication 3, Fig.8C*). In the Avr MP946, this suggests reinforcement of transcriptional repression through NO depletion. Interestingly, the vr MP977, which lacked detectable PifHDAC3 occupancy at this *locus* under control and GSNO-treated conditions, showed transparent recruitment of PifHDAC3 upon cPTIO application. This newly observed binding corresponds to genome-wide ChIP-seq data, which show increased PifHDAC3 association with gene regulatory regions following NO scavenging in the virulent genotype. Overall, these data support a model in which NO acts as a key epigenetic signal, promoting *P. infestans* pathogenicity by modulating PifHDAC3 binding at effector gene promoters in a genotype- and *locus*-specific manner.

Summarizing the third part of the results, in the context of the Avr MP946 isolate, NO was found to derepress virulence genes, thereby shifting the balance toward pathogenicity. In contrast, the vr MP977 isolate of *P. infestans* exhibits adaptive chromatin remodeling, which facilitates sustaining the infection during an NO burst generated by the host (Floryszak-Wieczorek *et al.*, 2007; Arasimowicz-Jelonek and Floryszak-Wieczorek, 2013; Drozda *et al.*, 2022*b*). This dynamic redistribution of PifHDAC3 binding likely provides virulent genotypes with an evolutionary advantage, consistent with previous reports of epigenetic plasticity in *P. infestans* (Haas *et al.*, 2009; Vetukuri *et al.*, 2011; Kronmiller *et al.*, 2023). Such adaptability, which historically contributed to devastating events like the Irish Potato Famine, continues to pose a threat to global potato production (Goodwin *et al.*, 1994). Targeting NO-driven HDAC3 activity could therefore represent a promising strategy for developing durable resistance in crops.

CONCLUSIONS

Based on the study, the following conclusions were drawn:

1. Endogenous NO generation was accelerated during *P. infestans* sporulation phase (*in vitro*) and host colonization (*in planta*). The generation of NO was accompanied by the $ONOO^-$ formation, indicating nitrosative conditions within the pathogen's cellular environment. Moreover, a relatively high production of NO detected in zoospores provided evidence that NO is an inherent signal of the sporulation phase, crucial to the oomycete's life cycle.

2. NO/RNS supplementation resulted in a marked increase in global acetylation of histones H3 and H4 in *P. infestans*, with the highest levels observed in sporulating hyphae. In the vr MP977 isolate, NO donor significantly enhanced histone H3 acetylation across all monitored developmental stages. In contrast, both RNS donors primarily promoted histone H4 acetylation, with the most potent effects observed in sporulating hyphae and zoospores. In the Avr MP946 isolate, RNS exposure accelerated global acetylation of histones H3 and H4 in sporulating hyphae and zoospores, with the most pronounced changes induced by the $ONOO^-$ donor. The results confirmed a functional role for NO/RNS in developmental regulation.

3. NO/RNS differentially modified histone acetylation marks (H3ac, H4ac, H3K36ac, H3K56ac, H4K5ac, and H4K16ac) in *P. infestans* sporulating hyphae. In both Avr/vr isolates RNS-mediated H3 acetylation and hyperacetylation of H3K56 were observed. NO donor primarily enhanced acetylation of H3 and accumulation of the site-specific acetylation marks H3K36 and H3K56 in the vr MP977 isolate, while the $ONOO^-$ donor significantly increased H4ac and H4K16ac across isolates. Importantly, the accumulation levels of H3K56ac and H4K16ac in *P. infestans* significantly increased during the *in planta* phase compared to *in vitro* growth.

4. NO/RNS promoted the deposition of H3K56ac and H4K16ac marks at the promoter regions of essential developmental and pathogenicity-related genes (*CesA1-3*, *sPLD-like1*, *PiCAT2*) in *P. infestans*. Specifically, both RNS donors enhanced H3K56ac levels at the promoter regions of *CesA1-3* and *sPLD-like1* in Avr/vr *P. infestans*. In

turn, RNS-mediated increase in H4K16ac levels was observed at the promoter regions of *sPLD-like1* and *PiCAT2*, with significantly higher enrichment of this epigenetic mark at the *PiCAT2* promoter in Avr isolates.

5. *P. infestans* exposure to a nitrosative environment accelerated histone architecture remodeling, marked by enriched accumulation of H3K56ac and H4K16ac at the promoter regions of biotrophic growth markers *Avr3a* and *Hmp1*. This epigenetic shift facilitated the transcriptional upregulation of these key genes related to pathogenicity.

6. Hyperacetylation of H3K56 and H4K16 observed in response to nitrosative environment created by RNS donors correlated with transcriptional activation of specific histone acetyltransferases *PifHAC3* and *PifHAM1*, catalyzing acetylation of H3K56 and H4K16, respectively.

7. Among the identified NO/RNS-sensitive *HDACs* (*PifHDAC1*, *PifHDAC3*, and *PifHDAC5*), *PifHDAC3* revealed the highest transcript accumulation in response to NO donor in both Avr MP946 and vr MP977 *P. infestans*. It exhibited a genotype-dependent temporal pattern of expression.

8. The PifHDAC3 protein was highly abundant in nitrosative environments (both *in vitro* and *in planta*), demonstrating its association with the colonization of the host by *P. infestans*. Immunoanalysis and *in vitro* enzymatic activity assay of recombinant PifHDAC3 revealed that the protein does not undergo S-nitrosation mediated inhibition.

9. Genome-wide ChIP-seq profiling revealed that PifHDAC3 regulates a vast network of genes involved in metabolism, stress responses, and pathogenicity linked to the pathogen's virulence pattern. Under control conditions, Avr MP946-specific PifHDAC3 targets were significantly enriched in pathways associated with RNA processing, nucleotide metabolism, fatty acid metabolism, and various carbohydrate metabolic processes. In contrast, the binding of PifHDAC3 in vr MP977 was explicitly linked to peroxisome function and nucleotide sugar metabolism. Upon NO treatment, PifHDAC3 binding was essentially reprogrammed in a genotype-

dependent manner, implicating a role for PifHDAC3 in stabilizing the pathogen's genome under nitrosative stress.

10. NO-dependent redox changes modulated PifHDAC3 binding at the *Avr3a* effector *locus*. ChIP-seq and ChIP-qPCR analyses demonstrated that NO availability promotes the release of PifHDAC3 from the *Avr3a* promoter, potentially facilitating gene activation and contributing to increased pathogen virulence. Upon NO scavenging, PifHDAC3 enrichment at the −1571 bp site was enhanced in both Avr/vr *P. infestans*. In the Avr MP946, this suggests reinforcement of transcriptional repression through NO depletion. In the vr MP977, which lacked detectable PifHDAC3 occupancy at this *locus* under control and nitrosative conditions, showed transparent recruitment of PifHDAC3 upon NO scavenger application.

11. **The obtained results indicate that the dynamic interplay between RNS and HATs/HDACs is vital in influencing the expression of diverse *P. infestans* genes and document NO as an essential epigenetic signal in the pathogen biology. By changing the histone (de)acetylation status, NO/RNS trigger transcriptional reprogramming of genes related to metabolic, developmental and offensive strategies, what may promote high adaptability to new (micro)environments, contributing enhanced invasiveness of *P. infestans*.**

SUMMARY

The presented dissertation provided the first insight into the histone acetylation status in the *P. infestans* structures in the face of a nitrosative challenge created by various (micro)environments, including the host plant. The multifaceted approach integrating aspects and techniques of molecular biology, phytopathology, and epigenetics allowed to explain how NO, as an abundant signaling molecule in the pathogen, may regulate the epigenetic control of gene expression in a genotype-dependent manner.

The research hypothesis was confirmed through the RNS-dependent transcriptional reprogramming of *P. infestans*, achieved by altering the status of histone (de)acetylation, which resulted in modifications to the expression patterns of genes involved in developmental and defense strategies. Specifically, pharmacologically induced nitrosative stress led to an overall increase in H3/H4 acetylation and specific histone acetylation marks, particularly in sporulating hyphae of Avr/vr isolates and during potato colonization by vr *P. infestans*. This effect was linked to the transcriptional upregulation of the acetyltransferases *PifHAC3* and *PifHAM1*, which catalyze the acetylation of H3K56 and H4K16, respectively. Additionally, RNS-induced modifications were associated with the deposition of H3K56 and H4K16 marks on the promoters of pathogenicity-related genes (*CesA1*, *CesA2*, *CesA3*, *sPLD-like1*, *Hmp1*, and *Avr3a*), resulting in the enhanced expression of key genes related to the biotrophic phase of the pathogen (*Hmp1* and *Avr3a*). Moreover, NO was shown to impact the PifHDAC3 gene, encoding a histone deacetylase that becomes overaccumulated during *P. infestans* growth *in planta*. Genotype-specific PifHDAC3-targeted genes, essential for the pathogen's aggressive strategies, have also been identified (Figure 1).

To summarize, epigenetic regulation of gene expression *via* histone (de)acetylation mediated by NO/RNS provides a dynamic mechanism for generating phenotypic diversity in *P. infestans*. This flexible regulatory system underscores the need to further elucidate the complexity of epigenetic variation in *P. infestans*, particularly in the context of climate change, which accelerates pathogen evolution and poses increasing challenges to host immunity. By confirming that NO plays a strategic role in the aggressor’s development and operation under environmental pressure, the results provide a foundation for enhanced host protection not only against the cause of late blight but also against the entire devastating pathogenic genus *Phytophthora*.

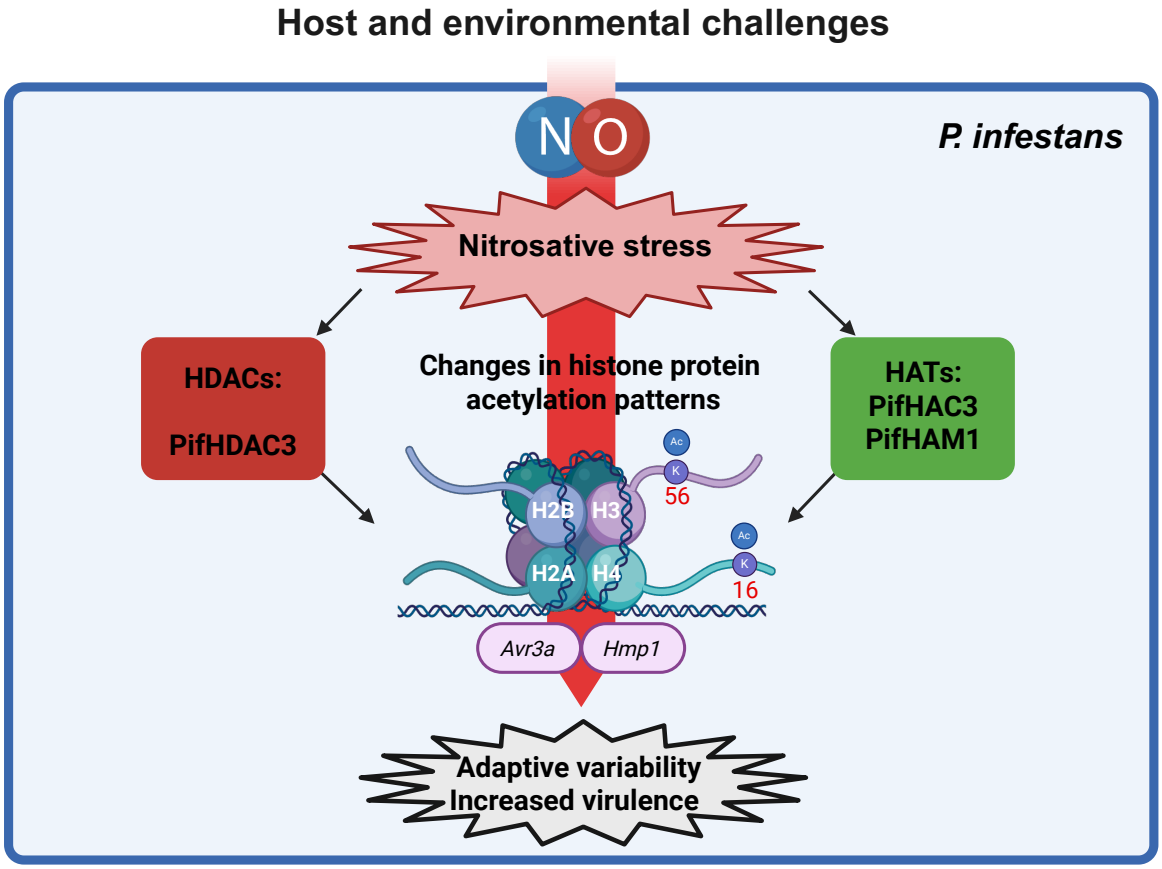


Figure 1. The sequence of events leading to changes in histone (de)acetylation status as a result of nitric oxide production induced by host and/or environmental factors - determined based on the obtained results. NO - nitric oxide; HDACs – histone deacetylases; HATs – histone acetyltransferases. Figure created using BioRender (https://app.biorender.com).

LITERATURE

Ageeva-Kieferle A, Georgii E, Winkler B, *et al.* 2021. Nitric oxide coordinates growth, development, and stress response via histone modification and gene expression. Plant Physiology **187**, 336–360.

Arasimowicz-Jelonek M, Floryszak-Wieczorek J. 2013. Nitric oxide: an effective weapon of the plant or the pathogen? Molecular Plant Pathology **15**, 406–416.

Arasimowicz-Jelonek M, Floryszak-Wieczorek J. 2016. Nitric Oxide in the Offensive Strategy of Fungal and Oomycete Plant Pathogens. Frontiers in Plant Science **7**.

Babarinde S, Burlakoti RR, Peters RD, Al-Mughrabi K, Novinscak A, Sapkota S, Prithiviraj B. 2024. Genetic structure and population diversity of Phytophthora infestans strains in Pacific western Canada. Applied Microbiology and Biotechnology **108**, 237.

Bienz M. 2006. The PHD finger, a nuclear protein-interaction domain. Trends in Biochemical Sciences **31**, 35–40.

Bos JIB, Armstrong MR, Gilroy EM, *et al.* 2010. Phytophthora infestans effector AVR3a is essential for virulence and manipulates plant immunity by stabilizing host E3 ligase CMPG1. Proceedings of the National Academy of Sciences **107**, 9909–9914.

Brachmann CB, Sherman JM, Devine SE, Cameron EE, Pillus L, Boeke JD. 1995. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes & Development **9**, 2888–2902.

Chen H, Shu H, Wang L, *et al.* 2018. Phytophthora methylomes are modulated by 6mA methyltransferases and associated with adaptive genome regions. Genome Biology **19**.

Colussi C, Mozzetta C, Gurtner A, *et al.* 2008. HDAC2 blockade by nitric oxide and histone deacetylase inhibitors reveals a common target in Duchenne muscular dystrophy treatment. Proceedings of the National Academy of Sciences **105**, 19183–19187.

Dong S, Zhou S. 2022. Potato late blight caused by *Phytophthora infestans*: From molecular interactions to integrated management strategies. Journal of Integrative Agriculture **21**, 3456–3466.

Drozda A, Kurpisz B, Arasimowicz-Jelonek M, Kuźnicki D, Jagodzik P, Guan Y, Floryszak-Wieczorek J. 2022*a*. Nitric Oxide Implication in Potato Immunity to Phytophthora infestans via Modifications of Histone H3/H4 Methylation Patterns on Defense Genes. International Journal of Molecular Sciences **23**, 4051.

Drozda A, Kurpisz B, Guan Y, Arasimowicz-Jelonek M, Plich J, Jagodzik P, Floryszak-Wieczorek J. 2022*b*. Insights into the expression of DNA (de) methylation genes responsive to nitric oxide signaling in potato resistance to late blight disease. Frontiers in Plant Science **13**, 1033699.

Dupont C, Armant D, Brenner C. 2009. Epigenetics: Definition, Mechanisms and Clinical Perspective. Seminars in Reproductive Medicine **27**, 351–357.

Elías-Villalobos A, Fernández-Álvarez A, Moreno-Sánchez I, Helmlinger D, Ibeas JI. 2015. The Hos2 Histone Deacetylase Controls Ustilago maydis Virulence through Direct Regulation of Mating-Type Genes. PLOS Pathogens **11**, e1005134.

Feng J, Jing F, Fang H, Gu L, Xu W. 2011. Expression, purification, and *S*-nitrosylation of recombinant histone deacetylase 8 in *Escherichia coli* . BioScience Trends **5**, 17–22.

Floryszak-Wieczorek J, Arasimowicz M, Milczarek G, Jelen H, Jackowiak H. 2007. Only an early nitric oxide burst and the following wave of secondary nitric oxide generation enhanced effective defence responses of pelargonium to a necrotrophic pathogen. The New Phytologist **175**, 718–730.

Forbes GA. 2012. Using Host Resistance to Manage Potato Late Blight with Particular Reference to Developing Countries. Potato Research **55**, 205–216.

Fry W. 2008. Phytophthora infestans: the plant (and R gene) destroyer. Molecular Plant Pathology **9**, 385–402.

Fry WE, Birch PRJ, Judelson HS, *et al.* 2015. Five Reasons to Consider Phytophthora infestans a Reemerging Pathogen. Phytopathology® **105**, 966–981.

Gajewska J, Floryszak-Wieczorek J, Kosmala A, Perlikowski D, Żywicki M, Sobieszczuk-Nowicka E, Judelson HS, Arasimowicz-Jelonek M. 2023. Insight into metabolic sensors of nitrosative stress protection in Phytophthora infestans. Frontiers in Plant Science **14**, 1148222.

Gerardy-Schahn R, Oelmann S, Bakker H. 2001. Nucleotide sugar transporters: Biological and functional aspects. Biochimie **83**, 775–782.

Goodwin SB, Cohen BA, Fry WE. 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Proceedings of the National Academy of Sciences **91**, 11591–11595.

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.

Haas BJ, Kamoun S, Zody MC, *et al.* 2009. Genome sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. Nature **461**, 393–398.

Han J, Zhou H, Horazdovsky B, Zhang K, Xu R-M, Zhang Z. 2007. Rtt109 Acetylates Histone H3 Lysine 56 and Functions in DNA Replication. Science **315**, 653–655.

Hardham AR, Suzaki E. 1990. Glycoconjugates on the surface of spores of the pathogenic fungus Phytophthora cinnamomi studied using fluorescence and electron microscopy and flow cytometry. Canadian Journal of Microbiology **36**, 183–192.

Ischiropoulos H. 2003. Biological selectivity and functional aspects of protein tyrosine nitration. Biochemical and Biophysical Research Communications **305**, 776–783.

Izbiańska K, Floryszak-Wieczorek J, Gajewska J, Gzyl J, Jelonek T, Arasimowicz-Jelonek M. 2019. Switchable Nitroproteome States of Phytophthora infestans Biology and Pathobiology. Frontiers in Microbiology **10**.

Janiszewska M, Sobkowiak S, Stefańczyk E, Śliwka J. 2021. Population Structure of Phytophthora infestans from a Single Location in Poland Over a Long Period of Time in Context of Weather Conditions. Microbial Ecology **81**, 746–757.

Kronmiller BA, Feau N, Shen D, *et al.* 2023. Comparative Genomic Analysis of 31 Phytophthora Genomes Reveals Genome Plasticity and Horizontal Gene Transfer. Molecular Plant-Microbe Interactions® **36**, 26–46.

Lee J, Lee J-J, Jeon J. 2019. A histone deacetylase, MoHOS2 regulates asexual development and virulence in the rice blast fungus. Journal of Microbiology **57**, 1115–1125.

Leipe DD, Landsman D. 1997. Histone deacetylases, acetoin utilization proteins and acetylpolyamine amidohydrolases are members of an ancient protein superfamily. Nucleic Acids Research **25**, 3693–3697.

Lin C, Cao X, Qu Z, Zhang S, Naqvi NI, Deng YZ. 2021. The Histone Deacetylases MoRpd3 and MoHst4 Regulate Growth, Conidiation, and Pathogenicity in the Rice Blast Fungus Magnaporthe oryzae. mSphere **6**, 10.1128/msphere.00118-21.

Liu S, Wang X, Tang X, Fang W. 2024. Histone deacetylase HDAC3 regulates ergosterol production for oxidative stress tolerance in the entomopathogenic and endophytic fungus Metarhizium robertsii. mSystems **9**, e00953-24.

Marmorstein R, Berger SL. 2001. Structure and function of bromodomains in chromatin-regulating complexes. Gene **272**, 1–9.

Mengel A, Ageeva A, Georgii E, Bernhardt J, Wu K, Durner J, Lindermayr C. 2016. Nitric Oxide Modulates Histone Acetylation at Stress Genes by Inhibition of Histone Deacetylases. Plant Physiology **173**, 1434–1452.

Mengel A, Ageeva-Kieferle A, Lindermayr C. 2017. Redox regulation of histone deacetylases in plants. Plant Physiology **174**, 1452–1462.

Michalska AM, Sobkowiak S, Flis B, Zimnoch-Guzowska E. 2016. Virulence and aggressiveness of Phytophthora infestans isolates collected in Poland from potato and tomato plants identified no strong specificity. European Journal of Plant Pathology **144**, 325–336.

Narita T, Weinert BT, Choudhary C. 2019. Functions and mechanisms of non-histone protein acetylation. Nature Reviews Molecular Cell Biology **20**, 156–174.

Nitsch S, Zorro Shahidian L, Schneider R. 2021. Histone acylations and chromatin dynamics: concepts, challenges, and links to metabolism. EMBO reports **22**.

Nott A, Watson PM, Robinson JD, Crepaldi L, Riccio A. 2008. S-nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. Nature **455**, 411–415.

Okuda K, Ito A, Uehara T. 2015. Regulation of Histone Deacetylase 6 Activity *via S*-Nitrosylation. Biological & Pharmaceutical Bulletin **38**, 1434–1437.

Prats E, Carver TLW, Mur LAJ. 2008. Pathogen-derived nitric oxide influences formation of the appressorium infection structure in the phytopathogenic fungus Blumeria graminis. Research in Microbiology **159**, 476–480.

Samalova M, Johnson J, Illes M, Kelly S, Fricker M, Gurr S. 2013. Nitric oxide generated by the rice blast fungus Magnaporthe oryzae drives plant infection. New Phytologist **197**, 207–222.

Sterner DE, Berger SL. 2000. Acetylation of Histones and Transcription-Related Factors. Microbiology and Molecular Biology Reviews **64**, 435–459.

Tang Y, Holbert MA, Wurtele H, *et al.* 2008. Fungal Rtt109 histone acetyltransferase is an unexpected structural homolog of metazoan p300/CBP. Nature Structural & Molecular Biology **15**, 738–745.

Tirado CA. 2014. Epigenetics. Pathobiology of Human Disease, 3399–3407.

Turrion-Gomez JL, Benito EP. 2011. Flux of nitric oxide between the necrotrophic pathogen Botrytis cinerea and the host plant. Molecular Plant Pathology **12**, 606–616.

Verdone L. 2006. Histone acetylation in gene regulation. Briefings in Functional Genomics and Proteomics **5**, 209–221.

Vetukuri RR, Avrova AO, Grenville-Briggs LJ, Van West P, Söderbom F, SAVENKOV EI, WHISSON SC, DIXELIUS C. 2011. Evidence for involvement of Dicer-like, Argonaute and histone deacetylase proteins in gene silencing in Phytophthora infestans. Molecular Plant Pathology **12**, 772–785.

Villota-Salazar NA, Ramos-García VH, González-Prieto JM, Hernández-Delgado S. 2023. Effects of chemical inhibition of histone deacetylase proteins in the growth and virulence of *Macrophomina phaseolina* (Tassi) Goid. Revista Argentina de Microbiología **55**, 296–306.

Wang X-W, Guo L-Y, Han M, Shan K. 2016. Diversity, evolution and expression profiles of histone acetyltransferases and deacetylases in oomycetes. BMC Genomics **17**, 927.

Wang J, Higgins VJ. 2005. Nitric oxide has a regulatory effect in the germination of conidia of Colletotrichum coccodes. Fungal Genetics and Biology **42**, 284–292.

Wang X-W, Lv J-L, Shi Y-R, Guo L-Y. 2020*a*. Comparative Transcriptome Analysis Revealed Genes Regulated by Histone Acetylation and Genes Related to Sex Hormone Biosynthesis in Phytophthora infestans. Frontiers in Genetics **11**.

Wang G, Song L, Bai T, Liang W. 2020*b*. BcSas2-Mediated Histone H4K16 Acetylation Is Critical for Virulence and Oxidative Stress Response of Botrytis cinerea. Molecular Plant-Microbe Interactions® **33**, 1242–1251.

Wang F, Yuan Q, Chen F, Pang J, Pan C, Xu F, Chen Y. 2021. Fundamental Mechanisms of the Cell Death Caused by Nitrosative Stress. Frontiers in Cell and Developmental Biology **9**.

Watson PMD, Riccio A. 2009. Nitric oxide and histone deacetylases. Communicative & Integrative Biology **2**, 11–13.

Weinhold B. 2006. Epigenetics: The Science of Change. Environmental Health Perspectives **114**.

van West P, Shepherd SJ, Walker CA, Li S, Appiah AA, Grenville-Briggs LJ, Govers F, Gow NAR. 2008. Internuclear gene silencing in Phytophthora infestans is established through chromatin remodelling. Microbiology **154**, 1482–1490.

Wu E, Wang Y, Yahuza L, He M, Sun D, Huang Y, Liu Y, Yang L, Zhu W, Zhan J. 2019. Rapid adaptation of the Irish potato famine pathogen Phytophthora infestans to changing temperature. Evolutionary Applications **13**, 768–780.

Zhao W, Wang T, Liu S, Chen Q, Qi R. 2015. The histone acetyltransferase PsGcn5 mediates oxidative stress responses and is required for full virulence of Phytophthora sojae. Microbial Pathogenesis **87**, 51–58.

PUBLICATIONS INCLUDED IN THE DISSERTATION

PUBLICATION 1

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle.

*Plant Physiology and Biochem*istry, 216, p.109129.

Doi: 10.1016/j.plaphy.2024.109129

https://www.sciencedirect.com/science/article/pii/S0981942824007976

PUBLICATION 2

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology.

Molecular Plant Pathology, 25(7), p.e13497.
Doi: 10.1111/mpp.13497
https://bsppjournals.onlinelibrary.wiley.com/doi/full/10.1111/mpp.13497

PUBLICATION 3

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

PLOS Pathogens

PLOS Pathogens

Genotype-specific transcriptional reprogramming of Phytophthora infestans by histone deacetylase PifHDAC3 under nitrosative stress

--Manuscript Draft--

Manuscript Number:	
Full Title:	Genotype-specific transcriptional reprogramming of Phytophthora infestans by histone deacetylase PifHDAC3 under nitrosative stress
Short Title:	Nitric oxide regulates Phytophthora infestans pathogenicity-related genes by targeting PifHDAC3
Article Type:	Research Article
Section/Category:	Plant Pathogens
Keywords:	nitric oxide, reactive nitrogen species, nitrosative stress, Phytophthora infestans, oomycetes, histone deacetylases, potato.
Corresponding Author:	Magdalena Arasimowicz-Jelonek Adam Mickiewicz University Faculty of Biology: Uniwersytet im Adama Mickiewicza w Poznaniu Wydzial Biologii Poznań, POLAND
Corresponding Author's Institution:	Adam Mickiewicz University Faculty of Biology: Uniwersytet im Adama Mickiewicza w Poznaniu Wydzial Biologii
First Author:	Yufeng Guan
Order of Authors:	Yufeng Guan
	Szymon Kubala
	Joanna Gajewska
	Ewa Sobieszczuk-Nowicka
	Dawid Perlikowski
	Arkadiusz Kosmala
	Jolanta Floryszak-Wieczorek
	Magdalena Arasimowicz-Jelonek
Abstract:	This study provides new insights into how nitric oxide (NO) and the nitrosative environment influence the network of histone deacetylases (HDACs), leading to genotype-specific transcriptional reprogramming of the most devastating potato pathogen, Phytophthora infestans (Mont.) de Bary. Among the nuclear HDAC genes identified as sensitive to reactive nitrogen species were PifHDAC1, PifHDAC3 and PifHDAC5. PifHDAC3 showed the highest level of transcript accumulation in response to NO in both avirulent (Avr) MP946 and virulent (vr) MP977 P. infestans, with an expression pattern that varied according to the genotype. The PifHDAC3 protein was also found to be abundant in nitrosative stress environments (both in vitro and in planta); however, it did not undergo S-nitrosation. To evaluate whether NO and the subsequent shift in the redox environment could affect PifHDAC3's recruitment to chromatin, we conducted chromatin immunoprecipitation sequencing (ChIP-seq) profiling. The insight into the key pathways regulated by PifHDAC3 in Avr/vr P. infestans exposed to nitrosative environment revealed PifHDAC3-targeted genes involved in the pathogen's offensive strategies, including Avr3a. We found that the availability of NO led to the displacement of PifHDAC3 from the Avr3a promoter. This loss of repressive chromatin structure enabled the transcriptional activation of Avr3a, demonstrating the connection between NO-dependent redox changes and the pathogen genotype. We also identified genes with different levels of PifHDAC3 enrichment that are involved in various cellular pathways. The recruitment of PifHDAC3 in transcriptional reprogramming under nitrosative stress emphasizes the link between a complex network of targeted epigenetic modifications and the virulence of the pathogen.

Suggested Reviewers:	
Opposed Reviewers:	
Additional Information:	
Question	Response
Government Employee Are you or any of the contributing authors an employee of the United States government? Manuscripts authored by one or more US Government employees are not copyrighted, but are licensed under a CC0 Public Domain Dedication, which allows unlimited distribution and reuse of the article for any lawful purpose. This is a legal requirement for US Government employees. This will be typeset if the manuscript is accepted for publication.	No - No authors are employees of the U.S. government.
Financial Disclosure Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS Pathogens* for specific examples. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate.	Yes

Funded studies Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript? Did you receive funding for this work?	
Please add funding details. as follow-up to "**Financial Disclosure** Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS Pathogens* for specific examples. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate. **Funded studies** Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript? Did you receive funding for this work?"	National Science Centre – project no. NCN 2018/31/B/NZ9/00355.
Please select the country of your main research funder (please select carefully as in some cases this is used in fee calculation).	POLAND - PL

as follow-up to "**Financial Disclosure** Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS Pathogens* for specific examples. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate. **Funded studies** Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript? Did you receive funding for this work?"	
Competing Interests On behalf of all authors, disclose any competing interests that could be perceived to bias this work. This statement will be typeset if the manuscript is accepted for publication. Review the instructions link below and PLOS Pathogens' competing interests policy to determine what information must be disclosed at submission.	The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Data Availability Provide a **Data Availability Statement** in	All relevant data are within the manuscript and its Supporting Information files. The ChIP-Seq read data are available from the Sequence Read Archive database via accession number SRP055716 (http://www.ncbi.nlm.nih.gov/Traces/sra)

the box below. This statement should detail where the data used in this submission can be accessed. This statement will be typeset if the manuscript is accepted for publication. Before publication, authors are required to make all data underlying their findings fully available, without restriction. Review our PLOS Data Policy page for detailed information on this policy. Instructions for writing your Data Availability statement can be accessed via the Instructions link below.	

COVER LETTER

Dear Editors,

We would be grateful if the Editors of *PLOS Pathogens* would consider accepting our proposal entitled: "Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress" as a research article for the Journal.

- Why is this manuscript suitable for publication in *PLOS Pathogens*?

It is known that epigenetic mechanisms can help pathogens adapt to new (micro)environments, yet the molecular processes underlying the high plasticity of *Phytophthora infestans* (Mont.) de Bary, one of the most devastating potato pathogen, are not fully understood. Our manuscript offers new insights into how nitric oxide (NO), a signaling molecule, and the resulting nitrosative environment—which the pathogen encounters during host colonization—affect the network of histone deacetylases (HDACs) in the pathogen. This leads to genotype-specific transcriptional reprogramming. We identified PifHDAC3 as highly abundant in nitrosative environments and found that redistribution of PifHDAC3 binding specific to genotypes exhibited epigenetic flexibility related to the pathogen's virulence pattern. Using chromatin immunoprecipitation sequencing (ChIP-seq) profiling, we found among others that NO-dependent redox changes modulate PifHDAC3 binding at the Avr3a effector locus, contributing to the virulence of *P. infestans*.

- Why will your study inspire other members of your field, and how will it drive research forward?

Our results demonstrate for the first time that the dynamic interplay between reactive nitrogen species and histone deacetylases is vital in influencing the expression of diverse pathogen genes. This opens exciting avenues for further research into (patho)biology of *P. infestans*. As nitrosative stress leads to the unique recruitment of PifHDAC3 to transcriptionally and metabolically active regions based on genotype, our study indicates that various *P. infestans* exhibit distinct transcriptional and metabolic responses to nitrosative environments (including those found in host plant). Moreover, our findings suggest that targeting PifHDAC3 driven by nitric oxide could disrupt virulence patterns, potentially providing strategies for developing crops resistant to pathogens.

In accordance with the Guide for Authors, we have enclosed our manuscript and submitted it online.

We look forward to hearing from you.

Yours faithfully,

Magdalena Arasimowicz-Jelonek and co-authors

Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress

Yufeng Guan[1], Szymon Kubala[2], Joanna Gajewska[1], Ewa Sobieszczuk-Nowicka[3], Dawid Perlikowski[4], Arkadiusz Kosmala[4], Jolanta Floryszak-Wieczorek[5], Magdalena Arasimowicz-Jelonek[1,*]

[1] Department of Plant Ecophysiology, Faculty of Biology, Adam Mickiewicz University; Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland

[2] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland

[3] Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University; Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland

[4] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland,

[5] Department of Plant Physiology, Poznań University of Life Sciences; Wołyńska 35, 60-637 Poznań, Poland

* arasim@amu.edu.pl

Abstract

This study provides new insights into how nitric oxide (NO) and the nitrosative environment influence the network of histone deacetylases (HDACs), leading to genotype-specific transcriptional reprogramming of the most devastating potato pathogen, *Phytophthora infestans* (Mont.) de Bary. Among the nuclear HDAC genes identified as sensitive to reactive nitrogen species were PifHDAC1, PifHDAC3 and PifHDAC5. PifHDAC3 showed the highest level of transcript accumulation in response to NO in both avirulent (Avr) MP946 and virulent (vr) MP977 *P. infestans*, with an expression pattern that varied according to the genotype. The PifHDAC3 protein was also found to be abundant in nitrosative stress environments (both *in vitro* and *in planta*); however, it did not undergo S-nitrosation. To evaluate whether NO and the subsequent shift in the redox environment could affect PifHDAC3's recruitment to chromatin, we conducted chromatin immunoprecipitation sequencing (ChIP-seq) profiling. The insight into the key pathways regulated by PifHDAC3 in Avr/vr *P. infestans* exposed to nitrosative environment revealed PifHDAC3-targeted genes involved in the pathogen's offensive strategies, including *Avr3a*. We found that the availability of NO led to the displacement of PifHDAC3 from the Avr3a promoter. This loss of repressive chromatin structure enabled the transcriptional activation of Avr3a, demonstrating the connection between NO-dependent redox changes and the pathogen genotype. We also identified genes with different levels of PifHDAC3 enrichment that are involved in various cellular pathways. The recruitment of PifHDAC3 in transcriptional reprogramming under nitrosative stress emphasizes the link between a complex network of targeted epigenetic modifications and the virulence of the pathogen.

Author summary

Given the pressing challenges posed by climate change on plant disease epidemiology, our research demonstrates that *Phytophthora infestans* (Mont.) de Bary—the notorious pathogen behind the Irish famine and the most economically devastating potato pathogen worldwide—exhibits an extraordinary capacity for generating phenotypic diversity without permanent alterations to its DNA. This flexibility enables this fungus-like microorganism to swiftly adapt to changing environmental conditions. Our study uncovers the pivotal role of nitric oxide (NO), a potent signaling molecule that accumulates extensively in the pathogen during critical developmental transitions and stressful conditions. We reveal that NO regulates transcriptional reprogramming through histone (de)acetylation. Specifically, we have identified the influence of NO on the PifHDAC3 gene, which encodes a key histone deacetylase, and pinpointed genotype-specific PifHDAC3-targeted genes essential for the pathogen's aggressive strategies. These findings illuminate a crucial link between NO signaling and histone (de)acetylation in phytopathogens, fundamentally transforming our understanding of NO's role as a direct or indirect epigenetic regulator of gene expression throughout the tree of life. This research not only advances our knowledge but also underscores the urgent need to address the implications of these adaptive mechanisms in managing plant disease in a rapidly changing climate.

Introduction

The oomycete *Phytophthora infestans* (Mont.) de Bary is a hemibiotrophic and heterothallic phytopathogen that attacks both potato and tomato, causing late blight disease [1]. The coexistence of two mating types named A1 and A2, necessary for the completion of the sexual cycle of *P. infestans*, results in the increased genetic variability and rapid adaptation to climate changes observed recently in many new geographical areas [2–4]. Consequently, a short

epidemic cycle, rapid evolution, and high adaptability make *P. infestans* the most dangerous pathogen in both potato and tomato crops [5].

To date, the molecular mechanisms underlying the high plasticity of *P. infestans* remain poorly understood. However, epigenetic mechanisms can contribute to the pathogen's adaptation to new (micro)environments [6,7]. Importantly, 5-methylcytosine (5-mC), a form of DNA methylation that plays a critical role in regulating gene expression in eukaryotes, is not identified in *Phytophthora* species; however, 6-adenine methylation (6mA) is widely distributed across their genomes [8,9]. Additionally, histone (de)acetylation represents a general and effective means of controlling transcriptional reprogramming in oomycete representatives. The balancing action of two enzymes, which belong to superfamilies of histone acetyltransferases (HATs) and histone deacetylases (HDACs), regulates the dynamic lysine acetylation of histone proteins. Histone acetyltransferases are responsible for adding an acetyl group on the N-terminal histone tail, and HDACs are responsible for removing this group [10,11]. Histone acetylation results in a more open chromatin state, promoting gene expression; whereas deacetylation of histone proteins may lead to opposite regulatory effects [12,13]. In *P. infestans*, there are five families of HATs, comprising a total of 33 members, and three classes of HDACs, totaling 11 members [10]. Two classes of HDACs, classes I (PifHDAC1, 3, 6, and 8) and II (PifHDAC2,4,5, and 7), have 4 members each, while class III (PifSir2.1, 2.2, and 2.3) has 3 members [14].

Reports concerning the histone acetylation status in various fungal phytopathogens have shown that some HDACs may be crucial for the virulence of microorganisms [15–18]. In *Ustilago maydis* (DC.) Corda, the HDAC Hos2, was found to be required for the dimorphic switch and pathogenic development [15]. In turn, MoHOS2 of *Magnaporthe oryzae* B.C. Couch was essential for the formation of infection structures, such as conidia and appressoria [16,18]. Two additional genes encoding HDAC in *M. oryzae*, namely MoRPD3 (reduced potassium

dependency 3) and MoHST, were found to be involved in pathogenicity-related processes. MoHST4 was required for the proper growth of the mycelium and full pathogenicity in rice seedlings, whereas overproduction of the MoRPD3 gene led to a loss of pathogenicity [17]. Interestingly, the protein sequence of *P. infestans* HDAC3 is 57.27% similar to MoRPD3, and the DNA sequence similarity between these two HDACs is 78.21%. This can suggest that PifHDAC3 plays a crucial role in regulating the reproduction and pathogenicity of the fungal-like microorganism [14]. Trichostatin A (TSA)-mediated inhibition of HDACs in *Macrophomina phaseolina* (Tassi) Goid reduced the growth and size of microsclerotia, affected the colony morphology, and attenuated virulence in relation to common bean [19]. Concerning *Phytophthora* species, some studies have shown the contribution of histone (de)acetylation in developmental-related processes and responses to various stress conditions [10,20–22]. For example, all HDACs of *P. infestans* were expressed differentially across ten developmental stages in response to abiotic stimuli and during host colonization, indicating their engagement in the entire pathogen life cycle [10]. Moreover, *P. infestans* transformants in which selected HDAC genes were silenced (HDST43 and H7ST20) exhibited abnormal hormone production accompanied by slower growth and defective asexual and sexual structure formation [21].

The excessive formation of nitric oxide (NO) signal and other reactive nitrogen species (RNS) creates nitrosative conditions in the cellular environment promoting covalent modification of different biomolecules, including histone proteins and their modifiers, as documented so far in several studies on plants and animals [23–28]. Thus, HDACs may constitute essential elements in redox-signaling cascades, which directly or indirectly translate the production of NO/RNS into epigenetic responses [23,29–34]. In *P. infestans*, NO has been recognized as an abundant signal generated during the saprophytic and parasitic phases of the pathogen [22,35]. RNS overaccumulation during pathogen growth *in vitro* and *in planta* was associated with global hyperacetylation of H3 and H4, as well as increased levels of some

histone acetylation marks enrichment. These changes correlated with the transcriptional upregulation of acetyltransferases *PifHAC3* and *PifHAM1*, which catalyze H3K56 and H4K16 acetylation, respectively [22]. Accordingly, a functional link between NO signaling and HATs associated with the reversible deposition of transcription activation marks at the promoters of pathogenicity-related genes was revealed [22].

The current study provides the first insight into how nitrosative stress affects the network of histone deacetylases, resulting in transcriptional reprogramming in the avirulent/virulent (Avr/vr) *P. infestans*. We found that the PifHDAC3 gene is regulated by NO and NO-dependent redox changes, which play a role in the pathogen's offensive strategy. Next, to gain an understanding of the key pathways regulated by PifHDAC3 in *P. infestans*, we conducted chromatin immunoprecipitation sequencing (ChIP-seq) profiling. This approach enabled us to identify PifHDAC3-targeted genes involved in the pathogen's offensive strategies, including *Avr3a*. We also identified genes with varying levels of PifHDAC3 enrichment that were affected explicitly by nitrosative stress and involved in diverse cellular pathways, depending on the *P. infestans* genotype.

Results and discussion

Nitrosative environment affects the expression pattern of *PifHDACs* in a genotype-dependent manner

Our recent findings indicate that *P. infestans* accelerates the generation of nitric oxide (NO) and reactive nitrogen species (RNS) during both sporulation and *in planta* phases. This process influences histone architecture through acetylation mediated by PifHAC3 and PifHAM1, leading to the deposition of acetylation marks H3K56ac and H4K16ac on the promoter regions of key pathogenicity-related genes [22].

To further investigate the role of NO and RNS in the epigenetic regulation of transcriptional reprogramming in the Avr/vr *P. infestans*, we evaluated the expression patterns of selected genes encoding nuclear PifHDACs. Analysis of *PifHDAC1*, *PifHDAC2*, *PifHDAC3*, *PifHDAC5*, and *PifHDAC7* during the sporulation phase of *P. infestans* exposed to nitrosative stress revealed that *PifHDAC1*, *PifHDAC3*, and *PifHDAC5* are particularly sensitive to RNS. However, a distinct expression pattern was observed between the two tested pathogen genotypes (Fig 1). In the Avr MP946 isolate of *P. infestans*, a significant upregulation of *PifHDAC1*, *PifHDAC3*, and *PifHDAC5* was noted starting 48 h after treatment with RNS donors (Figs 1A, 1C, and 1E). Notably, *PifHDAC3* showed the highest increase, approximately a 6-fold rise in transcript levels at 72 h following GSNO application (Fig 1C). In contrast, in the vr MP977 *P. infestans*, increased levels of *PifHDAC1* and *PifHDAC3* transcripts were observed only early, at 2 h post-donor treatment (Figs 1B and 1D).

PifHDAC1 exhibited approximately a 1.5-fold and 2.5-fold increase in response to GSNO or SIN-1, respectively (Fig 1B). For *PifHDAC3*, increases of 3-fold and 1.5-fold were noted after treatment with GSNO or SIN-1, respectively (Fig 1D). GSNO was also effective in upregulating *PifHDAC5*; however, in the Avr *P.infestans* isolate, NO-dependent expression occurred at 48 and 72 h after donor administration (Fig 1E). In the vr isolate, this effect was observed only at 24 h post-treatment (Fig 1F). Moreover, *in vitro* cultures of *P. infestans* co-treated with GSNO/cPTIO or SIN-1/ebselen showed diminished gene expression or no significant changes compared to control cultures treated with water.

For *PifHDAC2* and *PifHDAC7*, no specific RNS-dependent expression patterns were identified. *PifHDAC2* showed significantly increased expression only 2 h after RNS donor treatment (S1 Fig). Meanwhile, *PifHDAC7* displayed contrasting expression patterns in both analyzed isolates under nitrosative conditions (S1 Fig).

In both plant and animal systems, NO has been shown to regulate specific histone deacetylases (HDACs) at the protein level through a process of S-nitrosation. This regulation affects enzyme activity and localization, ultimately impacting gene expression [24,26,36,37]. However, the regulation of individual HDACs by NO and the nitrosative environments encountered by pathogens throughout their life cycles has not been experimentally verified.

Nonetheless, various stress conditions—including nitrogen, carbon, and water starvation, heat treatment, and low nutritional content in the growing medium—have been documented to significantly alter the transcript levels of all HDACs in *Phytophthora infestans* [10]. This study also demonstrates that nitrosative stress impacts the expression patterns of nuclear *PifHDACs*. While several HDACs are dependent on NO or redox conditions at the protein level, little is known about the redox mechanisms regulating their expression [34].

Moreover, changes in cellular levels of ROS and RNS, which modulate the redox balance, can influence each stage of gene transcription—from initiation to elongation and termination [38]. The core transcriptional machinery contains redox-sensitive cysteine residues within several complexes, and redox-sensitive transcription factors can undergo S-nitrosation or other redox-dependent modifications [39].

Among the identified RNS-sensitive *HDACs* (*PifHDAC1*, *PifHDAC3*, and *PifHDAC5*), *PifHDAC3* shows the highest transcript accumulation in response to GSNO in both Avr MP946 (*ca*. 6-fold increase) and vr MP977 (*ca*. 3-fold increase) of *P. infestans*, exhibiting a genotype-dependent temporal pattern of expression. Interestingly, phylogenetic analysis reveals that PifHDAC3 clusters closely with human HDAC1 and HDAC2, which primarily catalyze the deacetylation of H3K56 (S2 Fig). Additionally, PifHDAC3 shares conserved domains with its human homologs, including the Hist_deacetyl and AcuC domains. These findings suggest that PifHDAC3 may regulate the deacetylation of lysine 56 on histone H3 in *P. infestans*, similar to the roles of HDAC1 and HDAC2 in other eukaryotes. Given that nitrosative and host

environments promote hyperacetylation of H3K56 in both Avr and vr *P. infestans* [22], PifHDAC3 may play a crucial role in regulating the transcriptional status of key developmental and pathogenicity-related genes. Moreover, it is noteworthy that HDAC3 has been implicated in the oxidative stress response, particularly by regulating ergosterol production in the entomopathogenic and endophytic fungus *Metarhizium robertsii* (Metchnikoff) Sorokin [40]. The deletion of *Hdac3* has been shown to reduce *M. robertsii's* tolerance to oxidative stress arising from insect and plant cellular environments [40]. This modification highlights the significance of HDAC3 in regulating transcriptional reprogramming during nitro-oxidative stress responses in various phytopathogens.

PifHDAC3 is highly abundant in environments with nitrosative stress, yet it does not undergo S-nitrosation

To investigate whether the protein encoded by *PifHDAC3* is involved in the previously observed hyperacetylation of H3K56 in *P. infestans* structures due to the inhibition of NO-dependent activity [22], we obtained a specific antibody against the fungus-like protein HDAC3 (anti-PifHDAC3) along with a recombinant protein. First, we monitored the accumulation pattern of PifHDAC3 in response to RNS donors (Fig 2A). Artificial nitrosative stress induced by GSNO resulted in approximately a 40% increase in PifHDAC3 protein accumulation in Avr/vr *P. infestans* structures (Fig 2A). Next, we investigated the relationship between RNS-mediated S-nitrosation and the enzymatic activity of PifHDAC3 by measuring the *in vitro* enzymatic activity of the recombinant enzyme, purified from *Escherichia coli*, in the presence of RNS donors or a potent inhibitor of HDACs from classes I and II, trichostatin A, TSA. Neither of the RNS donors was effective in modulating PifHDAC3 activity (Fig 2B). However, *in-silico* analyses suggested that PifHDAC3 could be a potential target for S-nitrosation (S3

Fig). The supplementary Figure S3 illustrates that there are two potential S-nitrosation sites in the PifHDAC3 protein (C168 and C273). Based on this prediction, we performed immunoprecipitation of biotinylated proteins, coupled with Western blot analysis, using an anti-PifHDAC3 antibody. However, we did not detect any signals of S-nitrosated PifHDAC3 (S4 Fig), indicating that PifHDAC3 does not undergo NO-mediated S-nitrosation. Previous research found that exogenous NO, in the form of sodium nitroprusside, did not affect the catalytic activity of HDA8, indicating that a specific structural interaction is required for transferring NO [41]. In turn, analysis of recombinant HDA1, HDA2, and HDA3 revealed that only HDA2 was highly sensitive to NO donors; HDA1 displayed only a slight reduction in protein activity, which was not caused by S-nitrosation, and the enzymatic activity of HDAC3 remained unaffected by NO [42]. Thus, the authors found that NO present in C2C12 myoblasts regulates the enzymatic activity of HDAC2 by S-nitrosation but not that of HDAC1 and HDAC3 [42]. Interestingly, although HDAC2 in neurons was also found to be S-nitrosated, this modification did not alter its catalytic activity; instead, it was essential for HDAC2's release from DNA and the subsequent increase in histone acetylation [23]. In endothelial cells, the S-nitrosation of protein phosphatase 2A resulted in the formation of a large protein complex that included HDAC4, HDAC5, and HDAC3, which was subsequently shuttled into the nucleus [43]. These studies imply that animal HDACs play central roles in redox-signaling cascades, translating the production of NO into epigenetic responses.

PifHDAC3 activity was not inhibited by RNS (Fig 2B), suggesting that it does not participate in the observed earlier RNS-mediated H3K56 hyperacetylation in *P. infestans*. However, RNS donors significantly accelerated both transcript (Figs 1C and 1D) and protein accumulation (Fig 2A), indicating that PifHDAC3 is involved in transcriptional reprogramming under artificial nitrosative stress. To gain insight into whether the host-derived nitrosative environment also stimulates PifHDAC3, the transcript and protein accumulation levels during

241 the vr MP977 *P. infestans* growth *in planta* were monitored. The accumulation of PifHDAC3
242 was observed in the later phase of disease development, specifically starting from 72 h post-
243 infection (Fig 2C). In subsequent hours, the abundance of PifHDAC3 continued to increase,
244 showing approximately a 12-fold increase by 120 hpi (Fig 2C). Notably, a comparison of
245 protein accumulation in the vr MP977 isolate growing *in vitro* versus *in planta* revealed a
246 significantly higher accumulation (about 3-fold) of PifHDAC3 when interacting with host
247 tissues (Fig 2D). Furthermore, monitoring PifHDAC3 expression throughout disease
248 development (0-96 hpi) revealed that protein accumulation *in planta* was preceded by a
249 significant increase in transcript accumulation, reaching approximately a 40-fold increase by
250 48 hpi compared to the inoculation start point (0 hpi) (Fig 2E). In the hours that followed,
251 specifically at 72 and 96 hpi with vr *P. infestans*, we noted a more than 300-fold increase in
252 PifHDAC3 transcript accumulation (Fig 2E).

253 Wang *et al.* [10] previously reported that all histone deacetylases (HDACs) in *P.*
254 *infestans* were expressed during various stages of infection. Consistent with our findings, the
255 expression of *PifHDAC3* was up-regulated during the early stages of disease development,
256 peaking at 2 days post-inoculation. This suggests a potential role for PifHDAC3 in the
257 pathogen's offensive strategy. Several studies have demonstrated the functional role of different
258 phytopathogen HDACs during plant invasion [15–19]. The deletion or mutation of specific
259 *HDACs* has resulted in alterations in fungal growth and metabolism [44]. In the fungus *Ustilago*
260 *maydis*, the HDAC Hos2 was found to be essential for full virulence, as the Δhos2 mutants
261 showed decreased plant death and smaller tumor sizes [15]. Similarly, in *Magnaporthe oryzae*,
262 MoHOS2 is necessary for the proper formation of appressoria, and Hos2 deletion mutants fail
263 to induce disease symptoms. Compared to the wild-type fungus, ΔMohos2 demonstrated
264 greater resistance to H_2O_2 treatment. It showed increased expression of genes encoding ROS-
265 detoxifying enzymes such as catalase, superoxide dismutase, and heme peroxidase [16].

Interestingly, two other HDACs in *M. oryzae*, namely MoRPD3 and MoHST4, were identified as significant for hemibiotrophic pathogenicity. Both transcripts were up-regulated at 12 hpi in barley plants; however, the overexpression strain of MoRPD3 was nonpathogenic [17]. In *Botrytis cinerea*, overexpression of the BcRPD3 gene resulted in decreased acetylation of H3 and H4. The changes were accompanied by impaired infection structure formation and significantly reduced disease lesion development in tomato, as compared to the wild-type strain [45].

Nitrosative stress leads to the recruitment of PifHDAC3 to transcriptionally and metabolically active regions in a genotype-specific manner

Previous experiments identified PifHDAC3 as a gene sensitive to NO and redox conditions, demonstrating its association with the colonization of potato by *P. infestans*. This association may lead to transcriptional changes in response to nitrosative stress derived from the host. Based on these findings, we conducted a ChIP-seq analysis on both Avr MP946 and vr MP977 genotypes of *P. infestans* under two conditions: control (non-stressed) and nitrosative stress. Our goal was to evaluate whether the recruitment of PifHDAC3 to chromatin is affected by reactive nitrogen species. Based on PifHDAC3 protein accumulation *in vitro* analysis (Fig 2A), GSNO was chosen as a physiological NO donor to simulate the cellular nitrosative environment.

To identify similarities and differences in PifHDAC3 chromatin recruitment between the analyzed genotypes, we conducted a comparative analysis of PifHDAC3-bound genes in the Avr MP946 and vr MP977 genotypes of *P. infestans* under control conditions (Fig 3). In the Avr genotype, we identified a total of 1,562 PifHDAC3-bound *loci*. In contrast, the vr

MP977 genotype showed PifHDAC3 association with 545 genomic regions. Overall, PifHDAC3 bound to 427 genes in both genotypes (Fig 3). These shared targets were enriched in functions related to proteasome activity, biosynthesis of cofactors, tyrosine metabolism, and the production of secondary metabolites, indicating a crucial regulatory role in metabolic maintenance and protein turnover. This finding aligns with the study by Haas *et al.* [46], who identified core metabolic genes as essential for the survival of *P. infestans* during interactions with its host. Avr MP946-specific PifHDAC3 targets (1,135) were significantly enriched in pathways associated with RNA processing (e.g., spliceosome, RNA degradation), nucleotide metabolism, fatty acid metabolism, and various carbohydrate metabolic processes. In contrast, the binding of PifHDAC3 in vr MP977 (118 genes) was linked to peroxisome function and nucleotide sugar metabolism. These differences suggest that MP977 *P. infestans* may adapt epigenetically to enhance its virulence, potentially by modulating responses to nitro-oxidative stress. Peroxisomes are associated with managing RNS and ROS, which are critical signaling factors in host-pathogen interactions. Furthermore, nucleotide sugar metabolism plays a vital role in the synthesis of glycoconjugates on cell surfaces and the biosynthesis of various polysaccharides. This metabolism is essential for the pathogen's virulence and pathogenicity [47,48]. Overall, these findings indicate that while PifHDAC3 maintains a conserved functional core across the analyzed genotypes of *P. infestans*, the specific chromatin interactions associated with each genotype reflect distinct transcriptional and metabolic strategies. These strategies may contribute to the pathogen's virulence and its ability to adapt to various stress conditions.

In the Avr genotype, nitrosative disorder resulted in 531 PifHDAC3-bound *loci*. Of these 446 were shared with the control, while 85 were specific to GSNO exposure (Fig 4A). GSNO exposure reduced the overall number of PifHDAC3 binding events but redirected PifHDAC3 to *loci* enriched in fatty acid degradation, inositol phosphate metabolism, and amino

sugar/nucleotide sugar metabolism. This highlights a transcriptional reprogramming toward lipid and carbohydrate metabolism. The overlapping genes were enriched in core metabolic processes, including proteasome function, the tricarboxylic acid (TCA) cycle, nucleotide biosynthesis, and amino acid metabolism. Collectively, these results suggest that PifHDAC3 binding is responsive to nitrosative signals, coordinating transcriptional programs that are specific to the Avr genotype. When nitric oxide was scavenged through the co-application of cPTIO and GSNO, PifHDAC3 binding was reduced to 430 genomic *loci*. Among these, 373 peaks were shared with the control, while 57 were unique to cPTIO-treated hyphae (Fig 4B). KEGG enrichment of control-specific PifHDAC3 targets revealed involvement in mRNA surveillance, mismatch repair, RNA degradation, nucleotide metabolism, and glutathione metabolism, indicating a regulatory role for PifHDAC3 in genome integrity, redox control, and proteostasis under basal conditions.

In contrast, cPTIO-specific targets were enriched in proteasome function, phagosome formation, and cyanoamino acid metabolism, suggesting a redirection of PifHDAC3 toward protein turnover and stress response pathways. Notably, shared targets between the control and cPTIO were enriched in peroxisome-related genes, supporting a conserved role for PifHDAC3 in redox and lipid metabolism. After GSNO treatment of the vr *P. infestans* isolate, PifHDAC3 was found to bind to 492 *loci*, with 274 of these overlapping with targets identified in control conditions (Fig 5A). The peaks specific to the control group were enriched in functions related to peroxisomes, purine metabolism, and nucleotide excision repair. Conversely, the targets unique to GSNO treatment were associated with DNA replication, 2-oxocarboxylic acid metabolism, nucleocytoplasmic transport, and mismatch repair. These observations align with trends noted in the Avr *P. infestans* genotype and suggest that PifHDAC3 may play a role in stabilizing the genome under nitrosative stress. The shared subset of targets included those related to proteasome function, indicating a conserved role in protein degradation. In contrast,

treatment with the NO scavenger cPTIO alongside GSNO significantly increased PifHDAC3 recruitment to 709 *loci* in vr *P. infestans*. This included 379 unique peaks and 330 that were also present in the control group (Fig 5B). Targets specific to cPTIO treatment were enriched in pathways such as glycolysis/gluconeogenesis, amino acid biosynthesis, and nucleocytoplasmic transport, highlighting an increased involvement of PifHDAC3 in energy production and biosynthesis under conditions with depleted NO. The binding of PifHDAC3 in the control condition was linked with antioxidant defense and carbohydrate metabolism, specifically involving pathways such as the ascorbate/aldarate and amino sugar metabolism pathways. Notably, the overlap between the cPTIO and control groups encompassed genes related to aminoacyl-tRNA biosynthesis and cyanoamino acid metabolism.

Redistribution of PifHDAC3 binding specific to genotypes under nitrosative stress demonstrates epigenetic flexibility linked to the pathogen's virulence pattern

Next we analyzed the spatial distribution of PifHDAC3 across gene bodies and determine how its occupancy is affected by nitrosative stress (Fig 6). Under control conditions, both genotypes displayed strong PifHDAC3 enrichment within gene bodies (Fig 6A). In Avr MP946 *P. infestans*, PifHDAC3 binding was sharply concentrated at the transcription start site (TSS) and persists with a fluctuating pattern of multiple distinct peaks along the gene body, suggesting a high degree of regulation at both promoter and intragenic regions. This pattern aligns with observations of HDAC activity in other oomycetes, where precise binding controls gene silencing during host interaction [49]. In contrast, vr MP977 *P. infestans* exhibits a broader and smoother distribution of PifHDAC3 occupancy, with symmetrical peaks flanking the TSS and moderate enrichment throughout the gene body, indicating a potentially different chromatin

landscape or regulatory mechanism. Following GSNO treatment, PifHDAC3 binding is substantially diminished in both genotypes (Fig 6B). In Avr MP946, there is a pronounced reduction in signal intensity across the entire region, with loss of the sharp peaks seen in the control, and a notable central dip in the gene body, indicating significant disruption of PifHDAC3 recruitment or stability in response to elevated NO. In contrast, MP977 *P. infestans* retains much of its PifHDAC3 occupancy, although the pattern becomes redistributed, with enrichment shifting toward the TSS (Fig 6). This promoter-proximal accumulation, despite global NO exposure, suggests that vr MP977 maintains PifHDAC3 recruitment under nitrosative stress but undergoes a spatial retargeting of the deacetylase. NO scavenging via cPTIO/GSNO co-application partially recovers PifHDAC3 binding (Fig 6C). In Avr MP946, the profile broadens and regains general gene body occupancy, although without fully restoring the distinct control peaks. Remarkably, in vr MP977, PifHDAC3 signal intensifies specifically near the transcription termination site (TTS), rather than across the gene body. This TTS-biased accumulation suggests that a lack of NO induced a shift in PifHDAC3 targeting toward the 3′ end of genes, potentially reflecting changes in co-transcriptional regulation, mRNA processing, or termination-coupled chromatin remodeling in this genotype. These results demonstrate that NO signaling modulates PifHDAC3 chromatin occupancy in a genotype-dependent and position-specific manner. The Avr MP946 genotype is more sensitive to NO, exhibiting a marked global loss of PifHDAC3 binding upon GSNO treatment and only partial recovery in response to cPTIO. In contrast, vr MP977 maintains robust PifHDAC3 occupancy under NO stress, with redistribution toward the transcription start site (TSS), and further shifts binding to the transcription termination site (TTS) upon NO scavenging (Fig 6). These dynamic, localized changes in PifHDAC3 binding suggest flexible and resilient chromatin regulation in the virulent genotype. Importantly, Guan *et al*. [22] noted elevated acetyltransferase expression (e.g., PifHAC3) under nitrosative stress, which could counterbalance PifHDAC3 activity, supporting

this plasticity. Interestingly, cPTIO treatment in the vr MP977 genotype intensifies HDAC3 binding near the TTS rather than the gene body or transcription start site TSS, suggesting a role in post-transcriptional regulation or mRNA processing. This unique response may enhance the pathogen's adaptability under low RNS conditions, consistent with our previous reports showing that *P. infestans* employs multiple NO detoxification systems to maintain homeostasis during infection [50].

Compared to Avr MP946, the vr MP977 *P. infestans* is known to carry a more complex virulence factor set (1, 2, 3, 4, 6, 7, and 10). Thus, its ability to maintain PifHDAC3 occupancy under nitrosative stress may reflect a mechanism of epigenetic resilience contributing to its pathogenic potential. The observed NO-induced redistribution of PifHDAC3, particularly toward the TSS, may enhance the expression of pathogenicity-associated genes by facilitating promoter-specific chromatin remodeling. These findings support the assumption that NO positively regulates the pathogen's offensive strategy, at least in part by modulating PifHDAC3 positioning and function, and that genotype-specific epigenetic responses to NO may underlie differential virulence patterns. Although direct studies on HDAC3 in oomycetes are limited, Vetukuri *et al.* [49] demonstrated that epigenetic modifications, including histone acetylation and methylation, differ between *P. infestans* strains, supporting the idea that a virulent genotype may possess greater chromatin plasticity, which could potentially enhance virulence.

NO-dependent redox changes modulate PifHDAC3 binding at the Avr3a effector *locus*

The *P. infestans* genome contains numerous potential effector genes; however, the Avr3a effector gene is a key component of the pathogen's virulence, as silencing Avr3a significantly reduces its ability to cause disease [51]. Additionally, redox-dependent changes

resulting from the overproduction of RNS enhance the presence of the H3K56ac and H4K16ac marks in the promoter region of Avr3a following plant infection. This enhancement correlates positively with Avr3a expression over time [22]. In the present study, ChIP analysis allows us to assess PifHDAC3 occupancy upstream of the Avr3a transcription start site (TSS) in Avr MP946 and vr MP977 *P. infestans* genotypes exposed to nitrosative conditions (Fig 7A). In the Avr MP946, PifHDAC3 was detected at −1586 bp upstream of the Avr3a TSS under control conditions, indicating that the gene is maintained in a transcriptionally repressed state under basal circumstances. Upon GSNO treatment, PifHDAC3 binding shifted slightly to −1736 bp, and overall signal intensity decreased, suggesting that NO signaling induces PifHDAC3 displacement from the Avr3a promoter. This loss of repressive chromatin architecture may facilitate transcriptional activation of Avr3a, supporting another link between NO-dependent redox changes and pathogen virulence (Fig 7).

This aligns with findings by Guan *et al.* [22], who showed that NO alters histone acetylation profiles in *P. infestans*, increasing H3/H4 acetylation under nitrosative stress and potentially derepressing virulence genes. The reduction in PifHDAC3 binding under nitrosative conditions suggests that NO disrupts its repressive function, facilitating transcriptional activation of pathogenicity-related *loci*. Moreover, Boss *et al.* [51] demonstrated that Avr3a stabilizes the host E3 ligase CMPG1, suppressing cell death during the biotrophic phase and aiding infection, a process that could be facilitated by both host- and pathogen-derived NO via NO-mediated Avr3a derepression. Conversely, cPTIO treatment restored PifHDAC3 binding to the −1586 bp position, with a modest increase in signal intensity compared to the control, reinforcing chromatin repression and indicating that scavenging of NO enhances PifHDAC3 recruitment, likely silencing Avr3a expression. In the vr MP977 genotype, PifHDAC3 was not detected at the Avr3a promoter under control or GSNO-treated conditions, consistent with gene activation or structural variation at this *locus*. However, cPTIO treatment led to detectable

PifHDAC3 enrichment at −1586 bp upstream of the TSS, suggesting that NO depletion can trigger the de novo recruitment of PifHDAC3, even at a *locus* otherwise inactive in this genotype. Quantification of ChIP-seq signal (Fig 7B) highlights the inverse relationship between NO availability and PifHDAC3 binding in the Avr MP946. GSNO-mediated NO formation correlates with decreased PifHDAC3 occupancy, potentially enabling Avr3a expression and enhanced virulence. In contrast, cPTIO-mediated NO depletion increases PifHDAC3 recruitment and reinforces transcriptional repression. These effects are largely absent in the vr MP977, which lacks functional Avr3a regulation. Interestingly, in GSNO-treated vr MP977 samples, although PifHDAC3 was not bound at the Avr3a locus, increased expression of PifHDAC3 was observed (Figs 1D and 2A). This data suggests that PifHDAC3 may be redistributed to alternative genomic regions, where it could participate in chromatin remodeling of other genes implicated in virulence. Such re-targeting of PifHDAC3 in response to NO signaling may contribute to broader transcriptional reprogramming and promote virulence potential through epigenetic activation or repression of additional effector or pathogenicity-related genes. It should be noted that vr MP977 exposed to GSNO showed enhanced levels of H4K16ac and H3K56ac accumulation on the promoter region of *Avr3a* correlated with *Avr3a* expression [22]. Overall, these data support a model in which NO acts as a key epigenetic signal, promoting *P. infestans* pathogenicity by modulating PifHDAC3 binding at effector gene promoters in a genotype- and locus-specific manner.

Additionally, ChIP-qPCR validation of PifHDAC3 enrichment at the promoter region of the Avr3a effector gene in Avr/vr *P. infestans* was performed (Fig 8). Multiple primer sets were used to interrogate sites upstream of the Avr3a transcription start site (TSS), focusing on key regulatory positions revealed by genome-wide ChIP-seq analysis. Under control conditions, PifHDAC3 was significantly enriched at the −1571 bp region upstream of the Avr3a TSS in the Avr MP946, indicating repressive chromatin configuration at this effector locus.

This observation is consistent with the genome-wide ChIP-seq dataset, in which PifHDAC3 was predominantly bound to promoter regions of numerous genes under basal conditions in the Avr genotype. In contrast, the vr MP977 exhibited no significant PifHDAC3 occupancy at this locus, both in ChIP-qPCR and ChIP-seq analyses, suggesting a lack of transcriptional repression at Avr3a, possibly due to genotype-specific regulatory differences or promoter rearrangement. Following GSNO treatment, a marked decrease in PifHDAC3 binding was detected at the −1571 bp site in the Avr *P. infestans*, with a slight shift in enrichment to −1740 bp. This redistribution of PifHDAC3 correlates with the global decrease in PifHDAC3 chromatin occupancy observed in ChIP-seq analysis under nitrosative stress.

These findings support the hypothesis that NO promotes the release of PifHDAC3 from the Avr3a promoter, potentially facilitating gene activation and contributing to increased pathogen virulence. Upon cPTIO treatment, PifHDAC3 enrichment at the −1571 bp site was enhanced in both Avr and vr genotypes. In the Avr MP946, this suggests reinforcement of transcriptional repression through NO depletion. Interestingly, the vr MP977, which lacked detectable PifHDAC3 occupancy at this locus under control and GSNO-treated conditions, showed clear recruitment of PifHDAC3 upon PTIO application. This newly observed binding corresponds to genome-wide ChIP-seq data showing increased PifHDAC3 association with gene regulatory regions following NO scavenging in the virulent genotype. Together, these results confirm that NO dynamically regulates PifHDAC3 binding to the Avr3a promoter and is tightly correlated with broader chromatin remodeling patterns observed in the ChIP-seq analysis. The data highlight that NO-driven displacement of PifHDAC3 from key effector loci in the Avr genotype may enable transcriptional activation of virulence genes. In contrast, the vr genotype displays a more flexible PifHDAC3 recruitment pattern, reflecting a potentially adaptive epigenetic response to host-derived nitrosative signals. The observed inverse relationship between NO levels and PifHDAC3 occupancy reflects NO’s role as an epigenetic

modulator of *P. infestans* offensive strategy. This data is combined with results of Armstrong *et al.* [52], who identified Avr3a allelic variation (e.g., AVR3aKI vs. AVR3aEM) as a determinant of virulence, with virulent strains possibly bypassing R3a-mediated immunity. However, the absence of NO, induced by cPTIO treatment, led to *de novo* PifHDAC3 binding at the promoter in vr MP977, indicating that NO depletion can reintroduce repression, adding complexity to epigenetic control. Additionally, Guan *et al.* [22] identified NO-dependent acetylation of H3K56 at genes related to pathogenicity, such as *Avr3a*, further supporting this dynamic interplay.

PifHDAC3 driven by NO contributes to the virulence of *Phytophthora infestans*

The dynamic interplay between reactive nitrogen species and histone deacetylases is vital in influencing the expression of diverse pathogen genes, opening exciting avenues for research. This research highlights NO as a key epigenetic signal in the (patho)biology of *P. infestans* (Fig 9). Specifically, in the context of the Avr MP946 isolate, NO was found to derepress virulence genes, thereby shifting the balance toward pathogenicity. In contrast, the vr MP977 isolate of *P. infestans* shows adaptive chromatin remodeling, which facilitates sustaining infection during an NO burst generated by the host. This ability to adapt, driven by the redistribution of PifHDAC3 binding, likely enhances the pathogen's offensive strategy, especially when compared to the response of an avirulent pathogen directed to a specific host genotype. Vetukuri *et al*. [49] suggested that epigenetic adaptations enable *P. infestans* to evade host defenses. This mechanism becomes more pronounced in virulent genotypes under nitrosative stress, as noted by Gajewska *et al.* [50]. The adaptability of *P. infestans* highlights its historical impact, as seen in the Irish Potato Famine, and continues to pose a significant

threat to global potato production today [53]. The study proposes that the ability of virulent genotypes to remodel chromatin in response to nitrosative stress provides them a significant evolutionary advantage. Earlier, Haas *et al*. and Kronmiller *et al.* [46,54] also highlighted this genomic plasticity of the pathogen. Therefore, targeting HDAC3, driven by nitric oxide, could disrupt these virulence patterns, offering potential strategies for developing crops resistant to pathogens.

Materials and methods

Pathogen culture and growth conditions

Phytophthora infestans (Mont.) de Bary – the avirulent (Avr) isolate MP946 (race 1.3.4.7.10.11) and the virulent (vr) MP977 (race 1.2.3.4.6.7.10) in reference to the potato cv. Sarpo Mira was kindly obtained from the Plant Breeding and Acclimatization Institute (IHAR), Research Division in Młochów, Poland. For *in vitro* studies, the pathogen was grown on a pea agar medium up to 3 weeks. Then it was treated with reactive nitrogen species (RNS) donors or scavengers as described in the section *RNS donors and scavengers treatment***.**

For *in planta* analyses, the potato tubers were inoculated by spraying with 3 ml of a freshly prepared suspension of sporangia and zoospores (5.0×10^5 sporangia per ml) and incubated in sterile boxes for 9 days at 16°C and 95% relative humidity in the darkness. For analyses during disease development, potato leaves were inoculated by spraying leaves with a zoospore suspension and kept overnight at 18°C and ~90% in sterile boxes. Control leaves were sprayed with sterile water. Samples were collected at 0, 2, 24, 48, 72, 96, and 120 h after inoculation (hpi).

Plant material

Potato plant *Solanum tuberosum* L. cultivar Sarpo Mira (carrying the R genes: *R3a, R3b, R4, Rpi-Smira1,* and *Rpi-Smira2*), was kindly obtained from the Potato Gene bank (Plant Breeding and Acclimatization Institute IHAR-PIB, Bonin, Poland). Plants from *in vitro* culture were transferred to the sterile soil, and the growth was performed in a phytochamber with 16 h of light (180 μmol m^{-2} s^{-1}) at 18 ± 2°C and 60% humidity.

RNS donors and scavengers treatment

Nitrosative stress conditions were selected based on our previous findings as described by Gajewska *et al.* [50]. *In vitro* pathogen culture was treated by spraying with reactive nitrogen species (RNS) donors, *i.e.,* S-nitrosoglutathione (GSNO; Sigma-Aldrich, Germany) at a concentration of 400 μM and 3-morpholinosydnonimine (SIN-1; Calbiochem, Germany) at a concentration of 5 mM. Moreover, RNS scavengers, *i.e.,* 500 μM 2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl 3-oxide (PTIO; Sigma-Aldrich, Germany) and 200 μM ebselen (Cayman Chemicals, USA), were used. Control cultures were treated with sterile water. Hyphae after treatment were collected at 0, 2, 24, 48, and 72 h after treatment and used directly or frozen in liquid nitrogen and stored at -80°C for further analysis.

RNA extraction and gene expression analysis

For RNA isolation, 150 mg of frozen hyphae were ground to a fine powder, and total RNA was extracted using TRIzol Reagent (Thermo Fisher Scientific, USA) as per the manufacturer's instructions. The total RNA obtained was purified using DNase I, RNase-free endonuclease (Thermo Fisher Scientific, USA). For reverse transcription 1 μg of RNA was processed with the Reverse Transcription Kit (Thermo Fisher Scientific, USA) according to the

manufacturer's instructions. The real-time PCR reactions were performed on a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, USA). The reaction mixture contained 0.1 µM of each primer (S1 Table), 1 µL of 5× diluted cDNA, 5 µL of Power SYBR Green PCR Master mix (Applied Biosystems, USA), and DEPC-treated water to a total volume of 10 µL. The PCR reaction consisted of denaturation at 95°C for 10s, primer annealing at 56 °C for 20s, and primer extension at 72°C for 30s. For the entire qRT-PCR reaction, 55 cycles were performed. The reaction specificity and CT values for individual samples were determined using the real-time PCR Miner Program [55]. The relative gene expression was calculated using the Pfaffl mathematical model [56].

Cloning of *PiHDAC3* and generation of a custom polyclonal antibody

The coding region of Histone deacetylase 3 (HDAC3) was amplified from the cDNA of both isolates with specific primers (S1 Table) designed based on the reference sequence PITG_04499.1 (Genbank accession number: XM_002905236.1). The PCR product was purified using the QIAEXII Gel Extraction Kit (Qiagen, Netherlands) and ligated into the pGEM-T Easy vector (Promega, USA). The *Escherichia coli* strain XL1 Blue was transformed with the ligation mixture. The selected clones (X-Gal and IPTG) carrying an appropriate PCR product were sequenced (Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznań). The obtained sequences were processed with the BioEdit software (ver 7.2.5). According to the consensus sequence obtained for both strains, a homologous region encoding peptide, (H_2N)- RDREDDDQHMMDVSGE-($CONH_2$), was selected. The peptide was used further as the antigen to produce the specific antibody in the rabbit host (Davids Biotechnologie, Germany).

Recombinant expression of PifHDAC3 in *Escherichia coli*

To construct *pPifHDAC3_ET302/NT-His* recombinant protein lines, the full length of the *PifHDAC3* CDS sequence was removed including the stop codon, and amplified by using specific gene primers (S1 Table). The PCR product was purified and cloned into pET302/NT-His by using ClonExpress II One Step Cloning Kit (Vazyme, China). After construction of the *pPifHDAC3_ET302/NT-His* vector, the vector was transformed into *E. coli* (DH5a), and the plasmid DNA was extracted using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, USA). The construct was validated by sequencing to confirm the accuracy of the inserted sequence. The validated *pPifHDAC3_ET302/NT-His* vector was subsequently transformed into *E. coli* (BL21) and IPTG (A&A Biotechnology, Poland) with a final concentration of 0.5mM was used to induce the protein expression. After induction, the cells were harvested, lysed, and purified by HisPur Ni-NTA Magnetic Beads (Thermo Fisher Scientific, USA) for further enzyme activity analysis.

Total protein isolation and protein concentration determination

For total protein extraction, *P. infestans* hyphae (250 mg) was homogenized with 500 µl extraction buffer (100 mM Tris–HCl pH=7.5, 300 mM sucrose, 2 mM EDTA, 1 mM PMSF, and 1% protease inhibitor cocktail). After centrifugation at 10 000 g for 15 min at 4 °C the supernatant was collected and stored at –80°C until use. The protein concentration in all protein-related experiments was determined by the Bradford method with bovine serum albumin (BSA) as the standard protein [57]. For all western blot experiments, the proper amount (µg) of proteins (described detailed in the section *Western blot analysis*) were mixed with sample buffer (62.5 mM Tris–HCl, pH=8.5, 10% sucrose, 2% SDS, 0.025% bromophenol blue, 0.1 M dithioerythritol) and then incubated at 95°C for 3 min.

Histone-enriched protein isolation for western blot

Histone-enriched proteins were isolated from *S. tuberosum* 'Sarpo Mira' leaves by the method proposed by Moehs *et al.* [58] with some modifications described previously by Drozda *et al.* [28].

HDACs Activity Quantification

The HDACs activity was measured using the Epigenase™ HDAC Activity/Inhibition Direct Assay Kit (Epigentek Cat. No. P-4034) following the manufacturer's instructions. Briefly, for general HDAC activity, 250 ng of recombinant protein was used. The final HDAC activity was expressed as OD/min/mg protein.

Biotin switch assay

For the biotin switch assay, 0.2 g of frozen *P. infestans* mycelium was homogenized in 400 µl of HENT buffer (100 mM HEPES-NaOH pH=7.4, 10 mM EDTA, 0.1 mM Neocuprine, 1% Triton X-100, EDTA-free Protease inhibitor) and incubated on ice for 15 min. After centrifugation at 12,000 × g for 10 min at 4°C, the supernatant was collected, and protein concentration was determined using the Bradford method [57]. Then, samples were adjusted to 2µg/µl using HENT buffer to a final volume of 150 µl. The following steps of the procedure were performed in a dark room illuminated with red lights to protect light-sensitive nitrosothiols from degradation. Then, 450 µl of NEHS buffer (225 mM HEPES-NaOH pH=7.4, 0.9 mM EDTA, 0.1 mM Neocuprine, 2.5% SDS) containing 60 mM NEM was added, and samples were incubated at 37°C for 30 min. Moreover, an additional control sample was incubated with HENS buffer without NEM (negative control sample) and with HENS buffer with NEM together with 100 µM GSNO (positive control sample). Next, samples were mixed with 1.4 ml

of cooled acetone and incubated at -20°C for 30 min. Proteins were pelleted by centrifugation at 12,000 × g for 10 min at 4°C, supernatant was removed, and then the pellet was dried for 10 min on ice. Then 40 µl of NEHS buffer without NEM, 10 µl of 150 mM sinapic acid, and 16.6 µl of 4 mM Biotin-HPDP was added to each sample. Additionally, a negative control (without biotin) for all samples was prepared. After incubation for 1 h at 25°C with gentle agitation, 200 µl of cooled acetone was added, followed by sample incubation for 30 min at -20°C. Then, protein was pelleted by centrifugation at 12,000 × g for 10 min at 4°C and depending on further analyses resuspended in 40 µl of non-denaturing sample buffer (samples for western blot with anti-biotin antibody) or 40 µl of HENS buffer (samples for purification with neutravidin agarose beads).

Biotinylated protein purification

Probes obtained during the biotin switch procedure, resuspended in 40 µl of HENS buffer, were mixed with 80 µl of neutralizing buffer (20 mM HEPES-NaOH pH=7.7, 0.9 mM EDTA, 100 mM NaCl, 0.5% Triton X-100) and 20 µl of 50% Neutravidin Agarose beads (Thermo Fisher Scientific, USA) prepared in neutralizing buffer. Then the samples were incubated for 1h at RT with gentle rotation. After incubation, biotinylated proteins were eluted from the beads with 40 µl of elution buffer (20 mM HEPES-NaOH pH=7.7, 0.9 mM EDTA, 100 mM NaCl, 0.5% Triton X-100, 100 mM β-me), and 10 µl of eluates were collected.

Protein immunoprecipitation

Protein immunoprecipitation was performed according to the protocol described by Zhao *et al.* [59] with some modifications. Mycelium of *P. infestans* (0.8 g) was homogenized in 800 µl of binding buffer (50 mM Tris-HCl pH=8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40, 1% protease inhibitor cocktail. The samples were then sonicated on ice for 3 × 10 s at 40%

of power. Then samples were centrifuged at 16000 × g for 10 min, and the supernatants were collected, and protein concentration was measured by the Bradford method (Bradford, 1976). Protein samples (2 mg) were incubated overnight at 4°C with gentle rotation with an anti-nitrotyrosine polyclonal antibody (Thermo Fisher Scientific, USA) at conc. 4 µg/1 mg of protein in a total volume of 450 µl. Simultaneously, protein samples without the tested antibody were incubated as a negative control. The next day, 500 µl of 50% protein G beads (Merck Group, Darmstadt, Germany) in PBS buffer were added to each sample, and samples were incubated overnight at 4°C with gentle rotation. After incubation, supernatants were removed by washing the protein G as follows: 1x with 1.25 ml of binding buffer, 2x with 1.25 ml of washing buffer no. 1 (50 mM Tris HCl pH=8.0, 100 mM NaCl, 1 mM EDTA), 1x with 1.25 ml of washing buffer no. 2 (50 mM Tris pH= 8.0, 100 mM NaCl, 1 mM EDTA, 10% acetonitrile [ACN]) and 1x with 1.25 ml of sterile water. Nitrotyrosine-containing proteins were eluted from the beads with 1 ml of low-pH acetonitrile solution (0.5% TFA, 25% ACN) and collected in 10 fractions. Based on SDS-PAGE analysis, the most protein-abundant fractions were selected and combined. The protein concentration was calculated based on SDS-PAGE analysis by summing the intensity of the pixels within each protein band image with BSA as the standard protein.

Western blot analysis

Standard SDS-PAGE separated equal amounts of all protein samples in 15% polyacrylamide gels and electrotransferred them onto a PVDF membrane. In the case of analyses of PifHDAC3 accumulation after RNS donors treatment and during disease development, 4 µg of histone-enriched proteins were used. For immunoprecipitated protein, 0.2 µg of nitrotyrosine-containing proteins were used. Immunostaining was performed overnight at 4ºC with antibody against HDAC3 diluted at 1:2000 (4.4 µg/ml) in 1% BSA/TBS-T.

Antigen–antibody complexes were detected using a secondary anti-rabbit IgG–horseradish peroxidase conjugate (Sigma-Aldrich, Germany) diluted at 1:20,000 in TBS-T and incubated for 2h at RT. To visualize the results, Chemiluminescent substrate Clarity Western ECL Substrate (BioRad, USA) was used.

In the case of Western blot after the biotin switch procedure, 10 µg of biotinylated protein was used. Immunostaining was performed overnight at 4ºC with anti-biotin mouse monoclonal antibody conjugated with alkaline phosphatase (Sigma-Aldrich, Germany) diluted at 1:5000 in TBS-T. The next day a signal was detected using Fast NBT/BCIP developing solution (Sigma-Aldrich, Germany). The reaction was carried out until the bands were visible and stopped by rinsing the membrane with water several times.

All western blot results were quantified using Image Lab™ software (Bio-Rad, USA).

Chromatin immunoprecipitation

For chromatin immunoprecipitation assay (ChIP) 2 g of *P. infestans* hyphae were crosslinked by vacuum infiltration in a crosslinking buffer (1% formaldehyde, 400 mM sucrose, 10 mM P/K buffer pH=7.4, 2 mM EDTA). Then samples were ground in liquid nitrogen and resuspended in nuclei isolation buffer (250 mM sucrose, 10 mM Tris-HCl pH=7.5, 5 mM $MgCl_2$, 60 mM KCl, 15 mM NaCl, 40% glycerol, 1% Triton X-100, 5 mM EDTA, 1 mM PMSF, 5 mM β-me) followed by 30 min incubation on ice. Then samples were filtered through Miracloth and centrifuged at 3,220 × g for 20 min at 4°C. After centrifugation, the pellet was resuspended in 350 µl of nuclei lysis buffer (10 mM Tris-HCl pH=7.5, 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.8% SDS, 1 mM EDTA, 1 mM PMSF, 5 mM β-me). Subsequently, probes were sonicated at 4°C for 13 cycles (30s on / 30s off) until DNA fragments of 300–700 nt were obtained. After sonication, each sample was treated with RNaseA (10 mg/ml, Roche) for 15 min on ice, followed by centrifugation at 13 800 × g for 10 min at

4°C. Then, an input sample (20 µl) was collected to check the quality of the sample on an agarose gel. The remaining solution was separated into the test sample to which the antibody of interest was added: H3 (Abcam; cat no. ab1791), or anti-HDAC3. As a control IgG (CellSignaling; cat. no. 2729) was added. Chromatin with antibodies was incubated overnight at 4°C with mixing. After overnight incubation, 110 µl of protein A agarose beads (Merck) were added, followed by incubation for at least 2h at 4°C with mixing. Then, the samples were washed (150 mM NaCl, 10 mM Tris-HCl pH-7.5, 5 mM EDTA) and decrosslinked overnight with high-salt elution buffer (300 mM NaCl, 10 mM Tris-HCl pH=7.5, 1% SDS) at 65°C with shaking. The following day, probes were incubated for 1h at 55°C with proteinase K (20 mg/ml) to digest proteins. Then, DNA isolation was prepared with a DNA purification kit (Cell Signaling Simple ChIP® DNA Purification Buffers and Spin Columns) according to the manufacturer's instructions.

Chip-seq library preparation, sequencing, and data analysis

Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) was performed to investigate protein-DNA interactions in *Phytophthora infestans*. Immunoprecipitated DNA was purified and used for library preparation with the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA), following the manufacturer's protocol optimized for low-input DNA. Briefly, 5–50 ng of ChIP DNA was subjected to end repair and A-tailing in a single enzymatic reaction to produce blunt-ended fragments with a 3' adenine overhang. Subsequently, NEBNext adapters containing a complementary thymine overhang were ligated to the DNA fragments. Adapter-ligated DNA was purified using AMPure XP beads (Beckman Coulter) with a dual size-selection strategy to enrich for fragments in the 200–500 bp size range. Libraries were then amplified by limited-cycle PCR (~8 cycles), using NEBNext Ultra II Q5 Master Mix and unique dual index primers.

Final libraries were quantified using a Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific, USA) and assessed for fragment size distribution and quality using the Agilent 2100 Bioanalyzer with the High Sensitivity DNA Kit. High-throughput sequencing of the libraries was performed on an Illumina HiSeq platform, generating paired-end reads of 200 bp in length. Sequencing was carried out at the Genome Core Facility Centre of New Technologies, University of Warsaw.

Raw sequencing reads were subjected to quality control using FastQC [60], and low-quality reads or adapter contamination were filtered and trimmed when necessary. High-quality reads were aligned to the *Phytophthora infestans* reference genome (Ensembl database release; FASTA format, Genome assembly: ASM14294v1, [46,61]) using Bowtie2 (Galaxy Version 2.3.4.1; [62]), employing default parameters optimized for sensitive local alignment of paired-end reads. Aligned reads were processed with SAMtools to generate sorted BAM files, which were subsequently used for peak detection.

Peak calling was performed using MACS2 (Model-based Analysis of ChIP-Seq; Galaxy Version 2.1.1.20160309.6) with default parameters. Input DNA samples were used as controls to normalize background signals and enhance the specificity of enriched region detection. Peaks were called using a q-value threshold of 0.05 to ensure statistical significance. The effective genome size was adjusted based on *P. infestans* genome parameters. Identified peaks were then annotated relative to genomic features (e.g., promoters, gene bodies) using SeqMonk (Version 1.46.0), incorporating gene annotation data from the Ensembl GFF3 files for *P. infestans*. For functional enrichment analysis, genes associated with significant ChIP-seq peaks were extracted based on proximity to peak summits and gene promoters or coding regions. These gene lists were subjected to KEGG pathway enrichment analysis using the Gene Ontology and KEGG Pathway Analysis tool (https://bioinformatics.sdstate.edu/go/). The study was performed with default settings, which apply a hypergeometric test to assess enrichment and

correct for multiple testing using the false discovery rate (FDR). Pathway enrichment results were used to identify biological processes and molecular pathways potentially regulated by the protein-DNA interactions captured in the ChIP-seq experiments.

3D modelling of PifHDAC3 and *in silico* analyses of potential S-nitrosation sites

The three-dimensional structure of PifHDAC3 was generated by Phyre2 (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index), visualized by Pymol (https://pymol.org/). To predict the secondary structure of PifHDAC3 protein, SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) was used with default parameters. Furthermore, the 3D structure of PifHDAC3 was inspected by the SAVES server (https://saves.mbi.ucla.edu/). The potential S-nitrosation sites were determined by IBS (http://ibs.biocuckoo.org/index.php) with default parameters.

Statistical Analysis

All results are based on three biological replicates derived from three independent experiments. For each experiment, the means of the obtained values (n = 9) were calculated along with standard deviations. To estimate the statistical significance between means, the data were analyzed with using one-way analysis of variance (ANOVA) followed by Dunnett's test at the level of significance $\alpha = 0.05$.

Supporting information

S1 Fig. Gene expression of *PifHDAC2* and *PifHDAC7*. Gene expression of *PifHDAC2* (A, B), *PifHDAC7* (C, D) in avirulent (Avr) MP946 and virulent (vr) MP977 *Phytophthora infestans* growing *in vitro*. The RT-qPCR gene expression was analyzed at selected time points (0–72 h) after the culture's treatment with water (control), S-nitrosoglutathione (GSNO), 3-morpholinosydnonimine (SIN-1), 2-(4-Carboxyphenyl)-4,4,5,5,-tetramethylimidazoline-1-oxyl 3-oxide (cPTIO), or ebselen, respectively. As a reference S3a was used. The results are averages from three independent experiments (n = 9) ± SD. Asterisks indicate values that differ significantly from the water treated samples (control) *P. infestans* culture at each time point at $p < 0.05$ (*).

S2 Fig. Phylogenetic analysis and conserved domain distribution of PifHDACs with selected human orthologs. The tree was constructed using the neighbor-joining method with 1000 bootstrap replications in MEGA 7. The conserved domain of PifHDACs and selected human orthologs was identified using NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). TBtools was used to visualize the obtained results.

S3 Fig. *In-silico* analyses of PifHDAC3 as a potential target for S-nitrosation. The three-dimensional structure of the protein encoded by PifHDAC3 was generated by Phyre2 (https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The protein structure was visualized by cyan color from N to C terminus. Coils and smooths represent alpha helices and beta sheets, respectively. Potential S-nitrosation sites, predicted using GPS-SNO [63], are highlighted at cysteine residues C168 and C273, marked in red.

S4 Fig. Immunoprecipitation of biotinylated proteins coupled with western blot analysis using anti-PifHDAC3 antibody. (A); Detection of S-nitrosated proteins in *Phytophthora infestans* using biotin

switch method. Lanes: Control, GSNO, SIN-1, cPTIO, ebselen (ebs), and negative control (without biotin). (B); Coomassie blue staining of SDS-PAGE gel. Lanes: Biotin-labeled SNOs, control (GSNO), SIN-1, cPTIO, ebselen (ebs), and negative control (without biotin). The band at approximately 38 kDa is marked as PifHDAC3. (C); Western blot analysis using anti-HDAC3 antibody confirming the absence of PifHDAC3 in biotin-labeled SNOs. Lanes: Biotin-labeled SNOs, control, GSNO, SIN-1, cPTIO, ebs, and negative control (without biotin). Bands at approximately 38 kDa indicate S-nitrosated PifHDAC3.

S5 Fig. Procheck Ramachandran plot analysis of PifHDAC3 3D modeling.

S1 Table. List of primers used in the study.

S2 Table. Prediction of secondary structure of PifHDAC3 protein.

Acknowledgments

This work was supported by the grant of National Science Centre – project no. NCN 2018/31/B/NZ9/00355. We thank Przemysław Jagodzik, Ph.D., for participation in the western-blot analysis which results are presented in Figure 2.

Author contributions

Conceptualization: MAJ, JFW

Data curation: MAJ

Formal analysis: YG, SK, JG, DP

Funding acquisition: MAJ

Investigation: YG, SK, JG

Methodology: MAJ, JFW, ESN, SK, AK

Project administration: MAJ

Resources: MAJ

Supervision: MAJ, JFW, ESN, AK

Writing – original draft: MAJ, JFW, SK, YG, JG

Writing – review & editing: MAJ, JFW, SK, ESN, AK

Literature

1. Ludwiczewska M, Janiszewska M, Yin Z, Śliwka J. Populations of Phytophthora infestans in northern and eastern Europe. Eur J Plant Pathol. 2025;171: 81–95. doi:10.1007/s10658-024-02933-x

2. Fry WE, Birch PRJ, Judelson HS, Grünwald NJ, Danies G, Everts KL, et al. Five Reasons to Consider Phytophthora infestans a Reemerging Pathogen. Phytopathology®. 2015;105: 966–981. doi:10.1094/phyto-01-15-0005-fi

3. Wu E, Wang Y, Yahuza L, He M, Sun D, Huang Y, et al. Rapid adaptation of the Irish potato famine pathogen Phytophthora infestans to changing temperature. Evolutionary Applications. 2019;13: 768–780. doi:10.1111/eva.12899

4. Babarinde S, Burlakoti RR, Peters RD, Al-Mughrabi K, Novinscak A, Sapkota S, et al. Genetic structure and population diversity of Phytophthora infestans strains in Pacific

western Canada. Appl Microbiol Biotechnol. 2024;108: 237. doi:10.1007/s00253-024-13040-6

5. Leesutthiphonchai W, Vu AL, Ah-Fong AMV, Judelson HS. How Does Phytophthora infestans Evade Control Efforts? Modern Insight Into the Late Blight Disease. Phytopathology®. 2018;108: 916–924. doi:10.1094/PHYTO-04-18-0130-IA

6. Kasuga T, Gijzen M. Epigenetics and the evolution of virulence. Trends in Microbiology. 2013;21: 575–582. doi:10.1016/j.tim.2013.09.003

7. Weiner AKM, Katz LA. Epigenetics as Driver of Adaptation and Diversification in Microbial Eukaryotes. Front Genet. 2021;12. doi:10.3389/fgene.2021.642220

8. van West P, Shepherd SJ, Walker CA, Li S, Appiah AA, Grenville-Briggs LJ, et al. Internuclear gene silencing in Phytophthora infestans is established through chromatin remodelling. Microbiology. 2008;154: 1482–1490. doi:10.1099/mic.0.2007/015545-0

9. Chen H, Shu H, Wang L, Zhang F, Li X, Ochola SO, et al. Phytophthora methylomes are modulated by 6mA methyltransferases and associated with adaptive genome regions. Genome Biology. 2018;19. doi:10.1186/s13059-018-1564-4

10. Wang X-W, Guo L-Y, Han M, Shan K. Diversity, evolution and expression profiles of histone acetyltransferases and deacetylases in oomycetes. BMC Genomics. 2016;17: 927. doi:10.1186/s12864-016-3285-y

11. Narita T, Weinert BT, Choudhary C. Functions and mechanisms of non-histone protein acetylation. Nature Reviews Molecular Cell Biology. 2018;20: 156–174. doi:10.1038/s41580-018-0081-3

12. Nitsch S, Zorro Shahidian L, Schneider R. Histone acylations and chromatin dynamics: concepts, challenges, and links to metabolism. EMBO reports. 2021;22. doi:10.15252/embr.202152774

13. Chen C, Liu J. Histone acetylation modifications: A potential targets for the diagnosis and treatment of papillary thyroid cancer. Front Oncol. 2022;12. doi:10.3389/fonc.2022.1053618

14. Guan Y, Gajewska J, Floryszak-Wieczorek J, Tanwar UK, Sobieszczuk-Nowicka E, Arasimowicz-Jelonek M. Histone (de)acetylation in epigenetic regulation of Phytophthora pathobiology. Molecular Plant Pathology. 2024;25: e13497. doi:10.1111/mpp.13497

15. Elías-Villalobos A, Fernández-Álvarez A, Moreno-Sánchez I, Helmlinger D, Ibeas JI. The Hos2 Histone Deacetylase Controls Ustilago maydis Virulence through Direct Regulation of Mating-Type Genes. PLOS Pathogens. 2015;11: e1005134. doi:10.1371/journal.ppat.1005134

16. Lee J, Lee J-J, Jeon J. A histone deacetylase, MoHOS2 regulates asexual development and virulence in the rice blast fungus. Journal of Microbiology. 2019;57: 1115–1125. doi:10.1007/s12275-019-9363-5

17. Lin C, Cao X, Qu Z, Zhang S, Naqvi NI, Deng YZ. The Histone Deacetylases MoRpd3 and MoHst4 Regulate Growth, Conidiation, and Pathogenicity in the Rice Blast Fungus Magnaporthe oryzae. mSphere. 2021;6: 10.1128/msphere.00118-21. doi:10.1128/msphere.00118-21

18. Izawa M, Takekawa O, Arie T, Teraoka T, Yoshida M, Kimura M, et al. Inhibition of histone deacetylase causes reduction of appressorium formation in the rice blast fungus

Magnaporthe oryzae. The Journal of General and Applied Microbiology. 2009;55: 489–498. doi:10.2323/jgam.55.489

19. Villota-Salazar NA, Ramos-García VH, González-Prieto JM, Hernández-Delgado S. Effects of chemical inhibition of histone deacetylase proteins in the growth and virulence of *Macrophomina phaseolina* (Tassi) Goid. Revista Argentina de Microbiología. 2023;55: 296–306. doi:10.1016/j.ram.2023.04.002

20. Zhao W, Wang T, Liu S, Chen Q, Qi R. The histone acetyltransferase PsGcn5 mediates oxidative stress responses and is required for full virulence of Phytophthora sojae. Microbial Pathogenesis. 2015;87: 51–58. doi:10.1016/j.micpath.2015.07.015

21. Wang X-W, Lv J-L, Shi Y-R, Guo L-Y. Comparative Transcriptome Analysis Revealed Genes Regulated by Histone Acetylation and Genes Related to Sex Hormone Biosynthesis in Phytophthora infestans. Frontiers in Genetics. 2020;11. doi:10.3389/fgene.2020.00508

22. Guan Y, Gajewska J, Sobieszczuk-Nowicka E, Floryszak-Wieczorek J, Hartman S, Arasimowicz-Jelonek M. The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle. Plant Physiology and Biochemistry. 2024;216: 109129. doi:10.1016/j.plaphy.2024.109129

23. Nott A, Watson PM, Robinson JD, Crepaldi L, Riccio A. S-nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. Nature. 2008;455: 411–415. doi:10.1038/nature07238

24. Watson PMD, Riccio A. Nitric oxide and histone deacetylases. Communicative & Integrative Biology. 2009;2: 11–13. doi:10.4161/cib.2.1.7301

25. Okuda K, Ito A, Uehara T. Regulation of Histone Deacetylase 6 Activity *via S*-Nitrosylation. Biological & Pharmaceutical Bulletin. 2015;38: 1434–1437. doi:10.1248/bpb.b15-00364

26. Mengel A, Ageeva A, Georgii E, Bernhardt J, Wu K, Durner J, et al. Nitric Oxide Modulates Histone Acetylation at Stress Genes by Inhibition of Histone Deacetylases. Plant Physiology. 2016;173: 1434–1452. doi:10.1104/pp.16.01734

27. Ageeva-Kieferle A, Georgii E, Winkler B, Ghirardo A, Albert A, Hüther P, et al. Nitric oxide coordinates growth, development, and stress response via histone modification and gene expression. Plant Physiology. 2021;187: 336–360. doi:10.1093/plphys/kiab222

28. Drozda A, Kurpisz B, Arasimowicz-Jelonek M, Kuźnicki D, Jagodzik P, Guan Y, et al. Nitric Oxide Implication in Potato Immunity to Phytophthora infestans via Modifications of Histone H3/H4 Methylation Patterns on Defense Genes. International Journal of Molecular Sciences. 2022;23: 4051. doi:10.3390/ijms23074051

29. Kaya C, Adamakis I-DS. Redox-Epigenetic Crosstalk in Plant Stress Responses: The Roles of Reactive Oxygen and Nitrogen Species in Modulating Chromatin Dynamics. International Journal of Molecular Sciences. 2025;26: 7167. doi:10.3390/ijms26157167

30. Dimauro I, Paronetto MP, Caporossi D. Exercise, redox homeostasis and the epigenetic landscape. Redox Biology. 2020;35: 101477. doi:10.1016/j.redox.2020.101477

31. Pérez-Torres I, Soto ME, Castrejón-Tellez V, Rubio-Ruiz ME, Manzano Pech L, Guarner-Lans V. Oxidative, Reductive, and Nitrosative Stress Effects on Epigenetics and on Posttranslational Modification of Enzymes in Cardiometabolic Diseases. Oxidative Medicine and Cellular Longevity. 2020;2020: 8819719. doi:10.1155/2020/8819719

32. Wu R, Li S, Hudlikar R, Wang L, Shannar A, Chou RPPJ, et al. Redox Signaling, mitochondrial metabolism, epigenetics and redox active phytochemicals Mini-review. Free Radic Biol Med. 2022;179: 328–336. doi:10.1016/j.freeradbiomed.2020.12.007

33. Auverlot J, Dard A, Sáez-Vásquez J, Reichheld J-P. Redox regulation of epigenetic and epitranscriptomic gene regulatory pathways in plants. J Exp Bot. 2024;75: 4459–4475. doi:10.1093/jxb/erae165

34. Li B, Ming H, Qin S, Nice EC, Dong J, Du Z, et al. Redox regulation: mechanisms, biology and therapeutic targets in diseases. Sig Transduct Target Ther. 2025;10: 72. doi:10.1038/s41392-024-02095-6

35. Izbiańska K, Floryszak-Wieczorek J, Gajewska J, Gzyl J, Jelonek T, Arasimowicz-Jelonek M. Switchable Nitroproteome States of Phytophthora infestans Biology and Pathobiology. Front Microbiol. 2019;10. doi:10.3389/fmicb.2019.01516

36. Gould N, Doulias P-T, Tenopoulou M, Raju K, Ischiropoulos H. Regulation of Protein Function and Signaling by Reversible Cysteine S-Nitrosylation *. Journal of Biological Chemistry. 2013;288: 26473–26479. doi:10.1074/jbc.R113.460261

37. Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. Biochemical and Biophysical Research Communications. 2004;315: 240–245. doi:10.1016/j.bbrc.2004.01.046

38. Dard A, Van Breusegem F, Mhamdi A. Redox regulation of gene expression: proteomics reveals multiple previously undescribed redox-sensitive cysteines in transcription complexes and chromatin modifiers. Journal of Experimental Botany. 2024;75: 4476–4493. doi:10.1093/jxb/erae177

39. Mengel A, Chaki M, Shekariesfahlan A, Lindermayr C. Effect of nitric oxide on gene transcription – S-nitrosylation of nuclear proteins. Front Plant Sci. 2013;4. doi:10.3389/fpls.2013.00293

40. Liu S, Wang X, Tang X, Fang W. Histone deacetylase HDAC3 regulates ergosterol production for oxidative stress tolerance in the entomopathogenic and endophytic fungus Metarhizium robertsii. mSystems. 2024;9: e00953-24. doi:10.1128/msystems.00953-24

41. Feng J, Jing F, Fang H, Gu L, Xu W. Expression, purification, and *S*-nitrosylation of recombinant histone deacetylase 8 in *Escherichia coli* . BioScience Trends. 2011;5: 17–22. doi:10.5582/bst.2011.v5.1.17

42. Colussi C, Mozzetta C, Gurtner A, Illi B, Rosati J, Straino S, et al. HDAC2 blockade by nitric oxide and histone deacetylase inhibitors reveals a common target in Duchenne muscular dystrophy treatment. Proc Natl Acad Sci USA. 2008;105: 19183–19187. doi:10.1073/pnas.0805514105

43. Illi B, Russo CD, Colussi C, Rosati J, Pallaoro M, Spallotta F, et al. Nitric Oxide Modulates Chromatin Folding in Human Endothelial Cells via Protein Phosphatase 2A Activation and Class II Histone Deacetylases Nuclear Shuttling. Circulation Research. 2008;102: 51–58. doi:10.1161/CIRCRESAHA.107.157305

44. Cuperlovic-Culf M, Culf AS. Role of Histone Deacetylases in Fungal Phytopathogenesis: A Review. International Journal of Modern Botany. 2014;4: 48–60.

45. Zhang N, Yang Z, Zhang Z, Liang W. BcRPD3-Mediated Histone Deacetylation Is Involved in Growth and Pathogenicity of Botrytis cinerea. Front Microbiol. 2020;11. doi:10.3389/fmicb.2020.01832

978 46. Haas BJ, Kamoun S, Zody MC, Jiang RHY, Handsaker RE, Cano LM, et al. Genome
979 sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. Nature.
980 2009;461: 393–398. doi:10.1038/nature08358

981 47. Hardham AR, Suzaki E. Glycoconjugates on the surface of spores of the pathogenic
982 fungus Phytophthora cinnamomi studied using fluorescence and electron microscopy and
983 flow cytometry. Can J Microbiol. 1990;36: 183–192. doi:10.1139/m90-032

984 48. Gerardy-Schahn R, Oelmann S, Bakker H. Nucleotide sugar transporters: Biological and
985 functional aspects. Biochimie. 2001;83: 775–782. doi:10.1016/S0300-9084(01)01322-0

986 49. Vetukuri RR, Avrova AO, Grenville-Briggs LJ, Van West P, Söderbom F, SAVENKOV
987 EI, et al. Evidence for involvement of Dicer-like, Argonaute and histone deacetylase
988 proteins in gene silencing in Phytophthora infestans. Molecular Plant Pathology. 2011;12:
989 772–785. doi:10.1111/j.1364-3703.2011.00710.x

990 50. Gajewska J, Floryszak-Wieczorek J, Kosmala A, Perlikowski D, Żywicki M,
991 Sobieszczuk-Nowicka E, et al. Insight into metabolic sensors of nitrosative stress
992 protection in Phytophthora infestans. Front Plant Sci. 2023;14: 1148222.
993 doi:10.3389/fpls.2023.1148222

994 51. Bos JIB, Armstrong MR, Gilroy EM, Boevink PC, Hein I, Taylor RM, et al. Phytophthora
995 infestans effector AVR3a is essential for virulence and manipulates plant immunity by
996 stabilizing host E3 ligase CMPG1. Proceedings of the National Academy of Sciences.
997 2010;107: 9909–9914. doi:10.1073/pnas.0914408107

998 52. Armstrong MR, Whisson SC, Pritchard L, Bos JIB, Venter E, Avrova AO, et al. An
999 ancestral oomycete locus contains late blight avirulence gene Avr3a, encoding a protein

1000 that is recognized in the host cytoplasm. Proceedings of the National Academy of
1001 Sciences. 2005;102: 7766–7771.

1002 53. Goodwin SB, Cohen BA, Fry WE. Panglobal distribution of a single clonal lineage of the
1003 Irish potato famine fungus. Proceedings of the National Academy of Sciences. 1994;91:
1004 11591–11595.

1005 54. Kronmiller BA, Feau N, Shen D, Tabima JF, Ali SS, Armitage AD, et al. Comparative
1006 Genomic Analysis of 31 Phytophthora Genomes Reveals Genome Plasticity and
1007 Horizontal Gene Transfer. MPMI. 2023;36: 26–46. doi:10.1094/MPMI-06-22-0133-R

1008 55. Zhao S, Fernald RD. Comprehensive Algorithm for Quantitative Real-Time Polymerase
1009 Chain Reaction. J Comput Biol. 2005;12: 1047–1064. doi:10.1089/cmb.2005.12.1047

1010 56. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR.
1011 Nucleic Acids Research. 2001;29: 45e–445. doi:10.1093/nar/29.9.e45

1012 57. Bradford MM. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities
1013 of Protein Utilizing the Principle of Protein-Dye Binding. Analytical biochemistry. 1976
1014 May 7;72(1-2):248-54. doi: 10.1016/0003-2697(76)90527-3

1015 58. Moehs CP, McElwain EF, Spiker S. Chromosomal proteins of Arabidopsis thaliana. Plant
1016 Molecular Biology. 1988;11: 507–515. doi:10.1007/bf00039031

1017 59. Zhao Y, Zhang Y, Sun H, Maroto R, Brasier AR. Selective Affinity Enrichment of
1018 Nitrotyrosine-Containing Peptides for Quantitative Analysis in Complex Samples. J
1019 Proteome Res. 2017;16: 2983–2992. doi:10.1021/acs.jproteome.7b00275

1020 60. Simon Andrews F, Segonds-Pichon A, Biggins L, Krueger C, Wingett S. FastQC: a
1021 quality control tool for high throughput sequence data. 2010.

61. Henriquez MA, Daayf F. Identification and Cloning of Differentially Expressed Genes Involved in the Interaction Between Potato and Phytophthora infestans using a Subtractive Hybridization and cDNA-AFLP Combinational Approach. Journal of Integrative Plant Biology. 2010;52: 453–467. doi:10.1111/j.1744-7909.2010.00943.x

62. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9: 357–359. doi:10.1038/nmeth.1923

63. Xue Y, Liu Z, Gao X, Jin C, Wen L, Yao X, et al. GPS-SNO: Computational Prediction of Protein S-Nitrosylation Sites with a Modified GPS Algorithm. PLOS ONE. 2010;5: e11290. doi:10.1371/journal.pone.0011290

Funding

This work was supported by the grant of the National Science Centre – project no. NCN 2018/31/B/NZ9/00355.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement:

All relevant data are within the manuscript and its Supporting Information files. The ChIP-Seq read data are available from the Sequence Read Archive database via accession number SRP055716 (http://www.ncbi.nlm.nih.gov/Traces/sra)

Figure 1

Click here to access/download;Figure;Fig 1.tif

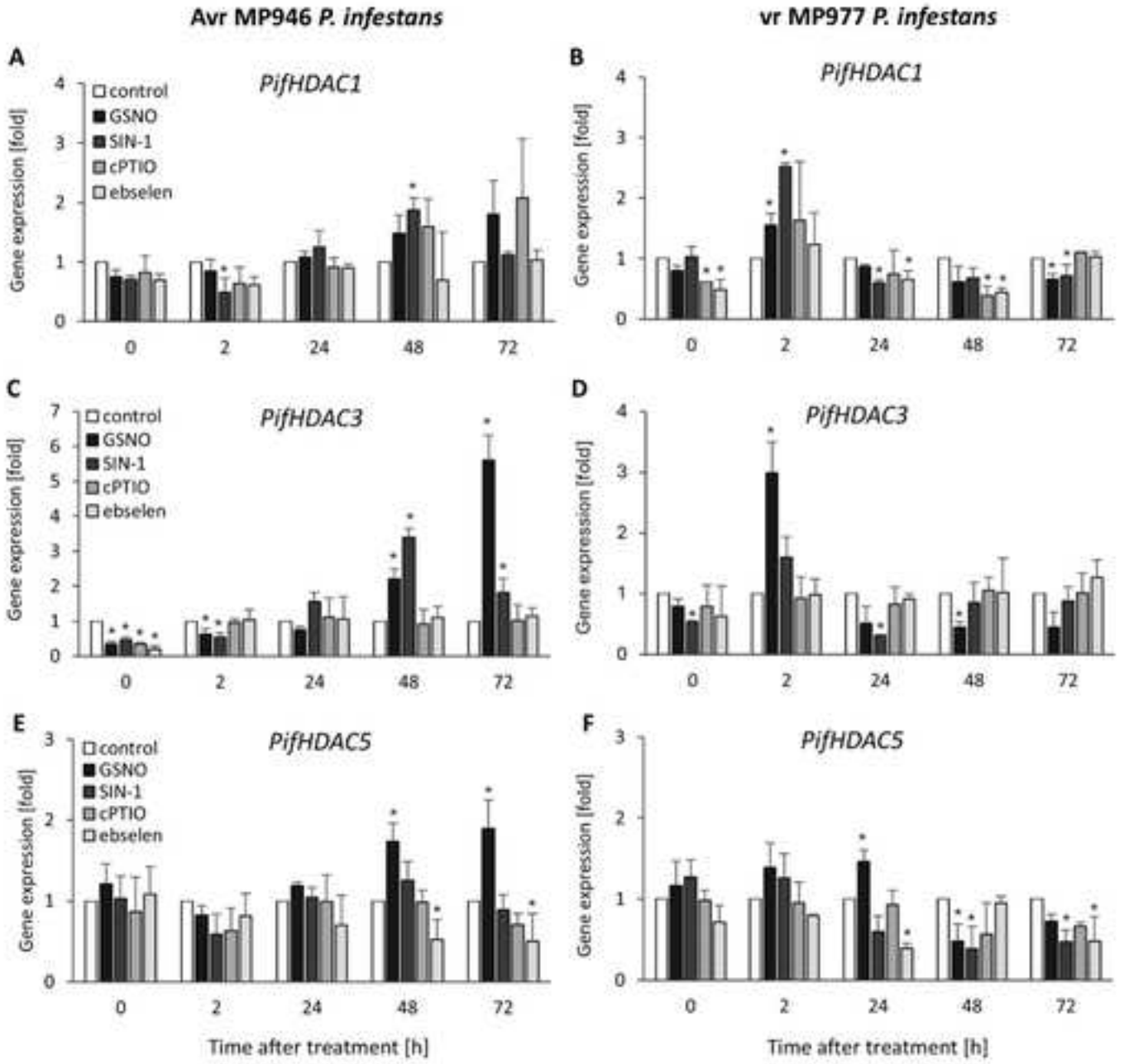


Figure 2

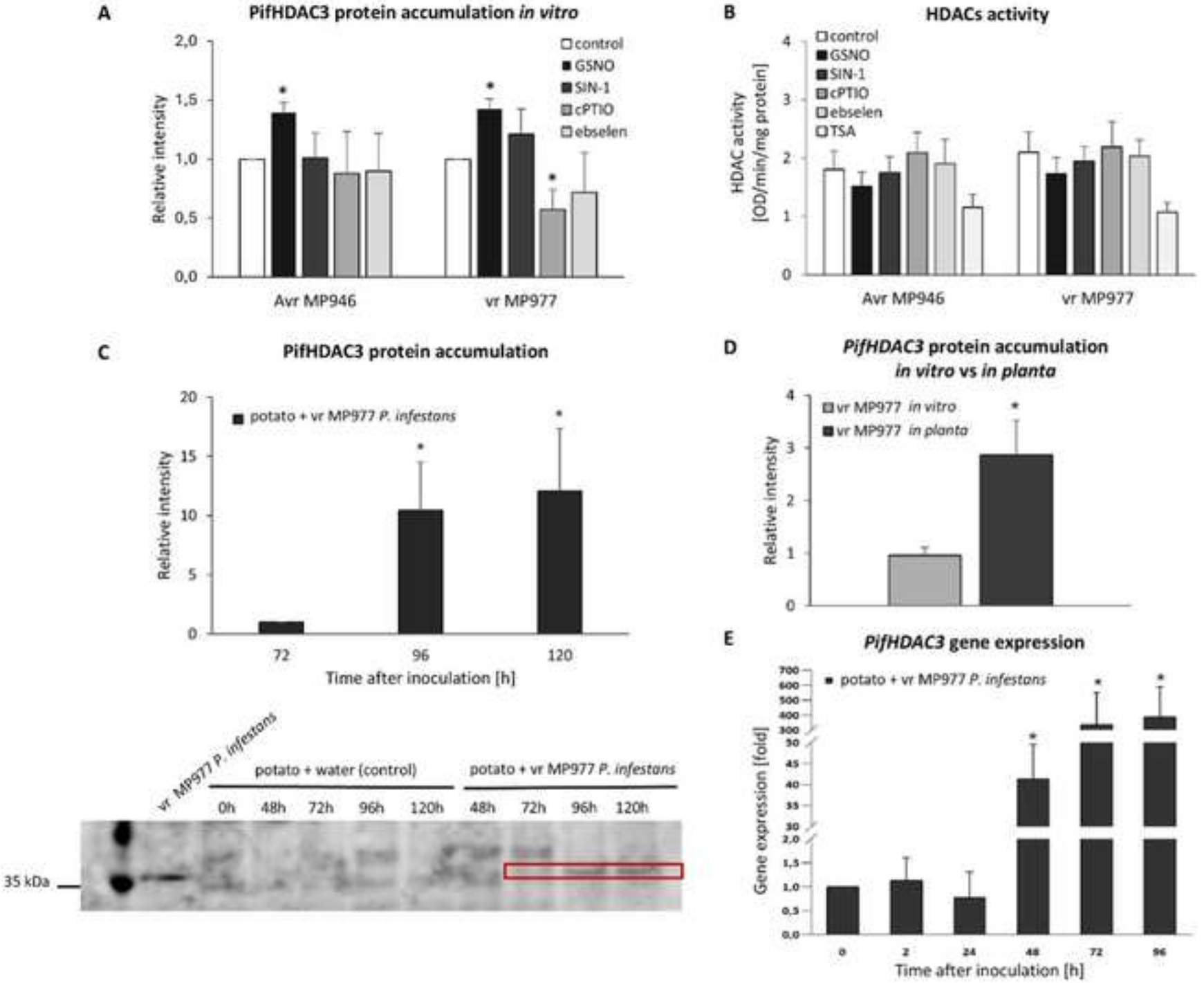


Figure 3

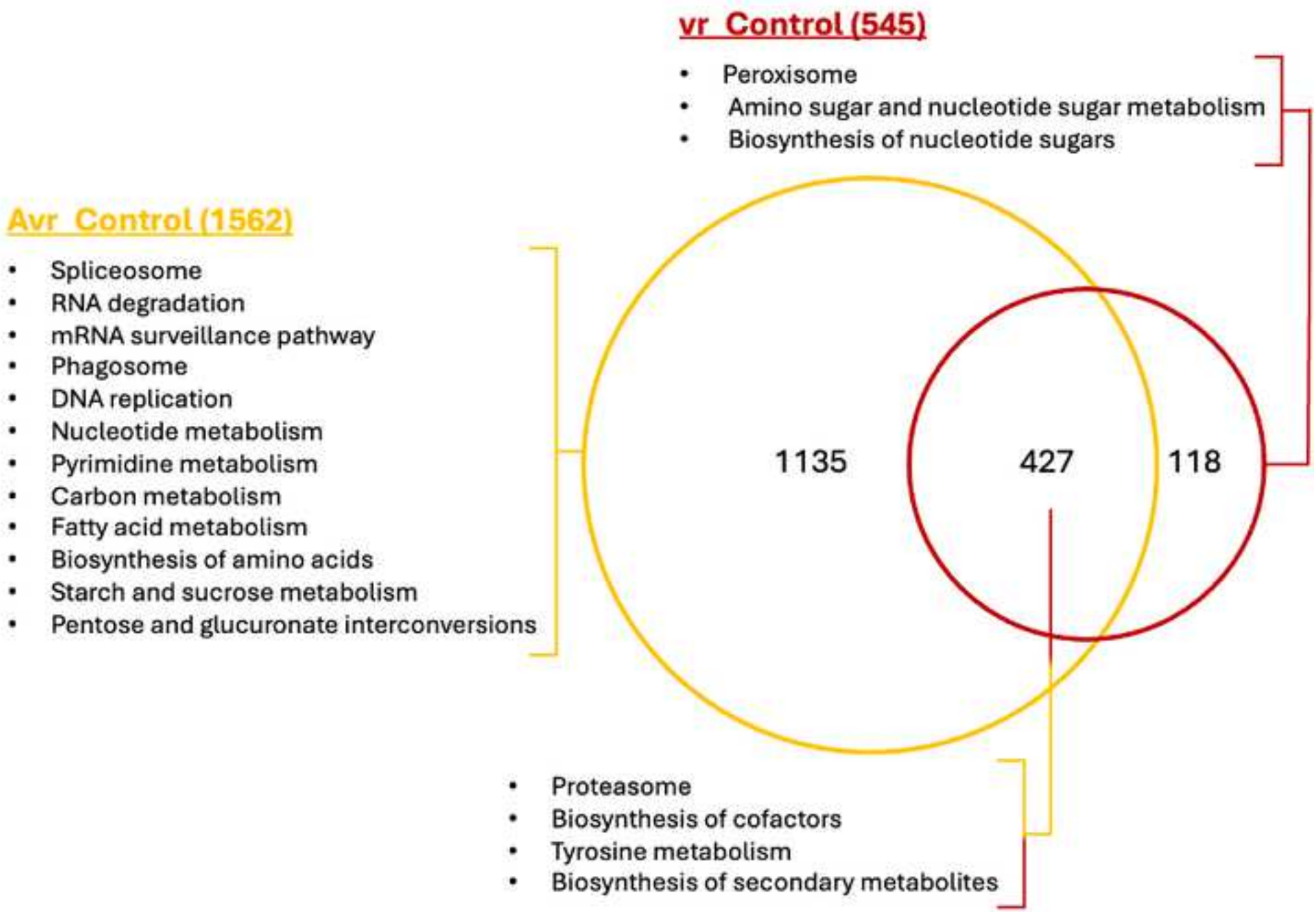


Figure 4

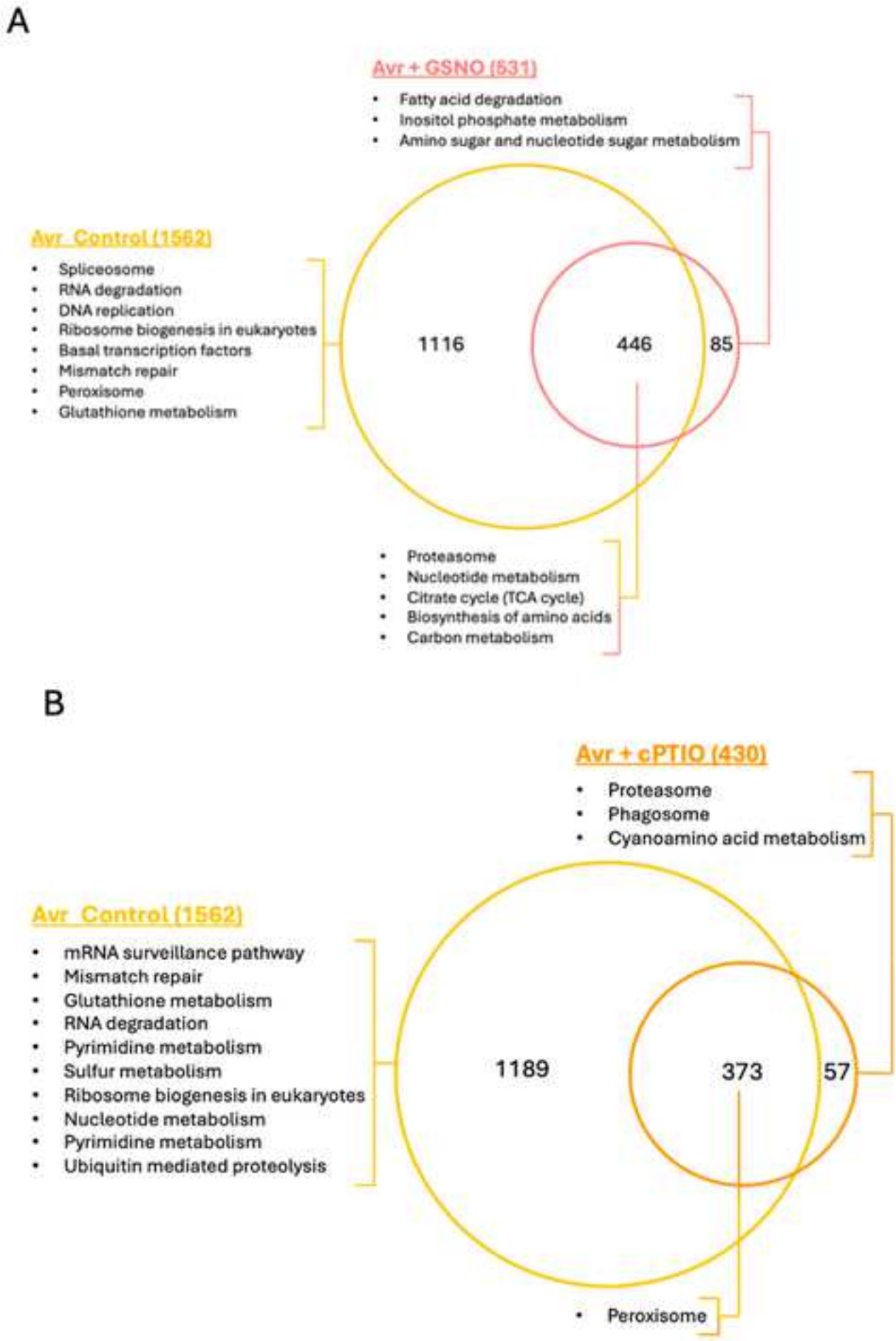


Figure 5

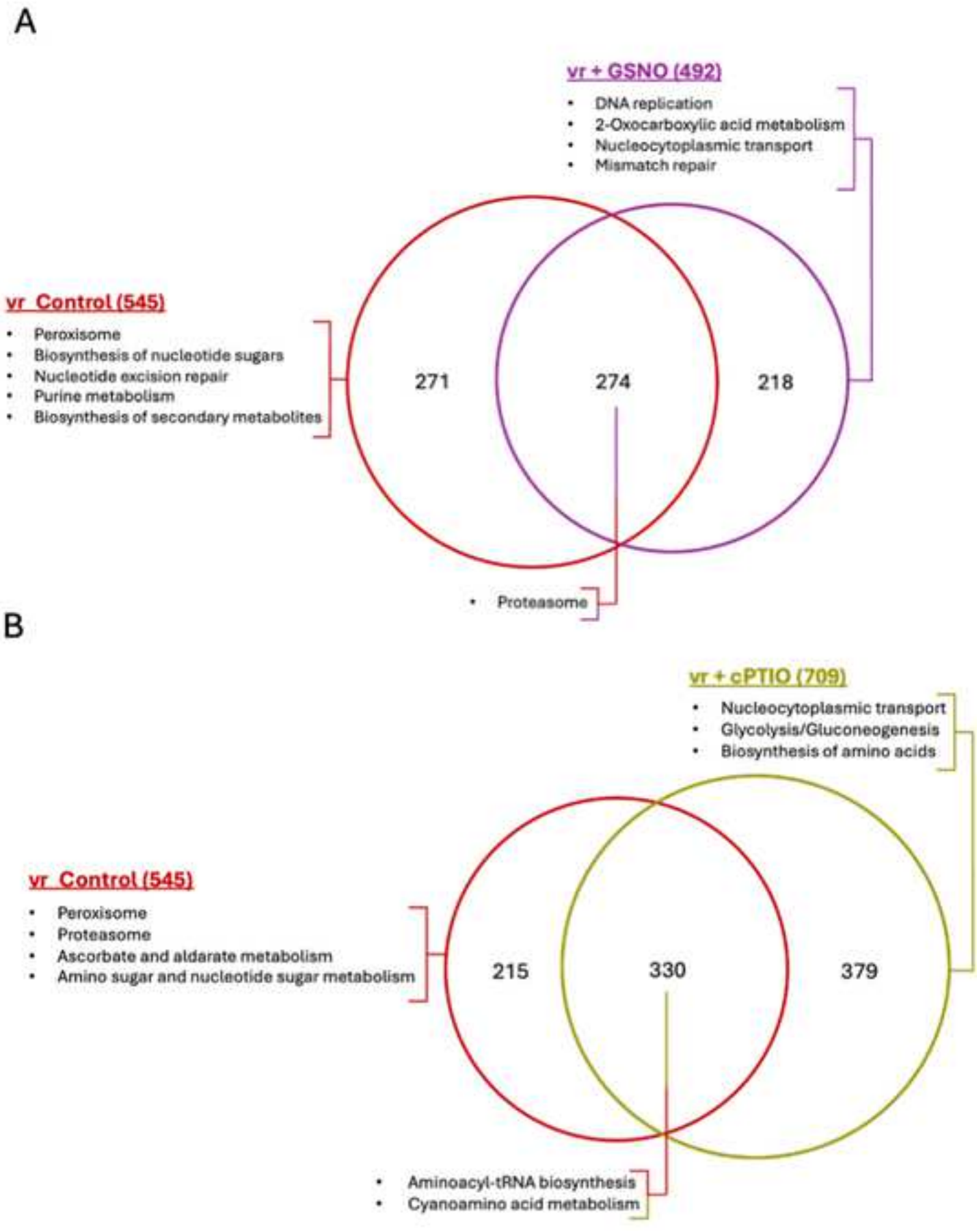


Figure 6

Click here to access/download;Figure;Fig 6.tif

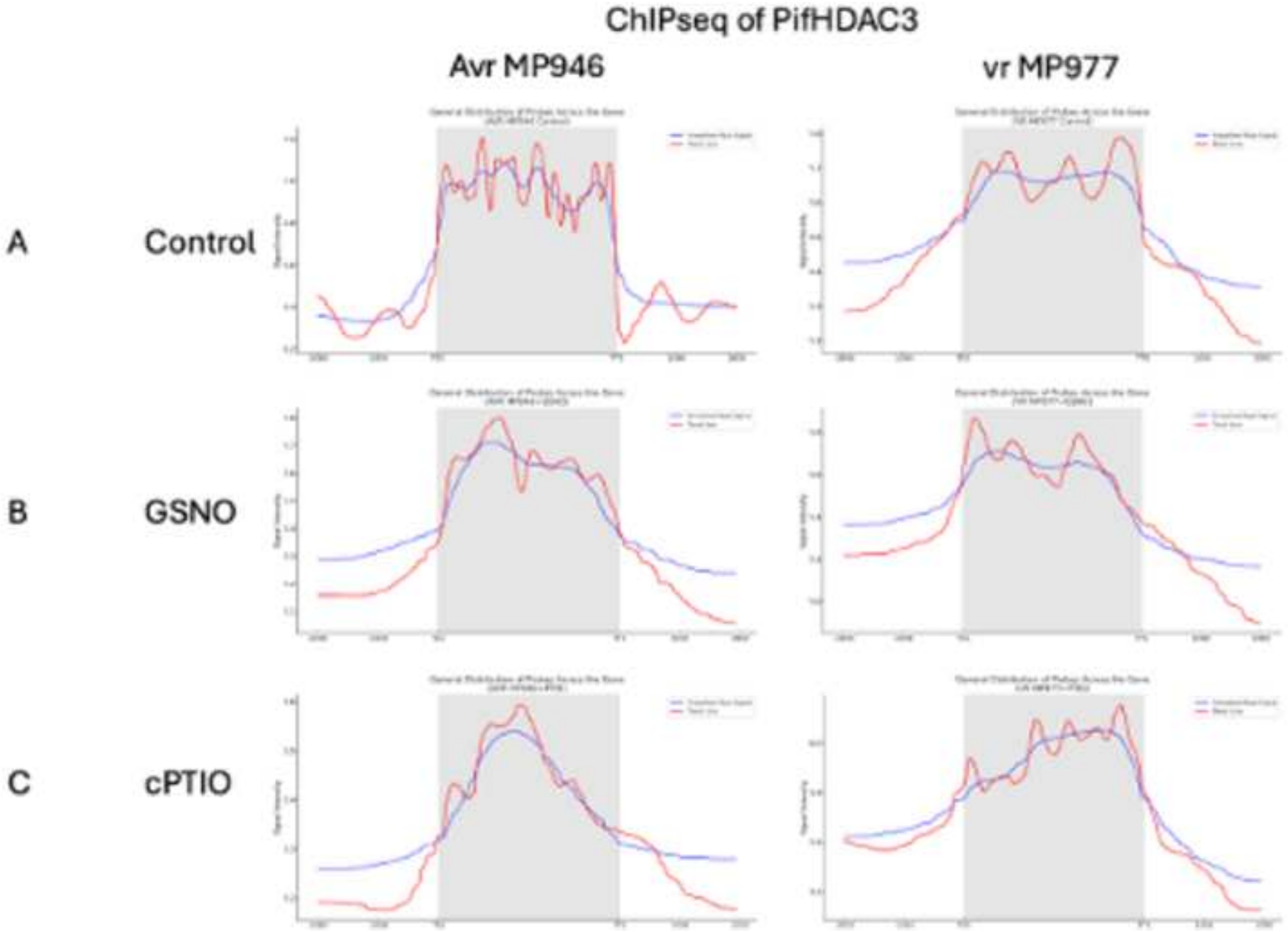
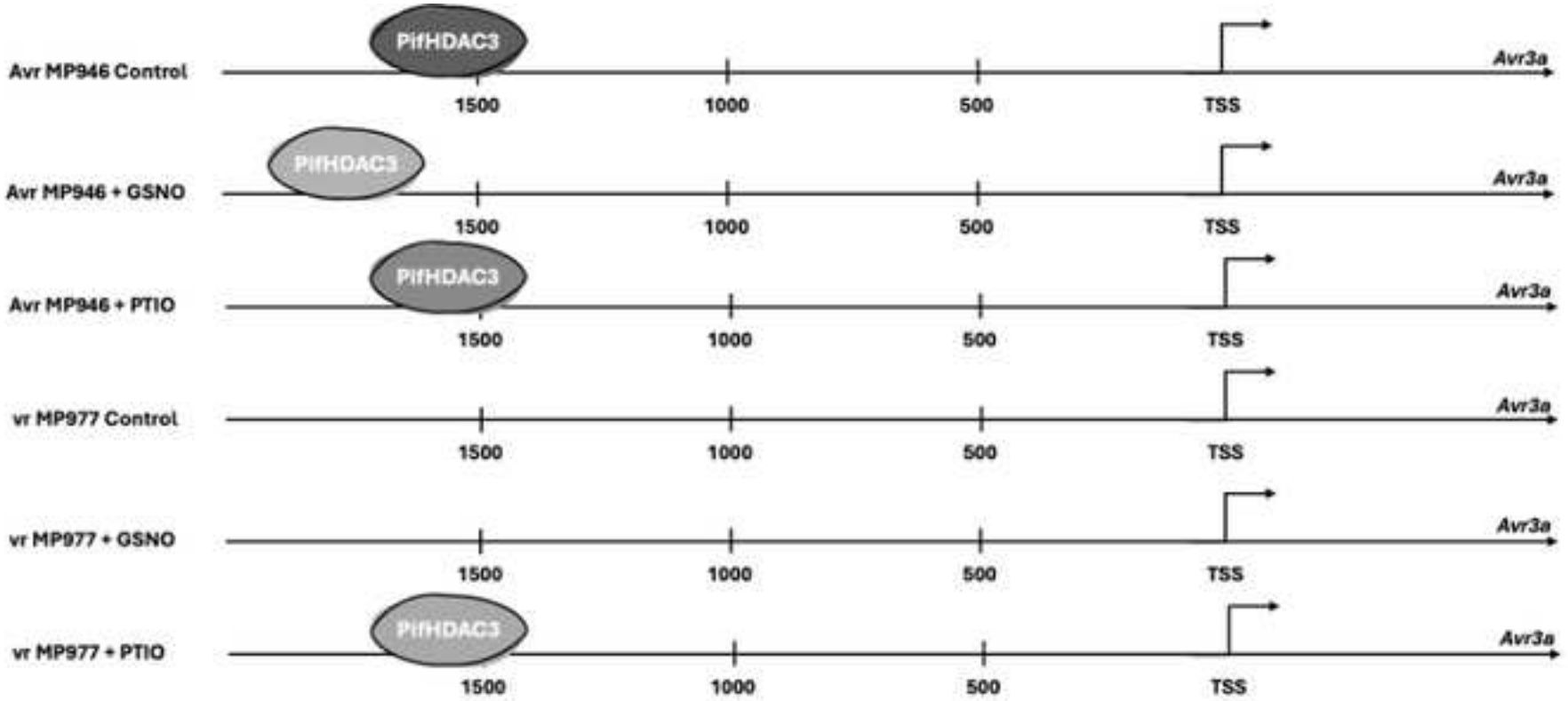


Figure 7

A



B

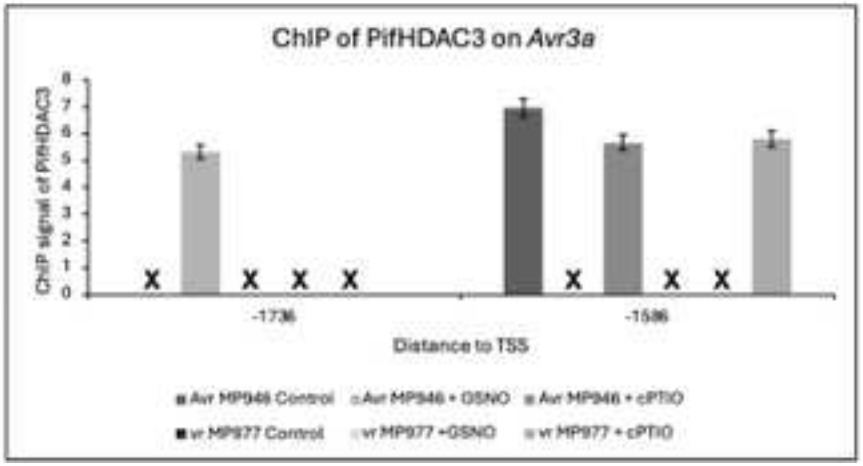


Figure 8

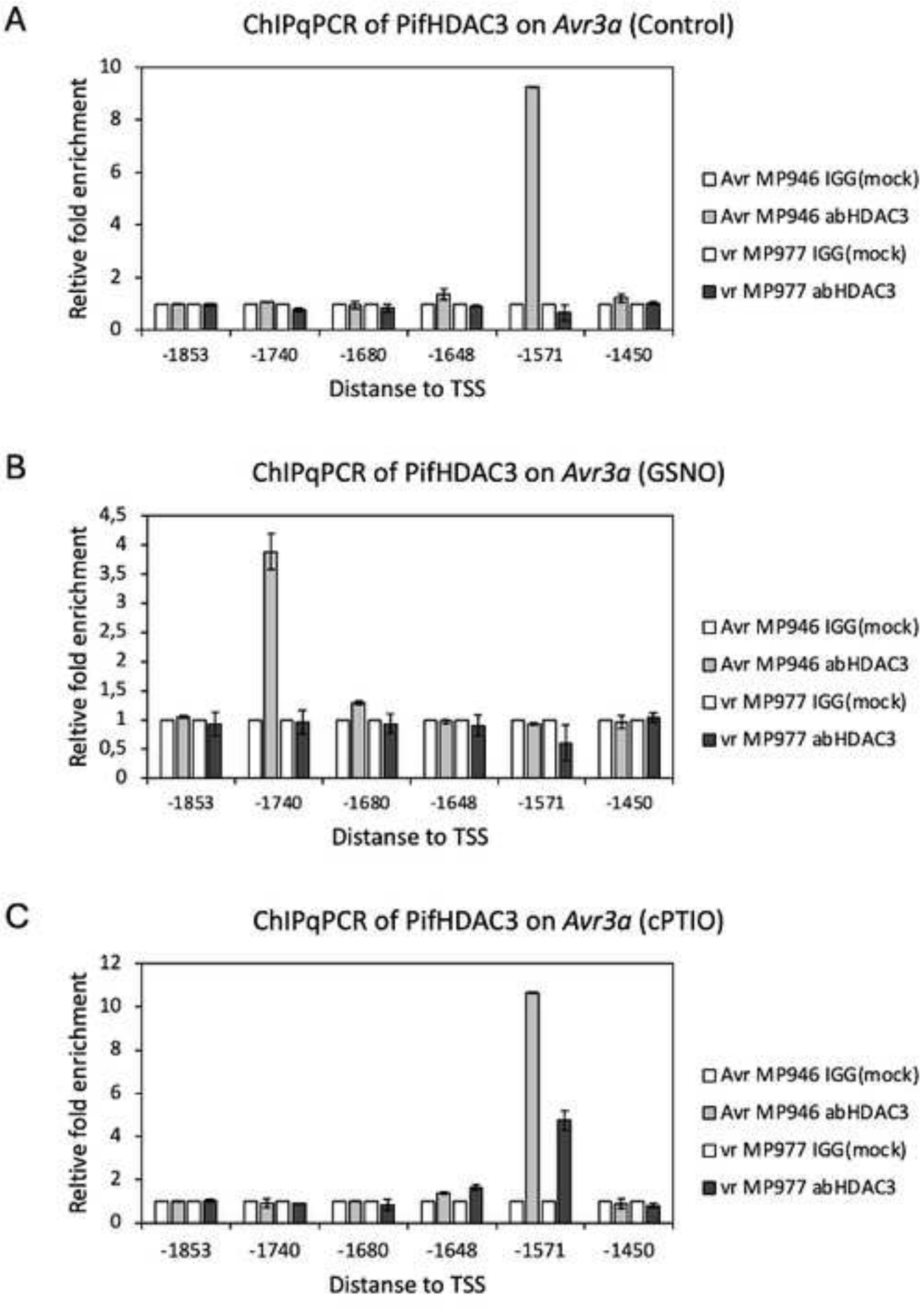
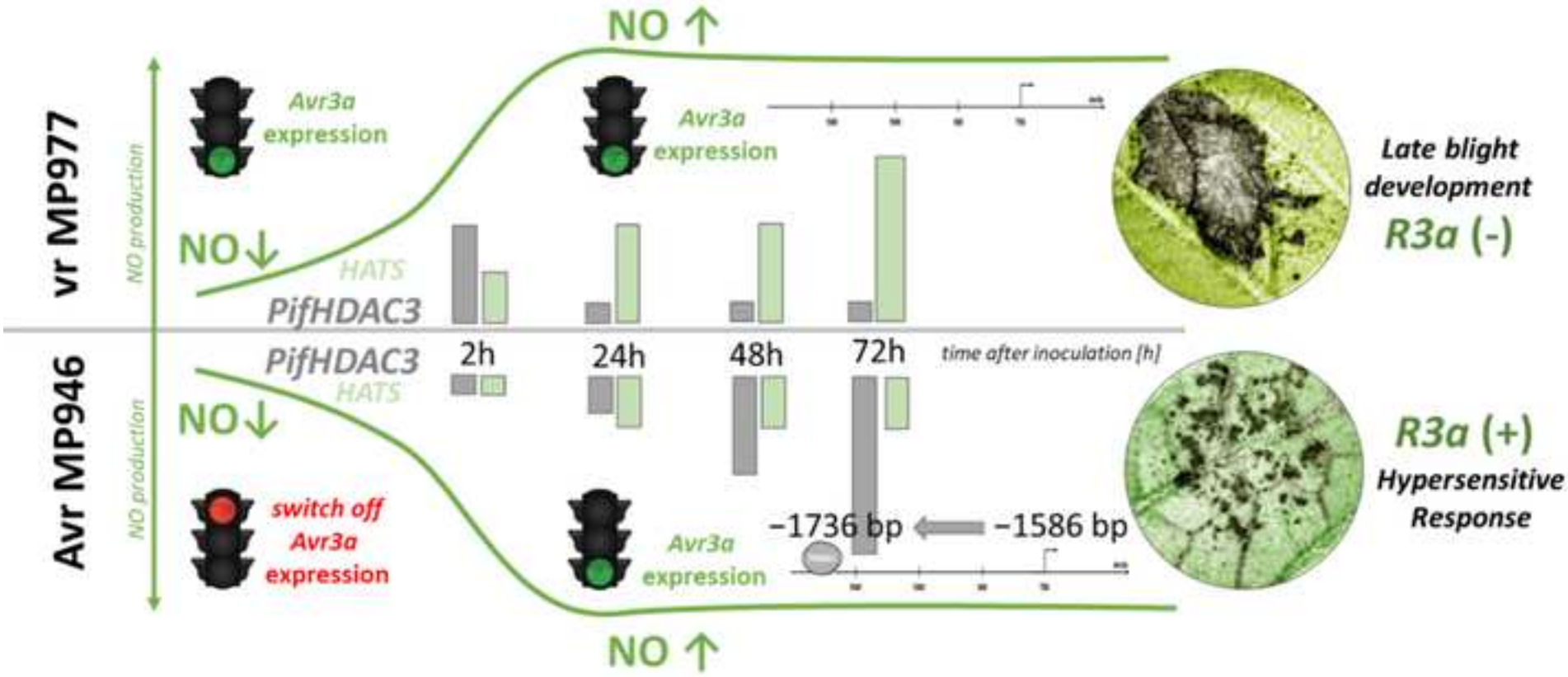
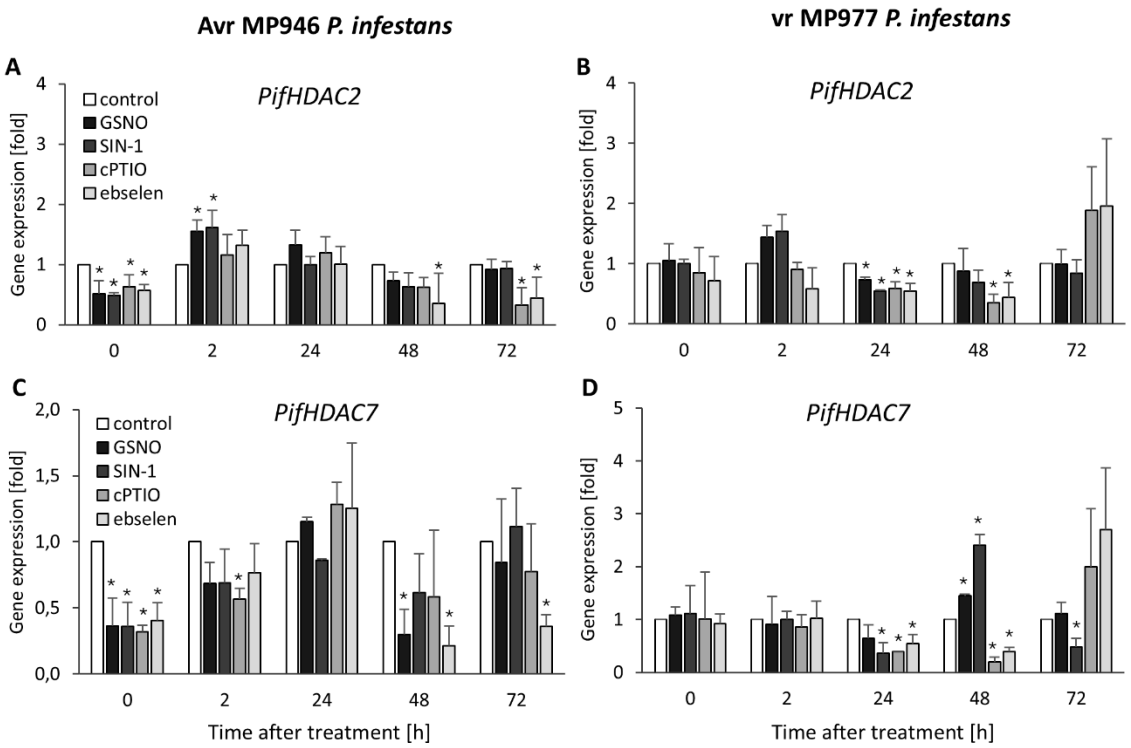


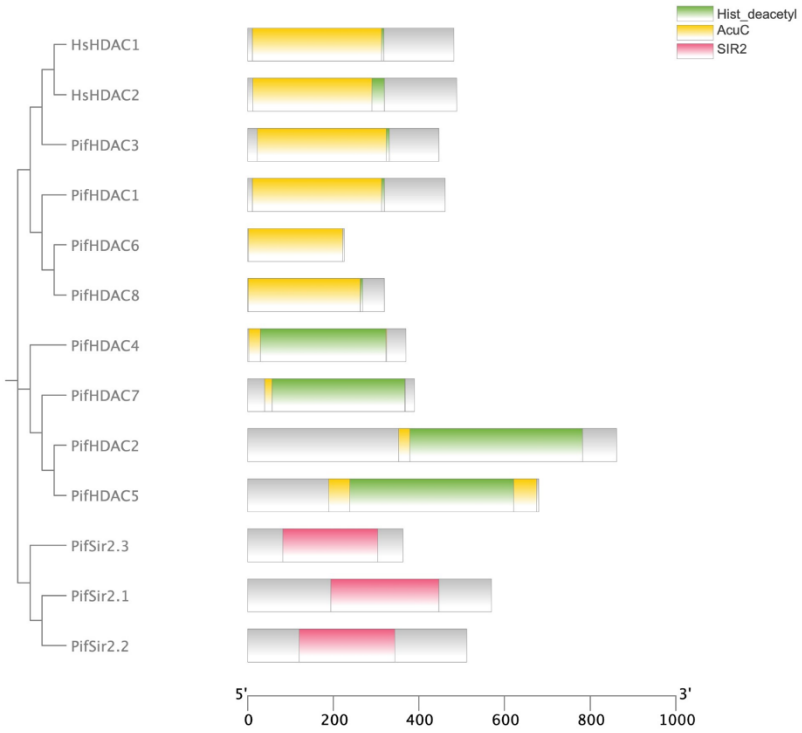
Figure 9

Click here to access/download;Figure;Fig 9.tif

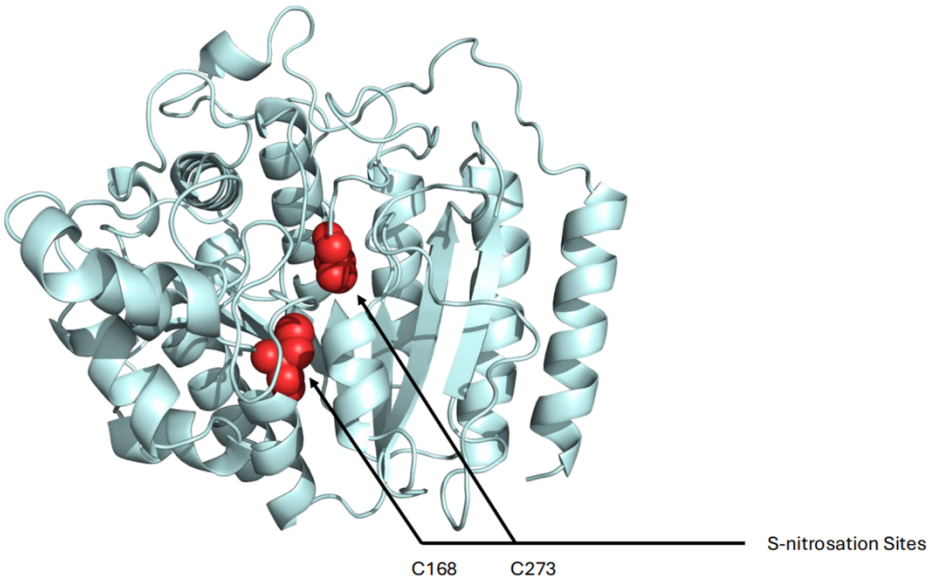




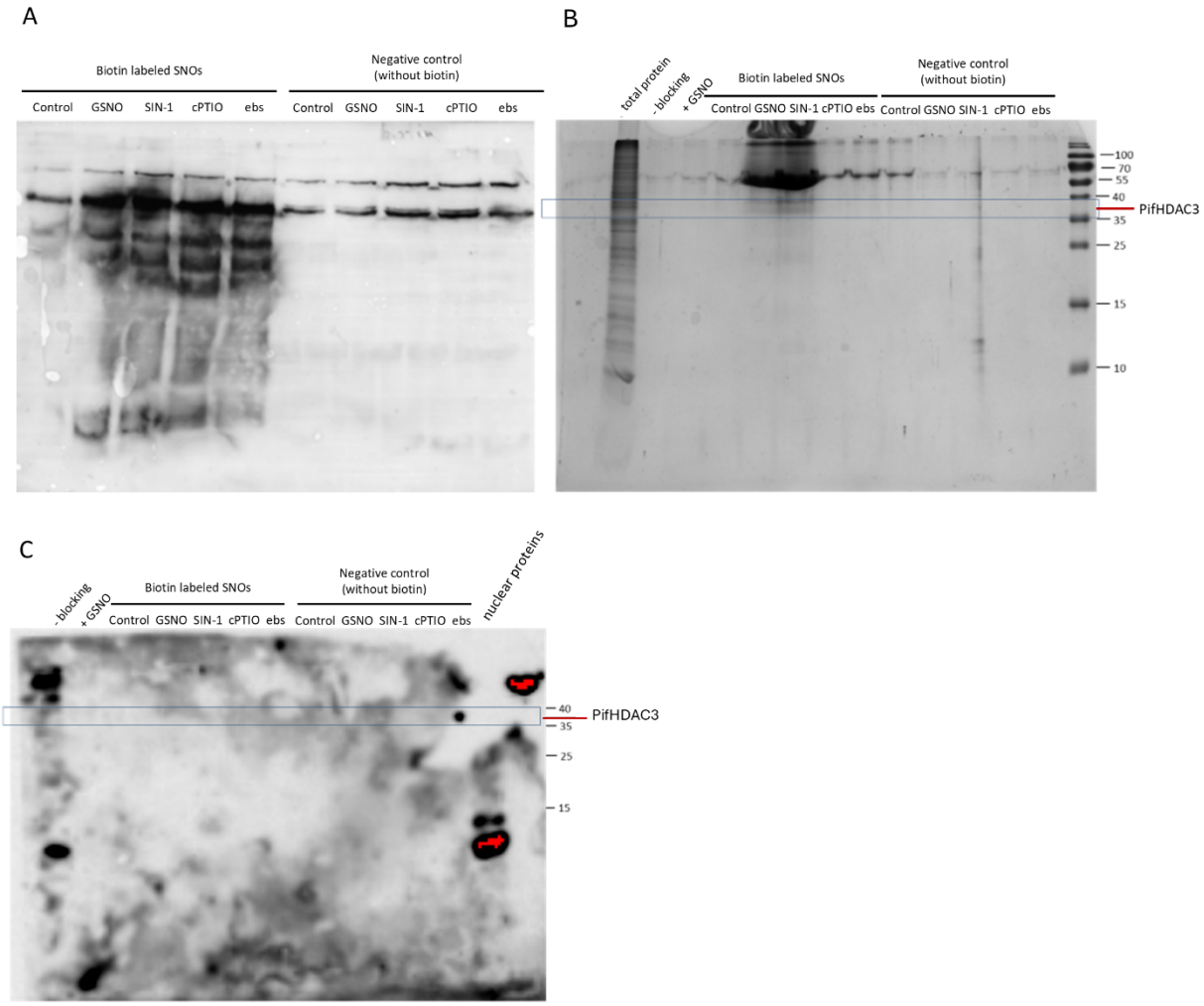
S1 Fig. Gene expression of *PifHDAC2* (A, B), *PifHDAC7* (C, D) in avirulent (Avr) MP946 and virulent (vr) MP977 *Phytophthora infestans* growing *in vitro*. The RT-qPCR gene expression was analyzed at selected time points (0–72 h) after the culture's treatment with water (control), S-nitrosoglutathione (GSNO), 3-morpholinosydnonimine (SIN-1), 2-(4-Carboxyphenyl)-4,4,5,5,-tetramethylimidazoline-1-oxyl 3-oxide (cPTIO), or ebselen, respectively. As a reference S3a was used. The results are averages from three independent experiments (n = 9) ± SD. Asterisks indicate values that differ significantly from the water treated samples (control) *P. infestans* culture at each time point at $p < 0.05$ (*).



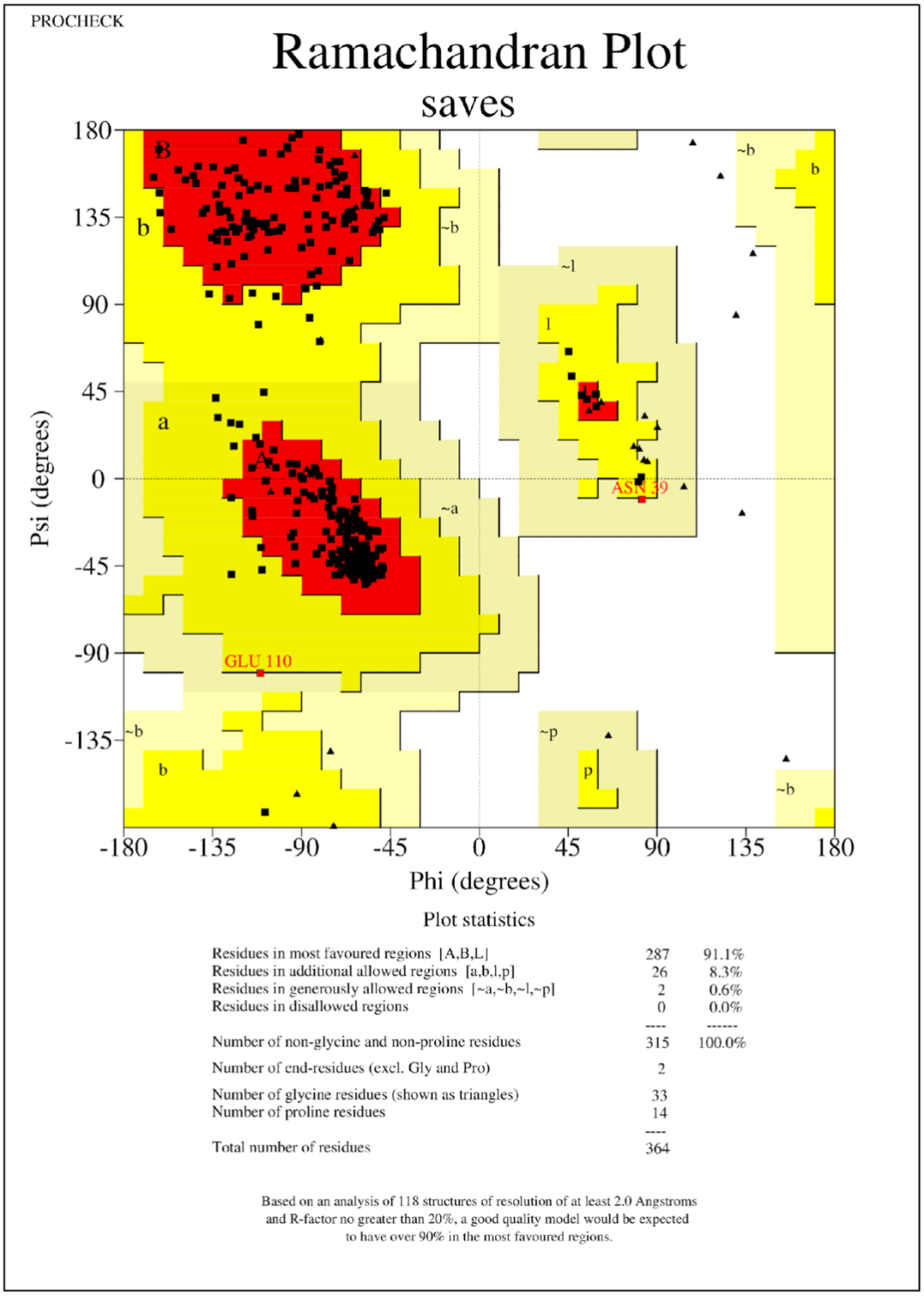
S2 Fig. Phylogenetic analysis and conserved domain distribution of PifHDACs with selected human orthologs. The tree was constructed using the neighbor-joining method with 1000 bootstrap replications in MEGA 7. The conserved domain of PifHDACs and selected human orthologs was identified using NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). TBtools was used to visualize the obtained results.



S3 Fig. *In-silico* analyses of PifHDAC3 as a potential target for S-nitrosation – The three-dimensional structure of the protein encoded by PifHDAC3 was generated by Phyre2 (https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The protein structure was visualized by cyan color from N to C terminus. Coils and smooths represent alpha helices and beta sheets, respectively. Potential S-nitrosation sites, predicted using GPS-SNO [62], are highlighted at cysteine residues C168 and C273, marked in red.



S4 Fig. Immunoprecipitation of biotinylated proteins coupled with western blot analysis using anti-PifHDAC3 antibody. (A); Detection of S-nitrosated proteins in *Phytophthora infestans* using biotin switch method. Lanes: Control, GSNO, SIN-1, cPTIO, ebselen (ebs), and negative control (without biotin). (B); Coomassie blue staining of SDS-PAGE gel. Lanes: Biotin-labeled SNOs, control (GSNO), SIN-1, cPTIO, ebselen (ebs), and negative control (without biotin). The band at approximately 38 kDa is marked as PifHDAC3. (C); Western blot analysis using anti-HDAC3 antibody confirming the absence of PifHDAC3 in biotin-labeled SNOs. Lanes: Biotin-labeled SNOs, control, GSNO, SIN-1, cPTIO, ebs, and negative control (without biotin). Bands at approximately 38 kDa indicate S-nitrosated PifHDAC3.



saves_01.ps

S5 Fig. Procheck Ramachandran plot analysis of PifHDAC3 3D modeling.

S1 Table. List of primers used in the study.

Gene	Gene symbol	Primer sequence
PifHDAC1	PITG_01897	F: CAGATGAGTCAAGCACCTCCCACG R: CTTCCTCCTGAGTAGAACCGTTGGCT
PifHDAC2	PITG_08237	F: TCAGTGCTGGAGGGAGGCTACAACTT R: TCAGTCTTACTGCGCTTCTTCGTCTGC
PifHDAC3	PITG_04499	F: ACTTGCCCGTGAGCAACATGGAAAAC R: TAAAATTCAACAGGATGACGGGGAGCGT
PifHDAC5	PITG_05176	F: ACGACTTCTACTACTTCCTGAGTGAGGA R: TAATGGAAGTTGGAGATAGCACTCTTACGC
PifHDAC7	PITG_15415	F: TATTAGAGAGAGATTCCCACGACTACCGA R: TCATGAGAGTCATATCGTCCCCCAGTT
S3a	PITG_11766	F: GGACGCCTTTCCTTCCTTCA R: CTCTGGTGGCCGTCTGTAAG
PifHDAC3_cDNA		F: ATGAGCAGTACCAACGGCAGTT R: TTAGTCGTAAAATTCAACAGGATG
pPifHDAC3_ET302/NT-His		F: GGATAACAATTCCCCTCTAGAATGAGCAGT ACCAACGGCAGTT R: TCGAATATCATCGATCTCGAGGTCGTAAAA TTCAACAGGATGACG

S2 Table. Prediction of secondary structure of PifHDAC3 protein.

Alpha helix (Hh): 140 is 23.29%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 39 is 6.49%
Beta turn (Tt): 18 is 3.00%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 404 is 67.22%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%

CO-AUTHORS' STATEMENTS

PUBLICATION 1

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle.

*Plant Physiology and Biochem*istry, 216, p.109129.
Doi: 10.1016/j.plaphy.2024.109129

Poznań, 01.09.2025

Mgr. Yufeng Guan

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

I declare that in the publication:

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle. *Plant Physiology and Biochem*istry, 216, p.109129. DOI: 10.1016/j.plaphy.2024.109129. **(Publication 1)**

My participation included:

- conducting a culture of the Avr MP946 and vr MP977 *P. infestans* for experimental purposes (*in vitro* and *in planta*),
- preparation of *P. infestans* spore suspension,
- preparation of reactive nitrogen species modulators and *P. infestans* treatment,
- collection of material for analyses,
- preparation and implementation of experiments involving:
    - measurement of nitric oxide emission (NO) using the NO chemiluminescence analyzer,
    - detection of peroxynitrite formation,
    - RNA isolation and gene expression measurement,
    - chromatin immunoprecipitation (ChIP) and the following ChIP-qPCR analyses,
    - immunoblot analyses of histone global and site-specific acetylation marks,
    - phylogenetic analysis and conserved domain distribution of PifHAM1 and its orthologs,
    - statistical analyses,
- participation in the analysis concerning isolation of histone-enriched protein for immunoassays and determination of histone H3 and H4 total acetylation levels,
- participation in the preparation of the first version of the manuscript (preparation of tables and figures - excluding Figure 9. manuscript formatting).

I estimate my contribution at: 60%

Yufeng Guan

Prof. dr hab. Magdalena Arasimowicz-Jelonek

Poznań, 01.09.2025

Dr. Joanna Gajewska

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle. *Plant Physiology and Biochem*istry, 216, p.109129. DOI: 10.1016/j.plaphy.2024.109129. **(Publication 1)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in Avr MP946 and vr MP977 *P. infestans* cultivation for experimental purposes (*in vitro* and *in planta*),
- participation in collection of material for analyses,
- preparation and implementation of experiments involving isolation of histone-enriched protein for immunoassays and determination of histone H3 and H4 total acetylation,
- preparation of the Figure 9.

I estimate my contribution at: 12.5%

Gajewska

Poznań, 01.09.2025

Prof. UAM dr hab. Ewa Sobieszczuk-Nowicka

Department of Plant Physiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle. *Plant Physiology and Biochem*istry, 216, p.109129. DOI: 10.1016/j.plaphy.2024.109129. **(Publication 1)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in chromatin immunoprecipitation (ChIP) assay (protocol supervision),
- participation in the interpretation of the obtained results,
- participation in reviewing and editing the first version of the manuscript and version following the reviewers' comments and remarks.

I estimate my contribution at: 7.5%

Ewa Sobieszczuk-Nowicka

Poznań, 01.09.2025

Prof. dr hab. Jolanta Floryszak-Wieczorek

Department of Plant Physiology

Poznań University of Life Sciences

ul. Wołyńska 35

60-637 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

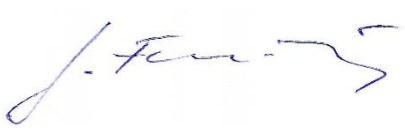
Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle. *Plant Physiology and Biochem*istry, 216, p.109129. DOI: 10.1016/j.plaphy.2024.109129. **(Publication 1)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in conceptualization and planning the experiments,
- participation in the interpretation of the obtained results,
- participation in the preparation of the first version of the manuscript (manuscript writing),
- participation in the preparation of the manuscript version following the reviewers' comments and remarks, including participation in preparing responses to the reviews.

I estimate my contribution at: 5%



Poznań, 01.09.2025

Jun. Prof. Dr. Sjon Hartman

Plant Environmental Signalling and Development

Faculty of Biology

University of Freiburg

79104 Freiburg, Germany

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and 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, p.109129. DOI: 10.1016/j.plaphy.2024.109129. **(Publication 1)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in reviewing and editing the first version of the manuscript and version following the reviewers' comments and remarks.

I estimate my contribution at: 5%

Johannes (Sjon) Hartman

Digitally signed by Johannes (Sjon) Hartman
DN: cn=Johannes (Sjon) Hartman, o=University of Freiburg, ou=Faculty of Biology, email=johannes.hartman@biologie.uni-freiburg.de
Date: 2025.09.02 09:01:19 +02'00'

Poznań, 01.09.2025

Prof. dr hab. Magdalena Arasimowicz-Jelonek

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., Hartman, S. and Arasimowicz-Jelonek, M. (2024). The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle. *Plant Physiology and Biochem*istry, 216, p.109129. DOI: 10.1016/j.plaphy.2024.109129. **(Publication 1)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- conceptualization and planning the experiments,
- substantive supervision during the research,
- interpretation of the obtained results,
- participation in the preparation of the first version of the manuscript (manuscript writing),
- participation in the preparation of the manuscript version following the reviewers' comments and remarks, including participation in preparing responses to the reviews.

I estimate my contribution at: 10%

PUBLICATION 2

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology.

Molecular Plant Pathology, 25(7), p.e13497.
Doi: 10.1111/mpp.13497

Poznań, 01.09.2025

Mgr. Yufeng Guan

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

I declare that in the publication:

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology. *Molecular Plant Pathology*, 25(7), p.e13497. DOI: 10.1111/mpp.13497. **(Publication 2)**

My participation included:

- participation in conceptualization,
- participation in the preparation of the first version of the manuscript (conducting *in silico* analysis on gene structure, protein sequence, and protein–protein interaction network of HDACs and HATs in *P. infestans*; participation in manuscript writing; manuscript formatting),
- participation in the preparation of the manuscript version following the reviewers' comments and remarks, including participation in preparing responses to the reviews,
- preparation of tables and figures (excluding Figure 3).

I estimate my contribution at: 55%

Yufeng Guan

Prof. dr hab. Magdalena Arasimowicz-Jelonek

Poznań, 01.09.2025

Dr. Joanna Gajewska

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology. *Molecular Plant Pathology*, 25(7), p.e13497. DOI: 10.1111/mpp.13497. **(Publication 2)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in conceptualization,
- participation in the preparation of the first version of the manuscript (participation in manuscript writing),
- preparation of the Figure 3.

I estimate my contribution at: 12.5%

Gajewska

Poznań, 01.09.2025

Prof. dr hab. Jolanta Floryszak-Wieczorek

Department of Plant Physiology

Poznań University of Life Sciences

ul. Wołyńska 35

60-637 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

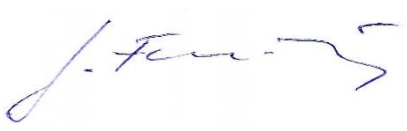
Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology. *Molecular Plant Pathology*, 25(7), p.e13497. DOI: 10.1111/mpp.13497. **(Publication 2)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in conceptualization,
- participation in the preparation of the first version of the manuscript (participation in manuscript writing),
- participation in reviewing and editing the first version of the manuscript and version following the reviewers' comments and remarks, including participation in preparing responses to the reviews.

I estimate my contribution at: 7.5%



Poznań, 01.09.2025

Dr. Umesh Kumar Tanwar

Legume Genomics Team

Institute of Plant Genetics

Polish Academy of Sciences

ul. Strzeszyńska 34

60-479 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology. *Molecular Plant Pathology*, 25(7), p.e13497. DOI: 10.1111/mpp.13497. **(Publication 2)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in conceptualization,
- participation in the preparation of the first version of the manuscript (participation in manuscript writing and conducting *in silico* analysis of the family distribution of HDACs and HATs across oomycete species),
- participation in reviewing and editing the first version of the manuscript and version following the reviewers' comments and remarks, including participation in preparing responses to the reviews.

I estimate my contribution at: 7.5%

Poznań, 01.09.2025

Prof. UAM dr hab. Ewa Sobieszczuk-Nowicka

Department of Plant Physiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology. *Molecular Plant Pathology*, 25(7), p.e13497. DOI: 10.1111/mpp.13497. **(Publication 2)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in conceptualization,
- participation in the preparation of the first version of the manuscript (participation in manuscript writing),
- participation in reviewing and editing the first version of the manuscript and version following the reviewers' comments and remarks, including participation in preparing responses to the reviews.

I estimate my contribution at: 7.5%

Ewa Sobieszczuk-Nowicka

Poznań, 01.09.2025

Prof. dr hab. Magdalena Arasimowicz-Jelonek

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Gajewska, J., Floryszak-Wieczorek, J., Tanwar, U.K., Sobieszczuk-Nowicka, E. and Arasimowicz-Jelonek, M. (2024). Histone (de) acetylation in epigenetic regulation of *Phytophthora* pathobiology. *Molecular Plant Pathology*, 25(7), p.e13497. DOI: 10.1111/mpp.13497. **(Publication 2)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in conceptualization,
- participation in the preparation of the first version of the manuscript (participation in manuscript writing),
- substantive supervision during the preparation of the manuscript,
- participation in the preparation of the manuscript version following the reviewers' comments and remarks, including participation in preparing responses to the reviews.

I estimate my contribution at: 10%

PUBLICATION 3

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

PLOS Pathogens

Poznań, 01.09.2025

Mgr. Yufeng Guan

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

I declare that in the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

My participation included:

- conducting a culture of the Avr MP946 and vr MP977 *P. infestans* for experimental purposes (*in vitro* and *in planta*),
- preparation of *P. infestans* spore suspension,
- preparation of reactive nitrogen species modulators and *P. infestans* treatment,
- collection of material for analyses,
- preparation and implementation of experiments involving:
    - RNA isolation and gene expression measurement,
    - recombinant expression of PifHDAC3 in *E. coli*,
    - quantification of HDACs activity,
    - construction of the 3D modelling of PifHDAC3 and *in silico* analyses of its potential S-nitrosation sites,
    - phylogenetic analysis and conserved domain distribution of PifHDAC3 and its orthologs,
    - statistical analyses,
- participation in analysis concerning chromatin immunoprecipitation (ChIP) assay, ChIP-seq data analysis; ChIP-qPCR validation of the obtained ChIP-seq results,
- participation in the preparation of the first version of the manuscript (participation in writing the manuscript, preparation of tables and figures, manuscript formatting).

I estimate my contribution at: 40%

Yufeng Guan

Yufeng Guan

Prof. dr hab. Magdalena Arasimowicz-Jelonek

Poznań, 01.09.2025

Dr. Szymon Kubala

Laboratory of Gene Expression Regulation

Institute of Biochemistry and Biophysics

Polish Academy of Sciences

ul. Pawinskiego 5A

02-106 Warsaw, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in chromatin immunoprecipitation (ChIP) assay (protocol supervision),
- preparation of the ChIP-seq library, sequencing and supervision of data analysis,
- ChIP-qPCR validation of the obtained ChIP-seq results,
- participation in the preparation of the first version of the manuscript (participation in writing the manuscript),
- participation in reviewing and editing the first version of the manuscript.

I estimate my contribution at: 20%

Poznań, 01.09.2025

Dr. Joanna Gajewska

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in Avr MP946 and vr MP977 *P. infestans* cultivation for experimental purposes (*in vitro* and *in planta*),
- cultivation of potato plants,
- participation in collection of material for analyses,
- participation in chromatin immunoprecipitation (ChIP) assay,
- preparation and implementation of experiments involving isolation of histone-enriched protein for immunoassays, western blot analysis of PifHDAC3 and immunoprecipitation of biotinylated proteins coupled with western blot analysis using anti-PifHDAC3 antibody,
- participation in the preparation of the first version of the manuscript (participation in writing the manuscript).

I estimate my contribution at: 13%

Gajewska

Poznań, 01.09.2025

Prof. UAM dr hab. Ewa Sobieszczuk-Nowicka

Department of Plant Physiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in the interpretation of the obtained results,
- participation in reviewing and editing the first version of the manuscript.

I estimate my contribution at: 5%

Ewa Sobieszczuk-Nowicka

Poznań, 01.09.2025

Dr. Dawid Perlikowski

Department of Plant Physiology

Institute of Plant Genetics

Polish Academy of Sciences

ul. Strzeszyńska 34

60-479 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in analysis of cDNA sequences coding the fungus-like protein HDAC3 (PifHDAC3); designing the sequence for anti-PiHDAC3 generation; evaluation of quality and functionality of serum and antibody during/and after production.

I estimate my contribution at: 4%

Signed by / Podpisano przez:
Dawid Bogdan Perlikowski
Date / Data:
2025-09-07 10:33

Poznań, 01.09.2025

Prof. dr hab. Arkadiusz Kosmala

Department of Plant Physiology

Institute of Plant Genetics

Polish Academy of Sciences

ul. Strzeszyńska 34

60-479 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- analysis of cDNA sequences coding the fungus-like protein HDAC3 (PifHDAC3); designing the sequence for anti-PiHDAC3 generation; evaluation of quality and functionality of serum and antibody during/and after production.
- participation in reviewing and editing the first version of the manuscript.

I estimate my contribution at: 4%

Signed by / Podpisano przez:

Arkadiusz Tomasz Kosmala

Date / Data: 2025-09-05 09:23

Poznań, 01.09.2025

Prof. dr hab. Jolanta Floryszak-Wieczorek

Department of Plant Physiology

Poznań University of Life Sciences

ul. Wołyńska 35

60-637 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- participation in conceptualization and planning the experiments,
- participation in the interpretation of the obtained results,
- participation in the preparation of the first version of the manuscript (participation in writing the manuscript),
- participation in reviewing and editing the first version of the manuscript.

I estimate my contribution at: 4%

Poznań, 01.09.2025

Prof. dr hab. Magdalena Arasimowicz-Jelonek

Department of Plant Ecophysiology

Faculty of Biology

Adam Mickiewicz University in Poznań

ul. Uniwersytetu Poznańskiego 6

61-614 Poznań, Poland

STATEMENT

regarding co-authorship in the publication with Yufeng Guan which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Guan, Y., Kubala, S., Gajewska, J., Sobieszczuk-Nowicka, E., Perlikowski, D., Kosmala, A., Floryszak-Wieczorek, J., and Arasimowicz-Jelonek, M. (2025). Genotype-specific transcriptional reprogramming of *Phytophthora infestans* by histone deacetylase PifHDAC3 under nitrosative stress. *PLOS Pathogens* (Under review) **(Publication 3)**

of which I am a co-author, has been included in the doctoral thesis of Yufeng Guan.

I contributed to:

- conceptualization and planning the experiments,
- substantive supervision during the research,
- interpretation of the obtained results,
- participation in the preparation of the first version of the manuscript (manuscript writing),
- participation in reviewing and editing the first version of the manuscript.

I estimate my contribution at: 10%

LIST OF OTHER ACHIEVEMENTS

Publications and monographs

1. **Guan, Y.,** Tanwar, U. K., Sobieszczuk-Nowicka, E., Floryszak-Wieczorek, J., & Arasimowicz-Jelonek, M. (2022). Comparative genomic analysis of the aldehyde dehydrogenase gene superfamily in *Arabidopsis thaliana*–searching for the functional key to hypoxia tolerance. Frontiers in Plant Science, 13, 1000024.

2. Stolarska, E., Tanwar, U. K., **Guan, Y.,** Grabsztunowicz, M., Arasimowicz-Jelonek, M., Phanstiel IV, O., & Sobieszczuk-Nowicka, E. (2023). Genetic portrait of polyamine transporters in barley: insights in the regulation of leaf senescence. Frontiers in plant science, 14, 1194737.

3. Drozda, A., Kurpisz, B., **Guan, Y.,** Arasimowicz-Jelonek, M., Plich, J., Jagodzik, P., Kuźnicki, D., & Floryszak-Wieczorek, J. (2022). Insights into the expression of DNA (de) methylation genes responsive to nitric oxide signaling in potato resistance to late blight disease. Frontiers in Plant Science, 13, 1033699.

4. Drozda, A., Kurpisz, B., Arasimowicz-Jelonek, M., Kuźnicki, D., Jagodzik, P., **Guan, Y.,** & Floryszak-Wieczorek, J. (2022). Nitric oxide implication in potato immunity to *Phytophthora infestans* via modifications of histone H3/H4 methylation patterns on defense genes. International Journal of Molecular Sciences, 23(7), 4051.

Participation in scientific conferences

International conferences

1. **Guan Y.,** Floryszak-Wieczorek J., Sobieszczuk-Nowicka E., Suarez AS., Hartman S., Arasimowicz-Jelonek M. 10th Plant Nitric Oxide International Meeting, Warsaw (Poland) 09-11/07/2025

2. **Guan Y.,** Floryszak-Wieczorek J., Sobieszczuk-Nowicka E., Arasimowicz-Jelonek M. Aldehyde dehydrogenase as a metabolic sensor of nitroxyl in *Arabidopsis*. Plant Biology Europe 2025, Budapest (Hungary) 25-28/06/2025

3. **Guan Y.,** Gajewska J., Sobieszczuk-Nowicka E., Floryszak-Wieczorek J., Hartman S., Arasimowicz-Jelonek M. Reactive nitrogen species are involved in *Phytophthora infestans* life cycle *via* modifications of histone H3 and H4 acetylation. 5th Epicatch meeting, Bordeaux (France) 10-12/07/2024 (Oral presentation)

4. Gajewska J., **Guan Y.,** Kosmala A., Sobieszczuk-Nowicka E., Floryszak-Wieczorek J., Arasimowicz-Jelonek M. HDAC3 activation in *Phytophthora infestans* structures in response to host-derived nitrosative stress. 5th Epicatch meeting, Bordeaux (France) 10-12/07/2024

5. Gajewska J., **Guan Y.,** Kosmala A., Sobieszczuk-Nowicka E., Floryszak-Wieczorek J., Arasimowicz-Jelonek M. Host-derived nitrosative stress activates *Phytophthora infestans* HDAC3. 9th Plant nitric oxide international meeting, New Delhi (India) 28-29/02/2024

6. Gajewska J., **Guan Y.,** Floryszak-Wieczorek J., Arasimowicz-Jelonek M. Does copper stress modify the offensive strategy of *Phytophthora infestans* towards potato? International Conference of the French Society of Plant Biology, Marseille (France) 03/06/-06/06/2023

7. Sobieszczuk-Nowicka E., Tanwar U.K., Stolarska E., **Guan Y.,** Grabsztunowicz M., Arasimowicz-Jelonek M. Genome-wide exploration of the genetics of transporters of biogenic polyamines in barley for nitrogen-remobilization crop improvement. International Conference of the French Society of Plant Biology, Marseille (France) 03/06/-06/06/2023

8. **Guan Y.,** Gajewska J., Floryszak-Wieczorek J., Sobieszczuk-Nowicka E., Arasimowicz-Jelonek M. Diversity and evolution of histone deacetylases in

phytopathogenic *Phytophthora infestans*. Epigenetic mechanisms of crop adaptation to climate change, Sofia (Bulgaria) 30/05-01/06/2023

9. Tanwar U.K., Stolarska E., **Guan Y.,** Grabsztunowicz M., Arasimowicz-Jelonek M., Phanstiel O., Gregersen P. L., Sobieszczuk-Nowicka E. Unraveling the genetics of polyamine transporters in barley for senescence-related crop improvement. At the forefront of plant research, Barcelona (Spain) 08-10/05/2023

10. Drozda A., **Guan Y.,** Kuźnicki D., Kurpisz B., Arasimowicz-Jelonek M., Floryszak-Wieczorek J. Is NO regulation of potato gene expression related to *Phytophthora infestans* resistance epigenetic in nature? 8th Plant Nitric Oxide International Meeting, Szeged (Hungary) 7 – 9/07/2021

11. Drozda A., Kurpisz B., **Guan Y.,** Kuźnicki D., Arasimowicz-Jelonek M., Floryszak-Wieczorek J. The impact of nitric oxide and peroxynitrite on dna(de)methylation gene expression in potato to *phytophthora infestans*. Current problems of plant physiology and genetics, Kyiv (Ukraine) 17/06/2021

12. Drozda A., Kurpisz B., **Guan Y.,** Kuźnicki D., Arasimowicz-Jelonek M., Floryszak-Wieczorek J. Nitric oxide as a regulator of diverse histone methylations involved in potato immunity to *Phytophthora infestans*. Plants Stress and Adaptation, Kharkiv (Ukraine) 25 – 26/02/2021

National conferences

1. **Guan Y.,** Floryszak-Wieczorek J., Sobieszczuk-Nowicka E., Suarez A S., Hartman S., Arasimowicz-Jelonek M. Nitroxyl as a new regulator of hypoxia response in Arabidopsis. VII Warsztaty Naukowe Instytutu Biologii Eksperymentalnej Uniwersytetu im. Adama Mickiewicza w Poznaniu, Poznań (Poland) 14/06/2024

2. Pawłowski T.A., Alipour S., Kurpisz B., Jagodzik P., Suszka J., Chmielarz P., Marczak Ł., **Guan Y.,** Arasimowicz-Jelonek M. Protein modifications associated with seed germination of European beech originating from different climate

conditions. 11th Conference of the Polish Society of Experimental Plant Biology, Poznań (Poland) 19 – 22/09/2023

3. Rajek M., Robakowski M., **Guan Y.,** Floryszak-Wieczorek J., Sobieszczuk-Nowicka E., Arasimowicz-Jelonek M. „Pep-13 acts as potential inducer of systemic acquired resistance in potato" 11th Conference of the Polish Society of Experimental Plant Biology, Poznań (Poland) 19 – 22/09/2023

4. Stolarska E., Tanwar U.K., Paluch-Lubawa E., Grabsztunowicz M., **Guan Y.,** Mattoo A.K., Arasimowicz-Jelonek M., Gregersen P.L., Phanstiel IV O., Sobieszczuk-Nowicka E. 11th Conference of the Polish Society of Experimental Plant Biology, Poznań (Poland) 19 – 22/09/2023

5. **Guan Y.,** Floryszak-Wieczorek J., Sobieszczuk-Nowicka E., Arasimowicz-Jelonek M. Aldehyde Dehydrogenase as a Metabolic Sensor of Nitroxyl in Arabidopsis. 11th Conference of the Polish Society of Experimental Plant Biology, Poznań (Poland) 19 – 22/09/2023 (Oral presentation)

6. Gajewska J., **Guan Y.,** Kosmala A., Sobieszczuk-Nowicka E., Floryszak-Wieczorek J., Arasimowicz-Jelonek M. *Phytophthora infestans* HDAC3 challenge during *in planta* nitrosative stress. 11th Conference of the Polish Society of Experimental Plant Biology, Poznań (Poland) 19 – 22/09/2023

7. **Guan Y.,** Gajewska J., Floryszak-Wieczorek J., Sobieszczuk-Nowicka E., Arasimowicz-Jelonek M. Różnorodność i ewolucja acetylotransferaz i deacetylaz histonowych w rodzaju *Phytophthora*. Ogólnopolska Konferencja Naukowa z okazji 10-lecia Polskiego Towarzystwa Mykologicznego, Poznań (Poland) 26-28/09/2022

8. Gajewska J., **Guan Y.,** Floryszak-Wieczorek J., Arasimowicz-Jelonek M. Fitopatogeny reprezentujące różne style życia - *Phytophthora infestans* i *Botrytis cinerea* - w obliczu stresu miedziowego. Ogólnopolska Konferencja Naukowa z okazji 10-lecia Polskiego Towarzystwa Mykologicznego, Poznań (Poland) 26-28/09/2022

9. **Guan Y.,** Drozda A., Kuźnicki D., Kurpisz B., Arasimowicz-Jelonek M., Floryszak-Wieczorek J. Influence of nitric oxide on the expression patterns of *mi482b* and *R3a* genes in potato leaves (*Solanum tuberosum* L. 'Sarpo Mira') COMPAS: the future of interdisciplinary science, Poznań (Poland) 22-24/09/2021