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EDITGRASS4FOOD

KO GENERATION AND STUDIES

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WHO ARE WE?

PLANT-PATHOGEN INTERACTIONS RESEARCH GROUP

- Cecilia Sarmiento (PI)
- Merike Sõmera (senior researcher)
- Ferenz Sustek (PhD student)
- Lenne Nigul (Technician)
- Signe Nõu (Technician)

- 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.



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

WP3. NEED FOR SEQUENCES AND DOMAINS FROM WP1

Frost tolerance	Drought resistance
Gene	Gene
VIN3	PHYB
QM	MYB41
TPT	NAC038
IRI1	MYB94
CBF6	WSD11
DHN1	TSO1
GI	MYB4
LEA-14	HSL1
PRR95	WRKY49
EF-CAX	PRR5

- Candidate genes
 - gRNA design, the better the sequence the lesser off-targets.
 - Sequences from selected genotypes (cultivars/breeding lines)
 - Reference genome, to avoid high amount of off-target indels
 - *Lolium perenne* draft genome?

WP3. PLANS

- Protoplasts experiments
 - Establishment of de novo protocol (High yield and high transformation efficiency)
 - Microfluidics for mutants sorting
 - Tissue culture
 - Embryogenic calli protocol development
 - GRF4-GIF1 chimera (*A. tumefaciens* EHA105)
 - Training at Teagasc (Carlow, Ireland)
 - Constructs design (GoldenBraid approach)
 - Training in ETKI (*A. tumefaciens* AGL1) (Jõgeva, Estonia)
- Cas9 optimized for Lolium?

Description	Name
L1P1 hygromycin selection cassette	pICSL11059
L1P2 Cas9 expression cassette	pICSL11056
L1P3 guide acceptor	pHvL1P3GA
L1P4 guide acceptor	pHvL1P4GA
L2 acceptor	pAGM8031



THANK YOU FOR YOUR ATTENTION!

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