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**Thesis Topic: Identification of novel and ABA-regulated miRNA and characterization of its target gene-*AtBro1* upon growth and abiotic stress response in *Arabidopsis thaliana***

(Temat pracy: Identyfikacja nowego i regulowanego przez ABA miRNA oraz charakterystyka jego genu docelowego - *AtBro1* w procesach wzrostu i w odpowiedzi na stres abiotyczny u *Arabidopsis thaliana*).

Crop growth and productivity can be perturbed by multiple environmental factors, such as climate change, which are associated with higher ambient temperatures, increased salinity and harsh drought. The core abscisic acid (ABA) signaling pathway regulates plant growth and development by controlling gene expression. ABA also affects the abundance of several microRNAs (miRNAs), which control downstream genes in plants. miRNAs are involved in various biological processes, including adaptive responses to abiotic stress.

The first aim of my doctoral study was to identify novel miRNAs involved in the response to ABA; such ABA-responsive miRNAs were identified by small-RNA sequencing in wild-type (WT) *Arabidopsis thaliana*, as well as in *abi1td*, *mkkk17* and *mkkk18* mutants. I identified 10 novel miRNAs in WT after ABA treatment, while in the *abi1td*, *mkkk17* and *mkkk18* mutants, three, seven and nine known miRNAs, respectively, were differentially expressed after ABA treatment. One novel miRNA (*ath-miRn-8*) was differentially expressed in the *mkkk17* mutant. I validated the sequencing results by quantitative RT-PCR of several known and novel miRNAs in all genotypes and predicted potential target genes of the miRNA panel using psRNATarget. Of the predicted targets of novel miRNAs, I verified cleavage sites in target genes *AT1G73390* (encoding a Bro1-like domain-containing protein) for *ath-miRn-1*, *AT5G40550* (*SGF29b*) for *ath-miRn-2*, *AT3G14070* (*CAX9*) for *ath-miRn-4*, *AT1G56650* (*MYB75*), *AT5G58490* for *ath-miRn-6*, *AT3G15570* (encoding a phototropic-responsive *NPH3* family protein) for *ath-miRn-8*, and *AT2G29140* (*PUM3*) for *ath-miRn-9* using 5' RLM-RACE. Gene Ontology analyses showed the potential target genes of ABA-responsive known and novel miRNAs to be involved in diverse cellular processes in plants, including development and stomatal movement. These results suggest that a number of the identified miRNAs have important roles in plant responses to environmental stress and might have common regulatory functions in the core ABA signaling pathway.

The next part of the study focused on characterization of the novel miRNA target gene, *AtBro1* (initially known as *AT1G73390*; see above), and its role in the response to abiotic stress in *Arabidopsis*. *AtBro1* was upregulated in plants treated with salt, ABA and mannitol. *AtBro1*-overexpression lines demonstrated robust tolerance to drought and salt stress. Furthermore, ABA stimulated resistance responses in a loss-of-function *bro1* mutant and *AtBro1* positively regulated drought resistance in *Arabidopsis*. When the *AtBro1* promoter was used to drive the  $\beta$ -glucuronidase (GUS) gene in transgenic plants, GUS was expressed mainly in rosette leaves and

floral clusters, especially in anthers. Introduction of an AtBro1-GFP fusion protein construct into Arabidopsis protoplasts showed that AtBro1 protein is localized in the plasma membrane. Global RNA-sequencing analysis showed that early transcriptional responses prompted by ABA treatment exhibit specific quantitative differences at different time points, suggesting that ABA stimulates resistance responses in *bro1* mutant plants. Additionally, transcript levels of *MOP9.5*, *MRD1*, *HEI10* and *MIOX4* were altered in loss-of-function mutant plants in response to different stress conditions. Collectively, it can be concluded that AtBro1 likely plays a significant role in the regulation of the plant transcriptional response to ABA and the induction of resistance responses to abiotic stress.

In conclusion, the results presented have allowed the identification of new mechanisms regulating the response of plants to abiotic stress and related to ABA signaling. The most important result is the identification of 10 unknown miRNAs and a deeper characterization of the function of one of them - ath-miRn-1 - in connection with the identification of a new effector in the plant response to stress - the previously unknown BRO1. The results of this work will shed light on the mechanisms underlying stress tolerance in plants.

**Key Words:** Abscisic acid, abiotic stress, novel miRNAs, AtBOR1, seed germination, Arabidopsis