

Expression of genes is regulated by epigenetic processes and among them a process of histone acetylation has a key role. In general, the acetylation state is related to gene activity, whereas the deacetylation state is associated with gene silencing. Acetylation of lysine residues of H3 and H4 histones is catalyzed by HAT and HDAC enzymes of acetyltransferase and deacetylase activity and the antagonistic function of these enzymes results in condensation/looseness of the chromatin that controls gene expression. **It is believed that histone acetylation among other epigenetic processes controls regenerative potential of plant tissue cultured *in vitro* but experimental evidences supporting this hypothesis are very few.** The aim of the project is to verify the hypothesis about the involvement of histone acetylation in plant regeneration induced *in vitro*. To this end, a **model in plant genomics, *Arabidopsis thaliana* (L.) Heynh and a crop plant, *Hordeum vulgare* (L.), will be studied.** The experiments will be focused on relation between histone acetylation and expression of the genes involved in a process of somatic embryogenesis (SE) induced *in vitro* in culture of *Arabidopsis* explants. The SE process of *Arabidopsis*, which in standard method is induced by auxin treatment, provides a model in studies on developmental plasticity of somatic plant cells. The research conducted in the applicant lab indicated that SE induction can be also promoted on an auxin-free medium supplemented with an inhibitor of histone deacetylases, trichostatin A (TSA). This embryogenic effect of TSA treatment suggests that SE is under histone acetylation regulation. In accordance with this postulate, we found the TSA treatment to stimulate the transcription of the genes of documented key role in SE and accumulation of auxin, a key inducer of embryogenic transition.

The study on a role of histone acetylation in SE induction in *Arabidopsis* will aimed at:

- (1) identification of genes involved in SE which expression is regulated by histone acetylation;**
- (2) determination whether SE induction triggered by 2,4-D treatment is related to histone acetylation;**
- (3) indication of changes in H3 and H4 histone acetylation regulating transcription factor genes of a key role in SE;**
- (4) identification of SE-involved genes encoding acetylases and deacetylases, *HAT* and *HDAC* and analysis of *HAT* and *HDAC* enzymes activity during SE.**

Beside *Arabidopsis*, also a crop plant, *H. vulgare*, of highly genotype-limited capacity for plant regeneration, will be used in the project. **TSA treatment will be applied to improve efficiency of plant regeneration in genotypes with poor regenerative potential *in vitro*. Moreover, homologs of *Arabidopsis* genes that are involved in SE and display histone acetylation-controlled expression will be searched in the barley genome.**

Arabidopsis and *H. vulgare* tissue cultured *in vitro* will be studied with the use of versatile analytical tools of plant genomics including, RNA-seq, ChIP-qPCR, ELISA, immunohistochemical and bioinformatic analysis. The RNA-seq analysis will be used to comparison the transcriptomes representing *Arabidopsis* explants subjected SE induction with use of TSA *versus* auxin treatment. The results of the RNA-seq will enable to identify the SE-involved genes of *Arabidopsis* that are controlled by histone acetylation. In addition, the *HAT* and *HDAC* genes engaged in histone acetylation during SE will be found and the activity of *HAT* and *HDAC* enzymes during SE will be studied with ELISA. The barley homologs of the *Arabidopsis* genes identified in the RNA-seq analysis will be identified with the use of bioinformatic tools and the expression profiles of these homologs will be studied during barley regeneration *in vitro* using RT-qPCR. The changes in acetylation of H3 and H4 associated with the SE induction will be studied at the tissue and gene levels. Accordingly, the spatio-temporal changes in acetylation of H3 and H4 histones during SE will be analysed in: (1) the SE-induced explants (immunohistochemical analyses) and in (2) chromatin regions associated with the transcription factor genes with essential role in SE (ChIP-qPCR analysis).

The research of the project will broaden our knowledge on epigenetic control of the developmental reprogramming of plant somatic cells. The results will have the practical impact on plant biotechnology and might contribute to the improvement of the plant *in vitro* regenerative capacity of the recalcitrant plants as barley via modification of epigenetic processes.