

Description for the general public

Unfavorable environmental conditions leads to **loss of seed vigor and ability to germination**, what is connected with **accelerating ageing**. This has a negative impact on agriculture, horticulture and biodiversity preservation. Nitric oxide (NO) belongs to plant regulators of bimodal function, which can be described using the model of “nitrosative doors”. Physiological concentration of NO is a “golden key” needed to “open the door” – initiation of signaling cascades. Contrary higher content of this compound starts pathophysiological events including uncontrolled protein and nucleic acid nitration. Ageing of seeds is caused by over accumulation of reactive oxygen species (ROS). Action of these compounds is similar to NO (depends on concentration). An increase of ROS is linked to protein oxidation (formation of carbonylated proteins), oxidation of nucleic acids (generation of 8-hydroxyguanosine) or lipid peroxidation. The results of last published data indicated that some diseases like diabetes or Alzheimer disease are characterized by generation of toxic isomer of tyrosine *meta*-tyrosine, which is non-enzymatically formed under high ROS concentration. This amino acid can be incorporated into protein structure in the place of phenylalanine, what leads to formation of abnormal proteins and disturbance of metabolism. There is no data revealed presence and potential accumulation of *m*-Tyr in seeds, especially during prolong storage or ageing.

The aim of proposed project is to indicate the role of short term (signaling) treatment of NO in improvement of aged seed quality, using as a laboratory model apple (*Malus domestica* Borkh.) embryos isolated from warm stratified seeds. In this case we will focus on analyzing of quantity and quality of carbonylated and nitrated proteins in embryonic axes of warm stratified (natural procedure of seed ageing) and cold stratified (natural treatment leading to break deep dormancy) seeds and in axis of embryos treated with NO after warm stratification. Furthermore, we want to connect proteomic research with estimation of gene expression encoding antioxidant proteins and proteins involved in seed longevity (e.g. L-isoaspartyl methyltransferase involved in repair pathway of damaged proteins or kinase - target of rapamycin, regulating cell proliferation). Moreover our goal is to show the presence and alteration in concentration of *meta*-tyrosine in embryonic axes isolated from warm stratified seeds, and warm stratified seeds shortly treated with NO.

This project will be performed using different techniques of molecular biology, biochemistry and plant physiology. Qualitative analysis of proteins will be done by two dimensional gel electrophoresis (2D) separation after immunoprecipitation (the technique of an affinity purification of proteins using specific antibodies) and further mass spectrometry identification. Quantitative protein estimation will be performed by enzyme-linked immunosorbent assay (ELISA) test using specific antibodies, and molecular analyzes of gene expression - the quantification by real-time polymerase chain reaction (RT-PCR). Estimation of *meta*-tyrosine concentration during ageing of apple seeds and after dormancy removal will be done by high performance liquid chromatography (HPLC), this technique allows to detect very small quantities of the metabolite. Apple embryo quality will be verified after measurement of malondialdehyde (the marker of lipid peroxidation) content, evaluation of DNA fragmentation, measurement of 8-hydroxyguanosine and 8-nitroguanine (molecular markers of nitro-oxidative stress) concentration.

Research hypothesis assumes that short-time treatment of apple embryos isolated from warm stratified seeds with NO improves their ability to germination by alteration of modified proteins (carbonylated or nitrated) quality and quantity, by modification of gene expression and by lowering of *meta*-tyrosine concentration. Obtained data will fill the gap in knowledge concerning molecular mode of action of NO in seed biology, especially in the aspect of the prevention of seed ageing. Estimation of *meta*-tyrosine content and potent accumulation in seeds will allow to create another important marker of seed quality not only used in agriculture but also in seeds collections.