



# **Germplasm Conservation: Instrumental in Agricultural Biodiversity—A Review**

Veerala Priyanka<sup>1</sup>, Rahul Kumar<sup>1</sup>, Inderpreet Dhaliwal<sup>2</sup> and Prashant Kaushik<sup>3,\*</sup>

- <sup>1</sup> Department of Genetics and Plant Breeding, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut 250110, India; veeralapriyanka@gmail.com (V.P.); rahul.gujjar.3009@gmail.com (R.K.)
- <sup>2</sup> Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, India; dhaliwalinderpreet@gmail.com
- <sup>3</sup> Instituto de Conservacióny Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, 46022 Valencia, Spain
- \* Correspondence: prakau@doctor.upv.es; Tel.: +34-963-877-000

Abstract: Germplasm is a valuable natural resource that provides knowledge about the genetic composition of a species and is crucial for conserving plant diversity. Germplasm protection strategies not only involve rescuing plant species threatened with extinction, but also help preserve all essential plants, on which rests the survival of all organisms. The successful use of genetic resources necessitates their diligent collection, storage, analysis, documentation, and exchange. Slow growth cultures, cryopreservation, pollen and DNA banks, botanical gardens, genetic reserves, and farmers' fields are a few germplasm conservation techniques being employed. However, the adoption of in-vitro techniques with any chance of genetic instability could lead to the destruction of the entire substance, but the improved understanding of basic regeneration biology would, in turn, undoubtedly increase the capacity to regenerate new plants, thus expanding selection possibilities. Germplasm conservation seeks to conserve endangered and vulnerable plant species worldwide for future proliferation and development; it is also the bedrock of agricultural production.

Keywords: germplasm; plant genetic resources; preservation; propagation; in vitro

## 1. Introduction

Humans comprehended the economic utility of plants and initiated domestication of wild species about 10,000 years ago. They started saving seeds or vegetative propagules of plants from one season to the next, even while migrating from place to place. The art of seed conservation was taught and enacted in parts of India and China as far back as 700 BC. This has been a crucial factor in the development of agriculture throughout the world and for the introduction of genetic variability into populations through natural hybridizations with wild and weedy relatives, coupled with spontaneous mutations. To ensure nutritional and economic security, mankind is reliant on the continuous availability of a diverse pool of plant genetic resources for agriculture. Capturing natural and existing genetic diversity through pre-breeding with crop wild relatives (CWRs) is critical for global food security. Their natural selection in the wild accumulated a rich set of useful variations that can be introduced into crop plants by crossing, providing a base for further changes. The CWRs not only constitute a valuable germplasm resource for improving agricultural production but are also central for maintaining sustainable agro-ecosystems. Therefore, an understanding of the pattern of variation and the places of its existence is imperative for conserving and utilizing germplasm resources.

The sum total of all allelic sources that influence a range of traits of a crop constitutes its plant genetic resources (PGRs) and germplasm is the genetic material passed from one generation to the next [1]. This genetic diversity may have been drawn from related wild plant species, that are direct or distant ancestral predecessors of cultivated



Citation: Priyanka, V.; Kumar, R.; Dhaliwal, I.; Kaushik, P. Germplasm Conservation: Instrumental in Agricultural Biodiversity—A Review. *Sustainability* **2021**, *13*, 6743. https:// doi.org/10.3390/su13126743

Academic Editor: Alejandro Rescia

Received: 18 April 2021 Accepted: 10 June 2021 Published: 15 June 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). species, currently cultivated or domesticated or semi-domesticated cultivars as well as their component cultivars that are currently in use or have become obsolete, or landraces or historic varieties [2]. Despite their existence, significant hurdles are faced in mobilizing these allelic resources for effective and sustainable use [3]. Even though many gene banks now exist worldwide, only about 30 countries have opted for safe long-term storage of their germplasm in them because of a shortage of long-term maintenance provisions for such gene banks [4]. Further, the 7.5 million accessions in these gene banks are primarily crops on which humans and animals rely for food and nutrition, including their diversified wild relatives and landraces. Still, there are locally important crops and underutilized species that need to be protected [5].

Germplasm conservation helps preserve knowledge about extinct, wild, and other living species of a crop plant since human interference has led to the erosion of genetic diversity by increasing favored genes and totally eliminating the less desirable, effecting the extinction of the historic genetic material. It is mainly concerned with ensuring the secure handling and proper preservation of germplasm of commercially valuable plants by collecting each taxon's propagules [6]. Plant breeding and habitat regeneration of ecosystems for livestock, horticulture, and forestry are a few applications of germplasm protection and include PGRs for food and agriculture (PGRFA) and PGRs for non-food utilization such as medicinal plant species, wood and fuel plant species, ornamental species, and recreation and amenity species (Figure 1). However, the utilization of available genetic resources for crop improvement is being neglected [7]. There is a significant gap between actual germplasm utilization and the number of collections available in gene banks for any given crop species [8,9]. The very aim of establishing vast germplasm collections is thus negated as plant breeders still extensively use fewer and closely related parents and their derivatives in crop improvement programs [10]. Introgression of desirable attributes from wild relatives to high yielding cultivars is one way of developing climate-resilient crops that are better adjusted to particular growing conditions [11]. Although the germplasm accessions seem to be genotypic duplicates, they are indispensable tools for studying plant development and gene functions [12].



Figure 1. Overall representation of global plant genetic diversity.

## 2. Conservation of Plant Genetic Resources: A Brief History

Alphonse de Candolle, a botanist, was the first to attempt and locate the origin of crop plants and his work is published as a book titled 'Origin of Cultivated Plants' in 1882, reprinted in 1959. N.I. Vavilov, a Russian explorer, geneticist, and plant breeder, was the first to explore and recognize the diversity present in crop plants. In 1926, he proposed the concept of 'centers of origin' of crop plants, which may be defined as a geographical area that has the maximum genetic diversity for a crop, and identified eight distinct centers of origin of crop plants (1951) [13]. He further observed that for some crops, the centers of diversity did not include their wild relatives and explained this pattern in the form of a distinction between primary center (a geographical region where a crop originated and had maximum diversity) and secondary center (wild relatives of crops migrated to other places from their center of origin where they were domesticated and evolved independently). In 1965, Zhukovsky [14] further modified the Vavilovian centers of origin into eight mega gene centers of crop diversity and four micro gene centers of crop wild relatives. However, Harlan (1970) contested that the centers of origin of some crops are so diffused in time and space that this problem can never be solved. Therefore, Harlan and De Wet (1971) [15] gave the concept of gene pools. They categorized the whole genetic variation at different levels as primary, secondary, and tertiary gene pools based on the degree of relationship between species, which is less taxonomical but very helpful in crop improvement:

- (i) The primary gene pool (GP1): Crossing among individuals is possible with normal seed set, segregation, and recombination such that gene transfer is possible through routine breeding. It includes both cultivated and wild races of a crop.
- (ii) Secondary gene pool (GP2): It includes biological species which have some barriers of crossability with the crop (GP1), resulting in sterile hybrids, as chromosome pairing is not normal; hence, transfer of genes is restricted. Overcoming barriers of crossability can lead to normal seed development
- (iii) Tertiary gene pool (GP3): More distant to GP2, crosses of GP3 with a crop (GP1) result in lethal or sterile hybrids due to abnormality in embryo development. Normal gene transfer is not possible but special tissue culture techniques can be deployed to produce hybrid embryos.

With technology developments in genetic engineering, the concept of a fourth gene pool (GP4), 'the Gene Ocean', has been made available, which has enabled the transfer of genes from different kingdoms into one another through recombinant DNA technology.

In 1948, the International Union for Conservation of Nature (IUCN) was established as the first global environmental union that brought together governments and civil society organizations for the protection of nature. However, the genetic security of crops gained worldwide momentum in the 1960s, when FAO (1961) organized the first international technical meeting on plant exploration and introduction. The Crop Ecology and Genetic Resources Unit of FAO (established in 1968) held international technical conferences in cooperation with the International Biological Program (established in 1963) that emphasized locating the germplasm, its survey, classification, evaluation, preservation, documentation, and coordination for the management of PGRs at international level. The technical advisory committee of Consultative Group on International Agricultural Research (CGIAR) recommended the creation of network of crop specific regional genetic resource centers. The Rockefeller Foundation and World Bank together established International Agricultural Research Centers (IARCs) in many developing countries, which have also established their own germplasm collections. Realizing the danger of genetic resources being eroded, the United Nations Conference on Human Environment, held in Stockholm in 1972, recommended the conservation of habitats that are rich in genetic diversity. In 1974, the International Board of Plant Genetic Resources (IBPGR) was established at FAO headquarters in Rome, with a specific mandate to promote and coordinate an international network of genetic resource centers for collecting, conserving, documenting, evaluating, and using plant germplasm for the welfare of people [16]. In 1992, after a decade of including research and training into its mandate, this international gene bank organization

was transformed into an autonomous organization by the name of International Plant Genetic Resources Institute (IPGRI). Many countries engaged in plant breeding activities have established their own national plant genetic resource management organizations in sync with the policies and programs of IPGRI such as the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, that monitors the activities related to plant genetic resources in India.

The 1992 Convention on Biological Diversity (CBD) gave a boost to the conservation of PGRs, as it aimed at the "conservation and sustainable use of biological diversity and fair and equitable sharing of benefits arising out of the utilization of genetic resources". The1996 FAO Global Plan of Action for Conservation and Sustainable Use of PGRFA aimed to "ensure the conservation of plant genetic resources for food and agriculture as the basis of food security, promote sustainable use of plant genetic resources to foster development and reduce hunger and poverty, promote fair and equitable sharing of benefits arising from the use of PGRs, assist countries and institutions to identify priorities for action, strengthen existing programs and enhance institutional capacity" [17]. In 2001, the International Treaty on Plant Genetic Resources for Food and Agriculture was signed by 136 countries for the conservation and sustainable use of PGRs for food and agriculture and the fair and equitable sharing of benefits derived from their use, in harmony with CBD for sustainable agriculture and food [18]. The Global Strategy for Plant Conservation (GSPC) became a CBD weapon in the early 2000s, when it declared a "positive, sustainable future in which human activities promote the diversity of plant life" [19]. By 2020, the initiative intended to keep a meager 75% of endangered plant species in ex situ stocks, feasibly in their native habitats, and at least 20% under biodiversity conservation drives. About 70% of plant genetic diversity, including wild relatives and few economic species, has been retained for honoring, protecting, and achieving sustainability [20]. The Nagoya Protocol of the CBD is an additional arrangement that encourages the signatories to share the rewards of using genetic resources equally [21].

#### 3. Need for Germplasm Conservation: Genetic Erosion and Genetic Vulnerability

Each crop enhancement program is aimed at increasing production which narrows down the genetic diversity. Harlan (1931) outlined the limited diversity available in barley [22]. Similarly, Vavilov also chronicled the shrinking crop diversity due to modern agriculture breeding approaches. Since then, scientists have been concerned about the eroding genetic diversity and have realized that CWRs and landraces are a rich source of essential variability. Assessing the genetic loss in cereals [23-25] led to the conclusion that cultivated crops have become less varied after domestication, due to selection pressures and dispersal bottlenecks [26]. Guarino refers to genetic erosion as a "loss of individual genes or combinations of genes, such as those found in locally adapted landraces, over time in a given region, and persistent reduction of common localized alleles" [27]. The definition suggests that a significant event in genetic erosion is the number and frequency of depletion of regionally adapted specific alleles. When geographical diversity reduces, the overall gene pool becomes more vulnerable to depletion and extinction, thereby reducing global equality and wealth [28]. According to the FAO, the key causes of genetic erosion are the direct replacement of local varieties, overexploitation of species, overgrazing, reduced fallow and changing agricultural systems, indirect land clearing, population pressure, environmental degradation, legislation/policy change, pest/weed/disease infestation, civil strife, and climate change making the PGRs more vulnerable to extinction. Plant species are also deemed endangered due to sudden changes in environmental conditions. They are either few in number or at risk of extinction [29]. It has been reported that about 12.5% (34,000 species) of vascular plants worldwide have been at threat (Table 1).

Category	Status
EX—Extinct	122
EW—Extinct in the wild	42
CR—Critically endangered	4674
EN—Endangered	8593
VU—Vulnerable	8459
LR/cd—Lower risk: Conservation dependent	157
NT or LR/nt—Near threatened	3181
LC or LR/lc—Least concern	24,810
DD—Data deficient	4090

Table 1. International Union for Conservation of Nature (IUCN) Red List for the year 2019–2020 [17].

A reduction in diversity may not generally lead to genetic erosion on a comprehensive scale in a certain region. A study on Australian wheat reported no national shift in diversity, although in some countries, the genetic base of wheat has narrowed [30]. A parallel study on barley reported a decrease in allelic diversity in some of the surveyed countries in the Baltic region although overall diversity was preserved [31].

## Global Germplasm Conservation Programs

The conservation of germplasm, in response to the CBD and GSPC, particularly in seed banks, has not only improved conventional germplasm preservation for major crop species, but has also conquered concerns regarding habitat destruction, climate change, and genetic erosion [20]. Germplasm preservation has now been expanded to include non-crop species, including native flora, such as CWRs and threatened species. The Global Crop Trust supports main crops in over 80 countries and has established a 'backup' seed bank underneath the Arctic ice in Svalbard, Norway [32]. The Millennium Seed Bank Partnership (MSBP) has played a pivotal role in establishing a global network for the conservation of native plants (including CWRs) and capacity building [33]. The main strength of such cooperative programs is the duplication of collections that ensure a backup if one gene bank fails. For example, because of the civil war in Syria, ICARDA seed bank collections were not accessible to plant breeders in 2015 [18]. The backup collections at Svalbard were made available to start cereal cultivation activities in dry-arid regions. A self-explanatory general pipeline of any conservation program is given in Figure 2. The curator mainly identifies and streamlines the possibility of duplicate accessions (Duplicates), which are an elementary concern for germplasm conservation. For instance, the ICAR-NBPGR created a software package "PGR dup" in the R environment, which works on passport information to exclude duplicate accessions from the existing gene bank collection [34]. On the other hand, duplication of genetically identical subsamples of accessions (Safety Duplicates) reduces the potential of moderate to severe destruction from natural or manmade disasters. These are also referred to as the second most original sample [35] that includes both the duplication of content and its relevant information and are deposited in the base collection at various locations, probably in another country.



Figure 2. General pipeline of a germplasm conservation program.

#### 4. Methods of Germplasm Storage and Conservation

Accessions are generally stored as different kinds of collections. Core collections [36] serve as an initial point for efficient germplasm utilization in crop breeding and refer to a subset of the base (large) collection or a limited number of accessions from an existing large collection of germplasm [37]. The core collection is used as a working collection and is closely reviewed, while reserve collections are accessions that do not form part of the core collection [38]. The vast number of base collections and a lack of accurate data on their economically important characteristics explain the underuse of genetic resources. ICARDA has made a core hybrid collection of 1000 entries of barley which reflects the genetic wealth of the entire world [39,40]. The two fundamental storage approaches, ex situ and in situ conservation [41], employed for germplasm storage are explained in Figure 3. PGRFAs need ex situ protection for safety from their natural environments. Samples may be stored as live plant specimens in field gene banks/botanic gardens/arboreta and can also be conserved as seed/pollen/explants/DNA in specialized artificial environments [42]. On the other hand, in situ conservation entails on-site survival of the species in its natural habitat ensuring sustainability of the environment and ecosystem, and in case of domesticated or cultivated species, storage within the ecosystem under which their distinctive characteristics developed.

#### 4.1. Ex Situ Conservation

Ex situ management is a straight forward, cost-effective approach that involves regular material viability testing and timely recovery, depending on the crop and its reproductive biology [43]. The collection of wild species in seed banks are projected to play a key role in preserving and restoring biodiversity [44]. It is necessary to efficiently manage these collections to obtain adequate viable seeds for their optimal utilization. The Global Crop Diversity Trust (GCDT) plays a key role in improving ex situ conservation techniques and managing global crop diversity [45]. As per their storage capacity, seeds are divided into two classes [46]:

- (i) Orthodox seeds: Such seeds can tolerate drying (5% RH) and freezing (very low temperatures) but remain viable. The vast majority of plants fall into this category whose seeds can thus be easily preserved for long periods of time [47]. Examples include citrus, guava, capsicum, cashew, and most grains and legumes.
- (ii) Recalcitrant seeds: Such seeds cannot tolerate drying and freezing. They lose viability significantly if the moisture content goes below 30–50%. Examples include a number of tropical trees and fruits, such as pineapples, cocoa, coffee, oil palm, mango, jackfruit, etc. Such seeds can be stored at temperatures of 0–10 °C briefly (1 to 5 years) while retaining their viability.



Figure 3. Schematic outline of germplasm conservation approaches.

Generally, for every decrease of 1% seed moisture content, the life of the seed doubles; this rule is applicable when moisture content is 5–14%. For every decrease of 5 °C in storage temperature, the life of seed doubles; this rule applies between 0 °C and 50 °C temperature [48].

The handling of a crop species depends upon whether the material being stored is seed or clone and on whether the seed is orthodox or recalcitrant. The conservation approaches that demand special techniques such as tissue culture/cold storage/liquid nitrogen storage/cryopreservation/in vivo conservation would cost much more compared to the storage of orthodox seed [49]. Another method of ex situ conservation is the desiccation and storage of embryos, but only by using somatic embryos and shoot tips [50].

#### 4.1.1. In Vitro Conservation

The behavior of storage for different species is still experimental [51–53]. In vitro conservation was first proposed in mid-1970 [54,55]. Vegetatively propagating species such as potato, species that do not produce viable seeds such as banana, or species that often produce recalcitrant seeds need to be preserved by other means [56]. For such species, in vitro plant conservation is the solution which utilizes the fundamentals of plant tissue culture involving the separation of a cell/tissue from the donor plant under aseptic conditions and producing it on a synthetic medium in a proper container under a controlled environment [57]. The tissue cultivation protocol for a test plant begins by searching for an already-known protocol of a plant in the same taxa and sharing near affinity due to common physiological and biochemical characteristics. For protection of such conserved species from viral infections, either meristem cultivation [58] or cryotherapy can be undertaken [59].

4.1.2. Methods Involved in the In Vitro Conservation of Germplasm

Among various in vitro conservation strategies, two have proven successful:

- (i) IVAG: In vitro active gene bank (in vitro conservation under slow growth) is widely used by a range of national and international research centers such as NBPGR, IITA (International Institute of Tropical Agriculture), and CIP (International Potato Centre) [60]. This technique can only be used as a short- to medium-term conservation strategy, meaning that it is impossible to preserve extensive collections using this process.
- (ii) Cryopreservation: This strategy makes use of solid carbon dioxide  $(-79 \degree C)$ , minimum temperature deep freezers (-80 °C), vapor nitrogen (-150 °C), or liquid nitrogen  $(-196 \,^{\circ}\text{C})$  for preserving cells and tissues in a frozen state at extremely low temperatures. The cell can be preserved for a prolonged period of time when it is inactivated at such low temperatures. Plant tissues that can be cryopreserved include meristems, eggs, endosperms, ovules, plants, plant cells, plant protoplasts, and calli [61]. Two advanced cryopreservation methods are employed that focus on the mitigation of cell damage caused by the production of ice crystals. One approach includes vitrifying cellular water with cryoprotective products, whereas the other involves encasing specimens in alginate gel and then dehydrating them. When a specimen is vitrified, a cryoprotective fluid is infused, facilitating the conversion to a non-crystalline vitreous solid of mostly cellular water [62]. Encapsulation involves embedding the specimen into an alginate gel [63], either in the form of a shoot tip or somatic embryo, to provide an artificial seed that is dehydrated. This procedure involves many actions, of which freezing, thawing, and reculturing are the most significant. Shoots, leaves, floral parts, immature embryos, hypocotyl bits, or cotyledons can be used as explants [64], thus requiring the establishment of systematic protocols. The cryopreservation of dormant buds and in vitro shoot tips is an alternate solution for long-term protection [65,66]. Progress is now underway in the perception of best practices for the cryopreservation of a range of commercially significant plants, including apples, grapes, and citrus [67,68]. For example, in some cases, it provides alternate ways to save entire species. Second, the transfer of germplasm is facilitated. Third, approaches to molecular biology are used to address germplasm control and use-related issues. The fourth impact stems from the growing demands of biotechnologists for germplasm and conservation resources. Biotechnological techniques, including in vitro culture, cryopreservation, and molecular markers, would be beneficial to plant diversity research and genetic resource control studies and in turn eventual restoration [69]. However, because of their high susceptibility to desiccation, systemic sophistication, and heterogeneity, it is far less sophisticated for recalcitrant seed species. Many scientific barriers prevent the use of cryopreservation regularly for plant meristems, pollens, and plant cells. Although many scientific collections and germplasm banks conduct cryopreservation experiments, none use cryopreservation for the storage of non-seed germplasm.

Other in vitro methods include:

(i) Slow-growing cultures: This is a viable alternative to cryopreservation as it is costeffective and straightforward and contamination through gene alterations are usually minimized [70]. Subculture cycles may be stretched up to 1 or 2 years, shortening the time, effort, and equipment needed to maintain them. Slower growth lessens the rate of cell division; thus, spontaneous mutation in culture is multiplied a number of times. Collections preserved under in vitro for slow growth are often susceptible to genetic instability and infection. All variables that affect culture development include temperature, nutritional constraint, growth regulation, and osmotic concentration. Other factors include oxygen concentration, the form of the propagation vessel used, and the light needed by cultures. In addition, stress variables may have different effects on the genotype of the population, some preferring somaclonal variants over others [71]. This could contribute to a cell population change and the genetic integrity of the original clonal material not being maintainable, as in shooting cultures of banana [72].

- (ii) DNA storage: Establishment of a DNA storage facility as a backup to traditional ex situ storage has been suggested [73] but is not widely used, since the use of stored genes for PGRFA is restricted because these need to be isolated, cloned, and then utilized through the production of a transgenic plant. DNA storage mainly complements germplasm conservation, as it forms a basis of genomic material that explains species origin or population diversity.
- (iii) Cold storage: This is a form of short-term storage, slow-growth preservation process where the germplasm is stored at a moderate, non-freezing temperature (1–9 °C). The prominent benefit of this method is that it accelerates plant growth in cold storage rather than stopping it during cryopreservation, so that plants are protected from cryogenic damage [74]. In addition, this technique is useful, inexpensive, and produces germplasm with higher rates of survival. Cold storage of the in vitro collection provides additional security while keeping the plants available for study or distribution. Many excellent reports exist on cold storage, such as virus-free strawberry plants that could be stored at 10 °C for around six years, certain grape plants that could be stored for about 15 years (by moving them to fresh medium every year) have recently been published [75].
- (iv) Low-pressure and low-oxygen storage: Under low-pressure storage (LPS), the atmospheric pressure surrounding the plant material is decreased which yields a partial reduction of the pressure exerted by the gases around the germplasm. The lowering of partial pressure decreases the in vitro growth of plants (of organized or unorganized tissues). Low-pressure storage systems are essential for both the short- and long-term storage of plant materials. The short-term storage is specifically useful to enhance the shelf life of many plant materials like fruits, vegetables, cut flowers, or plant cuttings. The storage of germplasm grown in cultures can be done long term under low pressure. In addition to germplasm preservation, LPS decreases the activity of pathogenic organisms and prevents spore germination in the plant culture systems. In low-oxygen storage, the oxygen concentration is decreased, but the atmospheric pressure (260 mm Hg) is maintained by the addition of inert gases (specifically nitrogen). There is a reduction in plant tissue growth if the partial pressure of oxygen is below 50 mm Hg. This is because, with less availability of  $O_2$ , the production of  $CO_2$ is low. As a result, the photosynthetic activity is decreased, thereby halting the plant tissue growth and dimension.

Seeds of many tropical and subtropical plants are recalcitrant and because of their shorter lifespan, it is difficult to save them for extended periods [76]. The seeds of coconut, cocoa, and several tree species are physiologically unripe, high in moisture content, cannot resist a lot of dehydration, are vulnerable to frost, and can only be preserved at low temperatures. Other species such as coffee and oil palm can be stored for a limited time only and indeed their long-term survival is not possible. For several limitations like seed dormancy, short life span, seed-borne diseases, and high cost of operation and labor, alternate storage strategies are necessary. Thus, modern in vitro techniques such as freezing the tissues and cells at -196 °C and cold storage were developed [77].

In vitro conservation provides various advantages like adaptability and stability in addition to freedom from the threat of contamination. Contamination of these cultures is influenced by various factors, including age: older tissues are more prone to viruses than younger ones [78]; position: the sterilization of underground tissues with high levels of endogenous contaminants is challenging [79]; complex tissue: in vegetative and floral buds, pathogens in complex tissue may protect even foreign microorganisms from surface sterilant [80]; and atmosphere: the environment may influence contamination [81,82].

### 4.1.3. Ex Situ Conservation in Field Gene Banks, Botanic Gardens, and Arboreta

The field gene banks have traditionally been responsible for conserving recalcitrant and vegetative species, such as fruit, tubers, and plantation crops. Germplasm is grown in field nurseries at varying levels above the sea depending on the species [83]. The whole plant collection can also be preserved using either of the two other ex situ management strategies, viz., botanical gardens and arboreta. These places have living specimens of plants for exhibitions or educational purpose, economic exploitation, or scientific use. Worldwide, there are about 1700 botanical gardens, with more than 3.2 million live accessions of 100,000 species. Ten to fifteen percent of these species are reported to be at risk in nature, with some form of conservation policy being deployed for saving about half of them [84]. The first botanical garden was founded in Pisa, Italy, in the 17th century and thereafter several others have served as study sites on plant taxonomy and horticultural development (Table 2).

**Table 2.** Major ex situ collections of crops and wild species held in gene banks throughout the world and the percentage of world germplasm they represent.

Crop	Number of Accessions	Major Gene Bank	Country	Percentage of World Germplasm Represented
Wheat (Triticum)	856,168	CIMMYT	Mexico	13
Rice (Oryza)	773,948	IRRI	Philippines	14
Maize (Zea)	327,932	CIMMYT	Mexico	8
Bean (Phaseolus)	261,963	CIAT	Colombia	14
Apple (Malus)	59,922	GEN (USA167)	USA	12
Palm (Elaeis)	21,103	INFRA	D.R. Congo	84
Medicago	91,922	AMGRC (AUS006)	Australia	30
Cacao (Theobroma)	12,373	ICGT	Trinidad	19

Source: FAO second report.

#### 4.2. In Situ Conservation

The genetic diversity of PGRFA is preserved in the natural world, whether in the wild or in a traditional agricultural or local environment [85]. The nature reserves and national parks/gene sanctuaries protect wildlife species/ecosystems/landscapes rather than individual PGRFAs. One of the strengths of in situ management is that it allows for species continuity while still evolving new recombinant types. The lack of protection in the absence of managed surveillance, the potential for multiple environmental contaminants to degrade the germplasm, and the high cost of retaining a huge number of genotypes are all disadvantages of in situ conservation [86–89]. Furthermore, the conserved substance is not immediately usable, and the longevity of the germplasm being conserved is unknown. Turkey claims to be the prime country in developing this kind of ideal strategy for protecting genetic diversity [90].

## 4.2.1. Natural Reserves or Genetic Reserves

The aim of the conservation process is to raise genetic diversity with a bare minimum number of genetic reserves. To do so, data on the target taxas' genetic diversity, population composition, breeding mechanism, habitat requirements, and geographical distribution are needed. The location, classification, maintenance, and monitoring of genetic diversity in a specific natural location should therefore be included in the conservation of the wild species component of the PGRFA. The basic model for establishing a natural reserve conservation involves planning the reserve, assessment of site and socioeconomic and political factors, design of the reserve, assessment of taxon and reserve sustainability, management plan formulation, managing and monitoring the reserve, initiation of the reserve management plan, establishment of the reserve, use of reserve traditionally or professionally, and linkage to ex situ conservation (complimentary) research programs and educational organizations. A comprehensive example of setting up and monitoring a natural reserve is provided by the Ammiad experiment in Israel that focuses on naturally occurring diversity in wild *T. turgidum* species [18].

## 4.2.2. On-Farm and Home Garden Conservation

Common crop varieties under various cropping schemes are maintained by farmers within traditional farming systems and form a part of these conservation techniques. Landraces, for example, are sown and harvested, and the farmer often saves a portion of the harvested seed for resowing in subsequent seasons. In this scenario, it is the farmer who is saving the germplasm, whether deliberately or accidentally. The conservationist can keep an eye on things but is not involved in the actual conservation [91]. While it is beneficial to preserve landraces in this manner, it is risky in the sense that farmers can still switch from evolving landraces to modern cultivars, and we thus lose an important resource for the future [92].

## 5. Status of Germplasm Conservation

By the end of 2019, gene banks worldwide held 5.43 million accessions [93] and only 5.8% of these accessions are retained as living field collections; the rest are cryopreserved and deposited as DNA [94]. Up to December 2019, 290 gene banks across the globe managed to safeguard 96,000 of around 1700 species with a critical concern for IUCN, including wild relatives of crops that are vital for domestic and global food stability (http://www.fao.org/sustainable-development-goals/indicators/251a/en/2020 (accessed on 15 March 2021)) [95]. The USDA-ARS National Plant Germplasm System is the world's largest provider of plant genetic capital, with 595,451 accessions covering 15,970 plants. However, the majority of them are annual species held as seeds, with the National Small Grains Set accounting for 25% of all accessions [96,97] while woody perennials are less represented [98].

The USDA collections at Geneva, New York, Davis, Central America, and Riverside hold 73% of all accessions, including economically important crops like apple, grape, kiwifruit, walnut, pomegranate, mandarin, almond, and other related plants [98]. All these principal collections of annual fruit crops are conserved at institutes that include the National Fruit Collection in the United Kingdom (http://www.nationalfruitcollection. org.uk/ (accessed on 15 March 2021)) [99], the N.I. Vavilov All-Russian Science Research Institute of Plant Industry's fruit collection (http://www.vir.nw.ru/unu-kollektsiya-vir/ (accessed on 15 March 2021)) [100], and the Foreign Centre for Research in Agronomy (http://www.vir.nw.ru/unu-kollekts (accessed on 15 March 2021)) [101,102]. The Crop Trust's CGIAR Gene bank Platform allows CGIAR gene banks to meet their fiduciary duties under the International Treaty on PGRFA to sustain and provide more accessions of crops and trees [103]. The 11 CGIAR gene banks are ideally situated as crop diversity hotspots, ensuring that germplasm acquisitions and distributions are global in scope, with a diverse range of partners and users [93] (Table 3) and the overall conservation trend depicted in Figure 4.



**Figure 4.** Representation of total conserved accessions among various gene banks since 2016–2019, where years are represented by *x*-axis and accession numbers by the *y*-axis.

In field gene banks across 44 countries, covering six geographic regions, the ICRAF platform alone has 11,000 accessions of 60 industrially valuable tree and nut species, mainly from Africa and Asia. Around one third of all recognized plant species (over 120,000) are found in botanical gardens worldwide [103,104]. Most botanical gardens began as medicinal plant collections or horticultural exhibits and since, many have developed into world-class research institutions dedicated to the preservation of global plant biodiversity [105]. In response to a request from the XVI International Botanical Congress to safeguard the world's endangered plant diversity, Botanic Gardens Conservation International (BGCI) was established in 2000 [106]. Millions of accessions worldwide can be found on online databases like Genesys (www.genesys-pgr.org (accessed on 15 March 2021)) [107], BGCI's Plant Search (https://www.bgci.org/plant search.php (accessed on 15 March 2021)) [108], and the FAO's Global Knowledge and Early Alert System on Plant Genetic Tools for Food and Agriculture (WIEWS) website (http://www.fao.org/wiews (accessed on 15 March 2021)) [109]. Forages are underrepresented in ex situ collections compared to food crops [110], with only about 182,000 accessions covering about 1000 species of grasses, legumes, and fodder trees disbursed in 80 national and international gene banks are enrolled in Genesys, compared to about 7.4 million plant accessions saved in around 1750 gene banks worldwide [111].

International Institutes	Number of Accessions under Corresponding Crops as Per 2019–2020	
IITA- International Institute of Tropical Agriculture, Ibadan, Nigeria (my.iita.org/accession2/ (accessed on 15 March 2021)) (https://www.genebanks.org/genebanks/iita/(accessed on 15 March 2021)) [112]	African Yam Bean-324, Groundnut-1890, Cassava-3184, Cowpea-15923, Maize-1561, Banana & Plantain-393, Soyabean-1575, Vigna-1878, Yam-5839	
CIAT- International Centre for Tropical Agriculture, Cali, Colombia (https://ciat.cgiar.org/ (accessed on 15 March 2021)) (https://www.genebanks.org/genebanks/ciat/(accessed on 15 March 2021)) [113]	Bean-37938, Cassava-6155, Forage-22694	
CIMMYT- International Maize and Wheat Improvement Centre, Mexico City, Mexico (https://www.genebanks.org/genebanks/cimmyt/(accessed on 15 March 2021)) [114]	Maize-28746, Wheat-155325	
CIP- International Potato Centre, Lima, Peru (https://www.genebanks.org/genebanks/international-potato-centre/ (accessed on 15 March 2021)) [115]	Andean roots and tubers-2526, Potato-7224, Sweet potato-8080	
ICARDA- International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria (https://www.genebanks.org/genebanks/icarda/(accessed on 15 March 2021) [116]	Barley-31392, Chickpea-13299, Fababean-8736, Forages-24632, Grasspea-3992, Lentil-13128, Pea-4159, Wheat-40,843	
ICRISAT- International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Hyderabad (https://www.genebanks.org/genebanks/icrisat/(accessed on 15 March 2021)) [117]	Chickpea-20764, Groundnut-15699, Pearl millet-24514, Pigeon pea-13783, Small millets-11797, Sorghum-41889	
AfricaRice- Africa Rice Centre, Abidjan, Côte d'Ivoire (https://www.genebanks.org/genebanks/africarice/ (accessed on 15 March 2021)) [118]	Rice- 21300	
Bioversity International, Rome, Italy (https://www.genebanks.org/genebanks/biodiversity-international/(accessed on 15 March 2021)) [119]	Musa-1617	
ICRAF- World Agro forestry, Nairobi, Kenya (https://www.genebanks.org/genebanks/icraf/(accessed on 15 March 2021)) [120]	Fruits-8246, Multipurpose trees-6456	
ILRI- International Livestock Research Institute, Nairobi, Kenya (https://www.genebanks.org/genebanks/ilri/(accessed on 15 March 2021)) [121]	Forage grasses and legumes-18662	
IRRI- International Rice Research Institute, Los Baños, Philippines (https://www.genebanks.org/genebanks/irri/(accessed on 15 March 2021)) [122]	Rice-132661	

Table 3. The CGIAR gene banks with number of accessions among respective crops as per 2019–2020.

Through germplasm introduction from varied research centers situated in foreign countries and germplasm collection from within the country and around the world, the National Bureau of Plant Genetic Resources (NBPGR) figured prominently in the betterment of numerous crop plants, diversification, and intensification of agriculture in India and conservation thereof containing the most significant number of 452,212 accessions, including in vitro—1916, cryopreservation—11,932, and DNA gene bank—2194 accessions that belong to 1762 species of plants (http://www.nbpgr.ernet.in/Research-Projects/Base\_ Collection accessed on 15 March 2021) [77,123]. The most significant number of species was preserved by germplasm banks such as the US National Plant Germplasm System (USDA), EMBRAPA (Brazil), and IBONE (Argentina), with about 48, 51, and 72 species, respectively [124]. The conservation of tropical and subtropical fruits genetic resources is handled by EMBRAPA, which has 24 field gene banks. This system has around 300 species and over 10,000 accessions under conservation, including duplications and several other subspecies collections [125]. The germplasm documentation has been updated through a national information system named the Brazilian Genetic Resources Information System-SIBRARGEN [126]. Users of Plant Genetic Resources (PGRs) get to use these capabilities to boost the efficiency and effectiveness of their efforts to preserve, explore, and use novel qualities in PGRs, as well as contribute to the achievement of the Sustainable Development Goals (SDGs) [127]. The UN Sustainable Development Goals (SDGs) call for the preservation of genetic diversity of seeds through well-managed seed and field gene banks at national and global scales as a critical step against world hunger [128].

## 6. Conclusions and Future Prospects

For the most part, agricultural production is focused on germplasm. Germplasm collection entails leveraging theoretical and empirical community sampling knowledge to achieve a good grasp on plant diversity, the environment, and farming's socioeconomic and cultural aspects. It contributes towards global efforts to ensure food security in the future by retrieving natural diversity and springing up crop diversity for cultivating agricultural crops. It is also critical for forestry and horticulture, as well as for the restoration of degraded lands and the preservation of ecosystem resources across the landscape. Biotechnology has contributed greatly to the betterment of plant genetic resource management and utilization. Rapid advancements have aided plant germplasm survival using in vitro culture technology, cryopreservation, and molecular markers, which provide a valuable alternative to plant diversity studies and genetic resource management. In vitro culture technology is used to increase the number of germplasm specimens in gene banks around the world, and it is particularly useful in plant species that produce recalcitrant seeds or reproduce asexually. Adjustments in gene bank protocols would be needed to reap the full benefits of cryopreservation. There is a compelling need for improved and robust data handling mechanisms for collection, recovery, and sequence comparisons. Since germplasm serves as the raw material for breeders to grow various crops, the gathering and storage of germplasm materials has taken on a new urgency in recent years. These efforts will gradually build a 'knowledge bank' based on genomics, digital phenotyping, and technological innovations, allowing for a more data-driven adoption of crop diversity.

**Author Contributions:** P.K. conceived of and designed the project; P.K. supervised the study; V.P., R.K. and I.D. wrote the paper; P.K. checked and corrected the final draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are thankful to the anonymous reviewers for their careful read.

Conflicts of Interest: The authors declare that no conflict of interest exists.

#### References

- 1. Wilkes, G. In Situ Conservation of Agricultural Systems. In *Biodiversity; Culture, Conservation and Eco Development;* Oldfield, M., Alcorn, J., Eds.; West View Press: Boulder, CO, USA, 1991; pp. 86–101.
- Rao, N.K. Plant genetic resources: Advancing conservation and use through biotechnology. *Afr. J. Biotechnol.* 2004, *3*, 136–145. [CrossRef]
- Baute, G.J.; Dempewolf, H.; Rieseberg, L.H. Using genomic approaches to unlock the potential of CWR for crop adaptation to climate change. In *Crop Wild Relatives and Climate Change*; Redden, R., Yadav, S.S., Maxted, N., Dulloo, M.E., Guarino, L., Smith, P., Eds.; Wiley Blackwell: Hoboken, NJ, USA, 2015.
- Kell, S.; Marino, M.; Maxted, N. Bottlenecks in the PGRFA use system: Stakeholders' perspectives. *Euphytica* 2017, 213, 170. [CrossRef]
- 5. Fao/Ipgri/Onu. *Genebank standards for Plant Genetic Resources for Food and Agriculture,* Rev. ed.; FAO: Rome, Italy, 2014; p. 182. ISBN 9290432365.
- 6. Gosal, S.S.; Wani, S.H.; Kang, M.S. Biotechnology and crop improvement. J. Crop. Improv. 2010, 24, 153–217. [CrossRef]
- De Wet, J.M.J. Cereals for the semi-arid tropics. In *Plant Domestication by Induced Mutation, Proceedings of the Advisory Group* Meeting on The Possible Use of Mutation Breeding for Rapid Domestication of New Crop Plants, Vienna, Austria, 17–21 November 1986; Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture: Rome, Italy, 1989; pp. 79–88.
- 8. Wright, B.D. Crop genetic resource policy: The role of ex situ gene banks. Aust. J. Agric. Resour. Econ. 1997, 41, 81–115. [CrossRef]
- Upadhyaya, H.D.; Gowda, C.L.L.; Buhariwalla, H.K.; Crouch, J.H. Efficient use of crop germplasm resources: Identifying useful germplasm for crop improvement through core and mini-core collections and molecular marker approaches. *Plant Genet. Resour.* 2006, *4*, 25–35. [CrossRef]
- 10. Debouck, D.G. Managing Plant Genetic Diversity. Crop Sci. 2003, 43, 749–750. [CrossRef]

- 11. Warschefsky, E.; Penmetsa, R.V.; Cook, D.R.; Von Wettberg, E.J.B. Back to the wilds: Tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *Am. J. Bot.* **2014**, *101*, 1791–1800. [CrossRef] [PubMed]
- 12. Foster, T.M.; Aranzana, M.J. Attention sports fans! the far-reaching contributions of bud sport mutants to horticulture and plant biology. *Hortic. Res.* 2018, 5. [CrossRef] [PubMed]
- 13. Vavilov, N.I. Origin and Geography of Cultivated Plant; Cambridge University Press: Cambridge, UK, 1992; ISBN 0521404274.
- 14. Zhukovsky, P.M. Main gene centres of cultivated plants and their wild relatives within the territory of the U.S.S.R. *Euphytica* **1965**, *14*, 177–188. [CrossRef]
- 15. Harlan, J.R.; De Wet, J.M.J. Toward a Rational Classification of Cultivated Plants. Taxon 1971, 20, 509–517. [CrossRef]
- 16. Haussmann, B.I.G.; Parzies, H.K.; Presterl, T.; Miedaner, T. Plant genetic resources in crop improvement. *Plant Genet. Resour. Charact. Util.* **2006**, *2*, 3–21. [CrossRef]
- 17. IUCN RED LIST 2019-20. Source-IUCN Official Website. Available online: https://www.iucnredlist.org/ (accessed on 15 March 2021).
- Offord, C.A. Germplasm Conservation. In *Encyclopedia of Applied Plant Science*, 2nd ed.; Thomas, B., Murray, B.G., Murphy, D.J., Eds.; Academic Press: Cambridge, MA, USA, 2017; Volume 2, pp. 281–288; ISBN 9780123948083. [CrossRef]
- 19. Crop Trust. Securing Crop Diversity for Sustainable Development; Global Crop Diversity Trust: Bonn, Germany, 2015.
- Offord, C.; Makinson, R. Options and major considerations for plant germplasm conservation. In *Guidelines for Germplasm Conservation in Australia: Strategies and Guidelines for Developing, Managing and Utilizing Ex Situ Conservation Collections*; Offord, C.A., Meagher, P.F., Eds.; Australian Network for Plant Conservation: Canberra, Australia, 2009; pp. 11–34.
- 21. Malhotra, N.; Panatu, S.; Singh, B.; Negi, N.; Singh, D.; Singh, M.; Chandora, R. *Genetic Resources: Collection, Conservation, Characterization and Maintenance*; Elsevier Inc.: Amsterdam, The Netherlands, 2018; ISBN 9780128135228.
- 22. Harlan, H.V. The Origin of Hooded Barley. J. Hered. 1931, 22, 265–272. [CrossRef]
- Martos, V.; Royo, C.; Rharrabti, Y.; Garcia Del Moral, L.F. Using AFLPs to determine phylogenetic relationships and genetic erosion in durum wheat cultivars released in Italy and Spain throughout the 20th century. *Fields Crop. Res.* 2005, *91*, 107–116. [CrossRef]
- Rocha, F.; Bettencourt, E.; Gaspar, C. Genetic erosion assessment through the re-collecting of crop germplasm. Counties of ArcodeValdevez, Melgaço, Montalegre, Ponte da Barca and Terras de Bouro (Portugal). *Plant Genet. Resour. Newsl.* 2008, 154, 6–13.
- 25. Ruiz, M.; Rodriguez-Quizano, M.; Metakovsky, E.V.; Vazquez, J.F.; Carrillo, J.M. Polymorphism, variation and genetic identity of Spanish common wheat germplasm based on gliadins alleles. *Field Crops Res.* **2002**, *79*, 185–196. [CrossRef]
- Ross-Ibarra, J.; Morrell, P.L.; Gaut, B.S. Plant domestication, a unique opportunity to identify the genetic basis of adaptation. *Proc. Acad. Natl. Sci. USA* 2007, 104 (Suppl. 1), 8641–8648. [CrossRef] [PubMed]
- Guarino, L. Approaches to measuring genetic erosion. PGR Documentation and Information in Europe—Towards a sustainable and user-oriented information infrastructure. In Proceedings of the EPGRIS Final Conference Combined with a Meeting of the ECP/GR Information and Documentation Network, Prague, Czech Republic, 11–13 September 2003.
- Upadhyaya, H.D.; Gowda, C.L.L. Managing and Enhancing the Use of Germplasm—Strategies and Methodologies; Technical Manual No. 10; ICRISAT: Patancheru, India, 2009.
- 29. Brodie, J.F.; Aslan, C.E.; Rogers, H.S.; Redford, K.H.; Maron, J.L.; Bronstein, J.L.; Groves, C.R. Secondary Extinctions of Biodiversity. *Trends Ecol. Evol.* 2014, 29, 664–672. [CrossRef] [PubMed]
- 30. Brennan, J.P.; Fox, P.N. Impact of CIMMYT varieties on the genetic diversity of wheat in Australia. *Aust. J. Agric. Res.* **1993**, *49*, 175–178. [CrossRef]
- Kolodinska, B.A.; Von Bothmer, R.; Dayeg, C.; Rashal, I.; Tuvesson, S.; Weibull, J. Inter simple sequence repeat analysis of genetic diversity and relationships in cultivated barley of Nordic and Baltic region. *Hereditas* 2004, 14, 186–192. [CrossRef]
- 32. Castaneda-Alvarez, N.P.; Khoury, C.K.; Achicanoy, H.A.; Bernau, V.; Dempewolf, H.; Eastwood, R.J.; Guarino, L.; Harker, R.H.; Jarvis, A.; Maxted, N.; et al. Global conservation priorities for crop wild relatives. *Nat. Plants* **2016**, *2*, 16022. [CrossRef]
- 33. Secretariat of the Convention on Biological Diversity. *The Convention on Biological Diversity Plant Conservation Report: A Review of Progress in Implementing the Global Strategy of Plant Conservation (GSPC);* Secretariat of the Convention on Biological Diversity: Montreal, QC, Canada, 2009.
- Aravind, J.; Radhamani, J.; Srinivasan, K.; Subhash, B.A.; Tyagi, R.K.; Aubrey, M.; Atkinson, K.; Philips, L. Package 'PGRdup' Title Discover Probable Duplicates in Plant Genetic Resources Collections. 2017. Available online: https://cran.rproject.org/ package=PGRdup (accessed on 15 March 2021).
- 35. Engels, J.; Visser, L. A Guide to Effective Management of Germplasm Collections; Bioversity International: Maccarese-Stazione, Italy, 2003; Volume 59, ISBN 9290435828.
- 36. Frankel, O.; Brown, A. Current Plant Genetic Resources: A Critical Appraisal; Oxford & IBH Publ. Co.: New Delhi, India, 1984.
- 37. Upadhyaya, H.D.; Ortiz, R. A mini-core collection for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theor. Appl. Genet.* **2001**, 102, 1292–1298. [CrossRef]
- Haupt, M.; Schmid, K. Combining focused identification of germplasm and core collection strategies to identify genebank accessions for central European soybean breeding. *Plant Cell Environ.* 2020, 43, 1421–1436. [CrossRef]
- 39. Furman, B.J. Methodology to establish a composite collection: Case study in lentil. Plant Genet. Resour. 2006, 4, 2–12. [CrossRef]
- 40. Azough, Z.; Kehel, Z.; Benomar, A.; Bellafkih, M.; Amri, A. Predictive Characterization of ICARDA Gene bank Barley Accessions Using FIGS and Machine Learning. *Intell. Environ.* **2019**, 121–129.

- 41. Maxted, N. Ex Situ, In Situ Conservation. In *Encyclopedia of Biodiversity*; Levin, S.A., Ed.; Academic Press: Cambridge, MA, USA, 2001; pp. 683–695; ISBN 9780122268656. [CrossRef]
- 42. Ifeanyi, N.; Okorie, N.; Marshall, U.E. Germplasm Preservation and Propagation; The Foundation of Agricultural Development— A Review. *IOSR J. Pharm. Biol. Sci.* **2016**, *11*, 70–73. [CrossRef]
- 43. International Seed Testing Association. International Rules for Seed Testing 2018; ISTA: Bassersdorf, Switzerland, 2018.
- 44. Roberts, E.H. Predicting the storage life of seeds. *Proceedings* 1973, 1, 499–514.
- 45. Hawkes, J.G.; Maxted, N.; Ford-Lloyd, B.V. *The Ex Situ Conservation of Plant Genetic Resources*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000.
- 46. Harrington, J.F. Thumb Rules of Drying Seed. *Crops Soils* **1960**, *13*, 16–17.
- 47. Merritt, D.; Dixon, K. Restoration Seed Banks—A Matter of Scale. Science 2011, 332, 424–425. [CrossRef]
- 48. Kholina, A.B.; Voronkova, N.M. Seed Cryopreservation of Some Medicinal Legumes. J. Bot. 2012, 2012, 186891. [CrossRef]
- 49. Horna, D.; Smale, M. Evaluating Cost-Effectiveness of Collection Management: A Methodological Framework. 2010. Available online: http://cropgenebank.sgrp.cgiar.org/images/file/management/DST/framework\_dst.pdf (accessed on 14 April 2021).
- 50. Hay, F.R.; Probert, R.J. Advances in seed conservation of wild plant species: A review of recent research. *Conserv. Physiol.* **2013**, *1*, cot030. [CrossRef]
- Xia, K.; Daws, M.I.; Hay, F.R.; Chen, W.Y.; Zhou, Z.K.; Pritchard, H.W. A comparative study of desiccation responses of seeds of Asian Evergreen Oaks, *Quercus* subgenus *Cyclobalanopsis* and *Quercus* subgenus *Quercus*. S. Afr. J. Bot. 2012, 78, 47–54. [CrossRef]
- 52. Han, C.Y.; Sun, W.B. Seed storage behaviour of Magnolia odoratissima. Seed Sci. Technol. 2013, 41, 143–147. [CrossRef]
- 53. Jayasuriya, K.M.G.G.; Wijetunga, A.S.T.B.; Baskin, J.M.; Baskin, C.C. Seed dormancy and storage behaviour in tropical Fabaceae: A study of 100 species from Sri Lanka. *Seed Sci. Res.* **2013**, *23*, 257–269. [CrossRef]
- 54. Henshaw, G.G. Technical aspects of tissue culture storage for genetic conservation. In *Crop Genetic Resources for Today and Tomorrow;* Cambridge University Press: Cambridge, UK, 1975; pp. 349–358.
- 55. Morel, G. Meristem culture techniques for the long-term storage of cultivated plants. In *Crop Genetic Resources for Today and Tomorrow;* Frankel, O.H., Hawkes, J.G., Eds.; Cambridge University Press: Cambridge, UK, 1975; pp. 327–332.
- 56. Engelmann, F. Use of biotechnologies for the conservation of plant biodiversity. *In Vitro Cell. Dev. Biol. Plant* **2011**, 47, 5–16. [CrossRef]
- 57. Ding, F.; Jin, S.; Hong, N.; Zhong, Y.; Cao, Q.; Yi, G.; Wang, G. Vitrification-cryopreservation, an efficient method for eliminating *Candidatus Liberobacter asiaticus*, the citrus Huanglongbing pathogen, from in vitro adult shoot tips. *Plant Cell Rep.* **2008**, *27*, 241–250. [CrossRef] [PubMed]
- 58. El-Dougdoug, K.A.; El-Shamy, M.M. Plant Tissue Culture: Current Status and Opportunities. Afr. J. Microbiol. 2011. [CrossRef]
- 59. Bettoni, J.C.; Costa, M.D.; Gardin, J.P.P.; Kretzschmar, A.A.; Pathirana, R. Cryotherapy: A new technique to obtain grapevine plants free of viruses. *Rev. Bras. Frutic.* **2016**, *38*, 833. [CrossRef]
- 60. Valerie, C.P.; Jorge, A.S.; Victor, M.V.A.; Florent, E. *In Vitro Collecting Techniques for Germplasm Conservation*; IPGRI Technical Bulletin No. 7; International Plant Genetic Resource Institute: Rome, Italy, 2002; p. 250.
- 61. Kaviani, B. Conservation of plant genetic resources by cryopreservation. Aust. J. Crop Sci. 2011, 5, 778–800.
- Sakai, A.; Engelmann, F. Vitrification, encapsulation-vitrification and droplet-vitrification: A review. *CryoLetters* 2007, *28*, 151–172.
  West, T.P.; Preece, J.E. Use of acephate, benomyl and alginate encapsulation for eliminating culture mites and fungal contamination from in vitro cultures of hardy hibiscus (*Hibiscus moscheutos* L.). *In Vitro Cell. Dev. Biol. Plant* 2006, *42*, 301–304. [CrossRef]
- Paunescu, A. Biotechnology for Endangered Plant Conservation: A Critical Overview. *Rom. Biotechnol. Lett.* 2009, 14, 4095–4103.
- Wang, M.-R.; Chen, L.; da Silva, J.A.T.; Volk, G.M.; Wang, Q.-C. Cryobiotechnology of apple (*Malus* spp.): Development, progress and future prospects. *Plant Cell Rep.* 2018, 37, 689–709. [CrossRef]
- 66. Bettoni, J.C.; Bonnart, R.; Shepherd, A.; Kretzschmar, A.A.; Volk, G.M. Cryopreservation of grapevine (*Vitis* spp.) shoot tips from growth chamber-sourced plants and histological observations. *Vitis* **2019**, *78*, 71–78. [CrossRef]
- 67. Jenderek, M.M.; Reed, B.M. Cryopreserved storage of clonal germplasm in the USDA National Plant Germplasm System. *In Vitro Cell. Dev. Biol. Plant* 2017, *53*, 299–308. [CrossRef]
- 68. Normah, M.N.; Sulong, N.; Reed, B.M. Cryopreservation of shoot tips of recalcitrant and tropical species: Advances and strategies. *Cryobiology* **2019**, *87*, 1–14. [CrossRef]
- 69. Dixit, S.; Mandal, B.B.; Ahuja, S.; Srivastava, P.S. Genetic stability assessment of plants regenerated from cryopreserved embryogenic tissues of *Dioscoreabulbifera* Using RAPD, biochemical and morphological analysis. *Cryoletters* **2003**, *24*, 77–84.
- 70. Chauhan, R.; Singh, V.; Quareshi, A. In Vitro Conservation Through Slow-Growth Storage. *Synth. Seeds Germplasm Regen. Preserv. Prospect.* **2019**, *1*, 482. [CrossRef]
- Silva, R.L.; Ferreira, C.F.; Ledo, C.A.S.; Souza, E.H.; Silva, P.H.; Costa, M.A.P.C.; Souza, F.V.D. Viability and genetic stability of pineapple germplasm after 10 years of in vitro conservation. *Plant Cell. Tissue Organ Cult.* 2016, 127, 123–133. [CrossRef]
- 72. Kulkarni, V.M.; Ganapathi, T.R. A simple procedure for slow growth maintenance of banana (*Musa* spp.) embryogenic cell suspension cultures at low temperature. *Curr Sci.* **2009**, *96*, 1372–1377.
- 73. Dulloo, M.E.; Hunter, D.; Borelli, T. Ex situ and in situ conservation of agricultural biodiversity: Major advances and research needs. *Not. Bot. Horti Agrobot. Cluj Napoca* **2010**, *38*, 123–135. [CrossRef]
- 74. West, T.P.; Ravindra, M.B.; Preece, J.E. Encapsulation, cold storage, and growth of Hibiscus moscheutos nodal segments. *Plant Cell. Tissue Organ Cult.* **2006**, *87*, 223–231. [CrossRef]

- 75. Kovalchuk, I.; Lyudvikova, Y.; Volgina, M.; Reed, B.M. Medium, container and genotype all influence in vitro cold storage of apple germplasm. *Plant Cell Tissue Organ Cult.* **2009**, *96*, 127–136. [CrossRef]
- 76. Berjak, P.; Pammenter, N.W. Implications of the lack of desiccation tolerance in recalcitrant seeds. *Front. Plant Sci.* **2013**, *4*, 478. [CrossRef]
- 77. Walters, C.; Berjak, P.; Pammenter, N.; Kennedy, K.; Raven, P. Preservation of recalcitrant seeds. *Science* 2013, 339, 915–916. [CrossRef]
- 78. Bernstein, M.E.; Carroll, G.C. Internal fungi in old-growth Douglas fir foliage. Can. J. Bot. 1977, 55, 644–653. [CrossRef]
- 79. Smith, R.H.; Burrows, J.; Kurten, K. Challenges associated with micropropagation of *Zephyranthes* and *Hippeastrum* sp. (Amaryllidaceae). *In Vitro Cell. Devel. Biol. Plant* **1999**, *35*, 281–282. [CrossRef]
- 80. Merkle, S.A.; Bailey, R.L.; Pauley, B.A.; Neu, K.A.; Kim, M.K.; Rugh, C.L. Somatic embryogenesis from tissues of mature sweetgum trees. *Can. J. For. Resour.* **1997**, *27*, 959–964. [CrossRef]
- 81. Pence, V.C. In vitro collection (IVCN). In *A Colour Atlas of Plant Propagation and Conservation;* Bowes, B., Ed.; CRC Press: Boca Raton, FL, USA, 1999; ISBN 1840765410.
- 82. MacKay, W.A. Micropropagation systems for the Mexican red bud (*Cercis canadensisvar.mexicana* L.) and other woody plants of the Chichuahuan desert. *In Vitro Cell. Devel. Biol. Plant* **1999**, *35*, 283–284. [CrossRef]
- 83. De, M. Field Gene Banks: The Living Repositories of Plant Genetic Resources. Harvest 2017, 2, 21.
- O'Donnell, K.; Sharrock, S. Botanic gardens complement agricultural gene bank in collecting and conserving plant genetic diversity. *Biopreserv. Biobank.* 2018, 16, 384–390. [CrossRef] [PubMed]
- 85. Cruz-Cruz, C.A.; Gonzalez-Arnao, M.T.; Engelman, F. Biotechnology and conservation of plant biodiversity. *Resources* **2013**, *2*, 73–95. [CrossRef]
- 86. Hoyt, E. Conserving the Wild Relatives of Crops; International Board for Plant Genetic Resources: Rome, Italy, 1988.
- 87. IBPGR. *Ecogeographical Surveying and In Situ Conservation of Crop Relatives*; International Board for Plant Genetic Resources: Rome, Italy, 1985.
- Meir, N.; Anikster, Y.; Waldman, M.; Ashri, A. Population dynamics research for in situ conservation: Wild wheat in Israel. *Plant Genet. Resour. Network*. 1989, 75/76, 9–11.
- 89. Plucknett, D.L.; Smith, N.J.H. Gene Banks and The World's Food; Princeton University Press: Princeton, NJ, USA, 2014; ISBN 1400858119.
- Kaya, Z.; Kün, E.; Güner, A. National Plan for In Situ Conservation of Plant Genetic Diversity in Turkey; Ministry of Environment, Ministry of Agriculture and Rural Affairs and Ministry of Forestry: Ankara, Turkey, 1998; pp. 98–113. Available online: https://agris.fao.org/agris-search/search.do?recordID=XF2015046805 (accessed on 25 March 2021).
- 91. Maxted, N.; Guarino, L.; Myer, L.; Chiwona, E.A. Towards a methodology for on-farm conservation of plant genetic resources. *Genet. Resour. Crop Evol.* **2002**, *49*, 31–46. [CrossRef]
- Swaminathan, M.S. The past, present and future contributions of farmers to the conservation and development of genetic diversity. In *Managing Plant Genetic Diversity, Proceedings of an International Conference, Kuala Lumpur, Malaysia, 12–16 June 2000;* CABI: Wallingford, UK; pp. 23–31. [CrossRef]
- 93. Halewood, M.; Jamora, N.; Noriega, I.L.; Anglin, N.L.; Wenzl, P.; Payne, T.; Ndjiondjop, M.N.; Guarino, L.; Lava Kumar, P.; Yazbek, M. Germplasm acquisition and distribution by cigar gen ebanks. *Plants* **2020**, *9*, 1296. [CrossRef] [PubMed]
- 94. FAO. WIEWS: World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture; FAO: Rome, Italy, 2019; Available online: http://www.fao.org/wiews (accessed on 10 April 2021).
- 95. FAO. 2020. Available online: http://www.fao.org/sustainable-development-goals/indicators/251a/en/2020 (accessed on 13 April 2021).
- 96. Byrne, P.F.; Volk, G.M.; Gardner, C.; Gore, M.A.; Simon, P.W.; Smith, S. Sustaining the Future of Plant Breeding: The Critical Role of the USDA-ARS National Plant Germplasm System. *Crop Sci.* **2018**, *468*, 451–468. [CrossRef]
- 97. GRIN-Global. U.S. National Plant Germplasm System. GRIN-Glob. Web Version 11040. 2019. Available online: https://npgsweb.ars-grin.gov/gringlobal/query/summary.aspx (accessed on 2 April 2021).
- Migicovsky, Z.; Warschefsky, E.; Klein, L.L.; Miller, A.J. Using living germplasm collections to characterize, improve, and conserve woody perennials. Crop Sci. 2019, 59, 2365–2380. [CrossRef]
- 99. National Fruit Collection in the United Kingdom. Available online: http://www.nationalfruitcollection.org.uk/ (accessed on 20 March 2021).
- 100. N.I. Vavilov All-Russian Science Research Institute of Plant Industry's Fruit Collection. Available online: http://www.vir.nw.ru/ unu-kollektsiya-vir/ (accessed on 15 March 2021).
- 101. Foreign Centre for Research in Agronomy. Available online: http://www.vir.nw.ru/unu-kollekts (accessed on 10 April 2021).
- 102. CGIAR Genebank Platform. 2019 Genebank Platform Annual Report; Global Crop Diversity Trust: Bonn, Germany, 2020.
- 103. CWR Project. Annual Report; Global Crop Diversity Trust: Bonn, Germany, 2019.
- 104. Miller, A.J.; Novy, A.; Glover, J.; Kellogg, E.A.; Maul, J.E.; Raven, P.; Jackson, P.W. Expanding the role of botanical gardens in the future of food. *Nat. Plants.* **2015**, *1*, 15078. [CrossRef]
- 105. Cibrian-Jaramillo, A.; Hird, A.; Oleas, N.; Ma, H.; Meerow, A.W.; Francisco-Ortega, J.; Griffith, M.P. What is the conservation value of a plant in a botanic garden. Using indicators to improve management of ex Situ collections. *Bot. Rev.* 2013, 79, 559–577. [CrossRef]

- 106. Jackson, P.W.; Kennedy, K. The global strategy for plant conservation: A challenge and opportunity for the international community. *Trends Plant Sci.* 2009, 14, 578–580. [CrossRef]
- 107. Genesys Database. Available online: www.genesys-pgr.org (accessed on 22 March 2021).
- 108. BGCI's Plant Search. Available online: https://tools.bgci.org/plant\_search.php (accessed on 22 March 2021).
- 109. Available online: http://www.fao.org/home/en/ (accessed on 26 March 2021).
- 110. Hanson, J.; Ellis, R.H. Progress and challenges in ex situ conservation of forage germplasm: Grasses, herbaceous legumes and fodder trees. *Plants* **2020**, *9*, 446. [CrossRef]
- 111. Yu, X.; Li, X.; Guo, T.; Zhu, C.; Wu, Y.; Mitchell, S.E.; Roozeboom, K.L.; Wang, D.; Wang, M.L.; Pederson, G.A.; et al. Genomic prediction contributing to a promising global strategy to turbo charge gene banks. *Nat. Plants.* **2016**, *2*, 16150. [CrossRef]
- 112. IITA-International Institute of Tropical Agriculture. Available online: https://www.genebanks.org/genebanks/iita/ (accessed on 9 April 2021).
- 113. CIAT-International Centre for Tropical Agriculture. Available online: https://www.genebanks.org/genebanks/ciat/ (accessed on 9 April 2021).
- 114. CIMMYT-International Maize and Wheat Improvement Centre. Available online: https://www.genebanks.org/genebanks/ cimmyt/ (accessed on 3 April 2021).
- 115. CIP-International Potato Centre. Available online: https://www.genebanks.org/genebanks/international-potato-centre/ (accessed on 9 April 2021).
- 116. ICARDA-International Centre for Agricultural Research in the Dry Areas. Available online: https://www.genebanks.org/genebanks/icarda/ (accessed on 3 April 2021).
- 117. ICRISAT-International Crops Research Institute for the Semi-Arid Tropics. Available online: https://www.genebanks.org/genebanks/icrisat/ (accessed on 3 April 2021).
- 118. AfricaRice-Africa Rice Centre. Available online: https://www.genebanks.org/genebanks/africarice/ (accessed on 9 April 2021).
- 119. Bioversity International. Available online: https://www.genebanks.org/genebanks/biodiversity-international/ (accessed on 9 April 2021).
- 120. ICRAF-World Agro Forestry. Available online: https://www.genebanks.org/genebanks/icraf/ (accessed on 3 April 2021).
- 121. ILRI-International Livestock Research Institute. Available online: https://www.genebanks.org/genebanks/ilri/ (accessed on 9 April 2021).
- 122. IRRI-International Rice Research Institute. Available online: https://www.genebanks.org/genebanks/irri/ (accessed on 3 April 2021).
- 123. National Bureau of Plant Genetic Resources (NBPGR). Available online: http://www.nbpgr.ernet.in/Research\_Projects/Base\_ Collection\_in\_NGB.aspx (accessed on 13 April 2021).
- 124. Acuna, C.A.; Martínez, E.J.; Zilli, A.L.; Brugnoli, E.A.; Espinoza, F.; Marcon, F.; Urbani, M.H.; Quarin, C.L. Reproductive Systems in Paspalum: Relevance for Germplasm Collection and Conservation, Breeding Techniques, and Adoption of Released Cultivars. *Front. Plant Sci.* 2019, 10, 1377. [CrossRef] [PubMed]
- Nass, L.L.; Sigrist, M.S.; Ribeiro, C.S.C.; Reifschneider, F.J.B. Genetic resources: The basis for sustainable and competitive plant breeding. Crop Breed. Appl. Biotechnol. 2012, 12, 75–86. [CrossRef]
- 126. Ferreira, F.R.; Pinto, A.C.Q. Germplasm Conservation and Use of Genebanks for Research Purposes of Tropical and Subtropical Fruits in Brazil. *Acta Hortic.* 2010, *864*, 21–28. [CrossRef]
- 127. Halewood, M.; Chiurugwi, T.; Sackville Hamilton, R.; Kurtz, B.; Marden, E.; Welch, E.; Michiels, F.; Mozafari, J.; Sabran, M.; Patron, N.; et al. Plant genetic resources for food and agriculture: Opportunities and challenges emerging from the science and information technology revolution. *New Phytol.* **2018**, *217*, 1407–1419. [CrossRef]
- 128. Smale, M.; Jamora, N. Valuing genebanks. Food Sec. 2020, 12, 905–918. [CrossRef]