Review

Germplasm Conservation: Instrumental in Agricultural Biodiversity-A Review

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Abstract: Germplasm is a valuable natural resource in plant diversity that is crucial for its potential use. It provides knowledge about a species genetic composition. Germplasm protection strategies are not just planting hope threatened with extinction, they preserve medicinal and other essential plants on which survival rests. The successful use of genetic plant resources necessitates diligent collection, storage, analysis, documentation, and germplasm exchange. Slow growth cultures, cryopreservation, pollen and DNA banks, botanic gardens, genetic reserves and farmer's fields are few conservation techniques. However, usage of an in vitro procedure with any chance of genetic instability leads to the destruction of the entire substance. Improved understanding of basic regeneration biology would, in turn, undoubtedly increase the capacity to regenerate plants from in vitro harvested explants, 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, Genetic plant resources, Preservation, Propagation, in vitro

1. Introduction

Germplasm is a genetic substance that can be passed from one generation to another, sexually or somatically [1]. Plant genetic diversity must be maintained, and a well-planned discovery and collection expedition is needed to accomplish this. It would primarily concern about species found among any wild plant genetic resources, currently cultivated, domesticated, or semi-domesticated plant species, as well as their component cultivars (currently in use or obsolete) and "landraces" or older varieties as well as related wild species, which may be direct or distant ancestral predecessors to cultivated species [2].

Plant breeding from crop wild relatives (CWRs) with novel genetic variants is critical for global food security. To ensure our nutrition and economic safety, mankind is reliant on the continuous availability of a diverse pool of plant genetic resources for food and agriculture (PGRFA), despite their existence, we face significant hurdles in mobilizing them for effective and sustainable use [3]. International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) was signed, for a very sustainable use of PGRFA, as well as the fair and equitable sharing of advantages accruing from their usage, in accordance with the 1992 Convention on Biological Diversity (CBD). It designates PGRFA as "any genetic material of plant origin, including repro-

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ductive and vegetative propagating material, comprising functionalities of heredity with food and agriculture potential [4]. Despite the fact that many gene banks around the world now exist, only about 30 countries have safe long-term storage because of a shortage of long-term maintenance provisions for gene banks. The 7.5 million accessions in the world's gene banks are primarily crops on which humans and animals rely for food and nutrition, which include diversified wild relatives and landraces, but there are also locally important crops and underutilized species [5]. Plant breeding and habitat regeneration of ecosystems for livestock, horticulture and forestry are few applications of germplasm protection that even includes PGRFA and other medicinal plants, wood & fuel plant species, ornamental species, and recreation & amenity species (PGR for nonfood utilization) (Figure 1).

Germplasm conservation is a key factor in preserving knowledge about extinct, wild, or other living species because genetic diversity leads to the extinction of older generation genetic material. It is mostly concerned with ensuring the secure handling and proper preservation of germplasm from commercially valuable plants by collecting each taxon's seed [6]. Introgression of seed related attributes from wild relatives to high yielding cultivars that are better adjusted to current growing conditions is one way for developing climate-resilient crops [7]. Although these accessions may seem to be genotypic duplicates, particularly when the comparison is made with just a few genetic markers, they are indeed helpful tools for plant developmental and gene function studies [8]. The International board for Plant Genetic Resources (IBPGR) created in 1974, renowned for its germplasm selection, improvement, and preserving plant genetic resources [9, 10].

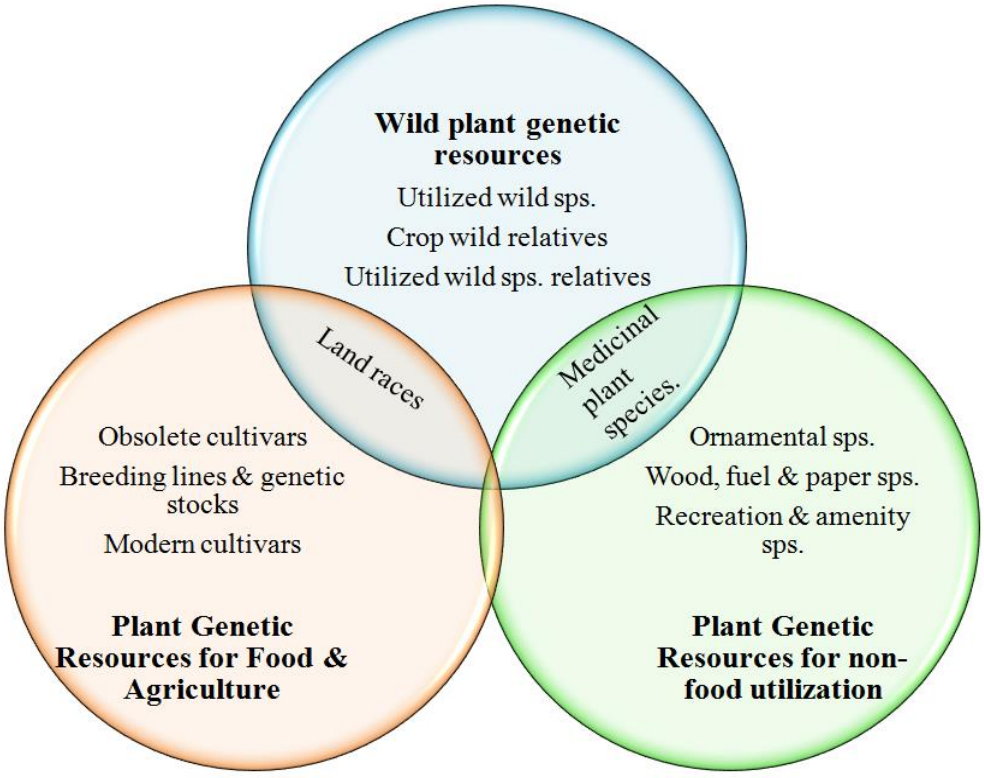

Fig 1. Overall representation of Global plant genetic diversity.

Harlan and De Wet (1971) [11] categorised the primary, secondary and tertiary gene pools on the basis of the degree of relationship, that is less taxonomical but useful for improving crops. i. The primary gene pool (GP1) is defined as the gene pool in which simple intermingling occurs and results in fertile hybrids. Between lines, the genes are usually transferred by regular crosses. ii. Secondary gene pool (GP2): Comprised of plant species with partial fertility when crossing with GP1. Genes from such

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content can, however complicated, possible to transfer to primary gene pools. (ii) Tertiary gene pool (GP3): genetic material that creates sterile variants by combining primary gene pools. It contains GP1-crossable stuff, but the hybrids are sterile. The most neglected aspect of germplasm conservation is the utilisation of available genetic resources for crop improvement [12]. There is a significant gap between actual germplasm utilisation and collection availability in gene banks [13, 14]. The very aim of establishing vast germplasm collections contravenes the extensive use of fewer and closely related parents and their crop improvement derivatives. [15].

2. Brief History of Germplasm or Genetic Resources Conservation

In 1926, N.I. Vavilov [16], a Russian geneticist and plant breeder, first centered focus on the diversity observed in crop plants and proposed the concept of 'centers of diversity,' which describes that characters found in one species also exist in other related species. Besides, eight main origin centers of origin were suggested, which were later regrouped into 11 centers in 1935. Zhukovsky [17] discovered 12 mega gene centers of crop plant diversity in 1965 and a set of micro gene centers of crop wild relatives. The Eastern Himalayas and the Western Ghats are two biodiversity hotspots on the Indian subcontinent, where around 147 agri-horticultural crop species are thought to have originated.

The genetic security of crops and related seeds gained momentum worldwide in the 1960s. In the 1970s, the UN Food and Agriculture Organization, and even the IPGR and its successor, were under the control of the Biodiversity International, previously International Plant Genetic Resources Institute (IPGRI) [18]. The 1992 Convention on Biological Diversity (CBD) gave a boost to its goals, which included "protecting biological diversity, having an equal and proportional share of the advantages of genetic resources and making beneficial use of its elements". Article 1 of the FAO International Plant Genetic Resource Undertaking (adopted in 2001) reflects CBD language for three major goals, in collaboration with the FAO, the introduction of a complementary plant-genetic capital management strategy for food and agriculture, in the context of both ex-situ and in situ strategies (PGRFA) [19]. Breeders with links to publicly owned crops and wild relatives were aided by the International Treaty on Plant Genetic Capital of 2004, which has about a hundred countries as signatories [20]. The Global Plant Conservation Strategy (GSPC) became a CBD weapon in the early 2000s. It declares a "positive, sustainable future in which human activities promote the diversity of plant life." It says (including the endurance of plant genetic diversity, plant species and habitats survival, and their surrounding landscapes and ecological associations) [21].

By 2020, the initiative intends to keep a meager 75 percent of endangered plant species in ex situ stocks, feasibly in their native countries, and at least 20 percent in biodiversity conservation ingenuities. 70 percent of plant genetic diversity, including wild relatives and few socio-economic ones, has been retained for honouring, protecting, and achieving sustainability [22]. The Nagoya Protocol on the biological diversity convention is an additional arrangement that encourages the CBD to share the rewards of using genetic resources equally [23].

3. Global Germplasm Conservation Programs

The conservation of the germplasm, in particular the seed banking, has grown to improve conventional germplasm preservation for major cropping species, as a response to the CBD and GSPC, together with the overarching concerns regarding habitat destruction, climate change and genetic erosion [22]. Recently, germplasm preservation has 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

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countries and has established a "backup" seed bank underneath the Arctic ice in Svalbard (Norway) [24]. In establishing a global network for the conservation of native plants (including CWR) and capacity building, the Millennium Seed Bank Partnership (MSBP) has played an essential role [25]. The central strength of such cooperative programmes is the duplication of collections that insure towards failure of one gene bank. Because of the civil war in Syria, ICARDA seed bank collections were not accessible to plant breeders in 2015 [20]. The backup collections of Svalbard were made available to start cereal cultivation activities in dry-arid regions. The general pipeline of the conservation program is given in Figure 2. Duplicates- The curator's primary duty is to identify and streamline the possibility of duplicate accessions, a primary concern for germplasm conservation. ICAR-NBPGR created a software package "PGR dup" R, which works on passport information, to exclude duplicated accessions from the existing gene bank collection [26]. Safety Duplicates- Duplication of genetically identical subsamples of accessions shall reduce the potential of moderate to severe destruction from natural or manmade disasters. They are alluded to as the second most original sample [27] that further include both the duplication of content and its relevant information and are deposited in the base collection at various locations, probably in another country.

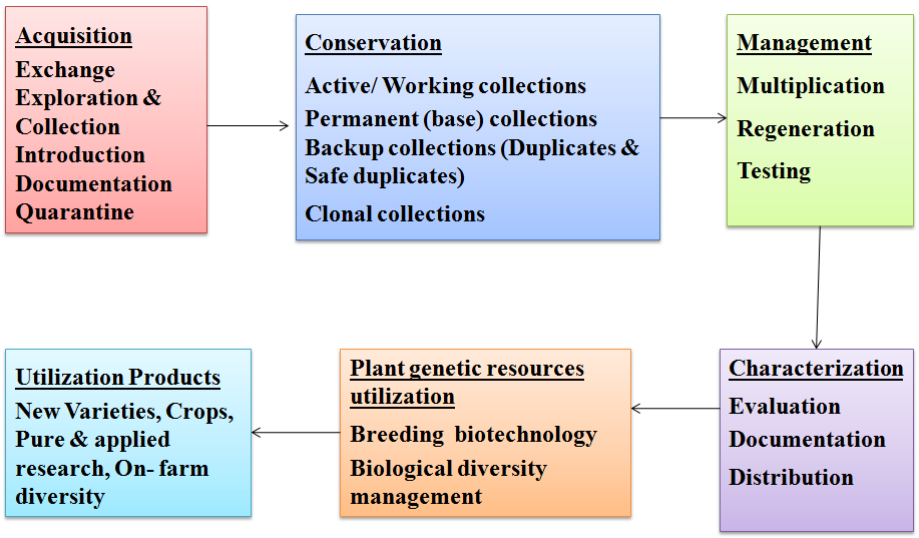

Figure 2. General Pipeline of Germplasm Conservation Program

Plant species which, due to sudden changes in environmental conditions, are either few in numbers or under risk of extinction are deemed endangered [28] and it reported that about 12.5% (34,000 species) of vascular plants worldwide have been at threat. The IUCN red list for the year 2019-20 is presented in Table 1.

Table 1. IUCN RED LIST 2019-20 [29].

Category	Global
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	24810

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DD - Data Deficient	4090

3.1. Need for Germplasm conservation: Genetic Erosion and Genetic Vulnerability

Each crop enhancement programme is aimed at increasing genetic diversity. In the 1930's, H.V. Harlan, a reputable geneticist, warns of the limited barley diversity in modern agriculture hands [30]. Since scientists have recognized that genetic diversity is eroding and land races are a rich source of essential features, genetic loss in cereals has been assessed [31- 33]. Briefly crops are less varied after domestication, due to selection constraints and dispersal bottlenecks [34]. Guarino refers to genetic erosion as "prolonged combination loss over time in a given region, or persistent wealth reduction or uniformness of common localized alleles [35]. The definition suggests that a major event in genetic erosion is the number and frequency depletion of specific regionally adapted alleles. When geographical diversity is reduced, the overall gene pool is more vulnerable to depletion and extinction, thereby reducing global equality and wealth [36]. Key causes of genetic erosion (FAO report) are either Direct- Replacement of local varieties, Overexploitation of species, Overgrazing, Reduced fallow and changing agricultural systems or may be Indirect- Land clearing, Population pressure, Environmental degradation, Legislation/policy change, Pests/weeds/diseases, Civil strife, and Climate change.

A reduction in diversity does not generally lead to genetic erosion on a more comprehensive regional scale in a certain area. There has been no national shift in diversity in Australian wheat (using parenting coefficient), although in some countries, the genetic basis has been narrowed [37]. A parallel study on barley diversity has been undertaken and found a decrease in allelic diversity in some of the surveyed countries in the north and Baltic countries, although overall diversity is preserved [38]. Germplasm collections maintaining a variety of clones are ideal for further research on epigenomic variations. There can be epigenetic modifications even in situations where accessions are genetically similar [39, 40]. Finally, germplasm collections provide an excellent stuff for developing methods for high-throughput (HT) phenotyping. For example, Color Quant was built up as an automated process to retrieve and quantify colour phenotypes from plant images on the Apple Biodiversity Collection (ABC) Kentville Orchard, NS, Canada, which consists of >1,000 unique apple accessions (http://www.cultivatingdiversity.org/) [41].

4. Method of Germplasm Storage

Ex-situ and in situ, each with different methods, are two fundamental storage approaches [42] given in Figure 3. PGRFAs need ex-situ protection for their safety from their natural environments. Samples are kept as live plant specimens in the field gene banks, botanic gardens or plant samples and conserved as seeds, pollen, explants, or DNA, in specialized artificial environments [43]. However, in situ presupposes the survival of entire organisms and natural ecosystems and genetic capital within their natural environments and in the event of domestications or developed species within ecosystem under which their distinctive properties were created.

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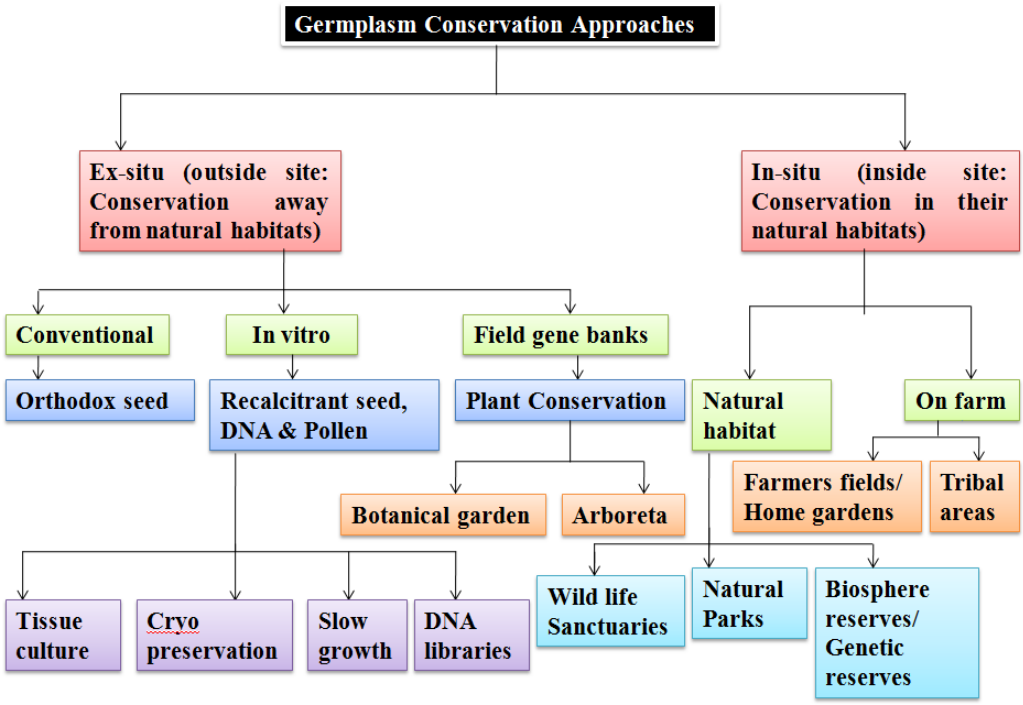

Figure 3. Schematic outline of germplasm conservation approaches.

4.1. Ex-situ Conservation

Ex-situ management is the simplest, cost-efficient approach that involves regular material viability testing and timely recovery, depending on crop and reproductive systems [44]. The Global Crop Diversity Trust (GCDT) plays a key role in improving ex-situ conservation techniques and in management of global crop diversity [45]. The collections of seed banks of wildlife species are projected to play a key role for preserving and restoring biodiversity [46]. Therefore, it is necessary to efficiently manage the collections of wild species for utilization and multiplication of adequate viable seeds.

The seeds were divided into two classes by Roberts [47] as per their storage capacity in 1973. Orthodox seeds: They can be dried up to 5 % moisture content and harvested at low temperatures without losing viability. The vast majority of plants are in this category, whose seeds can easily be preserved for long periods, and as a result of lower humidity and storage temperature, their lifespan is increased due to their resistance to drying and freezing of seed [48]. Examples of these include wheat, rice, maize, sunflowers, chickpeas, and other grains and legumes. Recalcitrant seeds: The viability of this type of seed is significantly reduced if the moisture content is between 12 and 30 %. It encompasses seeds from a number of tropical trees and fruits, including pineapples, cocoa, coffee, oil palm, mango, jackfruit, etc. This seed can be stored at low temperatures (0-10^{0}C) for a brief span of time, about 1 to 5 years. The handling of crops varies as to whether the content is seed or not (clonal material). The odds of survival are approximately doubled for every 50°C drop in temperature or 1% decrease in moisture content, indeed the Harrington's "thumb rule" [49]. The certain approaches to preserve vegetatively propagated materials or recalcitrant seeds, which demands special techniques of tissue culture, cold storage, fluid nitrogen, cryopreservation and *in vivo* conservation, results in higher costs than the orthodox seed storage [50]. Another method of germplasm protection is the desiccating and storing of embryos as artificial plants. These have been shown to be a successful remedy, but only in particular conditions by using somatic embryos and shoot tips [51].

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The core selection concept was developed by Frankel and Brown, as an initial point for efficient control of germplasm utilization in crop breeding, in the 1980s [52], which refer to a subset of the base collection representing a large collection or a limited number of accessions from an existing collection of germplasm [53]. It showed approximately 10% of the total selection and the highest heterogeneity in the whole sample size, but this will vary between 5% and 20% depending on the variety and size of the collection. The core selection must be used as a working collection and closely reviewed while reserve collections are accessions that don't form part of the core collection [54]. The vast number of collections and a lack of accurate data on economically important characteristics reveal strong interactions between genotype and environment that are thought to be core explanations for the underuse of genetic resources. ICARDA has made a hybrid collection of 1000 entries of barley reflecting the genetic wealth of the entire world [55, 56].

This process lowers the atmospheric pressure (under 50mm Hg reduces tissue development). The accumulation of oxygen enclosed by the plant material reduces production of O_2, CO_2, thereby decreasing photosynthetic activity and inhibiting plant tissue growth and size. This reduces the concentration and the concentration in the plant material. These conditions will slow the growth and reduce the plant growth content, which helps to improve fruit, vegetables, and flora's shelf life [57]. Seeds of diverse plants, mostly tropical or subtropical, are of a recalcitrant or medium class; their shorter lifespan is difficult to save them for extended periods [58]. Coconut, cocoa, and several tree species seeds are physiologically unripe, high in humidity, cannot resist a lot of dehydration, are vulnerable to frost, and can be preserved only at low temperatures. Other kinds including coffee spp. and oil palm (*Elaieis guineansis*) are stored for a limited time only, and long-term survival is not possible. For several limitations like seed dormancy, shorter plant life, seed-borne diseases, high costs and labor inputs, alternative growth is necessary. Thus, modern in vitro techniques such as freezing the tissues and cells at –196°C and cold storage were developed [59].

4.1.1. Ex Situ: In Vitro Conservation

The establishing of a DNA storage facility as a complementary "back-up" to traditional ex situ collections has been suggested [60], yet not widely used. In this way, the present use of stored genes for PGRFA is restricted to their isolation, cloning, and conversion through the production of a transgenic plant. It is known mainly to be complementary to germplasm conservation, as it forms a basis of genomic material to explain species origin or population diversity. DNA Banking creates the source of recorded material to carry out comparisons through partnership such as the Barcode of Life (BOL). Cost-lowering and enhanced genetic sequence strength through next-generation techniques is useful for choice of comparison from databases such as Genbank [3]. There seem to be genetic resources for which seed storage is not relevant or even feasible, such as vegetatively propagated (e.g., potatoes), or that don't produce viable seed (e.g., bananas), or that produce often quite short-lived seeds (recalcitrant), must be preserved by other means [61]. Such slow-growth storage has proved extremely successful for shoot cultures of potato, cassava (*Manihot spp*.), fruit crops, such as banana, apple (*Malus pumila*), pear (*Pyrus communis*), and strawberry (*Fragaria ananassa*), and few other horticultural species. The behaviour of seed storage for different species is still experimental [62-64].

The in vitro plant collection utilizes the fundamentals of plant tissue culture that involves separation of a cell/tissue from the donor plant under aseptic conditions and producing it on a synthetic medium in a proper container in a controlled environment [65]. The statement of cell theory implies totipotency for individual cells of an organism [66]. The tissue cultivation protocol for a test plant is the most needed thing, which begins by searching for an already known protocol of a plant in the same taxa those

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shares near affinity due to common physiological and biochemical characteristics. Virus eradication from infected plants is accomplished through meristem cultivation [67] and by cryotherapy (storing under cryopreservation) [68]. The thinning of plant explants reduces room needs and hence labour costs in maintaining germplasm collections and recently, significant advantages of exchanging this plant germplasm across countries through embryonic cultures have come in to limelight [69].

4.1.2. Methods Involved in the in vitro Conservation of Germplasm

Germplasm in vitro conservation was first proposed in the mid-1970 [70, 71]. Among in vitro conservation strategies (IVBG- in vitro base gene bank), two interventions that proved successful were in vitro conservation under slow growth (IVAG- in vitro active gene bank) and cryopreservation. In vitro slow growth is used in a diverse range of national and international research centers (NBPGR-National Bureau of Plant Genetic Resources, IITA-International Institute of Tropical Agriculture, CIP-International Potato Centre) [72].This technique can only adapt to a short to medium-term conservation strategy, meaning that extensive collections using this process are impossible to preserve. **Slow-growing cultures**: This is a viable alternative to cryopreservation as treatment is cost-effective and simple and the contamination and gene alteration are usually minimized [73]. Subculture cycles may be stretched up to 1 or 2 years, shortening the time, effort, and equipment needed to maintain crops. Slower growth lessens the rate of cell division; as a result, spontaneous mutation in culture is multiplied by the number of times. Collections preserved under in vitro for slow growth are often susceptible to genetic instability and infection. All variables that affect cultural 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 population, preferring some somaclonal variants over others [74]. This could contribute to a cell population change and the genetic integrity of the original clonal material that cannot be maintained, particularly in shooting cultures of banana [75].

It includes: **Cold Storage** – This is a form of short term storage, slow-growth preservation process where the germplasm is held at a moderate, non-freezing temperature (1–9 ^{0}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 [76]. 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 [77]. Many excellent reports on cold storage, for example, virus-free strawberry plants could be stored at 10^{0}c for around six years, while certain grape plants could be stored about 15 years (by moving them to fresh medium every year) have recently been published.

Cryopreservation: The use of solid carbon dioxide (–79°C), minimal temperature deep freezers (–80°C), vapor nitrogen (–150°C) and liquid nitrogen (–196°C), for preserving cells and tissues in frozen state at quite low temperatures. The cell can be preserved for a prolonged period of time when it is inactivated at this temperature. All examples of plant tissues that can be cryopreserved are meristems, eggs, endosperms, ovules, plants, plant cells, plant protoplasts and calli [78]. It contained two advanced cryopreservation methods focusing 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 most cellular water [79]. Encapsulation involves embedding the specimen into an alginate gel [80], may be in

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the form of shoot tip or somatic embryo, to provide an artificial seed that is dehydrated until cooling in hot air. This procedure involves many actions, of which freezing, thawing and reculture are the most significant. This method entails several moves, the most important of which are freezing, thawing, and re-culturing. Shoots, leaves, floral parts, immature embryos, hypocotyl bits, or cotyledons are the main sources of explants [81], requiring the establishment of systematic protocols. The cryopreservation of dormant buds and in vitro shoot tips is an alternate solution for long-term protection [82, 83]. Progress is now under way in the perception of best practices for the cryopreservation of a range of commercially significant plants, including apples, grapes, and citrus [84, 85].

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 may be 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 [86]. 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 cryopreservation on a regular basis for plant meristems, pollens, and plant cell crops. However, many scientific collections and germplasm banks conduct cryopreservation experiments, none use cryopreservation currently for the storage of non-seed germplasm.

In vitro collection provides various advantages like adaptability and ability. Plant conservation efforts have traditionally been divided into two areas. Contamination of In vitro collected cultures are influenced by various factors, including *Age*: Older tissues are more prone to viruses than younger one [87], *Position*: Sterilization of underground tissues with high levels of endogenous contaminants is challenging [88], *Complex tissue*: In vegetative and floral buds, pathogens in complex tissue may protect even foreign microorganisms from surface sterilant [89], *Atmosphere*: The environment may influence contamination. [90]. Desert trees, on the other hand, have less bacteria and fungi on their surfaces and are easier to handle, than tissue from moister environments [91].

4.1.3. Ex Situ: Field Gene banks, Botanic Gardens and Arboreta

The field gene banks have traditionally been responsible for recalcitrant and vegetative plants conservation such as few fruit, tuber, and plantation crops. Germplasm is grown in field nurseries of varying levels above sea level, including fruit trees, potatoes, and grasses [92]. The whole plant collection is normally preserved using two other ex situ management strategies, botanical gardens, and arboreta. There are living specimens of public show plants, educational gain, economic exploitation, and scientific inquiry. Worldwide there are about 1700 botanical gardens, with more than 3,2 million live accessions of 1,00,000 species. 10-15 percent of these species have been recorded to be at risk in nature, with some form of conservation policy in about half of them [93]. After the arrival of the first botanical garden in Pisa, Italy, in the 17th century, they served as study sites on plant-taxonomy and horticultural development (Table 3).

Table 3. Few examples of major ex situ collections of crops and wild species held in gene banks throughout the world and the percentage of world germplasm (FAO second report).

Crop	No. of world	Major gene	Country	% of world

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	accessions	bank		germplasm
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

4.2. In Situ Conservation

It is defined as preserving PGRFA's genetic diversity in the natural world, whether in the wild or in a traditional agricultural or local environment [94]. Whilst the existing nature reserves and new national parks/gene sanctuaries are meant to protect wildlife species, ecosystems, or landscapes rather than individual PGRFAs. One of the strengths of in-situ management is that it allows for species continuity, while still planning for the arrival of 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 [95-98]. 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 to develop a strategic strategy for protecting in-situ genetic diversity [99].

4.2.1. Natural Reserves or Genetic Reserves

Aim of the conservation process must be to raise genetic diversity within a bare minimum number of genetic reserves. To do so, data on the target taxa's genetic diversity, population composition, breeding mechanism, habitat requirements, and geographical distribution is 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; Basic model for natural reserve conservation- Plan and establish the reserve- Assessment of site and socioeconomic and political factors, Design of reserve, Assessment of taxon and reserve sustainability, Management plan formulation, Manage and monitor reserve, Initiation of reserve management plan, Use of reserve traditionally or professionally, Linkage to ex situ conservation (complimentary), research programs, educational organizations. A comprehensive example of setting and monitoring a natural reserve is provided by the 'Ammiad' experiment in Israel that focused upon naturally occurring wild *T. turgidum* species [20].

4.2.2. On-Farm and Home-Garden Conservation

Common crop varieties or cropping schemes are maintained by farmers or gardeners within traditional farming systems as 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 can save germplasm, whether deliberately or accidentally. The conserva-

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tionist can keep an eye on things, but they will not be involved in the actual conservation [100]. While it is beneficial to preserve landraces in this manner, it is risky in the sense that farmers will still be able to switch from developing landraces to modern cultivars, and could do so in the future [101]

5. Status of germplasm conservation

At the end of 2019, gene bank holdings were 5.43 million accessions [102] and only 5.8% of accessions are retained in living field collections; the rest are cryopreserved and deposited as DNA [103]. Until December 2019, 290 gene banks across the globe managed to safegourd 96,000 of around 1,700 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) [104] 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 [105, 106] but woody perennials are less represented [107].

The USDA collections in Geneva, New York, Davis, Central America, and Riverside hold 73 percent of all accessions, including economically important crops like apple, grape, kiwifruit, walnut, pomegranate, mandarin, almond, and other related plants [106]. All these principal collections of annual fruit crops comprehend the National Fruit Collection in the United Kingdom (http://www.nationalfruitcollection.org.uk/) [108], the N.I. Vavilov All-Russian Science Research Institute of Plant Industry's fruit collection (http://www.vir.nw.ru/unu-kollektsiya-vir/) [109], and the Foreign Centre for Research in Agronomy (http://www.vir.nw.ru/unu-kollekts) [110]. The Crop Trust's CGIAR Gene bank Platform allows CGIAR gene banks to meet their fiduciary duties under the TPGRFA to sustain and provide more accessions of crops and trees [111]. The 11 CGIAR gene banks are ideally situated in crop diversity hotspots, ensuring that germplasm acquisitions and distributions are global in scope, with a diverse range of partners and users [102] listed in Table 4 and the overall conservation trend depicted in Figure 4.

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 Asian continents. Around one-third of all recognized plant species (over 120,000 species) are found in botanical gardens worldwide [112, 113]. About the fact that most botanical gardens began as medicinal plant collections or horticultural exhibits, many have developed into world-class research institutions dedicated to the preservation of global plant biodiversity [114]. 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 organized in 2000 [115]. Millions of accessions that are linked worldwide, can be found in online databases like Genesys (www.genesys-pgr.org) [116], BGCI's Plant Search (https://www.bgci.org/plant search.php) [117], 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) [118]. Forages are underrepresented in ex situ collections compared to food crops [119], with only about 182,000 accessions trying to cover about 1000 species of grasses, legumes, and fodder trees disbursed in 80 national and international gene banks enrolled in Genesys, compared to about 7.4 million plant accessions saved in around 1750 gene banks worldwide [120].

Wild species, crop wild relatives and forage seeds do not have the same high intra-seed lot homogeneity, which can cause problems while using standard protocols [121]. Through germplasm introduction from varied research centers situated in foreign countries and germplasm collection from within the country and around the

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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 4,52,212 accessions, including invitro-1916, cryopreservation-11932 and DNA gene bank-2194 accessions that belongs to 1,762 species of plants (http://www.nbpgr.ernet.in/Research-Projects/Base_Collection 2021) [122]. The greatest number of species was preserved by germplasm banks such as U.S. National Plant Germplasm System (USDA), EMBRAPA (Brazil) and IBONE (Argentina) with about 48, 51, and 72 species respectively [123]. 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 germplasm collections. [124]. The germplasm documentation has been updated through a national information system named Brazilian Genetic Resources Information System-SIBRARGEN [125]. Users of Plant Genetic Resources (PGR) get the potential to use these capabilities to boost the efficiency and effectiveness of their efforts to preserve, explore and use novel qualities in PGR, as well as contribute to the achievement of the Sustainable Development Goals (SDGs) [126]. Target 2.5 of the UN Sustainable Development Goals (SDGs) calls 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 [127].

Table 4. The CGIAR gene banks with number of accessions among respective crops as per 2019-20.

International Institutes	**Number of accessions in corresponding crops as per 2019-20 reports**
IITA- International Institute of Tropical Agriculture (my.iita.org/accession2/) (https://www.genebanks.org/genebanks/iita/) [128]	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 (https://ciat.cgiar.org/) (https://www.genebanks.org/genebanks/ciat/) [129]	Bean-37938, Cassava-6155, Forage-22694
CIMMYT- International Maize and Wheat Improvement Centre (https://www.genebanks.org/genebanks/cimmyt/) [130]	Maize-28746, Wheat-155325
CIP- International Potato Centre (https://www.genebanks.org/genebanks/international-potato-centre/) [131]	Andean roots & tubers-2526, Potato-7224, Sweet potato-8080
ICARDA- International Centre for Agricultural Research in the Dry Areas (https://www.genebanks.org/genebanks/icarda/) [132]	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 (https://www.genebanks.org/genebanks/icrisat/) [133]	Chickpea-20764, Groundnut-15699, Pearl millet-24514, Pigeon pea-13783, Small millets-11797, Sorghum-41889

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AfricaRice- Africa Rice Centre (https://www.genebanks.org/genebanks/africarice/) [134]	Rice- 21300
Bioversity International (https://www.genebanks.org/genebanks/biodiversity-international/) [135]	Musa-1617
ICRAF- World Agro forestry (https://www.genebanks.org/genebanks/icraf/) [136]	Fruits-8246, Multipurpose trees- 6456
ILRI- International Livestock Research Institute (https://www.genebanks.org/genebanks/ilri/) [137]	Forage grasses and legumes- 18662
IRRI- International Rice Research Institute (https://www.genebanks.org/genebanks/irri/) [138]	Rice- 132661

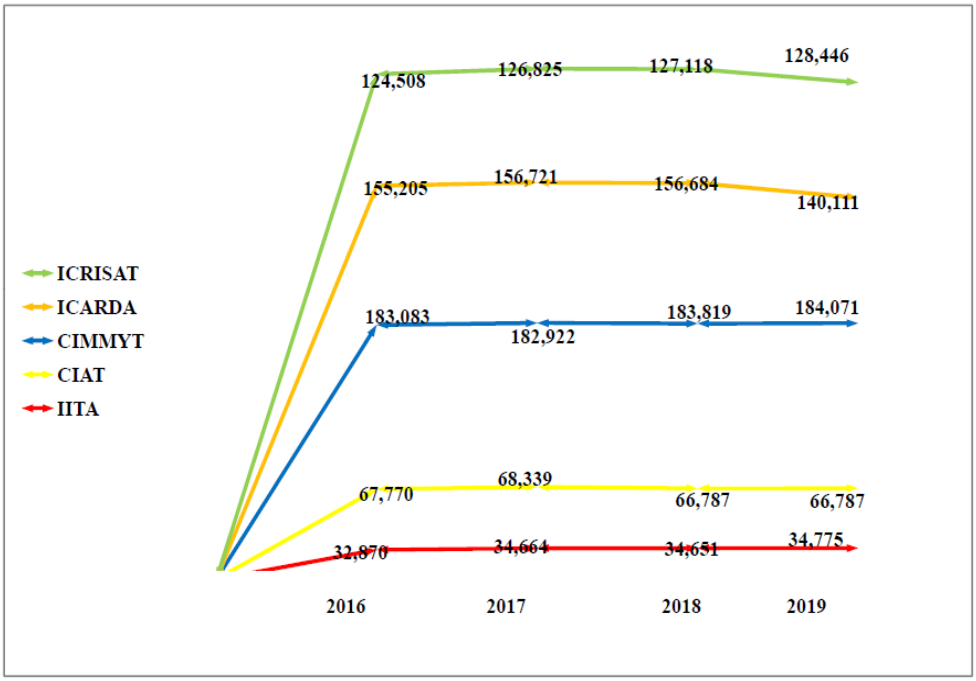

Figure 4. Representation of total conserved accessions among various gene banks since 2016-2019

6. Conclusion and 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 of plant diversity, the environment, and farming's socioeconomic and cultural aspects. It contributes to global efforts to ensure food security in the future by retrieving natural and springing up crop diversity and cultivating new agricultural crops. It's also critical for forestry and horticulture, as well as 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. Plant germplasm survival has been aided by rapid advancements in invitro culture technology, cryopreservation, and molecular markers, which

provide a useful alternative to plant diversity studies and genetic resource management. Invitro cultures technology is used for increasing the number of germplasm specimens in gene banks around the world, and it's particularly useful in plant species that produce recalcitrant seeds or reproduce asexually. Adjustments to the gene bank's protocols would be needed to reap the full benefits of cryopreservation. There is a compelling necessity for improved robust data handling mechanisms for collection, recovery, and sequence comparisons. Recently, since germplasm serves as the raw material for breeders to grow various crops, the gathering and storage of germplasm materials has taken on new urgency. The environment friendly facility will gradually build a "knowledge bank" based on genomics, digital phenotyping, and technological innovations, allowing for a more data-driven adoption of crop diversity. Future Seeds will also be a great platform for scientists looking to enhance biodiversity as a source of agricultural development.

Supplementary Materials:

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References

1. Wilkes, G. In Situ Conservation of Agricultural Systems. In M. Oldfield and J. Alcorn, eds. *Biodiversity; Culture, Conservation and Eco development* **1991**. West view Press, Colo. Boulder. Pp. 86–101
2. Kameswara Rao, N. Plant genetic resources: Advancing conservation and use through biotechnology. *African J. Biotechnol.* **2004**, *3*, 136–145, doi:10.5897/ajb2004.000-2025.
3. 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, New Jersey. **2015**.
4. Kell, S.; Marino, M.; Maxted, N. Bottlenecks in the PGRFA use system: stakeholders' perspectives. *Euphytica* **2017**, *213*, 1–24, doi:10.1007/s10681-017-1935-z.
5. Fao/Ipgri/Onu. Genebank standards for Plant Genetic Resources for Food and Agriculture; Rev. ed. Rome. **2014;** 182. ISBN 9290432365.
6. Gosal, S.S.; Wani, S.H., Kang, M.S., 2010. Biotechnology and crop improvement. J. Crop. Improv. 24 (2), 153_217.
7. Warschefsky, E.; Varma Penmetsa, R.; 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, doi:10.3732/ajb.1400116.
8. 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*, doi:10.1038/s41438-018-0062-x.
9. Thormann, I.; Engels, J.M.M.; Halewood, M. Are the old International Board for Plant Genetic Resources (IBPGR) base collections available through the Plant Treaty's multilateral system of access and benefit sharing? A review. *Genet. Resour. Crop Evol.* 2019, *66*, 291–310.
10. Mathur, S. Conservation of Biodiversity Through Tissue culture. *Res. Rev. J. Microbiol. Biotechnol.* **2013**, *2*, 2–7.
11. Harlan, J.R.; De Wet, J.M.J. Toward a Rational Classification of Cultivated Plants Published by : International Association for Plant Taxonomy (IAPT) Stable URL : http://www.jstor.org/stable/1218252 . *Taxon* **1971**, *20*, 509–517.

12. De Wet, J.M.J. 1989. Cereals for the semi-arid tropics. In: Plant Domestication by Induced Mutation. Proceedings of an Advisory group meeting on the possible use of mutation breeding for rapid domestication of new crop plants. Joint FAO/IAEA Division of Nuclear techniques in Food and Agriculture, 17–21 Nov. 1986, Vienna, p. 79–88.
13. Wright, B.D. Crop genetic resource policy: The role of ex situ genebanks. *Aust. J. Agric. Resour. Econ.* **1997**, *41*, 81–115, doi:10.1111/1467-8489.00005.
14. 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, doi:10.1079/pgr2006107.
15. Debouck, D.G. Managing Plant Genetic Diversity. *Crop Sci.* **2003**, *43*, 749–750, doi:10.2135/cropsci2003.749a.
16. Vavilov, N.I. *Origin and geography of cultivated plants.*; Cambridge University Press: Cambridge, 1992; ISBN 0521404274.
17. 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, doi:10.1007/BF00038985.
18. 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, doi:10.1079/pgr200430.
19. Commission on genetic resources for food and agriculture – FAO. The Second Report on the state of the world's plant genetic resources organization. **2010**.
20. Offord, C.A. *Germplasm Conservation*, Editor(s): Brian Thomas, Brian G Murray, Denis J Murphy, Encyclopedia of Applied Plant Sciences (Second Edition), Academic Press, 2017, Vol 2, Pages 281-288, ISBN 9780123948083, https://doi.org/10.1016/B978-0-12-394807-6.00046-0.
21. Crop Trust. Securing Crop Diversity for Sustainable Development; Global Crop Diversity Trust: Bonn, Germany, 2015.
22. Offord C.; Makinson R. Options and major considerations for plant germplasm conservation. In: Offord C. A.; Meagher P. F. (eds) Guidelines for germplasm conservation in Australia: Strategies and guidelines for developing, managing and utilising *ex situ* conservation collections. Australian Network for Plant Conservation, Canberra, pp 11–34; 2009.
23. Malhotra, N.; Panatu, S.; Singh, B.; Negi, N.; Singh, D.; Singh, M.; Chandora, R. *Genetic resources: Collection, conservation, characterization and maintenance*; Elsevier Inc., 2018; ISBN 9780128135228.
24. 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, doi:10.1038/nplants.2016.22.
25. 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. 2009.
26. 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 from: https://cran.rproject.org/package=PGRdup.
27. Engels, J.; Visser, L. *A guide to effective management of germplasm collections.*; 2003; Vol. 59; ISBN 9290435828.
28. Vinson Kurian, The Hindu Businessline; Monday, Nov 04, 2002
29. IUCN RED LIST 2019-20. Source- IUCN official website, Available online: https://www.iucnredlist.org/ (accessed on 15 March 2021).
30. Harlan, H.V. The Origin Of Hooded Barley. *J. Hered.* **1931**, *22*, 265–272, doi:10.1093/oxfordjournals.jhered.a103496.
31. 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. *F. Crop. Res.* **2005**, *91*, 107–116, doi:10.1016/j.fcr.2004.06.003.
32. 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). *PlantGenet. Resour. News***. 2008.** *154*, 6–13.
33. 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.
34. Ross-Ibarra, J.; Morrell, P.L.; Gaut, B.S. Plant domestication, a unique opportunity to identify the genetic basis of adaptation; Proceedings of the National Academy of Sciences May 2007, 104 (suppl 1) 8641-8648; DOI: 10.1073/pnas.0700643104.
35. Guarino, L. Approaches to measuring genetic erosion. PGR Documentation and Information in Europe – towards a sustainable and user-oriented information infrastructure. EPGRIS Final Conference combined with a meeting of the ECP/GR Information and Documentation Network, Prague, Czech Republic, 2003. pp. 11–13.
36. Upadhyaya, H.D.; Gowda, C.L.L. Managing and Enhancing the Use of Germplasm – Strategies and Methodologies. Technical Manual No. 10. 2009. ICRISAT, Patancheru.

37. Brennan, J.P.; Fox, P.N. Impact of CIMMYT varieties on the genetic diversity of wheat in Australia. *Aust. J. Agric. Res.***1993.** *49* (2), 175–178.
38. 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.
39. Douhovnikoff, V.; Dodd, R.S. Epigenetics: A potential mechanism for clonal plant success. *Plant Ecol*. **2015.** *216*:227–233. doi:10.1007/s11258-014-0430-z
40. Gollin, D. Conserving genetic resources for agriculture: economic implications of emerging science. *Food Sec.* **2020**. 12, 919–927. https://doi.org/10.1007/s12571-020-01035-w
41. Li, M.; Frank, M.H.; Migicovsky, Z. ColourQuant: A high-throughput technique to extract and quantify colour phenotypes from plant images. 2019. arXiv 190301652. http://arxiv.org/abs/1903.01652.
42. Maxted, N. Ex Situ, In Situ Conservation, Editor(s): Simon Asher Levin, Encyclopedia of Biodiversity, Elsevier, 2001, Pages 683-695, ISBN 9780122268656, https://doi.org/10.1016/B0-12-226865-2/00115-2.
43. 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, doi:10.9790/3008-1104017073.
44. International Seed Testing Association. International Rules for Seed Testing 2018; ISTA: Bassersdorf, Switzerland, 2018.
45. Hawkes, J.G; Maxted, N.; Ford-Lloyd, B.V.; The Ex Situ Conservation of Plant Genetic Resources. 2000. Dordrecht: Kluwer Academic Publishers.
46. Roberts, E. H. Predicting the storage life of seeds. In Proceedings. *Seed Science and Technology*, 1973. 1, 499-514.
47. Harrington, J.F. Thumb Rules of Drying Seed. *Crops and Soils*. **1960**, *13*: 16-17.
48. Merritt, D.; Dixon, K. Restoration Seed Banks—A Matter of Scale. *Science*. **2011,** *332*, 424-425. 10.2307/29784114.
49. Kholina, A.B.; Voronkova, N.M. Seed Cryopreservation of Some Medicinal Legumes. *J. Bot.* **2012**, *2012* 1–7, doi:10.1155/2012/186891.
50. 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 April 14, 2021).
51. Hay, F.R.; Probert, R.J. Advances in seed conservation of wild plant species: A review of recent research. *Conserv. Physiol.* **2013**, *1*. [CrossRef] [PubMed]
52. Frankel, O.; Brown, A. Current plant genetic resources: A critical appraisal. 1984. Oxford & IBH Publ. Co., New Delhi.
53. 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.
54. 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. https://doi.org/10.1111/pce.13761
55. Furman, B.J. Methodology to establish a composite collection: case study in lentil. *PlantGenet. Resour*. **2006,** *4*, 2–12.
56. Azough, Z.; Kehel, Z.; Benomar, A.; Bellafkih, M.; Amri, A. Predictive Characterization of ICARDA Genebank Barley Accessions Using FIGS and Machine Learning. *Intell. Environ*. **2019**, 121–129. [CrossRef]
57. Kaczmarczyk, A.; Funnekotter, B.; Menon, A.; Phang, P.Y.; Al-Hanbali, A.; Bunn, E.; Mancera, R.L. Current issues in plant cryopreservation, *Curr Front Cryobiol*, In: Prof. Igor Katkov, editor. 2012, Available online: http://www.intechopen.com/books/currentfrontiers-in-cryobiology/current-issues-in-plant-cryopreservat (accessed on 11 April 2021).
58. Berjak, P.; Pammenter, N.W. Implications of the lack of desiccation tolerance in recalcitrant seeds. *Front. Plant Sci.* **2013**, *4*, 1–9, doi:10.3389/fpls.2013.00478.
59. Walters, C.; Berjak, P.; Pammenter, N.; Kennedy, K.; Raven, P. Preservation of recalcitrant seeds. *Science* **2013**, *(80) 339*, 915–916, doi:10.1126/science.1230935.
60. 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, doi:10.15835/nbha3824878.
61. Engelmann, F. Use of biotechnologies for the conservation of plant biodiversity. *InVitro Cell. Dev. Biol. Plant* **2011**, *47*, 5-16.
62. 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. *South African J. Bot.* **2012**, *78*, 47–54, doi:10.1016/j.sajb.2011.05.001.
63. Han, C.Y.; Sun, W.B. Seed storage behaviour of Magnolia odoratissima. *Seed Sci Technol***. 2013,** *41*: 143–147.

64. 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, doi:10.1017/S0960258513000214.
65. 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, doi:10.1007/s00299-007-0467-8.
66. Hussain, A.; Ahmed, I.; Nazir, H.; Ullah, I. Plant Tissue Culture: Current Status and Opportunities. *Recent Adv. Plant Vitr. Cult.* **2012**, 1–28, doi:10.5772/50568.
67. El-Dougdoug, K.A.; El-Shamy, M.M.; Plant Tissue Culture: Current Status and Opportunities. *Afr. J. Microbiol*. **2011.** Res. 53259235932.
68. 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. Available from: https://doi.org/10.1590/0100-29452016833
69. Gonzalez-Arnao, M.T.; Panta, A.; Roca, W.M.; Escobar, R.H.; Engelmann, F. Development and large scale application of cryopreservation techniques for shoot and somatic embryo cultures of tropical crops. *Plant Cell Tiss Org Cult.* **2008,** *92*, 1-13.
70. Henshaw, G.G. Technical aspects of tissue culture storage for genetic conservation. Crop genetic resources for today and tomorrow, Cambridge University Press, UK, 1975, 349-358.
71. Morel, G. Meristem culture techniques for the long-term storage of cultivated plants. In Crop Genetic Resources for Today and Tomorrow, O. H. Frankel and J. G. Hawkes, eds. 1975. New York: Cambridge University Press, Pp. 327–332.
72. Valerie, C.P.; Jorge, A.S.; Victor, M.V.A.; Florent, E. *In vitro* collecting techniques for germplasm conservation. IPGRI technical bulletin No7. International Plant Genetic Resource Institute, Rome, Italy. 2002, 250.
73. Chauhan, R.; Singh, V.; Quareshi, A. In Vitro Conservation Through Slow-Growth Storage. *Synth. Seeds Germplasm Regen. Preserv. Prospect.* **2019**, *1*, 482, doi:10.1007/978-3-030-24631-0.
74. 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.
75. 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
76. 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, doi:10.1007/s11240-006-9155-6.
77. 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 Culture* **2009,** *96*, 127–136.
78. Kaviani, B. Conservation of plant genetic resources by cryopreservation. *Aust. J. Crop Sci.* **2011**, *5*, 778–800.
79. Sakai, A.; Engelmann, F. Vitrification, encapsulation-vitrification and droplet-vitrification: a review. *CryoLetters* **2007,** *28*, 151–172.
80. 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
81. Paunescu, A. Biotechnology for Endangered Plant Conservation: A Critical Overview. *Romanian Biotechnological Letters*. **2009**, *14*, 4095-4103.
82. Wang, M.-R.; Chen, L.; Teixeira da Silva, J.A.; Volk, G.M.; Wang, Q.-C. Cryobiotechnology of apple (*Malus* spp.): development, progress and future prospects. *Plant Cell Rep.* **2018**, *37*, 689–709, doi:10.1007/s00299-018-2249-x.
83. 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, doi:10.5073/vitis.2019.58.71-78.
84. 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. doi:10.1007/s11627-017-9828-3
85. 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. doi:10.1016/j.cryobiol.2019.01.008
86. Dixit, S.; Mandal, B.B.; Ahuja, S.; Srivastava, P.S. Genetic stability assessment of plants regenerated from cryopreserved embryogenic tissues of *Dioscorea bulbifera* Using RAPD, biochemical and morphological analysis. *Cryoletters* **2003**, *24*, 77-84.
87. Bernstein, M.E.; Carroll, G.C. Internal fungi in old-growth Douglas fir foliage. *Canadian Journal of Botany* **1977**, *55*, 644-653.
88. Smith, R.H.; Burrows, J.; Kurten, K. Challenges associated with micropropagation of Zephyranthes and Hippeastrumsp. (Amaryllidaceae). *In vitro Cell. Devel. Biol. Plant* **1999**, *35*, 281-282.

89. 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. *Canadian Journal of Forest Resources* **1997**, *27*, 959-964.
90. Pence, V.C. In vitro collection (IVCN). In: Bowes, B. *A colour atlas of plant propagation and conservation*; CRC Press, 1999; ISBN 1840765410.
91. MacKay, W.A. Micropropagation systems for the Mexican red bud (*Cercis canadensis var. mexicana* L.) and other woody plants of the Chichuahuan desert. *In vitro Cell.Devel. Biol. Plant* **1999**, *35*, 283-284.
92. De, M. Field Gene Banks: The Living Repositories Of Plant Genetic Resources. *Harvest* (Online); Bi-Annual Field Gene Banks. **2017**, Volume 2; Page 21. ISSN 2456-6551
93. O'Donnell, K.; Sharrock, S. Botanic gardens complement agricultural gene bank in collecting and conserving plant genetic diversity. *Biopreserv. Biobank.* **2018**, *16*, 384–390, doi:10.1089/bio.2018.0028.
94. Cruz-Cruz, C.A.; Gonzalez-Arnao, M.T.; Engelman, F. Biotechnology and conservation of plant biodiversity. *Resources* **2013**, *2*, 73–95.
95. Hoyt, E. Conserving the Wild Relatives of Crops. 1988, International Board for Plant Genetic Resources, Rome.
96. IBPGR. Ecogeographical Surveying and In Situ Conservation of Crop Relatives. 1985, International Board for Plant Genetic Resources, Rome:
97. Noy-Meir.; Anikster, Y.; Waldman, M.; Ashri, A. Population dynamics research for in situ conservation: Wild wheat in Israel. *Plant Genet. Resources Newsl*. **1989,** *75/76*, 9–11.
98. Plucknett, D.L.; Smith, N.J.H. *Gene banks and the world's food*; Princeton University Press, 2014; ISBN 1400858119.
99. Kaya, Z.; Kün, E.; Güner, A. National plan for in situ conservation of plant genetic diversity in Turkey. **1998**.
100. 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, doi:10.1023/A:1013896401710.
101. Swaminathan, M.S. The past, present and future contributions of farmers to the conservation and development of genetic diversity, 2009. *Manag. plant Genet. Divers. Proc. an Int. Conf. Kuala Lumpur, Malaysia, 12-16 June 2000*, 23–31, doi:10.1079/9780851995229.0023.
102. 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 cgiar genebanks. *Plants* **2020**, *9*, 1–31, doi:10.3390/plants9101296. (Cross ref)
103. FAO 2019. WIEWS: World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture. FAO, Rome. Available online: http://www.fao.org/wiews (accessed on 10 April 2021).
104. FAO 2020. Available online: http://www.fao.org/sustainable-development-goals/indicators/251a/en/2020 (accessed on 13 April 2021).
105. 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. **2018**, *468*, 451–468, doi:10.2135/cropsci2017.05.0303.
106. GRIN-Global. 2019. U.S. National Plant Germplasm System. GRIN-Glob. Web version 11040. GRIN-Global. Available online: https://npgsweb.ars-grin.gov/gringlobal/query/summary.aspx (accessed on 2 April 2021).
107. 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.
108. National Fruit Collection in the United Kingdom. Available online: http://www.nationalfruitcollection.org.uk/ (accessed on 20 March 2021).
109. 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).
110. Foreign Centre for Research in Agronomy. Available online: http://www.vir.nw.ru/unu-kollekts (accessed on 10 April 2021).
111. CGIAR Genebank Platform. 2019 Genebank Platform Annual Report; Global Crop Diversity Trust: Bonn, Germany, 2020.
112. CWR Project. Annual Report; Global Crop Diversity Trust: Bonn, Germany, 2019.
113. Miller, A.J.; Novy, A.; Glover, J.; Kellogg, E.A.; Maul, J.E.; Raven, P.; Wyse Jackson, P. Expanding the role of botanical gardens in the future of food. *Nat. Plants.* **2015**, *1*, 15078. doi:10.1038/nplants.2015.78
114. 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. doi:10.1007/s12229-013-9120-0
115. Wyse Jackson, P.; Kennedy, K. The global strategy for plant conservation: A challenge and opportunity for the international community. *Trends Plant Sci.* **2009**, *14*, 578–580. doi:10.1016/j.tplants.2009.08.011
116. Genesys database. Available online: www.genesys-pgr.org (accessed on 22 March 2021).
117. BGCI's Plant Search. Available online: https://www.bgci.org/plant search.php (accessed on 22 March 2021).

118. FAO's Global Knowledge and Early Alert System on Plant Genetic Tools for Food and Agriculture (WIEWS) website. Available online: http://www.fao.org/wiews (accessed on 26 March 2021)..
119. Hanson, J.; Ellis, R.H. Progress and challenges in ex situ conservation of forage germplasm: Grasses, herbaceous legumes and fodder trees. *Plants* **2020**, *9*. (Cross ref) (Google scholar).
120. 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] [PubMed]
121. Walters, C.; Berjak, P.; Pammenter, N.; Kennedy, K.; Raven, P. Preservation of recalcitrant seeds. *Science (80).* **2013**, *339*, 915–916, doi:10.1126/science.1230935.
122. 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).
123. 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*, 1–17, doi:10.3389/fpls.2019.01377.
124. 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, doi:10.1590/s1984-70332012000500009.
125. 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. DOI: 10.17660/ActaHortic.2010.864.1. (CrossRef Full Text) | (Google Scholar)
126. 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, https://doi.org/10.1111/nph.14993 .
127. Smale, M.; Jamora, N. Valuing genebanks. *Food Sec*. **2020,** *12*, 905–918, https://doi.org/10.1007/s12571-020-01034-x
128. IITA- International Institute of Tropical Agriculture. Available online: my.iita.org/accession2/ , https://www.genebanks.org/genebanks/iita/ (accessed on 09 April 2021)
129. CIAT- International Centre for Tropical Agriculture. Available online: https://ciat.cgiar.org/, https://www.genebanks.org/genebanks/ciat/ (accessed on 09 April 2021).
130. CIMMYT- International Maize and Wheat Improvement Centre. Available online: https://www.genebanks.org/genebanks/cimmyt/ (accessed on 03 April 2021).
131. CIP- International Potato Centre. Available online: https://www.genebanks.org/genebanks/international-potato-centre/ (accessed on 09 April 2021).
132. ICARDA- International Centre for Agricultural Research in the Dry Areas. Available online: https://www.genebanks.org/genebanks/icarda/ (accessed on 03 April 2021).
133. ICRISAT- International Crops Research Institute for the Semi-Arid Tropics. Available online: https://www.genebanks.org/genebanks/icrisat/ (accessed on 03 April 2021).
134. AfricaRice- Africa Rice Centre. Available online: https://www.genebanks.org/genebanks/africarice/ (accessed on 9 April 2021).
135. Bioversity International. Available online: https://www.genebanks.org/genebanks/biodiversity-international/ (accessed on 09 April 2021).
136. ICRAF- World Agro forestry. Available online: https://www.genebanks.org/genebanks/icraf/ (accessed on 03 April 2021).
137. ILRI- International Livestock Research Institute. Available online: https://www.genebanks.org/genebanks/ilri/ (accessed on 09 April 2021).
138. IRRI- International Rice Research Institute. Available online: https://www.genebanks.org/genebanks/irri/ (accessed on 03 April 2021).