

Due to the growing population of the world and the global problem of climate change, the main challenge of this century is the development of crops yielding high yields and nutritional values. The improvement in crop yielding efficiency can be achieved by regulating the senescence process, both towards its delay and acceleration. (1) Delaying senescence of the leaves and thereby prolonging their period of intense photosynthesis increases the starch content in the grains. (2) However, in crops intended for animal feed, the level of nitrogen in the green mass and grains can be increased by accelerating the senescence process. Broadening the knowledge on the signaling role of PA should lead to a better understanding of the aging mechanisms in plants and provide new knowledge about autophagy mechanisms, also in organisms with distant systematic positions, on the molecular and cellular level. Polyamines and their analogs due to easy application and no negative impact on the natural environment can be successfully used in agriculture. These compounds can be applied individually or in combinations, as an aqueous solution, administered by foliar application in the form of spraying and/ or to soil to plants after induction of the senescence process. Thanks to the PA application, it is possible to increase the tolerance of crops to stress.

**According to our hypothesis, the interaction of polyamines (PAs) with signaling molecules such as nitric oxide (NO) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) can be part of the NO-PA-H<sub>2</sub>O<sub>2</sub> signal transduction, which conditions the metabolic reprogramming of cells and introduces it to the senescence pathway.**

The senescence process is regulated by the action of endogenous signals, among which hormones are mainly described. It has been shown that the regulators of the senescence process are also polyamines (PA). We postulate that PA participate in signalization in senescence process *inter alia* due to the fact that PA homeostasis in cell is strictly regulated. Changes in PA concentration in properly functioning cell are very unlikely. Induced senescence disturbs their homeostasis, which contributes successively to senescence-dependent metabolic changes. PA catabolism was proposed as a process promoting senescence mainly because H<sub>2</sub>O<sub>2</sub> production. In *Arabidopsis* PA back-conversion oxidase (PAO4(bc)) mutants, which had inactivated PAO4(bc) delayed entering in dark-induced senescence, the consequence of which is reduced production of reactive oxygen species and increased levels of nitric oxide (NO) was found. Recently published paper by Groß et al. (2017) connects PA catabolism (mainly putrescin – Put) with NO synthesis. Sobieszczuk-Nowicka et al. (2016) suggested participation of Put catabolism in senescence-dependent changes occurring in cell. However they considered primarily  $\gamma$ -amino butyric acid (GABA) synthesis from Put. Put catabolism in senescence may lead to NO generating or scavenging towards Put synthesis. Therefore NO-PA-H<sub>2</sub>O<sub>2</sub> may be component of signal transduction propagating initiation of leaf cells to senescence pathway.

In the project developed by us dark-induced barley leaf (cv Golden Promise) senescence procedure will be used (Sobieszczuk i in. 2015, 2016). **In order to accurately verify our hypothesis of PA interaction with signaling molecules such as NO and H<sub>2</sub>O<sub>2</sub>, inhibitors of PA metabolism and NO synthesis will be used.** Broadening the knowledge on the signaling role of PA will lead to a better understanding of the mechanisms of senescence in plants and will allow the development of new agrotechnical methods that can increase the tolerance of crops to stress.

Barley genes of PA metabolism have not been sufficiently known. Thanks to the analysis of *in silico* identification (which we have already done) it is possible to design starters and determine the senescence-dependent changes in the level of PA metabolism gene transcripts such as: *ADC*, *ODC*, *SAMDC*, *SPDS*, *DAO* i *PAObc*, using Real-time PCR. Analysis of the expression of selected genes of control plants will tell us which part of PA metabolism and NO synthesis will be important for further analysis, and thus which inhibitors will be used in subsequent tasks. The aim of the research will be achieved thank to: 1) comparison of phenotypes of plants treated with inhibitors and control plants under stress conditions, using measurements of: chlorophyll *a* fluorescence, the relative content of chlorophyll and flavonoids and morphometric parameters. 2) Analysis of free polyamines using HPLC. 3) Determination of NO and H<sub>2</sub>O<sub>2</sub> level. Cytochemical visualization of NO will be conducted using specific fluorochromes such as DAF-2DA, together with a quantitative measurement by the fluorimetric method. Control preparations will be treated with a NO scavenger (cPTIO). Since the source of NO synthesis in the senescence process has not been defined yet, in addition a pool of nitrites, a direct substrate for NR in NO synthesis will be determined. In addition, NO can be formed from nitrites via a non-enzymatic route. Measurements of H<sub>2</sub>O<sub>2</sub> level will be carried out using a spectrophotometric technique with the use of titanium compounds, as well as a H<sub>2</sub>O<sub>2</sub> cytochemical visualization using a specific fluorochrome DCFH-DA will be conducted.