Plant Tissue Culture a valuable technique for the conservation of endangered Mexican cactus species

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Introduction

Mexico's most representative plants are, no doubt, the Cactaceae. Many species in this family find suitable habitats in our country (Hunt, 1992). Regrettably, the great majority of the endangered species of cacti are also in Mexico (Hernández y Godínez 1994).

Cactus diversity in México is represented by, referring to the most conservative taxonomic view, 50 genera and 550 species (sensu Hunt 1999). This family takes first place for exclusiveness: 72% of the species and 36% of the genera are endemic to México (Rzedowski 1991). Unfortunately, the indigenous cactus populations have been continuously plundered and their habitats destroyed. These are the two main factors contributing to the extinction and increasing rarity of many Mexican cacti (Toledo 1988).

Mexican law, specifically Mexican Official Norm 059, published March 6, 2002, list species of flora and fauna threatened with extinction. A total of 285 species are now listed (Semarnat 2002) (30 Endangered (P), 89 Threatened (A) and 166 under special protection (Pr)).Fifteen years ago, in 1991, Mexican legislation included 111 cacti as being at risk. Mexican cacti are also included in some international listings, for example CITES red list (Franco 1997).

From nearly fifteen years (from 1986 to 2001) the Instituto Tecnológico y de Estudios Superiores de Monterrey, Queretaro Campus (ITESM-Campus Querétaro), was engaged in research activities aimed at propagating Mexican cactus in danger of extinction as an ex situ strategy for their conservation. This poster briefly presents an overview of the cactus propagation achievements made at ITESM, particularly those attained when using micropropagación techniques, in the Plant Tissue Culture Lab of this Institute Botanic Garden.

Figure 1. The botanic garden of the ITESM-Campus Queretaro.

Propagation techniques

ITESM- Campus Queretaro developed appropriate technology to achieve efficient reproduction of Mexican Cactaceae in danger of extinction. Species were propagated by seed, cuttings and by in vitro micropropagation. In vitro propagation techniques were used for those species whose culture was difficult or when seeds were scarce. Such difficulty may be due to a diminishing population, slow growth or high mortality.

The selected tissue culture methodology for regeneration was axillary shoot production (ASP). Axillary shoot proliferation allows that lateral meristems (areola) are stimulated to break and grow. The 4 stages propagation procedure can be outlined as follows:

Stage I. Establishment. Explants (usually seeds) were established in aseptic culture. Disinfestation was made with commercial bleach plus a few drops of a surfactant (liquid soap); sometimes fungicides were also used. Different disinfectant dosage and agitation times were tested. Culture medium was Murashige & Skoog (1962), half strength (MS/2).

Stage II. Shoot Multiplication. The objective of this stage was to optimize axillary shoot production. Appropriate hormone levels (Cytokinin to Auxin ratio) were found. Murashige & Skoog (1962), full or half strength, was the selected medium. In many cases, multiplication was possible using only the Murashige & Skoog medium, added with activated charcoal. 6-benzylaminopurine (BAP) turned out to be the more useful synthetic cytokinin for cactus shoot proliferation. Gibberellic acid was rarely added to the culture medium.

Stage III. In Vitro Rooting. Plants were rooted combining different concentrations of Indolebutyric acid (IBA) plus Naphthaleneacetic acid (NAA); however, sometimes, rooting was spontaneous. Growing medium was, mainly, the above mentioned.

Stage IV. Acclimation. Shoots were rooted primarily in 100% peat medium, in a warm high humidity greenhouse.

Cultures were grown in a chamber with cool white fluorescent lighting (2,115 Lux), 16/8 photoperiod, and a temperature of 26+- 1ºC.

It is worth mentioning that Professor Paulino Martínez Vara was the leader of this project, assisted by technician Genaro Ruiz Campos.

Figure 2. Genaro Ruiz Campos, main technician at the ITESM Plant Tissue Culture Lab

Figure 3. Turbinicarpus spp. in Stage .II

Results.

After 15-year-research activities at the ITESM-Campus Queretaro Plant Tissue Culture Lab, more than 50 endangered or critically endangered taxa were micropropagated. Box 1 shows a summary (alphabetical order) of some of the reproduced species and the degree of success for these propagation procedures.

Endangered Mexican cactus species micropropagated at the ITESM- Campus Queretaro Lab

Scientific Name	Mexican protection status NOM-059-ECOL-2001	Propagation Efficiency	
		Multiplication Rate (times per unit time)	Soil establishment (%)
Ariocarpus fissuratus subsp. bravoanus	P	2-fold every 6 weeks	Unrecorded
Ariocarpus kotschoubeyanus	Pr	2-fold every 6 weeks	98
Astrophytum asterias	P	2-fold every 6 weeks	Unrecorded
Astrophytum ornatum	A	4-fold every 4 weeks	95
Aztekium ritteri	A	2-fold every 4 weeks	Unrecorded
Cephalocereus senilis	A	5-fold every 4 weeks	100
Coryphantha jalpanensis	Locally endangered	4-fold every 4 weeks	100
Epithelantha micromeris	Pr	4-fold every 4 weeks	98
Geohintonia mexicana	Pr	10 fold every 4 weeks	90
Leuchtenbergia principis	A	2-fold every 4 weeks	97
Mammillaria guelzowiana	A	3-fold every 4 weeks	90
Mammillaria hahniana	A	5 fold every 4 weeks	99
Mammillaria hernandezii	Pr	2 fold every 4 weeks	Unrecorded
Mammillaria herrerae	P	8-fold every 4 weeks	100
Mammillaria laui	P	5 fold every 4 weeks	99
Mammillaria luethyi	Internationally listed as endangered	3-fold every 4 weeks	100
Mammillaria mathildae	P	5-fold every 4 weeks	100
Mammillaria microhelia	Pr	4-fold every 4 weeks	100
Mammillaria muehlenpfordtii	Locally endangered	3-fold every 4 weeks	100
Mammillaria plumosa	A	5-fold every 4 weeks	100
Mammillaria sanchez-mejoradae	P	2-fold every 4 weeks	Unrecorded
Mammillaria crinita subsp. scheinvariana	Locally endangered	6-fold every 4 weeks	100
Mammilloydia candida	A	2-fold every 4 weeks	Unrecorded
Neobuxbaumia polylopha	Locally endangered	2-fold every 4 weeks	100
Strombocactus disciformis	A	10 fold every 4 weeks	85
Thelocactus heterochromus	A	4-fold every 4 weeks	100
Turbinicarpus spp. (several species including some Gymnocactus)	P, A, Pr	4-fold every 4 weeks	100
Turbinicarpus zaragozae	Internationally listed as endangered	5-fold every 4 weeks	95

Key to Names: P: Endangered, A: Threatened, Pr: Under special protection

Figure 4. Mammillaria herrerae, a highly threatened species, being cultivated in the acclimation chamber.

Figure 5. Greenhouse grown cactus species, regenerated through micropropagation procedures.

In Box 2, as an example, a synthesis of an in vitro propagation procedure is presented for the case of Mammillaria herrerae, a highly menaced taxon and endemic to the state of Queretaro (México).

Box 2 Micropropagation Method for Mammillaria herrerae (Cactaceae)

Method	Explant	Sterilization	Propagation	Acclimation	Environmental conditions
ASP	Seed	15% Household bleach (Cloralex) with 0.5% wetting agent (liquid soap). Seeds were aseptically sown in MS/2 added with 1.5 g L^{-1} activated charcoal	MS/2 plus 1.5 g L^{-1} activated charcoal (no hormone needed)	Shoots were rooted in 100% peat moss medium under greenhouse conditions	16/8 hours photoperiod (2,115 Lux); 26+-1ºC. pH medium adjusted to 5.8

Conclusions

Mexico's Botanical Gardens have been working hard toward the very complex task of endangered species conservation. Many of them have used ex situ artificial propagation as a strategy for assuring preservation of highly menaced species. Some others have conducted research to increase effectiveness. Cactus tissue culture techniques are the result of this research, which must be applied to the ultimate goal of maintaining wild populations in their habitat.

During 15 years of work, the ITESM-Campus Querétaro Plant Tissue Culture Lab, as an important part of the institute botanic garden, participated in the propagation of Mexican cactus in order to contribute to the preservation of this world heritage. Today part of its legacy (techniques and plants) is under the custody of the Cadereyta Regional Botanic Garden (CRBG), a government institution where some of the former ITESM-Querétaro Campus crew was transferred to continue its mission.

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Abstract.

The Plant Tissue Culture Lab of the ITESM-Querétaro Campus (Monterrey's Technological and Higher Studies Institute), as a part of the Botanical Garden of this University, was engaged with research activities with the primary goal of developing in vitro techniques for the efficient artificial reproduction of species in the Cactacean family. After 15 years of work, the Lab micropropagated more than 50 endangered and critically endangered taxa from all over this country and from afar. Even though this program is now closed, thousands of in vivo plantlets were delivered to Botanical Gardens in México and are now useful in diverse conservation activities. Part of this hoard is currently under the custody of the Cadereyta´s Botanical Garden, a government institution where the former ITESM-Querétaro Campus crew was transferred to continue its mission.

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