EEA-RESEARCH-64

Improving adaptability and resilience of perennial ryegrass for safe and sustainable food systems through CRISPR-Cas9 technology (EditGrass4Food)

Improving adaptability and resilience of perennial ryegrass for safe and sustainable food systems through CRISPR-Cas9 technology (EditGrass4Food)

EEA-RESEARCH-64

Principal Investigator: Nils Rostoks

Promoter: University of Latvia

Partners:

- Norwegian University of Life Sciences, NMBU, Norway
- Tallinn University of Technology, TalTech, Estonia
- Lithuanian Research Centre for Agriculture and Forestry, LAMMC, Lithuania

From **01.05.2021** to **30.04.2024** (36 months)

Website: https://www.editgrass4food.lu.lv/en/

Project partners

- University of Latvia
- Norwegian University of Life Sciences
- Lithuanian Research Centre for Agriculture and Forestry
- Tallinn Technical University

Lolium perenne

- *Lolium perenne* (perennial ryegrass)
- Native to Southern Europe, the Middle East and North Africa
- Important pasture and forage plant, extensively used in seed mixes
- High yield in fertile soil
- Lacks adaptation to climate conditions in Nordic and Baltic region, but due to the climate change this situation can change
- For cultivation in Nordic and Baltic countries perennial ryegrass needs improved freezing and drought tolearance

Lolium perenne

- *Lolium perenne* exhibits perennial growth habit
- *L. perenne* is an outcrossing, wind-pollinated species
- Selfing is largely prevented by a gametophytic, two-locus incompatibility system (*SZ*)
- Genome is heterozygous and the varieties consist of a mixture of related genotypes
- Genotypes exhibit different efficiency of *Agrobacterium*-mediated transformation (CRISPR/Cas constructs) and variable regeneration capacity

Project aims and objectives

EEA-RESEARCH-64

Aim of the project is to utilize transcriptomics and functional genomics to increase sustainability in agriculture through improvement of perennial ryegrass with better adaptation to frost and drought for current and future climates.

1. Establish a diverse perennial ryegrass core association panel by utilization of data from ongoing projects (WP1),
2. Screen the association panel in order to detect haplotype-resolved single-nucleotide variants and structural variation in the targeted genes/alleles for freezing and drought genes (WP1),
3. Identify novel genes and characterize drought and freezing tolerance genes by comparing their expression for pathway related genes in non-edited and mutant plants (WP2),
4. Develop CRISPR-Cas9 constructs and generate CRISPR-edited perennial ryegrass mutants for freezing and mild drought tolerance (WP3),
5. Validate and characterize the role of the genes and their sequence variations in the freezing and drought mechanisms (WP4).

Work packages

WP1. Establishment and screening of perennial ryegrass association panel for freezing and drought related traits. Coordinator: NMBU; Involved partners: NMBU, LAMMC

WP2. Transcriptome regulation of freezing and drought tolerance in perennial ryegrass. Coordinator: NMBU; Involved partners: NMBU, LAMMC

WP3. Functional characterization of frost and drought candidate genes in perennial ryegrass by CRISPR-Cas9. Coordinator: TalTech; Involved partners: LU, NMBU

WP4. Validation of improved freezing and water shortage tolerance. Coordinator: LAMMC; Involved partners: TalTech, NMBU, LU

WP5. Management and coordination of research activities and dissemination of results. Coordinator: LU; Involved partners: TalTech, NMBU, LAMMC

Deliverables

- Publications

  4 papers and 1 book chapter (dedicated funding for open access publishing)

- Project meetings:

  kick-off meeting (2021, Latvia), annual meeting (2022 in Estonia), workshop (2023, Lithuania), final conference (2024, Latvia)

- Scientific achievements

  association mapping panel, transcriptome sequences, protoplast cultures, gene edited plants (candidate genes for freezing and drought) assessed for abiotic stress tolerance tolerance, 4 PhD students

- Joint application for international funding
- Project interim/final reports – June 2022, June 2023, May 2024

Current progress of project activities

- EEA-RESEARCH-64

10:10 Dr. Cecilia Sarmiento «The regulation of New Genomic Techniques in the EU»
11:00 Dr. Kristina Jaškūnė „Abiotic stress response in ryegrasses: old problems and new opportunities»
11:20 M.Sc. Akhil Reddy Pashapu «Transcriptome profiling reveals insight into the cold response of perennial ryegrass»
11:40 M.Sc. Ferenz Sustek Sánchez «Genome editing in perennial ryegrass protoplasts»
12:00 Dr. Anete Borodušķe «Genetic transformation of perennial ryegrass using ectopic expression of morphogenic regulators»

Current progress of project activities – WP5

EEA-RESEARCH-64

Management and coordination of research activities and dissemination of results (Coordinator: LU; Involved partners: TalTech, NMBU, LAMMC)

Project meetings:

- Kick-off meeting in Riga – October 7 – 8 2021 public event, steering committee meeting, internal progress report
- Annual project meeting – October 27 – 28 2022 in Tallinn public event, steering committee meeting, internal progress report
- Extra project meeting – March 30 – 31 2023 in Riga steering committee meeting and internal progress report
- Annual project meeting – October 26 - 27 2023 at LAMMC public event, steering committee meeting, internal progress report
- Final project conference – April 2024 in Riga, public event
- Online project meetings

Current progress of project activities – WP5

EEA-RESEARCH-64

Management and coordination of research activities and dissemination of results (Coordinator: LU; Involved partners: TalTech, NMBU, LAMMC)

Project publications:

- Book chapter in 34th Meeting of the EUCARPIA Fodder Crops and Amenity Grasses
- Review article - Sustek-Sánchez F, Rognli OA, Rostoks N, Sõmera M, Jaškūnė K, Kovi MR, Statkevičiūtė G, Sarmiento C (2023) Improving abiotic stress tolerance of forage grasses – prospects of using genome editing. Frontiers in Plant Science 14
- Publication plan established in 2023
- Research articles under preparation – 1) protoplast protocols and transformation; 2) tolerance to freezing and transcriptome response; 3) haplotype association with freezing tolerance in candidate genes
- Genome edited plants require further characterization of mutations in candidate gene sequences and phenotyping for changes in abiotic stress tolerance (publications to be prepared after the end of the project)

Current progress of project activities – WP5

EEA-RESEARCH-64

Management and coordination of research activities and dissemination of results (Coordinator: LU; Involved partners: TalTech, NMBU, LAMMC)

Conferences:

- 2nd PlantEd Conference (COST Action CA18111) in Lecce, Italy, 20-22 September 2021 (Cecilia Sarmiento)
- Mendel Early career symposium in Viena, Austria May 2022 (Ferenz Sustek)
- 100th Anniversary of Plant Breeding in Lithuania conference in Akademija, Lithuania, 8 – 9 June (Nils Rostoks)
- FEBS3+ conference in Tallinn, Estonia 15 – 17 June 2022 (Nils Rostoks)
- 3rd PlantEd Conference (COST Action CA18111) in Dusseldorf, Germany, 5 – 7 September 2022 (Cecilia Sarmiento)
- EUCARPIA Fodder Crops and Amenity Grasses Conference in Brno, Czech Republic, September 10-14, 2023 (Ferenz Sustek)
- 4th PlantEd Conference (COST Action CA18111) in Porto, Portugal, 18 – 20 September 2023 (Cecilia Sarmiento, Nils Rostoks)

Current progress of project activities – WP5

EEA-RESEARCH-64

Management and coordination of research activities and dissemination of results (Coordinator: LU; Involved partners: TalTech, NMBU, LAMMC)

Student theses:

- Anneta Kļujeva (University of Latvia), bachelor thesis «Preparation and functional analysis of plasmid constructs for genome editing of abiotic stress tolerance candidate gene in *Lolium perenne*»
- Mari Talgø Syvertsen (Norwegian University of Life Sciences), master thesis «Establishing efficient transformation technologies for CRISPR/Cas9 genome editing in *Lolium perenne* L.»
- Dāvis Dūcis (University of Latvia), master thesis under preparation on genome editing of a CBF6 candidate gene
- Ferenz Sustek (Tallinn University of Technology), PhD thesis
- Potentially several more theses on characterization of genome edited plants

Current progress of project activities – WP5

EEA-RESEARCH-64

Management and coordination of research activities and dissemination of results (Coordinator: LU; Involved partners: TalTech, NMBU, LAMMC)

Publicity and social media:

- Project website https://www.editgrass4food.lu.lv/en/
- Twitter - @foodedit
- ResearchGate (closed)
- Interview for the Norwegian TV (Odd Arne Rognli)

NordPlus project proposal

- NPHE-2024/10036; Plant genome editing network (P-GEN2024) for higher education

Main results achieved – based on indicators

EEA-RESEARCH-64

Indicators	Achieved/planned
Researchers (8 PhD, 4 PhD students) supported	13 / 12
Joint peer-reviewed scientific publications	1 / 4
Joint applications for further funding	1 / 1
Jointly registered applications for Intellectual Property Protection	0 / 0

Lessons learned from implementation

EEA-RESEARCH-64

- COVID – 19 pandemic (problems related to travel, attending conferences, as well as increased delivery times for consumables)
- Difficulties to recruit PhD students (have been solved, but caused delay in the project)
- Research challenges:
    - Difficulty to obtain and propagate perennial ryegrass *in vitro* cultures for AMT
    - Difficulty to identify and re-sequence abiotic stress candidate genes (highly heterozygous genome)
    - Ryegrass genotypes recalcitrant to AMT and regeneration

Added value of programme

EEA-RESEARCH-64

Benefits from the implementation of the project:

- Collaboration among project partners
- Shared plant material, gene sequences, plasmid constructs etc.
- Possibilities for training students

Iceland
Liechtenstein
Norway grants

Norway
grants

QUESTIONS?

EEA-RESEARCH-64

EDIT
GRASS
4FOOD