

Copy number variation (CNV) refers to differences in the genomic sequence of individuals of the same species, resulting from the gain or loss of certain DNA segments by duplication or deletion, respectively. These segments often overlap protein coding genes. Additionally, numerous studies indicate that in humans, microRNA genes are also present in the polymorphic (copy number variable) DNA segments. These genes are hereafter named CNV-microRNA genes.

MicroRNAs are small RNA molecules that are not translated. Their function is the post-transcriptional regulation (silencing) of certain protein genes' expression. In this way, microRNAs participate in the regulation of numerous cellular processes. Duplication or deletion of the microRNA genes, just like in the case of polymorphic protein coding genes, may lead to a change in their expression level and consequently to the increase or decrease in functional mature microRNAs. As a result, differences in the number of copies of the CNV-microRNA genes can translate into the diversification in expression of their protein coding targets and this in turn may result in phenotypic differences. Indeed, in humans, certain CNV-microRNA variants have been linked, inter alia, to various types of cancer, male infertility, or mental disorders.

In plants, so far, variation in the number of copies of the microRNA genes has not been studied. Taking into account the involvement of these small, non-coding RNAs in controlling both growth and development processes as well as in response to stress, the copy number polymorphism of microRNA genes may affect any stage of plant development and contribute to plant adaptation e.g. to environmental conditions. Therefore, the main goal of this project is to investigate the extent to which CNV affect microRNA genes in the population of *Arabidopsis thaliana* model plant. Furthermore, it will be determined, whether the protein coding genes, regulated by the CNV-microRNAs identified within this project exhibit differences in expression levels that are correlated to differences in the number of microRNA gene copies .

*A. thaliana* is a well suited model for performing the scheduled research tasks. For this species, extensive sequencing data are available, including genomes, transcriptomes and epigenomes for over a thousand of *A. thaliana* natural lines (ecotypes). By the analysis of this genomic data we were recently able to identify a group of microRNA genes that are potentially copy number variable. By utilizing the CNV genotyping methods that we previously optimized for *A. thaliana*, we will be able to experimentally verify computational predictions and obtain a list of confirmed CNV-microRNA genes in this species. For these CNV-microRNAs, population-based analyses will be performed using the MLPA (multiplex ligation-dependent probes amplification) to evaluate the level of polymorphism in a large population (about 1,000 different ecotypes to be tested)

The final step in the analysis will include bioinformatics and experimental evaluation of the CNV effect on the target genes regulated by CNV-microRNAs. The special focus will be put on targets of microRNAs present in some ecotypes but absent from the others. Performing this task will allow us to get some conclusions about the existence and significance of regulatory dependences between such microRNA-target pairs. Consequently, it may contribute to identification of the true target genes for relatively young microRNAs, which role or functionality, remains unconfirmed. The target gene expression assay will cover a number of plant tissues and will be made using the ddPCR technique that allows a precise measurement of the absolute number of molecules of interest (in this case cDNA) in the sample. In addition, publicly available transcriptomes sequencing (RNA-Seq) data for the *A. thaliana* ecotypes will be analysed.

The results of this project will shed light on the previously unexplored CNV-microRNAs in plants. By determining CNV effect on altering the target genes' expression, it will be possible to further conclude about the microRNA functions and the significance of their polymorphism for the adaptation and evolution of *A. thaliana*.