SEP

POLIBOTÁNICA

ISSN 1405-2768

Julio 2023 Núm. 56 POLIBOTÁNICA

CONAHCYT

SEP
SECRETARÍA DE EDUCACIÓN PÚBLICA
Instituto Politécnico Nacional
"La Técnica al Servicio de la Patria"
DIRECCIÓN DE PUBLICACIONES

CONTENIDO

PÁG.

1 Riqueza y distribución altitudinal de hepáticas epifitas del bosque mesófilo de montaña de Hidalgo, México
Richness and altitudinal distribution of epiphytic liverworts from the cloud forest of Hidalgo, Mexico
José Francisco Juárez López | Arturo Sánchez González | Maritza López Herrera | Dorismilda Martínez Cabrera

17 Descripción e ilustración del desarrollo morfogénico de los gametófitos y esporófitos jóvenes de *Asplenium blepharophorum* Bertol. (Aspleniaceae-Polypodiidae) en tres sustratos naturales
Description and illustration of the morphogenic development of the young gametophytes and sporophytes of Asplenium blepharophorum Bertol. *(Aspleniaceae-Polypodiidae) in three natural substrates*
Adriana Rojas Cano | María de la Luz Arreguín Sánchez | Rafael Fernández Nava | David Leonor Quiroz García

39 Análisis morfométrico de *Agave sensu stricto* (Asparagaceae: Agavoideae) en Veracruz y áreas adyacentes de Puebla, México
Morphometric analysis of Agave sensu stricto *(Asparagaceae: Agavoideae) in Veracruz and adjacents areas of Puebla, Mexico*
Carlos Rafael Arzaba Villalba | Miguel Cházaro Bazáñes | Mario Luna Cavazos | Edmundo García Moya

61 Variación clinal de caracteres fenotípicos y fisiológicos en *Pinus hartwegii* Lindl., para la Estación Forestal Experimental Zoquiapan, México
Clinal variation of phenotypic and physiological characters in Pinus hartwegii Lindl., *for the Zoquiapan Experimental Forest Station, Mexico*
Adrián López López | María Isabel Palacios Rangel | Cuauhtémoc Sáenz Romero | Villanueva Morales Antonio | Victoria Pacheco Almaraz

81 Composición, estructura y estado de la regeneración arbórea en un gradiente altitudinal en un bosque templado de Guadalupe y Calvo, Chihuahua
Composition, structure and status of tree regeneration in an altitudinal gradient in a temperate forest of Guadalupe y Calvo, Chihuahua
Samuel Alberto García García | Eduardo Alanís Rodríguez | Oscar Alberto Aguirre Calderón | Eduardo Javier Treviño Garza | Luis Gerardo Cuéllar Rodríguez | Alejandro Collantes Chávez Costa

101 Efectos del manejo forestal en la emisión de CO_2 de un suelo umbrisol en bosques de Durango, México
Effects of forest management on the soil CO_2 emission of an umbrisol in forests from Durango, Mexico
Erik Orlando Luna Robles | Israel Cantú Silva | Francisco Javier Hernández | Silvia Janeth Béjar Pulido

115 Influencia del conocimiento ecológico tradicional y la altitud en la estructura y diversidad arbórea de los cercos vivos del corredor biológico Chichinautzin, México
Influence of traditional ecological knowledge and altitudinal gradient on richness, structure and tree diversity of live fences in the Chichinautzin biological corridor, Mexico
Emir Basurto García | Hortensia Colin Bahena | Rafael Monroy Ortiz | Alejandro García Flores | Leonardo Beltrán Rodríguez

151 Efecto del medio de cultivo y escotoperiodo en la germinación de semillas y crecimiento *in vitro* de *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins (Orchidaceae)
Effect of culture media and skotoperiod on the germination of seeds and growth in vitro *of* Guarianthe skinneri *(Bateman) Dressler & W.E. Higgins (Orchidaceae)*
Fabiola Hernández Ramírez | Leobardo Iracheta Donjuan | Anne Damon | Sylvia Patricia Fernández Pavía | Karina Guillén Navarro

171 Plant regeneration from indirect somatic embryogenesis of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* using zygotic embryo obtained by in-casa pollination as explants
*Regeneración de plantas por embriogénesis somática indirecta de A*gave salmiana *Otto ex Salm-Dyck subsp.* salmiana *usando embriones cigóticos obtenidos por polinización In-casa como explantes*
Blanca Vianey Angeles Vázquez | Jorge Alvarez Cervantes | Xóchitl Tovar Jiménez | Benjamín Rodríguez Garay

183 Flavonoids, anthocyanins and total macronutrients in handmade products of blackberry (*Rubus* sp.) from Atecaxil, Veracruz, Mexico
*Flavonoides, antocianinas y macronutrientes totales en productos artesanales de zarzamora (*Rubus *sp.) de Atecáxil, Veracruz, México*
Vianey del Rocio Torres Pelayo

203 Composición química, actividad antioxidante, antiinflamatoria y antiproliferativa del extracto de callos derivado de *Acalypha californica* Bentham
Chemical composition, antioxidant, antiinflammatory an antiproliferative activity of callus extract derived from Acalypha californica Bentham
Lesyanny Hechavarría Pérez | Luisa Alondra Rascón Valenzuela | Armando Tejeda Mansir | José Alberto Pérez Burgos | Gloria Irma Ayala Astorga

225 Dinámica de la Etnobotánica médica de los pobladores de Córdoba, Argentina. Aportes de la Encuesta Nacional de Folklore (1921) a la comprensión de los cambios en el uso y percepción de plantas medicinales
Dynamics of the medical Ethnobotany of the inhabitants of Córdoba, Argentina. Contributions of the National Survey of Folklore (1921) to understanding changes in the use and perception of medicinal plants
Cecilia Trillo | Bárbara Arias Toledo

249 Ethnobotany of medicinal plants Used for healers of the Chol ethnic group from Tila, Chiapas, Mexico
Etnobotánica de plantas medicinales usadas por curanderos del grupo étnico Chol de Tila, Chiapas, México
José del Carmen Rejón Orantes | Sabina Andrea Sánchez-Cartela | Wilbert Gutiérrez-Sarmiento | Oscar Farrera-Sarmiento | Miguel Pérez de la Mora

265 Estudio de plantas medicinales utilizadas en San José Iturbide, Guanajuato, México
Study of medicinal plants used in San Jose Iturbide, Guanajuato, Mexico
Eduardo Alberto Lara Reimers | Carlos Omar Hernández Robledo | Pablo Preciado Rangel | Oscar Sariñana Aldaco

287 Percepción y significados del guaje rojo *Leucaena esculenta* (DC.) Benth. en la cultura ngiwa de Puebla, México
Perception and meanings of the red guaje Leucaena esculenta *(DC.) Benth. in the ngiwa culture from Puebla, Mexico*
Guadalupe García Maceda | Arturo Hernández Montes | María Carmen Ybarra Moncada | Rocío Guadalupe Casañas Pimentel

311 Intergenerational transmission of traditional ecological knowledge about medicinal plants in a riverine community of the Brazilian Amazon
Transmisión intergeneracional del conocimiento ecológico tradicional sobre las plantas medicinales en una comunidad ribereña de la Amazonía Brasileña
Rogério Lima Mota | Iani Dias Lauer-Leite | Jaílson Santos de Novais

POLIBOTÁNICA

Núm. 56 ISSN electrónico: 2395-9525 Julio 2023

Portada

Jerzy Rzedowski Rotter (1926-2023). Considerado uno de los botánicos más influyentes de México. Incursionó en diversas disciplinas botánicas como taxonomía, florística, fitogeografía y ecología. Formó varios herbarios institucionales y recolectó muestras de la flora mexicana, logrando una colección que superó los 50,000 ejemplares. Trabajó en la Flora Fanerogámica del Valle de México y en la Flora del Bajío y Regiones Adyacentes; también escribió el libro La Vegetación de México, obra clásica de la literatura botánica mexicana. A lo largo de su carrera, se dedicó además a la enseñanza y formación de botánicos. Su obra incluye la publicación de 7 libros, 47 capítulos de libros, 128 artículos en revistas científicas y 31 fascículos de floras. Descubrió alrededor de 190 nuevas especies de plantas mexicanas y más de 85 especies de hongos, plantas y animales mexicanos recibieron su nombre en su honor.

Jerzy Rzedowski Rotter (1926-2023). Considered one of the most influential botanists in Mexico. He ventured into several botanical disciplines such as taxonomy, floristics, phytogeography, and ecology. He formed several institutional herbaria, and collected samples of Mexican flora, achieving a collection that exceeded 50,000 numbers. He worked on the Phanerogamic Flora of the Valley of Mexico and the Flora of the Bajio and Adjacent Regions; he also wrote the book The Vegetation of Mexico, a classic work of Mexican botanical literature. Throughout his career, he was also dedicated to teaching and training botanists. His work includes the publication of 7 books, 47 book chapters, 128 articles in scientific journals, and 31 fascicles of floras. He discovered about 190 new species of Mexican plants and more than 85 species of Mexican fungi, plants, and animals were named in his honor.

por/by **Rafael Fernández Nava**

POLIBOTÁNICA, Año 28, No. 56, julio 2023, es una publicación semestral editada por el Instituto Politécnico Nacional, a través de la Escuela Nacional de Ciencias Biológicas. Unidad Profesional Lázaro Cárdenas, Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tomas C.P. 11340 Delegación Miguel Hidalgo México, D.F. Teléfono 57296000 ext. 62331. http://www.herbario.encb.ipn.mx/, Editor responsable: Rafael Fernández Nava. Reserva de Derechos al Uso Exclusivo del Título No. 04-2015-011309001300-203. ISSN impreso: 1405-2768, ISSN digital: 2395-9525, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, Unidad de informática de la ENCB del IPN, Rafael Fernández Nava, Unidad Profesional Lázaro Cárdenas, Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tomas CP 11340 Delegación Miguel Hidalgo México, D.F.

Toda correspondencia relacionada con la revista deberá ser dirigida a:

Dr. Rafael Fernández Nava
Editor en Jefe de

POLIBOTÁNICA

Departamento de Botánica
Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional
Apdo. Postal 17-564, CP 11410, Ciudad de México

Correo electrónico:
polibotanica@gmail.com
rfernan@ipn.mx

Dirección Web
http://www.polibotanica.mx

POLIBOTÁNICA es una revista indexada en:

CONAHCYT, índice de Revistas Mexicanas de Investigación Científica y Tecnológica del Consejo Nacional de Humanidades, Ciencia y Tecnología.

SciELO - Scientific Electronic Library Online.

Google Académico - Google Scholar.

DOAJ, Directorio de Revistas de Acceso Público.

Dialnet portal de difusión de la producción científica hispana.

REDIB Red Iberoamericana de Innovación y Conocimiento Científico.

LATINDEX, Sistema regional de información en línea para revistas científicas de América Latina, el Caribe, España y Portugal.

PERIODICA, Indice de Revistas Latinoamericanas en Ciencias.

CONAHCYT
CONSEJO NACIONAL DE HUMANIDADES CIENCIAS Y TECNOLOGÍAS

Polibotánica
ISSN electrónico: 2395-9525
polibotanica@gmail.com
Instituto Politécnico Nacional
México
http://www.polibotanica.mx

PLANT REGENERATION FROM INDIRECT SOMATIC EMBRYOGENESIS OF *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* USING ZYGOTIC EMBRYO OBTAINED BY *in-casa* POLLINATION AS EXPLANTS

REGENERACIÓN DE PLANTAS POR EMBRIOGÉNESIS SOMÁTICA INDIRECTA DE *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* USANDO EMBRIONES CIGÓTICOS OBTENIDOS POR POLINIZACIÓN *in-casa* COMO EXPLANTES

Angeles-Vázquez, Blanca Vianey; Jorge Alvarez-Cervantes; Xochitl Tovar-Jiménez y **Benjamín Rodríguez-Garay**

PLANT REGENERATION FROM INDIRECT SOMATIC EMBRYOGENESIS OF *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* USING ZYGOTIC EMBRYO OBTAINED BY *in-casa* POLLINATION AS EXPLANTS

REGENERACIÓN DE PLANTAS POR EMBRIOGÉNESIS SOMÁTICA INDIRECTA DE *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* USANDO EMBRIONES CIGÓTICOS OBTENIDOS POR POLINIZACIÓN *in-casa* COMO EXPLANTES

POLIBOTÁNICA
Instituto Politécnico Nacional

Núm. **56**: 171-182 México. Julio 2023
DOI: 10.18387/polibotanica.56.9

Núm. 56: 171-182 Julio 2023 ISSN electrónico: 2395-9525

Plant regeneration from indirect somatic embryogenesis of *Agave salmiana* 0tto ex Salm-Dyck subsp. *salmiana* using zygotic embryo obtained by *in-casa* pollination as explants

Regeneración de plantas por embriogénesis somática indirecta de *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* usando embriones cigóticos obtenidos por polinización *in-casa* como explantes

Blanca Vianey Angeles-Vázquez
https://orcid.org/0000-0003-4650-4098
Maestría en Biotecnología, Universidad Politécnica de Pachuca, Carretera Pachuca-Cd. Sahagún km 20, Ex Hacienda de Santa Bárbara, Zempoala, Hidalgo, México, CP-43830

Jorge Alvarez-Cervantes / jorge_ac85@upp.edu.mx
https://orcid.org/0000-0002-0379-5588
Xochitl Tovar-Jiménez
https://orcid.org/0000-0002-6626-6237
Universidad Politécnica de Pachuca, Carretera Pachuca - Cd. Sahagún km 20 Ex-Hacienda de Santa Bárbara, Zempoala Hidalgo, México CP-43830.

Benjamín Rodríguez-Garay / agavero01@hotmail.com
https://orcid.org/0000-0003-4307-6622
Unidad de Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Camino el Arenero No. 1227, Colonia El Bajío del Arenal, Zapopan Jalisco, México CP-45019

ABSTRACT: The use of somatic embryogenesis can be an alternative to produce a large number of somatic seedlings in less time at low cost; however, the viability and success of this method depend on several factors such as the type of explant used, the hormonal balance, among others. In this sense, the present research work evaluated the use of immature zygotic embryos obtained by *in-casa* pollination as explants of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* obtained from different municipalities of the state of Hidalgo, Mexico. After 30-40 days of having pollinated inflorescences or panicles, a total of 89 fruits were obtained, of which 1608 zygotic embryos were rescued in vitro, and from these somatic embryos were generated in a nutrient medium (MS) by Murashige and Skoog, supplemented with vitamins L2, 9 µM of the auxin 2,4-dichlorophenoxyacetic (2,4-D) and 1.3 µM of the cytokinin 6-6 benzylaminopurine (BAP). Calli were used in two experiments in the presence of 2,4-D auxins and α-naphthaleneacetic acid (NAA) in combination with the cytokinin BAP. After 40 days of incubation, it was observed that a large number of embryos and embryogenic calli were generated using 4.5-9.0 µM 2,4-D in combination with 0.4-1.3 µM BAP. *In-casa* pollination allows the formation of viable zygotic embryos, and these can be used as explants in somatic embryogenesis, this being an alternative to help the genetic conservation of *Agave* plants in Mexico.
Key words: *In-casa* pollination, embryo rescue, zygotic embryos, auxins

RESUMEN: El uso de embriogénesis somática puede ser una alternativa para producir en menos tiempo un gran número de plántulas somáticas a bajo costo; sin embargo, la viabilidad y el éxito de este método dependen de varios factores como el tipo de explante utilizado, el balance hormonal entre otros. En este sentido, el presente trabajo de investigación evaluó el uso de embriones cigóticos inmaduros obtenidos por polinización-cruzada *in-casa* como explantes de *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* obtenidos de diferentes municipios del estado de Hidalgo, México.

Angeles-Vázquez, Blanca Vianey; Jorge Alvarez-Cervantes; Xochitl Tovar-Jiménez y Benjamín Rodríguez-Garay

PLANT REGENERATION FROM INDIRECT SOMATIC EMBRYOGENESIS OF *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* USING ZYGOTIC EMBRYO OBTAINED BY *in-casa* POLLINATION AS EXPLANTS
REGENERACIÓN DE PLANTAS POR EMBRIOGÉNESIS SOMÁTICA INDIRECTA DE *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* USANDO EMBRIONES CIGÓTICOS OBTENIDOS POR POLINIZACIÓN *in-casa* COMO EXPLANTES

POLIBOTÁNICA
Instituto Politécnico Nacional

Núm. 56: 171-182. Julio 2023

DOI: 10.18387/polibotanica.56.9

Núm. 56: 171-182 Julio 2023 ISSN electrónico: 2395-9525

Después de 30-40 días de haber polinizado inflorecencias o panículas se obtuvieron un total de 89 frutos, de los cuales se rescataron *in vitro* 1608 embriones cigóticos, y de estos se generaron embriones somáticos en un medio nutritivo (MS) de Murashige y Skoog, suplementado con vitaminas L2, 9 µM de la auxina 2,4-diclorofenoxiacético (2,4-D) y 1,3 µM de la citoquinina 6-6 bencilaminopurina (BAP). Los callos se utilizaron en dos experimentos con presencia de auxinas 2,4-D y el ácido αnaftalenoacético (NAA) en combinación con la citoquinina BAP. Después de 40 días de incubación, se observó que se generó una gran cantidad de embriones y callos embriogénicos utilizando 4,5-9,0 µM de 2,4-D en combinación con 0,4-1,3 µM de BAP. Después de 60 días de incubación, el porcentaje de germinación de embriones somáticos fue superior al 70% en todos los tratamientos, con una tasa de supervivencia superior al 95% en 21 días. La polinización cruzada *in-casa* permite la formación de embriones cigóticos viables, y estos pueden ser utilizados como explantes en la embriogénesis somática, siendo esto, una alternativa para ayudar a la conservación genética de plantas de *Agave* en México.
Palabras clave: Polinización-cruzada en casa, rescate de embriones, embriones cigóticos, auxinas.

INTRODUCTION

The genus *Agave* is endemic to America and is the largest and most diverse of the Asparagaceae family with 210 species of angiosperm and monocotyledonous plants; of these, 159 are native to Mexico (García-Mendoza A, 2002). An example is *Agave salmiana* that is distributed mainly in the Mexican states of Tlaxcala, Puebla, Mexico, and Hidalgo (MolinaVega *et al.,* 2021). This species is used in the elaboration of traditional foods, fibers production, pulque and mezcal, saponins, fructans and in obtaining biofuels (Álvarez-Chávez *et al.,* 2021). It is a semelparous species and takes 10 to 15 years to reproduce sexually. In comparison, asexual reproduction takes about five years with the formation of offshoots, being this form of propagation the most used for commercial purposes. However, this has led to the incidence of pests and diseases due to the decrease in genetic variability (Puente-Garza *et al.,* 2015). Plant tissue culture can be a biotechnological tool of great help to preserve the genetic diversity of this species and take advantage of its diverse uses. *In vitro* propagation of *Agave* by the proliferation of axillary buds has been reported in different species between them *A. salmiana* subspecies *crassispina* (Nava-Cruza *et al.,* 2015).

Somatic embryogenesis (SE) is an alternative to the mass culture of plants, which is characterized as a pathway for the regeneration of whole plants from a bipolar structure derived from somatic cells. This process is carried out through the induction of plant cells to dedifferentiate and enter a new biological development program and the subsequent expression and germination of the resulting embryos (Gutirrez-Mora *et al.,* 2012), by using growth regulators such as auxins and cytokinins. Auxins being an essential factor for the initial cell polarization and asymmetrical first division (Asghar *et al.,* 2023).

Some of the factors that have been studied to carry out the embryogenesis process in *Agave* are the type of explant used, the combination and hormonal concentration, osmotic conditions, concentration of amino acids and salts, etc. In the SE of *Agave spp*, different explants have been used: leaf segments, in *A. victoria-reginae* (Rodríguez-Garay *et al.,* 1996), *A. salmiana* (FloresBenítez *et al.,* 2007), *A. vera-cruz* (Tejavathi *et al.,* 2007) and *A. tequilana* Weber, blue cultivar (Portillo *et al.,* 2007; Rodríguez-Sahagún *et al.,* 2011); stem tissues in *A. victoria-reginae* Moore (Martínez-Palacios *et al.,* 2003) and *A. fourcroydes* Lem (Monja-Mio & Robert, 2016); tissue of bulbil apices in *A. sisalana* (Nikam *et al.,* 2003) zygotic embryos in *A. angustifolia* Haw (Arzate-Fernández & Mejía-Franco, 2011; Reyes-Díaz *et al.,* 2017) The combination of hormones is another of the parameters that has been manipulated to induce ES in different species of *Agave*, being 2,4-D auxin in combination with other hormones the common one of all. Such as the study carried out on *Agave marmorata* in which it was combined with BAP, using mature seeds as explants (Alvarez-Aragón *et al.,* 2020).

Therefore, the use of somatic embryogenesis using zygotic embryos as explants of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana*, a product of *in-casa* cross-pollination, could be a useful alternative in the production of plants, conservation of genetic diversity, and for the genetic improvement of this species.

MATERIALS AND METHODS

Plant material. In the April-June 2017 period, panicles and inflorescences of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* in the stage of sexual reproduction were collected in the municipalities El Arenal (N: 20º 10' 39.1" W: 98º 53' 56.1" 2217 m.s.n.m , N: 20º 10' 36.8" W: 98º 53' 54.5" 2239 m.s.n.m., N: 20º 10' 37.1" W: 98º 53' 51.5" 2246 m.s.n.m, N: 20º 10' 38.4" W: 98º 53' 53.1" 2267 m.s.n.m), Cardonal (N: 48º 78' 94.0" W: 22º 77' 97.3" 1961 m.s.n.m, N: 20º 36' 03.8" W: 99º 06' 40.0" 1953 m.s.n.m, N: 20º 36' 03.8" W: 99º 06' 40.0" 1953 m.s.n.m.), San Agustín Tlaxiaca (N: 51º 40' 99" W: 22º 18' 749" 2624 m.s.n.m, N: 50º 96' 66" W: 22º 25' 077" 2301 m.s.n.m) and Emiliano Zapata (N: 19º 39' 20.2" W: 98º 33' 10.4" 2502 m.s.n.m). The taxonomic classification was made according to dichotomous keys (Little & Gentry, 1983). The plant material was kept in the laboratory in containers with clean water (flower vases) at an average room temperature of 21-23 ºC.

Pollen viability tests. Mature flowers were emasculated, and anthers were collected and placed on a sheet of bond paper at room temperature, for three days, to carry out the pollen harvest and its subsequent viability test. The viability of pollen grains was observed through the formation and growth of the pollen tube. The pollen grains were placed in Petri dishes (100 mm x 15 mm) with 25 mL of culture medium containing 102.69 gl^{-1} sucrose, 0.02 g L^{-1} boric acid, 0.287 g L^{-1} calcium nitrate and 5 g L^{-1} agar, subsequently incubated for 30 minutes at 25 ºC (López Díaz & Rodríguez Garay, 2008).

***In-casa* cross-pollination.** After checking the viability of pollen and when the flower was receptive with the presence of a pollen drop in the stigma, pollination was carried out as described by Rodríguez-Garay *et al.,* (2018).

Callus formation. After 30 to 40 days post-pollination, the fruits were collected from the panicles and disinfected with 96% v/v alcohol and flamed within a laminar flow hood, then opened with a sterile scalpel and extracted the immature viable seeds (black color). The immature zygotic embryos were extracted with the help of a stereoscopic microscope, fine forceps, and a scalpel under aseptic conditions (Rodríguez-Garay *et al.,* 2018). These immature zygotic embryos were used as explants. Embryos were placed in Petri dishes (100 mm x 15 mm) containing 25 mL of MS medium; supplemented with 30 g L^{-1} sucrose, L_2 vitamins (Phillips & Collins, 1979), 8 g l^{-1} agar, 9.0 µM 2,4-D (Sigma-Aldrich D8407) and 1.3 µM of BAP (Sigma-Aldrich B3408) (Portillo *et al.,* 2007). The pH was adjusted to 5.8 prior sterilization in an autoclave. Five explants (immature zygotic embryos) were seeded in each Petri dish and incubated at 27 °C ± 2 °C for 40 days under total darkness.

Experimental method

To increase the number of genotypes that could form somatic embryos, the explants that formed calluses were distributed in two experiments that differed by the use of plant growth regulators and their concentration.

Experiment 1: A 3 × 3 bifactorial experiment was designed: The first factor was 2,4-D in concentrations of 4.5, 9.0, and 13.5 µM, in combination with the second factor BAP in concentrations of 0.4, 1.3 and 2.2 µM (Portillo *et al.,* 2007).

Experiment 2: A 3 × 3 bifactorial experiment was designed, using the auxin αnaphthaleneacetic acid (NAA) (Sigma-Aldrich N0640) in concentrations of 1.34, 2.7 and 4.06 µM, and the cytokinin BAP in concentrations of 2.5, 5.0 and 7.05 µM (Flores-Benítez *et al.,* 2007).

Induction medium
For each of the experiments, MS medium (Murashige & Skoog, 1962), supplemented with 30 g L^{-1} sucrose, L_2 vitamins (Phillips & Collins, 1979) and 8 g l^{-1} agar was used, with plant growth regulator, respectively.

Each experiment resulted in nine treatments, with eight and seven replications respectively for both experiments. Each experimental replication consisted of four calluses per Petri dish (nodular calluses, reliable and beige in color). They were incubated at 27ºC ± 2ºC for 40 days under total darkness. After this period, the number of calluses that could express somatic embryogenesis was quantified, as well as the number of somatic embryos formed in each callus before its maturation and germination.

Statistical analysis. A unifactorial analysis of variance (ANOVA) with a level of significance of 0.05 was performed, using the PAleontological STatistics program version 3.20 (Hammer & Harper, Norway).

The response surface methodology was used with the help of Design-expert software version 7.1.5 (Stat-Ease, Minneapolis), adjusting the data to a Miscellaneous 3^2 design with 4 points in the center to estimate the optimal conditions within the experimental area, (Table 1).

Table 1. Experimental range of the levels of the two independent variables in terms of real values in the experiments of induction of somatic embryogenesis in *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana*.

Variables	Simbol	Coded variable levels		
		-1	0	+1
6-bencilaminopurine (BAP)	X1	0.4 µM	1.3 µM	2.2 µM
2,4-diclorofenoxiacetic acid (2,4-D)	X2	4.5 µM	9.0 µM	13.5 µM

Maturation and germination of somatic embryos. The somatic embryos generated in both experiments were transferred to Petri dishes (100 x 15 mm) containing 25 mL of MS medium with the addition of 30 g L^{-1} sucrose, 500 mg L^{-1} glutamine, 250 mg L^{-1} casein hydrolysate and supplemented with L_2 vitamins, and 6 g L^{-1} phytagel (Sigma P8169) (Santacruz-Ruvalcaba *et al.,* 1998). For this purpose, eight embryos per Petri dish were incubated at 27ºC ± 2ºC with a photoperiod of 16 h light for 60 days, to quantify the percentage of germination.

Propagation. Two germinated plants without roots were placed in each bottle containing 100 mL of MS medium supplemented with 30 g L^{-1} sucrose, 44.3 µM BAP, 0.18 µM 2,4-D, and 8 g L^{-1} agar (Santacruz-Ruvalcaba *et al.,* 1999). The pH was adjusted to 5.8 prior sterilization in an autoclave. The cultures were incubated at 27 ° C ± 2 ° C for 60 days with a photoperiod of 16 h light to generate the formation of multiple shoots.

For rooting, the same light and temperature conditions were used; however, the culture medium did not contain plant growth regulators and with a salts reduction of 50% of the MS medium. Finally, the rooted plants were transplanted to vessels made of expanded polystyrene (EPS) with an equivalent mixture of peat moss, vermiculite, and soil (1: 1: 1). The survival rate of plants was quantified 21 days after the initiation of incubation.

RESULTS

Pollen viability tests in most of the collected parent plants indicated that 90% of the collected pollen could form a pollen tube. In Figure 1, the general process of the formation of zygotic embryos is depicted. 89 fruits were formed after 30 to 40 days as a product of the *in-casa* pollination. 25 out of 89 fruits were selected randomly, of which 1608 immature zygotic embryos were rescued. Seven of the rescued embryos had two cotyledons. 744 (46.3%) out of 1608 immature zygotic embryos could form calli, and 30 (1.8%) of them expressed somatic embryogenesis after 40 days of incubation in the induction medium; however, it was observed that only explant formed somatic embryos within 20 days.

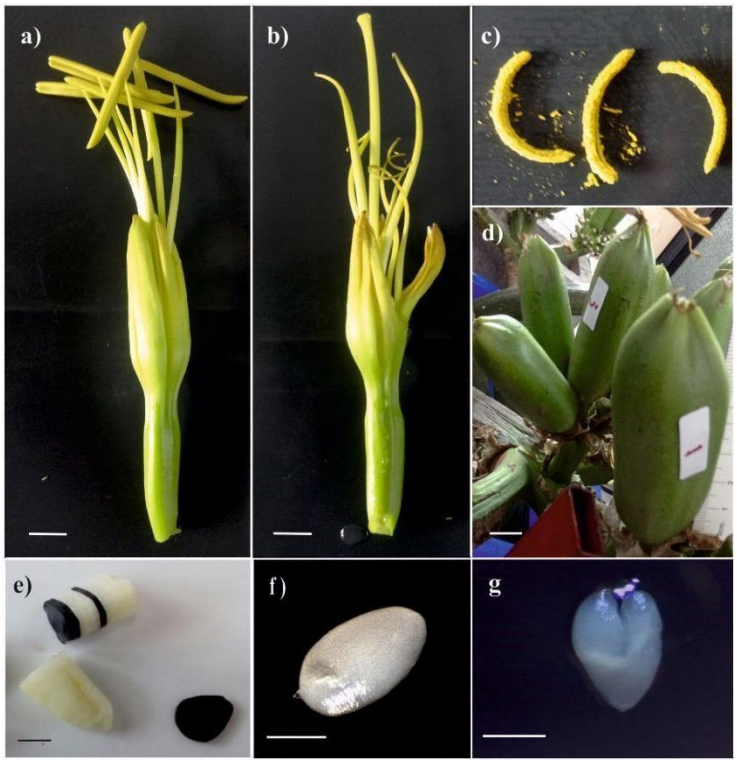

Fig. 1. Formation of zygotic embryos by *in-casa* cross-pollination of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana*. **a)** Flowers ready to be emasculated (Bar = 1cm). **b)** Mature flower ready to be pollinated (Bar = 1cm). **c)** Mature and open anthers for pollen harvest. **d)** Fruit ripening (Bar = 1cm). **e)** Viable (black color) and non-viable (white color) seeds (Bar = 5mm). **f)** Monocotyledon zygotic embryo (Bar = 1mm). **g)** Dicotyledon zygotic embryo (Bar = 0.5mm).

The analysis of variance (ANOVA) for the results of experiment 1 showed that there was a statistically significant difference between the treatments, after quantifying the number of calli that expressed somatic embryogenesis ($p = 0.040$) (Table 2). The response surface methodology predicted the number of calli expressed by somatic embryogenesis as a function of BAP and 2,4-D, and the results were adjusted to a second-order equation:

$$y = 1.098 - 0.148\,x1 + 0.218x2 - 0.007\,x1x2 - 0.058x12 - 0.014\,x22$$

Table 2. Number of calli that expressed somatic embryogenesis of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* in nine treatments with different concentrations of growth regulators: 2,4-D / BAP.

BAP / 2,4-D	4.5 µM	9.0 µM	13.5 µM
0.4 µM	1.750 ± 0.250 ^{a}	1.875 ± 0.479 ^{ab}	1.125 ± 0.227 ^{c}
1.3 µM	1.250 ± 0.250 ^{c}	1.500 ± 0.189 ^{ac}	1.125 ± 0.227 ^{c}
2.2 µM	1.250 ± 0.313 ^{c}	1.000 ± 0.267 ^{bc}	0.500 ± 0.189 ^{c}

*Number of calluses per Petri dish (Maximum value 4) ± standard error
*Values with the same letter do not have a statistically significant difference ($p < 0.05$) using the LSD test.

The model predicted that the optimal concentration of plant growth regulators within the experimental area was 0.44 µM BAP and 7.39 µM 2,4-D with a response of 1.812 calli expressing SE. According to the model, the factor with the highest level of significance was auxin 2,4-D ($p = 0.003$). Figure 2 shows the response surface as a function of different concentrations of BAP and 2,4-D. It was found that there was no significant statistical difference between treatments ($p = 0.456$) when quantifying the number of somatic embryos formed.

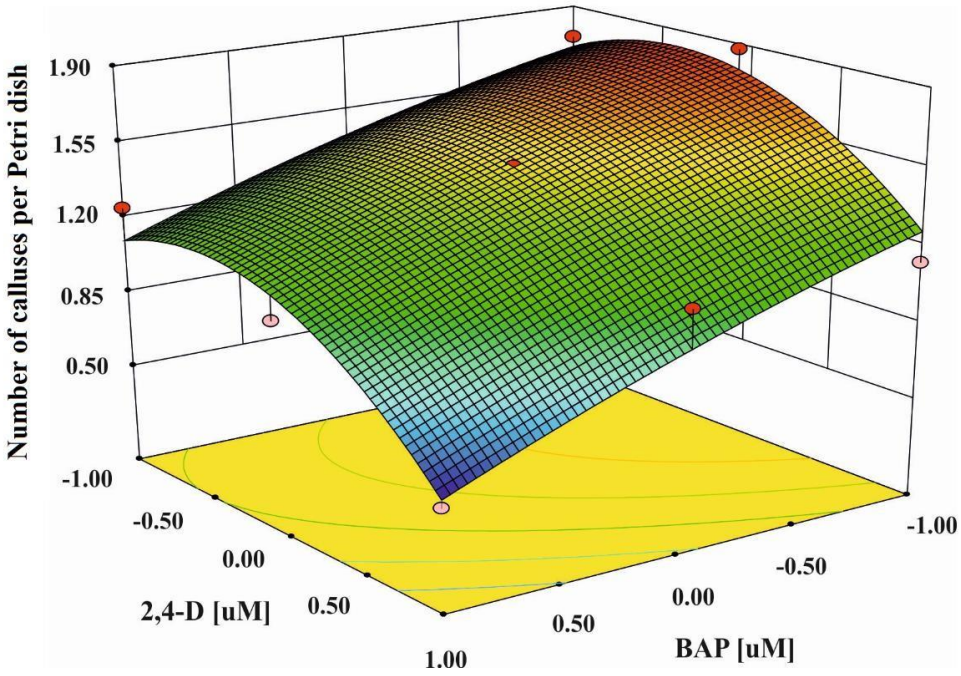

Fig. 2. Contour plot of the surface for the number of embryogenic calluses (Z) using the interaction of the variables (X1) cytokinin BAP and (X2) auxin 2,4-D. Having as answer the number of calluses formed per Petri dish (The coding of the data is shown in Table 1).

In experiment 2, the ANOVA demonstrated that there was no statistically significant difference between treatments when quantifying the number of calli that expressed somatic embryogenesis ($p = 0.315$), as well as in the number of somatic embryos formed ($p = 0.617$). However, when comparing the results of both experiments, a statistically significant difference was observed in the number of genotypes (each zygotic embryo) that expressed somatic embryogenesis ($p = 0.035$), and in the number of somatic embryos formed ($p = 0.000$) (Table 3).

Table 3. Comparison of two experiments with different auxin in combination with BAP for the SE of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana.*

Experiment	Number of calluses per Petri dish	Number of somatic embryos per callus
(1) 2,4-D / BAP	1.264 ± 0.137	42.419 ± 5.858
(2) NAA / BAP	0.873 ± 0.099	10.698 ± 1.925

Number of calluses per Petri dish have a Maximum value 4 ± standard error.

The germination percentage of the somatic embryos in experiments 1 and 2 was 97.11 ± 0.98% and 73.03 ± 5.43%, respectively. There was a 95% survival rate as a result of the acclimatization. Figure 3 shows some images of the process.

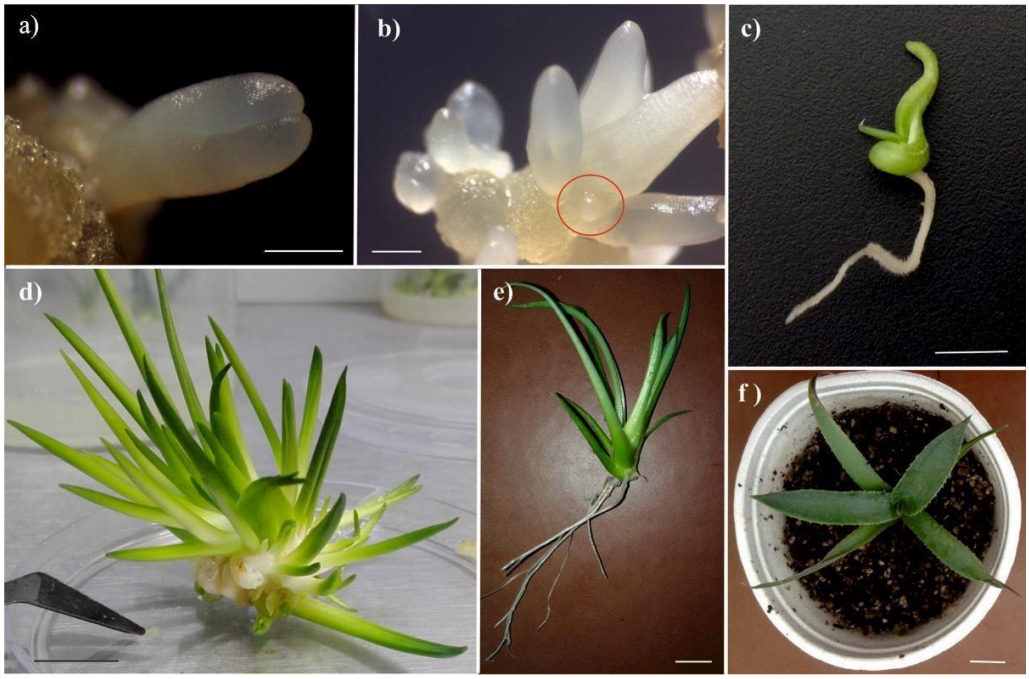

Fig. 3. *In vitro* propagation of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* by somatic embryogenesis. **a)** Dicotyledonar somatic embryo (heart) (Bar = 0.5 mm). **b**) Globular and torpedo structures seen under the stereo microscope on the surface of the embryogenic callus**.** (Bar = 0.5 mm). **c)** Germination of a somatic embryo (Bar = 5 mm). **d)** Bud formation (Bar = 1 cm). **e)** Rooted plant ready to be transplanted to soil (Bar = 1cm). **f)** Plant regenerated in substrate after 21 days (Bar = 1cm)

Núm. 56: 171-182 Julio 2023 ISSN electrónico: 2395-9525

DISCUSSION

In-casa cross-pollination could be a useful tool for the conservation of the genetic diversity of agave plants of the same species and variety. The previous procedure can be carried out in the laboratory under controlled conditions for the production of hybrids through sexual reproduction (Rodríguez-Garay *et al.,* 2018), avoiding open pollination of unknown species/varieties. The results obtained in this proposal allowed the rescue of zygotic embryos, which were used as explants in the process of somatic embryogenesis, facilitating the selection of phenotypes and the production of cell lines with genetic diversity of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* for conservation and propagation purposes (Angeles Vázquez *et al.,* 2018).

The genus *Agave* is composed of monocotyledonous plants, which are characterized by having only one cotyledon in their embryonic phase (Rodríguez-Garay, 2016). However, in this work, embryos with two cotyledons were found, similar to those reported for *Agave tequilana* with an incidence of 4% (Ayala-González *et al.,* 2014). The presence of more than one cotyledon in this genus represents an opportunity to study the mechanisms that gave rise to its unique cotyledon since the phylogenetic groups of monocots and dicots are closely related (Ayala-González *et al.,* 2014).

The embryogenic calli obtained in this work showed beige, yellow, and green colorations, with friable and compact textures, many of them nodular. These characteristics have been found in embryogenic calli of *Agave victoria-reginae* (Martínez-Palacios *et al.,* 2003), *Agave sisalana* (Nikam *et al.,* 2003), and *Agave tequilana* (Portillo *et al.,* 2007), showing a specific morphology for embryogenic callus where they are generally friable and nodular of a beige coloration. After 20 days of incubation in the induction medium, somatic embryos were observed in some of the explants. This incubation period is shorter than that reported for other *Agave* species such as *A. victoria*-reginae (Rodríguez-Garay *et al.*, 1996), *A. tequilana* (Rodríguez-Sahagún *et al.*, 2011), *A. fourcroydes* (Monja-Mio & Robert, 2013), *A. sisalana* (Carneiro *et al.*, 2014). This period is attributed to the type of explant used, in which the suspensor plays a significant role since several studies indicate that it is made up of totipotent cells that have the possibility of producing somatic embryos (Liu *et al.*, 2015). In this work, it was observed that in most of the explants (zygotic embryos), the formation of embryogenic callus was initiated in the suspensor. As can be seen in Figure 3. At the end of 40 days of incubation, indirect embryogenesis occurred, which has been described in most *Agave* species (Arzate-Fernández & Mejía-Franco, 2011).

Recent studies have reported the use of zygotic embryos as explants to carry out the process of somatic embryogenesis in the *Agave* genus, rescued from seeds formed by open pollination, such as the one reported for *A. angustifolia, A. cupreata* and *A. salmiana*, the which were subjected to osmotic stress induced by the application of compounds such as polyethylene glycol (PEG) and abscisic acid (ABA), reaching percentages of embryogenic callus formation above 50% (Hernández-Solis *et al.*, 2023). Other factors have also been explored, such as the concentration of vitamins in the induction medium, such as Thiamine, using the same type of explants (embryonic axes) in *A. angustifolia*, demonstrating that the higher the concentration of this compound, it is possible to obtain a greater number of embryos somatic (Reyes-Díaz *et al.*, 2020.)

The addition of plant growth regulators in the *in vitro* culture indirectly causes local variations in the auxin concentration of the explant, possibly triggering the de novo synthesis and relocation of the endogenous auxin. Also, it has been demonstrated that the auxin analog 2,4- D can act directly as an inducer of genes related to stress (Fehér, 2015). Besides, it has been shown that it causes hypermethylation of DNA, inducing the expression of the MET1 gene that leads to the reprogramming of the genome and the acquisition of embryogenic competence (Leljak-Levanić *et al.,* 2004).

The auxin 2,4-D is the most used analog in SE protocols in different species, including the genus *Agave* (Monja-Mio & Robert, 2016). Finally, the results obtained in this work show that the SE of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana* is favored by relatively high concentrations of 2,4-D with the addition of low concentrations of the cytokinin BAP, as established for other *Agave* species (Delgado-Aceves *et al.,* 2021).

CONCLUSIONS

The use of complete inflorescence of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana in-casa* cross-pollination allowed obtaining viable zygotic embryos after 30 days post-pollination, which presented embryogenic capacity when using an MS medium supplemented with vitamins L2, 2,4-D and ANA in combination with the cytokinin BAP. The presence of 2,4-D auxin compared to ANA, in a 40-day incubation period in the dark, increased the number of somatic embryos formed and the number of embryogenic calli. The proposal to carry out *in-casa* crosspollination facilitates the conservation of diversity through the controlled pollination of a certain species or variety of *Agave*. Furthermore, it is possible to induce and express the embryogenic capacity of zygotic embryos of *Agave salmiana* Otto ex Salm-Dyck subsp. *salmiana*, this being an alternative to help the genetic conservation of *Agave* plants in Mexico.

ACKNOWLEDGMENTS

The authors thank Mexico's Consejo Nacional de Ciencia y Tecnología (CONACYT) for the scholarship awarded to Blanca Vianey Angeles-Vázquez (No. 447330), to support her M.Sc. studies in Biotechnology at Universidad Politécnica de Pachuca. Also, to Red Temática Mexicana Aprovechamiento Integral Sustentable y Biotecnología de los Agaves (AGARED-CONACyT 2017 Mobility Plan) and the project "Industrial techniques for the production and exploitation of maguey" of the 2017 Centro de Investigación y Asistencia en Tecnología y Diseño del estado de Jalisco, A.C. (CIATEJ) Trust Fund.

LITERATURE CITED

Alvarez-Aragón, C., Martín Arzate-Fernández, A., Yarenssy Martínez-Martínez, S., & Martínez-Velasco, I. (2020). Regeneración de plantas de *Agave marmorata* Roezl, vía embriogénesis somática † [Regeneration of *Agave marmorata* Roezl Plants, by somatic embriogenesis]. *Tropical and Subtropical Agroecosystems*, *23*.

Álvarez-Chávez, J., Villamiel, M., Santos-Zea, L., & Ramírez-Jiménez, A. K. (2021). *Agave* By-Products: An Overview of Their Nutraceutical Value, Current Applications, and Processing Methods. *Polysaccharides*, *2*(3), 720–743. https://doi.org/10.3390/polysaccharides2030044

Angeles Vázquez, B. V., Álvarez Cervantes, J., Anducho Reyes, M. A., & Rodríguez Garay, B. (2018). Embriogénesis somática como método de conservación y propagación de *A. salmiana* var. *salmiana*. *SIRGEAC*, 407–410.
https://vun.inifap.gob.mx/VUN_MEDIA/BibliotecaWeb/_media/_memoria/1080_4764_XI_Simposio_Internacional_de_Recursos_Gen%c3%a9ticos_para_las_Am%c3%a9ricas_y_el_Caribe.pdf

Arzate-Fernández, A. M., & Mejía-Franco, R. (2011). Capacidad embriogénica de callos inducidos en ejes embrionarios cigóticos de *Agave angustifolia* Haw. *Revista Fitotecnia Mexicana*, *34*(2). https://doi.org/10.35196/rfm.2011.2.101

Asghar, S., Ghori, N., Hyat, F., Li, Y., & Chen, C. (2023). Use of auxin and cytokinin for somatic embryogenesis in plant: a story from competence towards completion. In *Plant Growth Regulation* (Vol. 99, Issue 3, pp. 413–428). Springer Science and Business Media B.V. https://doi.org/10.1007/s10725-022-00923-9

Ayala-González, C., Gutiérrez-Mora, A., & Rodríguez-Garay, B. (2014). The occurrence of dicotyledonar embryos in *Agave tequilana*. *Biologia Plantarum*, *58*(4). https://doi.org/10.1007/s10535-014-0456-z

Carneiro, F. dos S., Queiroz, S. R. de O. D., Passos, A. R., Nascimento, M. N. do, & Santos, K. S. dos. (2014). Embriogênese somática em *Agave sisalana* Perrine: indução, caracterização anatômica e regeneração. *Pesquisa Agropecuária Tropical*, *44*(3). https://doi.org/10.1590/s1983-40632014000300005

Delgado-Aceves, L., González-Arnao, M. T., Santacruz-Ruvalcaba, F., Folgado, R., & Portillo, L. (2021). Indirect somatic embryogenesis and cryopreservation of *Agave tequilana* weber cultivar 'chato.' *Plants*, *10*(2), 1–14. https://doi.org/10.3390/plants10020249

Fehér, A. (2015). Somatic embryogenesis - stress-induced remodeling of plant cell fate. In *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms* (Vol. 1849, Issue 4). https://doi.org/10.1016/j.bbagrm.2014.07.005

Flores-Benítez, S., Jiménez-Bremont, J. F., Rosales-Mendoza, S., Argüello-Astorga, G. R., Castillo-Collazo, R., & Alpuche-Solís, Á. G. (2007). Genetic transformation of Agave salmiana by *Agrobacterium tumefaciens* and particle bombardment. *Plant Cell, Tissue and Organ Culture*, *91*(3). https://doi.org/10.1007/s11240-007-9287-3

García-Mendoza A. (2002). Distribution of *Agave* (Agavaceae) in Mexico. *Cactus and Succulent Journal*. https://www.researchgate.net/profile/Abisai-GarciaMendoza/publication/303263665_Distribution_of_Agave_Agavaceae_in_Mexico/link s/5b3676cdaca2720785f8e251/Distribution-of-Agave-Agavaceae-in-Mexico.pdf

Gutirrez-Mora, A., Guillermina, A., Rodrguez-Garay, B., Ascencio-Cabral, A., & Li-Wei, L. (2012). Plant Somatic Embryogenesis: Some Useful Considerations. In *Embryogenesis*. https://doi.org/10.5772/36345

Hernández-Solis, M., Arzate-Fernández, A. M., Martínez-Martínez, S. Y., & Acosta-Villagrán, L. (2023). Effect of promoting compounds of indirect somatic embryogenesis in three *Agave* species. *Agrociencia*. https://doi.org/10.47163/agrociencia.v57i1.2858

Leljak-Levanić, D., Bauer, N., Mihaljević, S., & Jelaska, S. (2004). Changes in DNA methylation during somatic embryogenesis in *Cucurbita pepo* L. *Plant Cell Reports*, *23*(3). https://doi.org/10.1007/s00299-004-0819-6

Little, E. L., & Gentry, H. S. (1983). Agaves of Continental North America. *Bulletin of the Torrey Botanical Club*, *110*(3). https://doi.org/10.2307/2996199

Liu, Y., Li, X., Zhao, J., Tang, X., Tian, S., Chen, J., Shi, C., Wang, W., Zhang, L., Feng, X., & Sun, M. X. (2015). Direct evidence that suspensor cells have embryogenic potential that is suppressed by the embryo proper during normal embryogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(40). https://doi.org/10.1073/pnas.1508651112

López Díaz, S., & Rodríguez Garay, B. (2008). Simple methods for in vitro pollen germination and pollen preservation of selected species of the genus *Agave*. *E-Gnosis*, *6*(2). http://www.redalyc.org/articulo.oa?id=73011197002www.redalyc.orgwww.egnosis.udg.mx/vol6/art2

Martínez-Palacios, A., Ortega-Larrocea, M. P., Chávez, V. M., & Bye, R. (2003). Somatic embryogenesis and organogenesis of *Agave victoriae-reginae*: Considerations for its conservation. *Plant Cell, Tissue and Organ Culture*, *74*(2). https://doi.org/10.1023/A:1023933123131

Molina-Vega, A., Hernández-Domínguez, E. M., Villa-García, M., & Álvarez-Cervantes, J. (2021). *Comadia redtenbacheri* (Lepidoptera: Cossidae) and *Aegiale hesperiaris* (Lepidoptera: Hesperiidae), two important edible insects of *Agave salmiana* (Asparagales: Asparagaceae): a review. In *International Journal of Tropical Insect Science* (Vol. 41, Issue 3, pp. 1977–1988). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s42690-020-00396-1

Monja-Mio, K. M., & Robert, M. L. (2013). Direct somatic embryogenesis of Agave fourcroydes Lem. through thin cell layer culture. *In Vitro Cellular and Developmental Biology - Plant*, *49*(5). https://doi.org/10.1007/s11627-013-9535-7

Recibido:
18/abril/2023

Aceptado:
30/junio/2023

Monja-Mio, K. M., & Robert, M. L. (2016). Somatic embryogenesis in *Agave*: An overview. In *Somatic Embryogenesis: Fundamental Aspects and Applications*. https://doi.org/10.1007/978-3-319-33705-0_17

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, *15*(3). https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nava-Cruza, N. Y., Medina-Moralesa, M. A., Martineza, J. L., Rodrigueza, R., & Aguilara, C. N. (2015). *Agave* biotechnology: An overview. In *Critical Reviews in Biotechnology* (Vol. 35, Issue 4, pp. 546–559). Taylor and Francis Ltd. https://doi.org/10.3109/07388551.2014.923813

Nikam, T. D., Bansude, G. M., & Aneesh Kumar, K. C. (2003). Somatic embryogenesis in sisal (*Agave sisalana* Perr. ex. Engelm). *Plant Cell Reports*, *22*(3). https://doi.org/10.1007/s00299-003-0675-9

Phillips, G. C., & Collins, G. B. (1979). In Vitro Tissue Culture of Selected Legumes and Plant Regeneration from Callus Cultures of Red Clover 1. *Crop Science*, *19*(1). https://doi.org/10.2135/cropsci1979.0011183x001900010014x

Portillo, L., Santacruz-Ruvalcaba, F., Gutiérrez-Mora, A., & Rodríguez-Garay, B. (2007). Somatic embryogenesis in *Agave tequilana* Weber cultivar azul. *In Vitro Cellular and Developmental Biology - Plant*, *43*(6). https://doi.org/10.1007/s11627-007-9046-5

Puente-Garza, C. A., Gutiérrez-Mora, A., & García-Lara, S. (2015). Micropropagation of *Agave salmiana*: Means to production of antioxidant and bioactive principles. *Frontiers in Plant Science*, *6*(NOVEMBER), 1–9. https://doi.org/10.3389/fpls.2015.01026

Reyes-Díaz, J. I., Arzate-Fernández, A. M., Piña-Escutia, J. L., & Norman-Mondragón, T. H. (n.d.). *THE EFFECT OF INOSITOL, PYRIDOXINE AND THIAMINE ON SOMATIC EMBRYOGENESIS OF Agave angustifolia † [EL EFECTO DEL INOSITOL, PIRIDOXINA Y TIAMINA EN LA*.

Reyes-Díaz, J. I., Arzate-Fernández, A. M., Piña-Escutia, J. L., & Vázquez-García, L. M. (2017). Media culture factors affecting somatic embryogenesis in *Agave angustifolia* Haw. *Industrial Crops and Products*, *108*, 81–85. https://doi.org/10.1016/j.indcrop.2017.06.021

Rodríguez-Garay, B. (2016). Somatic embryogenesis in Agave spp. In *Somatic Embryogenesis: Fundamental Aspects and Applications*. https://doi.org/10.1007/978-3-319-33705-0_16

Rodríguez-Garay, B., Gutiérrez-Mora, A., & Acosta-Dueñas, B. (1996). Somatic embryogenesis of *Agave victoria-reginae* Moore. *Plant Cell, Tissue and Organ Culture*, *46*(1). https://doi.org/10.1007/BF00039700

Rodríguez-Garay, B., López-Díaz, S., Rodríguez-Domínguez, J. M., Gutiérrez-Mora, A., & Tapia-Campos, E. (2018). Application of in Casa Pollination and Embryo Rescue Techniques for Breeding of *Agave* Species. In *Methods in Molecular Biology* (Vol. 1815). https://doi.org/10.1007/978-1-4939-8594-4_20

Rodríguez-Sahagún, A., Acevedo-Hernández, G., Rodríguez-Domínguez, J. M., RodríguezGaray, B., Cervantes-Martínez, J., & Castellanos-Hernández, O. A. (2011). Effect of light quality and culture medium on somatic embryogenesis of *Agave tequilana* Weber var. Azul. *Plant Cell, Tissue and Organ Culture*, *104*(2). https://doi.org/10.1007/s11240-010-9815-4

Santacruz-Ruvalcaba, F., Gutierrez-Mora, a, & Rodriguez-Garay, B. (1998). Somatic embryogenesis in some cactus and agave species. *Journal of the Professional Association for Cactus Development*, *3*.

Santacruz-Ruvalcaba, F., Gutiérrez-Pulido, H., & Rodríguez-Garay, B. (1999). Efficient in vitro propagation of *Agave parrasana* Berger. *Plant Cell, Tissue and Organ Culture*, *56*(3). https://doi.org/10.1023/A:1006232911778

Tejavathi, D. H., Rajanna, M. D., Sowmya, R., & Gayathramma, K. (2007). Induction of somatic embryos from cultures of *Agave vera-cruz* Mill. *In Vitro Cellular and Developmental Biology - Plant*, *43*(5). https://doi.org/10.1007/s11627-007-9088-8