N^6-methyladenosine (m^6A) RNA modification as a putative metabolic switch between cell survival and cell death in induced barley leaf senescence

One of the major challenges facing the world today is the development of crops with high yields and nutritional value. Crop production is essential for cereal production, which accounts for 60% of the world's arable land. The improvement of the yield of cereals can be achieved by regulating the senescence process, both in the direction of its delay and acceleration. Delaying the senescence of the leaves extends the period of their intensive photosynthesis, which translates into a higher starch content in the grains. On the other hand, acceleration of senescence results in a greater amount of nitrogen in the green mass, which is a desirable feature of animal feed.

In recent years, many scientific works have attempted to describe the epitrancriptome, i.e. reversible RNA modifications, which constitute a dynamic, additional layer of gene expression regulation. The most frequently studied modifications include N^6-methyl adenosine, which appears as a result of the action of methyltransferase enzymes and removed by demethylating enzymes. N^6-methyl adneosine is involved in the regulation of such processes as: transcription, alternative splicing, alternative polyadenylation, nuclear export, stabilization and degradation of mRNA. The association of an increased level of m6A modification with senescence processes in the brain of mice has also been proven. The importance of m6A modification during leaf senescence remains an unrecognized issue.

DILS - our research model is a dark-induced series of transformations at the cytological, biochemical and molecular levels. Selective, senescence-dependent remobilization of macromolecules in this process is accompanied by autophagy mechanisms. The effects of DILS degradation processes are reversible even with advanced autophagy. The effective regulation of senescence processes is a symptom of the vitality of senescence cells, which must maintain homeostasis at every stage. In the model, we defined a critical stage in which it is still possible to reverse leaf senescence and stop cell death, but the mechanism of its control is unknown.

We have conducted studies, the preliminary results of which suggest that during DILS there is an increase in the expression of m^6A methylase enzymes ("writers") responsible for the appearance of m^6A modifications. We also demonstrated the presence of this modification with an appropriate antibody in the material isolated from barley leaves of plants subjected to DILS.

The conclusions from the research contributed to the formulation of the research hypothesis that the post-transcriptional modification of m^6A may constitute as so far undiscovered metabolic switch between the cell's ability to survive and programmed cell death.

The aim of the project is to investigate the epigenetic regulation of leaf senescence. This goal will be achieved through the following research tasks:

1. Analysis of changes in the expression level of selected genes encoding m^6A RNA methyltransferases, m6A RNA demethylase and proteins recognizing m^6A RNA in response to DILS using the qPCR method.

2. Identification of genes regulated by m^6A mRNA modification in response to DILS using the innovative, high-throughput MeRIP-seq method.

3. Quantitative evaluation of the level of m^6A modification in different RNA fractions from plants subjected to DILS by colorimetric analysis.

4. Analysis of the level of m^6A modification in various types of RNA isolated from plants subjected to DILS using the immuno-northern blot technique.

In light of the current research on the improvement of yield and quality of the crop, deepening the knowledge of the regulators of the stress-induced senescence process, as well as the molecular mechanism underlying it, seems to be a priority. The project will deliver new knowledge on epigenetic and epitrancriptomic regulation of the senescence process, enabling the development of environmentally friendly technologies to improve the efficiency of cereal crops.