Description for the general public

The goal of this project is to characterize the function of proteins or lncRNA (bioinformatically identified in the Department of Gene Expression) encoded by microRNA genes from *MIR444* gene family in barley.

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. The oldest known evidence of barley cultivation comes from the Middle East and is dated to the VII millennium BC. Today, barley is a fodder corn and a leading grain in brewing.

MicroRNAs are small molecules, usually 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. MicroRNAs from the *MIR444* gene family are special interesting, becasue they are present only in monocots. Preliminary results of a studies carried out by the Department of Gene Expression have shown that there are three *MIR444* genes in barley genome. It was shown that primary transcripts (pri-miRNAs) of these genes undergo alternative splicing events generating multiple isoforms that can be divided into two classes: functional (miRNA can be produced) and non-functional (microRNA cannot be produced). Bioinformatic analysis revealed that all pri-miRNAs contain open reading frames (ORFs). Using the polysome profiling technique, we revealed that some isoforms are functionally associated with ribosomes in the root under conditions of nitrogen excess. In addition we confirmed the presence of 168-aa protein (derived from *MIR444c* gene) in *Escherichia coli* using Western Blot. Recent literature reports reported that plant pri-miRNAs can encode proteins that stimulate microRNA biogenesis (Lauressergues i in., 2015). In the light of these data, our preliminary results indicate a new, non-identified yet, pri-miRNA function in barley.

The project is aimed to the experimental verification of the hypothesis that that primary microRNA transcripts from *MIR444* genes are exported to the cytoplasm, where they can be translated like mRNA or act like long non-coding RNAs. We plan to transform barley pollen in order to produce transgenic plants with deletion of the sequences encoding selected proteins (using the CRISPR/Cas9 technique) and with overexpression of genes encoding identified proteins. For this purpose we will use constructs prepared in cooperation with The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). We will observe plant growth as well as analyse the level of expression of candidate genes under control and nitrogen excess conditions. We will check whether the identified proteins have an effect on microRNA biogenesis (small RNA sequencing experiment). Transgenic lines with overexpression of genes encoding selected proteins will be used for a co-Immunoprecipitation (co-IP) experiment. Based on that, we will identify proteins interacting with selected proteins. Moreover, we plan to analyse transgenic plants response to the stress of nitrogen excess.

The results obtained during this project will let us determine the function and biological role of proteins encoded by *MIR444* genes in barley and in considerable way will contribute towards understanding developmental processes of such important plant which is barley.