CRYO2019

EXTENDED ABSTRACT FORM
STUDENT AWARDS AND TRAVEL GRANTS

APPLICANT INFORMATION	
Name: Lourdes Delgado-Aceves	
Degree Program: Ph. D. CS Biosystematics, Ecology and Management of Natural and Agricultural Resources	Expected Date of Completion: July 31th, 2021
University Name: University of Guadalajara	Country: Mexico

PRESENTATION/ABSTRACT TITLE

CRYOBIOTECHNOLOGY FOR SOMATIC EMBRYOS OF *Agave tequilana* cv. chato: AN ENDANGERED GENETIC RESOURCE

Background

Agave tequilana cv. chato is an important resource used to produce a very emblematic and popular Mexican beverage called mezcal; however, its wild populations are currently severely degraded due to overexploitation. The slow growth and old reproductive age (8-15 years) have made this resource particularly vulnerable, since the plants are usually exploited before the formation of the flower stalk, avoiding their reproduction and risking their permanence in the environment and the diversity of *Agave genus*. The application of tissue culture techniques emerges as a tool, which offers by somatic embryogenesis a regeneration system to dispense with the several years that it takes *Agave* to reach sexual maturity to produce seeds. In addition, somatic embryos represent a biotechnological product with important genetic information useful for breeding programs that can be safely cryopreservation for long periods. Cryogenic storage of complex structures like somatic embryos has been mostly successfully accomplished using different vitrification-based procedures.

Methods

Induction of indirect somatic embryogenesis (ISE)

To generate calli, segments (1 cm) of young leaves were cultivated for 40 days on MS basal solid medium with 10 $mg \cdot L^{-1}$ 4-amino-3, 5, 6-trichloro-2-pyridinecarboxylic acid (PIC) and 0.75 $mg \cdot L^{-1}$ benzylaminopurine (BA). Calli were transferred to basal MS medium with reduced (76%) concentration of NH_4NO_3 and supplemented with 500 $mg \cdot L^{-1}$ glutamine, 250 $mg \cdot L^{-1}$ casein hydrolysate, and solidified with 6 $g \cdot L^{-1}$ phytagel for ISE conversion (Fig. 1).

Cryopreservation

Experiments were performed following the V-cryoplate method (Yamamoto *et al*. (2011) using somatic embryos (1-3 mm) which were 1 d-precultured on MS solid medium with 0.3 M sucrose in dark, encapsulated over the cryoplate with calcium alginate (2%) containing 0.4 M sucrose, loaded in solution with 1 M sucrose and 2 M glycerol (15 min), and exposed to the vitrification solution PVS2 or PVS3 for 30 min prior to direct immersion in liquid nitrogen.

Rewarming took place in liquid medium with 1.2 M sucrose and samples were transferred to solid conversion medium in darkness for 7 days (Fig. 2).

Results and discussion

After 50 days of culture, an average of 30±5 somatic embryos per explant was regenerated from calli mass. Globular, scutellar, coleoptilar, and other stages as heart-shaped dicotyledonar embryos were observed as previously reported by Ayala-González et al. (2014) in a related cultivar. More than 90% of somatic embryos shown post-cryopreservation recovery after 15 days of reculture, irrespectively of the PVS used. Samples treated with PVS2 presented faster regeneration than treated with PVS3. Recovery was detected by the elongation of coleoptile and radicle (Fig. 3). In this work, we report the efficient application of in vitro procedures using an important *Agave* cultivar as a study case. Mass production of somatic embryos was achieved by ISE, and a cryopreservation protocol was defined for the long-term storage of these biotechnological products. Tissue culture allowed obtaining large amounts of actively growing embryos, which is a key requirement to achieve high recovery after LN exposure. The V-cryoplate method resulted in a practical and effective approach to safely cryopreserve Agave somatic embryos as a model for other exceptional *Agave* species that already hold a regeneration protocol. It allowed easy handling of explants throughout the process and produced high post-cryopreservation recovery. The high cooling and warming rates provided protection against freezing damage because the recovery was very similar before and after immersion in LN.

This is the first report on successful cryopreservation of somatic embryos from *Agave* genus.

References

Ayala-González, C. Gutiérrez-Mora, A., B. Rodríguez-Garay. (2014). The occurrence of dicotyledonar embryos in *Agave tequilana*. Biologia Plantarum. 58 (4): 788-791.

Yamamoto S, Rafique T, Priyantha WS, Fukui K, Matsumoto T, Niino T. (2011). Development of a cryopreservation procedure using aluminum cryo-plates. Cryo-Lett 32: 256-26.

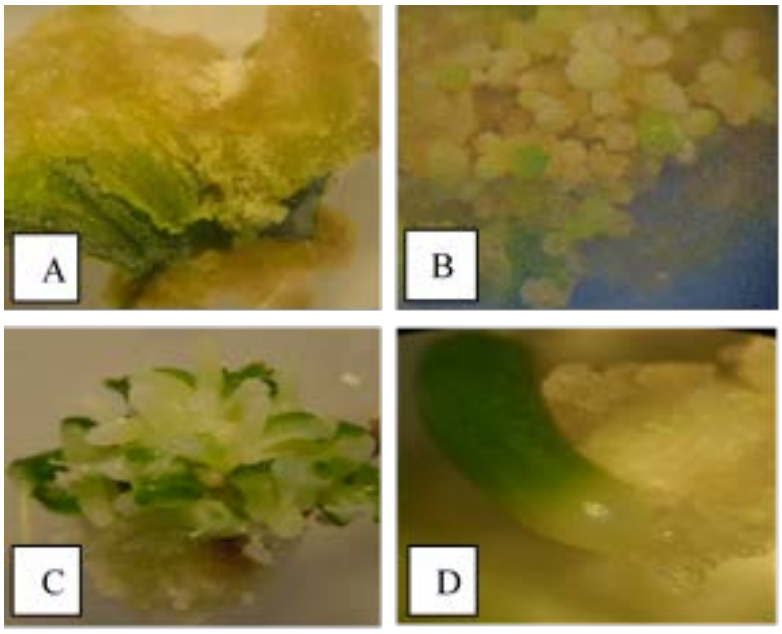

Figure 1. Indirect Somatic embryogenesis process in *Agave tequilana* cv. chato. A) Calli induction in segments of leaves after 40 d of culture; B) Globular stage of embryos (60 d); C) Embryos conversion to scutellar stage (80 d); and D) Coleoptilar stage after 90 d of culture.

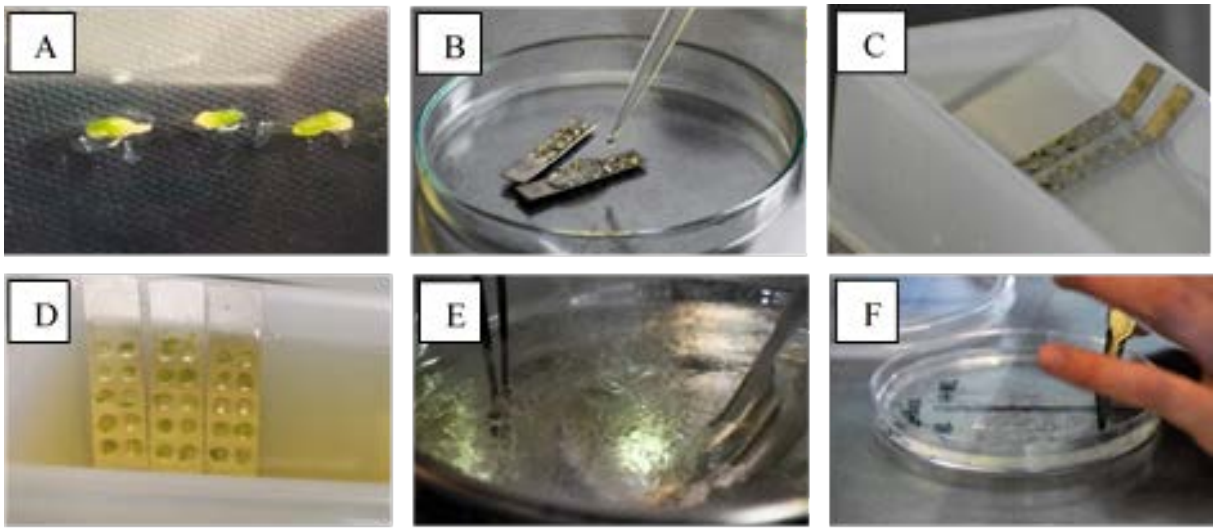

Figure 2. Cryopreservation of Agave somatic embryos using V-cryoplate method. A) Selection of somatic embryos cv. chato bar=2 mm; B) Mounting of embryos on cryoplates with calcium alginate (2%) containing 0.4 M sucrose; C) Loading treatment in solution with 1 M sucrose and 2 M glycerol (15 min); D) Exposure to vitrification solutions (PVS2 or PVS3) for 30 min; E) Direct immersion in liquid nitrogen; F) Transfer to solid conversion medium for reculture.

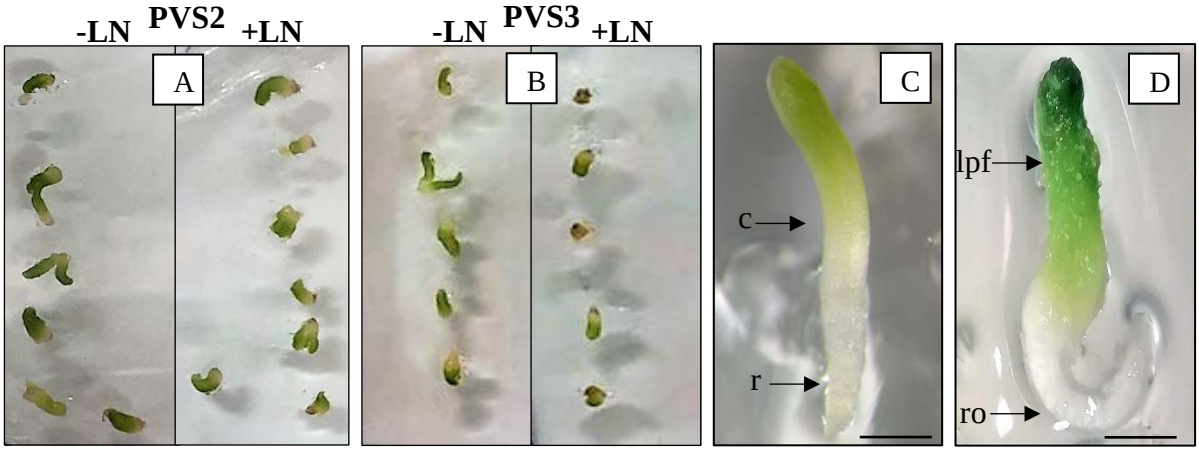

Figure 3. Culture of somatic embryos of *Agave tequilana* cv. chato. Non-cryopreserved (-LN) controls (A1, B1) of preconditioned and loaded embryos 15 d after reculture. Cryopreserved somatic embryos (+LN) after preconditioning, loading, and exposure to PVS2 (A2) or PVS3 (B2) solution 15 d after rewarming. C) Elongation of coleoptile (c) and radicle (r) after 50 d of reculture *bar=3 mm*. D) Somatic embryo showing leaf primordia formation (lpf) and root (ro) development at 65 d of reculture *bar=5 mm.*