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PERSPECTIVE

CHALLENGES AND RECENT PROGRESS ON THE USE OF CRYOBIOTECHNOLOGY FOR CONSERVING BRAZILIAN NATIVE PLANTS

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Abstract

Brazil is a megadiverse country with continental dimensions. It is long acknowledged as the richest country in plant diversity, encompassing approximately 20% of the world's flora, with more than 50,000 species of plants, algae and fungi distributed in six major biomes, including two biodiversity hotspots. However, significant environmental challenges, primarily driven by climate changes and intensive, non-sustainable land use practices, have led to widespread deforestation, habitat reduction and, consequently, shifts in species distribution, genetic erosion and increased vulnerability. Considering the high rates of endemism and the global economic value of numerous Brazilian native species as crops and wild relatives, ornamentals and medicinal plants, cryopreservation emerges as a fundamental ex situ complementary strategy to safeguard its plant genetic resources. This article aims to provide a comprehensive overview of cryopreservation of native plants in Brazil during the past decade, which shows that more than 85 species from 23 families have been cryopreserved. Methods for assessing cryoinjury at the morphophysiological, biochemical, molecular and metabolic levels are reviewed. The main challenges, as well as future perspectives for the cryopreservation of Brazilian floristic diversity, are also discussed.

Keywords: climate crisis; cryopreservation; deforestation; endemic species; in vitro conservation.

INTRODUCTION

Brazil is a megadiverse country of continental dimensions, with distinct climatic and geographical characteristics. It is long acknowledged as the richest country in plant diversity, encompassing approximately 20% of the world's flora, with more than 50,000 species of plants, algae and fungi, with a high level of endemism (1). This diversity is distributed over six major biomes: Amazon, Caatinga, Pantanal, Pampa (Subtropical Forest), Atlantic Forest, and Cerrado (Brazilian Savanna). Cerrado and the Atlantic Forest are two biodiversity hotspots (2).

Because of its great diversity, Brazilian flora plays a key role in maintaining the planet´s ecological balance, providing habitat and food for a vast array of wildlife. Despite their importance, Brazilian plant genetic resources are threatened by significant loss and fragmentation of native forests and other ecosystems, predominantly caused by intense non-sustainable land use practices for monocultures and livestock farming, along with illegal activities such as mining and trafficking of drugs and firearms. This critical scenario has been exacerbated by the environmental challenges driven by climate changes, which are leading to natural disasters, widespread deforestation, habitat reduction and, consequently, shifts in species distribution, genetic erosion, and increased vulnerability (3).

Until the 2000s, there were no accurate inventories of the plant, algal and fungal species in Brazil, and no updated information on their geographical distribution. In the last two decades, however, a massive association of Brazilian taxonomists and researchers from other countries led to a partnership of more than 200 institutions that resulted in the first online Brazilian Flora 2020. This initiative meets Target 1 of the Global Strategy for Plant Conservation (GSPC), which calls for a catalog of plant diversity, enhancing the freely available data and providing valuable information for the conservation, management, and sustainable use of Brazilian flora diversity (1).

Since then, significant progress has been made in the definition of priority areas for conservation and the creation of protected areas or conservation units (3). Given the heightened risks of species extinction and biodiversity loss from climate disturbances and human activities, ex situ conservation of whole plants, seeds, pollen, tissues and other propagules is both urgent and essential. Beyond providing protection, ex situ conservation raises awareness about the importance of biodiversity and serves as a reliable source of materials for reintroduction programs.

Current efforts that involve political and economic investments resulted in the establishment of national and international ex situ genebanks, mainly to encompass collections of crop diversity with orthodox seeds. As these collections remain exposed to natural disasters, human actions and technical problems, they are commonly duplicated in more than one bank. High-risk situations of genebanks due to wars or environmental calamities have proven how real this vulnerability is (4, 5). The Svalbard Global Seed Vault, created in 2008 in Norway, provided an additional level of security for crop diversity collections, maintaining over a million samples of orthodox seeds from almost 100 different countries stored at -18°C, represents one of the most significant protection initiatives of the 21st Century. Additional efforts have emerged to establish seed banks for wild species, such as the Millennium Seed Bank (UK) and the Australian Seed Bank Partnership (Australia), intending to achieve the GSPC's goal of securing 75% of threatened species in ex situ collections (6).

In Brazil, it is estimated that there are over 250 ex situ germplasm banks, distributed in more than 30 institutes, which accommodate approximately 370,000 accessions (7). These collections are primarily managed by the Brazilian Corporation for Agricultural Research (Embrapa), which holds the responsibility for the national base collections and focuses on the conservation of native species related to important crops, forest, medicinal and ornamental plants (8). Other significant ex situ collections are kept in the Agronomic Institute of Campinas (IAC), which manages active germplasm banks of various species related to Brazilian crops and plays a key role in research on accession characterization, evaluation and conservation (9).

However, genetic resources of several important crops and tropical species that produce recalcitrant, desiccation sensitive seeds or are short-lived in storage, together with those propagated vegetatively, cannot be maintained in seed banks. In vitro conservation techniques have been recognized as a complementary tool for these species, as well as for accessions from rare collections or those that are short-lived in conventional seed banks (10). These techniques are also currently acknowledged as important means for the conservation of wild, crop, ornamental and medicinal species, along with large-scale propagation, reintroduction of threatened species, and conserving virus-free miniaturized explants in an aseptic environment (11).

Cryopreservation, i.e. storage of biological samples in liquid nitrogen (LN, -196°C) or LN vapor (LNV, approx. -165 to -195 °C), is the only method available for the safe and cost-efficient long-term conservation of genetic material, since all cellular and metabolic processes are interrupted in these conditions. Moreover, plant materials can be stored in small volumes and protected from contamination, requiring very limited maintenance (12). Therefore, cryopreservation emerges as a fundamental complementary ex situ strategy, and several actions have already been developed for many species, including those with recalcitrant, dehydration-sensitive seeds, vegetatively propagated plants, rare and endangered species, in addition to biotechnological products such as clones and cell lines derived from elite genotypes and genetically transformed material (13).

A recent survey showed that many institutions are involved with plant cryopreservation in Brazil, developing protocols for the long-term preservation of species with distinct ecological and economic value. However, only 9% of the plant germplasm stored in ex situ collections is maintained in cryobanks, primarily located in research institutes and universities (14). The main Brazilian cryobank is located at Embrapa Genetic Resources and Biotechnology Research Center (Brasilia, DF), which stores a large number of accessions representative of the country's agrobiodiversity. This germplasm has been collected from fields, markets, small farms and other locations and maintained as seeds, cuttings, or sprouts. Although most of these accessions are from species of economic interest, efforts have been made to conserve plant materials from native species that are not preserved in seed banks and field collections.

The cryopreservation status of Brazilian native plants has been reviewed previously (15, 16). Although these works focused mainly on seed cryopreservation, a few reports on cryopreservation of clonal materials were also mentioned. In this review, we summarize the different approaches and advances in the cryobiotechnology of Brazilian native plants over the last 10 years. Our goal is to outline the state-of-the-art of applying cryopreservation to Brazilian endemic and endangered species, as well as to woody and forest species and horticultural plants used for food, ornamental, and medicinal purposes.

ENDEMIC AND ENDANGERED SPECIES

Among the Brazilian native plants, endemic species are the most threatened by climate change, deforestation, over-exploitation and habitat fragmentation, because of their specific environmental requirements and restricted distribution. Moreover, some of them occur as small populations and have reduced reproductive capacity, which enhances their vulnerability. Brazil harbors more than 25,000 endemic species, which represent over half of its flora diversity. The Atlantic Forest is the biome with the highest number of endemic species (36.5%), followed by the Amazon (27.8%) and Cerrado (27.3%) (1).

Recent initiatives have evaluated the conservation status of Brazilian plants and, so far, approximately 43% of the assessed species have been classified as threatened with extinction. According to the updated Official National List of Threatened Species of Flora (17) and the International Union for Conservation of Nature (IUCN) list of threatened species, there are more than 3,000 endangered plant species in Brazil, assigned to different threat categories, namely Near Threatened (NT), Vulnerable (VU), Endangered (EN), and Critically Endangered (CR). Hence, conservation efforts for these unique species, many of which have yet to be studied, should be the highest priority. In the last decade, numerous reports on cryopreservation of Brazilian native plants concerned rare and endangered species, most of them focusing on species from the Bromeliaceae, Cactaceae, Orchidaceae and Arecaceae families (Table 1), which include the largest number of threatened plants in the country.

Most of these studies focused on the cryopreservation of seeds and zygotic embryos, due to their high tolerance to drying and storage at low temperature. In the Bromeliaceae family, this behavior was associated with the presence of carbohydrates that can be altered during adaptation to dehydration and low temperature, providing protection to cell membranes and allowing high post-freezing recovery after direct immersion in LN (18).

Seeds of two endangered species of the Cactaceae family, *Melocactus violaceus* and *M. sergipensis*, were successfully cryopreserved by direct immersion in LN. Post-freezing germinability was higher in comparison to seeds stored at room temperature, with no deterioration, physiological damage, dormancy or morphological abnormalities in the seedlings (19).

Distinct cryopreservation approaches were developed for conserving species from the genus *Butia* (Arecaceae) endemic to the Atlantic Forest and Cerrado. In addition to predatory exploitation, these plants are only propagated by seeds that show dormancy and limitations for seedling establishment. Therefore, as a step toward cryobanking these species, seeds and zygotic embryos of *Butia eriospatha*, *B. capitata* and *B. yatay* were cryopreserved following desiccation, resulting in over 70% survival and post-freezing germination (20, 21, 22, 23).

Seeds of *Cattleya crispa*, an epiphytic orchid endemic to the Atlantic Forest included in the Red List of Brazilian Flora, were also cryopreserved by direct immersion in LN.

Table 1. Publications on the cryopreservation of Brazilian endemic and endangered species during the last decade (2014-2024). The threat category is based on information in the IUCN list of threatened species and/or the Official National List of Threatened Species of Flora (23).

Family (-aceae)	Species	Biome	Threat cat-egory	Plant material	Technique	Regrowth	Ref.
Araucari-	*Araucaria angustifolia**	Atlantic Forest, Pampa	EN	EC	Slow cooling	100%	(26)
				Shoot tips	Vitrification	35.3%	(29)
				ZE	Slow cooling	ND	(30)
Arec-	*Butia eriospatha**	Atlantic Forest	VU	ZE	Desiccation Droplet-vitrification	92%	(23)
	*Butia capitata**	Cerrado	VU	Seeds; ZE	Slow cooling	75 - 90%	(20)
			VU	ZE	Desiccation	70 - 86%	(22)
	*Butia yatay**	Atlantic Forest, Pampa	VU	ZE	Pregrowth	92%	(21)
Bignoni-	*Handroanthus impetiginosus**	Amazon, Atlantic Forest, Caatinga, Cerrado, Pantanal	NT	Seeds	Vitrification	76%	(31)
				Seeds	Direct freezing	89%	(32)
	*Handroanthus serratifolius**	Amazon, Atlantic Forest, Caatinga, Cerrado, Pantanal	NT	Seeds	Direct freezing	95%	(33)
				Seeds	Direct freezing	93%	(32)
	*Handroanthus spongiosus**	Atlantic Forest, Caatinga	EN	Seeds	Direct freezing	92%	(34)
Bromeli-	*Dyckia delicata*	Atlantic Forest	CR	Seeds	Vitrification	79%	(35)
	Hohenbergia castellanosii	Atlantic Forest	EN	Seeds	Desiccation	100%	(36)
	Vriesea reitzii	Atlantic Forest	NT	Seeds	Desiccation	65%	(37)
				Seeds	Desiccation	89.6%	(18)
Cact-	*Melocactus conoideus*	Caatinga, Cerrado	CR	Seeds	Direct freezing	10%	(38)
	Melocactus sergipensis	Caatinga	-	Seeds	Direct freezing	80%	(19)
	Melocactus violaceus			Seeds	Direct freezing	80-85%	(19)
	Micranthocereus flaviflorus	Caatinga, Cerrado	NT	Seeds	Direct freezing	58.8%	(38)
	Micranthocereus polyanthus	Caatinga, Cerrado	EN	Seeds	Direct freezing	22.5%	(38)

* Woody or forest species. EC – Embryogenic cultures; ZE – Zygotic embryos. ND – Not determined.

Table 1 (cont.). Publications on the cryopreservation of Brazilian endemic and endangered species during the last decade (2014-2024). The threat category is based on information in the IUCN list of threatened species and/or the Official National List of Threatened Species of Flora (23).

Family (-aceae)	Species	Biome	Threat category	Plant material	Technique	Regrowth	Ref.
Orchid-	*Catasetum atratum*	Atlantic Forest, Cerrado	NT	Seeds	Vitrification	65.4%	(25)
	Cattleya amethystoglossa	Atlantic Forest, Caatinga	NT	Seeds	Desiccation	94%	(39)
	Cattleya crispa	Atlantic Forest	VU	Seeds	Direct freezing	11%	(24)
	Cattleya granulosa	Atlantic Forest	VU	Seeds	Vitrification	60%	(40)
	Cattleya guttata	Atlantic Forest	VU	Seeds	Direct freezing	78.8%	(41)
	Cattleya intermedia	Atlantic Forest, Pampa	VU	Seeds	Direct freezing	ND	(42)
	Cattleya kautskyana	Atlantic Forest	CR	Seeds	Desiccation	100%	(39)
	Cattleya labiata	Atlantic Forest, Caatinga	VU	Seeds	Desiccation	95%	(43)
	Cattleya tigrina	Atlantic Forest	VU	Seeds	Vitrification	22.8%	(41)
				Seeds	Desiccation	99%	(39)
	Cattleya walkeriana	Amazonia Cerrado	VU	Seeds	Vitrification	66.33%	(44)

*Woody or forest species. EC – Embryogenic cultures; ZE – Zygotic embryos. ND – Not determined. Threat categories: NT– Near Threatened; VU– Vulnerable; EN– Endangered; CR– Critically Endangered.

Viability, germination, survival and protocorm weight of the cryopreserved seeds did not differ from the control (24). However, considering that many Brazilian species have recalcitrant seeds, which do not tolerate dehydration and storage at low temperature, many authors have employed different methods for reducing water content and inducing cryotolerance, including desiccation in silica gel or under the air flow of a laminar chamber and the use of sugars and other cryoprotectants. Suzuki et al. (25) evaluated the effect of different cryoprotectants (glycerol, sucrose, PVS2 and phloroglucinol, individually and combined) for the cryopreservation of seeds from *Catasetum atratum*. The highest post-freezing viability occurred in response to PVS2, glycerol + PVS2 or sucrose + PVS2 + 1% phloroglucinol, whereas the highest germination (65.4 %) was attained when seeds were exposed to PVS2 before immersion in LN.

Although seeds and zygotic embryos are considered suitable materials for germplasm conservation since they represent good sources of variability, they may also pose a significant challenge to the cryopreservation of endangered tropical species. Issues related to small population sizes and low seed production can hinder seed collection from the field and limit the use of these explants for long-term conservation purposes. Therefore, efforts must be driven to explore alternative plant materials for the cryopreservation of these species.

Table 2. Publications on the cryopreservation of Brazilian woody and forest species during the last decade (2014-2024).

Family (-aceae)	Species	Biome	Plant material	Technique	Regrowth	Ref.
Anacardi-	*Astronium fraxinifolium*	Amazon, Atlantic Forest, Cerrado	Seeds	Vitrification	86%	(47)
	Astronium urundeuva	Atlantic Forest, Caatinga, Cerrado, Pampa, Pantanal	Seeds	Vitrification	69%	(48)
	Myracrodruon urundeuva	Atlantic Forest, Caatinga, Cerrado	Seeds	Vitrification	83%	(47)
Arec-	*Acrocomia aculeata*	Amazon, Atlantic Forest, Cerrado	Zygotic embryos	Desiccation	81%	(49)
	Attalea vitrivir	Amazon, Cerrado	Seeds	Direct freezing	90%	(50)
	Bactris gasipaes	Amazon	Embryo-genic cluster	Vitrification	90%	(46)
	Butia catarinensis	Atlantic Forest	Zygotic embryos	Vitrification, Droplet-vitrification	77%	(51)
	Syagrus romanzoffiana	Atlantic Forest, Cerrado, Pampa	Zygotic embryos	Desiccation	93%	(52)
Bignoni-	*Handroanthus chrysotrichus*	Atlantic Forest, Cerrado, Pampa	Seeds	Vitrification	88%	(31)
	Tabebuia aurea	Amazon, Atlantic Forest, Caatinga, Cerrado, Pantanal	Seeds	Direct freezing	100%	(32)
	Tabebuia roseoalba	Amazon, Atlantic Forest, Caatinga, Cerrado, Pantanal	Seeds	Direct freezing	93%	(32)
Fab-	*Senegalia polyphylla*	Amazon, Atlantic Forest, Caatinga, Cerrado, Pantanal	Seeds	Direct freezing	42.5%	(53)
Malv-	*Chorisia speciosa*	Amazon, Atlantic Forest, Caatinga, Cerrado, Pampa, Pantanal	Seeds	Desiccation	63.3%	(28)
Po-	*Guadua chacoensis*	Atlantic Forest	Embryo-genic culture	Slow cooling	100%	(45)

An efficient cryopreservation protocol for embryogenic cultures of *Aracauria angustifolia*, an endangered woody species from the Atlantic Forest, was developed by Fraga et al. (26), who investigated the effects of cryoprotection exposure (0, 30, 60, 120 and 240 min) on both regrowth (%) and ultrastructural alterations of two cell lines (Cr01 and Cr02) after LN exposure. Although 100% regrowth was observed in both lines, they showed genotype-dependent responses to cryoprotectant treatment, as only Cr02 cells were sensitive to the duration of the cryotreatment. No cell damage or proliferation inhibition were associated with the cryopreservation procedure.

WOODY AND FOREST SPECIES

Besides their role in the ecological balance of ecosystems, woody and forest species have undeniable economic importance, providing timber and several non-wood products. Thus, the development of cryopreservation protocols is essential to the long-term conservation of their genetic diversity for breeding and research (27). In this regard, different cryopreservation approaches were applied to these species in Brazil during the last decade, using seeds and zygotic embryos, together with embryogenic cultures, which play an important role in in vitro conservation of species with long reproductive cycles and/or low seed production (Table 2).

Prudente et al. (28) developed a cryopreservation protocol for seeds of *Chorisia speciosa*, commonly known as *paineira-rosa* often used in landscaping and the construction industry, as well as for the restoration of degraded ecosystems and riparian forests. Seeds were desiccated in silica gel or under the airflow of a laminar chamber for different periods before LN storage for 24 h and, although both dehydration methods led to a reduction of approximately 50% of the initial water content, the highest post-freezing germination (63.3%) was achieved after desiccation in the laminar flow chamber, probably due to a more uniform dehydration.

The influence of exposure to the cryoprotectant solution, as well as the slow cooling technique, were investigated in embryogenic cultures of *Guadua chacoensis*, a lignified bamboo native to the Brazilian Atlantic Forest with a long-life cycle and irregular seed production (45). The authors reported that cryoprotective treatment for 120 min before freezing resulted in 100% survival, while phytotoxic effects occurred after 240 min cryoprotection.

Another interesting approach for embryogenic materials was reported by Ree & Guerra (46) for peach palm (*Bactris gasipaes*), a species native to the Amazon forest. They investigated the effects of sample size, diluted PVS3, addition of inorganic acids to PVS3, materials of strips for droplet-vitrification, partial dehydration before PVS3 exposure and rewarming conditions. The use of droplet-vitrification with aluminum or silver strips, larger embryogenic clusters, higher rewarming temperatures, or partial dehydration for 1 h in a laminar-flow chamber induced greater regrowth in cryopreserved samples compared to non-cryopreserved materials, while the addition of inorganic ions to the vitrification solution or the use of diluted PVS3 showed no significant benefits.

HORTICULTURAL PLANTS

Crops and wild relatives

Brazil is a major player in global agriculture, ranking among the largest producers and exporters of bioenergy, food, and fiber. Agribusiness is one of the pillars of the Brazilian economy, with various native species used as food, including cassava (*Manihot esculenta*), passion fruit (*Passiflora edulis*), peanuts (*Arachis hypogaea*), pineapple (*Ananas comosus*), Brazil nut (*Bertholletia excelsa*) and cocoa (*Theobroma cacao*), in addition to forage species such as *Paspalum* spp. and *Arachis pintoi* (54).

Besides its remarkable agrobiodiversity richness, which includes wild, domesticated and semi-domesticated species of native fruits, vegetables and grains, Brazil is also considered one of the largest holders of cultivated plants and wild relatives collections in the world (9). These plants are mainly secured ex situ in research institutes and universities or through in situ/on-farm collections managed by family farmers, maintaining a wide range of neglected and underutilized species with high nutritional value, which are important sources of genes for breeding programs (55).

However, their storage in ex situ collections still has gaps and needs, requiring efforts for the adoption of integrative conservation approaches, including cryopreservation, particularly for crop wild relatives threatened by the expansion of agriculture, anthropogenic pressure and disturbance of their natural habitats caused by the climate crisis (8, 9).

Among the cryopreservation protocols established during the last decade for Brazilian species with agronomic value, those developed for *Passiflora* species (passion fruit) using seeds and clonal materials stand out (Table 3). The main studies on seed cryopreservation aimed at determining the optimal water content and at inducing freezing tolerance (56, 57, 58, 59, 60, 61, 62). Although many species showed high

Table 3. Publications on the cryopreservation of Brazilian crops and wild relatives during the last decade (2014-2024).

Family (-aceae)	Species/cultivar	Plant Material	Technique	Regrowth	Ref.
Anacardi-	*Anacardium humile*	Achenes	Desiccation	96%	(70)
Apocin-	*Hancornia speciosa*	Shoot tips	Vitrification, Droplet-vitrification	over 70%	(67)
		Shoot tips	Droplet-vitrification	77%	(71)
		Lateral buds	Vitrification	73%	(68)
		Lateral buds	Encapsulation-vitrification	89%	(72)
		Seeds	Desiccation	0%	(66)
		Shoot tips	Droplet-vitrification	14 - 30%	(69)
Bromeli-	*Ananas comosus*	Shoot tips	Vitrification	100%	(73)
	Ananas comosus (wild and cultivated genotypes	Shoot tips	Droplet-vitrification	90 - 100%	(74)
	Ananas comosus 'MD-2'	Shoot tips	Vitrification	45%	(75)
	Ananas comosus varieties	Pollen	Desiccation	5 - 65%	(76)
	Ananas comosus varieties	Shoot tips	Droplet-vitrification	80 - 100%	(77)
	Ananas comosus varieties/ hybrids	Shoot tips	Droplet-vitrification	0 - 100%	(78)
	Ananas comosus 'MD-2'	Shoot tips	Droplet-vitrification	ND	(79)
	Ananas comosus cultivars	Shoot tips	Droplet-vitrification	96 - 100%	(80)
	Ananas comosus 'MD-2'	Shoot tips	Droplet-vitrification	ND	(81)
	Ananas comosus 'MD-2'	Shoot tips	Droplet-vitrification	90%	(82)
	Ananas comosus cultivars	Shoot tips	Droplet-vitrification	ND	(83)
	Ananas comosus 'MD-2'	Shoot tips	Droplet-vitrification	98%	(84)
Caric-	*Vasconcellea quercifolia*	Zygotic embryos	Desiccation	26%	(85)
Convolvul-	*Ipomoea cynanchifolia*	Seeds	Direct freezing	100%	(8)
Euphorbi-	*Manihot esculenta*	Nodal segments	Vitrification	66.6%	(86)

ND, not determined

germination after seed dehydration and LN storage, some were negatively affected, showing low germination after rewarming. For vegetative propagules, which were comparatively less studied, different parameters that affect post-freezing recovery, including pre-culture in high sucrose concentration, exposure to PVS2 and PVS3, along with distinct recovery conditions, were evaluated. The V-Cryo-plate technique was applied to *P. pohlii* roots (63) and *P. suberosa* shoot tips. The recovery of shoot tips (60%) was increased when compared with the previously obtained with the encapsulation-vitrification protocol (28%) (64). More recently, Ferreira et al. (65) reported protocols for pollen cryopreservation of wild *Passiflora* species, as influenced by genotype and dehydration methods.

Hancornia speciosa, a native fruit tree commonly known as *mangaba*, with great economic potential and social importance for the Northeast region of Brazil, was another species studied in the last decade, considering the intense reduction of its natural populations. Desiccation, vitrification, encapsulation-vitrification and droplet-vitrification protocols were successfully established for seeds, shoot tips and lateral buds. Santana et al. (66) evaluated the effects of desiccation on moisture content and germination capacity of cryopreserved seeds from mature and immature fruits, but no post-freezing recovery was achieved. On the other hand, Santos et al. (67) observed that preculture of shoot tips with 0.3 M sucrose was essential for improving regrowth, regardless of using vitrification or droplet-vitrification techniques. Prudente et al. (68) reported that adding proline or glycine betaine in this step of the protocol increased post-freezing recovery of lateral buds by reducing oxidative stress. Droplet-vitrification was applied for cryopreserving shoot tips of five accessions from the Mangaba Active Germplasm Bank to evaluate possible genotypic responses (69). The low recovery (14-30%) indicated the need for further adjustments to the protocol, especially the preculture and post-culture media.

Different techniques were also applied to cryopreserve cultivated and wild genotypes of pineapple (*Ananas comosus*), mainly using droplet-vitrification. Pineapple is one of the most consumed tropical fruits in the world and it is widely cultivated in Brazil, which is considered the most important center of origin of the genus. A cryopreservation protocol for shoot tips using droplet-vitrification resulted in high survival after exposure to PVS2 for 45 min, but some morphophysiological alterations were detected in the explants (74). More recently, Guerra et al. (78) showed that both genotype and dehydration methods had a significant influence on regrowth, indicating the need for standardization of the starting materials for protocol reproducibility. Additional reports focused on the cryopreservation of pollen grains, pursued to overcome asynchronous flowering. High viability and germination were obtained following pollen dehydration on silica gel before freezing, suggesting a positive effect of the cryopreservation conditions on breaking pollen dormancy (76, 96).

It is also worthy to mention the use of cryopreservation for pathogen elimination from infected plants of horticultural crops and their wild relatives (13). Shoot tip cryotherapy is currently a prevalent method for producing pathogen-free plants, providing certificated healthy plant materials. In Brazil, this technology has been successfully applied to eradicate the *Pineapple Mealybug Wilt-associated Virus* (PMWaV), which has been affecting pineapple accessions maintained in the Pineapple Active Germplasm Bank at Embrapa Cassava and Fruits. The production and conservation of virus-free plants reduced losses in the field, ensuring the phytosanitary quality of the accessions maintained in germplasm banks (77).

The field performance of plants derived from cryopreserved shoot tips was evaluated after storage for 24 h, 1, 2 and 3 years (82). There were no differences between control and cryopreserved plants regarding fruit morphology and nutritional content, confirming that cryopreservation is a suitable tool for long-term storage of pineapple germplasm.

Ornamentals

The global market of ornamental plants is a multi-billion-dollar industry, based on cut flowers, foliage, bulbs, dried flowers and garden and potted plants for landscaping. Brazil is one of the largest producers of ornamental plants, accounting for about 8% of the world's flower production. This industry generates approximately USD 2.2 billion and creates around 1 million direct and indirect jobs. The 15,600 ha of cultivated area are primarily concentrated in the South and Northeast regions of the country (97).

Table 4. Publications on the cryopreservation of Brazilian ornamental species during the last decade (2014-2024).

Family (-aceae)	Species	Explant	Technique	Regrowth	Ref.
Bromeli-	*Aechmea bicolor*	Pollen	Desiccation	92%	(101)
	Dyckia brevifolia	Seeds	Desiccation	92%	(35)
	Dyckia dusenii	Seeds	Vitrification	78%	(110)
	Dyckis kranziana	Seeds	Vitrification	73%	(110)
	Dyckis walteriana	Seeds	Vitrification	69%	(110)
	Encholirium spectabile	Seeds	Vitrification	97%	(111)
		Seeds	Desiccation	100%	(36)
	Tillandsia spp.	Seeds	Desiccation	45 - 90%	(100)
	Vriesea bahiana	Seeds	Desiccation	100%	(36)
	Vrisea philippocoburgii	Seeds	Desiccation	97%	(37)
Cact-	*Cereus fernambucensis*	Seeds	Direct freezing	80%	(19)
	Cereus gounellei	Seeds	Vitrification	95%	(103)
	Melocactus zehntneri	Seeds	Direct freezing	40%	(19)
		Seeds	Vitrification	89%	(103)
	Pilosocereus catingicola	Seeds	Direct freezing	80%	(19)
	Pilosocereus gounellei	Seeds	Direct freezing	40 - 45%	(19)
	Pilosocereus pachicladus	Seeds	Direct freezing	36%	(102)
Gesneri-	*Sinningia leucotricha*	Seeds	Vitrification	57%	(112)
Orchid-	*Cattleya forbessii*	Seeds	Vitrification	52.7%	(44)
	Cattleya harrisoniana × Cattleya walkeriana	Seeds	Vitrification	21.4%	(41)
	Cohniella cepula	Seeds	Direct freezing	90%	(113)
	Epidendrum ciliare	Seeds	Vitrification	93%	(104)
	Miltonia flavenscens	Seeds	Vitrification	71%	(114)
	Miltonia regnellii	Seeds	Desiccation	68%	(43)
Polypodi-	*Pleopeltis lepidopteris*	Spores	Direct freezing	97%	(115)

Brazilian ornamental plants, especially orchids and bromeliads, are highly appreciated in both domestic and international markets. However, the predatory exploitation associated with unsustainable extractivism, invasion of alien species and environmental disturbances caused by climate change has led to intense genetic erosion (98). Conservation of these species has been mainly carried out in ex situ collections distributed in universities, botanic gardens, private collections, cooperatives for agro-industrial activities and research institutes, which maintain accessions from all Brazilian biomes to provide diversification of the materials available to the current market, including cultivars with high productivity and resilience to climate alterations (99). Nonetheless, these efforts are still restricted to some target species, especially from groups of major economic importance, not reflecting the wide range of species variability (98).

Great progress has been observed in the development of biotechnological tools for propagating and conserving Brazilian ornamental species, reinforcing the relevance of in vitro systems as plant biofactories, as well as for the storage of high quality materials (99). As shown in Table 4, most cryopreservation approaches in the last decade used seeds as explants, generally due to their low moisture contents and small dimensions, such as those from the Bromeliaceae, Cactaceae and Orchidaceae families.

Many bromeliads suffer extractivism for ornamental purposes due to their exotic colorful flowers and are threatened by illegal collection and commercialization. Considering this situation, Oliveira et al. (100) studied the effect of seed desiccation on the germination of 20 *Tillandsia* species as influenced by morphometry, weight and water content. The storage at ultralow temperature after drying to moisture contents of approximately 7% did not impair germination level and the germination speed index, supporting the possibility of establishing a cryobank for the conservation of ornamental *Tillandsia*. Storage of pollen grains was explored for the conservation of *Aechmea bicolor*, a commercially valuable bromeliad from the Atlantic Forest, by Souza et al. (101). The authors evaluated different desiccation and conservation methods, and the best results were achieved with dehydration in silica gel for 3 h before immersion in LN, with normal pollen tube development and germination over 92%.

Cryopreservation by direct immersion of seeds in LN was also reported for different Cactaceae species native to the Caatinga and widely used as ornamentals. Bárbara et al. (102) evaluated the physiological quality of cryopreserved seeds of *Pilosocereus pachycladus* and observed no differences in germination (%), germination speed index and coefficient of uniformity of germination, in comparison to non-cryopreserved explants. More recently, Vendrame et al. (103) evaluated the efficiency of different vitrification solutions for the cryopreservation of seeds of *Melocactus zehntneri* and *Cereus gounellei*. They concluded that both species can be cryopreserved without the need for cryoprotectants, maintaining high germination (%).

Successful cryopreservation protocols were also developed for Orchidaceae species. Vettorazzi et al. (41) reported low germination (21.4%) of immature seeds of the *Cattleya harrisoniana* × *Cattleya walkeriana* hybrid, which was associated with the high moisture content of the seeds (65.6%). In contrast, for seeds of *Epidendrum ciliare*, an orchid often used for the creation of intergeneric ornamental hybrids, Pereira et al. (104) reported the need for PVS2 combined with phloroglucinol as cryoprotectants before immersion in LN to obtain post-cryo recovery. These studies corroborate the idea that successful regrowth after LN storage is species-specific, requiring adjustments in the cryopreservation procedures.

Medicinal plants

Brazil is rich in plants with medicinal properties and several species are included in different Pharmacopeias of the world (105). The popular tradition of using plants for therapeutic purposes can be attributed to the ethnopharmacological knowledge derived from the complex formation of the Brazilian population. Considering the wide use of medicinal plants, particularly by low-income populations, the Brazilian Health System launched a list of national plants with medicinal properties, bringing more value to traditional knowledge and stimulating phytotherapy. At the same time, this stimulated research for new products and innovative therapies by providing a foundation for bioeconomy-driven phytopharmaceutical drug development (106).

Unfortunately, the reduction of ecosystems and the predatory collection have resulted in the loss of medicinal species, many of them not yet characterized. It is important to keep in mind that unknown species may serve as sources of novel natural compounds and drug candidates that can be utilized to treat chronic and infectious diseases. This scenario is now aggravated by the climate crisis that impacts water availability and other environmental parameters such as soil and specific light-specific conditions, which influence not only plant growth but also the synthesis and accumulation of bioactive compounds (107). Therefore, cryopreservation approaches to ensure the availability of these germplasms as a source of materials for diverse pharmacological applications are critically important. Moreover, the interruption of metabolic events during storage at ultra-low temperature highlights the importance of cryopreservation as a sustainable strategy, securing the maintenance of the biosynthetic capacity of cryopreserved materials (107).

Over the last decade, the main approach adopted for the cryopreservation of Brazilian medicinal and aromatic plants was the combined

Table 5. Publications on the cryopreservation of Brazilian medicinal species during the last decade (2014-2024).

Family (-aceae)	Species	Plant material	Technique	Regrowth	Ref.
Amaranth-	*Pfaffia glomerata*	Shoot tips	Vitrification	65%	(116)
		Shoot tips	Droplet-vitrification	82%	(117)
Aster-	*Stevia rebaudiana*	Shoot tips	Droplet-vitrification, V-Cryo-plate	93%	(109)
Bignoni-	*Pyrostegia venusta*	Seeds	Desiccation	98%	(124)
Cleom-	*Tarenaya rosea*	Shoot tips	V-Cryo-plate	100%	(118)
		Adventitous roots	Vitrification	63.6%	(121)
		Adventtious roots	Vitrification	100%	(119)
		Adventitous roots	Encapsulation-vitrification	91%	(120)
Melastomat-	*Miconia ligustroides*	Seeds	Vitrification	70%	(125)
Piper-	*Piper aduncum*	Seeds	Vitrification	90.2%	(126)
	Piper hispidinervum	Seeds	Vitrification	98.5%	(126)

use of different cryoprotectant solutions and vitrification-based procedures (Table 5). Among them, droplet-vitrification and V-Cryo-plate techniques stand out, due to the high thermal conductivity of aluminum in foil strips and cryo-plates, which allow higher cooling and warming rates, reducing cell and tissue injury caused by intracellular ice crystal formation, thus enhancing post-freezing recovery (108).

For *Stevia rebaudiana* (Bertoni), an herbaceous perennial plant native to Brazilian Cerrado, which produces economically important diterpene glycosides (stevioside and rebaudiosides), shoot tips submitted to droplet-vitrification and V-Cryo-plate combined with optimal exposure to PVS2 showed 93% recovery, whereas the previous recovery observed with the vitrification technique reached only 68% (109).

Droplet-vitrification was also applied to shoot tips of *Pfaffia glomerata*, a native species commonly known as Brazilian ginseng, widely used in folk medicine as an energy booster and for the treatment of gastric disorders, inflammations, arthrosis and diabetes. The use of PVS3 significantly increased post-freezing recovery (81%) when compared to the vitrification standard procedure with the same cryoprotectant solution (42%) (116, 117).

Different cryopreservation protocols have been developed for *Tarenaya rosea*, previously named *Cleome rosea*, an endemic species from the Atlantic Forest with anti-inflammatory, antigenotoxic, antiviral and antibacterial activities. Vitrification and encapsulation-vitrification were employed for cryopreserving adventitious roots, which kept both their multiplication capacity and shoot regeneration ability after freezing (118, 119, 120). In addition, Cordeiro et al. (121) reported a protocol for the cryopreservation of shoot tips using the V-Cryo-plate technique with both PVS2 and PVS3 plus cytokinin supplementation in the recovery medium and maintenance of cryopreserved explants under dim light. The V-Cryo-plate technique was also used for the cryopreservation of *Passiflora pohlii* Mast. roots, which are rich in saponins and show high antioxidant potential. To adapt the aluminum plates for root explants, custom-made cryo-plates with one long and thin well were necessary. Exposure to PVS2 for 45 min induced cryotolerance, allowing high recovery (79%) and multiplication (63).

OCCURRENCE, MONITORING AND MITIGATION OF OXIDATIVE STRESS IN CRYOPRESERVATION

Plants from tropical climates, unlike those from temperate regions, do not have natural adaptation mechanisms against cold temperatures. As a result, many tropical species are recalcitrant to cryopreservation, with low or zero survival after direct immersion in LN (122). Therefore, for the successful induction of cryotolerance in these species, some critical parameters should be considered, including the reduction of intracellular water content, metabolic adjustments, optimization of post-freezing recovery conditions, and a deeper understanding of tissue-specific physiological and biochemical responses.

A critical factor in cryopreservation is the occurrence of reactive oxygen species (ROS) that result in oxidative stress during the different stages of the protocol, which include the establishment of stock cultures, explant excision, preculture, dehydration, cryoprotection, freezing-thawing, and post-culture recovery (123). While ROS play a vital role in cellular processes at controlled levels, acting in the signaling of various physiological processes, their overproduction leads to severe toxicity, causing damage to DNA, proteins, and membranes.

Damage resulting from the cryopreservation process is referred to as cryoinjury, which includes morphophysiological, biochemical and molecular changes that impact recovery efficiency. As the mechanisms of induction and action of cryonjury are not yet fully elucidated, the implementation of systems to monitor the stress induced by cryopreservation by determining the most critical stages of the protocols is of fundamental importance for managing oxidative stress and achieving methodological optimization (123). This can be carried out through the analyses of morphoanatomical, physiological and biochemical parameters, as well as through the evaluation of (epi)genetic and metabolic stability.

Morphophysiological and ultrastructural analyses

Morphophysiological analyses have been extensively used to assess and monitor cryoinjury in Brazilian native species in the past decade.

The extent of tissue and cellular damage during the different steps of the V-Cryo-plate and encapsulation-vitrification protocols were evaluated by Simão et al. (63) and Cordeiro et al. (120) in root explants of *Passiflora pohlii* and *Tarenaya rosea*, respectively. Regardless of the species or cryopreservation technique, both reports associated the occurrence of cryoinjury with cell size and localization. Major damage was observed primarily in cortical cells after loading and exposure to vitrification solutions, whereas pericycle and central cylinder cells displayed higher tolerance to the osmotic stress caused by dehydration, leading to the regeneration of new roots after rewarming.

The morphophysiological features of cryopreserved plants were also assessed after the acclimatization process. Villalobos-Olivera et al. (84) analyzed several physiological indicators such as mesophilic succulence index, chlorophyll contents, transpiration rate, and gas exchange in pineapple plants derived from cryopreserved shoot tips and did not detect anatomical or physiological modifications. All acclimatized plants shifted from C3 to Crassulacean Acid Metabolism (CAM) during the first phase of the acclimatization process, reflecting the adaptation to increased light intensity and temperature of the ex vitro environment.

Ultrastructural analyses employed for cellular characterization after cryopreservation did not reveal damage induced by the cryopreservation process of *Vriesea reitzii* (18). On the contrary, Fraga et al. (26) described increased levels of heterochromatin and changes in cell surface after freezing and rewarming of embryogenic cultures of *Araucaria angustifolia*, which were attributed to a protection mechanism of DNA against cleavage and thickening of the cell wall in response to osmotic stress.

A different approach was carried out by Faria et al. (60), who used X-ray analysis to detect freeze-induced damage in seeds of *Passiflora eichleriana*, *P. nitida* and *P. mucronata*. This analysis showed no deleterious effects on the seed tegument or embryos in cryopreserved seeds of the three species, corroborating the efficiency of the X-ray technique for visualizing mechanical injury caused by cryopreservation.

Biochemical analyses

Biochemical responses to stress conditions associated with cryopreservation can be assessed by measuring the products derived from intracellular oxidative reactions, evaluating the activity of antioxidant enzymes, or quantifying non-enzymatic antioxidant compounds. These analyses can be performed during the different stages of the cryopreservation protocol to guide further ROS mitigation actions.

The malondialdehyde (MDA) content, an end product of free-radical-mediated chain of reactions that result in the peroxidation of polyunsaturated fatty acids, is recognized as a marker to evaluate oxidative stress (127). Additionally, as stress conditions may also induce the activation of the enzymatic antioxidant system, the activities of the main antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), have been adopted as indicators of oxidative stress and acquisition of cryotolerance. SOD is responsible for the dismutation of $O_2^{\bullet -}$ in O_2 and H_2O_2, whereas CAT and APX are involved in the metabolism of H_2O_2 and O_2 (127, 128).

The importance of assessing oxidative stress during the different stages of cryopreservation was explored by Vianna et al. (64) and Polesi et al. (45). Both reports evaluated MDA production and the activity of antioxidant enzymes, indicating that osmoprotection and exposure to cryoprotectant solutions were the critical stages of the protocols.

Prudente et al. (68) measured MDA and H_2O_2 contents along with the activities of SOD, CAT and APX in cryopreserved lateral buds of *Hancornia speciosa* after preculture with proline and glycine betaine. A reduction in MDA production and an increase in SOD activity and H_2O_2 content, followed by activation of CAT and APX, occurred in response to the treatment with 0.1 M proline for 24 h, which helped overcome the adverse effects of dehydration by providing a suitable internal balance of the oxidative metabolism.

The role of polyamines (PAs) as osmoprotectants and cryotolerance inductors was another aspect evaluated for mitigating stress conditions during cryopreservation of Brazilian plants. These molecules have been implicated in diverse functions, including cell responses to biotic and abiotic stresses, taking part in the stabilization of membranes and proteins, as well as in ROS scavenging by stimulating the activity of antioxidant enzymes (129).

Pradella et al. (37) evaluated PAs content in seeds of two bromeliads, correlating the results with cellular alterations and germination capacity after cryopreservation. Seeds with high water content showed low PAs levels, and cell alterations such as plasmolysis, membrane rupture, and vacuolar cell death, whereas dehydrated seeds displayed a significant increase in putrescine (PUT) and spermidine (SPD) concentrations, as well as high germination, suggesting a protective role of these PAs during the cryopreservation process.

Goeten et al. (23) assessed PAs and amino acid profiles during the dehydration stage, to determine the suitable physiological requirements for the cryopreservation of jelly palm (*Butia eriospatha*) zygotic embryos. The combined role of high PUT concentrations with different amino acids, especially lysine, glutamic acid, leucine and glutamine, was strongly related to the high post-freezing recovery (90%). Considering that these molecules are involved in osmotic adjustments and ROS detoxification, they might act as protectants for membrane stability during dehydration, allowing post-freezing viability.

Evaluation of (epi)genetic) and metabolic stability

The (epi)genetic fidelity of cryopreserved Brazilian native plants has been evaluated with different approaches. Vettorazzi et al. (41) analyzed plants derived from cryopreserved seeds of two *Cattleya* species by flow cytometry and reported no changes in DNA content, indicating no differences in ploidy levels of cryopreserved plants when compared to the control samples.

The genetic stability of cryopreserved roots of *Cleome rosea* was assessed by Random Amplified Polymorphic DNA (RAPD) markers, revealing high similarity between cryopreserved explants and their donor plants (119). More recently, Villalobos-Olivera et al. (83) evaluated phenotypic features and genetic fidelity of pineapple plantlets derived from micropropagated and cryopreserved shoot tips, using Inter Simple Sequence Repeats (ISSR) markers. No phenotypic alterations or polymorphic bands were detected, validating both protocols and indicating the true-to-type status of the regenerants.

Methylation Sensitive Amplified Polymorphisms (MSAP) markers were used to evaluate the epigenetic profiles of cryopreserved somatic embryos of cocoa (*Theobroma cacao*) (88). Alterations in the DNA methylation pattern were higher when cryopreserved embryos gave rise to secondary somatic embryos after freezing. Additionally, this variability seemed to be reversible to some extent and was associated with adaptive responses to osmotic and oxidative stress during vitrification.

The evaluation of the metabolic stability after cryopreservation, albeit still insufficient, is essential for cryopreserved medicinal plants to ensure that their therapeutic properties are not compromised. Oliveira et al. (50) examined seed germinability and oil quality in cryopreserved seeds and zygotic embryos of *Attalea vitrivir*, a neotropical species known as babassu palm. The authors reported high germination after LN storage, with the oil retaining its quality and showing increased levels of fatty acids.

PERSPECTIVES ON THE USE OF CRYOBIOTECHNOLOGY FOR CONSERVING BRAZILIAN PLANT DIVERSITY

Brazil, like several other countries worldwide, requires continuous and effective conservation actions to preserve its plant biodiversity, particularly given that numerous species remain undiscovered. To put this into perspective, a report by WWF-Brazil, in collaboration with the Ministry of Science, Technology and Innovations (MCTI), revealed that between 2014 and 2015, 216 new plant species and 165 new animal species were discovered in the Amazon. This means that, on average, a new species was discovered every two days within just one of the country's six biomes (130).

As a signatory of GSPC, Brazil has already performed a risk assessment of part of its known flora, in line with Target 2, which states that countries must undertake risk assessments of their entire known plant species by 2020. Due to its large territory and to the enormous number of species, the goal has not yet been achieved, but this is expected to happen by 2030, depending on governmental initiatives, with the joint participation of the environmental and agriculture sectors, as well as of farmers, indigenous people and NGOs (9).

Successful actions also rely on the availability of research funding to all the areas related to in situ and ex situ conservation. Cryopreservation will play a critical role in this context, as a complementary method for the safe storage of Brazilian native species, especially for plants that do not produce adequate seeds for banking or seeds that are short-lived in storage, which are now included under a grouping known as ‘exceptional species’ (131). Notably, more than a quarter of threatened species in the world are projected to be made up of exceptional species, in particular those from tropical and subtropical regions (132). Moreover, cryopreservation could help Brazil meet GSPC Target 8, which states that 75% of threatened plant species be held in ex situ collections, preferably in their country of origin, with at least 20% available for recovery and restoration programs. During the past 10 years, several efficient cryopreservation protocols have been developed for Brazilian native plants, covering a wide range of both wild and cultivated species, and exploring almost all available vitrification-based techniques, cryoprotective agents and plant materials, besides investigating various preculture and post-freezing conditions. However, to this moment, only a few have been effectively implemented on a large scale for germplasm long-term storage in cryobanks.

It will be important to address the need for detailed information on Brazilian plant germplasm already stored in cryobanks, both nationally and internationally. This could prevent the unnecessary duplication of genetic resources across different banks and serve as a key strategy to efficiently combat the erosion of plant genetic resources (133). A more accurate assessment of the stored germplasm and of available protocols would also provide a clearer understanding of the species already studied. Other relevant aspects that should be carefully considered include the improvement to cryopreservation protocols, the use of alternative explants and different antioxidants, as well as further development of efficient cryopreservation protocols for a wider range of species.

A recent study commissioned by the Alliance of Biodiversity International, the Global Crop Diversity Trust, the International Potato Center (CIP) and the International Treaty of Plant Genetic Resources for Food and Agriculture emphasized the urgent need for a global cryopreservation network to safeguard some of the humanity's most vital crops, following the principles and policies of the Svalbard Global Seed Vault. In 2022, Brazil and some Latin American countries convened as a first step towards setting out the Latin American Cryonetwork (LAC), aiming at conserving crop species with vegetative propagation and/or with recalcitrant seeds. This network can significantly contribute to the conservation of Brazilian species by fostering collaborations and addressing current limitations in technical personnel and funding for cryopreservation research.

Given the vast diversity of Brazilian plants and ecosystems, it is also relevant to expand cryopreservation research to include non-agronomic species, as they play a key role in ecological stability, providing essential ecosystem services and genetic traits that could address future agricultural, medical and industrial challenges. Furthermore, future studies must concern highly endangered species due to deforestation, habitat loss and unprecedented climate change, especially those classified as critically endangered. These efforts require effective funding policies to support scientific and technological innovation. Encouragements to use cryopreserved plants to restore and reinforce degraded ecosystems, as well as for other direct applications such as breeding programs and the pharmaceutical industry are also necessary.

Developing and optimizing cryopreservation techniques for these unique species can be challenging due to their specific responses to dehydration and ultralow temperature, as well as to the limited understanding of their biology and physiology. Low recovery can result from underlying mechanisms that include the oxidative status of cells, freezing damage and changes in membrane structure and protein conformation (122). It is also important to note that morphophysiological and (epi)genetic changes during cryopreservation may affect the quality of recovered plants and their performance in reintroduction, commercial exploitation and breeding programs (13).

To enhance our knowledge of the molecular events associated with cryopreservation, to improve recovery growth and ensure trueness-to-type of recovered plants, it will be essential to analyze the cryopreserved materials using the conceptual framework of cryobiomics or cryobionomics proposed by Harding (134), which is based on genomics, transcriptomics, proteomics, and metabolomics (122).

Extending cryopreservation to threatened wild species yet to be studied, especially crop wild relatives, is another urgency in the country, due to the current accelerated biodiversity loss. The need for more basic research to encompass a wider range of species, in parallel with the development and improvement of protocols and a deeper understanding of tissues' response to freezing must be prioritized in conservation programs. Moreover, the implementation of new facilities that meet the high diversity and wide geographic range is imperative to protecting natural assets and safeguarding Brazilian biodiversity for future generations.

This review reported the latest advances in cryopreservation of Brazilian native species and highlighted key factors for the establishment of successful protocols. The main challenges to the effectiveness of protocols together with alternative measures to improve post-freezing recovery have been discussed. Preservation and sustainable use of the Brazilian flora resources should be of high priority, to guarantee their survival and the ecosystemic equilibrium, as well as to ensure food security and resilience against climate crisis.

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