Chloroplasts are characteristic plant organelles in the group of plastids surrounded by a double protein-lipid membrane. The inner membrane creates numerous pouches (thylakoids) which, stacked on top of each other, build structures termed grana. The grana contain chlorophyll, an active pigment, involved in the photosynthesis process, i.e. the transformation of water and carbon dioxide with the energy of sunlight into organic compounds. Chloroplasts contain an intensely green pigment which is most visible in plant leaves from early spring to fall. It is amazing that the seeds of plants produced in light-impermeable coats which darken during ripening can be green, for example peas or broad beans. Seeds are the most important form of plant reproduction and play an important role in their spread. Therefore, knowledge of seed biology contributes to the establishment of proper protocols to ensure the viability of seeds during their storage, in particular for plant species such as trees or shrubs that produce seeds at irregular intervals after several or several dozen years of growth.

Seed longevity is characteristic of seeds that are resistant to extreme dehydration (desiccation) thus termed orthodox (e.g. pine, spruce, Norway maple). In contrast, seeds sensitive to desiccation (recalcitrant category, e.g. sycamore maple, sessile oak) lose their viability very quickly and cannot be stored long-term. Despite the fact that they acquire resistance to desiccation, seeds of the intermediate category (e.g. beech, euonymus) lose their viability during long-term storage. The seeds of some of the mentioned species of woody plants are green, but it is not known whether they have active chloroplasts or not. Studies of the seeds of the model plant *Arabidopsis thaliana*, classified as orthodox, revealed that during seed maturation chloroplasts are first formed and then degenerated or dedifferentiated into primary plastids from which they were differentiated. Other studies have shown that orthodox seeds that retain active chloroplasts show reduced viability. Nothing is known about the fate of chloroplasts in seeds of the recalcitrant and intermediate categories. It will be of particular interest to study this phenomenon in green seeds of woody plants and to relate it to their viability during storage and subsequent aging. The proposed research will determine whether the strategy of preserving or dedifferentiating chloroplasts is related to the resistance or sensitivity of seeds to desiccation and their variation in longevity. There are three strategies for chloroplast dedifferentiation: 1) all chloroplasts can be absorbed into the vacuole by a phagocytic type mechanism (chlorophagy); 2) the components of the chloroplasts can be packed into bodies containing Rubisco (RCB) - vesicles with a diameter of 1 μm which are also transported to the vacuole; 3) stromal proteins or parts of the chloroplast can be transported to the senescence associated vacuole (SAV) located in the cytoplasm. The planned research will help to distinguish which strategy is used in green seeds representing different categories in terms of resistance to desiccation.

Chloroplasts are the site of metabolic processes that generate NADPH - nicotinamide adenine dinucleotide (NAD) phosphate in a reduced form. They are also the site of the main protein regenerating systems for NADPH-dependent reductases. Therefore, the preservation of at least the protein elements of the stroma in seeds would be favorable for the maintenance of homeostasis in reduction and oxidation (redox) reactions. NADPH also occurs in the oxidized form ($NADP^+$) and is formed from NAD^+, which is generated by a *de novo* synthesis pathway and by a salvage pathway. NADH and NADPH are the primary carriers of protons and electrons for almost all redox reactions. Our previous studies have shown that the concentrations of these nucleotides and their redox status contrast orthodox and recalcitrant seeds, hence they are the primary cause of their differently functioning metabolism and antioxidant systems. Therefore, it will be investigated whether the diametrically different concentrations of NAD(P) in orthodox and recalcitrant seeds is the result of different regulation of activity of the two pathways of NAD^+ synthesis or the activity of enzymes consuming and degrading NAD(P). This is extremely important because the NAD^+ precursor in the salvage pathway regulates cell lifespan, suggesting that the lack or low activity of the NAD^+ recovery pathway is related to seed viability, because all biological responses are redox-controlled. NAD and NADP are coenzymes involved in catabolic and anabolic reactions, respectively, shaping the entire metabolism. It will be interesting to investigate changes in metabolites to identify compounds that are potential new indicators of reduced seed viability.

The project aims to explain how green seeds differ in their responses to aging and what mechanisms and which mechanisms are acting to maintain their viability. The characterization of all possible pathways of NAD synthesis / recycling / utilization the origins of depleted NAD(P) levels in chloroembryos displaying poor quality. The discovery of a pivotal role of NADPH in seed longevity is expected. Identification of metabolites and thus gradually disturbed biochemical pathways will reveal a sequence of cause-and-effect events leading to cell death in aging chloroembryos. The expected results will contribute to the progress of science, mainly seed science and forestry, as they will provide knowledge useful for optimizing storage protocols in seed banks.