Symbiotic nitrogen fixation in legumes is important for food production and sustainable agriculture. It takes place in root nodules – specialized organs that develop upon perception of symbiotic bacteria (rhizobia) and become colonized by them. Temperate legumes including *Medicago truncatula*, which is a close relative to alfalfa, form indeterminate nodules, capable of constant growth, due to persistent presence of apical meristem. In indeterminate nodules, several specialized zones can be distinguished, like infection zone, where plant cells are being entered by rhizobia, interzone, where both plant and bacterial cells undergo differentiation and nitrogen fixing zone, where rhizobia in their terminal form of bacteroids fix atmospheric nitrogen and supply it to plants, in exchange for carbon sources. Thanks to symbiotic nitrogen fixation, legumes are capable of growing on soils with low abundance of mineral and organic nitrogen and fertilize them.

Although nodule development begins with mutual recognition of symbiotic partners and relies on simultaneous differentiation of bacterial and plant cells, spontaneous nodule formation in absence of rhizobia has been observed in *Medicago* and *Lotus* mutants, with deregulated plant signaling pathways. This indicates that nodule development is mainly under plant control. Therefore, understanding the molecular mechanisms that control the nodule developmental program is of high importance, because it may also increase our general knowledge about plant organogenesis.

It has been shown previously that expression of some genes important for nodule development is epigenetically controlled. More specifically, genomic DNA in regions harboring these genes is highly methylated in roots, but becomes demethylated in nodules. It is assumed that increased expression of these genes in nodules compared to roots, is an outcome of DNA demethylation. One of the primary role of DNA methylation is to silence transposable elements (TEs) - genetic elements present in the genome that have the ability to cut and insert or copy themselves to various locations in the genome. Interestingly, regions activated by DNA demethylation in nodules also contain numerous TEs. It is however not clear, whether their presence contributes to the activation of gene expression and how precisely it is controlled by the cell. It is also not known what are the genome-wide patterns of genomic DNA methylation in roots and nodules, apart form few studied regions, that together constitute only ~2% of the *M. truncatula* genome.

To better understand the role of reprogramming DNA methylation during nodule development, we plan to bioinformatically establish genomic localization of TEs and identify structural differences between the genomes of ~300 *M. truncatula* lines, using a wide range of genomic datasets from high throughput sequencing, available in public databases. We will then select 3 highly diverse lines and establish their genomic sequences and DNA methylation patterns, both in roots and nodules. We will also analyze transcriptomes in these organs. We will identify differentially methylated regions and evaluate whether links between methylation changes and gene activation/repression exist. We will also verify, whether natural changes in TE insertion sites affect the distribution of differentially methylated regions in the genome, thus contributing to variation at gene expression in nodules. By combining all the results obtained in this project we will increase our knowledge about *M. truncatula* intraspecies variation, discover possible new regulatory roles of genomic TEs, and get insight into the genetic mechanisms directing the complex process of nodule formation, during which the expression of hundreds of genes is altered.