CRISPR/Cas9 targeted mutagenesis technology allows precise genome editing and is therefore widely used in plant functional genomic studies. *Brachypodium distachyon* is a model organism for cereals and temperate grasses due to a number of beneficial biological features as well as an ever-growing repertoire of experimental tools and resources linked to this species. Another representative of the *Brachypodium* genus is the allotetraploid species *B. hybridum*, which is mainly used to study phenomena typical for allopolyploids.

The main purpose of this work was to optimise the methodology for editing *B. distachyon* and *B. hybridum* genomes using CRISPR/Cas9 system. Three types of vectors were used, two of which had only one site to clone the gRNA sequence, and one to enable the simultaneous editing of two genes. Transformation of *B. distachyon* and *B. hybridum* was performed using *Agrobacterium tumefaciens* on embryogenic callus induced from immature embryos.

Brachypodium distachyon mutants in *PDS*, *CDKG1* and *CDKG2* genes as well as *B. hybridum cdkg1* and *cdkg2* mutants were obtained. Mutations were detected using PCR combined with restriction digestion and confirmed by DNA sequencing. Small indels were usually observed, which resulted in frameshift mutation which brought a premature STOP codon into frame and, therefore, the formation of a shortened, non-functional proteins.

The *PDS* gene in *B. distachyon* was targeted as it encodes a phytoene desaturase in the chlorophyll biosynthesis pathway and its knockout leads to an albino phenotype. The use of this phenotypic marker enabled the initial assessment of the efficacy of the CRISPR/Cas9 technology in genome editing in *Brachypodium*, initially focusing on *B. distachyon*. The derived albino phenotypes confirmed the CRISPR/Cas9 approach was successful.

At the final stage of the study, cytomolecular analysis of chromosome pairing during meiotic division was performed in the wild-type plants as well as CRISPR/Cas9 *cdkg1* and *cdkg2 B. distachyon* and *B. hybridum* mutants. These assessments showed that CDKG1 and CDKG2 cyclin-dependent kinases as well as the increased ambient temperature do not affect chromosome pairing which was regular in every experiment.

In spite of the failure to influence chromosome pairing, this project has optimised of *B. distachyon* and *B. hybridum* genome editing methodology using CRISPR/Cas9 system. This will allow future comprehensive functional analyses of many other genes in these model grasses.