

The project aims to identify cell-specific genetic factors controlling somatic cells' pluripotency during *Arabidopsis thaliana* embryogenic transition. The process somatic embryogenesis (SE) manifests the unique developmental plasticity of plant cells. Totipotency is one of the most desirable features by humans, characteristic for plants and responsible for the ability of a single or a group of cells to restore a fully functional organism. For years, SE provides a useful system in studies on molecular factors determining somatic cells' pluripotency. SE plays a critical role in clonal propagation and is a powerful tool for synthetic seed production, germplasm conservation, and cryopreservation and material for genetic transformation.

However, the fundamental limitation in experimental approaches to identify molecular determinants of SE is the difficulty to separate and analyze exclusively those cells which undergo SE induction from the whole explant. To date, the analyses rely on RNA isolation techniques from cultures where embryogenic cells are only a small percentage. To answer the questions about transcriptomic changes during the formation of pluripotent stem cells during SE induction and somatic embryo development, we will perform the fluorescence-activated nuclear sorting (FANS) to isolate only cells, which undergoing SE.

The embryogenic direct SE culture, in which somatic embryos are produced rapidly and efficiently, will be used (Gaj, 2001). The analysis of several time points during SE culture with the FANS method will give us a unique opportunity to get insights into dynamic transcriptomic changes associated with embryogenic induction. To analyze the transcriptomes of the FANS-isolated nuclei of embryogenic cells, we will apply protocols for a low-input RNA and small RNA sequencing (RNA-seq; sRNA-seq).

To verify the role of candidate genes and small RNA molecules chosen by transcriptomic analyses transgenic lines with modulated activity of relevant genes will be used, e.g., insertional lines, lines with overexpression of analyzed genes, reporter lines, etc. The production of new constructs, crossing plants and genetic transformation to get new transgenic lines needed to perform functional analyses are planned within project.

The project results will extend knowledge of the mechanism controlling somatic cell pluripotency contributing to the progress in many areas of biotechnology and medicine. Implementing the FANS for studies on SE allows for pioneer analyses on specific cell fractions undergoing SE, which will significantly broaden the knowledge of the molecular basis of plant cell totipotency, including economically important species. Identification of the genetic regulators of SE is essential for further progress in plant regeneration protocols and the establishment of effective culture methods for plant micropropagation, production of artificial seed, and genetic modification of plants. Additionally, the comparison planned in the project may be a key element to the ongoing discussion on the convergence of genetic mechanisms involved in zygotic and somatic embryogenesis in plants.