

## **Spermidine Regulates Dark-Induced Leaf Senescence: eukaryotic translation factor 5A -Dependent and Independent Manner**

In plants, senescence is a highly controlled and active process requiring global metabolic reprogramming, aimed at organized disintegration and remobilization of valuable resources. **It is a fundamental aspect of plant development, necessary to optimize resource allocation and promote phenotypic plasticity to adapt to the environment under restricted conditions.** It has attracted investigations from different perspectives to identify the different players involved. Polyamines (PAs) are polycationic biogenic amines prevalent in all eukaryotes and are necessary for cell survival. The research carried out by our group, allowed to develop a model of PA action on DILS (Dark-Induced Leaf Senescence) program, indicating that the direction of PA metabolism, towards their anabolism or catabolism, plays a central role in metabolic reprogramming, that introduces or not senescing leaf into the programmed organ death.

DILS is a barley crop model for early and late events and identification of the critical time limit for reversal of the senescence process that stops the leaves reaching the cell death phase. The efficiency of regulation of the senescence process is a sign of the vitality of senescing cells, which at each stage must maintain their ability to maintain homeostasis. We have defined a critical moment in the model that determines the point of no return, but the mechanism of its control is unknown.

S-adenosylmethionine decarboxylase (SAMDC) and spermidine synthase (SPDS) gene family transcripts' expression in leaf during senescence undergoes significant changes. We have conducted studies which suggest that one of the SAMDC isoforms and SPDS gene involved in spermidine synthesis are key genes in PA metabolism that may condition this reprogramming. Further, our preliminary studies showed that SPD level raises significantly at the first hours of darkness and the expression patterns of *eIF5A* isoforms together with DHS and DOHH responsible for hypusination differ between dark-induced and developmental senescence.

The findings contributed to the research hypothesis that the spermidine regulates dark-induced leaf senescence in both eIF5A-independent and dependent manner.

**The project aims to fill the knowledge gap about the mode of action of SPD in induced senescence that may represent a hitherto undiscovered metabolic switch between senescence and programmed cell death, another words cell survival and cell death.**

To verify the hypothesis main objectives will be:

- to learn about the spermidine metabolism crosstalk with metabolic network that organizes the senescence process to assess if SPD metabolism will affect the process. All in barley during DILS and recovery (**WP1**)
- to learn about the role of SPD as a molecular sentinel of eIF5A activation by hypusination and eIF5A dependent translome in barley during DILS and recovery (**WP2**)

We will explore the aim by: **(i)** Tilling mutant lines with silenced the rate-limiting SPD biosynthetic genes, (*SAMDC* and *SPDS*) **(ii)** Testing barley SPD mutants for their adaptability to leaf senescence phenomenon using phenotyping: morphological parameters including yield, physiological, biochemical characteristic. **(iii)** Profiling of transcriptome of wild type plants vs mutants to establish dependences in metabolic networks between SPD metabolism and other pathways involved in senescence. **(iv)** Characterization of the molecular machinery responsible for eIF5A-activating hypusination **(v)** Profiling transcriptome vs translome of WT plants vs SPD mutants to provide a "snapshot" of all ribosomes active in a cell in barley leaf during DILS and at recovery. This information determine which proteins are being actively translated vis a vis eIF5A in a senescing cell.

All types of (a)botic stresses, limit plant growth and crop productivity. Thus, it is clear that increasing plant productivity, improving food quality and enhancing agricultural sustainability cannot be ignored. In this regard, research on PAs plays an important role, since reprogramming metabolic switches such that senescence can be altered for a particular pro-growth phenotype or where organ death is enhanced without affecting the energy-use efficiency of plants. Correlations between plant production parameters and PA contents have also been reported. If the key role of PAs in plant development and stress responses is to be better understood, it is important to consider not only the PA pool but also expression of genes encoding enzymes involved in the synthesis and catabolism, and other processes related to PA metabolism. Many of these regulatory mechanisms are broadly conserved in cells, suggesting that the regulation of the PA metabolism is of great importance. The delineation of the roles of PAs in senescence should lead to a better understanding of senescence-related cell death mechanisms and provide new knowledge about senescence and programmed cell death also in mammalian systems at the cellular and molecular level, since PAs are universal bioregulators of these processes across kingdoms.