



Waksman Institute
of Microbiology

Developing a Duck Weed Transformation Platform for Mass Production of Food and Feed

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Host: Prof. Jo Messing

Procedure

Identify a totipotent tissue of duckweed



Develop a Reproducible regeneration Protocol



Develop a Reproducible Transformation Protocol



Assay of transgene

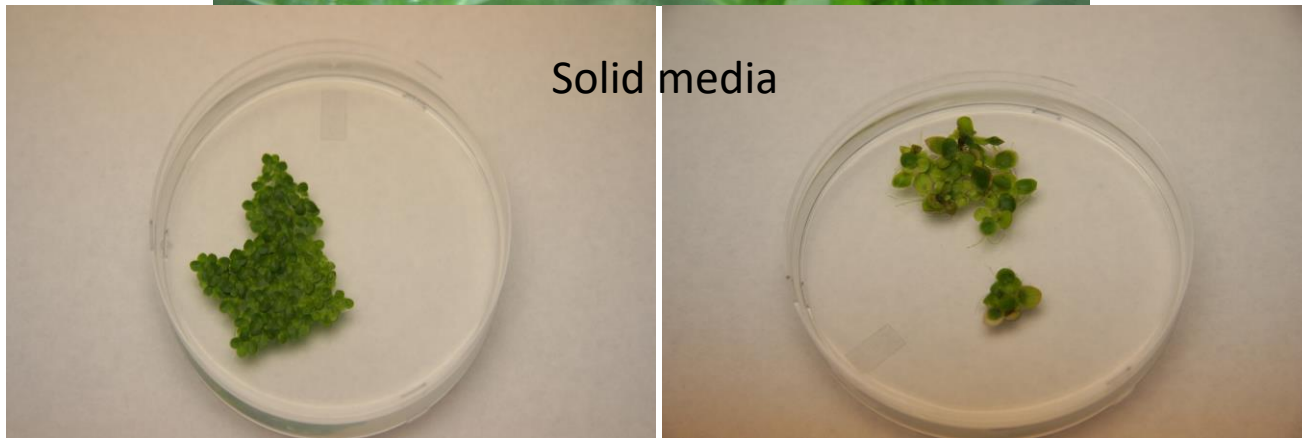
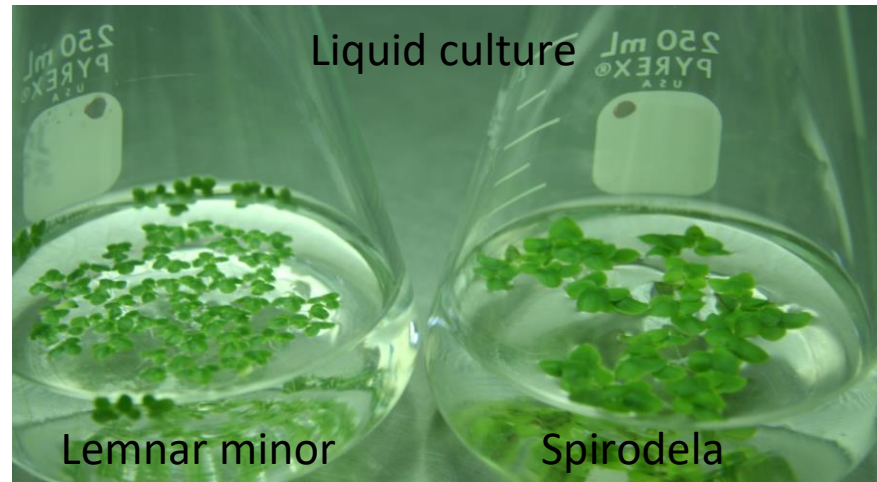


Propagation of duckweed

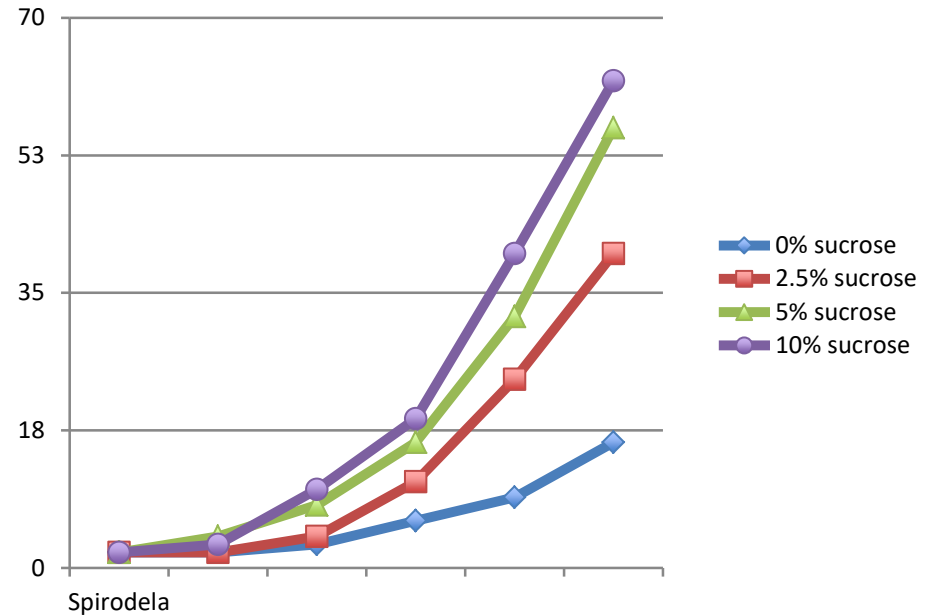
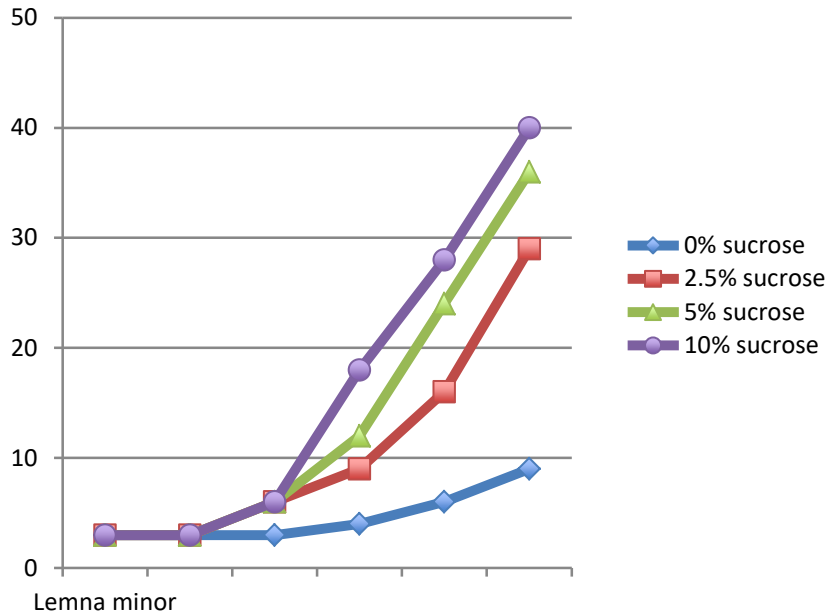
Introduction/Regeneration of Duck weed

- Duck weed belongs to family of Lemnaceae, a free floating monocot found in fresh water (Landolt 1986a)
- Its grouped in 5 genera
 - Spirodela
 - Landoltia
 - Lemna
 - Wolffia
 - Wolffia
- Spirodela polyrriza ww123-8403 and Lemna minor ww313-9223 are types of duck weed whose tissue culture protocol had not yet to been established
- AIM: To develop a repeatable regeneration and transformation protocol of the two **species** of duck weed

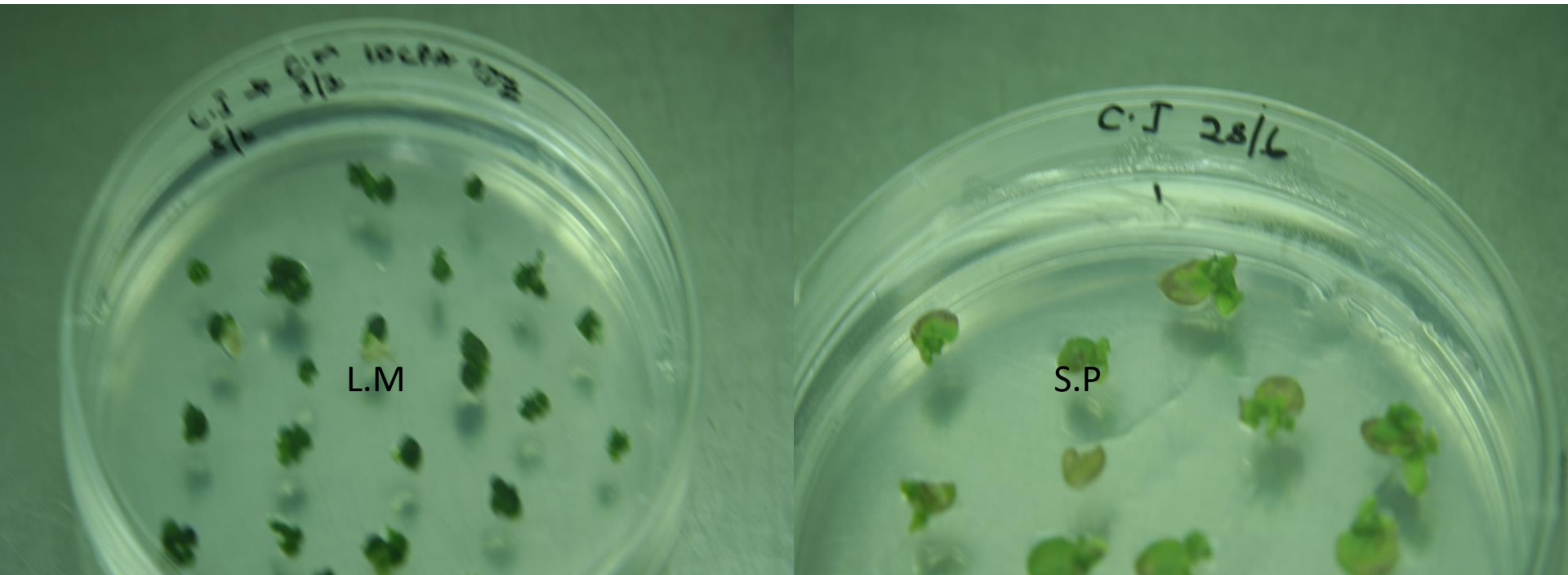
Grow Duck weed



Growth pattern of the two species on half strength Schenk Hildebrandt (SH) media+ 0,2.5,5 and 10g sucrose



Callus induction

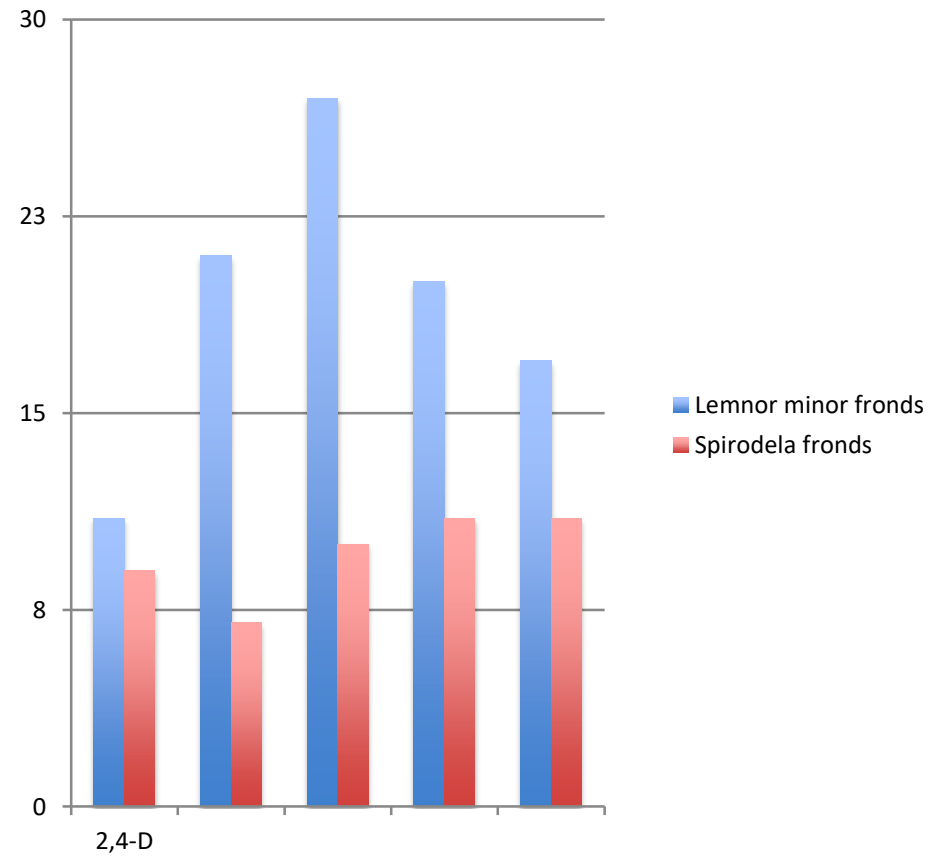
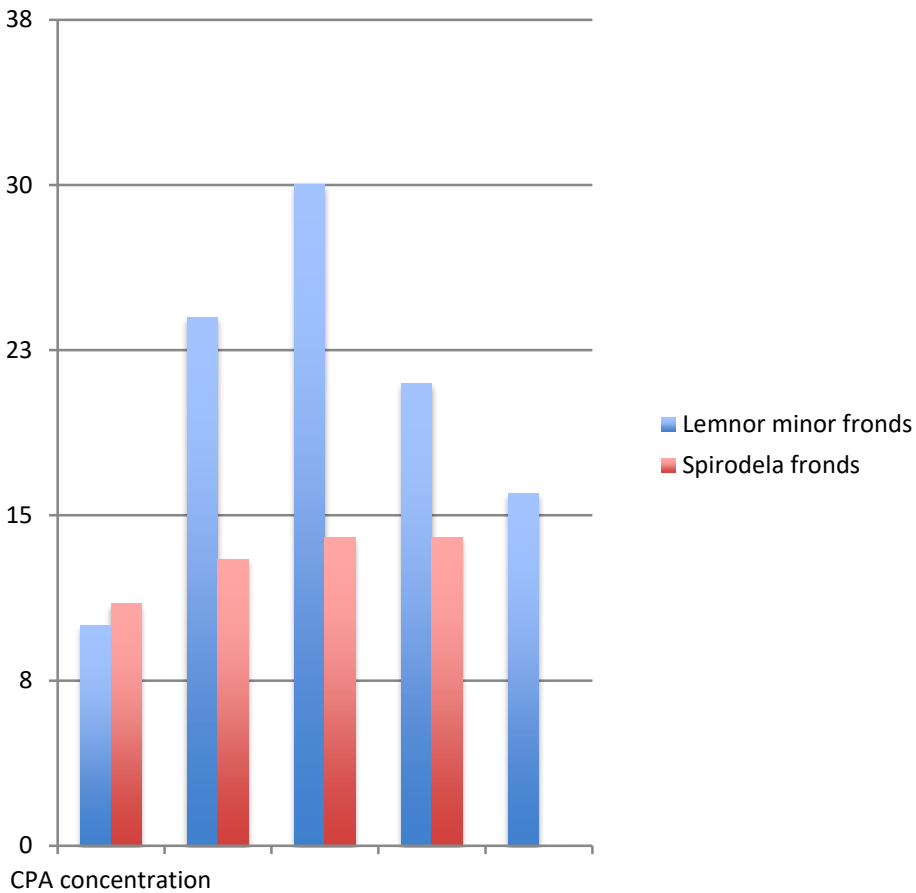


Callus induction

Callus induction

- J.Li et al 2004 used (Mc WP) **Mc Cown Woody** Plant (5mg/l CPA+ BA), **MS**(15mg/l dicamba+2mg/l iP) and B5(50mg/l dicamba+2mg/l BA) media formulation on SP and L gibba but did not use Mc Cown WP on Lemna minor
- Mc WP media, titrated p-chlorophenoxyacetic acid **CPA**(5,7.5,10,15 and 20mg/l)+0.5(thidiazuron)TDZ and 2,4-D **dichlorophenoxyacetic** acid 1,1.5,2.0 and 2.5mg/l+0.5TDZ

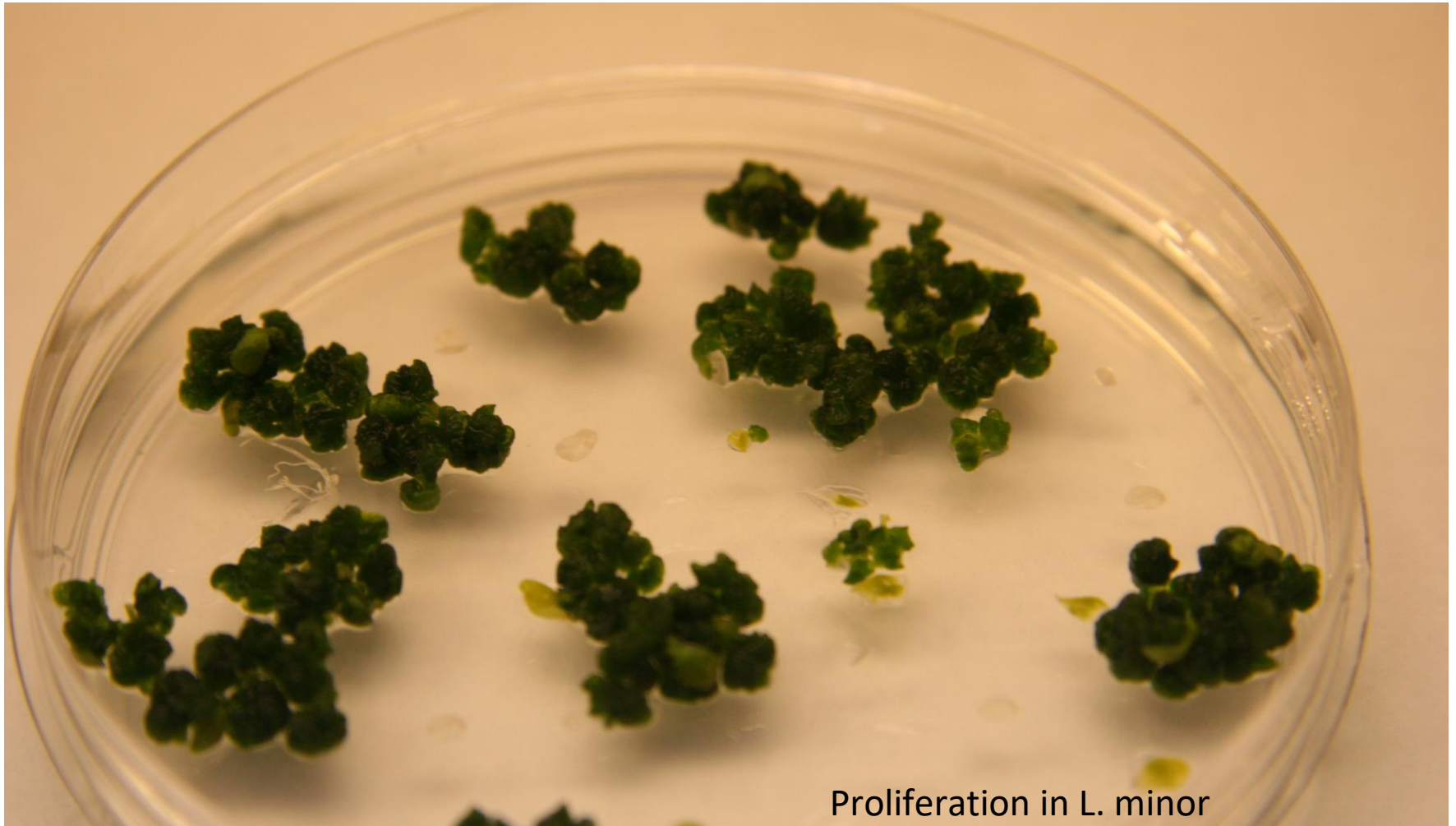
CPA and 2,4D effect on callus induct'n



Callus maintenance

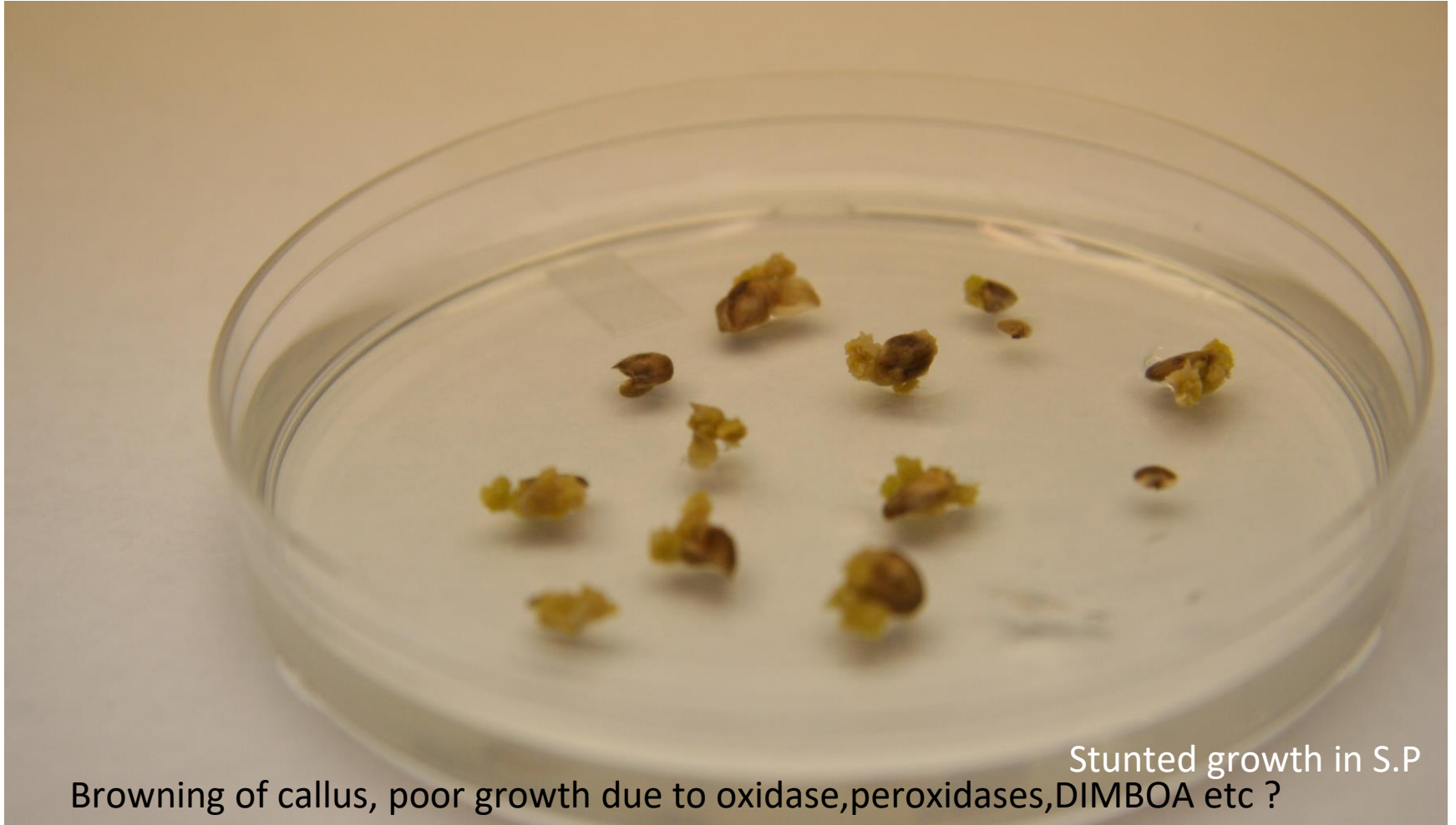
- **S-orbital**, **sucrose** and **Mannose** have been shown to influence callus maintenance in *S. orligorrhiza*, *S. punctata* and *L. gibba* var. *Hurfeish*. (J. Li et al., 2004)
- Using **optimal** concentration of hormones for induction plus titrating concentration of s-orbital, mannitol and sucrose

Callus maintenance/Proliferation



Proliferation in *L. minor*

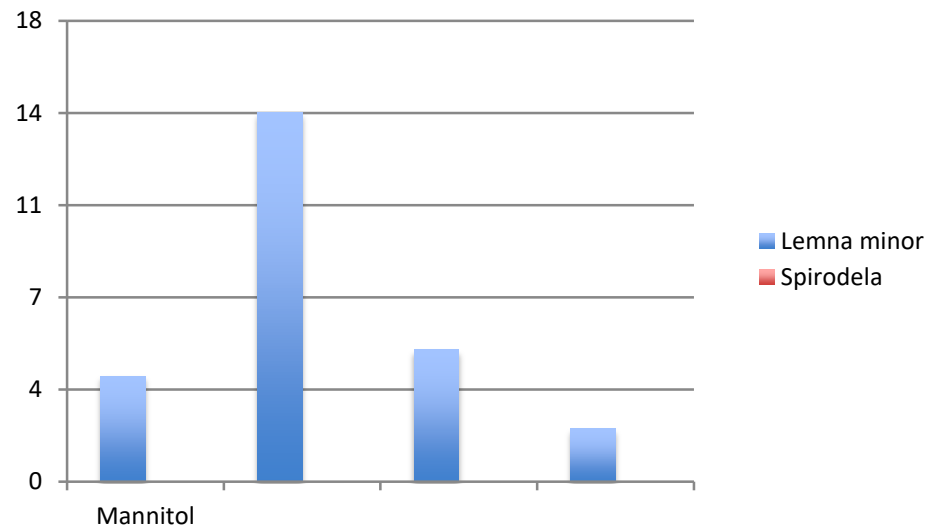
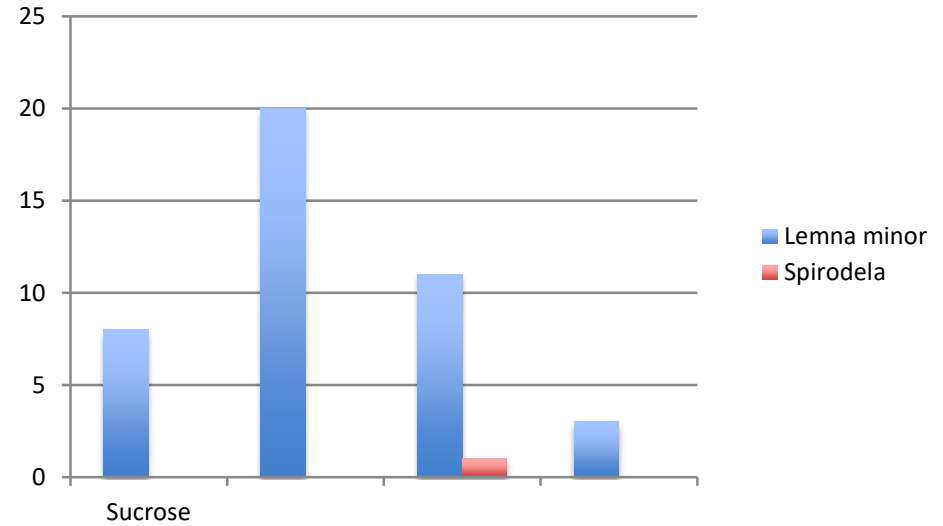
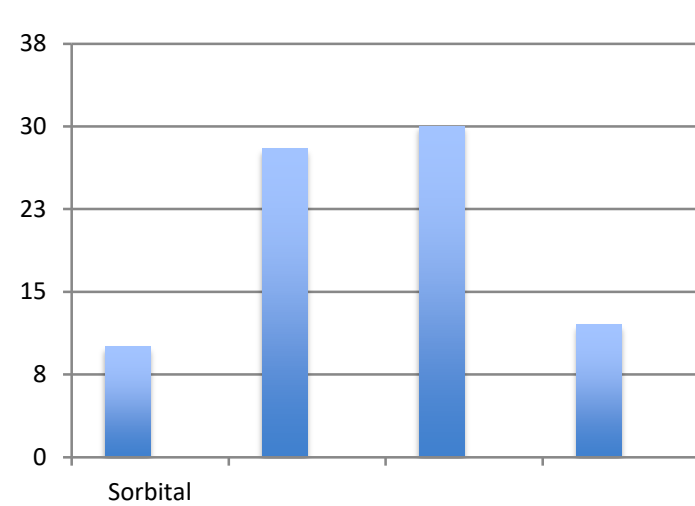
Callus maintenance/Proliferation



Browning of callus, poor growth due to oxidase, peroxidases, DIMBOA etc ?

Stunted growth in S.P

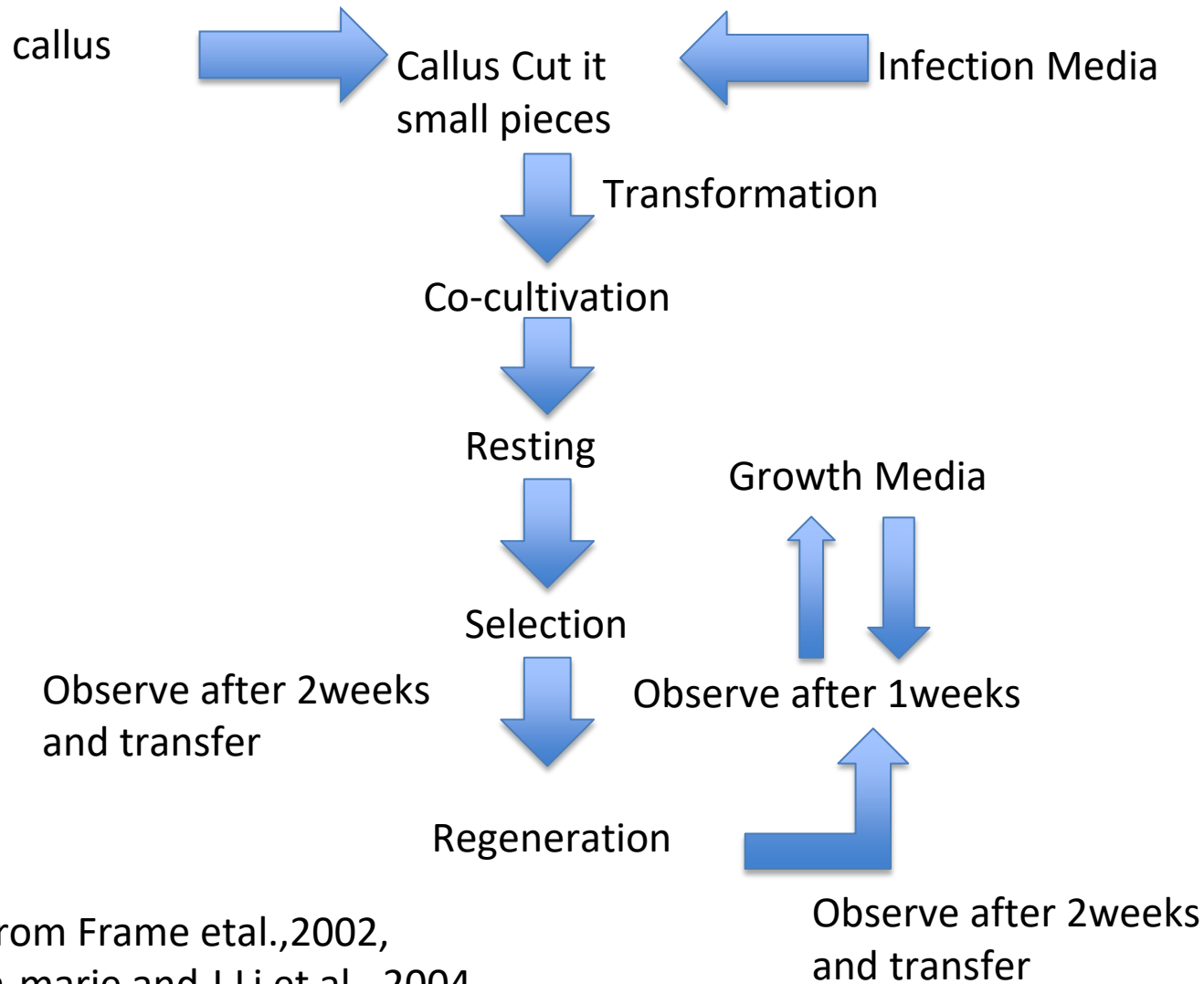
Callus proliferation



Lemnor minor 313 callus transformation

- The positive callus results set direction for Agrobacterium transformation of L.minor.
- J.Li et al.,2004, Ron vashu t al.,2007, Stomp Anne-marie EP20010967946 and Frame et al., 2002 guided the transformation protocol ref developed protocol

Agro- transformation/Selection/Regeneration

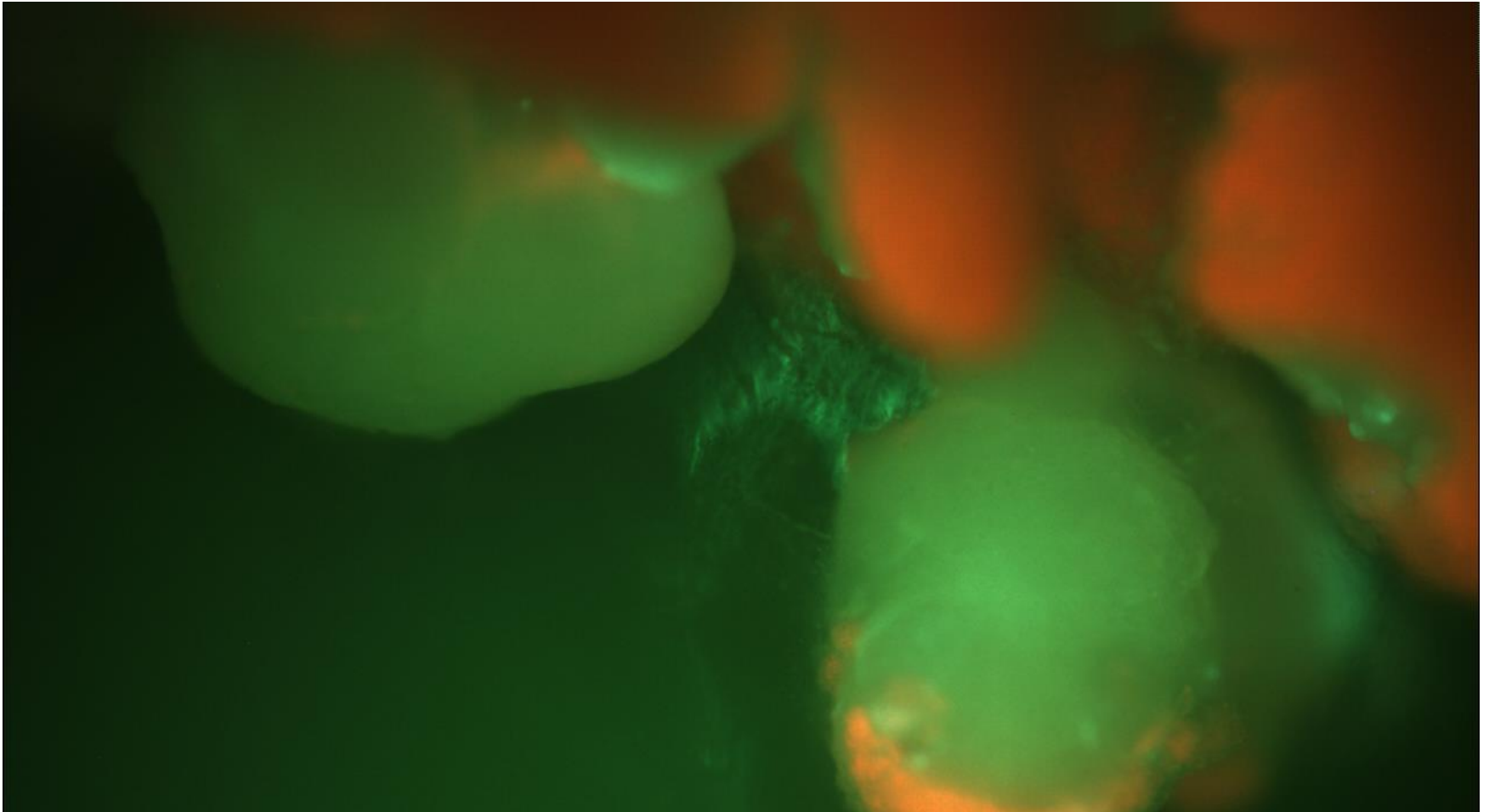


GFP Construct

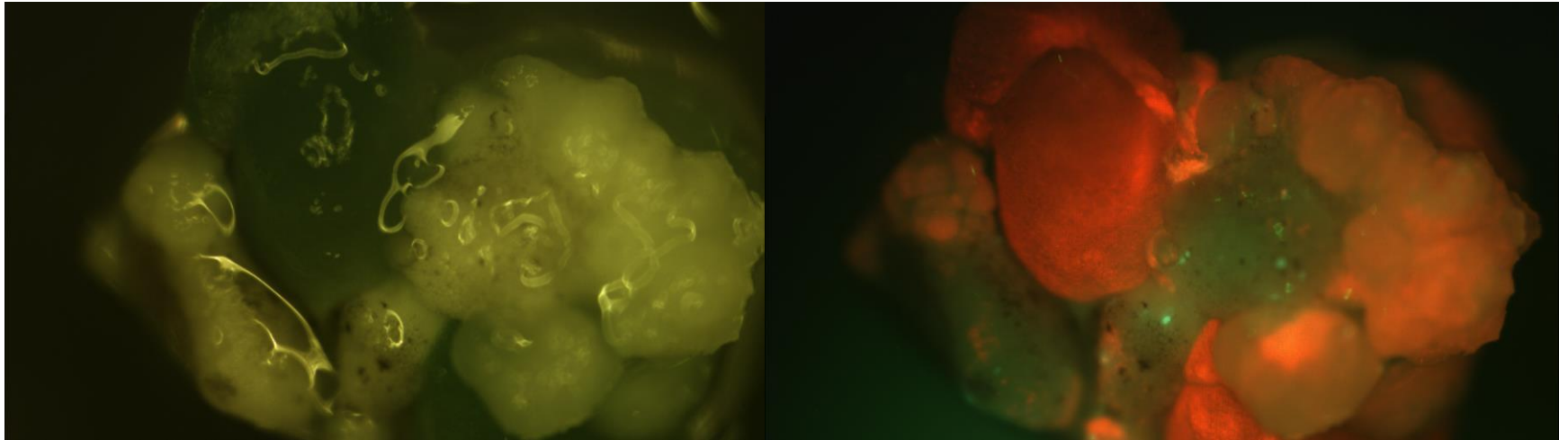


Yongrui construct

Transformed duckweed callus expressing GFP

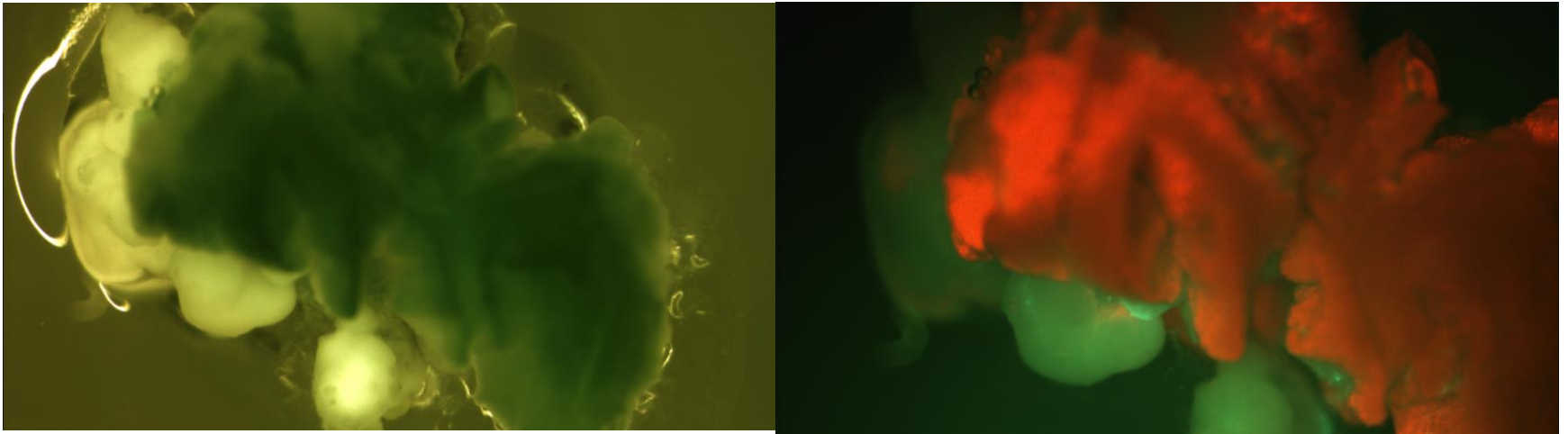


GFP positive on Selection



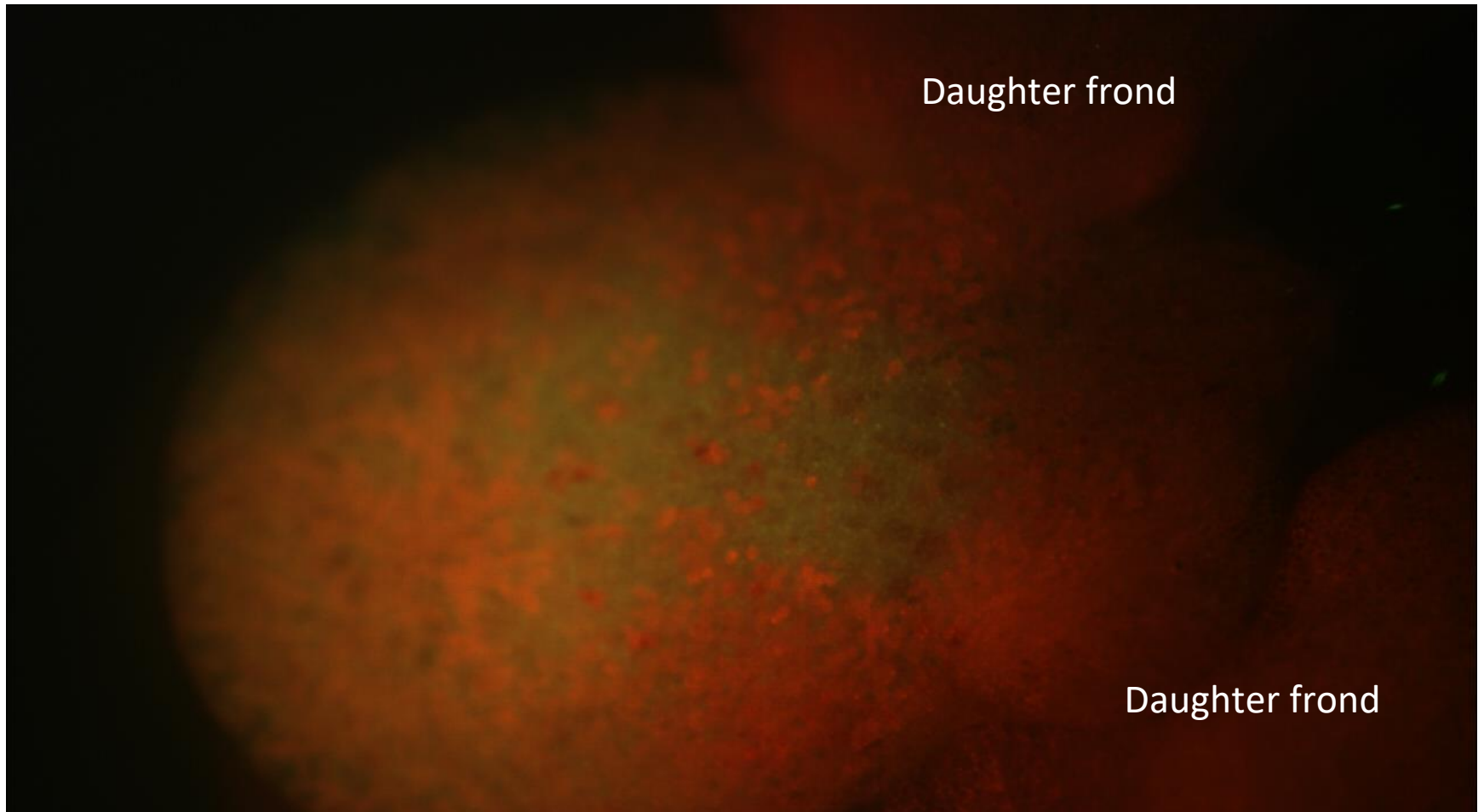
After 2 weeks on Selection

On Regeneration



After 2 weeks on regeneration media

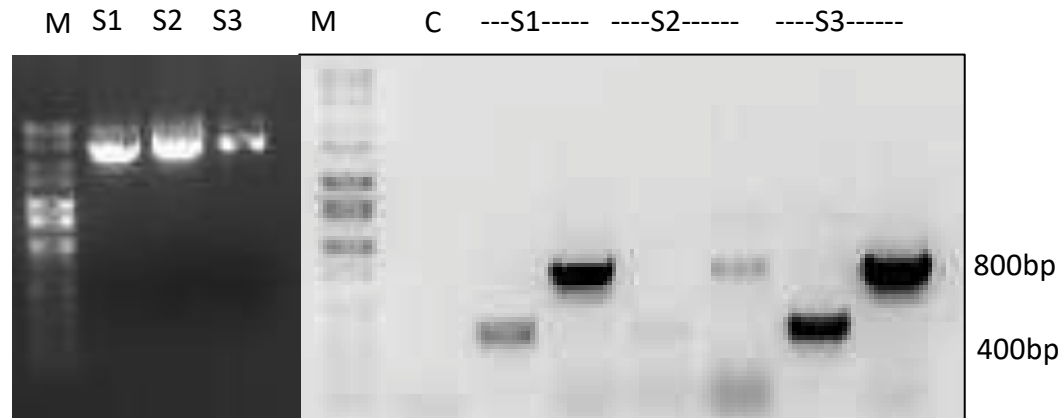
Transformed Duckweed



After 5 days on ½ Strength SH growth media

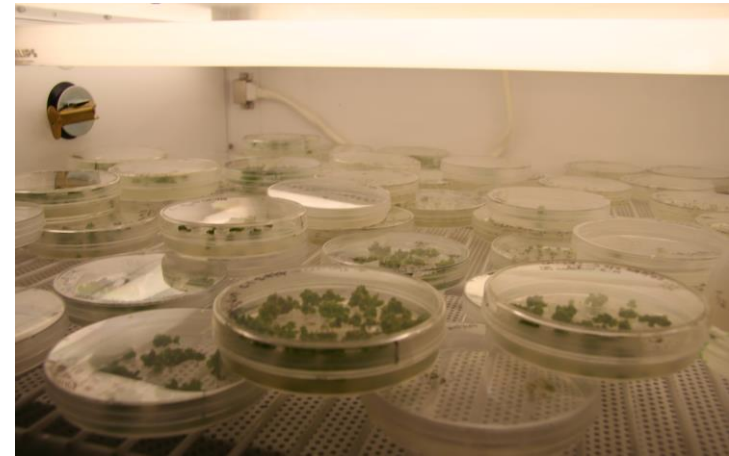
PCR results

Two sets of Primers encoding for different regions of the GFP cassette were used



Duck weed work

- Successful report
 - Regeneration efficiency of non transgenic Lemna minor 313 >95% observed
 - Transformation of >30 observed in some cases upto 60% in a plate
 - Transformation of regeneration of Lemna minor ww313-9332 achieved
 - Detected in daughter frond DNA by PCR.



Why Transform Duckweed?

- **High turn over**-Mass production of protein/essential aa supplements
- **Easy to transform**- direct/indirect methods possible
- **Genome is sequenced**- Research studies/track gene
- **Possible model plant?**

Absorption of Technology-Parabel Ltd UAE based Co. for Amino Acid Production

Parabel Completes the First Phase of Uganda Investment Project

Oct 30, 2013, 09:24 ET from Parabel, Inc.

ABU DHABI, United Arab Emirates, Oct. 30, 2013 /PRNewswire/ -- Parabel Ltd has successfully completed the first phase of a 7 million USD, high tech agro-investment that produces human food and animal feed in Entebbe, Uganda.

The project run by the Abu Dhabi-based company, Parabel Ltd, involves a unique technology designed to grow and process a locally available aquatic plant, Lemna, to create food and feed products.

This involved the large-scale growth and harvesting of local micro-crops in a demonstration facility outside Entebbe International Airport. At the minister's follow-up visit, Hon Bright Rwamirama, State Minister for Agriculture, Industry and Fisheries, pledged government support for the project.

Parabel's technology is recognized as part of the solution to the food security crisis with global estimates indicating that food demand will outstrip current supply with 30% more food needed in the next 40 years.

The sustainably grown micro-crop is produced without using expensive farmland and minimizing use of local resources. It is not genetically modified and is harvested all year around by local workforce. The company's solution provides a sustainable harvest and production providing local workforce with opportunities.

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- Home
- Authorities
- Educational Inst
- Events
- Finance & Insurance
- Fundamentals
- Govt Buganda
- Govt Uganda
- Media
- News Archive
- Products & Services
- Sales & Marketing
- Social
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PARABEL
Parabel Ltd
Tel: +1 (321) 409-7970

Location: 1901 S. Harbor City Blvd., 6th Floor Melbourne, FL 32901 USA

PARABEL Parabel completes the first phase of Uganda investment project
Parabel Ltd has successfully completed the first phase of a 7 million USD, high tech agro-investment that produces human food and animal feed in Entebbe, Uganda. The project run by the Abu Dhabi-based company, Parabel Ltd, involves a unique technology designed to grow, harvest and process a locally available aquatic plant, Lemna, to create food and feed products. [Read more](#)

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Duckweed Lagoon of Parabel Ltd
in UAE



biosafety bill
passed yesterday?

Uganda President visit USD7M
Project-2013



Harvesting of Duckweed



68% Protein- 50 times more than Soy

Natural advantages of duck weed?

- Like Water Hyacinth
 - Biofuel——bioethanol/biogas
 - Bioremediation
 - Sink of N, P, K and heavy metals
 - Nutrient recycle
 - Harvest and used as fodder
 - Compost manure

Thank You



Masinde Muliro University of
Science and Technology



Waksman Institute of Microbiology
Rutgers University