Summary

The research carried out in the presented doctoral thesis concerns the process of somatic embryogenesis (SE). In SE, already differentiated somatic cells undergo embryogenic reprogramming and develop into embryo-like structures capable of forming a complete plant. SE process manifests toti-/pluripotential properties of somatic cells and is a model for studying the molecular determinants of plant cell developmental plasticity.

Studies on SE in a model species for plant genomics, *Arabidopsis thaliana* (L.) Heynh., resulted in the identification of several genes, including those encoding transcription factors (TFs), of a central role in the embryogenic transition of cells. Much less is known how, in response to embryogenic treatment such as auxin treatment, these *TF* genes are controlled by epigenetic processes, including histone acetylation (Hac). In support of the role of Hac in SE, we indicated that explant treatment with a chemical inhibitor of HDAC, trichostatin A (TSA), activated the embryogenic pathway of development in Arabidopsis. Hence, the research conducted in the frame of the presented doctoral thesis aimed to get insights into the role of Hac in the regulation of SE induction. In particular, the involvement of Hac in regulating *TF* genes of the essential function in SE induction (so-called SE-*TF*s), including *LEC1* and *LEC2* (*LEAFY COTYLEDON 1*; *2*), *FUS3* (*FUSCA 3*), *BBM* (*BABY BOOM*), *WUS* (*WUSCHEL*), *AGL15* (*AGAMOUS-LIKE 15*) and *MYB118* (*MYB DOMAIN PROTEIN 118*). The involvement of Hac in the mechanism that regulated target genes for SE-TFs, including AGL15 and AP2 (APETALA 2), in SE was also investigated.

SE was induced in *in vitro* culture of explants, immature zygotic embryos of Arabidopsis, on two different media, EA and ET, supplemented with a synthetic auxin, 2,4-D (5 μM) and TSA (1 μM), respectively. In control culture, explants developed into seedlings on E0 medium without SE-inducers. Different genotypes of Arabidopsis were analyzed, including mutants and transgenic lines. Several genomic methods were used, including ELISA, immunohistochemistry, ChIP-qPCR, and gene expression analyses (RNA-seq and RT-qPCR). Hac was analyzed at H3K9/K14ac and H4K5/K8/K12/K16ac marks and involved Hac analyses at the global and gene-specific levels.

ELISA and immunohistochemical analysis results indicated spatio-temporal changes in Hac accumulation in the SE-induced explants. However, these changes were not specific to the explant areas involved in somatic embryo formation, SAM, and cotyledons. Thus, we hypothesized that rather not global but gene-specific de-/acetylation events limited to less

numerous embryogenic cells of the explant might play a vital role in the Hac-dependent mechanism of SE induction. In support of the role of Hac in epigenetic control of SE-*TF*s, significant Hac enrichment was observed in the chromatin associated with *LEC1*, *LE*C2, *FUS3*, and *MYB118* genes in ET culture, which was accompanied by increased transcription of these genes. It was suggested that in TSA-induced SE, Hac controls the expression of the analyzed SE-*TF*s via modifying chromatin bound to the TSS+300 bp gene fragment. In an auxin-induced culture (EA), despite the increased transcription of SE-*TF*s, no significant changes in Hac levels were found in the analyzed chromatin region. However, these results do not exclude auxin-induced Hac changes in other than analyzed chromatin region or/and lysine positions that control the expression of the SE-*TF*s in EA culture.

The results showed that SE induction was accompanied by the differentiated expression of nine *HAT* (*HAG1*/*GCN5*, *HAG2*, *HAG3*, *HAG4*, *HAG5*, *HAC2*, *HAC4*, *HAC5,* and *HAF2*) and twelve *HDAC* genes (*HDA5*, *HDA6*, *HDA8*, *HDA9*, *HDA15*, *HDA18*, *HDA19*, *HDT1*, *HDT2*, *HDT3*, *HDT4,* and *SRT1*). The results of the ELISA analysis also confirmed the HAT/HDAC involvement in SE induction. Evaluation of the *hat*/*hdac* mutant capacity for SE induction indicated *HAT* and *HDAC* of potentially regulatory function in embryogenic transition. Most of the *hat*/*hdac* lines displayed reduced SE response, implying the positive role of corresponding HAT/HDAC in SE regulation. The exceptions were mutants in *HAG1*/*GCN5* gene (*hag1-5* and *gcn5-1*) and the RNA interference-mediated *HDA19* silencing line (*HDA19:RNAi*), which showed increased embryogenic potential. These observations imply HAG1/GCN5 and HDA19 as negative regulators of SE. Further, it was demonstrated that HAG1/GCN5 and HDA19 might control SE by affecting the expression of SE-*TF*s genes, including repressive (*LEC1* and *LEC2*) and promoting (*BBM*) effect. Moreover, the contribution of HDA19 to Hac-mediated regulation of *LEC2* in SE was indicated.

In addition to Hac involvement in the regulation of SE-*TF*s, the results indicated the contribution of Hac in controlling targets of the SE-TFs during embryogenic response. It was shown that AGL15 TF via the Hac-dependent pathway controls transcription of the *DCL1* (*DICER-LIKE 1*) and *SERRATE* genes that are involved in the biogenesis of microRNA molecules (miRNAs) in SE. Moreover, another SE-involved TF, AP2, was found to regulate the expression of the *TF WUS* gene in SE via a Hac-related mechanism. It was postulated that both AGL15 and AP2 TFs might recruit a repressor complex with HDAC, which results in histone deacetylation and transcriptional silencing of target genes.

In conclusion, the research conducted in the doctoral thesis provided new experimental evidence for the involvement of Hac in controlling *TF* genes of master regulatory role in SE induction. Moreover, the contribution of Hac in the transcriptional regulation of SE-TFs target genes was revealed. Altogether, the doctoral thesis results indicated the essential role of Hac in regulating the complex gene network that controls SE induction. The results also provided future directions for research on the epigenetic regulation of embryogenic reprogramming of the *in vitro* cultured somatic plant cells.