



**TAL  
TECH**

# EDITGRASS4FOOD WP3 (TALTECH): PROGRESS AND FUTURE PLANS

Ferenz Sustek Sánchez  
Institute of Chemistry and Biotechnology  
Tallinn University of Technology

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# PLANT COLLECTION

No	Type	Country of origin	Cultivar name	Drought sensitivity
22	cultivar	RU	Leningradskij 809	tolerant
3177	ecotype	LT		tolerant
3618	cultivar	FI	Riikka	tolerant
3943	cultivar	NL	Kerdion	moderate
3948	cultivar	NL	Magella	moderate
3938	cultivar	NZ	Grasslands Nui	moderate-sensitive
3944	cultivar	DE	Kerem	moderate-sensitive
34551	ecotype			moderate-sensitive
3774	ecotype	UA		moderate-sensitive
3784	ecotype	LV		moderate-sensitive
3821	ecotype	UA		sensitive
3575	ecotype	LT		sensitive
3776	ecotype	SK		sensitive

**In-vitro culture**

**Vernalization**

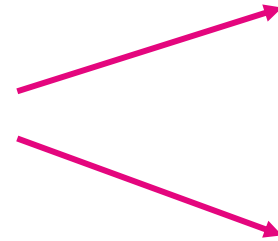
# IN-VITRO TISSUE CULTURE



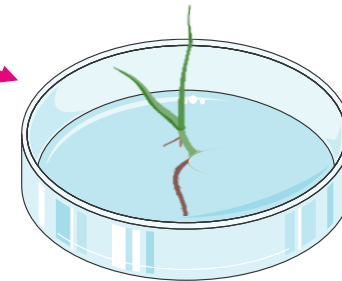
1 clone of each  
line grown in  
**soil**



**Sterilization of tillers**

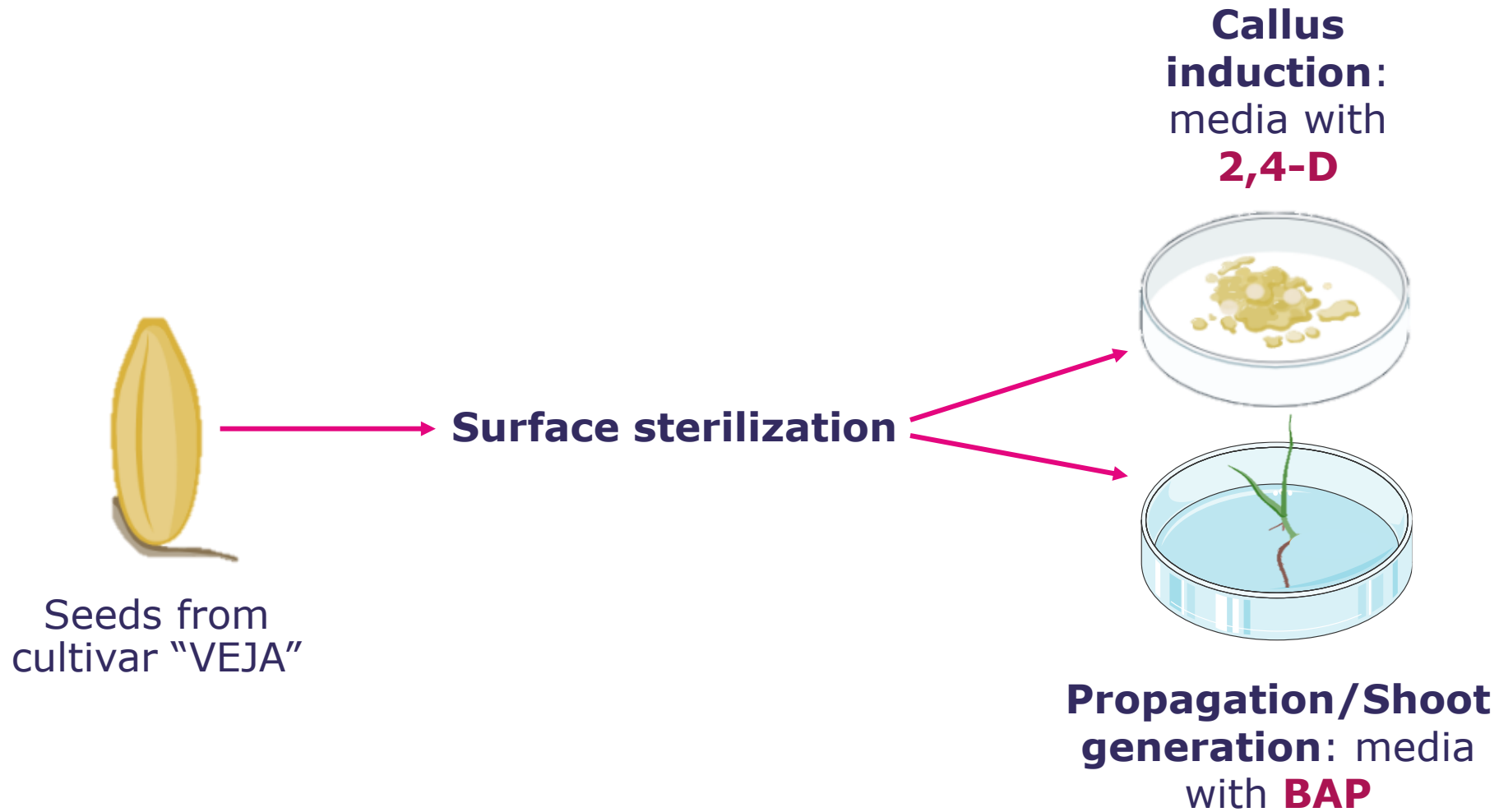


**Callus  
induction:**  
media with  
**2,4-D**



**Propagation/Shoot  
generation:** media  
with **BAP**

# IN-VITRO TISSUE CULTURE



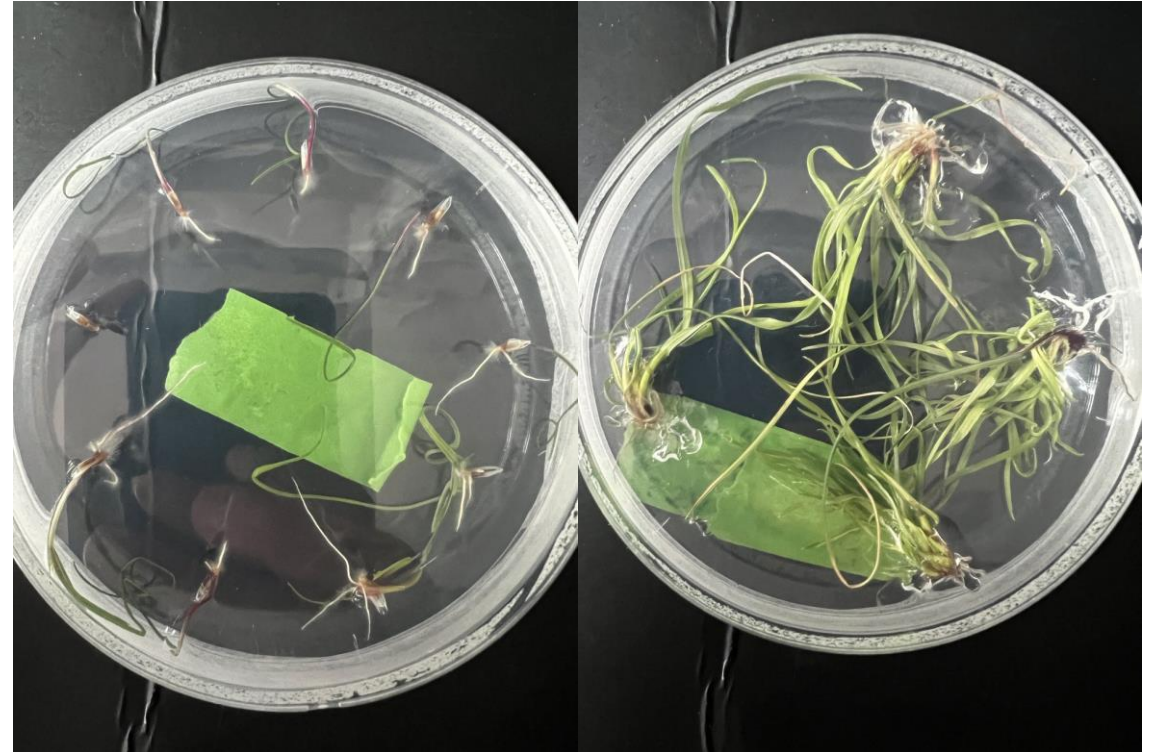


# IN-VITRO TISSUE CULTURE

From tillers



From seeds



# CANDIDATE GENES FOR CRISPR-CAS KNOCK OUTS

Gene	Denmark genome homologue	Identity	Coverage	Query
VIN3	Lp_chr4_0G13152.1	100	99.7	Lolium Mallik
QM	Lp_chr1_0G4562.1	89.3	90.9	Hordeum predicted
	Lp_chr3_0G20332.1	88.6	93.3	Hordeum predicted
IRI1	Lp_chr3_0G9338.1	96.1	100	Lolium perenne
	Lp_chr5_0G782.1	96.1	100	Lolium perenne
CBF6	Lp_chr5_0G16922.1	81	93.3	Hordeum predicted
DHN1	Lp_chr1_0G4004.1	92.4	53.3	Lolium Mallik
EF-CAX	Lp_chr2_0G4306.1	98.9	100	Lolium partial mRNA
	Lp_chr3_0G7610.1	86.3	93.8	Hordeum predicted
	Lp_chr6_0G8564.1	90.8	77.9	Hordeum predicted
GI	Lp_chr3_0G8870.1	90.3	92.9	Hordeum predicted
LEA-14	Lp_chr3_0G15594.1	90.3	66.2	Hordeum predicted
PRR95	Lp_chr5_0G17244.1	79	70.6	Oryza sativa
TPT	Lp_chr3_0G9158.1	87.4	100	Hordeum predicted
VRN1	Lp_chr4_0G5312.1	98	100	Lolium Mallik
CBP20	Lp_chr6_0G11740.1	93	97.8	Hordeum predicted

Gene	Denmark genome homologue	Identity	Coverage	Query
PHYB	Lp_chr4_0G21910.1	97.5	100	Lolium perenne
	Lp_chr4_0G21912.1	97.5	100	Lolium perenne
MYB41	Lp_chr2_0G18342.1	98.1	100	Lolium rigidum predicted
NAC038	Lp_chr5_0G15838.1	82.7	67.3	Hordeum vulgare morex
MYB94	Lp_chr2_0G4322.1	96.7	70.4	Lolium rigidum predicted
WSD11 /FOP1	Lp_chr2_0G20.1	94.2	100	Lolium rigidum predicted
TSO1	Lp_chr2_0G11088.1	83.8	90.4	Hordeum predicted
MYB4	Lp_chr2_0G17906.1	94.6	94.9	Lolium rigidum predicted
HSL1	Lp_chr5_0G12878.1	82.3	89.7	Hordeum predicted
WRKY49	Lp_chr3_0G17844.1	85.3	74.4	Hordeum vulgare
	Lp_chr3_0G17848.1	83.1	88	Hordeum vulgare
PRR5	Lp_chr1_0G13020.1	68	40	Hordeum predicted

## ❖ Design of gRNAs

❖ **CRISPOR**: using Swiss genome for off-targets ✓

❖ **GENIOUS**: using Danish genome for off-targets

# PROTOPLASTS EXPERIMENTS



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- Test different methods to **maximize yield** of isolation
- Optimize the **transformation** approach
  - transient expression of **fluorescent protein** e.g., **GFP**
- Test **efficiency** of CRISPR-Cas vectors and gRNAs

# PROTOPLASTS EXPERIMENTS

## Isolation methods

- Cutting leaves
- Blending leaves
- Using whole seedlings

## Different conditions

- Mannitol pretreatment
- Enzyme concentrations
- Vacuum infiltration
- Incubation duration



## EXPERIMENTAL CONDITIONS

<b>Mannitol (Molar)</b>	<b>Cellulase R-10 (% w/v)</b>	<b>Vacuum (MPa for 30 minutes)</b>	<b>Incubation (hours)</b>
0.6	3	0.07	20
0.5	2.5	0.05	16
0.3	2	0.03	12
0.2	1.5	0.01	8

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