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

"EditGrass4Food", ID No EEA-RESEARCH-64, Contract No EEZ/BPP/VIAA/2021/4

is financially supported by European Economic Area (EEA) grants

21.01.2022

Agenda

Administrative topics

- Next project report to be prepared by May 31st (partner information to be submitted by May 15)
- 1st report still under considerations after revisions were submitted on January 4

Steering committee meeting in April:

- Preparation for the 2nd project report

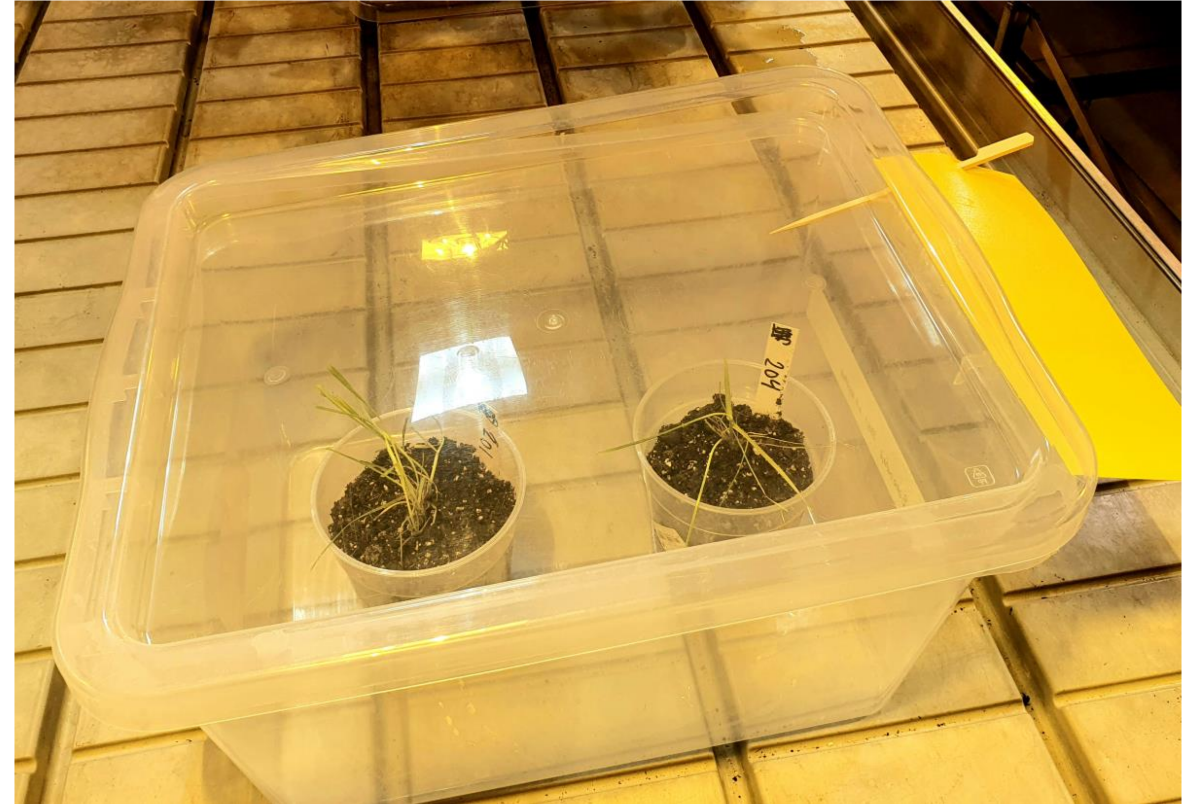
Next partner meeting on 13- 14th October, 2022 in Tallinn, Estonia

Scientific discussion

- Do we have a plan for publishing results?

Plant material

Genotypes from Lithuania
(vernalization @ ~7 ^{o}C)

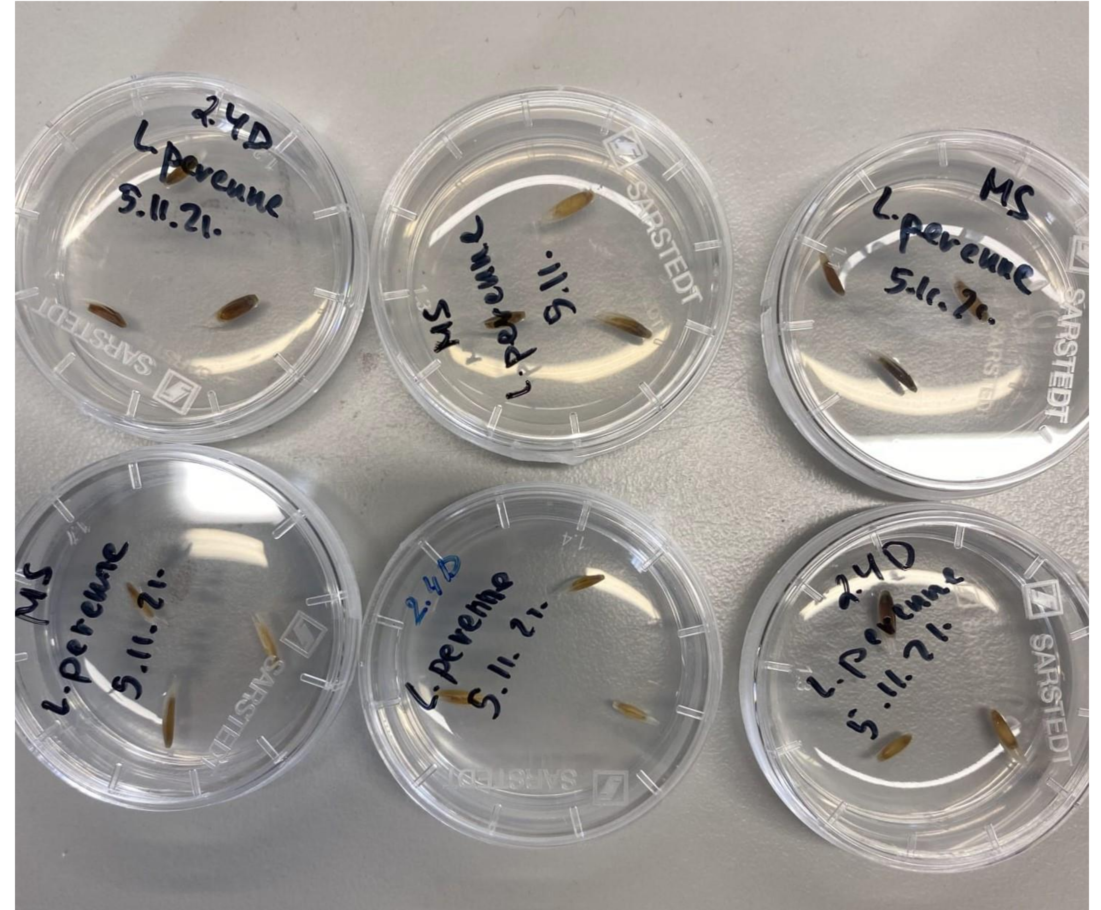


Genotypes from Norway
(glasshouse)

Seed surface sterilization

Sterilization agents:

- 70% EtOH
- 25% ACE



Seed germination

Culture medium:

100% Murashige & Skoog medium without growth regulators

Embryonic callus induction I

MS callus induction medium:

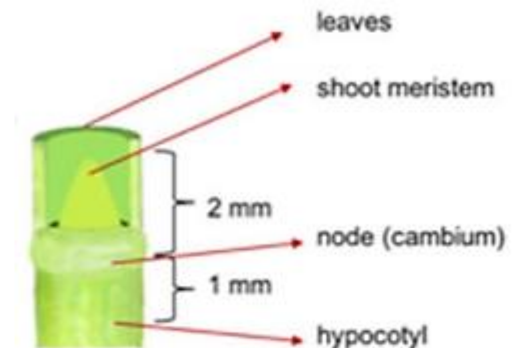
1. 1.5 mg/L 2,4-D
2. 5 mg/L 2,4-D; 0,5 mg/L BAP

Seed germination:

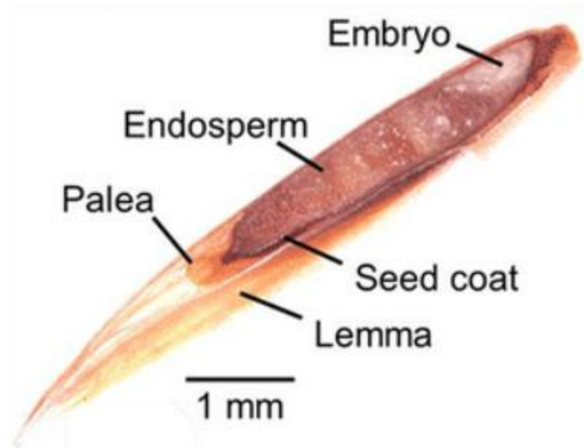
Shoot tip length - 4-6 cm

Cutting manner:

- 3 mm upward and downward from the node
- 2-4 mm downward the node



Embryonic callus induction II



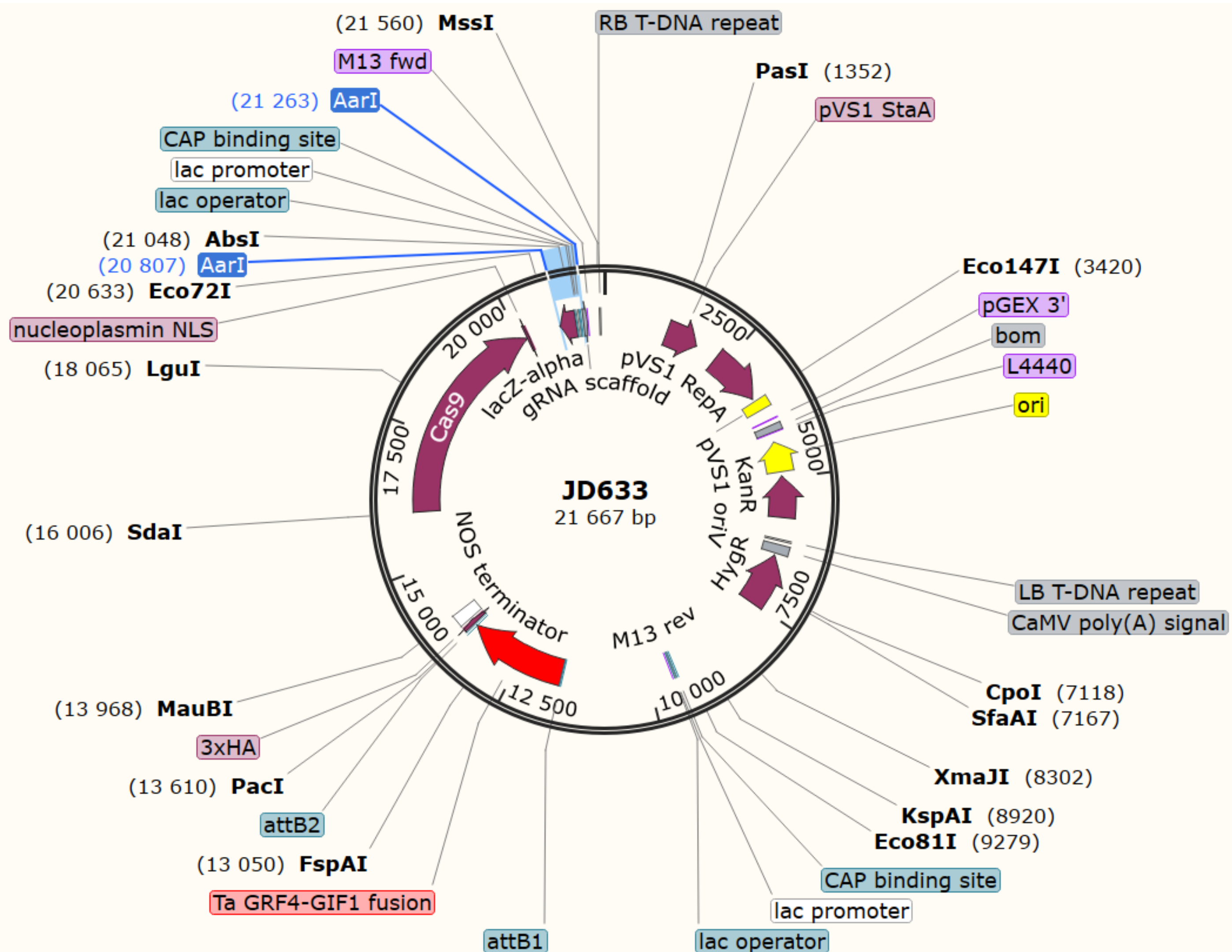
MS callus induction medium:

1. 2 mg/L 2,4-D
2. 5 mg/L 2,4-D; 0,5 mg/L BAP

Material:

- Mature seed embryos

CRISPR/Cas9 constructs (GRF4-GIF1)



To develop the JD635-*GRF4–GIF1/CRISPR–Cas9–gRNA–Q* vector, we amplified by PCR a cassette including the maize *UBIQUITIN* promoter, the *GRF4–GIF1* chimera and the Nos terminator (primers Fw_ZmUbi-AscI and Rev_NosTerm-AscI). The PCR product was gel purified and cloned by In-fusion (Takara Bio USA) into the AscI site of the pYP25F binary vector, which contains a wheat codon-optimized Cas9 (TaCas9) with two nuclear localization signals and is a modified version of pDIRECT_25F (Addgene, 91143) from the laboratory of D. Voytas (University of Minnesota). We validated the vector sequence by Sanger sequencing. Next, we cloned a gRNA construct targeting the coding region of gene *Q*[35] by GoldenGate reaction into two AarI sites of the vector and transformed it into chemically competent *Escherichia coli* DH5α. We validated the JD635-*GRF4–GIF1/CRISPR–Cas9–gRNA–Q* vector by Sanger sequencing and transformed it by electroporation into *Agrobacterium* strain EHA105.

https://www.addgene.org/160393/

CRISPR/Cas9 constructs (pDIRECT series)

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LARGE-SCALE BIOLOGY ARTICLE

A Multipurpose Toolkit to Enable Advanced Genome Engineering in Plants[OPEN]

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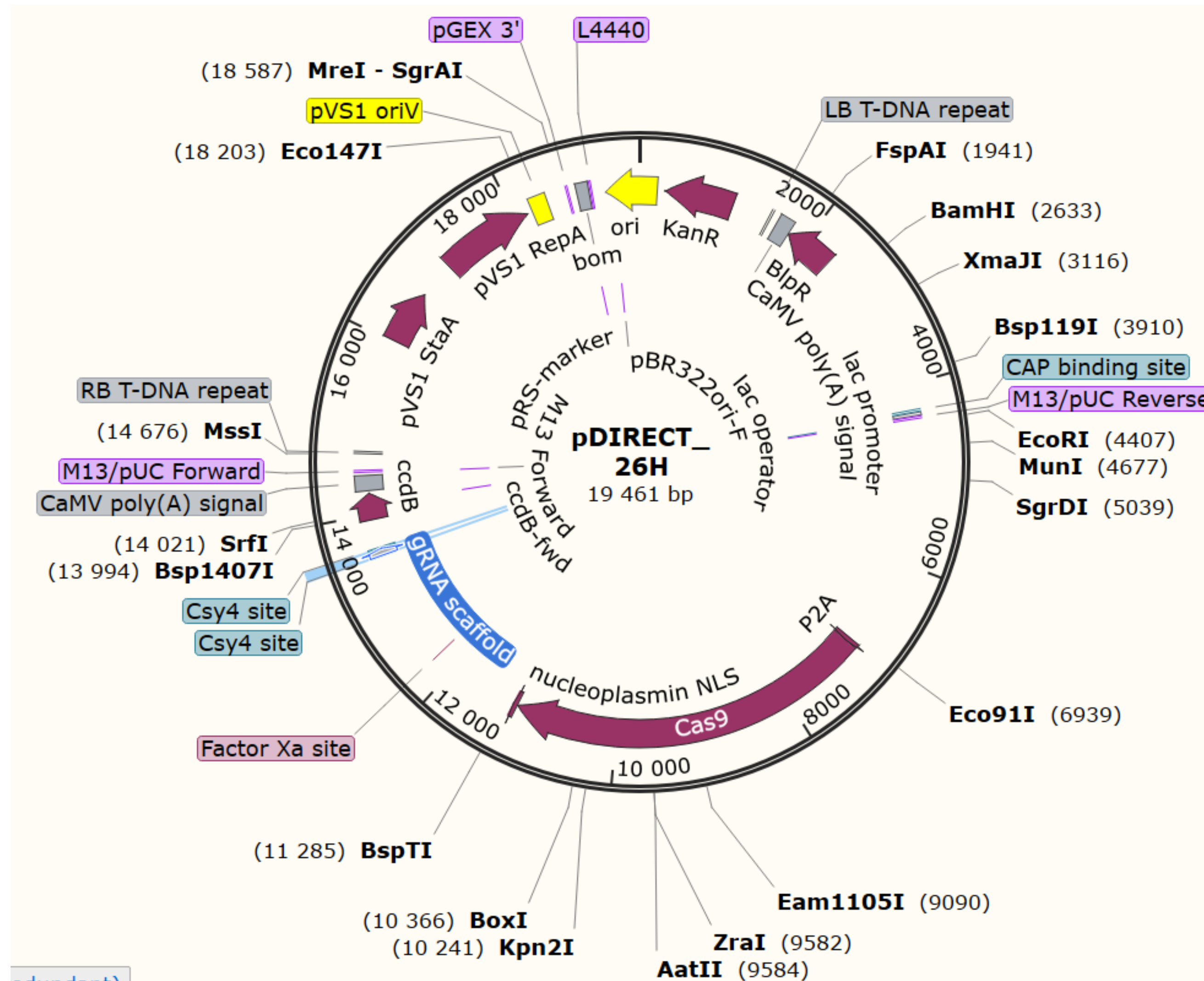
We report a comprehensive toolkit that enables targeted, specific modification of monocot and dicot genomes u
of genome engineering approaches. Our reagents, based on transcription activator-like effector nucleases (TAI
clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, are systematized for fast, mo
and accommodate diverse regulatory sequences to drive reagent expression. Vectors are optimized to create e
multiple gene knockouts and large chromosomal deletions. Moreover, integration of geminivirus-based ve
precise gene editing through homologous recombination. Regulation of transcription is also possible. A We
streamlines vector selection and construction. One advantage of our platform is the use of the Csy-type (CF

pDIRECT_25F
17 286 bp
(17 179) MssI
RB T-DNA repeat
M13 fwd
CAP binding site
lac promoter
lac operator
(16 667) AbsI
(16 252) Eco72I
(15 853) BspTI
nucleoplasmin NLS
(13 684) LguI
(13 216) OliI
(11 625) SdaI
(10 225) SgrDI
(9863) MunI
(9624) SgsI
(9593) EcoRI
M13 rev
lac operator
CAP binding site
(9279) Eco81I
(8920) KspAI
XmaJI (8302)
BamHI (7819)
SfaAI (7167)
CpoI (7118)
LB T-DNA repeat
PasI (1352)
Eco147I (3420)
pVS1 oriV
MreI - SgrAI (3804)
pGEX 3'
bom
L4440
lacZ-alpha
gRNA scaffold
pVS1 StaA
pVS1 RepA
ori
KanR
HygR
CaMV poly(A) signal
lac promoter
Cas9
2000
4000
6000
8000
10 000
12 000
14 000
16 000
redundant)

https://www.addgene.org/91143/

CRISPR/Cas9 constructs (pDIRECT series)



https://www.addgene.org/91150/

Genes

- *VRN1* (sequence from Mallik)
- *GIGANTEA* (GenBank DQ534010.3 or FN376855) *L. perenne* sequence from New Zealand and Whales. *GIGANTEA* promotes flower development in plants. In Arabidopsis, this gene is involved in CBF-independent freezing tolerance, and is responsive to cold in *Zea mays*. Also part of the circadian clock.
- Need to resequence both genes from the two genotypes sent by Mallik 201 and 204