SUMMARY OF DOCTORAL THESIS

Nitric oxide (NO) is a critical redox signaling molecule involved in numerous physiological and pathophysiological processes in plants. To date, there has been no information on the potential involvement of NO in the expression of genes involved in the stress response regulated epigenetically by changing the availability of chromatin for transcription factors. The publications in the doctoral dissertation concern the determination of the potential role of NO in the epigenetic control of the ETI-type resistance of potato 'Sarpo Mira' to avr *Phytophthora infestans* (Mont.) de Bary. The overarching goal of the research was the identification and functional analysis of biomolecules modified directly or indirectly by NO, with a significant impact on chromatin rearrangement and expression of genes related to the mentioned biotic stress (Drozda et al. 2022a; Drozda et al. 2022b).

Publication 1 (Drozda et al. 2022a) investigated the effect of biphasic NO generation regulated by the activity of S-nitrosoglutathione reductase (GNSOR) after inoculation of avr *P. infestans*. It was shown that in the biphasic NO burst after inoculation, the phase of periodic NO reduction at 6 hours after infection coincided with the increase in the expression of the defense strategy genes (*NPR1*, *WRKY1*, *PR1*) and the *R3a* resistance gene enriched in the active H3K4me3/*TrxG* mark in the promoter regions of these genes. Furthermore, the post-inoculation results obtained at the same time show that the PRMT5 arginine methyltransferase catalysing the symmetrical dimethylation of histone H4R3 (H4R3sme2) is required for potato resistance to avr *P. infestans*. Both pathogen inoculation and NO donor (GSNO) treatment altered the methylation status of the analysed genes by periodically reducing the repressive mark H4R3sme2 in the promoter of defense genes, *R3a* and the active nucleus death marker gene *HSR203J*, thereby enhancing their transcription. In confirmation, inhibition of PRMT5 under a selective inhibitor reduced the expression of *R3a* and the HR-type hypersensitivity reaction to the pathogen. Research shows that due to the activity of GSNOR, a periodic reduction in the level of NO (in the 6th hour after infection) is crucial for the increase in the expression of defense strategy genes and the *R3a* resistance gene, which are epigenetically regulated by changing the H3/H4 histone methylation pattern, which affects potato resistance to late blight.

The investigations conducted as part of **Publication 2** (Drozda et al. 2022b) are a continuation of the research initiated in the previous work, as they concern the analysis of the potential impact of NO on the expression of genes regulating the process of DNA (de)methylation, remaining in a functional dialogue with histone methylation in the

resistance of potato leaves on avr *P. infestans*. During the early phase after inoculation, with high levels of NO, a global increase in DNA methylation and increased expression of RdDM pathway genes (*DCL3*, *AGO4*, *DRM2*, and *miRNAs*) were found, which affected the inhibition of the expression of *R*-genes (*R3a* and *Rpi-phu1*) and defense strategy genes (i.e. *NPR1*, *WRKY1*, and *PR1*). The subsequent decrease in the bioavailability of NO at 6 hours (due to the activity of GSNOR reductase) resulted in a decrease in the transcription of selected miRNAs, which enabled an increase in the expression of targeted *R*-genes and defense strategy genes, with a low level of the inhibitory marker H3K9me2/*SUVH4* on the *R3a* promoter. The obtained results prove that the level of NO controlled by GSNOR after inoculation indirectly affecting the regulation of the RdDM pathway, which by controlling the expression of the *R3a* gene, affects potato resistance to avr *P. infestans*.

The results obtained as part of the presented dissertation enriched the knowledge about the previously unknown role of NO as an indirect epigenetic regulator of histone and DNA methylation (5-mC) in the resistance of potato leaves to late blight.

Keywords: nitric oxide, late blight, histone methylation, 5-mC DNA, RdDM pathway

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