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Cryopreservation of plant germplasm in Argentina

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Abstract: This review describes the current status of development of methods for cryopreservation (at -196ºC) of plants germplasm in Argentina. *Arachis pintoi,* a forage legume, has been maintained as seeds using vitrification method. Additionally, apical meristems, shoot tips, and somatic embryos have been cryopreserved using encapsulation-dehydration. Zygotic embryos, encapsulated and dehydrated, have permitted the cryopreservation of seven species of the genus *Ilex.* Various explants (apical meristems, uninodal segments, buds and somatic embryos) of *Melia azedarach* have been cryopreserved using the encapsulation-dehydration method. Protocols based in encapsulation-dehydration have also been developed for shoot tips of *Citrus sinensis*, seeds and protocorms of *Oncidum bifolium* and anthers of *Oryza sativa.* Vitrification protocols have been developed forcryopreservation of shoot tips of *Solanum tuberosum* and seeds of *Toona ciliata.*

1. Introduction

Argentina is an agricultural country with different climates. Many of its economically important plant species (such as corn, wheat, beans, cucumbers) have - from a germplasm preservation point of view - orthodox seeds and are maintained in conventional seedbanks (mostly belonging to the National Institute of Agriculture) distributed in 10 places of Argentina. However, seeds of other plant species (such as, peanuts, cotton, tea, "yerba mate", oranges, various plants used for timber), being either recalcitrant or suborthodox, are highly sensitive to desiccation and cannot be stored at low temperature. Many plant crops (such as potatoes, strawberry, cassava, sweet potato) have also been maintained by vegetative propagation. Genetic resources of these crops are actually maintained either *in situ* or *ex situ* as plants in the field; losses of plant material may occur due to diseases or accidents. Additionally, a great deal of labor and time is required for the maintenance of these field collections. Cryopreservation is an alternative strategy for efficient and economical long-term conservation of these plant species.

Research on cryopreservation of plant species in Argentina was initiated in 2001. At present, no collection is maintained under cryopreservation, but at least two institutions (IBONE, and the Facultad de Ciencias Agrarias, UNMP-INTA) are making efforts to develop protocols in order to establish long-term banks for some species of economical or ecological importance. The present paper provides an overview of the current status of the development of plant germplasm cryopreservation in Argentina.

2. *Arachis pintoi* ("pinto peanut")

Arachis pintoi, a novel and important forage legume in tropical and subtropical areas of the world, has two cytotypes, one diploid, 2n=2x=20 chromosomes, and one, triploid with 2n=3x=30 chromosomes. The former produces relatively few seeds and they show high loss of viability after a few months of storage. The triploid cytotype does not produce seeds and propagation by vegetative procedures is obligatory. Thus, it is necessary to maintain collections of plants in the field for conservation of its germplasm.

Table 1 summarizes the results obtained at IBONE with cryopreservation of different plant material of *Arachis pintoi*. Seeds of the diploid cytotype were easily maintained at -196ºC using vitrification method. As much as 90% of them germinated after rewarming (Rey and Mroginski, 2007).

Different *in vitro* cultured explants can also be employed for germplasm preservation of both diploid and triploid cytotypes, with survival percentages ranging from 17 to 60%, depending upon the explants. The method employed was encapsulation-dehydration. Encapsulation was carried out by the classical procedure of sodium alginate-calcium chloride. The enca-

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Table 1 - Cryopreservation of germplasm of *Arachis pintoi*

Material cryopreserved	Method	Cooling	Results (% survival)	
			Diploid	Triploid
Seeds	vitrification	rapid	90	---
Apical meristems	encapsulation-dehydration	rapid	17	20
Shoot tips	encapsulation-dehydration	rapid	57	60
Uninodal segments	encapsulation-dehydration	rapid	0	0
Somatic embryos	encapsulation-dehydration	rapid	27	30
	preculture-desiccation	rapid	24	14

psulated explants were pregrown for three days in liquid medium with progressively increasing sucrose concentration (0.5, 0.75 and 1 M of sucrose for 24 hr each). The beads with explants were dehydrated with silicagel for 5 hr to 25% moisture content and immersed in liquid nitrogen followed by rapid cooling. Between rewarming and reculture of the explants it was necessary to culture them for two days in liquid medium with 1 M sucrose for 24 hr followed by 0.75 M sucrose for 24 hr. It is interesting to note that using this method, it was not possible to employ uninodal segments. Preculture-desiccation was also successfully used for freezing somatic embryos (Rey, 2004).

3. *Ilex* species

The genus *Ilex* (Aquifoliaceae), which includes about 220 species, inhabits temperate and tropical regions of South America. The most interesting among them, in terms of economic importance, is *I. paragua - riensis* because of its value for making a stimulatory beverage called "*mate*". Seeds of most of the *Ilex* species contain rudimentary embryos that remain at the immature heart-shaped stage for a long time after the fruits reach maturity. An additional problem for seed conservation of these species is the fact that they are highly sensitive to desiccation and cannot be stored at low temperature. In other words, the seeds are recalcitrant and are therefore not suitable for long-term conservation using conventional seed storage methods. Thus, germplasm of *Ilex* spp. is maintained in the field as *ex situ* genebanks.

Two strategies for *in vitro* cryopreservation of *Ilex* species were tested, including cryopreservation of fruit or isolated embryos followed by embryo culture (Fig. 1). In the first case, it was only possible to regenerate plants from five out of eight species tested (Table 2) when the embryo were isolated from fruits that were cryopreserved using slow freezing (1°C min^{-1} till -40°C) with a cryoprotective solution containing 50% sucrose and 50% glycerol and stored in liquid nitrogen (Mroginski *et al.*, 2006). However, survival was low (3-23%), yet percentages increased (Table 3) when embryos were cryopreserved by using the encapsulation-dehydration and rapid cooling (Mroginski *et al.*, 2007).

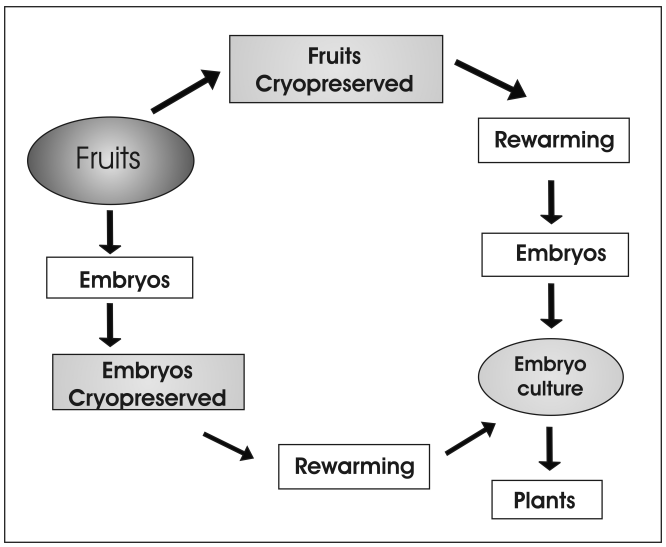

Fig. 1 - Strategies for cryopreservation of germplasm of *Ilex* spp.

Table 2 - *In vitro* germination (%) of zygotic embryos from cryopreserved fruits of *Ilex* species (modified from Mroginski *et al.*, 2006)

Plant spp.	Rapid cooling	Slow cooling
Ilex brasiliensis	0	3
I. brevicuspis	0	0
I. dumosa	0	13
I. intergerrima	0	0
I. microdonta	0	3
I. paraguariensis	0	10
I. pseudoboxus	0	23
I. theezans	0	0

Table 3 - Effect of cryopreservation (rapid cooling) by encapsulation/dehydration of *Ilex paraguariensis* rudimentary embryos on their *in vitro* germination after 60 days in culture on $^1/_4$ MS, 3% sucrose and 0.1 mg/l Zeatin

Plant spp.	Embryos forming plants (%)
Ilex brasiliensis	71
I. brevicuspis	0
I. intergerrima	40
I. dumosa	30
I. paraguariensis	57
I. pseudoboxus	33
I. taubertiana	10
I. theezans	39

4. *Melia azedarach* (Paradise tree)

The paradise tree, native to the Asiatic Middle East, is an important forest tree in Argentina with excellent adaptability to a wide range of soil and climate conditions. Table 4 summarizes the results obtained at IBONE using cryopreservation of different plant material of *Melia azedarach*. Various explants were encapsulated (Fig. 2 A), pretreated in liquid medium with sucrose concentration increased daily (0.5 M/0.75 M/1.0 M), dehydrated with silica gel to 21-26% moisture content, then frozen in liquid nitrogen using a rapid cooling or slow cooling method (Scocchi *et al*., 2004 b; Scocchi, 2005; Scocchi *et al*., 2007). The cryopreserved explants, when rewarming and recultured *in vitro*, regenerated shoots (Fig. 2 B), and whole plants (Fig. 2 C) which were successfully transplanted to pots (Fig. 2 D) and to the field (Fig. 2 E).

Table 4 - Cryopreservation of germplasm of *Melia azedarach* using the encapsulation-dehydration method

Plant material	Explants forming shoots (%)	
	Rapid cooling	Slow cooling
Apical meristems	43	60
Uninodal segments	23	57
Non-dormant buds	45	70
Dormants buds	40	52
Somatic embryos	0	36

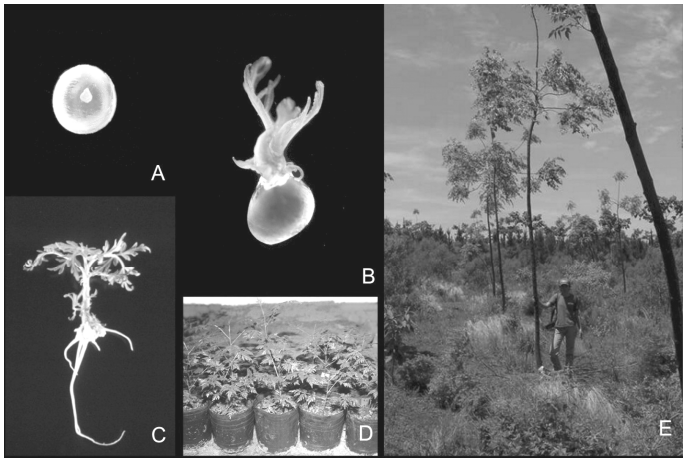

Fig. 2 - Cryopreservation of germplasm of *Melia azedarach*. A) Encapsulated meristems; B) Shoot regeneration of encapsulated meristems; C) Plant regeneration; D) Regenerated plants transferred to pots; E) Regenerated plants growing in field conditions.

5. Other plant species

Table 5 summarizes the results obtained with different plants, such as *Citrus sinensis*, when shoot tips were cryopreserved using the encapsulation-dehydration method (Dolce *et al.*, 2004) or seeds and protocorms of the orchid *Oncidium bifolium*, preserved by the same procedure (Flachsland *et al.*, 2006). Likewise, seeds of *Toona ciliata* (Scocchi *et al.*, 2004 a) and shoot-tips of *Solanum tuberosum* were stored at -196ºC through a vitrification protocol (Digilio, 2004). It is interesting to note that with rice (*Oryza sativa*) the objective of the work was to solve one of the limitations of the anther-culture technique, which is the short period of time during which flower explants are available (Marassi *et al*., 2006).

6. Conclusions

Cryopreservation has been considered an ideal alternative for germplasm conservation of plant species of economical importance for Argentina and for native endangered plant species. However, reduced financial support has characterized the small projects which started in 2001, yet, significant progress has been made with plant species of economic interest such as *Arachis pintoi, Citrus sinensis, Melia azedarach* and *Ilex paraguariensis.*

Undoubtedly, action needs to be taken to increase the cryopreservation research in Argentina.

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References

DIGILIO A., 2004 - *Crioconservación de variedades nativas de* Solanum tuberosum *spp.* andigenum *Juz.& Bukasov*. - M.S. Thesis, Universidad Nacional de Mar del Plata, Argentina.

DOLCE N., SCOCCHI A.M., MROGINSKI L.A., 2004 - *Criocon - servación de ápices de* Citrus sinensis. - FACENA, 20: 37-45.

FLACHSLAND E., TERADA G., SCOCCHI A.M., REY H.Y., MROGINSKI L.A., ENGELMANN F., 2006 - *Cryopreserva -*

Table 5 - Summary of results obtained with cryopreservation of other plant spp. in Argentina

Plant species	Material cryopreserved	Method [z]	Cooling	Results (% survival)	References
Citrus sinensis	Shoot tips	E-D.	slow [y]	65	Dolce *et al.*, 2004
Oncidium bifolium	Seeds	E-D.	rapid		Flachsland *et al.*, 2006
Oryza sativa	Protocorms	E-D.	rapid	11	Flachsland *et al.*, 2006
	Anthers	E-D.	slow	15	Marassi *et al.*, 2006
Solanum tuberosum	Shoot-tips	V	rapid	2-11	Digilio, 2004
Toona ciliata	Seeds	V	rapid	35	Scocchi *et al.*, 2004 a

[z] E-D= encapsulation dehydration; V= vitrification.
[y] Slow cooling (at 1ºC min^{-1} to -30ºC and then immersion in liquid nitrogen).

tion of seeds and in vitro-*cultured protocorms of* Oncidium bifolium *Sims. (Orchidaceae) by encapsulation-dehydration.* - CryoLetters, 27(4): 235-242.

MARASSI M.A., SCOCCHI A., GONZALEZ A.M., 2006 - *Plant regeneration from rice anthers cryopreserved by an encapsula - tion/dehydration technique.* - In Vitro Cell. Dev. Biol. Plant., 42: 31-36.

MROGINSKI L.A., SANSBERRO P.A., SCOCCHI A.M., LUNA C., REY H., 2006 - *Effect of fruit cryopreservation on* in vitro *germination of zygotic embryos of several species of* Ilex. - Acta Horticulturae, 725: 417-420.

MROGINSKI L.A., SANSBERRO P.A., SCOCCHI A.M., LUNA C., REY H.Y., 2007 - *A cryopreservation protocol for immatu - re zygotic embryos of species of* Ilex (*Aquifoliaceae*). - Biocell. (In press).

REY H.Y., 2004 - *Conservación* in vitro *de germoplasma de* Arachis Pintoi *(Leguminosae).* - Ph.D. Thesis, Universidad Nacional del Nordeste, Argentina.

REY H.Y., MROGINSKI L.A., 2007 - *Cryopreservation of* Arachis pintoi (*Leguminosae*) *seeds by vitrification*. - Seed Science & Tecnology (In press).

SCOCCHI A., 2005 - *Conservación* in vitro *de germoplasma de paraíso* (Melia azedarach). - Ph.D. Thesis, Universidad Nacional del Nordeste, Argentina.

SCOCCHI A.M., DIERINGER E., MROGINSKI E., MROGINSKI L.A., 2004 a - *Conservación de semillas de cedro austra - liano* (Toona ciliata). - Plant Genetic Resources Newsletter, 137: 1-4.

SCOCCHI A.M., FALOCI M., MEDINA R., OLMOS S., MROGINSKI L.A., 2004 b - *Plant recovery of cryopreserved apical meristem-tips of* Melia azedarach *L. using encapsulation/dehy - dration and assessment of their genetic stability.* - Euphytica, 135: 29-38.

SCOCCHI A.M., VILAS.K., MROGINSKI L.A., ENGELMANN F., 2007 - *Cryopreservation of somatic embryos of paradise tree* (Melia azedarach). - CryoLetters (In press).