

# Cryopreservation of avocado shoot tips for long term conservation.

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## Current Status

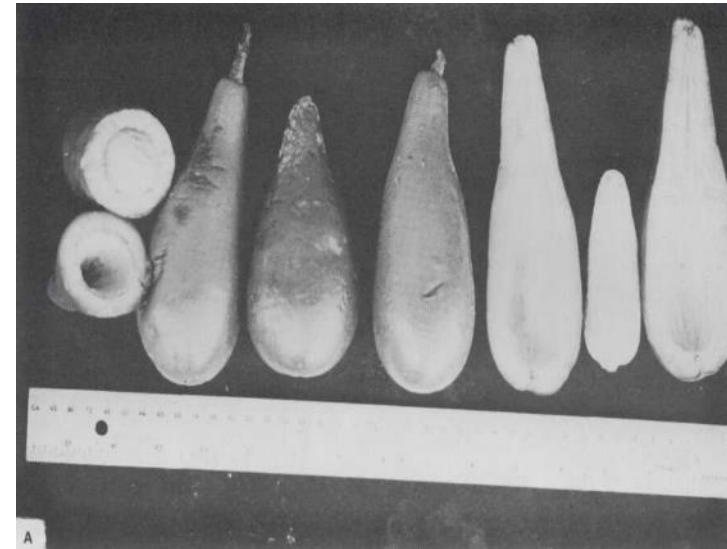
Over 1000 accessions across the globe are  
maintained  
only in **field repositories.**

Advantage:  
evaluation of trees for parameters such as yield, tree  
height and disease resistance.



# Disadvantages of field repositories are:

- Costly to maintain
- High inputs in the form of land and labour
- Exposed to natural disasters
- Pests and diseases
- Limitation of numbers

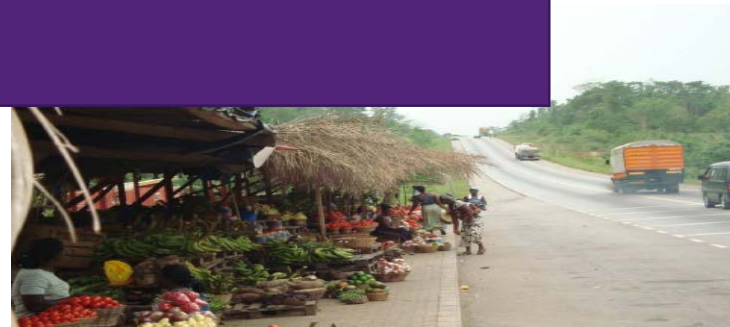


# Avocado Germplasm Diversity

- Drought tolerance (Guatemalan in Chiapas)
- Good fruit quality (Guatemalan in Chiapas)
- High oil content (Mexican in Veracruz)
- Tree longevity (Mexican in Yucatán)
- Two harvests a year (West Indian in Yucatán)



(pictures from George Nksanh)



Avocado seeds are  
recalcitrant and heterozygous.



- Recalcitrant seeds do not survive freezing and drying, therefore not amenable to seed storage.
- Avocados are open-pollinated crops and seeds are heterozygous and have limited conservation value.

# *In Vitro* Conservation

Tissue Culture



Cryopreservation

Protocols have been developed for plant species using

- Apices
- Calli
- Cell suspensions
- Seeds
- Somatic embryos
- Zygotic embryos



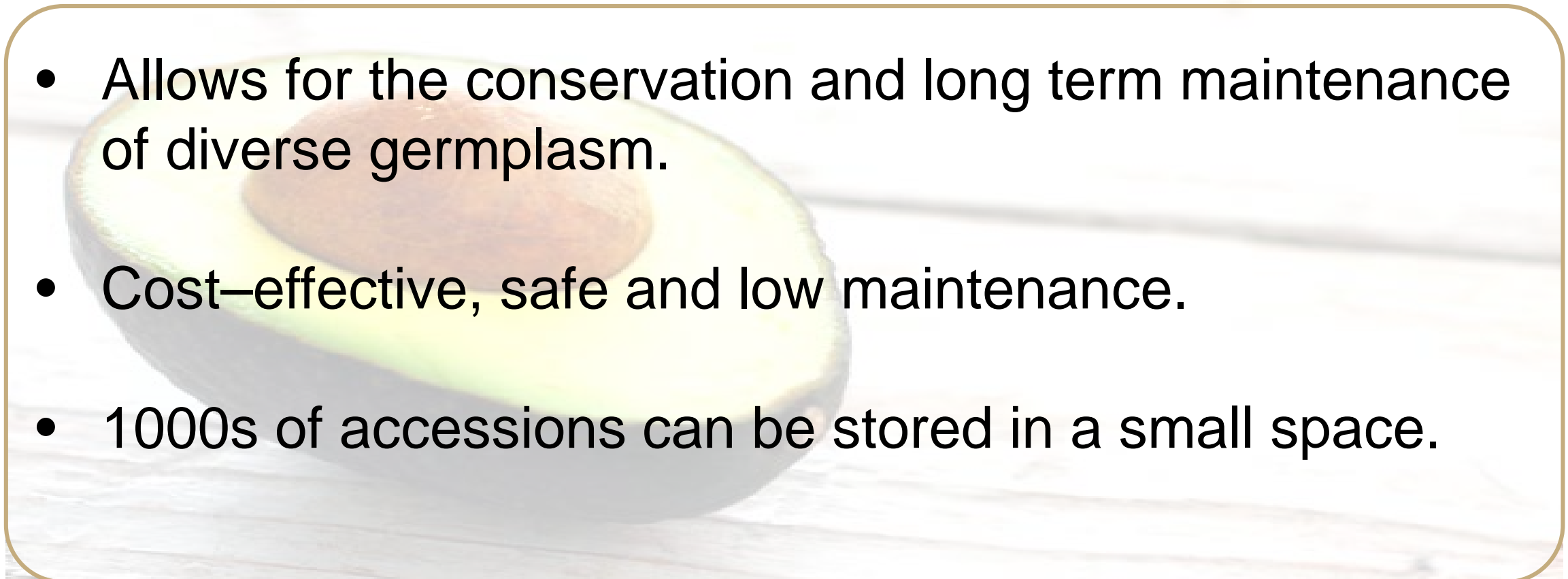
Engelmann 2011b

# What is Cryopreservation?

Cryopreservation is the storage of living tissues at ultra-low temperature in liquid nitrogen (-196 °C).



# Cryopreservation benefits

- 
- Allows for the conservation and long term maintenance of diverse germplasm.
  - Cost-effective, safe and low maintenance.
  - 1000s of accessions can be stored in a small space.



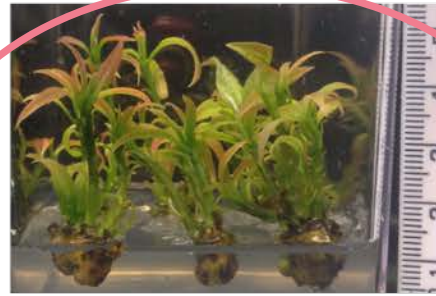
# World's first Avocado tissue culture system as developed by the Mitter lab



F1 ( Multiplication)



F2 (Multiplication)



S1 ( Shoot elongation)



Single shoots



Rooting (90% success at 4 weeks)



Acclimatised 6 wks (>95% success)



<1 year old



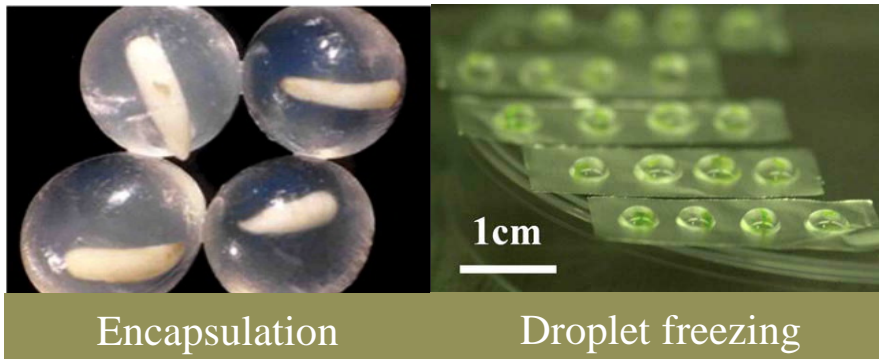
Nursery maintained plants

# Dissection of avocado shoot tips

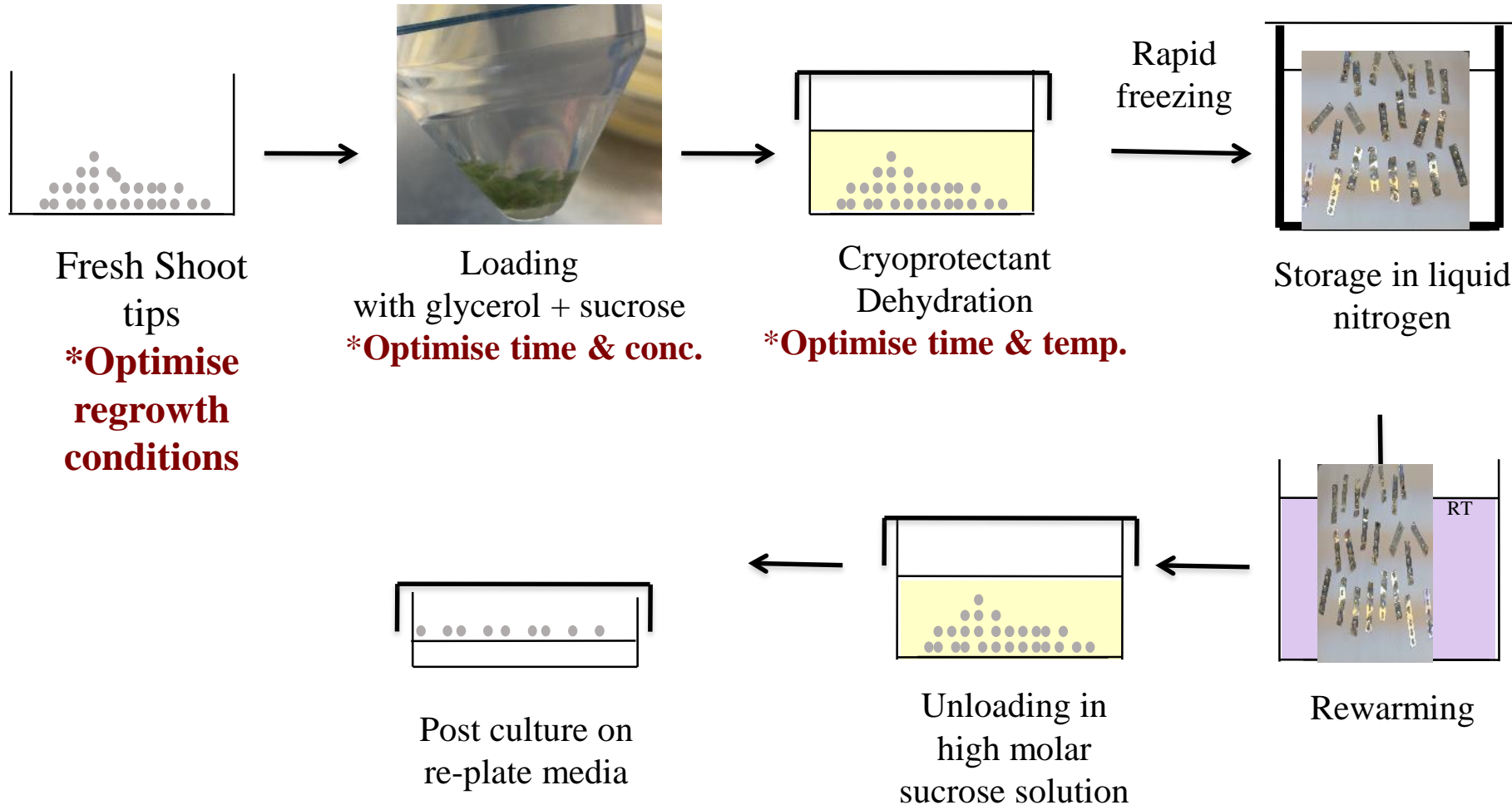


# Cryopreservation techniques

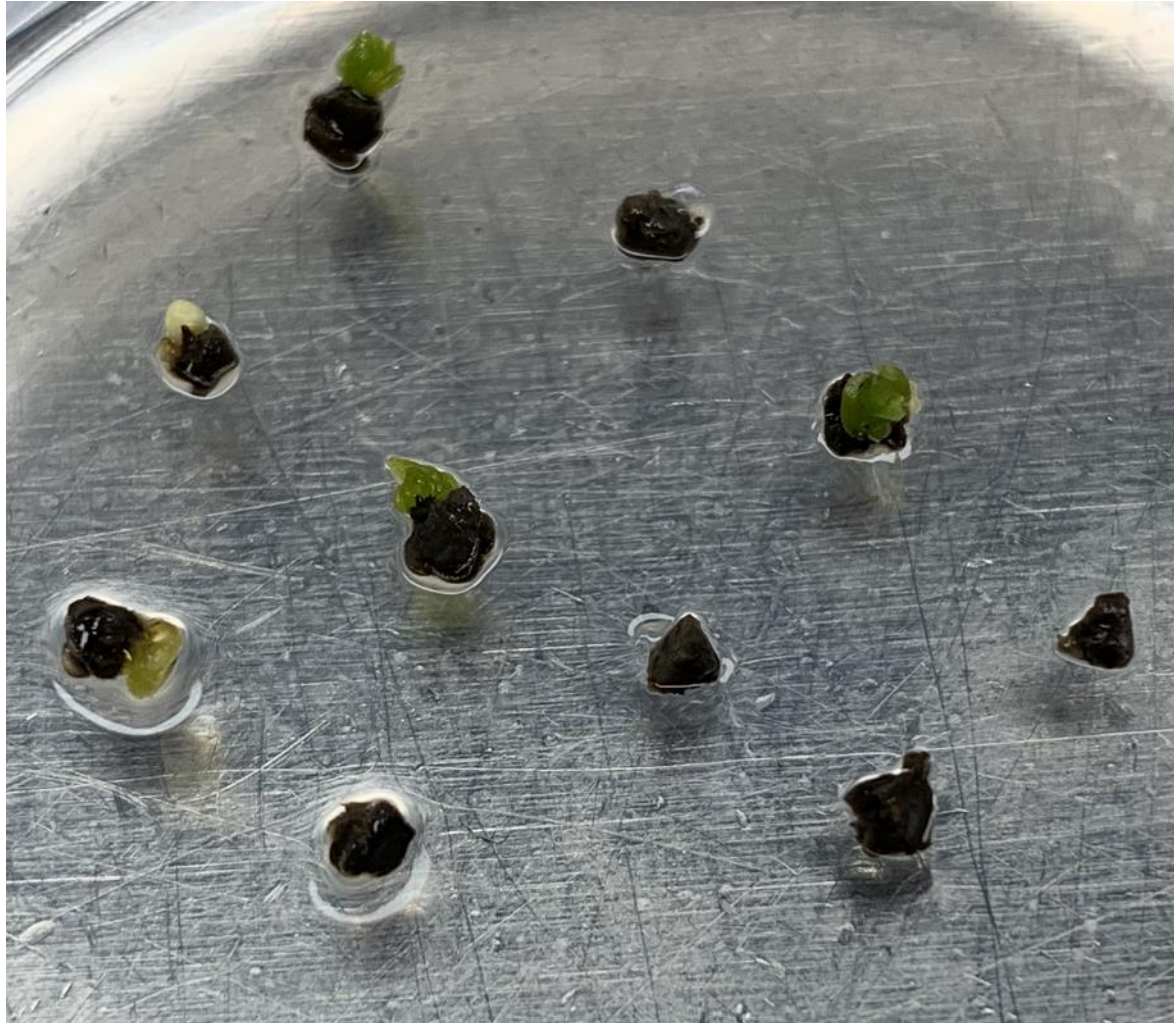
- Encapsulation-dehydration
- Encapsulation-vitrification
- Classical vitrification
- **Droplet-vitrification**
- Dehydration using DMSO



# Droplet vitrification



# Fresh cut





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## ISHS Acta Horticulturae 1285: XXX International Horticultural Congress IHC2018: II International Symposium on Micropropagation and In Vitro Techniques

### Developing a cryopreservation protocol for avocado (*Persea americana* Mill.) apical shoot tips using different antioxidants

**Authors:** C. O'Brien, J.C.A. Hiti-Bandaralage, R. Folgado, S. Lahmeyer, A. Hayward, N. Mitter

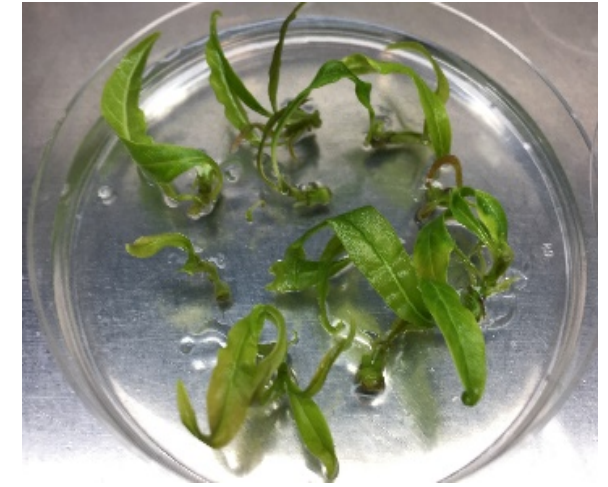
**Keywords:** ascorbic acid, PVP, citric acid, melatonin, avocado, browning

**DOI:** [10.17660/ActaHortic.2020.1285.3](https://doi.org/10.17660/ActaHortic.2020.1285.3)

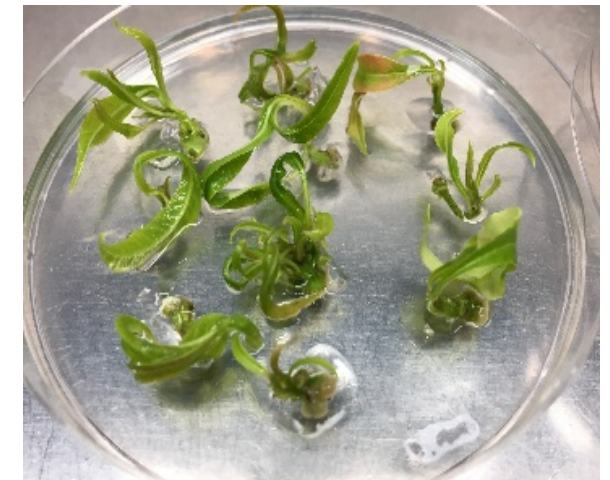
# Summary

- Shoot quality was affected by treatments in regrowth media; i.e. signs of vitrification, narrowing of leaves and stunted growth.

- Ascorbic acid at 100, 250 mg/L best controlled browning and displayed normal growth.



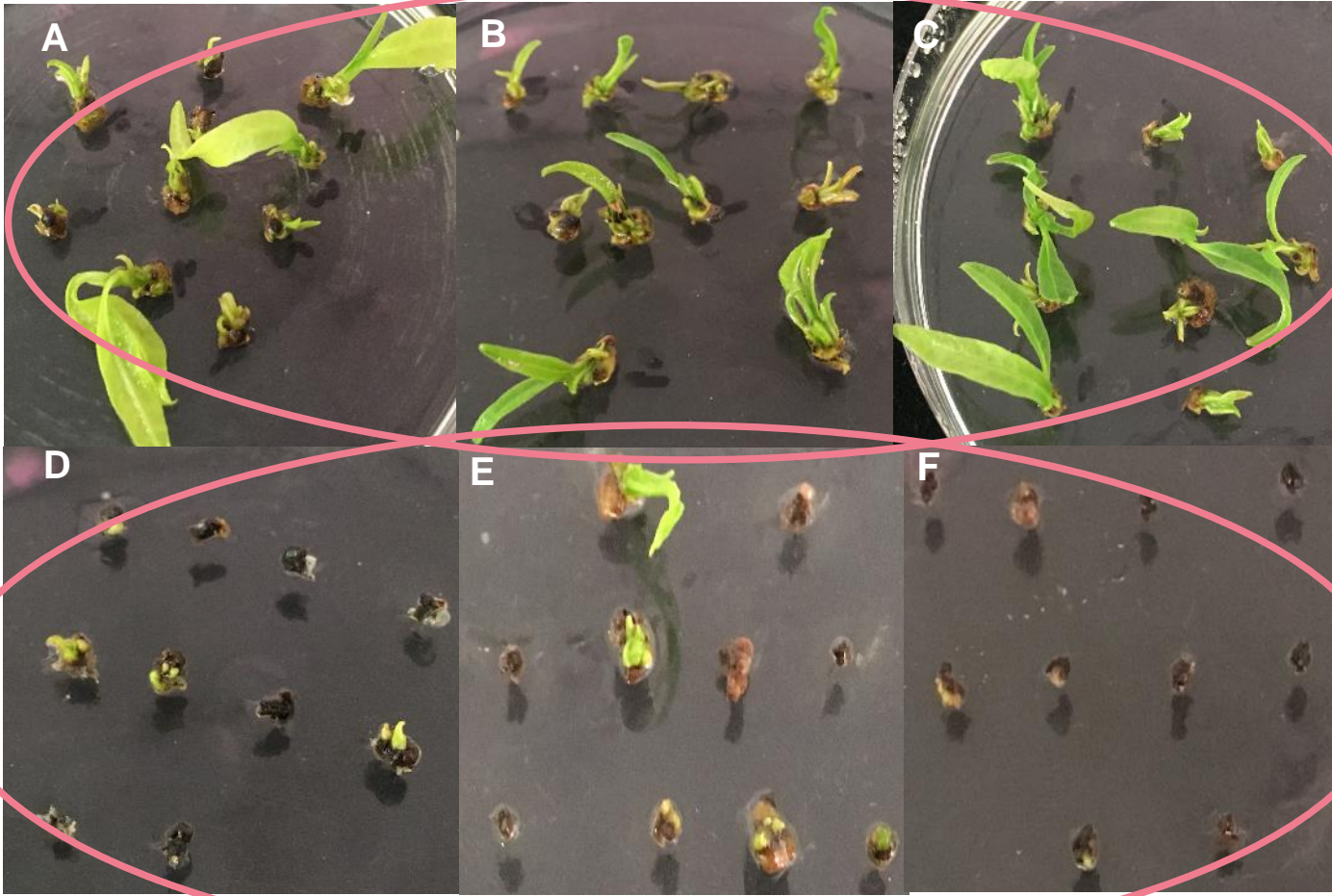
Ascorbic acid 100 mg/L



Ascorbic acid 250 mg/L

O'Brien, C. et al 2020

# Application of CPAs



A to C Loading only

D to F Loading + PVS2 0°C 20 min

Survival and regrowth was poor after being treated with CPAs



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## A method to increase regrowth of vitrified shoot tips of avocado (*Persea americana* Mill.): First critical step in developing a cryopreservation protocol



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# Results - Pre-treatment of donor plants

cv Velvick



O'Brien, C. et al 2020a

# Results - Pre-treatment of donor plants

cv Reed



O'Brien, C. et al 2020a

## Summary

- Pre-treatment is beneficial to increase tolerance of avocado shoot tips after being treated with cryoprotectants.
- Characteristics in field
  - 1) Velvick high salinity tolerance and poor cold tolerance
  - 2) Reed moderate cold tolerance and salinity tolerance
- Type of pre-treatment used is dependant on cultivar and seems to related to avocado race genetic attributes i.e. cold tolerance (10 °C) and salinity tolerance (0.3 M sucrose)

## Addition of LN step to best pre-culture method for each cultivar



Original Article | Published: 01 June 2020

# First report on cryopreservation of mature shoot tips of two avocado (*Persea americana* Mill.) rootstocks

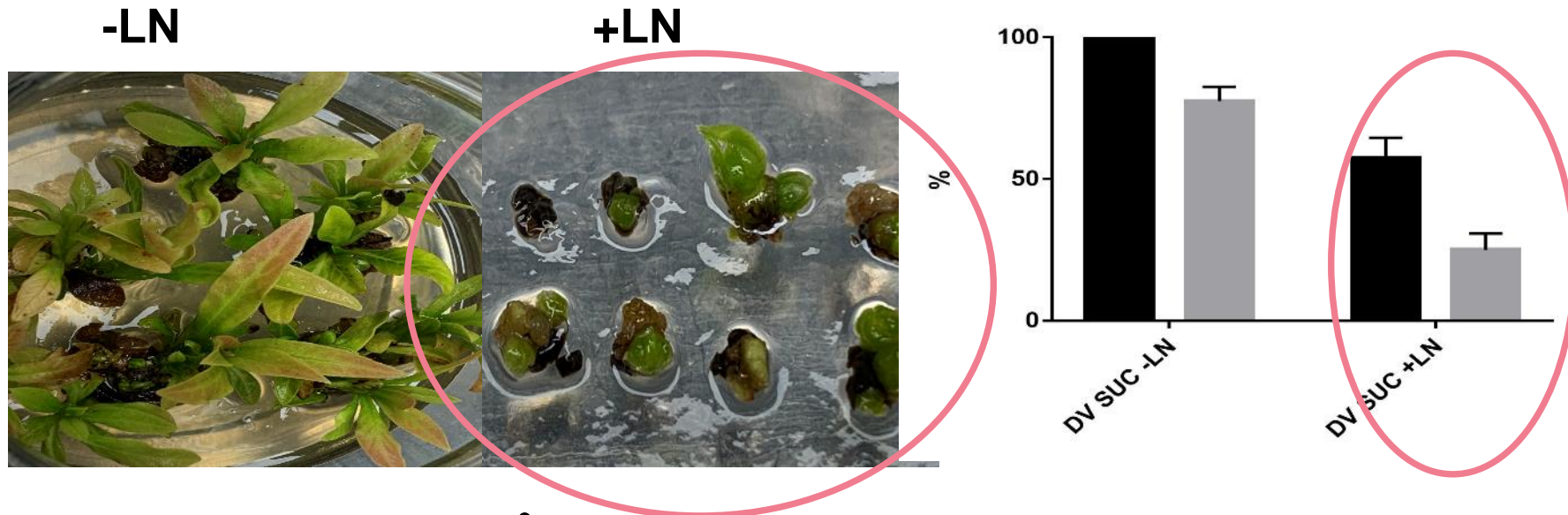
[C. O'Brien](#), [J. C. A. Hiti-Bandaralage](#), [R. Folgado](#), [S. Lahmeyer](#), [A. Hayward](#), [J. Folsom](#) & [N. Mitter](#) 

[Plant Cell, Tissue and Organ Culture \(PCTOC\)](#) (2020) | [Cite this article](#)

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# Velvick

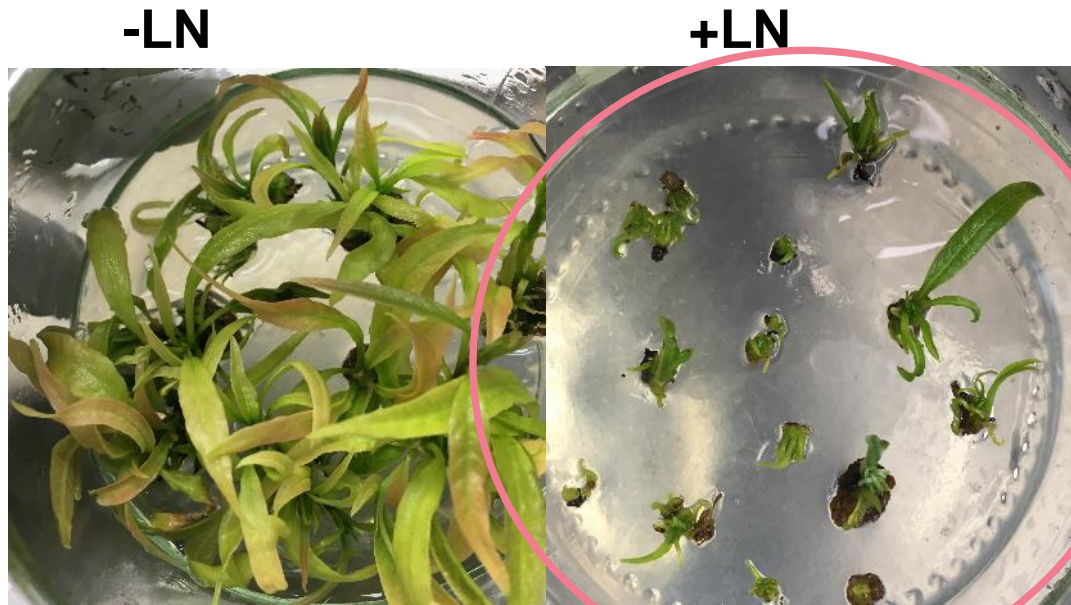
## Pre-culture donor plants with 0.3M sucrose 2 weeks +/-LN



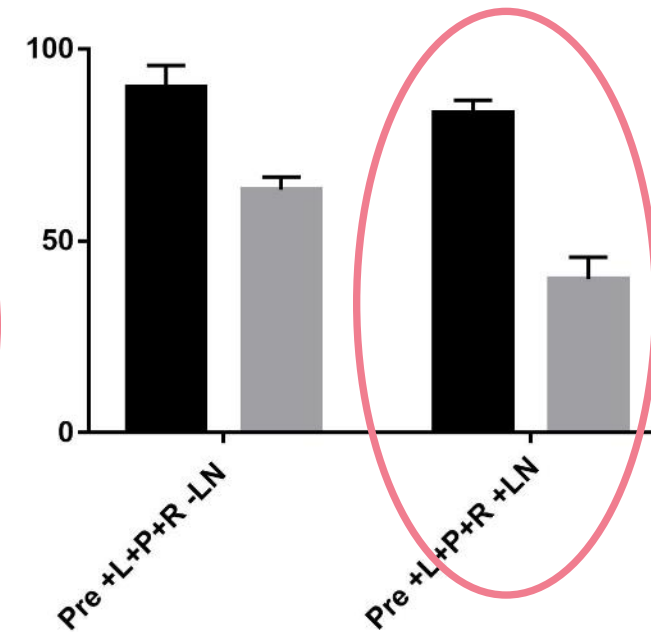
Survival after LN PVS2 0° C 20 min 60%  
and Regrowth of 35 % after 8 weeks

# Reed

## Pre-culture donor plants with 10 °C 2 weeks +/-LN

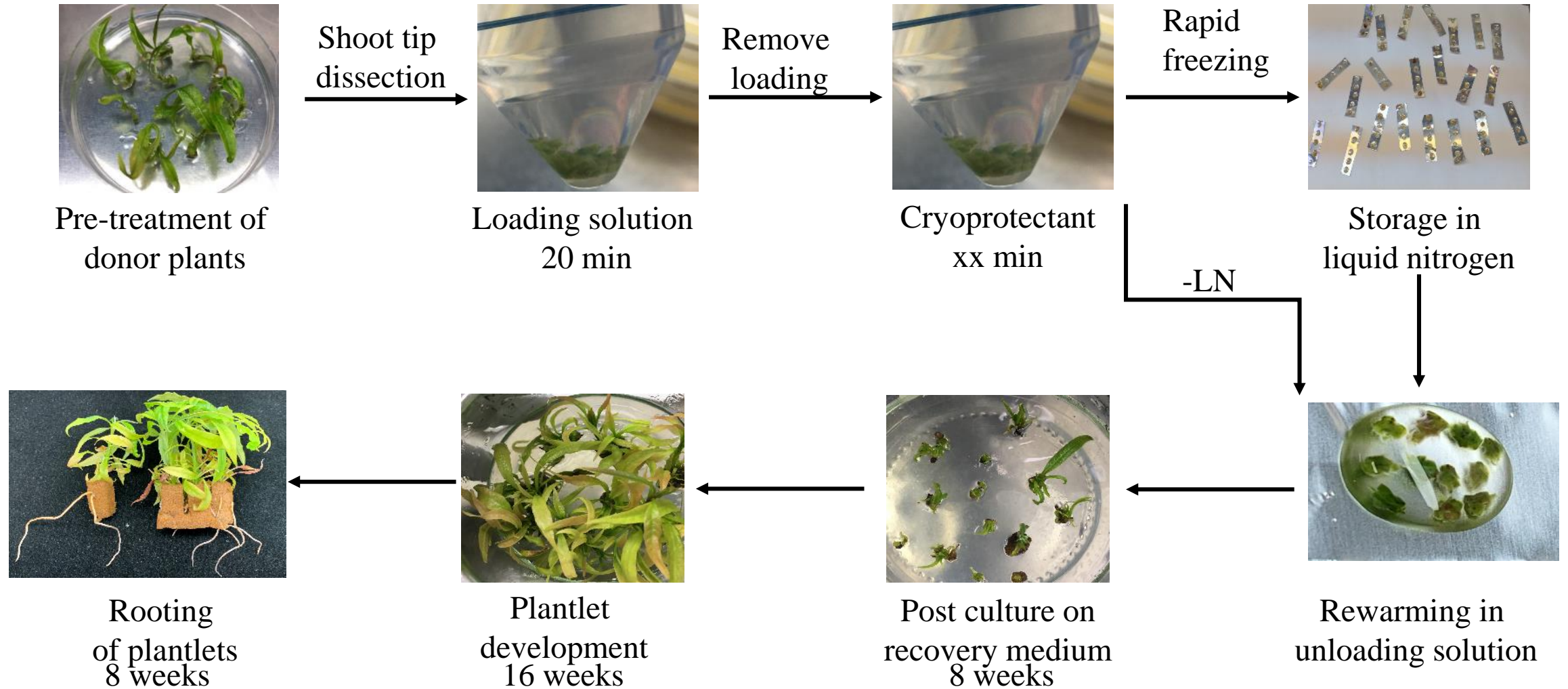


Survival after LN PVS2 0°C 20 min 90%  
and Regrowth of 40% after 8 weeks





# Flowchart of protocol for avocado



O'Brien, C. et al 2020b

# Acclimatization of Reed plants after LN





## Summary

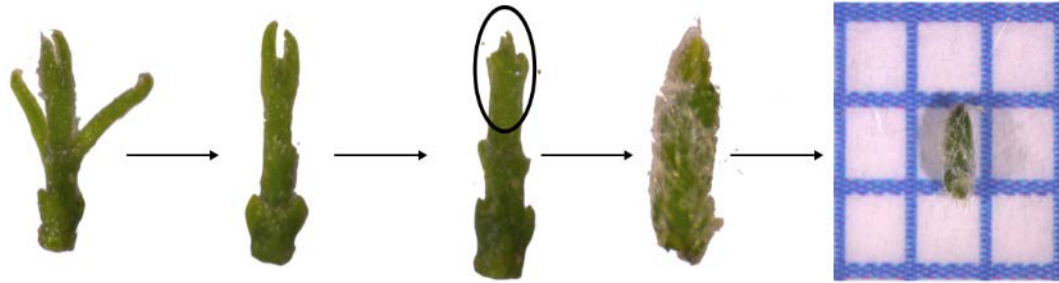
- This is the first report of successfully storage of avocado shoot tips in LN and regeneration into full plants.
- Achieved by using pre-culture to increase survivability following cryoprotectant incubation of avocado as well as cryoprotectant optimization.
- Cold pre-treatment not suitable for Velvick.
- Sucrose pre-treatment not suitable for Reed.

# Expanding to other Avocado Germplasm



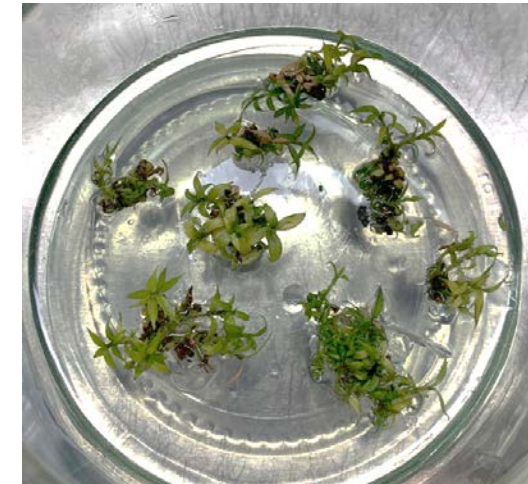
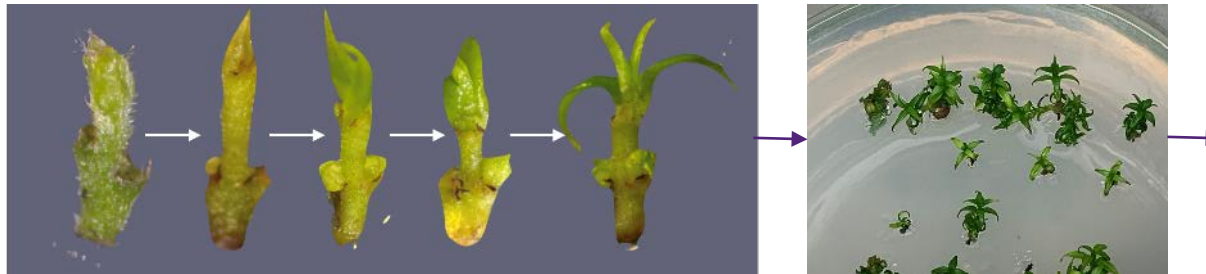
# Progress in Australian spp.

**Macadamia**  
(Xueying Li PhD)



Survival after cryoprotectant  
Now optimising LN2 exposure

**Gossia**  
(Jingyin Bao PhD  
Chris O'Brien)



40%  
regrowth  
after LN2  
exposure



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