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Bibliographic review

Current status of the conservation of coffee (*Coffea* spp.) plant genetic resources

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ABSTRACT

The coffee plant (*Coffea* spp.) is an agricultural crop of significant economic and social relevance in many countries, including Cuba. It is proposed that 60 % of its species are classified as threatened with extinction, so this literature review was proposed with the objective of analyzing the conservation status of coffee plant genetic resources in recent years. Deforestation, climate change and invasive species are some of the risks faced in their sites of origin. The *ex situ* conservation of coffee germplasm by means of seeds is not feasible for prolonged periods of time, due to the sensitivity of its tissues to desiccation and low temperatures. Germplasm collections in the field are exposed to different risks, from their location in ecological conditions that are not ideal for the survival of all the material, the aging of the specimens, inappropriate cultivation methods and the emergence of pests and diseases, to the lack of interest of local authorities. The biotechnological methods of minimal growth and cryopreservation are promising options for the medium and long term maintenance of coffee plant genetic resources, so it is essential to expand research in this direction, in order to complement the different alternatives for the conservation of this traditional genus.

Key words: plant biotechnology, climate change, in vitro cultivation, deforestation, species extinction, germplasm

INTRODUCTION

The coffee plant (*Coffea* spp.) plays an important economic role worldwide, since it represents one of the main sources of income in some 80 coffee producing countries [(1)]. World production is mainly based on two species: *Coffea arabica* L., which represents 60 % and *C. canephora* Pierre with the remaining 40 % [(2)], although some of the non-commercial species have been used locally or regionally as substitutes for *C. arabica* L. [(3)]. In the 18th century *C. arabica* was introduced in Latin America [(4)], where

approximately 60% of the international coffee export volume is produced, as the main source of income for many countries [5]. In this region, Brazil, Colombia, Honduras, Mexico and Peru, among others, are the main producers [5]. In Cuba, the coffee plant is a priority in the agricultural sector, since coffee is a beverage commonly consumed by the population, as well as an exportable item [6].

There are 124 species of *Coffea* spp. known to science, which naturally inhabit tropical Africa, the islands of the Indian Ocean (Madagascar, Comoros and Mascarene Islands), Asia and Australia [7]. All have in common the characteristic morphology of coffee beans [8]. In addition, these species show useful traits for genetic improvement, such as climatic tolerance [9], including drought tolerance, resistance to pests and diseases [10], low caffeine content [11] and sensory improvement [12].

However, the application of the Categories and Criteria of the Red List of the International Union for Conservation of Nature (IUCN) [13] to *Coffea* species has yielded alarming data regarding their conservation status. Sixty percent of the total (75 species) have been classified as threatened with extinction, including 13 Critically Endangered, 40 Endangered and 22 Vulnerable. Thirty-five species have been listed as non-threatened (Near Threatened or Least Concern) and 14 species have been considered Data Deficient [14].

On the other hand, it has been demonstrated that only approximately half (55 %) of all coffee plant species are maintained in ex situ germplasm collections. Seventy-two percent are found in at least one in situ protected area; 18 % have no *in situ* protection and 13 species are not known to exist in protected areas (including 11 Data Deficient species). *C. arabica*, *C. eugenioides*, *C. canephora* and *C. liberica* seem to occupy a more secure position than others, as they are included in at least one protected area and in germplasm collections. However, the genetic diversity in both protected environments (*in situ* and *ex situ*) is not adequately covered. Due to rapid deforestation, climate change and genetic erosion, the options for collecting plant material of wild *C. arabica* for use, for example, in genetic improvement, are decreasing [14], hence the importance of contributing to the study of its conservation.

Taking into account the above, the objective of this bibliographic review was to analyze the state of conservation of phytogenetic resources of coffee plants (*Coffea* spp.) in the last decade.

In situ conservation

The conservation of plant species in situ offers the possibility of maintaining a greater diversity of species and genes in a dynamic environment, allowing populations to continue to evolve. Wild coffee plants are found growing naturally as understory trees in the tropical forests of Africa, spanning a wide geographic range from Guinea in West Africa through Central Africa to East Africa [1]. Other centers of diversity include Madagascar, the Comoro Islands, and the Mascarene Islands in the Indian Ocean (Reunion and Mauritius), and with the inclusion of the genus *Psilanthus* in *Coffea*, the geographic distribution extends to tropical Asia and Australia [7].

The primary centers of origin and diversity of *C. arabica* are located in the highlands of southwestern Ethiopia, the Boma Plateau in South Sudan, and Mount Marsabit in Kenya [15]. In Ethiopia, for example, one of the key factors influencing the erosion of coffee genetic diversity is deforestation [16]. Between 1971 and 1977, about 235,400 ha of closed and lightly disturbed forests were deforested in the highlands of the southwestern Ethiopian plateau [17].

In an attempt to conserve the last coffee forests in Ethiopia and to halt biodiversity loss, several areas have been incorporated into the UNESCO (United Nations Educational, Scientific and Cultural Organization) World Biosphere Network under the Man and the Biosphere program. In 2010, Yayu and Kafa Biosphere Reserves were added, while in 2012 Sheka Forest joined. These represent some of the last remaining fragments of montane forest with wild populations of *C. arabica* on the planet [1]. The designation of these areas as UNESCO Biosphere Reserves is certainly an important step towards the implementation of an active land conservation approach in Ethiopia, which covers more than one million hectares among the three reserves [18].

Despite these advances, published studies show that the area occupied by coffee forests included in protected areas is still small and climate change is projected to have a substantially negative influence in places currently inhabited by indigenous Arabicas in Ethiopia and South Sudan. All available future projections for the species, based on multiple general circulation models, emission scenarios and emigration scenarios, have been used to predict changes in the extent of occurrence, area of occupancy and population numbers for wild *C. arabica*. Under the effects of climate change the results showed that population numbers could be reduced by 50 % or more (with some models showing up to 80 %) by 2088, while area of occupancy could decrease by 30 % in many cases [19].

The natural range of *C. canephora* covers a much wider geographic area, extending from West Africa to Cameroon, Central African Republic, Congo, the Democratic Republic of Congo, Uganda, and northern Tanzania to northern Angola, although it generally occurs as small, isolated populations with a small number of mother trees and very few seedlings scattered over areas of less than 1 ha [15].

In Uganda's Kibale National Park, the Wild Coffee Tree Project proposed to use a unique approach to conserve the genetic resources of *C. canephora* through a market-based approach. This project proposed to support nature conservation and local communities by marketing sustainable harvests of the wild coffee plant that grows naturally in the park [20]. Although it was not successful in gaining access to international coffee markets, the lessons learned provided useful guidance for other market-based efforts linking forest resource conservation, local communities and international trade [1].

In Mauritius, one of the Mascarene Islands located in the southwestern Indian Ocean, there is also experience with in situ conservation of the three *Coffea* species native to that territory: *C. mauritiana*

Lam. Rich. and *C. myrtifolia* (A.Rich. ex DC.) Leroy. The protected areas there (seven Natural Reserves and one National Park) have been established primarily to protect representative samples of the native vegetation, in such a way that they conserve only part of the range of the genetic diversity of the wild coffee plant, which has been affected by the drastic reduction of forested areas and the destruction of habitats by invasive exotic species. Additionally, reserves known as Conservation Management Areas have been established to conserve native flora and fauna and their habitats in an integrated manner. These areas were fenced to prevent animals such as deer and pigs, and exotic plants were manually removed. In this way, the quality of the forest has been significantly improved and plant and animal communities have recovered [(21)].

The erosion of the *in situ* gene pool of *Coffea* spp. has become a significant concern due to multiple threats to their natural habitats, such as deforestation, encroachment by agricultural activities, population pressures, and economic hardship for the local populations that depend on these forests [(15)]. Efforts to conserve coffee germplasm in its natural habitats have been very limited, with known examples only in Ethiopia, Mauritius and Uganda. Many areas, such as central Gabon and the Central African Republic, still remain unexplored and much work remains to be done in diversity hotspots in Madagascar and continental Africa, particularly Tanzania [(15)]. Therefore, conservation of these plant genetic resources in *ex situ* collections such as germplasm banks in the field becomes imperative as an alternative or backup strategy for *in situ* conservation measures [(15)].

Ex situ conservation

Conservation in germplasm banks in the field

Field genebanks that maintain significant collections of *C. arabica* are located in Africa (Cameroon, Côte d'Ivoire, Ethiopia, Kenya and Tanzania), Madagascar, India and in the Americas (Brazil, Colombia and Costa Rica). Field collections in Cameroon, Côte d'Ivoire, India and Madagascar also maintain a good representation of *C. canephora*. A large part of the uncultivated wild coffee species are conserved in germplasm banks in Madagascar, which maintains about 50 species, and Côte d'Ivoire, with about 30 species of African coffee plants [(22)].

In botanical gardens in 50 countries, some 445 accessions of coffee plants from 26 species and some interspecific hybrids are also conserved. *C. arabica*, *C. canephora* and *C. liberica* account for 81 % of these accessions in botanical gardens [(1)]. From 73 accessions with species designation, eleven are kept only in one botanical garden and four of these species have not been kept in any other collection. Thus, botanic gardens can be reservoirs of unique species or of a level of diversity of varieties that needs to be considered as part of the global system for ex situ conservation of coffee plants [(1)].

Over the years, substantial losses of coffee genotypes have occurred in several germplasm banks, resulting in the loss of entire accessions. Taking as an example the CATIE (Centro Agronómico Tropical

de Investigación y Enseñanza) collection in Costa Rica, one of the main challenges faced includes the aging of the trees (most were established in the 1970s, although there are collections that were initiated in the 1940s). Also influential are sub-optimal climatic conditions and elevation, the need for varied cultural practices due to the diverse nature of the collections, with cultivated and wild genotypes that differ in their needs for shade, pruning, fertilizers, as well as lack of funding and other resources. Some 53 accessions are considered to have been lost over the years due to aging and the effect of pests and diseases [1,22].

Cuba is not a country of origin of the coffee plant, so in situ conservation does not take place; however, there are areas dedicated to ex situ conservation, such as those belonging to the Central Station for Coffee and Cocoa Research (ECICC), located in Santiago de Cuba province, where 1597 accessions are conserved [1].

Coffee germplasm collections in the field are exposed to different risks, from extreme climatic events to the lack of priority from local authorities [1]. They are frequently located in ecological conditions that are not ideal for their maintenance or for the survival of all the material, causing genetic erosion [21]. Other causes of this are the loss of trees as a consequence of aging, inappropriate cultivation methods, as well as the emergence of pests and diseases [23]. Some of the preventive measures taken against biotic and abiotic stress include frequent monitoring of collections, adequate irrigation, fertilizer application, shading, chemical protection, and integrated pest and disease management [1]. From all this, the need to apply other alternatives to the field conservation of coffee plant genetic resources is evident.

Storage of coffee seeds

Based on their characteristics during storage, coffee seeds have been classified as intermediate, since they tolerate a certain level of desiccation, but do not survive total desiccation, nor the combined effects of desiccation and low temperatures [24]. It is known that the viability of C. arabica seeds decreases rapidly after four to six months at room temperature [25].

Although the most common way to produce coffee seedlings is by botanical seed, the loss of germination during storage is a limitation for the conservation and propagation of this genus [26]. Hence the need to study alternative forms of ex situ conservation, such as the application of biotechnological methods to the preservation of phytogenetic resources of the coffee plant.

Biotechnological methods

In vitro preservation

Among the multiple applications of in vitro culture is *in vitro* conservation, a valuable option for the preservation of germplasm of species with seeds of short viability, such as coffee plants. Minimal growth methods make it possible to achieve this objective [27].

Minimal growth methods consist of reducing the growth rate of plants, based on the reduction of cell division and metabolism, which is achieved by altering the optimal culture conditions. It is possible to vary the composition of the culture medium or modify the growth environment (temperature, light intensity, oxygen availability) [28]. In this way, it is feasible to conserve the plants in the short or medium term, which allows a reduction in the frequency of subcultures and an increase in the *in vitro* longevity of the plant material, as well as a minimum risk of genetic changes [27,29].

For the *in vitro* conservation of coffee plants, different variants of minimal growth have been studied [30], such as variations in culture temperature, in combination with a decrease in oxygen content [31,32], modification in the concentration of the carbon source [33,34] and the addition of osmotic growth regulators [35]. The most widely used has been the modification of the concentration of growth regulators in the culture medium [36-38]. Other contributions to these topics have been made in the last decade, as will be detailed below.

For *in vitro* conservation of *C. arabica* cv S. 795 germplasm, a protocol was developed consisting of inoculating zygotic embryos in MS medium [39], supplemented with sucrose (3.0 %) and different concentrations of abscisic acid (ABA) (0; 0.1; 0.5 and 1.0 mg L^{-1}). They were maintained at a temperature of 25±1 ºC and photoperiod of 16 h, for twelve months. For germination, embryos were subcultured in MS medium supplemented with different concentrations of kinetin (KIN) or gibberellic acid (AG3) (0; 0.1; 0.1; 0.5; 1.0 and 5.0 mg L_{-1}) and after 45 days were evaluated [40]. The results showed that zygotic embryos placed in culture medium with the different concentrations of ABA exhibited variable maturation responses.

In the control, embryos germinated after one week and the shoot and root developed longer, compared to the media with low concentrations, where very slow germination was induced. When embryos were grown in higher ABA concentrations, elongation was observed, followed by a color change from white to dark green at a two-week interval. These embryos reached full maturity and remained dormant, with no shoot or root development [40].

Of the various concentrations of KIN, the 0.1 mg L^{-1} treatment was more effective for seedling development, with maximum shoot length and the longest root with numerous lateral roots, compared with the higher concentrations of KIN, which resulted in complete inhibition of the development of this organ. Addition of AG3 at 0.1 and 0.5 mg L^{-1} increased shoot length compared to the control, whereas an increase in AG3 concentration did not have this effect. These results showed that KIN was more suitable than AG3 for developing vigorous seedlings [40].

A study conducted by Cuban researchers set out to determine the effect of decreasing the mineral content of the culture medium on the response of coffee plants (*C. arabica*) preserved in vitro for a period of two to six months. The plants, previously obtained in vitro, were grown in modified MS medium, with treatments consisting of a 75, 50 and 25 % reduction of their macro and microelements. Survival, number of leaf pairs, leaf abscission and percentage of plants with roots were evaluated. In the 50 % MS treatment, the survival percentages varied between 85 and 100 % and with respect to the rest of the treatments, intermediate values of leaf pairs and leaf abscission were obtained. In spite of the lack of nutrients, the plants developed roots that allowed them to survive under these conditions, so that with this treatment it is considered feasible to conserve coffee plants *in vitro* for up to six months [41].

Cryopreservation

Cryopreservation, the storage of biological material preferably at liquid nitrogen temperature (-196 °C), is an alternative to ex situ conservation of germplasm, which allows long-term, safe and cost-effective maintenance of plant genetic resources with minimal space requirements and routine maintenance [42]. In addition, cryopreservation eliminates the need for regular renewal of the collection, which reduces the risk of genetic erosion caused by pests, diseases, climatic conditions, contamination, and genetic variation [43].

Several studies have been carried out in the field of coffee plant cryopreservation [30], using different methods and types of explants: apices [44], somatic embryos and embryogenic callus [45-49], zygotic embryos [50-53], seeds [47,50,54-58] and have obtained diverse results. The research trend is mainly directed towards cryopreservation of seeds and zygotic embryos, a field currently led by Brazilian scientists.

Seed cryopreservation

The evaluation of the physiological quality of *C. arabica* seeds cryopreserved by direct immersion in liquid nitrogen, following rapid or slow drying, has recently been studied. Seeds of the cultivars Arara, Catiguá, Catuaí Amarelo and Mundo Novo were subjected to rapid drying using silica gel, and slow drying using a saturated NaCl solution (75 % relative humidity), until they reached a moisture content of 20 % (dry basis) in both variants. They were immersed in liquid nitrogen for 24 hours and then reheated in a water bath at 40 °C for two minutes. The physiological quality of the seeds after cryopreservation was evaluated by germination, tetrazolium and vigor tests. It was determined that even with different levels of tolerance, the seeds of these cultivars can be subjected to cryopreservation, with yellow Catuaí being the most tolerant and Arara the most sensitive, regardless of the speed of desiccation. Rapid drying in silica gel up to 20 % of moisture content, followed by direct immersion in

liquid nitrogen, allows obtaining higher percentages of normal seedlings, expanded cotyledonary leaves and dry mass and, therefore, favors the cryopreservation of coffee tree seeds [59].

Another research aimed to determine the water content, the cooling speed and the most adequate final temperature for cryopreservation of *C. arabica* seeds. These were dried with silica gel to water contents of 5, 10, 15, 15, 20, 30 and 40 % (wet basis), subjected to slow cooling treatments at rates of -1, -3 and -5 °C min^{-1} to final temperatures of -40, -50 and -60 °C and then immersed directly in liquid nitrogen (LN). After storage, the seeds were re-warmed at 40 °C for two minutes. The survival rate and viability of seeds and embryos were assessed by the tetrazolium and germination tests. The results of the tetrazolium test indicate that embryos extracted from cryopreserved seeds are less sensitive to cryopreservation than whole seeds. In general, water content of 20 % and the use of zygotic embryos led to the highest survival rate of coffee plant seeds, depending on the cooling rate and final pre-cooling temperature [60].

For *C. canephora* seeds dried in silica gel up to a water content of 0.25 g g^{-1}, slow cooling treatments identical to those cited above have been used [60]. The best result of these treatments was compared with rapid cooling, in which the seeds were directly immersed in liquid nitrogen and the physiological and biochemical alterations occurring in the seeds after cryopreservation were evaluated. Desiccation to 0.25 g g^{-1} of water content did not affect the viability of *C. canephora* seeds and they responded better to cryopreservation by rapid cooling compared to slow cooling. Catalase and esterase enzymes are good biochemical markers for cryopreserved coffee seeds and their activity is higher in those of better physiological quality [61].

The ideal physical and physiological conditions for cryopreservation of *C. canephora* seeds have also been investigated to reduce mortality caused by intracellular ice crystal formation and to avoid cell damage caused by excessive desiccation. Seeds were subjected to rapid desiccation in silica gel, and slow desiccation in saturated NaCl solution to moisture contents of 0.20, 0.25 and 0.28 g g^{-1} (dry basis), followed by direct immersion in liquid nitrogen for flash freezing. Physiological and biochemical analyses were performed to evaluate the quality of these explants before and after cryopreservation. Rapid drying at values close to 0.20 g g^{-1} does not cause a reduction in physiological quality, while moisture content of 0.25 g g^{-1} results in higher seed survival, after cryopreservation. The speed of drying affects physiological quality after cryopreservation, since rapid drying in silica gel is more favorable than slow drying in a saturated NaCl solution. The activity of catalase, esterase, glutamic oxaloacetic transaminase, and polyphenol oxidase enzymes is an indicator of the quality of *C. canephora* seeds subjected to cryopreservation [62].

Coffee plant (*C. arabica*) seeds have been subjected to different types of desiccation and stored in cold storage and cryopreservation, with the purpose of studying the subsequent development of the seedlings. The seeds were subjected to four drying treatments in a stationary dryer until they reached 12 and 32 % humidity and in saturated saline solution and silica gel, until they reached 17 % humidity. Those with

12 and 32 % moisture were stored in a cold, dry chamber (at 10 °C and 45 % relative humidity) and those with 17 % moisture were stored in cryogenic chambers (-196 °C) for a period of six months, after which they were planted in plastic bags in a nursery. Seedlings from seeds dried in silica gel showed vegetative development results similar to those of seedlings produced from seeds at 32 % humidity and stored for six months. The use of silica gel-dried and cryopreserved *C. arabica* seeds is considered a viable alternative for the production of vigorous seedlings [63] and a simpler method than the others tested in this study.

Embryo cryopreservation

Other explants frequently used for the cryopreservation of coffee plant genetic resources are zygotic and somatic embryos. In the case of *C. arabica* L. cv. Catuaí Vermelho IAC 144, different drying times in silica gel were tested for zygotic embryos (0; 15; 30; 60; 120; 240 min) and somatic embryos (0; 60; 120 min). Although a high germination percentage (98 %) was obtained for zygotic embryos at 120 min of desiccation, only 41 % of the regenerated seedlings were considered normal. On the other hand, for somatic embryos it was impossible to apply the cryopreservation treatment, since no germination was obtained due to the sensitivity of these explants to dehydration [64].

The vitrification method has also been used to develop a cryopreservation protocol for zygotic embryos of *C. arabica* L. cv. "Catuaí Vermelho" - IAC 144. For this purpose, embryos were immersed in PVS 2 [65] (Plant Vitrification Solution) (composed of 30 % glycerol, 15 % ethylene glycol, 15 % dimethyl sulfoxide and 0.4 M sucrose, in liquid MS medium), at different times (0, 10, 25, 50, 50, 100 and 250 min) and at two temperatures (0 and 25 °C). For thawing, different times in water bath were evaluated: 1, 3, 5 min or directly in recovery solution. An anatomical study was performed on embryos stored with or without the use of PVS 2, and not stored. It was determined that immersion in cryoprotective solution, for 100 min at 0 °C allows cryopreservation of embryos, which can be thawed directly in the recovery solution after storage in liquid nitrogen. It was observed that PVS 2 reduced the internal water content in the cells, which allowed the subsequent resumption of zygotic embryo growth [66].

Cuban researchers from the National Institute of Agricultural Sciences (INCA), in collaboration with scientists from the Gosling Research Institute for Plant Preservation (GRIPP), belonging to the University of Guelph in Canada, determined the effectiveness of two methods derived from vitrification, in the cryopreservation of zygotic embryos of C. *arabica*, with the use of PVS 3 [67] (50 % glycerol + 50 % sucrose w/v), with promising results for the conservation of phytogenetic resources of coffee plants by this method [68].

CONCLUSIONS

Achieving the complementarity of the different types of conservation: *in situ* (where appropriate) and *ex situ*, through field preservation and the use of biotechnological methods of minimal growth and cryopreservation, constitutes an important challenge to be overcome at a global level in the near future, with a view to the protection of coffee plant genetic resources.

BIBLIOGRAPHY

1. Bramel P, Krishnan S, Horna D, Lainoff B, Montagnon C. Global conservation strategy for coffee genetic resources. Crop Trust and World Coffee Research [Internet]. 2017;72. Available from: https://worldcoffeeresearch.org/media/documents/Coffee_Strategy_Low_Res.pdf

2. International Coffee Organization - What's New [Internet]. [cited 2/11/2021]. Available from: https://www.ico.org/

3. Wellman FL. Coffee: botany, cultivation, and utilization. Coffee: Botany, Cultivation, and Utilization. [Internet]. 1961; Available from: https://www.cabdirect.org/cabdirect/abstract/19640305741

4. Aguilar ME, Ortiz JL, Mesén F, Jiménez LD, Altmann F. Cafe Arabica *Coffea arabica* L. In: Jain SM, Gupta P, editores. Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants: Volume II [Internet]. Cham: Springer International Publishing; 2018 [cited 2/11/2021]. p. 39-62. (Forestry Sciences). doi:10.1007/978-3-319-79087-9_3

5. International Coffee Organization - Historical Data on the Global Coffee Trade [Internet]. [cited 2/11/2021]. Available from: https://www.ico.org/new_historical.asp?section=Statistics

6. González Vega ME, Rosales Jenqui P, Castilla Valdés Y, Lacerra Espino JÁ, Ferrer Viva M. Efecto del Bioenraiz® como estimulante de la germinación y el desarrollo de plántulas de cafeto (*Coffea arabica* L.). Cultivos Tropicales [Internet]. 2015;36(1):73-9. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362015000100009

7. Davis AP, Tosh J, Ruch N, Fay MF. Growing coffee: Psilanthus (Rubiaceae) subsumed on the basis of molecular and morphological data; implications for the size, morphology, distribution and evolutionary history of Coffea. Botanical Journal of the Linnean Society [Internet]. 2011;167(4):357-77. Available from: https://academic.oup.com/botlinnean/article/167/4/357/2418595?login=true

8. Davis AP, Bridson D, Rakotonasolo F, Keating RC, Hollowell VC, Croat T. A reexamination of *Coffea subgenus* Baracoffea and comments on the morphology and classification of Coffea and Psilanthus (Rubiaceae-Coffeeae). Monographs in Systematic Botany. 2005;104:399.

9. Davis AP, Govaerts R, Bridson DM, Stoffelen P. An annotated taxonomic conspectus of the genus Coffea (Rubiaceae). Botanical Journal of the Linnean Society [Internet]. 2006;152(4):465-512. Available from: https://academic.oup.com/botlinnean/article/152/4/465/2420564?login=true

10. Silva M do C, Várzea V, Guerra-Guimarães L, Azinheira HG, Fernandez D, Petitot A-S, et al. Coffee resistance to the main diseases: leaf rust and coffee berry disease. Brazilian journal of plant physiology [Internet]. 2006;18(1):119-47. Available from: https://www.scielo.br/j/bjpp/a/dTHqD9SqgdJtq9wHCf9Hp6R/abstract/?lang=en

11. Hamon P, Grover CE, Davis AP, Rakotomalala J-J, Raharimalala NE, Albert VA, et al. Genotyping-by-sequencing provides the first well-resolved phylogeny for coffee (Coffea) and insights into the evolution of caffeine content in its species: GBS coffee phylogeny and the evolution of caffeine content. Molecular phylogenetics and evolution [Internet]. 2017;109:351-61. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1055790317301288

12. Cheney RH. Coffee : a monograph of the economic species of the genus *Coffea* L. [Internet]. New York : New York University Press,; 1925. 270 p. doi:10.5962/bhl.title.66317

13. The IUCN Red List of Threatened Species [Internet]. IUCN Red List of Threatened Species. [cited 2/11/2021]. Available from: https://www.iucnredlist.org/en

14. Davis AP, Chadburn H, Moat J, O'Sullivan R, Hargreaves S, Lughadha EN. High extinction risk for wild coffee species and implications for coffee sector sustainability. Science Advances [Internet]. 2019;5(1):eaav3473. Available from: https://www.science.org/doi/10.1126/sciadv.aav3473

15. Krishnan S. Current status of coffee genetic resources and implications for conservation. CAB Reviews [Internet]. 2013;8(16):1-9. Available from: https://www.researchgate.net/profile/Sarada-Krishnan-3/publication/265160327_Current_status_of_coffee_genetic_resources_and_implications_for_conservation/links/540088710cf2bba34c1a36cc/Current-status-of-coffee-genetic-resources-and-implications-for-conservation.pdf

16. Labouisse J-P, Bellachew B, Kotecha S, Bertrand B. Current status of coffee (*Coffea arabica* L.) genetic resources in Ethiopia: implications for conservation. Genetic Resources and Crop Evolution [Internet]. 2008;55(7):1079. Available from: https://link.springer.com/article/10.1007%2Fs10722-008-9361-7

17. Gole TW, Denich M, Teketay D, Vlek PLG. Human impacts on the *Coffea arabica* genepool in Ethiopia and the need for its *in situ*. conservation'in JMM Engels, V. Ramanatha Rao, AHD Brown and MT Jackson (eds), Managing Plant Genetic Diversity. Rome: International Plant Genetic Resources

Institute (IPGRI) [Internet]. 2002; Available from: https://www.cabi.org/cabebooks/ebook/20023003589

18. Aerts R, Geeraert L, Berecha G, Hundera K, Muys B, De Kort H, et al. Conserving wild Arabica coffee: Emerging threats and opportunities. Agriculture, Ecosystems & Environment [Internet]. 2017;237:75-9. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0167880916306053

19. Moat J, Gole TW, Davis AP. Least Concern to Endangered: Applying climate change projections profoundly influences the extinction risk assessment for wild Arabica coffee. Global change biology [Internet]. 2019;25(2):390-403. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.14341

20. Lilieholm RJ, Weatherly WP. Kibale forest wild coffee: challenges to market-based conservation in Africa. Conservation Biology [Internet]. 2010;24(4):924-30. Available from: https://conbio.onlinelibrary.wiley.com/doi/abs/10.1111/j.1523-1739.2010.01527.x

21. Dulloo ME, Guarino L, Engelmann F, Maxted N, Newbury JH, Attere F, et al. Complementary conservation strategies for the genus Coffea: A case study of Mascarene Coffea species. Genetic Resources and Crop Evolution [Internet]. 1998;45(6):565-79. Available from: https://www.researchgate.net/profile/Mohammad-Dulloo/publication/226464325_Complementary_conservation_strategies_for_the_genus_Coffea_A_case_study_of_Mascarene_Coffea_species/links/54e3b41c0cf2b2314f5e8e0d/Complementary-conservation-strategies-for-the-genus-Coffea-A-case-study-of-Mascarene-Coffea-species.pdf

22. Vega FE, Ebert AW, Ming R. Coffee germplasm resources, genomics, and breeding [Internet]. 2008. Available from: https://worldveg.tind.io/record/22062/

23. Anthony F, Dussert S, Dulloo E. Coffee genetic resources. Complementary strategies for ex situ conservation of coffee [Internet]. 2007;12-22. Available from: https://core.ac.uk/download/pdf/39840068.pdf#page=23

24. Hong TD, Ellis RH. Optimum air-dry seed storage environments for arabica coffee. Seed Science and Technology [Internet]. 1992;20(3):547-60. Available from: http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=CAFE.xis&method=post&formato=2&cantidad=1&expresion=mfn=005738

25. Eira MT, Silva EA, De Castro RD, Dussert S, Walters C, Bewley JD, et al. Coffee seed physiology. Brazilian Journal of Plant Physiology [Internet]. 2006;18(1):149-63. Available from: https://www.scielo.br/j/bjpp/a/zZgRS3Mbwg8KjW8R9bn9X3L/?lang=en

26. Aleman E, Aguiler C, Olmedo J, Vega M, Boix Y, Dubois A. Effect of electromagnetic field on mineral content and chemical group during in vitro establishment amultiplication phases of coffee seedling. Rev. Cubana Quim [Internet]. 2016;28(2):692-702. Available from: http://scielo.sld.cu/scielo.php?pid=S2224-54212016000200012&script=sci_abstract&tlng=en

27. García-Águila L, de Feria M, Acosta K. Aspectos básicos de la conservación *in vitro* de germoplasma vegetal. Biotecnología Vegetal [Internet]. 2007;7(2). Available from: https://revista.ibp.co.cu/index.php/BV/article/view/359

28. González-Arnao MT, Dolce N, González-Benito ME, Martínez CRC, Cruz-Cruz CA. Approaches for *in vitro* conservation of woody plants germplasm. In: Biodiversity and Conservation of Woody Plants [Internet]. Springer; 2017. p. 355-419. Available from: https://link.springer.com/chapter/10.1007/978-3-319-66426-2_13

29. Morales MMB. Conservación *in vitro*: una perspectiva para el manejo de los Recursos Fitogenéticos. Revista de Investigación Agraria y Ambiental [Internet]. 2015;6(1):67-82. Available from: https://hemeroteca.unad.edu.co/index.php/riaa/article/view/1264

30. Castilla Valdés Y. Conservación de recursos fitogenéticos de cafeto (*Coffea* spp.) por métodos biotecnológicos: una alternativa para su preservación. Cultivos Tropicales [Internet]. 2012;33(4):29-39. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362012000400004&script=sci_arttext&tlng=pt

31. Jouve L, Engelmann F, Charrier A. Effets de l'hypoxie et de la temperature sur la conservation *in vitro* de pousses feuillées de *Coffea arabica* L. Café, Cacao, Thé (Francia) v. 35 (3) p. 205-210 [Internet]. 1991; Available from: https://www.researchgate.net/profile/Jouve-Laurent/publication/32979019_Effets_de_l'hypoxie_et_de_la_temperature_sur_la_conservation_in_vitro_de_pousses_feuillees_de_Coffea_arabica_L/links/02e7e51c0146eab293000000/Effets-de-lhypoxie-et-de-la-temperature-sur-la-conservation-in-vitro-de-pousses-feuillees-de-Coffea-arabica-L.pdf

32. Nassar AH. Slow growth storage of encapsulated germplasm of *Coffea arabica* L. Int J Agric Biol [Internet]. 2003;5:517-20. Available from: http://www.fspublishers.org/published_papers/68819_..pdf

33. Bertrand-Desbrunais A, Noirot M, Charrier A. Slow growth *in vitro* conservation of coffee (*Coffea* spp.). Plant Cell, Tissue and Organ Culture [Internet]. 1992;31(2):105-10. Available from: https://www.researchgate.net/publication/226595441_Slow_growth_in_vitro_conservation_of_coffee_Coffea_spp

34. Jouve L, Engelmann F, Noirot M, Charrier A. Evaluation of biochemical markers (sugar, proline, malonedialdehyde and ethylene) for cold sensitivity in microcuttingsof two coffee species. Plant Science [Internet]. 1993;91(1):109-16. Available from: https://www.sciencedirect.com/science/article/abs/pii/0168945293901945

35. González ME, Castilla Y, Hernández MM, Hernández A. Factibilidad del cultivo *in vitro* en la conservación de recursos fitogenéticos de cafeto. Revista Agrotecnia de Cuba [Internet]. 2007;31:4-7. Available from: https://www.grupoagricoladecuba.gag.cu/media/Agrotecnia/pdf/2007/Revista2/19.pdf

36. Kartha KK, Mroginski LA, Pahl K, Leung NL. Germplasm preservation of coffee (*Coffea arabica* L.) by in vitro culture of shoot apical meristems. Plant Science Letters [Internet]. 1981;22(4):301-7. Available from: https://www.sciencedirect.com/science/article/abs/pii/0304421181900754

37. Bertrand-Desbrunais A, Noirot M, Charrier A. Minimal growth *in vitro* conservation of coffee (*Coffea* spp.). Plant Cell, Tissue and Organ Culture [Internet]. 1991;27(3):333-9. Available from: https://www.researchgate.net/profile/Andre-Charrier/publication/225984801_Minimal_growth_in_vitro_conservation_of_coffee_Coffea_spp/links/541a9e030cf203f155ae3e10/Minimal-growth-in-vitro-conservation-of-coffee-Coffea-spp.pdf

38. Naidu MM, Sreenath HL. *In vitro* culture of coffee zygotic embryos for germplasm preservation. Plant cell, tissue and organ culture [Internet]. 1998;55(3):227-30. Available from: https://link.springer.com/article/10.1023/A:1006194405702

39. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum [Internet]. 1962;15(3):473-97. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1399-3054.1962.tb08052.x

40. Sangeetha N, Sankar R, Mercy S, Kumari AHS, Kavitha M, Ganesh D. *In vitro* conservation of zygotic embryos of *Coffea arabica* L. Journal of Plantation Crops [Internet]. 2010;38(1):82-6. Available from: https://www.cabdirect.org/cabdirect/abstract/20113073585

41. Castilla-Valdés Y, González-Vega ME, Espinosa-Torres L. Conservación *in vitro* de cafeto (*Coffea arabica* L.) mediante la disminución de sales minerales en el medio de cultivo. Cultivos Tropicales [Internet]. 2020;41(1). Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362020000100004

42. González-Arnao MT, Engelmann F. Consideraciones teóricas y prácticas para la crioconservación de germoplasma vegetal. en América Latina y el Caribe [Internet]. 2013;37. Available from: https://www.researchgate.net/profile/Maria-Gonzalez-Arnao/publication/280638888_Consideraciones_teoricas_y_practicas_para_la_crioconservacion_de_germoplasma_vegetal/links/5613f6ac08ae4ce3cc636447/Consideraciones-teoricas-y-practicas-para-la-crioconservacion-de-germoplasma-vegetal.pdf

43. Popova E, Shukla M, Kim HH, Saxena PK. Plant cryopreservation for biotechnology and breeding. In: Advances in plant breeding strategies: breeding, biotechnology and molecular Tools [Internet]. Springer; 2015. p. 63-93. Available from: https://link.springer.com/chapter/10.1007/978-3-319-22521-0_3

44. Mari S, Engelmann F, Chabrillange N, Huet C, Michaux-Ferrière N. Histo-cytological study of apices of coffee (*Coffea racemosa* and C. sessiliflora) in vitro plantlets during their cryopreservation using the encapsulation-dehydration technique Coffee genetic resources and breeding: recent scientific papers [Internet]. ORSTOM, Montpellier (Francia). Laboratoire de Ressources Génétiques et …; 1994.

Available from: https://iifiir.org/en/fridoc/histo-cytological-study-of-apices-of-coffee-coffea-racemosa-and-c-104173

45. Bertrand-Desbrunais A, Fabre J, Engelmann F, Dereuddre J, Charrier A. Reprise de l'embryogenèse adventive à partir d'embryons somatiques de caféier (*Coffea arabica* L.) après leur congélation dans l'azote liquide. Bulletin de la Société Botanique de France. Actualités Botaniques [Internet]. 1989;136(3-4):195-195. Available from: https://www.researchgate.net/profile/F-Engelmann/publication/32983244_Reprise_de_l'embryogenese_adventive_a_partir_d'embryons_somatiques_de_cafeier_Coffea_arabica_L_apres_leur_congelation_dans_l'azote_liquide/links/55e436b808ae2fac47215327/Reprise-de-lembryogenese-adventive-a-partir-dembryons-somatiques-de-cafeier-Coffea-arabica-L-apres-leur-congelation-dans-lazote-liquide.pdf

46. Mycock DJ, Wesley-Smith J, Berjak P. Cryopreservation of somatic embryos of four species with and without cryoprotectant pre-treatment. Annals of Botany [Internet]. 1995;75(4):331-6. Available from: https://academic.oup.com/aob/article-abstract/75/4/331/166928

47. Martínez M, González-Arnao MT, Urra C, Rojas R, Cuba M. Estudios preliminares sobre la crioconservación de callos embriogénicos y semilla botánica de *Coffea arabica* variedad 9722. Cultivos Tropicales [Internet]. 1995;16(3):74-6. Available from: https://ftp.inca.edu.cu/revista/1995/3/CT16317.pdf

48. Tessereau H, Florin B, Meschine MC, Thierry C, Pétiard V. Cryopreservation of somatic embryos: a tool for germplasm storage and commercial delivery of selected plants. Annals of Botany [Internet]. 1994;74(5):547-55. Available from: https://academic.oup.com/aob/article-abstract/74/5/547/2587365

49. Hatanaka T, Yasuda T, Yamaguchi T, Sakai A. Direct regrowth of encapsulated somatic embryos of coffee (*Coffea canephora*) after cooling in liquid nitrogen. Cryo-Letters (RU) v. 15 (1) p. 47-52 [Internet]. 1994; Available from: https://iifiir.org/en/fridoc/direct-regrowth-of-encapsulated-somatic-embryos-of-coffee-coffea-98610

50. Normah MN, Vengadasalam M. Effects of moisture content on cryopreservation of Coffea and Vigna seeds and embryos. Cryo Letters (Suiza) v. 13 (3) p. 199-208 [Internet]. 1992; Available from: http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=orton.xis&method=post&formato=2&cantidad=1&expresion=mfn=045366

51. Abdelnour-Esquivel A, Villalobos V, Engelmann F. Cryopreservation of zygotic embryos of Coffea spp. CryoLetters [Internet]. 1992;13(5):297-302. Available from: https://core.ac.uk/download/pdf/39860212.pdf

52. Martínez M, González-Arnao MT, Urra C, Rojas R, Cuba M. Estudios preliminares para la crioconservación de embriones cigóticos de *Coffea arabica* variedad 9722. Comunicación corta.

Cultivos Tropicales (Cuba) v. 17 (1) p. 79-81 [Internet]. 1996; Available from: http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=orton.xis&method=post&formato=2&cantidad=1&expresion=mfn=047171

53. Castilla Y, Dussert S, González ME, Engelmann F. Crioconservación de embriones cigóticos de *Coffea canephora* mediante vitrificación: primeras aproximaciones. In: Memorias del VIII Congreso Internacional de Biotecnología Vegetal, Bioveg [Internet]. 2011. Available from: https://www.researchgate.net/publication/282769616_Crioconservacion_de_embriones_cigoticos_de_Coffea_canephora_mediante_vitrificacion_primeras_aproximaciones

54. Becwar MR, Stanwood PC, Leonhardt KW. Dehydration effects on freezing characteristics and survival in liquid nitrogen of desiccation-tolerant and desiccation-sensitive seeds. Journal of the American Society for Horticultural Science [Internet]. 1983;108(4):613-8. Available from: https://www.cabdirect.org/cabdirect/abstract/19830317155

55. Dussert S, Chabrillange N, Engelmann F, Anthony F, Hamon S. Cryopreservation of coffee (*Coffea arabica* L) seeds: importance of the precooling temperature. Cryo-Letters [Internet]. 1997;18(5):269-76. Available from: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/pleins_textes_6/b_fdi_47-48/010011490.pdf

56. Dussert S, Chabrillange N, Vasquer N, Engelmann F, Anthony F, Guyot A, et al. Beneficial effect of post-thawing osmoconditioning on the recovery of cryopreserved coffee (*Coffea arabica* L.) seeds. Cryo letters [Internet]. 2000;21(1):47-52. Available from: https://pubmed.ncbi.nlm.nih.gov/12148064/

57. Vasquez N, Salazar K, Anthony F, Chabrillange N, Engelmann F, Dussert S. Variability in response of seeds to liquid nitrogen exposure in wild coffee (*Coffea arabica* L.). Seed Science and Technology [Internet]. 2005;33(2):293-301. Available from: https://www.ingentaconnect.com/content/ista/sst/2005/00000033/00000002/art00003

58. Dussert S, Chabrillange N, Rocquelin G, Engelmann F, Lopez M, Hamon S. Tolerance of coffee (*Coffea* spp.) seeds to ultra-low temperature exposure in relation to calorimetric properties of tissue water, lipid composition, and cooling procedure. Physiologia Plantarum [Internet]. 2001;112(4):495-504. doi:10.1034/j.1399-3054.2001.1120406.x

59. Coelho SVB, Rosa S, Fernandes JS. Cryopreservation of coffee seeds: A simplified method. Seed Science and Technology [Internet]. 2017;45(3):638-49. Available from: https://www.ingentaconnect.com/content/ista/sst/2017/00000045/00000003/art00010

60. Figueiredo MA de, Coelho SVB, Rosa SDVF da, Vilela AL, Silva LC. Exploratory studies for cryopreservation of *Coffea arabica* L. seeds. Journal of Seed Science [Internet]. 2017;39:150-8. Available from: https://www.scielo.br/j/jss/a/XYFbP89j9D8GmNXdgMMFL3J/?lang=en&format=html

61. Coelho SVB, Rosa SDVF da, Fantazzini TB, Baute JL, Silva LC. Cryopreservation in *Coffea canephora* Pierre seeds: Slow and fast cooling. Ciência e Agrotecnologia [Internet]. 2018;42(6):588-97. Available from: https://www.scielo.br/j/cagro/a/QSbWQWb4J4mvHQT6vSp9NPD/abstract/?lang=en

62. Coelho SVB, Sttelada Rosa DVF, Fantazzini TB, Silva LC. Cryopreservation of *Coffea canephora* Pierre ex A. Froehner seeds: importance of drying rate and moisture content. Australian Journal of Crop Science [Internet]. 2019;13(8):1335-42. Available from: https://search.informit.org/doi/abs/10.3316/informit.755572688870935

63. Ricaldoni MA, Rosa SDVF da, Figueiredo MA de, Carvalho MA de F, Fantazzini TB. Avaliação de parâmetros de desenvolvimento de mudas de *Coffea arabica* L. originadas de sementes criopreservadas. 2019; Available from: http://tot.dti.ufv.br/handle/123456789/12611

64. Pinto M de S, Paiva R, Silva DPC da, Santos PAA, Freitas RT de, Silva LC. Cryopreservation of coffee zygotic embryos: dehydration and osmotic rehydration. Ciência e Agrotecnologia [Internet]. 2016;40:380-9. Available from: https://www.scielo.br/j/cagro/a/XD48FV5D7xrSfXmt34kNMxj/abstract/?lang=en

65. Sakai A, Kobayashi S, Oiyama I. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. brasiliensis Tanaka) by vitrification. Plant Cell Reports [Internet]. 1990;9(1):30-3. Available from: https://link.springer.com/article/10.1007/BF00232130

66. de FREITAS RT, Paiva R, Sales TS, da SILVA DPC, de SOUZA AC. Cryopreservation of *Coffea arabica* L. zygotic embryos by vitrification. Notulae Botanicae Horti Agrobotanici Cluj-Napoca [Internet]. 2016;44(2):445-51. Available from: https://www.notulaebotanicae.ro/index.php/nbha/article/view/10553

67. Nishizawa S, Sakai A, Amano Y, Matsuzawa T. Cryopreservation of asparagus (*Asparagus officinalis* L.) embryogenic suspension cells and subsequent plant regeneration by vitrification. Plant Science [Internet]. 1993;91(1):67-73. Available from: https://www.sciencedirect.com/science/article/abs/pii/0168945293901897

68. Valdés YC, Shukla MR, Vega MEG, Saxena PK. Improved Conservation of Coffee (*Coffea arabica* L.) Germplasm via Micropropagation and Cryopreservation. Agronomy [Internet]. 2021;11(9):1861. Available from: https://www.mdpi.com/2073-4395/11/9/1861