The goal of this project is to characterize the nine novel barley miRNAs which have been identified in the Department of Gene Expression.

Barley is one of the most important cereal in the world. Barley ranks 4th in terms of global production right after maize, rice and wheat. Species grown today come from a wild cultivar - *Hordeum vulgare ssp. Spontaneum* that domestication occurred about 10 000 years ago in the area of the Nile Delta, known as the Fertile Crescent. Today, barley is a fodder corn and a leading grain in brewing.

MiRNAs are small molecules typically 21 nucleotides in length, which when incorporated into the protein complex RISC (RNA-induced silencing complex) regulate gene expression at the transcriptional and posttranscriptional level. The first plant miRNAs have been identified over a decade ago in the course of study on model organism *Arabidopsis thaliana*. As early as then, researchers speculated on the importance of miRNAs in plant development. Their assumptions have been confirmed since then, and the study of miRNAs extended to other species, including crops. Currently there are 71 mature miRNAs identified in barley. This number appears to be small when compared to the other cereals, in which miRNAs can be counted in hundreds. **That fact leads to the conclusion that the presence of many barley miRNAs remains undetected, and thus many regulatory mechanisms unknown. Our goal is to complement these differences and to identify and characterize previously undescribed miRNAs in barley and the processes governed by them.**

To achieve this goal we first conducted deep sequencing of sRNA libraries from five barley development stages and, in cooperation with Prof. Wojciech Karłowski form the Department of Computational Biology, we obtained ranking list of potentially novel miRNAs in barley. The presence of nine of them was confirmed by Northern hybridization. Then using the public databases we determine the structures of their precursors (pre-miRNAs) and explored expression levels of pri-miRNAs in five stages of barley development and in flower organs. We observed a high level of primary transcripts at the spike stage and interesting fluctuations of expression in flower organs. We especially focused our attention on those pri-miRNAs which expression was significantly increased in the stamens, suggesting the involvement in the function and development of this organ. We also looked at the counts of reads for our miRNAs in sRNA libraries derived from barley treated with drought stress. We noticed significant quantitative changes for some miRNAs between mild and severe stress conditions. These results led us to hypothesize that novel miRNAs are not only involved in the development of barley, especially at the stage of flower formation but also in response of this plant to environmental cues. To complete our characterization in the course of this project, we plan to analyze the expression of the mature miRNAs with a use of RT-qPCR with TaqMan probes and also we plan to analyze their expression in different environmental stresses using Northern blot hybridization. In addition, we want to identify target genes for all miRNAs studied, by analyzing degradome data. We will also learn about the structure of novel miRNA genes and determine the possible post-transcriptional regulation of miRNAs transcripts. The final step in our project would be to choose the most interesting for barley development miRNAs and prepare transgenic plants with miRNA abolished function in order to define received phenotype and ultimately confirm the metabolic pathways regulated by novel miRNAs in barley.