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**PROJECT TITLE: EPIGENETIC DISSECTION OF INDUCED BARLEY LEAF  
SENESCENCE AND ITS UTILIZATION IN CROP IMPROVEMENT**

**SONATA BIS no 2018/30/E/NZ9/00827**

## 1. Research project objectives/Research hypothesis

Chromatin remodelling through histone tail post-translational modifications, DNA methylation, and ATP-dependent nucleosome reorganization represents a ubiquitous mechanism to regulate gene expression. The epigenetic and epigenomic studies for the regulation of gene expression to a specific stress and the origin of this specificity in crop is still unknown. Genetic modifications have been used for crop improvement, however, using the epigenetic modifications are at their beginning.

The research carried out by the author of this project, allowed to develop a barley crop model for early and late events during the dark-induced leaf senescence (DILS) as well as for deciphering critical time limit for reversal of the senescence process that stops the leaves by reaching cell death phase. We also shown the most evident differences in gene medleys between DILS and developmental senescence that included inter alia DNA modifications active in DILS. This suggests the possibility of a yet-to-be discovered additional switch between cell survival - cell death.

The conclusion from the research contributed to the formulation of the current **research hypothesis: chromatin-remodelling mechanisms in response to induced-senescence as environmental stimuli control the rate of the leaf senescence process by: i) introduce leaf into senescence, (ii) control senescence-dependent remobilization, (iii) enter leaf from senescence into the death phase.**

**The aim of the project is to generate new knowledge about the epigenetic regulation of induced senescence and discuss their potential for crop improvement.**

## 2. Research project methodology

To decipher the comprehensive epigenetic DILS-related processes in barley, as a strategy for survival or cell death, its acclimation role, and the ability to inherit these mechanisms we aim to analyzed the epigenetic mechanism in DILS and also in developmental leaf senescence (DLS). We will achieve the goal by: **(i)** determining DILS- and DLS-associated changes in gene transcripts involved in DNA methylation and postranslational modification (PTMs), with the use of TaqMan Real-Time qPCR. **(ii)** Determining DILS and DLS control via DNA methylation - with the use of reduced representation bisulfite sequencing (RRBS). **(iii)** Determine DILS and DLS control via different histone modification by mapping histone modification and the location of DNA-binding proteins (Chip seq). **(iv)** Developing transgenic barley plants with defects in specific genes involve in either the DNA methylation machinery and/or histone modifiers using the RNAi approach and profiling of transcriptome of wild type plants vs transformants to learn about epigenetic mechanisms crosstalk with the metabolic network that organizes DILS using RNA-Seq followed by NGS. **(v)** To complement the issue and learn whether epigenetic marks in DILS are (1) mitotically inherited (within one generation) and/or (2) meiotically inherited (transgenerational inheritance) we aim: for (1) -

re-green senescing in darkness leaves by re-exposing them to the light, and measure their DNA methylation profile after re-greening, if DNA methylation will be maintained, the leaves will go repeated dark incubation and by phenotyping will be evaluated whether senescence is delay. For (2) - collect seeds from plant that went senescence in darkness and then were re-exposed to light. Grow them and measure their DNA methylation profile, if stress-induced epigenetic modifications will be maintained in F1, the leaves will go repeated dark incubation and by phenotyping will be evaluated whether senescence is delay.

### 3. Expected impact of the research project on the development of science

It is necessary to deepen our investigation of the epigenetic regulators in crops during stress-induced senescence and their underlying molecular mechanism. Understanding the mechanism of epigenetic regulators and their regulatory networks in this process in crops will be a potential tool for further exploitation toward sustainable agriculture (so call epibreeding). Moreover, it is desirable to design new breeding strategies in which the epigenetic variability should be taken into consideration. This seems even more realistic with the advancement of genomic technologies and cost lowering of next-generation sequencing. Like MAS (marker-assisted selection), epigenetic marker-assisted selection could also be initiated.