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INVITED REVIEW

In vitro and cryogenic preservation of plant biodiversity in Brazil

Fernanda Kokowicz Pilatti · Tarsis Aguiar · Thiago Simões · Erica E. Benson · Ana Maria Viana

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Abstract The Brazilian biomes (Amazon, Atlantic Forest, Cerrado, Caatinga, Pantanal, and Pampa) comprise one of the highest levels of plant diversity in the world; however, non-sustainable practices, deforestation, and land use have resulted in significant losses and fragmentation of the native forests. These ecosystems are now threatened and protection of their native plants through *ex situ* conservation is an urgent necessity. Cryopreservation and *in vitro* conservation are complementary options for securing and protecting Brazil's native plant species because their potential economic value is critically important to develop strategies that will (1) support their sustainable utilization, (2) protect against the over-exploitation of species growing in natural habitats, and (3) conserve the genetic diversity of germplasm from species of different provenances. Biotechnological approaches will help to address future economic and environmental demands placed upon already at-risk species. Conserving seed germplasm *ex situ* provides an additional safeguard against the risks (*e.g.*, loss due to disease, climate change) of field conservation. Moreover, seed banks and cryobanks permit the long-term conservation of a wider genetic base; this offsets the labor and space intensive costs of conserving in the active growing state. This paper is a compilation of the current status of strategies applied for conserving Brazilian native plant species.

E. E. Benson
Damar Research Scientists,
Drum Road, Cupar Muir,
Fife, Scotland KY155RJ, UK

F. K. Pilatti · T. Aguiar · T. Simões · A. M. Viana (✉)
Departamento de Botânica, Centro de Ciências Biológicas,
Universidade Federal de Santa Catarina,
Florianópolis, Santa Catarina 88040-970, Brazil
e-mail: amarna@mbox1.ufsc.br

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Introduction

The six major biomes of Brazil are the Amazon, Atlantic Forest, Pantanal, Cerrado, Caatinga, and Pampa. Totaling 8.5 million km^2, they comprise one of the greatest measures (20%) of biodiversity in the world (Ministério do Meio Ambiente, Brazil 2010; http://www.mma.gov.br/sitio/). Table 1 summarizes data on their location, areas in relation to the total country, actual remaining areas, and the approximate number of native plant species, climate, and vegetation. The Amazon and Pantanal are the most preserved areas (82–83% remaining), followed by Cerrado, Caatinga, and Pampa (41–45% remaining areas) and the Atlantic Forest (of which only 7.26% remains). In terms of species richness, the Amazon and the Atlantic Forest together contain >50% of native Brazilian species. Deforestation and burning of the Amazon forest are serious concerns for environmentalists. From 2000 to 2003, 77,000 km^2 of the Amazon forest was destroyed. In the last two decades, the Amazon forest contributed from 14% to 85% of the total wood used in Brazil (Ministry of Environment/The Brazilian Institute for Environment and Renewable Natural Resources (MMA/Ibama) 2003). The Atlantic Forest is the most endangered ecosystem due to urban occupation and exploitation as 70% of the Brazilian population lives in this biome (Instituto Brasileiro de Geografia e Estatística 2010; http://www.ibge.com.br). In the north of Parana state (south Brazil), forests of valuable hardwood have been devastated for coffee plantations (MMA/Ibama 2003). According to Ratter *et al.* (1997),

Table 1. The major Brazilian biomes

Biomes	Location	Area/total (%)[z]	Remaining area (%)[y]	Number of species[y]	Climate[x]	Vegetation[z]
Amazon	North	49.29	82	40,000	Tropical humid	Dense ombrophylous forest
Atlantic Forest	Atlantic Coast (south to northeast)	13.04	7.26	20,000	Tropical/temperate	Seasonal semideciduous forest, dense, and mixed ombrophylous forest
Pantanal	West/Paraguay basin river	1.76	83	3,500	Tropical/floods/abundant rain	Dense ombrophylous forest, savanna, xerophytic, grassland
Cerrado	Central–east	23.92	45	6–10,000	Tropical/strong dry season	Savanna
Caatinga	Northeast	9.92	41	932	Semiarid/low rain/high temperature	Savanna/xerophytic
Pampa	South	2.07	41	3,000	Temperate	Grassland

[z] The Brazilian Geographic Institute (IBGE; http://www.ibge.gov.br—accessed February 19, 2010)

[y] http://www.estadao.com.br—accessed February 19, 2010/IBGE, SOS Mata Atlantica/Conservation Internacional/Reserva da Biosfera da Caatinga

[x] The Brazilian Ministry of Environment (MMA; http://www.mma.gov.br—accessed February 19, 2010)

Cerrado (center Brazil) covers about 2 million km^2, and as an endangered biome (Palazetti and Shepherd 1999), it is estimated that it will be completely degraded in 30 yr (Centro Nacional Recursos Genéticos, Embrapa, Brazil 2010; http://www.cenargen.embrapa.br). Caatinga is a center of genetic diversity for aromatic species and several fruit trees, which are exported. Pantanal, located in the western states of Mato Grosso and Mato Grosso do Sul, is considered the largest wetland in the world by the MMA (Vieira 1999).

Brazilian Plant Species at Risk and Under Threat of Extinction

In 2005, Ibama and the Biodiversitas Foundation revised the red list of Brazilian native species in extinction or threatened by extinction; in 2008, a new list was published and 1,495 species were considered threatened by extinction. Subsequently, Ibama and MMA representatives evaluated 472 species on the verge of extinction and prioritized them for public political attention in Brazil (Biodiversitas Foundation 2010; http://www.biodiversitas.org.br). According to the Biodiversitas Foundation, the species considered vulnerable or at risk are distributed across Brazilian biomes as follows: 60 in the Amazon, 713 in the Atlantic rainforest, ten in Pantanal, 531 in Cerrado, 179 in Caatinga, and 61 in Pampa. The data in Table 2 were compiled from the official list of the Brazilian native species in danger of extinction published by the Ministry of Environment in 2008 (Instituto Brasileiro do Meio Ambiente 2010; http://www.ibama.gov.br/recursos-florestais/documentos/lista-oficial-de-especies-brasileiras-ameacadas-de-extincao/).

Bromeliaceae, Cactaceae, and Orchidaceae are the families with the highest number of endangered species. The following genus/species of economically important plants are on the verge of extinction: *Pfaffia* (Amarantaceae, medicinal); *Myracrodruon urundeuva* (Anacardiaceae); *Araucaria angustifolia* (Araucariaceae); *Bactris*, *Butia*, and *Euterpe edulis* (Arecaceae); *Lychnophora* (Asteraceae, medicinal); *Jacaranda* and *Tabebuia* (Bignoniaceae, medicinal); *Dyckia*, *Cryptanthus*, and *Vrisea* (Bromeliaceae); *Maytenus* (Celastraceae); *Dicksonia* (Dicksoniaceae); *Caesalpinia echinata*, *Dalbergia*, *Machaerium*, and *Mimosa* (Fabaceae); *Sinningia* (Gesneriaceae); *Ocotea* (Lauraceae, medicinal); *Bertholletia excelsa* (Brazilian nut tree; Lecythidaceae); *Cariniana* (Lecythidaceae); *Tibouchina* (Melastomataceae); *Swietenia macrophylla* (mahogany; Meliaceae); *Eugenia* and *Myrcia* (Myrtaceae); *Cattleya* (Orchidaceae); *Passiflora* (Passifloraceae); and *Pilocarpus* (Rutaceae, medicinal) (Instituto Brasileiro do Meio Ambiente 2010; http://www.ibama.gov.br/recursos-florestais/documentos/lista-oficial-de-especies-brasileiras-ameacadas-de-extincao/).

Constraints in Plant Conservation Practices

A great constraint in the amelioration of species loss in Brazil is the problem of establishing strategies for forest seed germplasm conservation, exacerbated by limited information available on seed storage behavior in the scientific literature. Practical restrictions arise for certain species that are difficult to collect, especially when the trees are located in the forest and for which there is an insufficient seed supply for conservation experiments. Tree scarcity in certain areas is due to intensive exploitation, and this also contributes to low seed availability and collection. There is a lack of information on flowering and seed production of certain Brazilian native forest trees, and seed production is irregular in different yr. For these reasons, most research on the conservation of Brazilian native

Table 2. Genus of native Brazilian plant species in danger of extinction

Family	Species[z]	Biome[z]
Acanthaceae	5	Atlantic Forest/Cerrado
Alstroemeriaceae	1	Atlantic Forest
Amaranthaceae	8	Atlantic Forest/Cerrado/Caatinga
Amaryllidaceae	3	Atlantic Forest/Cerrado
Anacardiaceae	2	Cerrado/Caatinga
Apocynaceae	7	Atlantic Forest/Cerrado/Caatinga
Araceae	4	Atlantic Forest
Araucariaceae	1	Atlantic Forest
Arecaceae	6	Atlantic Forest/Cerrado/Pampa
Arnelliaceae	1	Atlantic Forest
Aspleniaceae	6	Atlantic Forest/Cerrado
Asteraceae	16	Atlantic Forest/Cerrado/Pampa/Amazon
Begoniaceae	11	Atlantic Forest
Bignoniaceae	10	Atlantic Forest/Cerrado/Caatinga/Amazon
Blechnaceae	3	Atlantic Forest/Pampa
Bromeliaceae	38	Atlantic Forest/Caatinga/Pampa
Bruchiaceae	1	Atlantic Forest
Burseraceae	3	Atlantic Forest/Amazon
Cactaceae	28	Atlantic Forest/Cerrado/Caatinga/Pantanal
Celastraceae	2	Atlantic Forest/Cerrado
Chrysobalanaceae	7	Atlantic Forest/Amazon
Combretaceae	3	Atlantic Forest
Connaraceae	1	Atlantic Forest/Cerrado
Convolvulaceae	3	Amazon/Cerrado
Costaceae	3	Atlantic Forest/Amazon
Cyperaceae	5	Atlantic Forest/Cerrado/Caatinga
Dicksoniaceae	1	Atlantic Forest
Dicranaceae	3	Atlantic Forest
Dilleniaceae	1	Atlantic Forest
Ephedraceae	1	Atlantic Forest
Eriocaulaceae	14	Cerrado
Erytroxylaceae	10	Atlantic Forest/Cerrado/Caatinga
Fabaceae	21	Atlantic Forest/Cerrado/Pampa/Amazon
Geocalycaceae	1	Atlantic Forest
Gesneriaceae	8	Atlantic Forest
Grammitidaceae	2	Atlantic Forest
Heliconiaceae	5	Atlantic Forest
Iridaceae	7	Cerrado
Jungermanniaceae	1	Atlantic Forest
Lamiaceae	13	Cerrado/Caatinga/Pampa/
Lauraceae	12	Atlantic Forest/Cerrado/Amazon
Lecythidaceae	6	Atlantic Forest/Amazon
Lejeuneaceae	3	Atlantic Forest
Lentibulariaceae	1	Cerrado
Lepidoziaceae	1	Atlantic Forest
Lycopodiaceae	2	Cerrado
Lythraceae	11	Cerrado
Malpighiaceae	1	Atlantic Forest
Malvaceae	3	Atlantic Forest/Pampa
Melastomataceae	9	Atlantic Forest/Cerrado
Meliaceae	1	Atlantic Forest
Monimiaceae	8	Atlantic Forest
Moraceae	4	Atlantic Forest
Myrtaceae	14	Atlantic Forest
Ochnaceae	1	Atlantic Forest
Oleaceae	1	Atlantic Forest
Orchidaceae	34	Atlantic Forest/Cerrado/Amazon
Orobanchaceae	1	Atlantic Forest
Passifloraceae	5	Atlantic Forest/Cerrado
Phytolaccaceae	1	Atlantic Forest
Picramniaceae	1	Atlantic Forest
Plagiochilaceae	1	Atlantic Forest
Plantaginaceae	2	Atlantic Forest/Cerrado
Poaceae	14	Atlantic Forest/Cerrado/Pampa
Podostemaceae	2	Atlantic Forest/Amazon
Polygalaceae	1	Cerrado
Pottiaceae	2	Atlantic Forest
Proteaceae	1	Pampa
Pteridaceae	5	Atlantic Forest/Cerrado
Ricciaceae	1	Caatinga
Rubiaceae	15	Atlantic Forest/Cerrado
Rutaceae	10	Atlantic Forest/Cerrado/Amazon
Santalaceae	1	Atlantic Forest
Sapindaceae	1	Cerrado
Sapotaceae	1	Atlantic Forest
Scrophulariaceae	1	Atlantic Forest
Siparunaceae	1	Atlantic Forest
Solanaceae	10	Atlantic Forest
Symplocaceae	2	Atlantic Forest
Thelypteridaceae	1	Atlantic Forest
Theophrastaceae	1	Atlantic Forest
Trigoniaceae	1	Atlantic Forest
Verbenaceae	2	Cerrado/Caatinga
Violaceae	1	Caatinga
Vitaceae	1	Cerrado
Xyridaceae	10	Atlantic Forest/Caatinga/Cerrado

[z] Compiled from the official list of the species in danger of extinction published by the Brazilian Ministry of Environment in 2008

species is performed on orthodox seeds. However, in the last decades, major efforts have been made by Brazilian researchers to focus their studies on the collection and behavior characterization of seeds of native species from the Amazon and Atlantic Forest, their priority being the long-term conservation of species producing recalcitrant seeds (Table 3). Barbedo and Bilia (1998) estimated that 70% of the native species (*e.g.*, *Inga uruguensis*, *Theobroma cacao*, *Hevea brasiliensis*, *B. excelsa*, *E. edulis*, *Paullinia cupana*) from humid forests are recalcitrant or intermediate. Thus, the overall aims of this paper are to review the status of the different strategies currently applied for conserving Brazil's native plant species and to highlight the potential role that *in vitro* conservation may play in the future.

Overview of Plant Germplasm Conservation Strategies in Brazil

Although the Brazilian biomes are under strong environmental and anthropogenic pressure, the *in situ* conservation of native species in their natural environment is the most predominant approach for biodiversity protection and germplasm conservation. *In situ* conservation involves conservation in units (genetic reserves and national parks). Ibama (Instituto Brasileiro do Meio Ambiente 2010; http://www.ibama.gov.br) report a total of 728 distributed among

Table 3. Examples of economically important native Brazilian species producing recalcitrant seeds

Family	Species	Importance	References
Apocynaceae	*Hancornia speciosa*	Fruit	Oliveira and Valio (1992)
Araucariaceae	*Araucaria angustifolia*	Seeds	Barbedo and Bilia (1998)
Clusiaceae	*Calophyllum brasiliense*	Wood	Carvalho *et al*. (2006)
Euphorbiaceae	*Hevea brasiliensis*	Rubber	Barbedo and Bilia (1998)
Fabaceae	*Inga uruguensis*	Fruit	Bilia *et al*. (1999)
	Inga edulis	Fruit	Barbedo and Bilia (1998)
	Inga striata	Fruit	Barbedo and Bilia (1998)
	Inga vera subsp. *affinis*	Fruit	Faria *et al*. (2004)
Lauraceae	*Nectandra grandiflora*	Wood	Carvalho *et al*. (2008)
	Nectandra lanceolata	Wood	Carvalho *et al*. (2008)
	Nectandra oppositifolia	Wood	Carvalho *et al*. (2008)
	Ocotea corymbosa	Wood	Carvalho *et al*. (2008)
	Ocotea pulchella	Wood	Carvalho *et al*. (2008)
Lecythidaceae	*Bertholletia excelsa*	Seed	Silva *et al*. (2009)
Magnoliaceae	*Talauma ovata*	Medicinal	Carvalho *et al*. (2006)
	Magnolia ovata	Medicinal	Pupim *et al*. (2009)
Myristicaceae	*Virola surinamensis*	Fruit	Barbedo and Bilia (1998)
Myrtaceae	*Aulomyrcia venulosa*	Ecology	José *et al*. (2007)
	Calyptranthes lucida	Fruit	Carvalho *et al*. (2006)
	Eugenia brasiliensis	Fruit	Kohoma *et al*. (2006)
	Eugenia cerasiflora	Fruit	Delgado and Barbedo (2007)
	Eugenia involucrata	Fruit	Delgado and Barbedo (2007)
	Eugenia pyriformis	Fruit	Delgado and Barbedo (2007)
	Eugenia stipitata subsp. *sororia*	Fruit	Gentil and Ferreira (1999)
	Eugenia umbelliflora	Fruit	Delgado and Barbedo (2007)
	Eugenia uniflora	Fruit	Delgado and Barbedo (2007)
	Eugenia handroana	Fruit	Carvalho *et al*. (2006)
	Myrcianthes pungens	Fruit	Wielewick *et al*. (2006)
Palmae	*Bactris gasipaes*	Fruit	Carvalho and Muller (1998)
	Euterpe espiritosantensis	Heart of palm	Martins *et al*. (1999)
	Euterpe edulis	Heart of palm	Martins *et al*. (2000)
Rubiaceae	*Genipa americana*	Fruit	Carvalho *et al*. (1995)
	Ixora warmingii	Ecology	José *et al*. (2007)
Sapindaceae	*Allophylus edulis*	Ecology	José *et al*. (2007)
	Cupania vernalis	Medicinal	Carvalho *et al*. (2006)
Sterculiaceae	*Theobroma cacao*	Fruit	Barbedo and Bilia (1998)
	Theobroma grandiflorum	Fruit	Cruz (2007)

Amazon, Atlantic Forest, Cerrado, Caatinga, Pantanal, and Pampa. Of these, only 126 conservation units are completely protected and 602 are for sustainable use. The conservation units comprise 74 national forests, 62 national parks, 29 biological reserves, 50 extractivist reserves, 32 ecological stations, 31 environmental protect areas, and 429 private reserves; the Atlantic Forest has ca. 860 conservation units and Caatinga has 53.

Brazilian Agricultural Research Corporation (Embrapa) created the National Center for Genetic Resources and Biotechnology (Cenargen) which is responsible for *ex situ* conservation through active germplasm banks, seed banks, DNA banks, and *in vitro* germplasm banks. The Plant Genetic Resources National Network created by Embrapa integrates germplasm conservation programs and expands the genetic basis for plant improvement carried out by the Embrapa Centers and other Brazilian institutions (Centro Nacional Recursos Genéticos, Embrapa, Brazil 2010; http://www.cenargen.embrapa.br). The Brazilian System Information of Genetic Resources (Sibrargen)–Colbase Embrapa is an internet access information system-based data bank developed in 1996 by Embrapa Genetic Resources and Biotechnology. It has registered 214 genus, 677 species, and 109,154 accessions (http://www.cenargen.embrapa./recgen/sibrargen/sibrargen.html—accessed February 3, 2010). The network comprises a considerable number of plant germplasm banks, biological collections, and conservation nuclei, distributed in the north, northeast, center-west, southeast, and south Brazil. The germplasm banks have a diverse genetic base and they primarily sustain genetic improvement programs for developing new Brazilian cultivars of native tropical fruit trees, medicinal and aromatic plants, forest trees, palms, and other non-native economically important species.

The medium- and long-term strategies for *ex situ* conservation are supported by the Embrapa's seed germplasm bank, created in 1976; it has 107,000 accessions of 661 species; and its objective is to ensure the availability of genetic resources for food and agriculture. The main strategies for *ex situ* conservation are currently the storage of orthodox seeds at −20°C and minimal growth of *in vitro* collections applied to vegetatively propagated or highly heterozygotic species. Protocols are currently being established for the *in vitro* culture and cryopreservation of vegetative and reproductive structures derived from species producing storage recalcitrant or intermediate seeds. DNA conservation of endangered species ensures the availability of "molecular genetic diversity" which can be used to study phylogeny, molecular systematics, comparative genomics, and gene prospecting (Centro Nacional Recursos Genéticos, Embrapa, Brazil 2010; http://www.cenargen.embrapa.br). As a large number of native Brazilian species are classified as vulnerable or at risk of extinction, the following species are targeted by Embrapa Genetic Resources for the establishment of DNA banks: *Cedrela fissilis* and *Amburana cearensis* (Fabaceae), *Caesalpinia equinata* (Caesalpiniaceae), *Dalbergia nigra* (Fabaceae), *Cedrela odorata* (Meliaceae), *Manikara multifida* (Sapotaceae), *Ilex paraguariensis* (Aquifoliaceae), *A. angustifolia* (Araucariaceae), *Butia eriospatha* (Arecaceae), *Ocotea porosa* (Lauraceae), *B. excelsa* (Lecythidaceae), and *Hymenaea courbaril* (Caesalpiniaceae; Centro Nacional Recursos Genéticos, Embrapa, Brazil 2010; http://www.cenargen.embrapa.br).

Progress and Incentives for Recalcitrant Seed Conservation in Brazil

Barbedo and Bilia (1998) analyzed the progress of recalcitrant seed research in Brazil. It was initiated in 1950, focusing on recalcitrant seed identification and assessment of tolerance and responses to different storage parameters (low temperatures, humidity, packaging, chemical composition, viability, fungicide efficacy). Until the 1980s, the Brazilian scientific literature reported on only the few economically important recalcitrant-seeded species, including *H. brasiliensis*, *A. angustifolia*, and *T. cacao* (Table 3). Barbedo and Bilia (1998) report the study of recalcitrant seeds from the Brazilian native species: *Inga edulis*, *Inga striata*, *A. angustifolia*, *T. cacao*, *E. edulis*, *H. brasiliensis*, *Virola surinamensis*, *Ocotea* sp., *Nectandra* sp., and the Brazilian Palm family. From the 1990s, the classification of native species by seed behavior was stimulated by demand for plants to regenerate degraded areas and aid ecosystem conservation. This research has increased in recent years and fostered studies on seed storage and conservation using the protocol of Hong and Ellis (1996). Wielewick *et al*. (2006) studied 27 forest trees native to the Atlantic Forest, reporting seeds of *Myrcianthes pungens* as recalcitrant, as qualified by a water content of 39.6% on collection and dehydration intolerance. Carvalho *et al*. (2006) classified the seed storage behavior of 39 tree species native to the Atlantic Forest (see Table 3 for examples).

Oliveira and Valio (1992) report that seeds of *Hancornia speciosa* (Apocynaceae), a native of the Cerrado and economically important for fruit production, lost viability when dehydrated to moisture content <30%. José *et al*. (2007) carried out studies on the desiccation tolerance and storage of five tree species of the Atlantic Forest to support future *ex situ* conservation programs for *Miconia argyrophylla* (Melastomataceae), *Allophylus edulis* (Sapindaceae), *Ixora warmingii* (Rubiaceae), *Aulomyrcia venulosa* (Myrtaceae), and *Metrodorea stipularis* (Rutaceae). These species grow near small rivers and streams and are important for preserving local biodiversity. According to their studies, *A. edulis*, *I. warmingii*, and *A. venulosa* were classified as recalcitrant, as they completely lost viability when desiccated to moisture contents, respectively, of <16% (*A. edulis*) and <25% (*I. warmingii* and *A. venulosa*), whereas *M. argyrophylla* and *M. stipularis* tolerated storage at −20°C and had a moisture content of <5%. Carvalho *et al*. (2008) investigated the *Lauraceae* family, showing recalcitrant seed behavior in *Nectandra grandiflora*, *Nectandra lanceolata*, *Nectandra oppositifolia*, *Ocotea corymbosa*, and *Ocotea pulchella*. Species from the Lauraceae family occur in the Brazilian Amazon and Atlantic Forests. Some such as *Nectandra micranthera*, *Persea pedunculosa*, *Ocotea cryptocarpa*, *Ocotea cymbarum*, and *Ocotea serrana* are in danger of extinction (Instituto Brasileiro do Meio Ambiente 2010; http://www.ibama.gov.br/recursos-florestais/documentos/lista-oficial-de-especies-brasileiras-ameacadas-de-extincao/). The *Nectandra* and *Ocotea* are economically important for the production of valuable hardwood, and many species of these genera are in danger of extinction due to over-exploitation.

V. surinamensis (Myristicaceae), a native of the Amazon forest, is one of the urgent priorities in the Brazilian genetic resources conservation program, and its storage in germplasm banks is required. Rodrigues (1980) reports that *V. surinamensis* seeds must be kept hydrated in water immediately after harvesting to maintain viability for up to 4 mo. If the seeds dehydrate in their natural environment, viability is lost after 20 d, indicative of recalcitrant behavior. Cunha *et al*. (1992) showed that seed initial moisture content was ca. 25.4%, but when exposed to drying at 22°C and 53% relative humidity (RH) for 60 min, moisture content decreased to 20%, for which a maximum germination rate (69%) was achieved. However, a drying period ≥90 min decreased the moisture content to 18.8%, and germination rate decreased to 45%, reaching zero after 5 h dehydration. These studies confirm low desiccation tolerance and the need for *in vitro* conservation strategies. By comparison, Martins *et al*. (1999, 2000) and Carvalho and Muller (1998) reported that recalcitrant seeds of *Euterpe espiritosantensis*, *E. edulis*, and *Bactris gasipaes*, native palm species from the Atlantic Forest and Amazon, could be dehydrated to 46.6%, 33%, and 39%, respectively, without a decrease in germination rates.

According to Maluf and Pisciottano-Ereio (2005), the Myrtaceae family is one of the most economically important. It includes the genus (*e.g.*, *Eugenia*, *Campomanesia*, *Psidium*, and *Myrciaria*) of many edible fruit trees native to the Atlantic Forest. Delgado and Barbedo (2007) confirm Eugenia species as particularly important for fruit production; they are of medicinal importance in the pharmaceutical industries. However, there are constraints as to their use due to lack of information on seed management. Kohoma *et al*. (2006) showed that a seed moisture content <43.1% reduced germination rate for *Eugenia brasiliensis*, which is a native fruit tree from the Atlantic Forest. However, seeds with 48.9% moisture content could be stored for 180 d at 7°C with 60% viability. Gentil and Ferreira (1999) and Delgado and Barbedo (2007) studied several other *Eugenia* species (Table 3), concluding that, in general, seeds did not tolerate moisture contents <45% with a loss in their viability occurring at <15%. Similarly, Cruz (2007) evaluated desiccation tolerance in *Theobroma grandiflorum*, a native tree of the Amazon and economically important for the production of edible fruits, the seeds of which could be desiccated to 37.8% moisture content without viability loss. *Inga vera* subsp. *affinis* produces recalcitrant seeds unable to withstand dehydration to levels <35% (Faria *et al*. 2004). Similar seed behavior was reported for *I. uruguensis* (Bilia *et al*. 1999). However, Bonjovani and Barbedo (2008) showed that mature embryos of *I. vera* subsp. *affinis* desiccated to a hydric potential of −4 MPa became tolerant to −2°C, offering another approach for conservation. *Magnolia ovata* (Magnoliaceae) is a native forest tree used for recuperation of areas near rivers; it produces recalcitrant seeds (23.5% water content) that lose viability after 30 d storage. In contrast, viability was maintained for 60 d (starting at 30%, decreasing to 23%, compared to initial germination rate) in seeds dehydrated to 10.9% water content and stored at 15°C or 20°C (Pupim *et al*. 2009). Another Magnoliaceae, *Talauma ovata*, was also classified as recalcitrant by Carvalho *et al*. (2006). *B. excelsa* produces recalcitrant seeds; it is the “Brazil nut tree” native to the Amazon and, as the main export product of the region, is in danger of extinction due to over-exploitation. Silva *et al*. (2009) reported seeds of *B. excelsa* with a 14% moisture content before storage in wet sand; they were able to maintain viability when their water content was increased to 35% after 175 d of storage. Problems associated with the preservation of at risk and endangered Brazilian native species and especially those producing recalcitrant seeds (Table 3) have stimulated the exploration of *in vitro* and cryopreservation as alternative and complementary conservation strategies.

In Vitro Culture, Conservation, and Sustainability of Native Brazilian Plants

In vitro studies on native species have been mainly carried out in universities and research institutions and published primarily in Brazilian journals (Table 4). While much of this literature concerns micropropagation and plant production, it has important significance for the *in situ* and *ex situ* conservation of Brazil’s native plant species, particularly

Table 4. *In vitro* culture and conservation of native Brazilian plant species

Family	Species	Biome	Explant	Technique	References
Anacardiaceae	*Myracrodruon urundeuva*	Cerrado	Seeds, seedlings	*In vitro* germination, micropropagation	Andrade *et al.* (2000)
Apocynaceae	*Aspidosperma polyneuron*	Atlantic Forest	Nodal segments	Micropropagation	Ribas *et al.* (2005)
	Hancornia speciosa	Caatinga, Atlantic Forest, Amazon	Seeds	*In vitro* germination	Pinheiro *et al.* (2001)
	Hancornia speciosa		Apical and nodal segments	Micropropagation	Grigoletto (1997)
	Hancornia speciosa		Nodal segments	Direct organogenesis	Soares *et al.* (2007)
	Mandevilla velutina	Cerrado	Seeds, seedlings	Micropropagation	Biondo *et al.* (2007)
			Microshoots	*In vitro* storage 6 mo	Biondo *et al.* (2007)
Aquifoliaceae	*Ilex paraguariensis*	Atlantic Forest	Microshoots	*Ex vitro* rooting	Quadros (2009)
			Seeds	Zygotic embryo culture	Hu (1975)
Araucariaceae	*Araucaria angustifolia*	Atlantic Forest	Immature zygotic embryos	Somatic embryogenesis	Astarita and Guerra (2000)
Asteraceae	*Lychnophora pinaster*	Cerrado	Seeds, nodal segments	*In vitro* germination, micropropagation	Souza *et al.* (2003)
Bignoniaceae	*Tabebuia impetiginosa*	Atlantic Forest	Seeds, seedling nodal segments	*In vitro* germination, Micropropagation	Martins *et al.* (2009)
Bromeliaceae	*Ananas lucidus*	Caatinga	Seedling nodal segments	Micropropagation	Oliveira *et al.* (2007a)
	Cryptanthus sinuosus	Atlantic Forest	Stem–root axis from greenhouse plants	Micropropagation	Arrabal *et al.* (2002)
	Vriesea reitzii	Atlantic Forest	Young basal leaves	Micropropagation	Alves *et al.* (2006)
Cactaceae	*Arrojadoa* spp.	Caatinga	Seeds	*In vitro* germination	Dias *et al.* (2008)
Caryocaraceae	*Caryocar brasiliense*	Cerrado	Seeds, seedling nodal segments	*In vitro* germination, micropropagation	Oliveira (2000)
	Caryocar brasiliense	Cerrado	Leaf explants	Callus	Landa *et al.* (2000)
Cochlospermaceae	*Cochlospermum regium*	Cerrado	Seeds	*In vitro* germination	Camillo *et al.*(2009b)
	Cochlospermum regium		Nodal segments	*In vitro* storage 3 mo	Camillo *et al.*(2009b)
Clusiaceae	*Hypericum brasiliense*	Amazon	Axillary buds	*In vitro* germination	Velloso *et al.* (2009)
	Kielmeyera coriacea	Cerrado	Nodal segments	Micropropagation	Pinto *et al.* (1994)
Compositae	*Acmella oleracea*	Amazon	Adult plants in the greenhouse	Micropropagation	Malosso (2007)
			Microshoots	*In vitro* storage 6 mo	Malosso (2007)
Fabaceae	*Anadenanthera colubrina*	Caatinga	Seeds	*In vitro* germination	Nepomuceno *et al.* (2009)
	Mimosa caesalpiniaefolia	Caatinga	Axillary buds from adult plants raised in the greenhouse	Micropropagation	Oliveira *et al.* (2007b)
			Seeds, seedling apical shoots	*In vitro* germination, micropropagation	Oliveira *et al.* (2007b)
	Parkinsonia aculeata	Caatinga	Seeds	*In vitro* germination	Gomes (2007)
	Pterodon pubescens	Cerrado	Seeds	*In vitro* germination	Coelho *et al.* (2001)
	Schizolobium parahyba	Amazon	Seeds	*In vitro* germination,	Reis *et al.* (2007)

Table 4. (continued)

Family	Species	Biome	Explant	Technique	References
	var. *amazonicum*			callus	
Gesneriaceae	*Sinningia allagophylla*	Cerrado	Nodal segments	Micropropagation	Almeida and Shepherd (1999)
Lauraceae	*Ocotea catharinensis*	Atlantic Forest	Air dehydrated mature somatic embryos	Storage at 25°C in sealed Petri dishes for 3 mo	Santa-Catarina *et al.* (2004)
Malpighiaceae	*Byrsonima intermedia*	Cerrado	Seeds	*In vitro* germination	Nogueira *et al.*(2004)
Meliaceae	*Aniba rosaeodora*	Amazon	Seeds	Zygotic embryo culture	Handa *et al.* (2005)
	Aniba rosaeodora	Amazon	Seedling apical and axillary buds	*In vitro* germination	Handa *et al.* (2005)
	Cedrela fissilis	Atlantic Forest	Seeds, seedling nodal segments	*In vitro* germination, micropropagation	Nunes *et al.* (2002)
	Cedrela fissilis	Atlantic Forest	Alginate-encapsulated nodal segments	*In vitro* storage at 25°C for 6 mo	Nunes *et al.* (2003)
	Swietenia macrophylla	Amazon	Seeds, seedlings	*In vitro* germination, callus	Couto (2002)
Moraceae	*Brosimum gaudichaudii*	Cerrado	Seeds	*In vitro* germination	Fidelis *et al.* (2000)
Myrtaceae	*Eugenia dysenterica*	Cerrado	seeds	*In vitro* germination	Martinotto *et al.* (2007)
	Eugenia pyriformis	Atlantic Forest	Seeds, seedling nodal segments	*In vitro* germination, micropropagation	Nascimento *et al.* (2008)
	Myrciaria aureana	Atlantic Forest	Zygotic embryos	Somatic embryogenesis	Motoike *et al.* (2007)
Palmae	*Acrocomia aculeata*	Cerrado	Zygotic embryos	Somatic embryogenesis	Moura *et al.* (2008)
	Euterpe edulis	Atlantic Forest	Immature Zygotic embryos	Somatic embryogenesis	Saldanha (2007)
		Atlantic Forest	Immature Zygotic embryos	Zygotic embryo culture	Saldanha (2007)
Quillajaceae	*Quillaja brasiliensis*	Atlantic Forest	Seedling nodal segments	Micropropagation	Fleck *et al.* (2009)

those that are overexploited and at risk in their native habitats. *In vitro* and seed conservation strategies address three main issues. First, seed storage, seedling production, vegetative production, and micropropagation support the sustainable and managed cultivation of commercially important genotypes of native species. This offsets the need to continually source plants direct from the wild, a practice that has caused the over-exploitation of many native Brazilian species, leading to genetic erosion, biodiversity loss, and extinction. Second, knowledge of seed storage behavior and vegetative physiologies can be applied to develop short- and medium-term conservation measures, which, coupled with seedling propagation in nurseries and micropropagation, increases plant production capacity for habitat restoration and endangered species reintroduction. Third, experience gained from developing plant tissue culture protocols for economically important native Brazilian species can be used to assist their *in vitro* conservation. This is highly practicable because the technical challenges of tissue culture are common to all *in vitro* procedures. They involve the optimization of stepwise manipulations (see Table 4) used in commercial *and* conservation scenarios: mother plant status, explant selection, surface sterilization, *in vitro* germination, axenic culture, micropropagation (organogenesis, somatic embryogenesis), plant regeneration, rooting, acclimatization *ex vitro*, and issues pertaining to genetic stability. Callus and cell suspension cultures are used for somatic embryogenesis micropropagation and in various biotechnological applications to exploit the economic value of native plant biodiversity, all of which have a requirement for *in vitro* conservation, especially cryopreservation.

In vitro culture and conservation are being increasingly applied to endemic Brazilian species, but steps forward remain limited compared to crop species. Embrapa reports protocols for pineapple, yam, sweet potato, vanilla, oregano, strawberry, potato, cassava, mint, banana, asparagus, grape, and Stevia. For example, *in vitro* conservation of sugarcane germplasm for 12 mo was achieved for apical buds cultured on 20 g/L sucrose, abscisic acid at 15°C (Lemos *et al*. 2002). A summary of *in vitro* culture technologies applied to native and/or economically important Brazilian plants is shown in Table 4. It is important to highlight the relevance of using "collective know-how" gained from economic and conservation plant biotechnologies to underpin economically significant and sustainable practices and *in vitro* plant conservation. *In vitro* germination, micropropagation, somatic embryogenesis, zygotic embryo culture, and callus culture systems have been developed successfully for a substantial number of native Brazilian species. It is anticipated that knowledge gained from these systems can be potentially used to further *in vitro* germplasm conservation studies.

A focus on the Meliaceae: constraints and successes. The successful application of *in vitro* conservation protocols for native Brazilian plant species remains restricted to a few cases. This is largely due to a lack of species-specific physiological knowledge, particularly regarding germplasm behavior, a problem that is exacerbated by the wide range of plant diversity under study and limited resources. The authors have placed particular emphasis on developing *in vitro* conservation protocols for the Meliaceae. *C. fissilis* is a valuable, fast-growing timber tree, and as a member of Meliaceae native to the Brazilian Atlantic Forest, it is at risk in its native habitat. Nunes *et al*. (2002, 2003) reported the establishment of a consistent micropropagation protocol and the successful optimization of plant recovery and rooting from calcium alginate-encapsulated *C. fissilis* shoot tips, cotyledonary, and epicotyledonary nodal segments stored on substrate containing 0.4% (*w*/*v*) agar, at 25°C. This protocol is used for *in vitro* short-term storage for up to 6 mo.

One of the best-known species of the *Meliaceae* is *S. macrophylla* (mahogany), a native tree from the Amazon, which is now on the verge of extinction in the wild because it has been intensively exploited for its valuable hardwood. Couto (2002) developed a protocol for the *in vitro* germination of seeds from this species as well as protocols for callus culture and shoot formation from pre-existing buds. Shoot production was achieved when hypocotyl segments comprising original axillary buds were cultured on half-strength MS (Murashige and Skoog 1962) medium supplemented with 6-benzylaminopurine.

Aniba rosaeodora is a native tree from the Amazon forest, important economically for its oil which is used in the cosmetics industries. It can be propagated by seeds and vegetatively via root cuttings; however, seed production is low and irregular. To help overcome these constraints, Handa *et al.* (2005) developed an alternative protocol for *A. rosaeodora* zygotic embryo culture and achieved a germination rate of 53%. This required embryos to be cultured on MS medium supplemented with 5% (*w*/*v*) sucrose and 1.8 g/L Phytagel. The survival of apical and axillary bud cultures was in contrast, 48% on MS medium supplemented with 300 mg/L agrymicin, 3% (*w*/*v*) sucrose, and 0.7% (*w*/*v*) agar. The buds were excised from seedlings grown in the greenhouse, and antibiotics were necessary to control endogenous contamination in explants.

Cryopreservation of Economically Important Non-native and Native Brazilian Plant Species

The development of cryostorage protocols for plants native to Brazil remains limited, with most efforts targeted at economically important species. Endangered and at-risk plant groups present special challenges due to recalcitrance, limited germplasm supply and lack of knowledge pertaining to seed storage behavior, adaptive physiology, and culture responses. *In vitro* manipulation and cryoconservation of intermediate and orthodox seed germplasm provide examples of the most noteworthy advances.

Intermediate seed cryopreservation. The Rubiaceae and Palmae families both demonstrate intermediate seed behavior; cryopreservation combined with *in vitro* zygotic embryo culture can be successfully and consistently employed for their germplasm conservation. This section will overview the cryopreservation of two economically important, non-native crops cultivated in Brazil, experience of which can be applied to native species.

Rubiaceae—*Coffea arabica*, a native of Africa, is one of the most important economic species of Brazil. Santos *et al*. (2002) reported seed tolerance to dehydration (5–7% moisture content) as applied in conventional storage, but sensitivity to low temperatures (−18°C). *C. arabica* seeds are thus classified as intermediate. However, seeds can be conserved for a maximum of 24 mo when partially dehydrated and stored at 20°C (Naidu and Sreenath 1999). Germplasm conservation of *Coffea* species in Brazil is carried out for vulnerable germplasm banks in the field; thus, Santos *et al*. (2002) developed a simple protocol for cryopreserving embryonic axes without using chemical cryoprotectants or a programmable freezer. Seeds were immersed in liquid nitrogen (LN) for 17 h, thawed rapidly in a water bath at 40±2°C for 3 min, surface-sterilized, and immersed in water for 48 h, after which their embryonic axes were removed and cultured on Wood Plant Medium. High survival rates were observed in embryos removed from seeds with 12.6–22% moisture content; this decreased at water contents of 29.4%. The protocol is a viable alternative for long-term *C. arabica* germplasm conservation. Eira *et al*. (2005) successfully achieved cryostorage for 24 mo for coffee seeds after their partial dehydration to 20% moisture content (concurring with Dussert *et al*. 1997, 1998). Thawing was achieved in a water bath at 40°C. Dehydration was carried out according to Eira *et al*. (1999a, b) and involved applying a NaCl solution (75.5% RH) at 15°C. Eira *et al*. (2005) report that their protocol maintains seed viability throughout storage in contrast to previous literature (Dussert *et al*. 1997, 1998; Eira *et al*. 1999a). The method of Eira and colleagues was successfully applied to 30 *C. arabica* cultivars, endorsing the method for the long-term conservation of Brazilian and other *Coffea* species.

Palmae—*Elaeis guineensis* (oil palm), a native of Africa, has been incorporated in genetic improvement programs by Embrapa in Brazil, in the Amazon basin. According to Camillo *et al*. (2009a), maintenance of field genebanks is expensive and new strategies are needed. Oil palm seeds are intermediate according to Ellis *et al*. (1991). Camillo *et al*. (2009a) developed a protocol for seed cryopreservation of five *E. guineensis* genotypes in which seeds were immersed in LN, stored for 7 d, and slow thawed at room temperature (25±3°C) for 12 h. The seed tegument was removed following LN storage; the zygotic embryos were excised and inoculated on half-strength MS medium supplemented with 2 mg/L pantothenic acid, 3% (*w*/*v*) sucrose, and 0.25% (*w*/*v*) Phytagel. After 28 d, *in vitro* zygotic embryo germination rates varied from 73% to 100%, depending on the genotype. The advantages of this protocol are that cryoprotection is not necessary, and seeds can be cryopreserved with intact teguments.

Developing cryogenic storage protocols for orthodox seeds of native forest species. Seed cryopreservation is advantageous for the long-term conservation of tropical and subtropical forest species biodiversity, as it circumvents problems related to embryo isolation and *in vitro* handling. Even for orthodox and intermediate seeds, cryostorage offers the advantages of seed longevity so long as LN levels are maintained. Storage of dry seeds at −20°C in seed banks for long periods might also lead to physiological and genetic damage in the very long term (Stanwood and Ross 1979; Stanwood and Bass 1981).

Table 5 summarizes cryogenic storage methods applied to orthodox seeds of native species. Wetzel *et al*. (2003) found that seeds of 13 species collected from the biome Cerrado (center Brazil) had initial seed moisture contents that varied from 5.41% (*Mimosa setosa*) to 25.17% (*Qualea parviflora*). After drying for 15 d in a drying oven at 20± 3°C and 12±3% RH, seeds were tested for tolerance to dehydration before immersion in LN and after drying; moisture contents varied from 3.46% (*M. setosa*) to 8.46% (*Q. parviflora*). Although all seeds studied by Wetzel *et al*. (2003) were tolerant to LN, in the cases of *Cassia ferruginea*, *Platypodium elegans*, *Sclerobium aureum*, and *Roupala montana*, germination rates after LN immersion were lower than controls. However, successful cryostorage was achieved for most of the dehydration tolerant seeds.

Astronium urundeuva is a valuable wood and medicinal tree. Medeiros and Cavallari (1992) exposed their orthodox seeds to drying at 25°C and 10–15% RH for 24, 48, 72, 96, and 120 h before immersion in LN; thawing was at room temperature for 30 min. Seeds survived cryogenic storage even without desiccation (8.01% moisture content), but best germination was optimized by Medeiros *et al*. (1992) after 24, 72, and 96 h drying, after which the seeds reached respective moisture contents of 7.13%, 5.89%, and 5.96%.

Salomão (2002) studied the LN tolerance of orthodox seeds from 66 tropical species native to Cerrado and the

Table 5. Cryopreservation of seeds from native Brazilian species

Family	Species	Initial moisture content (% FW)[z]	Storage period[z]	References
Anacardiaceae	*Astronium urundeuva*	8.01	15 d	Medeiros *et al.* (1992)
	Astronium fraxinifolium	6.3	3 d	Salomão (2002)
	Astronium fraxinifolium	5.9	3 d	Lima *et al.* (2008)
	Myracrodruon urundeuva	12.0	3 d	Lima *et al.* (2008)
	Schinopsis brasiliensis	6.7	3 d	Salomão (2002)
	Schinopsis brasiliensis	7.3	3 d	Lima *et al.* (2008)
	Spondias mombin	4.1	3 d	Salomão (2002)
Apocynaceae	*Aspidosperma discolor*	5.7	3 d	Salomão (2002)
	Aspidosperma parvifolium	7.2	3 d	Salomão (2002)
	Aspidosperma pyrifolium	5.4	3 d	Salomão (2002)
	Aspidosperma pyrifolium	6.8	3 d	Lima *et al.* (2008)
Bombacaceae	*Cavanillesia arborea*	8.5	3 d	Lima *et al.* (2008)
	Chorisia speciosa	7.46	7 d	Wetzel *et al.* (2003)
	Chorisia pubiflora	8.5	3 d	Salomão (2002)
	Eriotheca gracilipis	6.5	3 d	Salomão (2002)
	Pseudobombax cf. *tomentosum*	7.0	3 d	Salomão (2002)
Boraginaceae	*Cordia trichotoma*	7.3	3 d	Lima *et al.* (2008)
Bignoniaceae	*Anemopaegma arvense*	6.3	3 d	Salomão (2002)
	Jacaranda cuspidifolium	8.5	3 d	Salomão (2002)
	Jacaranda decurrens	5.2	3 d	Salomão (2002)
	Jacaranda brasiliana	5.7	3 d	Lima *et al.* (2008)
	Tabebuia umbellata	8.97	7 d	Wetzel *et al.* (2003)
	Tabebuia aurea	7.0	3 d	Salomão (2002)
	Tabebuia aurea	6.7	3 d	Lima *et al.* (2008)
	Tabebuia impetiginosa	5.8	3 d	Salomão (2002)
	Tabebuia impetiginosa	7.5	3 d	Lima *et al.* (2008)
	Tabebuia roseo-alba	5.8	3 d	Salomão (2002)
	Tabebuia serratifolia	5.4	3 d	Salomão (2002)
	Zeyheria montana	5.5	3 d	Salomão (2002)
Bromeliaceae	*Encholirium heloisae*	19.0	24 h	Tarré *et al.* (2007)
	Encholirium magalhaesii	14.6	24 h	Tarré *et al.* (2007)
	Encholirium pedicellatum	13.7	24 h	Tarré *et al.* (2007)
	Encholirium reflexum	11.2	24 h	Tarré *et al.* (2007)
	Encholirium subsecundum	12.7	24 h	Tarré *et al.* (2007)
	Encholirium scrutor	24.0	24 h	Tarré *et al.* (2007)
	Dyckia sordida	13.4	24 h	Tarré *et al.* (2007)
	Dyckia ursina	28.2	24 h	Tarré *et al.* (2007)
Combretaceae	*Buchenavia tomentosa*	8.1	3 d	Salomão (2002)
Dioscoreaceae	*Dioscorea* sp.	12.4	3 d	Salomão (2002)
Fabaceae	*Acacia farnesiana*	6.3	3 d	Salomão (2002)
	Acacia polyphylla	9.4	3 d	Lima *et al.* (2008)
	Albizia sp.	6.3	3 d	Salomão (2002)
	Albizia lebbeck	9.2	7 d	Wetzel *et al.* (2003)
	Amburana cearensis	5.3	3 d	Salomão (2002)
	Amburana cearensis	8.9	3 d	Lima *et al.* (2008)
	Anadenanthera colubrina	7.1	3 d	Salomão (2002)
	Anadenanthera colubrina	8.8	3 d	Lima *et al.* (2008)
	Anadenanthera macrocarpa	7.42	7 d	Wetzel *et al.* (2003)
	Anadenanthera macrocarpa	7.42	7 d	Wetzel *et al.* (2003)

Table 5. (continued)

Family	Species	Initial moisture content (% FW)[z]	Storage period[z]	References
	Apuleia leiocarpa	3.5	3 d	Salomão (2002)
	Bauhinia sp.	13.78	7 d	Wetzel *et al*. (2003)
	Bauhinia sp.	6.9	3 d	Salomão (2002)
	Bauhinia acuruana	5.7	3 d	Salomão (2002)
	Bauhinia ungulata	5.2	3 d	Salomão (2002)
	Bowdichia virgilioides	5.9	3 d	Salomão (2002)
	Caesalpinia echinata	9.7	180 d	Zanotti *et al*. (2007)
	Camaecrista desvauxii	7.7	3 d	Salomão (2002)
	Cassia ferruginea	10.96	7 d	Wetzel *et al*. (2003)
	Copaífera langsdorffii	5.9	3 d	Lima *et al*. (2008)
	Copaífera langsdorffii	5.9	3 d	Lima *et al*. (2008)
	Crotalaria cf. *spectabilis*	15.0	3 d	Salomão (2002)
	Cyclolobium cf. *blanchetianum*	6.8	3 d	Salomão (2002)
	Dalbergia miscolobium	8.6	3 d	Salomão (2002)
	Dialium divaricatum	8.4	3 d	Salomão (2002)
	Dimorphandra mollis	8.4	3 d	Salomão (2002)
	Enterolobium contortisiliquum	7.3	3 d	Lima *et al*. (2008)
	Enterolobium contortisiliquum	7.7	3 d	Salomão (2002)
	Enterolobium gummiferum	7.3	3 d	Salomão (2002)
	Hymenaea courbaril var. *stilbocarpa*	5.8	3 d	Lima *et al*. (2008)
	Hymenaea stigonocarpa	10.46	7 d	Wetzel *et al*. (2003)
	Lonchocarpus montanus	5.3	3 d	Lima *et al*. (2008)
	Machaerium aculeatum	4.2	3 d	Salomão (2002)
	Machaerium cf. *acutifolium*	6.4	3 d	Salomão (2002)
	Machaerium brasiliensis	4.9	3 d	Salomão (2002)
	Machaerium scleroxylon	8.5	3 d	Lima *et al*. (2008)
	Melanoxylum brauna	9.9	3 d	Salomão (2002)
	Mimosa setosa	5.41	7 d	Wetzel *et al*. (2003)
	Mimosa somnians var. *viscida*	12.2	3 d	Salomão (2002)
	Mimosa sp.	7.3	3 d	Salomão (2002)
	Ormosia fastigiata	3.0	3 d	Salomão (2002)
	Peltogyne confertiflora	10.1	3 d	Salomão (2002)
	Platypodium elegans	12.46	7 d	Wetzel *et al*. (2003)
	Platypodium elegans	8.4	3 d	Salomão (2002)
	Pterodon emarginatus	5.3	3 d	Salomão (2002)
	Senna sp.	9.3	3 d	Salomão (2002)
	Senna alata	7.2	3 d	Salomão (2002)
	Sclerolobium aureum	9.3	7 d	Wetzel *et al*. (2003)
	Sclerolobium paniculatum	6.9	3 d	Salomão (2002)
	Stryphnodendron polyphyllum	5.1	3 d	Salomão (2002)
	Stryphnodendron pulcherrimum	13.0	3 d	Salomão (2002)
Guttiferae	*Kielmeyera coriacea*	8.7	3 d	Salomão (2002)
Lecythidaceae	*Cariniana estrellensis*	5.9	3 d	Salomão (2002)
	Cariniana legalis	7.2	3 d	Salomão (2002)
Lythraceae	*Lafoensia pacari*	9.2	3 d	Salomão (2002)
Malpighiaceae	*Byrsonima basiloba*	3.0	3 d	Salomão (2002)
Meliaceae	*Cedrela fissilis*	9.8	3 d	Salomão (2002)
	Cedrela fissilis	8.0	3 d	Lima *et al*. (2008)
Proteaceae	*Roupala montana*	12.82	7 d	Wetzel *et al*. (2003)

Table 5. (continued)

Family	Species	Initial moisture content (% FW)[z]	Storage period[z]	References
Rubiaceae	*Guettarda pohliana*	3.5	3 d	Salomão (2002)
	Tocoyena formosa	5.9	3 d	Salomão (2002)
Sapindaceae	*Magonia pubescens*	5.0	3 d	Salomão (2002)
Sterculiaceae	*Sterculia striata*	11.1	3 d	Salomão (2002)
	Sterculia striata	10.9	3 d	Lima *et al.* (2008)
Styracaceae	*Styrax camporum*	3.0	3 d	Salomão (2002)
Tiliaceae	*Apeiba tibourbou*	6.9	3 d	Salomão (2002)
	Luehea sp.	7.5	3 d	Salomão (2002)
Verbenaceae	*Aegiphila lhotzkiana*	8.21	7 d	Wetzel *et al.* (2003)
Vochysiaceae	*Qualea parviflora*	25.17	7 d	Wetzel *et al.* (2003)

[z] Medeiros *et al.* (1992), Salomão (2002), Wetzel *et al.* (2003), Lima *et al.* (2008), and Tarré *et al.* (2007)

Atlantic Forest and demonstrated cryopreservation as an alternative to conservation at −20°C, on the basis of enhancing longevity (Stanwood and Bass 1981). Salomão (2002) did not dehydrate seeds prior to their immersion in LN, in which they were stored for 3 d; thawing was carried out at room temperature (25°C) for 3 h. Hard coat seeds were scarified after LN exposure when failure to germinate in 30 d was observed; seed moisture contents varied from 3.0% to 15.0% on a fresh weight basis. All species tested were cryostorage tolerant; however, sensitivity to LN varied among species, and for some, seed viability was reduced as follows: *Dimorphandra mollis* and *Bauhinia acuruana* (from 95% to 65%), *Bauhinia ungulata* (from 98% to 82%), *Dalbergia miscolobium* (from 100% to 53%), *Sterculia striata* (from 100% to 85%), and *Styrax camporum* (from 90% to 36%). Salomão (2002) highlighted the need to optimize protocols for cryogenic storage for these species. In others, germination was apparently increased: *Schinopsis brasiliensis* (from 45% to 86%), *Spondias mombin* (from 11% to 53%), *Bowdichia virgilioides* (from 20% to 85%), *Pterodon emarginatus* (from 50% to 80%), *Mimosa somnians* var. *viscida* (from 65% to 90%), *Stryphnodendron polyphyllum* (from 50% to 90%), *Apeiba tibourbou* (from 22% to 54%), *Crotalaria* cf. *spectabilis* (from 73% to 94%), and *Cariniana estrellensis* (from 4% to 52%) after LN exposure. This interesting effect could be due to the LN and freeze/thaw cycles acting as a scarification treatment resulting in enhanced permeability and/or changes in seed physiology after cryogenic storage which result in an enhanced level of germination compared to controls.

Tarré *et al.* (2007) developed an efficient protocol for the cryostorage of eight Bromeliaceae species (six from the genus *Encholirium* and two from the genus *Dyckia*) native to the Atlantic Forest. Seed moisture contents varied from 11.2% to 28.2%; their protocol did not include seed dehydration prior to the immersion in liquid nitrogen, except for *Encholirium pedicellatum* (seeds were desiccated to 2.5% moisture content). Thawing was undertaken at

Table 6. Cryostorage of seeds from Brazilian native species

Family	Species	Uses	Moisture content (% FW)	Storage period (mo)	Germination (%)[z]	
					Control	LN
Cecropiaceae	*Cecropia glazioui*	Medicinal	10.3	4	33 a	42 a
Bignoniaceae	*Tabebuia avellanedae*	Medicinal	9.3	26	62 a	54 a
	Tabebuia heptaphylla	Ornamental	7.5	26	42 a	46 a
	Tabebuia impetiginosa	Ornamental	7.6	8	69 a	53 a
	Tabebuia pentaphylla	Ornamental	7.1	15	70 a	73 a
	Tabebuia roseo-alba	Ornamental	8.5	10	78 a	75 a
	Cybistax antisyphilitica	Medicinal	6.6	7	63 a	20 b
Meliaceae	*Cedrela fissilis*	Wood	10.0	15	85 a	84 a

[z] Means of five replicates (of 25 seeds each) in a line followed by different *letters* are significantly different at the 5% probability level according to the Student *t* test

room temperature and the survival rates observed were similar to controls. This protocol is highly relevant as six *Dyckia* species are in the red list of the Brazilian species in danger of extinction.

Lima *et al*. (2008) studied tolerance to LN of 19 species belonging to 11 genus of the deciduous forest of Paraná river valley in Goiás (center Brazil). Their protocol did not include prior dehydration. The seeds were immersed directly in LN and stored for 3 d, after which they were thawed at room temperature (25°C). Seed moisture content varied from 5.7% (*Jacaranda brasiliana*) to 12% (*M. urundeuva*). All species were tolerant to cryopreservation, but viability was reduced in *H. courbaril* (5.8% moisture content) and *Aspidosperma pyrifolium* (6.8% moisture content).

Studies of extended seed storage in LN. For the purpose of testing the cryogenic parameters required to achieve some level of survival, most research carried out on seed cryostorage in Brazil concerns short-term exposure (3 to 7 d) to LN (Table 5). This section reports outcomes for seeds maintained for longer periods in LN (see Table 6). *C. echinata*, a native of the Atlantic rainforest, was the first Brazilian tree to be exploited commercially; it is now on the verge of extinction. Its seeds are orthodox (9.7% moisture content), but they lose viability after 3 mo in their natural environment. Barbedo *et al*. (2002) showed that seeds tolerate desiccation to 7.6% moisture content and can be stored for 18 mo at 7°C, maintaining 80% viability. Hellmann *et al*. (2006) inform that seeds of *C. echinata* could be stored at −18°C for 24 mo, producing 80% normal seedlings; those stored at 7°C produced only 20% of normal seedlings. Zanotti *et al*. (2007) reported that seeds under cryostorage for 6 mo were viable and able to produce normal seedlings. Cryopreservation can thus expand storage time to ensure availability of seedlings for regeneration of natural populations.

Additional studies on seed cryostorage from 4 to 26 mo were carried out (Table 6) for *Cecropia glazioui* (Cecropiaceae); for the Bignoniaceae *Cybistax antisyphilitica*, *Tabebuia impetiginosa*, *Tabebuia pentaphylla*, *Tabebuia roseo-alba*, *Tabebuia avellanedae*, and *Tabebuia heptaphylla*; and for *C. fissilis* (Meliaceae). Initial seed moisture contents varied from 6.6% to 10.3%; seeds were not dehydrated before storage in LN, and thawing was achieved at room temperature (25°C) for 30 min. No significant differences in seed viability were detected between the treatments for most species except for *C. antisyphilitica* (Table 6). As its seeds tolerated 1 h immersion in LN without loss of viability, no significant differences were observed between the control seeds (46%) and those exposed to LN (44%), and a slight decrease in the germination did not indicate intolerance to cryostorage.

Studies performed on orthodox seed cryostorage will be used to progress the development of improved protocols for the more difficult to preserve intermediate and recalcitrant seed germplasm of Brazilian native species.

Conclusions

This review highlights the different strategies currently applied to conserve Brazil's native flora. The value of experience gained from the tissue culture and seed cryopreservation of economically important native and non-native species is also considered in the Brazilian context. However, there is now an urgent need to expand conservation research to a greater number of native species. Although studies on *in vitro* germination, zygotic embryo excision, micropropagation, and somatic embryogenesis of endangered species have increased considerably, knowledge gaps still remain. The development of *in vitro* conservation and cryopreservation methods for Brazil's endemic species producing recalcitrant seeds must be a research priority. Future studies requiring immediate attention concern the development of reproducible *ex situ* conservation protocols for the medium and long term (cryostorage) of critically endangered plants. In particular, it will be important to target those at high risk of over-exploitation through non-sustainable practices, climate change, deforestation, and habitat loss.

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References

Almeida V. P.; Shepherd S. L. K. *Sinningia allagophylla* (Gesneriaceae): *in vitro* cultivation of a native plant of the Brazilian cerrado. *Rev Bras Bot* 22: 381–384; 1999. doi:10.1590/s0100-84041999000300005.

Alves G. M.; Dal Vesco L. L.; Guerra M. P. Micropropagation of the Brazilian endemic bromeliad *Vriesea reitzii* through nodule clusters culture. *Sci Hortic* 110: 204–207; 2006.

Andrade M. W.; Luz J. M. Q.; Lacerda A. S.; Melo P. R. Micropropagação da aroeira (*Myracrodruon urundeuva* Fr. All). *Ciênc Agrotec* 24(1): 174–180; 2000.

Arrabal R.; Amancio F.; Carneiro L. A.; Neves L. J.; Mansur E. Micropropagation of endangered endemic Brazilian bromeliad *Cryptanthus sinuosus* (L.B. Smith) for *in vitro* preservation. *Biodivers and Conserv J* 11: 1081–1089; 2002.

Astarita L. V.; Guerra M. P. Conditioning of the culture medium by suspension cells and formation of somatic proembryos in *Araucaria angustifolia* (Coniferae). *In Vitro Cell Dev Biol Plant* 36: 194–200; 2000.

Barbedo C. J.; Bilia D. A. C. Evolution of research on recalcitrant seeds. *Sci Agric* 55: 121–125; 1998. doi:10.1590/s0103-90161998000500022.

Barbedo C. J.; Bilia D. A. C.; Figueiredo-Ribeiro R. C. Tolerância à dessecação e armazenamento de sementes de *Caesalpinia echinata* Lam. (pau-brasil), espécie da Mata Atlântica. *Rev Bras Bot* 25(4): 431–439; 2002.

Bilia D. A. C.; Filho M. J.; Novembre A. D. C. L. Desiccation tolerance and seed storability of *Inga uruguensis* (Hook. et Arn.). *Seed Sci Technol* 27(1): 77–89; 1999.

Biodiversitas Foundation. http://www.biodiversitas.org.br. Cited 8 January 2010; 2010.

Biondo R.; Souza A. V.; Bertoni B. W.; Soares A. M.; França S. C.; Pereira A. M. S. Micropropagation, seed propagation and germplasm bank of *Mandevilla velutina* (Mart.) Woodson. *Sci Agric* 64(3): 263–268; 2007.

Bonjovani M. R.; Barbedo C. J. Recalcitrant seeds: intolerant to low temperatures? Recalcitrant embryos of *Inga vera* Willd. Subsp. affinis (DC.) R. D. Penn. tolerant to subzero temperature. *Rev Bras Bot* 31: 345–356; 2008. doi:10.1590/s0100-84042008000200017.

Camillo J.; Luis Z. G.; Scherwinski-Pereira J. E. Tolerância de sementes de dendezeiro à criopreservação. *Pesq Agropec Bras* 44: 211–215; 2009a. doi:10.1590-204x2009000200015.

Camillo J.; Scherwinski-Pereira J. E.; Vieira R. F.; Peixoto J. R. Conservação *in vitro* de *Cochlospermum regium* (Schrank) Pilg.—Cochlospermaceae sob regime de crescimento mínimo. *Rev Bras PL Med* 11(2): 184–189; 2009b.

Carvalho J. E. U.; Muller C. H. Níveis de tolerância e letal de umidade em sementes de pupunheira *Bactris gasipaes*. *Rev Bras Frutic* 20 (3): 238–283; 1998.

Carvalho J. E. U.; Nascimento N. V. M.; Leão N. V. M. Sensibilidade de sementes de jenipapo (*Genipa americana* L.) ao dessecamento e ao congelamento. *Informativo Abrates* 5 (2): 170; 1995.

Carvalho L. R.; Davide A. C.; Silva E. A. A.; Carvalho M. L. M. Classificação de sementes de espécies florestais dos gêneros Nectandra e Ocotea (Lauraceae) quanto ao comportamento no armazenamento. *Rev Bras Sementes* 30: 1–9; 2008. doi:10.1590/s0101-31222008000100001.

Carvalho L. R.; Silva E. A. A.; Davide A. C. Classificação de sementes florestais quanto ao comportamento no armazenamento. *Rev Bras Sementes* 28: 15–25; 2006. doi:10.1590/s0101-31222006000200003.

Centro Nacional Recursos Genéticos, Embrapa, Brazil. http://www.cenargen.embrapa.br. Cited 8 Jan 2010; 2010.

Coelho M. C. F.; Pinto J. E. B. P.; Morais A. R.; Cid L. P. B.; Lameira O. A. Germinação de sementes de sucupira-branca (*Pterodon pubescens* (Benth.) Benth.) i*n vitro* and *ex vitro*. *Ciênc Agrotec* 25(1): 38–48; 2001.

Couto J. M. F. Germination and morphogenesis *in vitro* of mahogany (*Swietenia macrophylla* King). Dissertação de Mestrado. Universidade Federal de Viçosa, Lavras, MG, Brasil; 2002.

Cruz E. D. Drying and germination of cupuassu (*Theobroma grandiflorum* (Willd ex. Spreng.) K. Schum.) seeds. *Rev Bras Sementes* 29(3): 197–201; 2007.

Cunha R.; Santanna C. M. A.; CAF P. T. Efeito do dessecamento sobre a viabilidade de sementes de *Virola surinamensis* (Rol) Warb. *Rev Bras Sementes* 14: 69–72; 1992.

Delgado L. F.; Barbedo C. J. Desiccation tolerance of seeds of species of *Eugenia*. *Pesq Agropec Bras* 42: 265–272; 2007. doi:10.1590/s0100-204x2007000200016.

Dias M. M.; Nietsche S.; Pereira M. C. T.; Matrangolo C. A. R. Emergência e desenvolvimento da cactácea rabo-de-raposa (*Arrojadoa* spp) em diferentes meios de cultura e recipientes. *Rev Ceres* 55(2): 117–123; 2008.

Dussert S.; Chabrillange N.; Engelmann F.; Anthony F.; Hamon S. Cryopreservation of coffee (*Coffea arabica* L.) seeds: importance of precooling temperature. *Cryo Lett* 18: 269–276; 1997.

Dussert S.; Chabrillange N.; Engelmann F.; Anthony F.; Louarn J.; Hamon S. Cryopreservation of seeds of four coffee species (*Coffea arabica*, *C. costatifructa*, *C. racemosa* and *C. sessiliflora*): importance of water content and cooling rate. *Seed Sci Res* 8: 9–15; 1998.

Eira M. T.; Reis R. B.; Ribeiro F. N. S.; Ribeiro V. S. Banco de sementes de café em criopreservação: experiência inédita no Brasil. *Circular Técnica Embrapa* 42: 1–6; 2005.

Eira M. T. S.; Walters C.; Caldas L. S. Water sorption isotherms in *Coffea* spp. seeds and embryos. *Seed Sci Res* 9: 321–330; 1999b.

Eira M. T. S.; Walters C.; Caldas L. S.; Fazuoli L. C.; Sampaio J. B.; Dias M. C. L. L. Tolerance of *Coffea* spp. seeds to desiccation and low temperature. *Rev Bras Fisiol Veg* 11: 97–105; 1999a.

Ellis R. H.; Hong T. D.; Roberts E. H.; Soetisna U. Seed storage behaviour in *Elaeis guineensis*. *Seed Sci Res* 1: 99–104; 1991.

Faria J. M. R.; Van Lammeren A. A. M.; Hilhorst H. W. M. Desiccation sensitivity and cell cycle aspects in seeds of *Inga vera* subsp. affinis. *Seed Sci Res* 14: 165–178; 2004.

Fleck J. D.; Schwambach J.; Almeida M. E.; Yendo A. C. A.; Costa F.; Gosmann G.; Fett-Neto A. G. Immunoadjuvant saponin production in seedlings and micropropagated plants of *Quillaja brasiliensis*. *In Vitro Cell Dev Biol - Plant* 45: 715–720; 2009.

Fidelis I.; Castro E. M. C.; Pinto J. E. B. P.; Gavilanes M. L.; Santiago E. J. A. Características anatômicas de estruturas vegetativas de *Brosimum gaudichaudii* Tréc. desenvolvidas *in vitro* e *in vivo*. *Ciênc Agrotec* 24(2): 327–336; 2000.

Gentil D. F. O.; Ferreira S. A. Viabilidade e superação da dormência em sementes de araçá-boi (*Eugenia stipitata* ssp. Sororia). *Acta Amazôn* 29(1): 21–31; 1999.

Gomes M. L. O. Germinação *in vitro* de *Parkinsonia aculeata* L.: uma espécie de uso múltiplo ocorrente nas matas ciliares da caatinga. Dissertação de Mestrado. Universidade Federal de Pernambuco, Recife, PE, Brasil; 2007.

Grigoletto E. R. Micropropagação de *Hancornia speciosa* Gómez (Mangabeira). Dissertação de Mestrado. Instituto de Ciências Biológicas da Universidade Estadual de Brasília, Brasília, DF, Brasil; 1997.

Handa L.; Sampaio P. T. B.; Quisen R. C. Cultura *in vitro* de embriões e de gemas de mudas de pau-rosa (*Aniba rosaeodora* Ducke). *Acta Amazôn* 35(1): 29–33; 2005.

Hellmann M. E.; Mello J. I. O.; Figueiredo-Ribeiro R. C. L.; Barbedo C. J. Freezing tolerance in seeds of *Caesalpinia echinata* Lam. (brazilwood) as influenced by the initial water content. *Rev Bras Bot* 29: 93–101; 2006. doi:10.1590/s0100-84042006000100009.

Hong T. D.; Ellis R. H. A protocol to determine seed storage behaviour. *Int Plant Genet Resour Inst Tech Bull* 1: 1–55; 1996.

Hu C. Y. *In vitro* culture of rudimentary embryos of eleven *Ilex species*. *J Amer Soc Hort Sci* 100(3): 221–225; 1975.

Instituto Brasileiro de Geografia e Estatística. http://www.ibge.com.br. Cited 5 Jan 2010; 2010.

Instituto Brasileiro do Meio Ambiente. http://www.ibama.gov.br/recursos-florestais/documentos/lista-oficial-de-especies-brasileiras-ameacadas-de-extincao/. Cited 19 Feb 2010; http://www.ibama.gov.br. Cited 8 Jan 2010; 2010.

José A. C.; Silva E. A.; Davide A. C. Classificação fisiológica de sementes de espécies florestais. *Rev Bras Sementes* 29(2): 171–178; 2007.

Kohoma S.; Maluf A. M.; Bilia D. A. C.; Barbedo C. J. Drying and storage of *Eugenia brasiliensis* Lam. ("Grumixameira") seeds. *Rev Bras Sementes* 28: 72–78; 2006. doi:10.1590/s0101-31222006000100010.

Landa F. S. L.; Paiva R.; Paiva P. D. O.; Filho J. S. S. B. Indução *in vitro* de calos em explantes foliares de pequizeiro (*Caryocar brasiliense* Camb.). *Ciênc Agrotec* 24: 56–63; 2000.

Lemos E. E. P.; Ferreira M. S.; Alencar L. M. C.; Neto C. E. R.; Albuquerque M. M. Conservação *in vitro* de germoplasma de cana-de-açúcar. *Pesq Agropec Bras* 37(10): 1359–1364; 2002.

Lima V. V. F.; Vieira D. L. M.; Sevilha A. C.; Salomão A. N. Germinação de espécies arbóreas de floresta estacional decidual do vale do rio Paraná em Goiás após três tipos de armazenamento por até 15 meses. *Biota Neotrop* 8: 89–97; 2008. doi:10.1590/s1676-06032008000300008.

Malosso L. G. Micropropagação de *Acmella oleraceae* (l.) R. K. Jansen e estabelecimento de meio de cultura para a conservação desta espécie em Banco de Germoplasma *in vitro*. Tese de Doutorado, Universidade Federal do Amazonas, Coarim, AM, Brasil; 2007.

Maluf A. M.; Pisciottano-Ereio W. A. Desiccation and storage of cambuci seeds. *Pesq Agropec Bras* 40(7): 707–714; 2005.

Martins C. C.; Nakagawa J.; Bovi M. L. Tolerância à dessecação de sementes de palmito-vermelho (*Euterpe espiritosantensis* Fernandes). *Rev Bras Bot* 22: 391–396; 1999. doi:10.1590/s0100-84041999000300007.

Martins C. C.; Nakagawa J.; Bovi M. L. A. Desiccation tolerance of four seed lots from *Euterpe edulis* Mart. *Seed Sci Technol* 28(1): 101–113; 2000.

Martins J. P. R.; Batista T. R.; Rodrigues L. C.; Nogueira D. A.; Paiva R., Barbosa S., Santos B.R. Multiplicação *in vitro* de explantes de ipê-roxo (*Tabebuia impetiginosa* (Mart.) Standl.). Anais do IX Congresso de Ecologia do Brasil, São Lourenço, MG, Brasil; 2009.

Martinotto C.; Paiva R.; Santos B. R.; Soares F. P.; Nogueira R. C.; Silva A. A. N. Efeito da escarificação e luminosidade na germinação *in vitro* de sementes de cagaiteira (*Eugenia dysenterica*). *Ciênc Agrotec* 31: 1668–1671; 2007. doi:10.1590/s1413-70542007000600010.

Medeiros A. C.; Czarneski C. M.; Freitas G. F. Criopreservação de sementes de aroeira (*Astronium urundeuva* (Fr. All.) Engl.). *Rev Inst Florest* 4: 544–547; 1992.

Medeiros A. C. S.; Cavallari D. A. N. Conservação de germoplasma de Aroeira (*Astronium urundeuva* (Fr. All.) Engl.) I. Germinação de sementes após imersão em nitrogênio líquido (−196°C). *Rev Bras Sementes* 14(1): 73–75; 1992.

Ministério do Meio Ambiente, Brazil. http://www.mma.gov.br/sitio/. Cited 19 Feb 2010; 2010.

MMA/Ibama. Desmatamento. *Informativo Técnico* 1: 1–93; 2003.

Moura E. F.; Ventrella M. C.; Motoike S. Y.; Júnior A. Q. S.; Carvalho M.; Manfio C. E. Histological study of somatic embryogenesis induction on zygotic embryos of macaw palm (*Acrocomia aculeate* (Jacq.) Lodd. ex Martiuis). *Plant Cell Tissue Organ Cult* 95: 175–184; 2008.

Motoike S. Y.; Saraiva E. S.; MCo V.; Silva C. V.; Salomão L. C. C. Somatic embryogenesis of *Myrciaria aureana* (Brazilian grape tree). *Plant Cell Tissue Organ Cult* 89: 75–81; 2007.

Murashige T.; Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473–497; 1962.

Naidu M. M.; Sreenath H. L. *In vitro* culture of coffee zygotic embryos for germplasm conservation. *Plant Cell Tissue Organ Cult* 55: 227–230; 1999.

Nascimento A. C.; Paiva R.; Nogueira R. C.; Porto J. M. P.; Nogueira G. F.; Soares F. P. BAP e AIB no cultivo *in vitro* de *Eugenia pyriformis* Cambess. *Rev Acad Ciênc Agr Ambient* 6(2): 223–228; 2008.

Nepomuceno C. F.; Rios A. P. S.; Queiroz S. R. O. D.; Pelacani C. R.; Santana J. R. F. Respostas morfofisiológicas *in vitro* de plântulas de *Anadenanthera colubrina* (Vell.) Brenan var. cebil (Griseb) Altschul. *Rev Árvore* 33: 481–490; 2009. doi:10.1590/s0100-67622009000300010.

Nogueira R. C.; Paiva R.; Castro A. H.; Vieira C. V.; Abbade L. C.; Alvarenga A. A. Germinação *in vitro* de murici-pequeno (*Byrsonima intermedia* A. Juss.). *Ciênc Agrotec* 28(5): 1053–1059; 2004.

Nunes E. C.; Benson E. E.; Oltramari A. C.; Araujo P. S.; Moser J. R.; Viana A. M. *In vitro* conservation of *Cedrela fissilis* Vellozo (Meliaceae), a native tree of the Brazilian Atlantic Forest. *Biodivers and Conserv J* 12: 837–848; 2003.

Nunes E. C.; Castilho C. V.; Moreno F. N.; Viana A. M. *In vitro* culture of *Cedrela fissilis* Vellozo (Meliaceae). *Plant Cell Tissue Organ Cult* 70: 259–268; 2002.

Oliveira A. M. Avaliação de substâncias inibidoras no controle de fungos endofíticos em micropropagação *in vitro* de *Caryocar brasiliense* Camb. Dissertação de Mestrado. Universidade Federal de Goiás, Goiânia, GO, Brasil; 2000.

Oliveira F. F. M.; Silva K. M. B.; Oliveira G. F.; Dantas I. M.; Camacho R. G. V. Micropropagação de *Mimosa caesalpiniaefolia* Benth. A partir de segmentos nodais e ápices caulinares. *Rev Caatinga* 20(3): 152–159; 2007b.

Oliveira L. M. Q.; Valio I. F. M. Effects of moisture content on germination of seeds of *Hancornia speciosa* Gom. (Apocynaceae). *Ann Bot* 69: 1–5; 1992.

Oliveira M. K. T.; Neto F. B.; Câmara F. A. A.; Nunes G. H. S.; Oliveira F. A. Propagação *in vitro* da cultura do abacaxizeiro ornamental (*Ananas lucidus* Miller). *Rev Caatinga* 20(3): 167–171; 2007a.

Palazetti V. A.; Shepherd S. L. K. *Sinningia allagophylla* (Gesneriaceae): *in vitro* cultivation of a native plant of the Brazilian cerrado. *Rev Bras Bot* 22: 381–384; 1999. doi:10.1590/s0100-84041999000300005.

Pinheiro C. S. R.; Medeiros D. N.; Macedo C. E. C.; Alloufa M. A. I. Germinação *in vitro* de mangabeira (*Hancornia speciosa* Gomez) em diferentes meios de cultura. *Rev Bras Frutic* 23(2): 413–416; 2001.

Pinto J. E. B. P.; Arello E. F.; Pinto C. A. B. P.; Barbosa M. H. P. Uso de diferentes explantes e concentrações de benzilaminopurina na multiplicação *in vitro* de brotos de *Kielmeyera coriacea*. *Pesq Agropec Bras* 29(6): 867–873; 1994.

Pupim T. L.; Nobembre A. D. L.; Brancalion P. H. S.; Moraes M. H. D.; Mondo V. H. V.; Labonia V. D. S. Conservação de sementes de *Magnolia ovata* St. *Hil Rev Bras Sementes* 31: 96–105; 2009. doi:10.1590/s0101-31222009000300011.

Quadros K. M. Propagação vegetativa de erva-mate (*Ilex paraguariensis* Saint Hilaire). Dissertação de Mestrado. Universidade Federal de Santa Maria, Santa Maria, RS; 2009.

Ratter J. A.; Ribeiro J. F.; Bridgewater S. The Brazilian cerrado vegetation and threats to its biodiversity. *Ann Bot* 80: 22–230; 1997.

Reis N. R. S.; Lameira O. A.; Cordeiro I. M. C. C. Indução de calogênese em Paricá (*Schizolobium parahyba* var. amazonicum (Huber ex Ducke) Barneby) através da adição de AIB e BAP. *Rev Bras Biociências* 5(2): 501–503; 2007.

Ribas L. L. F.; Zanette F.; Kulchetscki L.; Guerra M. P. Micropropagation of *Aspidosperma polyneuron* from single node culture of juvenile material. *Rev. Árvore.* 29(4): 517–524; 2005.

Rodrigues W. A. Revisão taxonômica das espécies de Virola Aublet. (Myristicaceae) do Brasil. *Acta Amazonica* 15(1):1–127; 1980.

Saldanha C. W. Conservação *in vitro* de *Euterpe edulis* Martius através da embriogênese somática. Dissertação de Mestrado. Universidade Federal de Santa Maria, Santa Maria, RS, Brasil; 2007.

Santa-Catarina C.; Olmedo A. S.; Meyer G. A.; Macedo J.; Amorim W.; Viana A. M. Repetitive somatic embryogenesis of *Ocotea catharinensis* Mez. (Lauraceae): effect of somatic embryo developmental stage and dehydration. *Plant Cell Tissue Organ Cult* 78: 55–62; 2004.

Santos I. R. I.; Salomão A. N.; Mundim R. C.; Ribeiro F. N. S. Criopreservação de eixos embrionários zigóticos de café (*Coffea arabica* L.). *Comun Téc Embrapa* 69: 1–5; 2002.

Salomão A. N. Respostas de sementes de espécies tropicais à exposição ao nitrogênio líquido. *Braz J Plant Physiol* 14(2): 133–138; 2002. doi:10.1590/s1677-04202002000200008.

Silva A. N.; Coelho M. F. B.; Guimarães S. C.; Albuquerque M. C. F. Brazil nut seed germination stored in moist sand. *Pesq Agropec Bras* 44: 1431–1436; 2009. doi:10.1590/s0100-204x2009001100009.

Soares F. P.; Paiva R.; Alavarenga A. A.; Nogueira R. C.; Emrich E. B.; Martinotto C. Organogênese direta em explantes caulinares de mangabeira (*Hancornia speciosa* Gomes). Direct organogenesis in nodal explants of mangabeira (*Hancornia speciosa* Gomes). *Ciênc Agrotec* 31: 1048–1053; 2007. doi:10.1590/s1413-70542007000400016.

Souza A. V.; Pinto J. E. B. P.; Bertolucci S. K. V.; Corrêa R. M.; Castro E. M. Germinação de embriões e multiplicação *in vitro* de *Lychnophora pinaster* Mart. *Ciênc Agrotec (Edição Especial)* 27: 1532–1538; 2003.

Stanwood P. C.; Bass L. N. Seed germplasm preservation using liquid nitrogen. *Seed Sci Technol* 9: 423–437; 1981.

Stanwood P. C.; Ross E. E. Seed storage of several horticultural species in liquid nitrogen (−196°C). *HortScience* 14: 530–628; 1979.

Tarré E.; Pires B. B. M.; Guimarães A. P. M.; Carneiro L. A.; Forzza R. C.; Mansur E. Germinabilidade de sementes de espécies endêmicas de *Encholirium* Mart. ex Schult. & Schult. f. and *Dyckia* Schult. f. species (Bromeliaceae). *Acta Botânica Brasílica* 21: 777–783; 2007. doi:10.1590/s0102-33062007000400003.

Vieira R. F. Conservation of medicinal and aromatic plants in Brazil. In: Janick J. (ed) Perspectives on new crops and new uses. ASHS, Alexandria, pp 152–159; 1999.

Velloso M. A. L.; Abreu I. N.; Mazzafera P. Indução de metabólitos secundários em plântulas de *Hypericum brasiliense* Choisy crescendo *in vitro*. *Acta Amazônica* 39(2): 267–272; 2009.

Wetzel M. M. V. S.; Reis R. B.; Ramos K. M. Metodologia para criopreservação de sementes de espécies florestais nativas. *Circular Técnica Embrapa* 26: 1–5; 2003.

Wielewick A. P.; Leonhardt C.; Schlindwein G.; Medeiros A. C. S. Proposta de padrões de germinação e teor de água para sementes de algumas espécies florestais presentes na região sul do Brasil. *Rev Bras Sementes* 28: 191–197; 2006. doi:10.1590/s0101-31222006000300027.

Zanotti R. F.; Motta L. B.; Silva A. I. S.; Leite I. T. A.; Cuzzuol G. R. Crescimento inicial de plântulas jovens de *Caesalpinia echinata* Lam. (Pau-Brasil) provenientes de bancos de germoplasma. Anais do VIII Congresso de Ecologia do Brasil, Caxambu, MG; 2007.