Research project objectives/Hypothesis:

The presented project aims at analysis of activity of four micro RNA (miR160, miR166, miR167 and miR393) during embryogenic transition induced *in vitro* in somatic cells Arabidopsis.

The involvement of the candidate molecules in the induction of SE was hypothesized considering auxin (mostly 2,4-D) as a main inducer of embryogenic response of somatic cells under *in vitro* culture in various plant species including a model plant, Arabidopsis. Candidate miRNAs are involved in direct or indirect regulation of auxin regulatory pathway. miRNA160 and miR167 regulate expression of *AUXIN RESPONSE FACTORS* (*ARF6, ARF8, ARF10, ARF16, ARF17*) genes, while miR393 target the transcripts of genes encoding auxin receptors belonging to TAAR family: TRANSPORT INHIBITOR1 (TIR1) and AUXIN F-BOX PROTEINS (AFB1, AFB2, AFB3). miR166 regulate expression of *PHABULOSA* and *PHAVOLUTA*, positive regulators of *LEAFY COTYLEDON2* (*LEC2*) - genetic marker of SE involved in biosynthesis of endogenous auxin.

Research methodology:

In the presented study, a model plant of functional genomics, *Arabidopsis thaliana* (L.) Heynh. and the sensor lines produced in this project, will be used. The candidate miRNA to be analysed include four miRNAs molecules: miR160, miR166, miR167 and miR393, encoded in Arabidopsis by sixteen *MIRNA* genes. To verify the hypothesis on the involvement of the candidate miRNAs in the process of embryogenic transition of somatic cells of Arabidopsis, the transgenic sensor lines will be produced carrying a GFP-sensor construct. The sensor construct will enable detection of the activity of the studied miRNA in embryogenic cells. To this end, the target sites specific for the analysed miRNA will be placed between *LEC2* promoter of SE-associated expression and *GFP* gene sequence. In addition, analysis of the GFP signal in the sensor line-derived cultures will lead to the knowledge of the spatiotemporal pattern of miRNA expression in different stages of SE including: (i) explants subjected to the embryogenic induction; ii) the derived embryogenic culture and iii) the developed somatic embryos. The sensor lines produced in the project will provide us a unique analytical tool for the first such validation a role of the miRNAs during SE induced in somatic cells of Arabidopsis, a model plant in functional genomics of plants. SE will be induced under *in vitro* culture of immature zygotic embryos of Arabidopsis following the effective method of SE induction developed and successfully applied in the laboratory of a project supervisor. Analysis of spatiotemporal pattern of miRNA molecules activity will be carried out using a confocal microscope and optical sections on the surface or in the depths of explants. In order to verify the regulatory relations between miR160, miR166, their target genes (*ARF10, ARF16, PHB, PHV*) and *LEC2*, transgenic lines with impaired activity of relevant *MIRNA* genes and their targets will be used. The impact of the mutations in the studied genes on the embryogenic potential of the *in vitro* cultured explants will be analysed

Research project impact:

The results of the project will extend the knowledge of the genetic mechanism triggering the embryogenic program of development in somatic cells of plants. Numerous lines of evidences suggest the essential role of miRNA molecules in this mechanism. Taking into consideration that miRNA sequences are highly conserved in plants, the identification of the genetic determinants of embryogenic induction in Arabidopsis, will significantly broad the knowledge of molecular basis of plant cell totipotency, including economically important species. Moreover, identification of the key genetic factors involved in SE significantly stimulates the progress in plant biotechnology. Identification of the genetic regulators of SE is basic 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.