MicroRNAs (miRNA) are a class of small non-coding RNAs that regulate gene expression in eukaryotes. MiRNAs are crucial regulators of plant development as well as responses for biotic and abiotic stresses. In plants, complete blockage of miRNA biogenesis is lethal. Whereas disrupted miRNA biogenesis lead to strong pleiotropic defects. Primary miRNA precursors (pri-miRNAs) have hairpin stem loop structures in which miRNA sequences are embedded. In the first step of miRNA biogenesis stem loop structures are cut out from pri-miRNAs and form shorter precursors called pre-miRNAs. In the second step, short miRNA/miRNA* duplexes are produced. These short miRNA perform their functions as negative regulators of gene expression. **In this proposal we will investigate a role of three DEAD-box helicases in remodeling hairpin stem loop structure to prevent microRNA biogenesis.**

The proposal is divided into 8 tasks in which we plan to verify this hypothesis using genetic and molecular approaches. During these 8 tasks, we plan to prepared Arabidopsis mutants of studies helicases as well as transgenic plants with overexpression of study helicases. Next, in these plants, we will check global changes in mature miRNAs as well as pri-miRNAs level using high-throughput methods. Moreover, using special sequencing method (DMS-MaPseq) we plan to investigate the influence of DEAD-box helicases on the secondary structure of miRNA precursors.

There are several reports which have shown that DEAD-box helicases influence miRNA biogenesis. However, there are no reports describing how DEAD-box helicases and its helicase activity affect miRNA biogenesis. In this project we would like to focus on the above issue and investigate if DEAD-box helicases can remodel pri-miRNA secondary structure to affect miRNA biogenesis.