



THIRD INTERNATIONAL SYMPOSIUM ON THE FAMILY
MAGNOLIACEAE, CUBA 2016



Cryopreservation of *Magnolia macrophylla* var. *ashei* shoot tips by droplet vitrification

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12 gardens.
15,000 plant varieties.



The Huntington



Cryopreservation at The Huntington

Shoot tips

- Aloe
- Agave
- Magnolia
- Avocado
- Oak

Seeds

- Cacti
- Orchids
- Magnolia

Pollen

- Titan arum
- Cycads

Embryos

- Oaks
- Cycads

Provide protocols for long-term conservation of plants



Conservation of Plant Germplasm

- *In situ*: natural environment
 - (-) High risks of losses, highly exposed
- *Ex situ*: botanical gardens, research centres, seed and germplasm banks

Ex vitro: Traditional method (field collections)

- (-) Infection risks, labour-intensive

In vitro: Vegetatively propagated species

- Normal growth
- Slow growth (temp \nearrow , $O_2 \nearrow$, $H_2O \nearrow$, medium \sim)

(+) Sterile material, no risks by insects or weather conditions

- (-) Still labour-intensive, risks for contamination, human error or somaclonal variation

Cryopreservation (-196°C): Long-term germplasm conservation

- (+) Unlimited storage time, reduced costs & space, prevention of infection and genetic changes

Cryopreservation

Cryopreservation (Greek, krayos-frost) literally mean “in the frozen state”

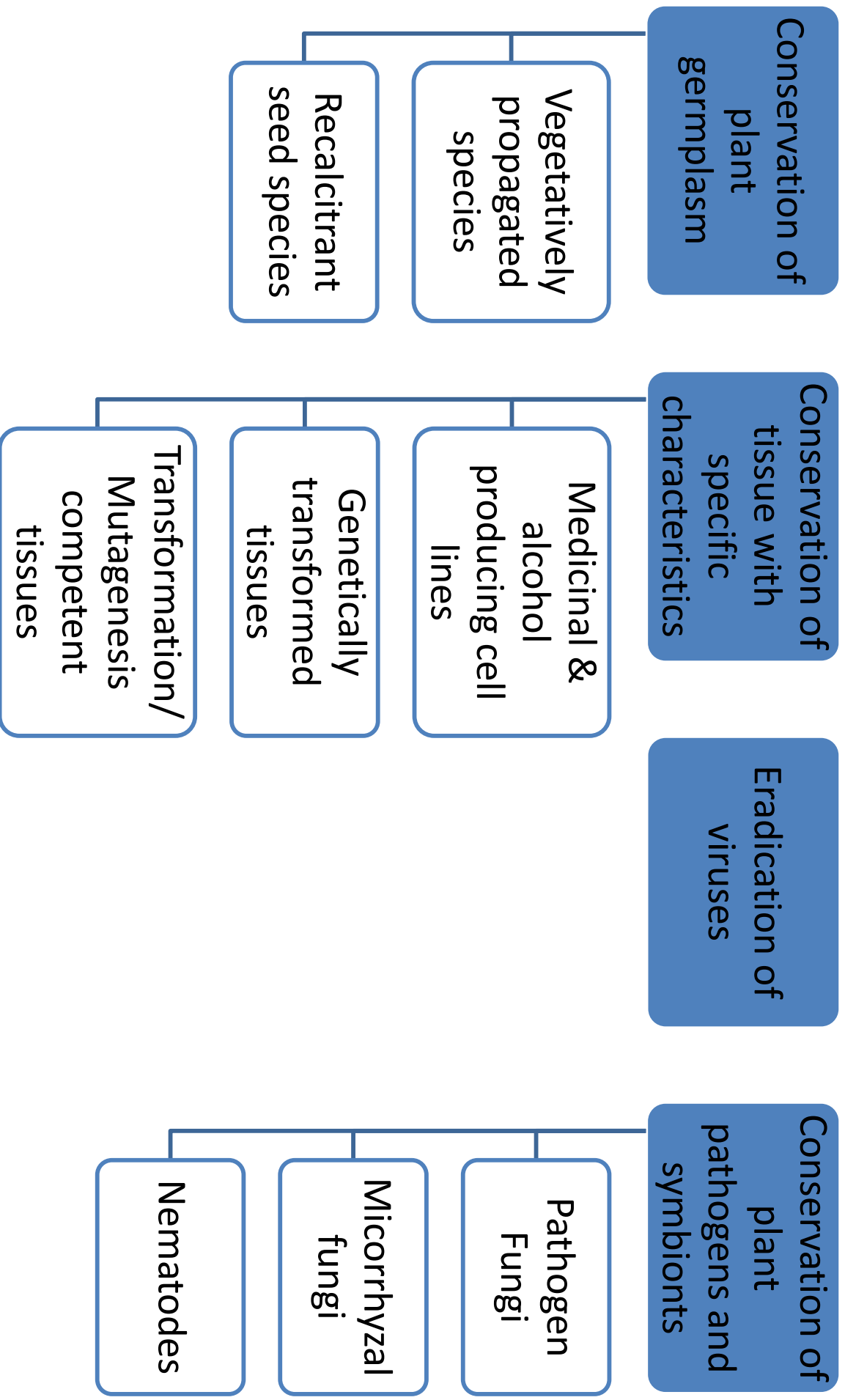
Process of cooling, or freezing, and storing cells, tissues or organs to ultra-low temperatures and keep them for a future use

- Over solid carbon dioxide (at -79°C)
- Low temperature deep freezers (at -80°C)
- In liquid nitrogen (at -196°C , gas phase -150°C)

At ultra-low temperature (such as -196°C), the metabolic processes and biological deteriorations in the cells/tissues are stopped.

Cryopreservation

Uses of cryopreservation



Ex situ conservation of magnolias using cryopreservation

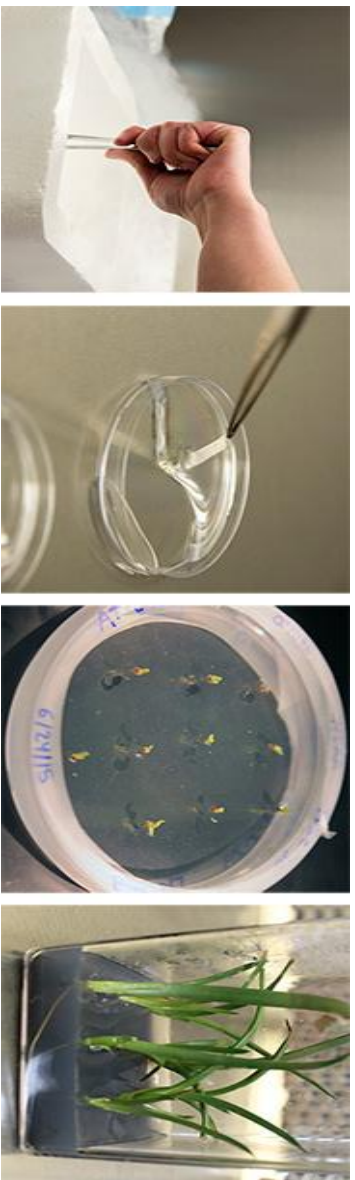
- ~1/2 of the Magnoliaceae taxa are threatened worldwide
- Development of efficient methods of ex situ conservation plays a crucial role
- Conservation of woody plants in field clonal banks is costly and risky
- Tissue culture helps to ensure the ex situ preservation of threatened Magnoliaceae
- Prior to the implementation of protocols for the cryopreservation of *Magnolia* spp., establishment of micropropagation from adult trees is needed
- The proliferation of *Magnolia* shoots is difficult due to the oxidation of phenolic compounds during micropropagation, recovery from cryopreservation and rooting before *ex vitro* acclimation

Cryopreservation by droplet vitrification



Photo by Kate Lain

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Photos by Kate Lain

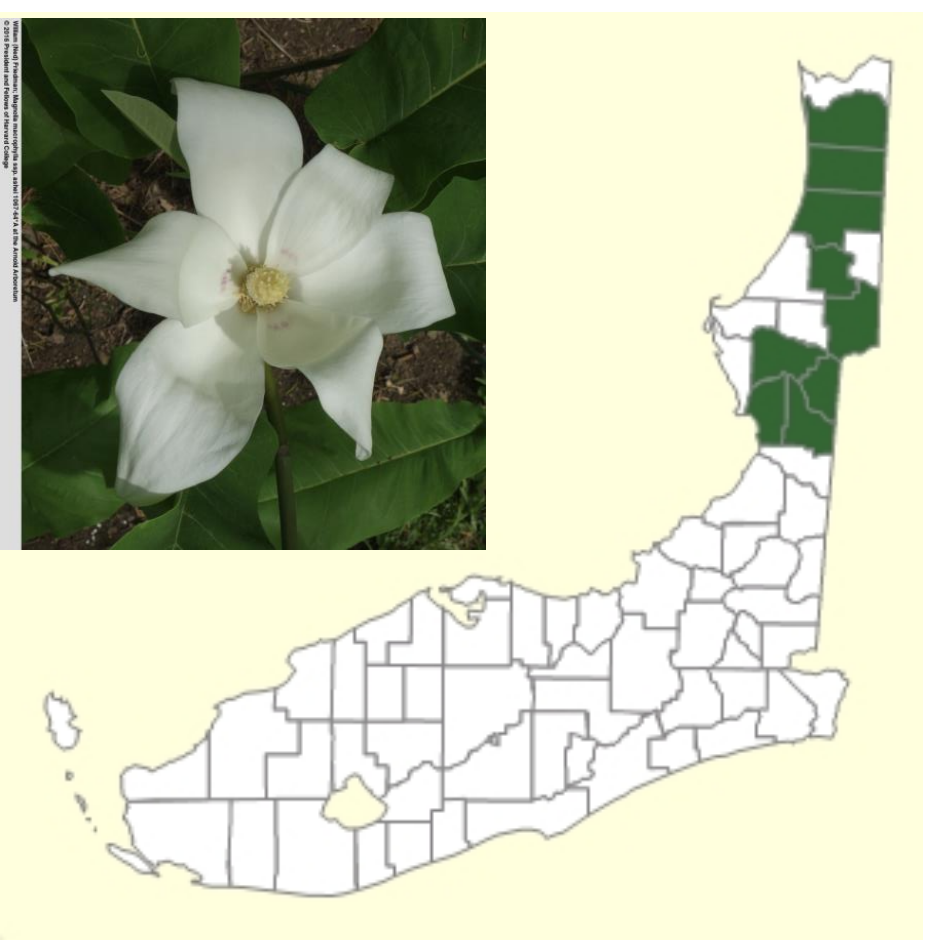
-Key: avoidance of the formation of intracellular ice

Vitrification: involve dehydration with highly concentrated cryoprotectant mixtures

Droplet-vitrification: reduced damage during cooling and re-warming

Ashe's Magnolia

- Small tree or large shrub, 15 - 30 feet tall
- Endemic to eight counties of the Florida Panhandle.

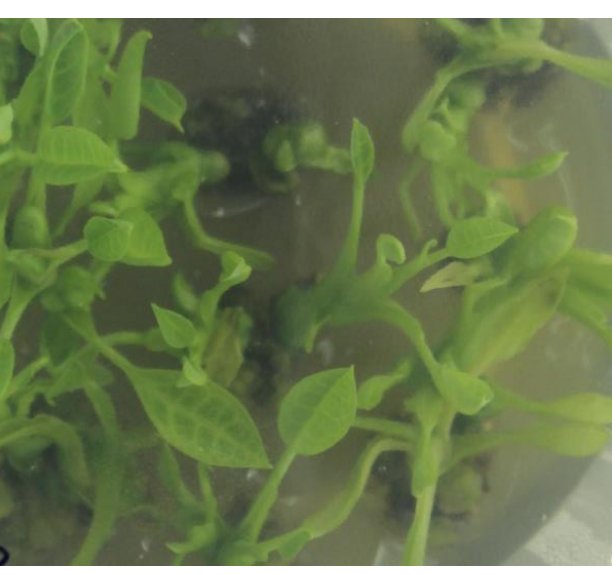
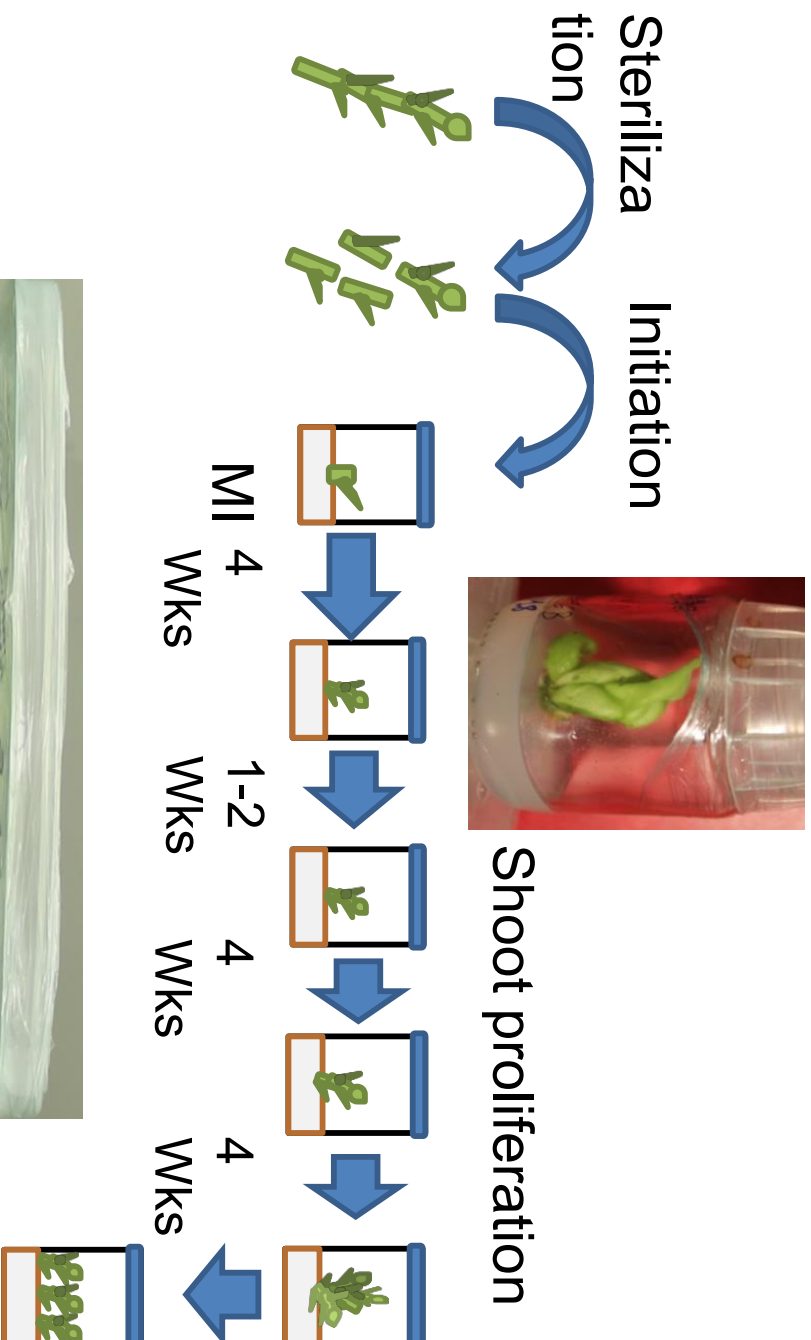


<http://floridata.com/Plants/Magnoliaceae/Magnolia%20macrophylla%20subsp.%20ashei/1071>

<http://florida.plantatlas.usf.edu/Plant.aspx?id=1241>

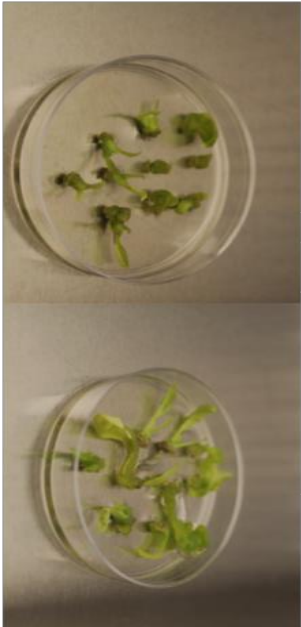
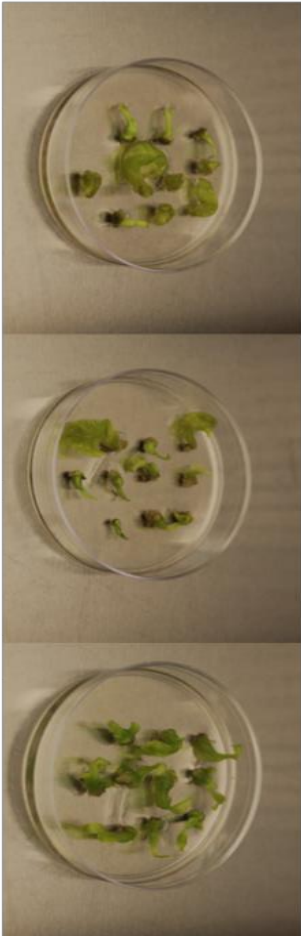
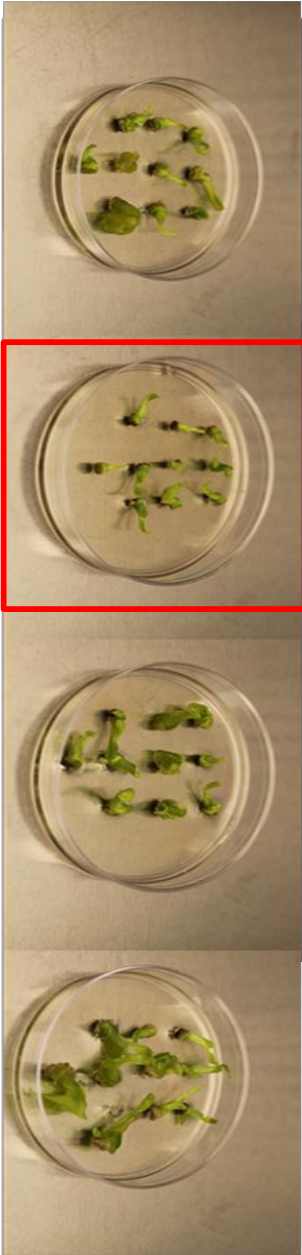
<http://www.arboretum.harvard.edu/plants>

Tissue culture of *Magnolia macrophylla* var. *ashei*



Optimization of medium for shoot tips recovery

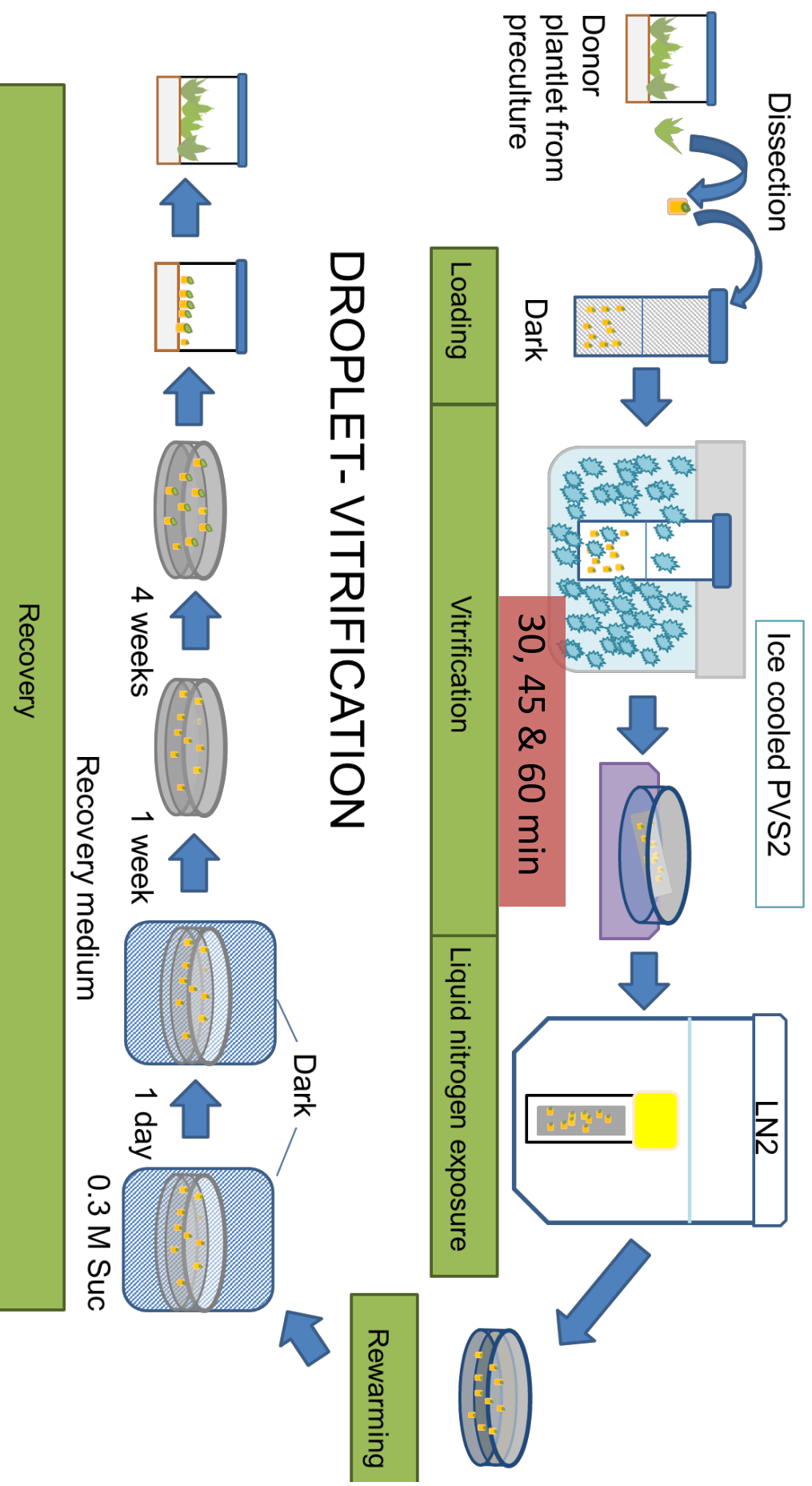
NAA			
higher			lower



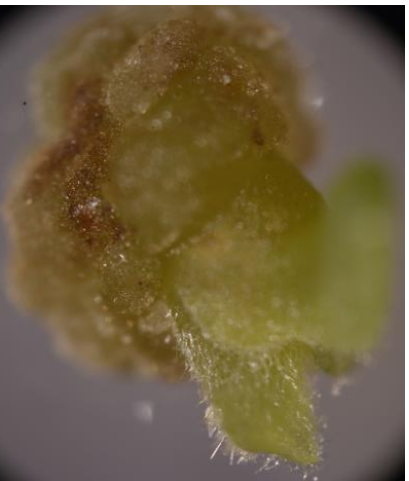
Shoot tip
growth, 4
weeks
after
dissection

BA	High
	Med
	Low

Cryopreservation using droplet-vitrification



Shoot-tip response to droplet-vitrification and LN exposure

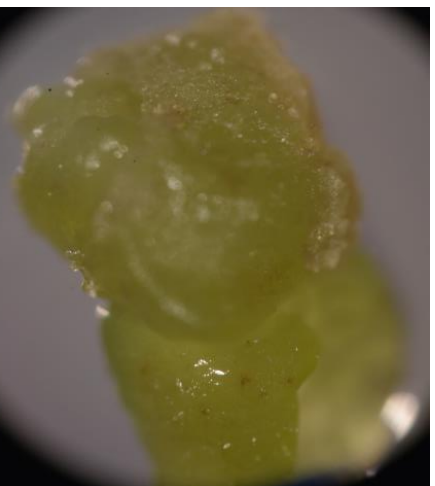


Normal

Post-rewarming regeneration rates were similar in explants treated 45 and 60 min



Callus



Hyperhydric

Lowest hyperhydricity was found in shoot tips exposed to PVS2 for 45 min

Conclusions

- Droplet-vitrification is a suitable method for the long-term conservation of *Magnolia macrophylla* var. *ashei*
- Recovery of plants from cryopreservation still needs to be optimized
- The recovery from cryopreservation depends on:
 - State of plantlets (preculture)
 - Use of antioxidants (before, during and/or after protocol)
 - Recovery medium (nutrients and hormones)

Outcome

- At The Huntington, we intend to provide new tools for plant conservation to other institutions and users, for the long-term conservation of magnolias
- In vitro repository at The Huntington:
 - M. acuminata* var. *subcordata*, *M. campbellii* spp *mollicomata*, *M. figo*, *M. garrettii*, *M. grandiflora*, *M. liliiflora*, *M. macrophylla*, *M. macrophylla* var. *ashei*, *M. macrophylla* var. *dealbata*, *M. officinalis*, *M. pacifica* spp *pacifica*, *M. pacifica* spp *tarahumara*, *M. sphaerantha*, *M. tamaulipana*, *M. x soulangiana*, *M. delavayii*,
- Cryopreservation technologies may be crucial to the conservation of Magnoliaceae diversity worldwide



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THANKS!

¡GRACIAS!

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