

The regulatory redox signaling network in plant cells detects every metabolic imbalance and modulates a rapid response at the level of transcription and translation. As a result, plants can quickly adapt to the changing environmental conditions and produce high-quality seeds. Preliminary experiments indicate that redox state regulation takes place already during seed development and can be closely linked with maintenance of seed viability during long-term storage, when seeds are exposed to viability loss and aging. It is claimed that for the effective conservation of genetic resources it is necessary to identify the causes of aging of seeds that differ in sensitivity to drying and long-term storage [1]. Because of their properties, seeds are divided into 3 categories: orthodox (tolerating drying to a moisture content <7% and storage at -10°C), recalcitrant (sensitive to drying to moisture content <27% and conventional storage conditions), and intermediate (losing viability relatively quickly, as compared to orthodox seeds). Research is conducted in the discipline of biological sciences and forestry. The planned study is aimed to analyze the network of processes of redox state control, involving the thiol proteins thioredoxins (Trxs) in association with NADPH-dependent thioredoxin reductase (TrxR) and peroxiredoxins (Prxs), in germination of seeds differing in sensitivity to water loss and belonging to 3 categories. Redox state is regulated by participation of Trx proteins and the enzyme TrxR in protein S-nitrosylation and denitrosylation. Trxs also participate in the control of Prxs activity. The Trx/TrxR/Prx system is also involved in the detection and transduction of stress signals, and participates in the adaptation of plant tissues to environmental conditions. We expect that the planned experiments will enable verification of 3 hypotheses: (1) Trx/TrxR and Prx proteins (identified by us in tree seeds) co-regulate redox conditions in seeds of orthodox, recalcitrant, and intermediate species in pre-harvest and post-harvest physiology. The activity of the Trx/TrxR/Prx system is the highest in desiccation-tolerant (orthodox) seeds, which can be stored for a long time. (2) The regulation of redox state involving the Trx/TrxR/Prx system takes place already at the stage of seed development and maturation, and affects seed viability after harvest, during storage. (3) The rate of seed aging during storage depends on the activity of the Trx/TrxR/Prx system during storage and is related to respiration rate in seeds and NO accumulation in cells. The research material include seeds of 3 species of trees, differing in sensitivity to water loss. Seeds of Norway maple (*Acer platanoides* L., category: orthodox) tolerate drying to moisture content <7% and storage at -10°C, seeds of sycamore (*Acer pseudoplatanus* L., category: recalcitrant) do not tolerate drying to moisture content <27% and conventional storage conditions, seeds of common beech (*Fagus sylvatica* L., category: intermediate) lose viability relatively quickly, as compared to orthodox seeds. For a detailed description of the network of interactions between the processes that affect redox state, the analyses will be performed at 10-15 time points, representing different developmental stages during seed maturation and in mature seeds during optimum storage and accelerated aging, as well as during germination. In order to achieve the main objective of the research and verify the hypotheses, we plan the following analyses: (1) evaluation of seed viability (viability assay according to ISTA); (2) identification of Trx proteins (Western-blotting, electrophoresis 2D-SDS-PAGE); (3) assays of the activity of TrxR and GSNOR (spectrophotometric methods; immunoblotting); (4) analysis of gene expression levels of Trx, TrxR, Prx, and GSNOR (RT-PCR, qPCR techniques); (5) identification of the target proteins of Trxs (affinity chromatography; mass spectrometry); (6) analysis of the level and location of NO and ONOO- (fluorescence measurements, location using confocal laser scanning microscope, CLSM); (7) determination of total thiol group and SNO content (spectrophotometric methods and electrophoresis SDS-PAGE); (8) assays of S-nitrosylation and denitrosylation of proteins (electrophoresis 2D-SDS-PAGE and mass spectrometry); (9) analysis of the intensity of seed respiration (Seahorse XF Extracellular Flux Analyzer); (10) assessment of oxidative damage DNA to seeds (measurement of 8-oxoG level by ELISA); and (11) statistical analyses of results (ANOVA, Tukey's test, analysis of the linear model). The proposed study is unique, as in contrast to a vast majority of available published studies, it will involve complex

investigations of seeds of 3 categories, differing in physiological characteristics (the planned study is not limited to orthodox seeds). Only such a complex approach can provide answers on the regulation of redox state in seeds and effects of the regulation during their development on seed viability during storage, which determines their quality and usefulness as sowing material. It should be noted that many species important from the ecological and economic standpoint produce seeds that are not orthodox (e.g. oaks, maples, beeches, poplars, citrus fruit trees, avocado). Molecular and enzymological methods will be used, which will be important for progress in in the discipline of biological sciences and forestry, progress seed science and technology, and will contribute to determination of new markers of seed viability, based on modern techniques.