Abstract for the general public

Progressive changes in environmental conditions, which are also the result of human activity, lead to the loss of vigour of seeds and their accelerated ageing. *Orthodox* type seeds, despite the mechanisms that allow them to obtain a deep dormancy state, are exposed to external factors, such as the increase in temperature or changes in the humidity of the ground. Consequently, the problem of seed ageing and biodiversity conservation concerns not only the environment itself (natural seed banks) but also has an economic aspect (negative impact on agricultural production, horticulture, and forestry). As natural ageing occur slowly, various seeds' accelerating ageing techniques are used, described in special protocols. The preservation of seed viability is influenced by reactive species, e.g., nitric oxide (NO). The effect of this regulatory molecule on seeds is described in the model of the "nitrosative door". Characteristic of a given plant species, the optimal NO concentration in the seeds is the "key" to breaking the dormancy and stimulating their germination. In turn, the high – pathophysiological concentration of NO promotes irreversible modifications of basic molecules in cells and, consequently, can lead to the organism's death. The results of studies published in recent years indicate that in the case of progressive loss of seed viability, a NO "deficiency" is observed. The treatment of artificially aged seeds with NO donors has a "remedy" effect. It is proposed that NO modulates the metabolism of reactive oxygen species (ROS), whose ageing effects are widely known, both in plants and animals, including humans. While the scarce information on NO's contribution to the preservation of seed viability mainly concerns the proposed mechanisms of action, there is a lack of data describing changes in the metabolism of this molecule. The most important enzyme associated with NO biosynthesis is nitrate reductase (NR), which during conditions of oxygen deficiency (occurring in the initial stages of germination / ageing) catalyzes the reaction of NO formation. Proteins that modulate NO concentration are also phytoglobins (Pgb), especially class 1 (Pgb1), responsible for transformation of NO into nitrate ions.

The proposed research aims to determine the change in NO's metabolism in the embryonic axes of *orthodox* type seeds subjected to artificial ageing for a different period compared to the NO alterations occurring in the axes of non-aged – dormant - seeds. The experimental material will be the embryonic axes of apple (*Malus domestica* Borkh.) seeds aged from 7 to 21 days (seeds mixed with sterile sand moistened with distilled water, placed at 33 °C). The embryonic axes isolated from dormant seeds, subjected to imbibition in water at room temperature, will serve as the control. Achieving the goal will involve research conducted at the transcriptomic, proteomic and metabolic levels. The project will use molecular biology techniques and standard biochemical and physiological analyses. In project it is planned to determine the level of transcripts encoding NR (*NIA*) and Pgb (*Pgb1*) (quantitative chain reaction of DNA polymerase in real-time – qRT-PCR), electrophoretic separations of protein extracts and labelling with specific antibodies anti: NR and Pgb (Western Blot), measurement of NR activity and total NO-dioxygenase activity of Pgb. Moreover, measurement of the content of metabolites (nitrate and nitrite ions – colourimetric methods) and ATP (bioluminescence) will be performed. Additionally, the goal is to determine the changes in the amount of NO (using the spectrofluorimetric technique) accompanying the modifications in the activity of the basic enzymes involved in anabolism and catabolism of NO.

The research hypothesis assumes that the ageing of the *orthodox* type seeds is accompanied by changes in the number of transcripts and the activity of proteins associated with NO alterations. These changes most likely depend on the duration of the seed ageing treatment and are associated with a modification of the number of metabolites that are either the source of NO or are the product of enzymatic reactions related to the metabolism of this molecule. **We propose, that by converting NO to nitrate ions, Pgb have and ageing-promoting effect, as they limit the availability of NO – molecule that regulates this phenomenon.**

The obtained results will allow supplementing the model of "nitrosative doors" in terms of NO's participation in maintaining the viability of seeds.