Nitroxyl's role in the signaling cross-talk between cell death and life during potato leaves' hypersensitive response to *Phytophthora infestans*

In the plant–pathogen interaction scenario, recognizing the pathogen's avirulence (Avr) signals by plant receptors (R) triggers host immunity through activation of cell death, also known as the hypersensitive response (HR), which arrests the pathogen's growth. The HR process is associated with a cascade of cellular changes, including *i.a.*, rapid reactive oxygen and reactive nitrogen species (RNS) generation, salicylic acid (SA) accumulation, lowering of cytosolic redox potential, activation of proteolytic enzymes leading to cellular components degradation, and finally, cell collapse. However, the HR process, which controls the range of cells dying and transduces signals to redox-sensing targets that effectively trigger distal defense responses, remains poorly understood in the plant immune system. Once a pathogen is recognized, the generation of nitric oxide (•NO) becomes a critical switch in the immune signaling network, triggering local and systemic defense responses. In our published results, when Avr *P. infestans* inoculates the potato, a biphasic •NO burst with SA-induced redox-heightened state forms other biologically active RNS (*e.g.* Arasimowicz-Jelonek i Floryszak-wieczorek 2015). Our recent pioneering discovery in living cells, related to the detection and visualization of endogenous nitroxyl (HNO) in Arabidopsis leaf cells subjected to abiotic stresses (dark-induced senescence or hypoxia) (Arasimowicz-Jelonek et al. 2022) prompted us to undertake further studies on the role of HNO in potato resistance to biotic stress. Although nitroxyl is the one electron-reduced and protonated congener of nitric oxide, HNO also operates differently than •NO bioactivity. It serves as an alternative or competitive molecule versus •NO and is suited to signaling in biological processes through a wide range of actions. Generally, HNO-published data confirm that thiols and thiol-containing proteins are potential targets by which HNO elicits biological responses producing non-cross-linked sulfinamides and disulfide linkages. **Our research proposal concerns recognizing nitroxyl (HNO) bioactivity in HR immunity of the *R3a* potato genotype to *Avr3a P. infestans*, a late blight disease pathogenic agent. The project will focus on the identification of direct molecular targets of HNO, providing yet unknown functional links between HNO-mediated signal generation in early HR sequence of events, including monitoring *in vivo* HNO and •NO production, SA accumulation, and measuring HNO-mediated Ca^{2+} and cGMP levels in potato leaves provoked by Avr *P. infestans*.** A critical question from the presented project is whether and how HNO regulates or blocks cell death during HR. It is reasonable to speculate that HNO could also function in the precise fine-tuning of the proteostasis network during HR execution and restriction. **Therefore, we will focus on cathepsin B (CathB) cysteine protease at the transcript, protein, and enzymatic activity levels**. CathB, responsible for the degradation of endogenous proteins and the regulation of active cell dying, represents one of the most recognized biological targets being inhibited by HNO in mammalian systems. **Other critical cysteine-harboring proteolytic enzymes, such as SAG12 - senescence-associated protease, StSBTc-3 - subtilisin-like protease, and ATG8 - ubiquitin-like protein, could also be sensitive target proteins for HNO, so their gene expression will be analyzed. In the final stage of the project, experiments involve implementing a series of advanced pathophysiological techniques and highly innovative technologies that will help identify spatial HNO targets in the cell death/life zone related to distal resistance signals.** So far, there are limited investigations of molecular HNO-sensor identification and HNO-dependent transcriptome landscape remodeling that promote or attenuate organismal immunity. At the same time, there is practically no evidence for such effects in plants.