Insight into the mechanism of the toxic effect of copper on *Phytophthora infestans* - the causative agent of potato late blight

PROJECT MEANING AND RESEARCH PURPOSE

It is generally known that every living organism is exposed to many adverse environmental factors, including heavy metals (HM), which can be toxic even at low concentrations. Heavy metals may be divided into two groups: essential and non-essential elements. One of the HMs classified as an essential element is copper (Cu), an important catalytic cofactor in the redox chemistry of proteins that carry out fundamental biological functions. Unfortunately, the anthropogenic origins, including global use of chemical biocides have contributed to the dispersal of the HM throughout different environmental compartments. Although the first registration of the Cu-containing pesticide was issued in 1956, there is a lack of experimental data on the effect of its continuous use and long-term exposure of economically important plant pathogens to Cu. Importantly, HMs accumulated in soil and hosts growing on contaminated soils may accelerate pathogen evolution since the microorganism in order to survive is able to dynamically adapt to the new (micro)environments. Thus, as a result of pathogen exposition to HMs, an increased pathogenicity can be observed. In light of the above, the research on the recognition of the molecular mechanism of Cu toxicity on phytopathogens needs to be a priority. Therefore, the main goal of this project is to determine the mechanism of Cu toxicity on *Phytophthora infestans* (Mont.) de Bary, the most dangerous oomycete plant pathogen on the global scale responsible for late blight disease. The planned research will focus mainly on the recognition of the key Cu-mediated changes at cellular and molecular levels which may directly reflect the toxicity of the widespread use of Cu-containing fungicides and the pathogen adaptive potential to environment contamination with copper.

RESEARCH PLAN

The planned experiments will be conducted on a model species for the *Oomycetes*, *i.e.* *Phytophthora infestans* (Mont.) de Bary. Moreover, a comparative analysis between two isolates differing in the degree of virulence, *i.e.* virulent (*vr*) and avirulent (*avr*) *P. infestans* isolate in reference to the potato (*Solanum tuberosum* L.) genotypes equipped with *R3a* gene are planned. The pathogen will be grown in *in vitro* conditions in the presence of two Cu concentrations reflecting moderate (5 mg/L) and sublethal (10 mg/L) HM stress. In the first stage, it is planned to clarify whether and to what extent Cu affects the integrity of *P. infestans* cell walls and membranes. Then the cellular nitro-oxidative status of *P. infestans* under the Cu stress will be recognized. During this step, a reactive nitrogen and oxygen species will be assessed followed by the determination of the activity of key enzymes of the antioxidant system including superoxide dismutase, catalase, and S-nitrosoglutathione reductase. The next step of the planned research will be focused on the detection Cu-dependent nitro-oxidative modifications at RNA and DNA levels in the form of 8-nitroguanine, 8-hydroxyguanosine and 8-hydroxydeoxyguanosine. Moreover, common markers of oxidative DNA damage in the form of AP-sites and double-strand breaks will be assayed in *P. infestans* exposed to Cu. In the last stage of the project, it is planned to determine whether and to what extent the observed changes caused by Cu at the molecular level affect *P. infestans* viability, developmental and pathogenicity-related events in the form of the pathogen ability to cause disease symptoms.

EXPECTED EFFECTS

It is expected that the obtained results will make it possible to verify the research hypothesis assuming that *P. infestans* exposure to Cu (i) results in nitro-oxidative homeostasis imbalance (ii) followed by changes at the RNA and DNA levels, which will affect *P. infestans* (patho)biology related events including disorders in development and pathogenicity.