Rola i regulacja na poziomie transkrypcyjnym i potranskrypcyjnym nowo zidentyfikowanych jęczmiennych mikroRNA z rodziny miR444
(The role and transcriptional/posttranscriptional regulation of newly identified barely microRNAs from miR444 gene family)

MicroRNAs are small eukaryotic molecules that regulate gene expression at the posttranscriptional level. They inhibit gene expression via targeting a RISC complex to a complementary mRNA and executing mRNA cleavage or translation inhibition. Thus, they are negative regulators of gene expression. The regulatory roles of miRNAs have been demonstrated in plant development, signal transduction, and protein degradation. Recent studies also have shown a crucial regulatory role of microRNAs in plant response to environmental cues.

Barley is an economically important monocotyledonous crop plant. However, little is known about barley miRNAs, their precursors and *MIR* gene structures. Our recent studies revealed the existence of three barley *MIR*444 genes from miRNA444 family that are monocot-specific (*MIR*444.1, *MIR*444.2, and *MIR*444.3). In all cases miR 444 is separated from its miR* by a long intron that must be spliced to allow the precursor to form a stem and loop structure containing miR444 and its cognate miR* in its stem part. Additionally, pri-miR444.1, pri-miR444.2, and pri-miR444.3 undergo extensive alternative splicing generating non-functional and functional isoforms of fully spliced pri-miRNAs. For miRNA 444.1 and miR444.3 we identified target mRNAs. These are transcription factors (TFs) from MADS box family (MADS 57 and MADS.3, respectively). However, for the miR444.2 we did not find any target mRNA. Moreover, genes encoded identified target mRNAs are encoded in the same locus at the opposite DNA strand. Our analysis also showed that miR444.1 is expressed in roots and aerial parts of the plant and is heat induced. During the heat stress target mRNA of the MADS 57 TF is strongly downregulated. A known phenomenon is that high temperature inhibits barley tillering. Tiller formation is a very important agronomic trait connected to grain yield. An orthologue rice MADS 57 TF is known to regulate rice tillering via repression of a DWARF 14 protein. Our experiments show that miR444.1-controlled downregulation of the MADS 57 TF mRNA is responsible for the upregulation of barley DWARF 14 mRNA and inhibits tillering.

Nothing is known about the expression regulation and function of barley miR444.2 and miR444.3. The aim of this study is to reveal the role and expression pattern of these two microRNAs. For this purpose RNA will be isolated from different barley developmental stages and organs to follow the expression pattern of both microRNAs. Analysis of promoter sequences of the *MIR*444.2 and *MIR*444.3 aiming to identify various stress responsive elements will be carried out. Appropriate abiotic stress conditions will be tested to examine if these molecules are stress responsive. In all stresses tested analysis of functional and nonfunctional pri-miR444.2 and pri-miR444.3 will be carried out using qRT-PCR. This will allow to reveal how alternative splicing is important for the final miR444.2 and miR444.3 product accumulation. Target mRNA for the miR444.2 is not known. We will use our degradome data to search for the miR444.2 target. Paralelly the level of known target for miR444.3 (MADS.3 TF mRNA) will be monitored. Since MADS 57 regulated by the miR444.1 affects important developmental traits in barley it will be also interesting to find out what is the biological role of the MADS.3 regulated by the miR444.3. Transgenic barley plants will be

produced with silenced MADS.3 gene using RNAi strategy. The Department of Gene Expression has expertise in barley embryo transformation. Silenced plants will be carefully examined for their phenotypic traits in control and selected abiotic stress conditions. Moreover, a transgenic barley plants carrying MADS.3 TF cDNA fused to c-Myc tag will be produced. In these lines CHIP experiments will be carried out and immunoprecipitated DNA library will be constructed and deep sequenced to identify MADS.3 regulated genes. If target mRNA for the miR444.2 will be find analogous experiments will be performed.

All these experiments will allow to decipher the role of the miR444 gene family in the regulation of barely plant development and response to environmental cues.