Current Master´s Thesis Topics for Academic Year 2024/2025

Specialization: Molecular Biology and Genetics

Thesis Title 1

Engineering chromosomal rearrangements in the *Cardamine hirsuta* genome using CRISPR/Cas

Annotation: This master's thesis focuses on inducing large-scale chromosomal rearrangements in the genome of *Cardamine hirsuta* using CRISPR/Cas technology. The student will be involved in designing and preparing CRISPR/Cas vectors and specific guide RNAs aimed at inducing significant chromosomal rearrangements, including deletions, inversions, and reciprocal translocations. Additional tasks will include performing plant transformation, selecting transformed lines, and optimizing PCR methods for detecting these rearrangements. The student will also conduct screening of T1 and T2 plant generations to verify the presence and heritability of chromosomal rearrangements. Furthermore, the impact of induced rearrangements on the phenotype of mutant plants will be investigated. The results of this work will enhance the understanding of the genetic and phenotypic consequences of large chromosomal rearrangements and provide new insights for further research into karyotype structure and evolution of plant genomes.

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Thesis Language: Czech or English

Thesis Title 2

Manipulating terminal NORs from subtelomeric to pericentromeric regions using CRISPR/Cas technique

Annotation: This master's thesis focuses on the translocation of terminal nucleolar organizing regions (NORs) from subtelomeric positions to pericentromeric regions of chromosomes using CRISPR/Cas technology. The model plant species studied are *Arabidopsis thaliana* and *Cardamine hirsuta*. The research includes the synthesis of CRISPR/Cas vectors and specific guide RNAs (gRNAs) designed to relocate NORs from the terminal regions of chromosomes to the pericentromeric regions. After vector preparation, plant transformation will be performed using the Agrobacterium-mediated floral dip method followed by the selection of positive transformants. Subsequently, PCR methods for detecting chromosomal rearrangements will be optimized, and Sanger sequencing and fluorescent in situ hybridization will be conducted to verify the NOR translocation. The next part of the work will focus on evaluating the impact of NOR relocation on their function and chromatin structure in mutant lines. The results of this study will contribute to a better understanding of the influence of chromosomal context on NOR activity and their role in cellular processes.

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Thesis Title 3

Transferring interstitial NORs from pericentromeric to subtelomeric regions using CRISPR/Cas technique

Annotation: The subject of this master's thesis is the translocation of interstitial nucleolar organizing regions (NOR, 35S rDNA) from pericentromeric to subtelomeric chromosomal regions using CRISPR/Cas technology. The model plants in this study are *Boechera* sp., *Draba nemorosa*, and *Thlaspi arvense*. The research involves the design and construction of CRISPR/Cas vectors and specific guide RNAs (gRNAs) to target paracentric inversions, causing the rotation of the chromosome arm carrying the NOR, thus relocating the NOR from the pericentromeric region to a subtelomeric position on the chromosome. After preparing these vectors, plant transformation will be conducted using the floral dip method, followed by the selection of transformants. The study includes optimizing PCR methods to detect induced paracentric inversions and verifying them through sequencing and cytogenetic methods. The impact of NOR relocation on their function and epigenetic modifications in mutant lines will also be assessed. The results of this work will provide new insights into how changes in chromosomal context affect interstitial NORs and their influence on genetic and cellular processes.

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Thesis Title 4

Evolution of chromosomal number in the genus *Trifolium*

Annotation: The agriculturally significant genus *Trifolium* from the legume family includes various clover species, which are important as forage crops and for their ability to fix atmospheric nitrogen, thereby improving soil quality. The ancestral chromosome number for this genus is n = 8. Throughout evolution, there have been several independent reductions in chromosome number (known as descending dysploidy) to n = 7 and n = 6. This thesis focuses on analyzing the karyotype structure of selected *Trifolium* species using advanced molecular cytogenetic methods, including chromosome-specific oligopainting and localization of tandem repetitive sequences. Due to the small mitotic chromosomes in Trifolium species, cytogenetic probes will be hybridized to meiotic chromosomes in the pachytene stage, which provides better resolution. The aim is to construct comparative cytogenetic maps that will clarify the evolution of chromosome number in *Trifolium* and identify mechanisms responsible for descending dysploidy. The results of this study will contribute to a better understanding of chromosome variability and evolutionary processes in plants, which could have significant implications for breeding and optimizing agricultural crops.

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