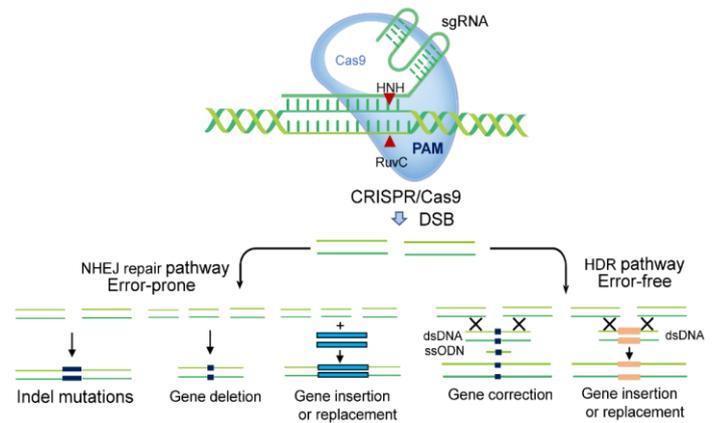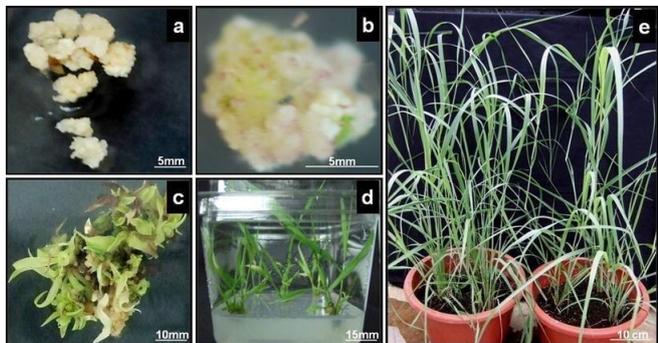**Topic/Title (English)** Develop CRISPR-Cas9 constructs and generate CRISPR-edited perennial ryegrass mutants for freezing and mild drought tolerance

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 of freezing and drought tolerant genotypes. Different transformation protocols for perennial ryegrass will be tested as part of the thesis work. Four candidate genes for freezing and drought related traits will be used to develop sgRNAs that will allow knock-out 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 is expected to travel to our collaborator lab in TalTech university, Estonia for training in transformation and developing Crispr constructs. After this project, you would expect to be familiar with tissue culture and transformation protocols and in designing crispr constructs

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

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