The main goal of the project is implementation of new genetic engineering techniques in barley for functional studies of genes controlling important agronomical traits. The research will be performed using the pioneering technology CRISPR/Cas (clustered regularly interspaced short palindromic repeats), which has not been applied to barley yet. This technology uses the directed mutagenesis process which allows to introduce mutations to virtually any gene with known sequence. By introduction of such mutations it is possible to block or change the function of a certain gene. The CRISPR/Cas system will be used to obtain plants with altered genotype and phenotype by induction of site-directed mutations in two genes, HvCKX1 and Nud, determining important agronomical traits.

The main research object in our project will be HvCKX1 gene in barley which encodes cytokinin oxidase/dehydrogenase enzyme. The CKX enzyme catalyzes the degradation of cytokinins, important group of plant hormones controlling many growth and developmental processes. In cereal species, the cytokinin hormones are important factor influencing seed yield. The research will be performed to better understand the physiological processes occurring in developing kernels under influence of cytokinin hormones. Using the CRISPR/Cas technique, the loss-of function mutation will be introduced to the HvCKX1 gene. The resulting physiological changes occurring in plants will be observed. We expect that as a result of HvCKX1 mutation, the activity of CKX enzyme will be reduced. Consequently, the increased cytokinin level in developing kernels can lead to a higher kernel mass and higher seed yield.

The second of barley genes that will be studied is Nud which controls the adhesion of husks to caryopses. In contrast to the most barley cultivars, plants carrying mutation in Nud gene form hulless (naked) caryposes. Because of known function and a distinct phenotypic effect of mutation, the Nud gene have been chosen to assess the effectiveness and efficiency of CRISPR/Cas system in barley.

The integral part of the project is also the analysis of specificity of CRISPR/Cas system which can be assessed by identification of a potential off-target mutations occurred in other than intended gene.

The CRISPR/Cas based genome editing technology has been developed for three years. The preliminary studies were performed mainly in model plant species such as Arabidopsis thaliana and tobacco. Therefore, it is necessary to expand these studies to crop plants where the CRISPR/Cas technology can be applied as a research tool in functional genomics or to obtain modified plants with desirable agronomic traits. The CRISPR/Cas technique can be an effective support for costly and time-consuming conventional breeding methods and may greatly contribute to agricultural progress.