

### **Description for the general public**

MicroRNA are key regulators of gene expression. In plants they control developmental processes as well as response to environmental cues. They are negative regulators of gene expression and they are often called “quick reaction forces” of a cell in response to internal and external stimuli.

MicroRNA444 family members were found exclusively in monocots and their expression profile and functions are not well known. In rice miR444 targets MADS box57 transcription factor (TF) mRNA (transcription repressor) and controls indirectly the level of DWARF14 protein. DWARF14 is a negative regulator of tillering in rice. Our preliminary results concerning the characterization of MIR444 family in barley resulted in the discovery of three *MIR444* genes that we temporarily called *MIR444.1*, *MIR444.2*, and *MIR444.3*. **The aim of this project is to reveal the function of all MIR444 family members in the regulation of barley development.** We discovered, that in 2-3 week old plants only miR444.1 is induced in heat stress. Simultaneously barley plants stop to produce new tillers. **The goal of the project is to reveal the new genes involved in metabolic pathway initiated by miR444.1 leading to tillering inhibition in heat stress.** We constructed transgenic barley plants with silenced MADS box 57 expression and with overexpression of MADS box57. Our results obtained so far point to MADS box57 TF as a regulator of tillering in barley upon heat stress. In the transgenic plants we will test the number and quality of tillers and identify genes which expression is affected. The results will allow us to construct a model of plant response to heat stress with the MADS box57 TF as a genetic interactions hub within this network.

The target for the second member of MIR444 family – miR444.2 remains unknown. We will perform experiments (e.g. results analysis of so called barley RNA degradome sequencing data obtained with high-throughput techniques) to identify a potential target for this miRNA.

We found that mir444.3 targets mRNA of another MADS box transcription factor that we called temporarily MADS box3. Functions of this TF remain unknown. We intend to construct analogous as described above barley transgenic plants and perform similar experiments to explain the role of this MADS box 3 TF and to understand the role of miR444.3 in the regulation of its level in barley.

**We also intend to study the regulation of expression of all MIR444 family members. All these genes have complex exon-intron structure. The most exciting observation is that in all barley MIR444 family genes microRNA and microRNA\* and located within neighbour exons separated by a long intron. We will test whether splicing and alternative splicing regulate the level of mature miR444 species in various growth conditions.** To obtain this goal we will use RT-PCR and RT-qPCR as well as NGS sRNA sequencing using RNA isolated from barley plants grown in control and selected abiotic stress conditions.

Results of this project will contribute to a better understanding of barley development. Barley belongs to the most economically important crop plants. Results will be published in highly visible peer-reviewed international scientific journals. They have also applicable potential because of the discovery of a new metabolic pathway leading to tillering inhibition in heat stress. There is a possibility to develop new modern breeding technologies to improve barley plants and minimize the grain yield loss during heat stress.