
# EDITGRASS4FOOD WP3 (TALTECH): PROGRESS

EDIT GRASS 4FOOD

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

07.11.2023

# OPTIMIZED *Lolium perenne* L. PROTOPLASTS ISOLATION AND TRANSFORMATION FOR CRISPR-CAS9 DOWNSTREAM APPLICATIONS

**Ferenz Sustek-Sánchez**
Department Of Chemistry And Biotechnology
Tallinn University Of Technology

In cooperation with **Anete Boroduška**
Department of Microbiology and Biotechnology
University Of Latvia

EDIT GRASS 4FOOD

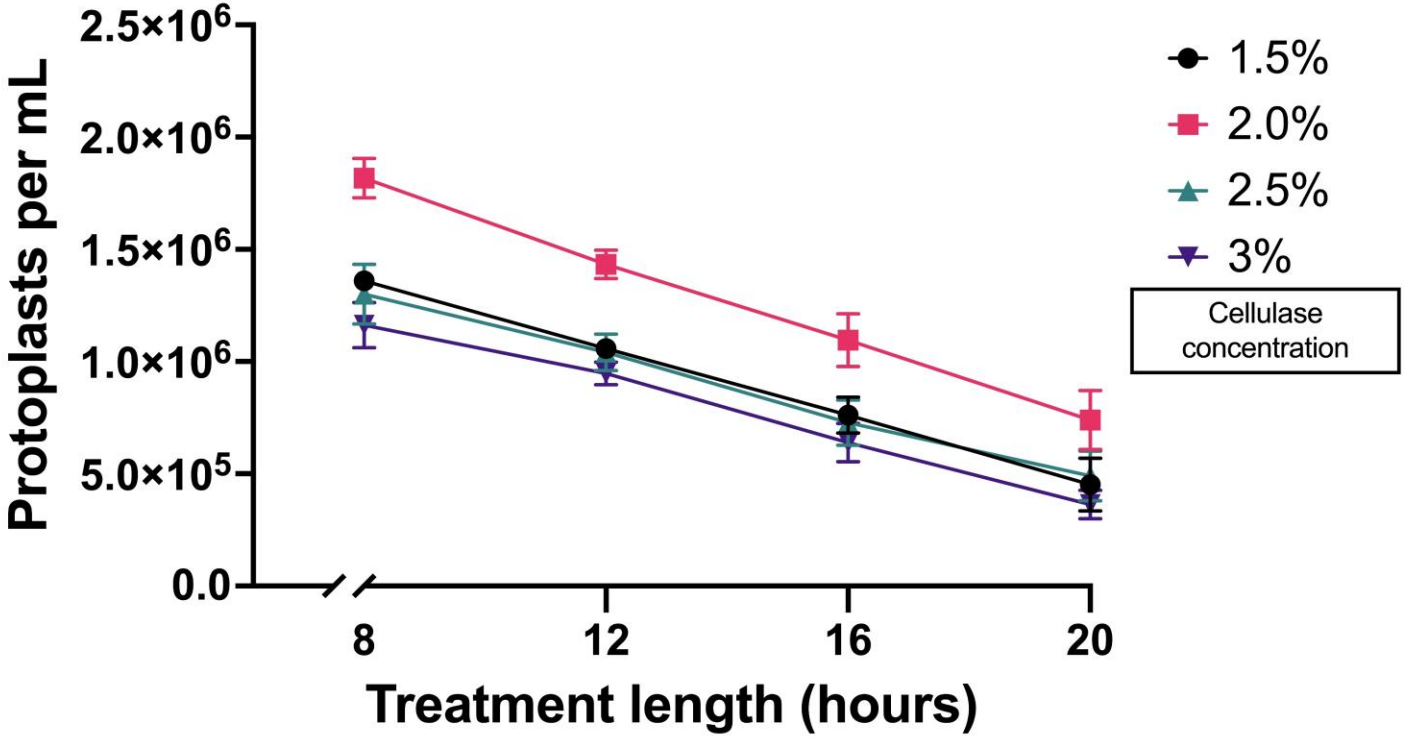
TAL TECH

UNIVERSITAS LATVIENSIS 19 19 SCIENTIAE ET PATRIAE
UNIVERSITY OF LATVIA

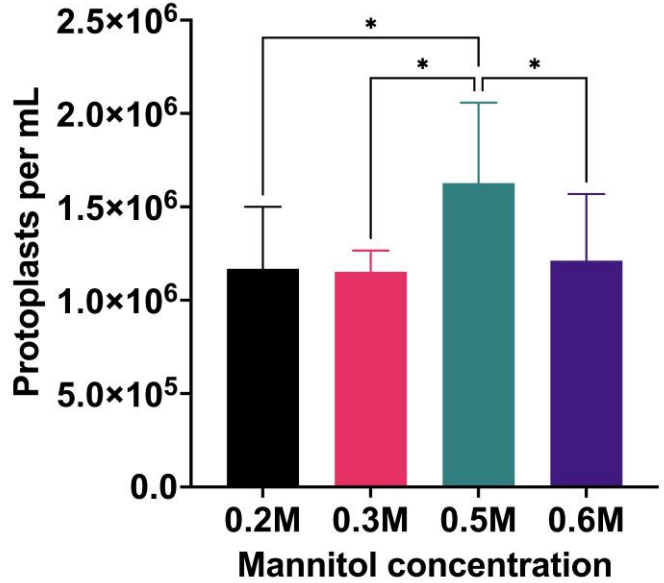
Iceland Liechtenstein Norway grants

Eesti Teadusagentuur
Estonian Research Council

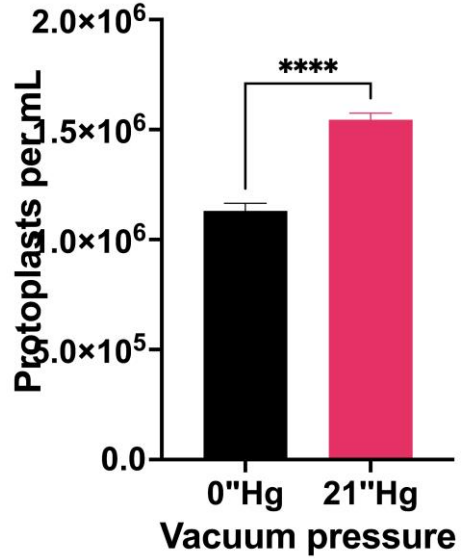
## Viable protoplasts after enzymatic treatment




## Viable protoplasts using different mannitol pretreatments

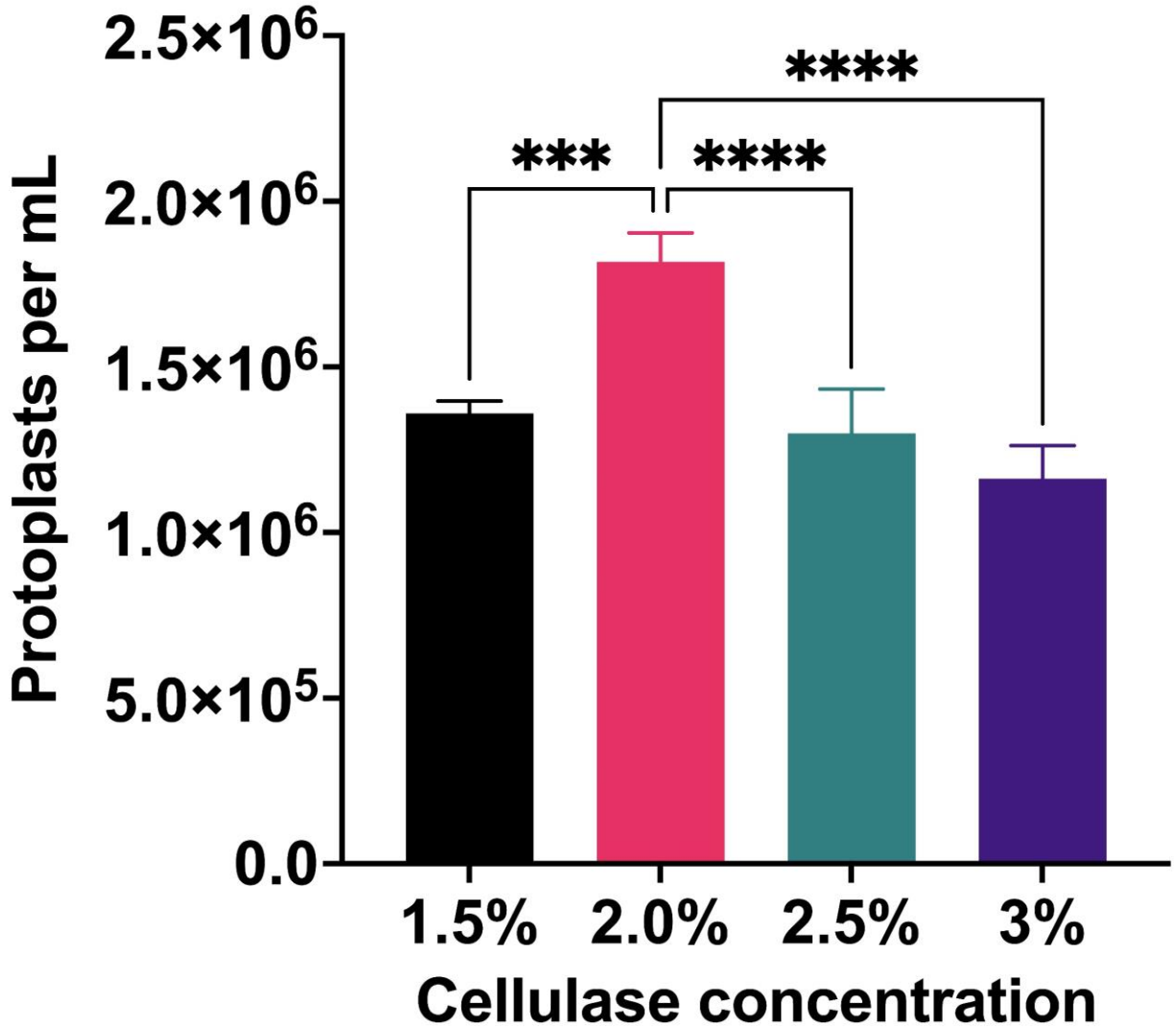



## Viable protoplasts after vacuum treatment



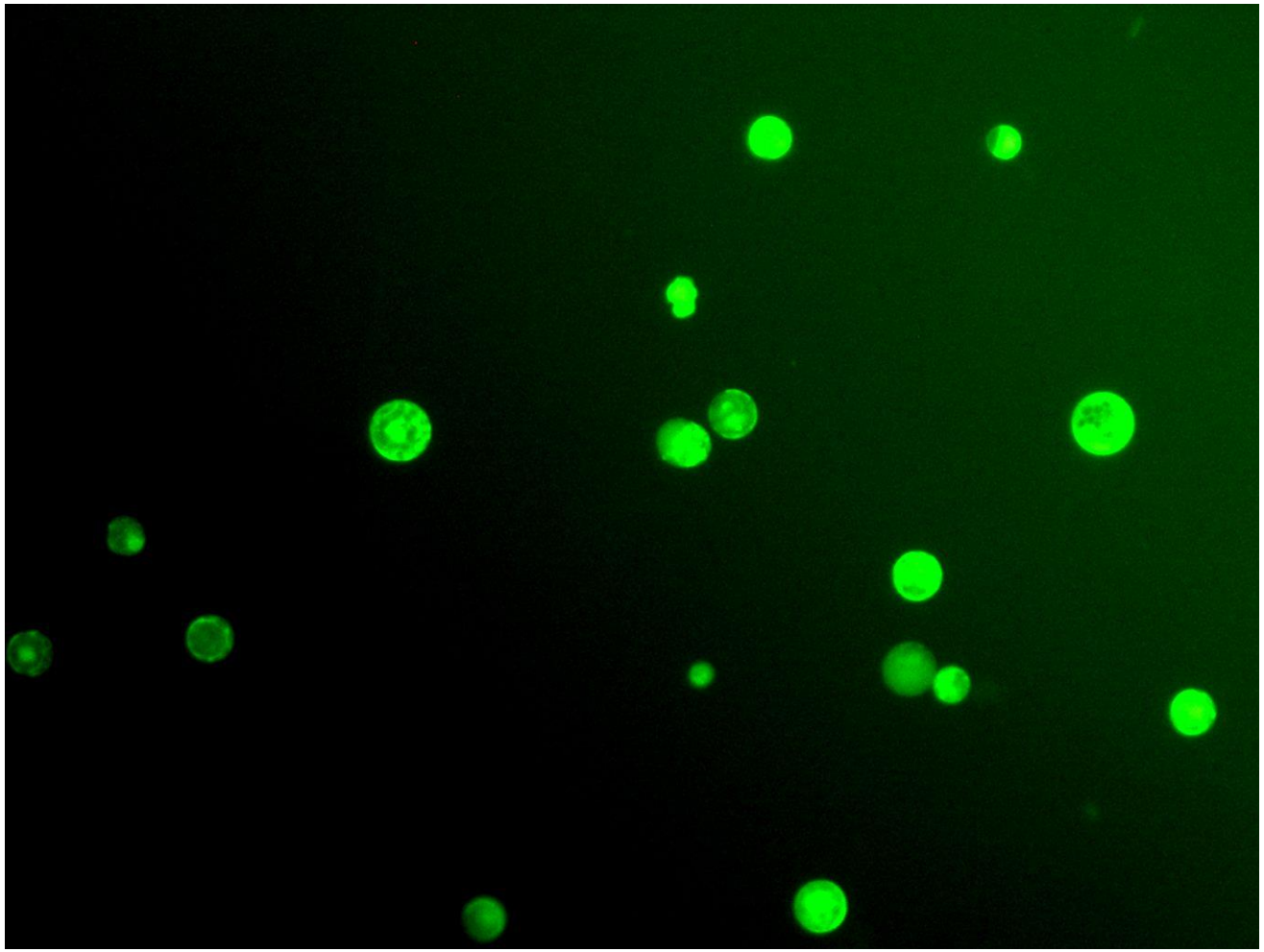

TAL TECH

# Viable protoplasts using different cellulase concentrations

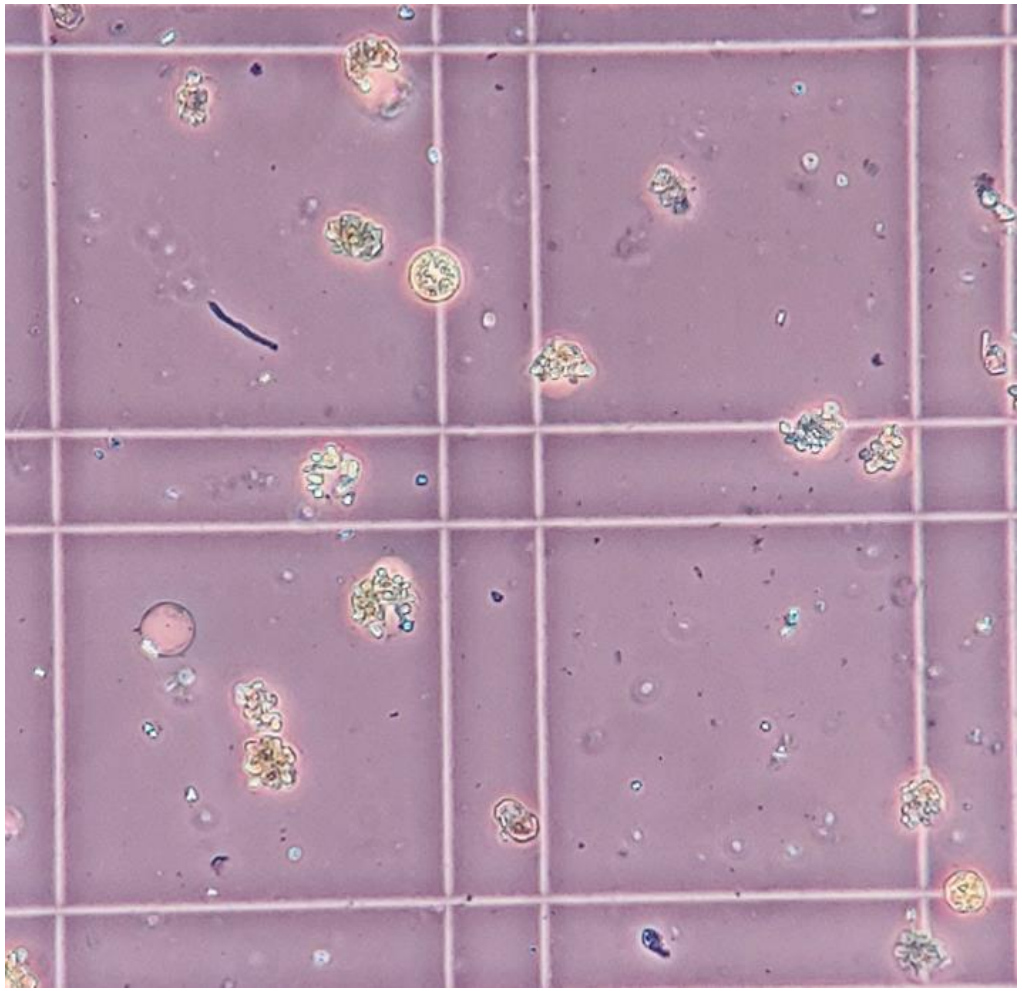



TAL TECH

# PROTOPLASTS VIABILITY ASSAY



Fluorescein diacetate (FDA)



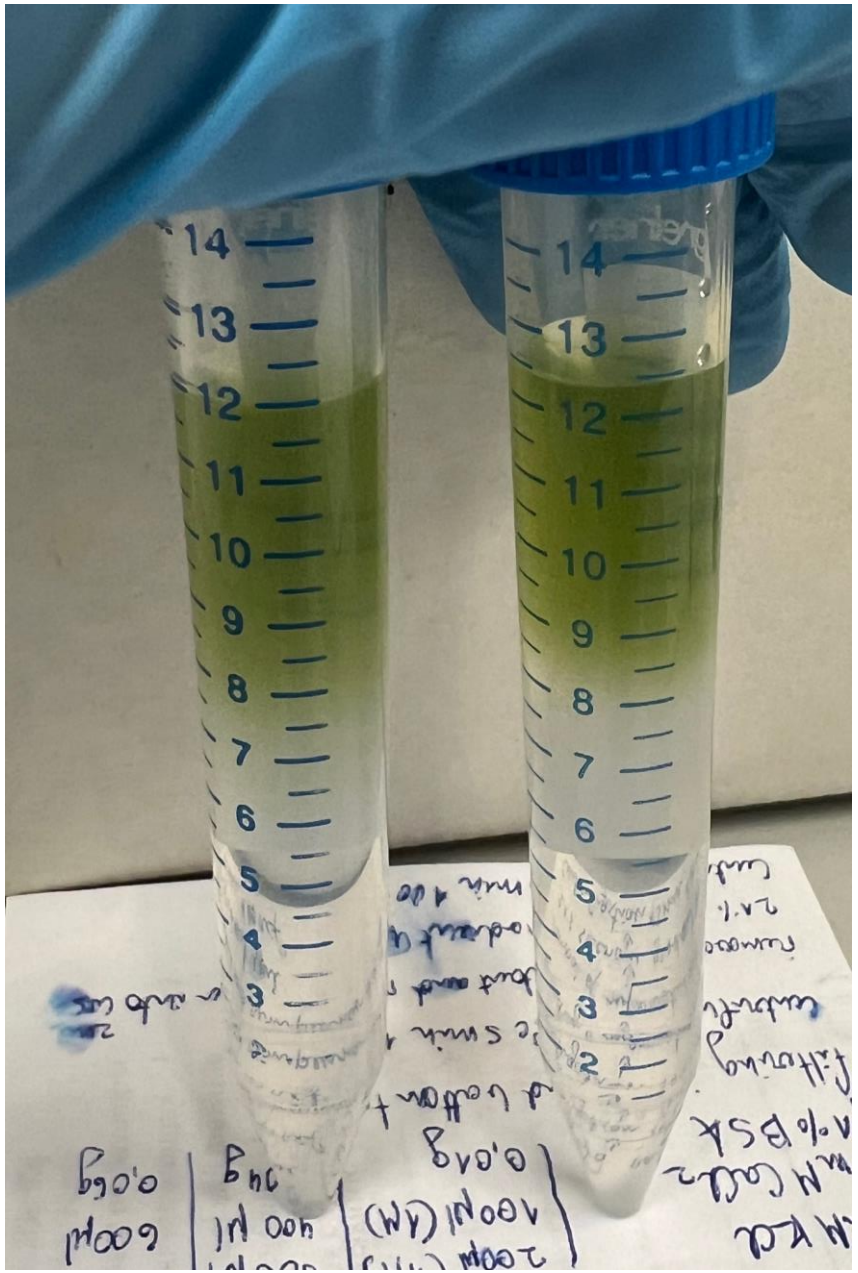
Trypan blue

TAL TECH

# REDUCING DEBRIS IN THE PROTOPLASTS SUSPENSION

- Using a 100 µm nylon filter
- Separating alive protoplasts using a sucrose cushion
  - Layering protoplasts suspension on top of 21% sucrose solution
  - Centrifugation 100g for 10 minutes at 11ºC

TAL TECH




TAL TECH

# PROTOPLASTS TRANSFORMATION

- Using PEG (Polyethylene glycol)
  - Most common method in recent papers.
  - Easy to set-up and doesn’t require specific equipment.
- PEG solution:
  - PEG 4000 40% (w/v)
  - D-mannitol 0.2M
  - $CaCl_2$ 0.1M

TAL TECH

Iceland Liechtenstein Norway grants

Eesti Teadusagentuur
Estonian Research Council

Euroopa Liit
Euroopa Regionaalarengu Fond

Eesti tuleviku heaks

EDIT GRASS 4FOOD

TAL TECH


- Cecilia Sarmiento
- Merike Sõmera
- Lenne Nigul
- Signe Nõu
- Erki Eelmets