Aims of the project. The project aims to broaden knowledge about auxin signaling in plant cells. Since auxin controls every aspect of plant development *in vivo* and *in vitro*, discovering how auxin regulates gene expression is crucial for further advancing biotechnology and translational genetics. We focus our research on unraveling the regulatory relationships between the transcription factor encoded by *MONOPTEROS* (*MP*) and its controlled target genes during the auxin-dependent somatic embryogenesis (SE) process.

Reasons for undertaking this research topic. Why are we focusing research on *MP*? The *MP's* role in SE is unquestioned because it is highly expressed during SE, active in explant regions where somatic embryos emerge, and *mp* mutants do not regenerate somatic embryos. In low auxin levels, MP interacts via their PB1 domain with BODENLOS (BDL), which recruits proteins responsible for the chromatin condensation and blocking the expression of auxin-responsive genes. In the presence of auxin, BDL is degraded, MP is released and creates a complex with chromatin remodelers, leading to chromatin unlocking and transcription of auxin response genes (Fig. 1). This canonical mode of MP action was valid until 2021 when MP11ir isoform was discovered. MP11ir is a truncated version of MP that doesn't contain the PB1 domain. MP11ir is insensitive to repression by BDL, others Aux/IAA, or itself. Preliminary studies of our teams led to the identification of the MP11ir isoform during the SE process. We confirmed a positive correlation between *MP11ir* transcript level and capacity to SE process. Both MP11ir and MP are necessary to rescue the *mpS319* mutant, but MP11ir/MP may act in different cells. We proved that MP11ir and MP alter endogenous local and global auxin levels during SE processes, which has not yet been discovered. We hypothesize that various genes may be under MP11ir/MP control. However, further global analyses are needed to fully understand the function of MP11ir and MP proteins. Why do we want to study the process of somatic embryogenesis? Because of an increasing human population, ongoing climate change reflected by decreased crop yields, and growing pollution, the importance of plant propagation using *in vitro* techniques, i.e., SE process, is increasing, inter alia, in ensuring food security. However, many plants still cannot be propagated in *in vitro* culture. Therefore, it is necessary to thoroughly explore the mechanism of auxin action, the primary inducer of embryogenic transition, to improve the efficiency of plant regeneration in the recalcitrant genotypes. To achieve the proposed scientific goals, a model system of Arabidopsis embryogenic culture will be examined.

Research description. Thus, the nature of *MP's* action appears more complex in the context of existing different MP isoforms. The proposed research will identify the mechanism of action of the recently discovered MP11ir isoform and MP canonical protein during the SE process (Fig. 1). Preliminary data raise the possibility that the action of MP11ir and MP may differ at the molecular level. Researchers will create an MP11ir/MP genetic network by identifying genes under their direct control during SE using a greenCUT&RUN technique (WP1).

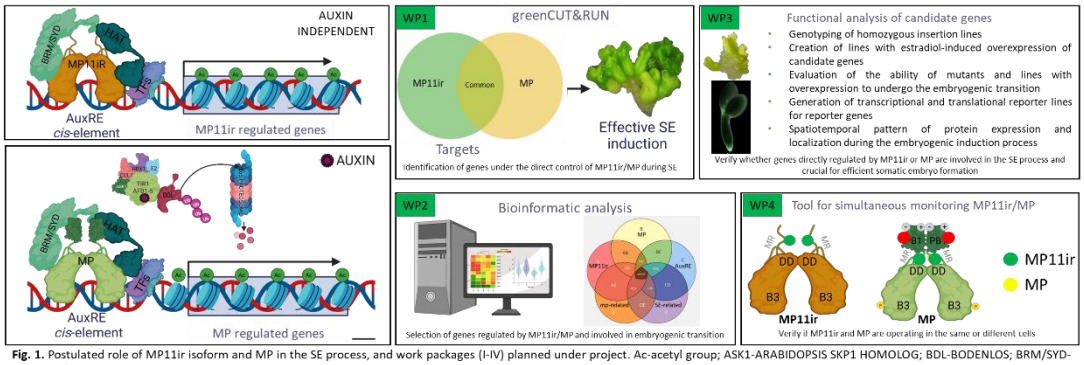

Fig. 1. Postulated role of MP11ir isoform and MP in the SE process, and work packages (I-IV) planned under project. Ac-acetyl group; ASK1-ARABIDOPSIS SKP1 HOMOLOG; BDL-BODENLOS; BRM/SYD-BRAHMA/SPLAYED; CUL1-CULLIN1; E2-UBIQUITIN-LIGASE; HAT-HISTONE ACETYLASES; HDA19-HISTONE DEACETYLASES19; MP-MONOPTEROS; MP11ir-MP isoform; RBX1- RING-BOX1; SE-somatic embryogenesis; TFs-TRANSCRIPTION FACTOR; TIR1/AFB-TRANSPORT INHIBITORRESISTANT1/AUXIN SIGNALING F-BOX; Ub-ubiquitin; Created with BioRender.com

A bioinformatic analysis will find candidate genes with MP11ir- or MP-dependent expression and, at the same time, differentially expressed during embryogenic transition. For this purpose, we will integrate the obtained greenCUT&RUN data with existing SE-related transcriptomic and ChIP-seq data (WP2). To confirm the involvement of candidate genes in the SE process, their expression profile will be analyzed; we will estimate the embryogenic capacity of mutant and transgenic lines with inducible overexpression; we will examine the expression pattern of candidate genes using transcriptional and translational reporter lines during SE (WP3). We will also develop a molecular tool to simultaneously monitor MP11ir and MP in plant cells (WP4). Thus, we will determine whether MP11ir and MP act in the same or different cells during the induction of the SE process.

Main effects of the research. The project will lead to identifying genes directly regulated by MP11ir/MP and the detailed characterization of selected, new genes with a key role in embryogenic transitions. Genes that may in the future contribute to overcoming resistance to plant regeneration in *in vitro* cultures. The proposed analysis will expand knowledge of the importance of alternative splicing and the role of isoforms in plant development. So far, insight into the molecular mechanism of isoform action has been residual.