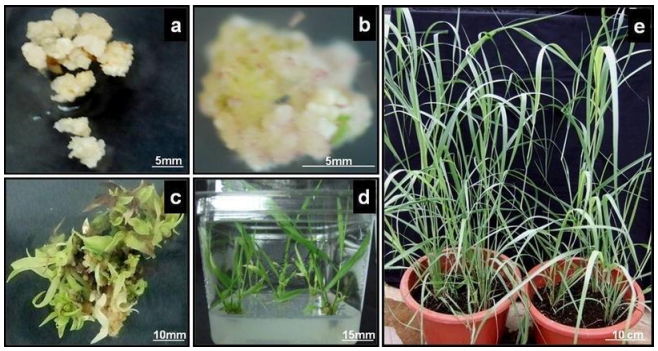
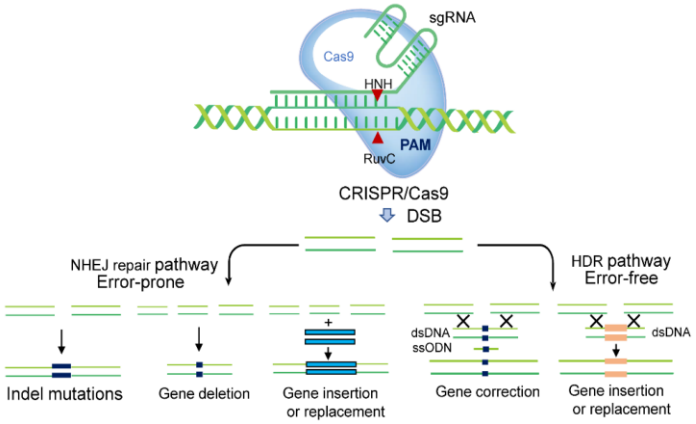
Master thesis BIOVIT 2023/24

Topic/Title (English) Develop CRISPR-Cas9 constructs and generate CRISPR-edited perennial ryegrass mutants for Nitrogen uptake efficiency





Grassland-based forage is pivotal for Norway's milk, meat, and farm economy, with permanent grasslands occupying 70-80% of its agricultural land. Climate change's rising temperatures allow high-yielding perennial ryegrass to grow further north. However, its expansion, predominantly as monocultures requiring extensive nitrogen fertilization, increases GHG emissions and nitrogen leaching. Traditional breeding for high yield and low nitrogen input is lengthy and costly. The NitroGenEdit preliminary project tests Norwegian perennial ryegrass cultivars for nitrogen uptake and its yield effects in hydroponic systems. It will also establish transformation protocols, utilizing CRISPR genome editing to target genes related to nitrogen transporters.

The master thesis project objectives is:

1) to characterize perennial ryegrass genotypes under varied nitrogen levels
2) to develop CRISPR methods for modifying nitrogen-regulating genes in two cultivars

The project focuses on the fundamental aspects of agricultural biotechnology by proposing to develop CRISPR/Cas genome editing system for perennial ryegrass to foster the breeding for efficient nitrogen uptake of genotypes. Different transformation protocols for perennial ryegrass will be tested as part of the thesis work. Two to three nitrogen transporter genes crucial for nitrogen uptake efficiency will be used to develop sgRNAs that will allow knock-out and over express of the selected genes. sgRNAs will be designed using tools like CCTop (Labuhn et al., 2017) or CRISPOR (Haeussler et al., 2016). Efficiency of designed sgRNAs to target candidate genes will be tested in transient protoplast assays. We will use either modular pTRANS or direct pDIRECT series of T-DNA vectors designed by Voytas lab. Delivery of Cas9-sgRNA or similar complexes into the plant will be achieved by Agrobacterium-mediated transformation. Protocols for selecting edited embryogenic callus cultures without marker genes will be developed using PCR tests and sequencing. Regeneration protocols from embryogenic calli will be optimized for selected Lolium genotypes.

The candidate working in this project might have an opportunity to attend the training in using nano chips in hydroponics to track nitrogen uptake at Austria. After this project, you would expect to be familiar with hydroponics system, tissue culture and transformation protocols and in designing crispr constructs. The knowledge gained in this master thesis project can generate ample career

opportunities both in industry (career in plant breeding company like Graminor etc) and academia (PhD positions)

Type of thesis work: laboratory work, literature study

Subject area Plant Science, Genome Science, Biology

Language thesis English

Master thesis

Credits 60 ETC

Project/company Dept. of plant Science, BIOVIT, NMBU

Please contact

Dr. Mallikarjuna Rao Kovi (mallikarjuna.rao.kovi@nmbu.no)

Dr Rómulo Sacramento Sobral (romulo.sacramento.sobral@nmbu.no)

Dr. Manikandan Veerabagu (manikandan.veerabagu@nmbu.no)