Germplasm Conservation

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Contributor: Prashant Kaushik

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.

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 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].

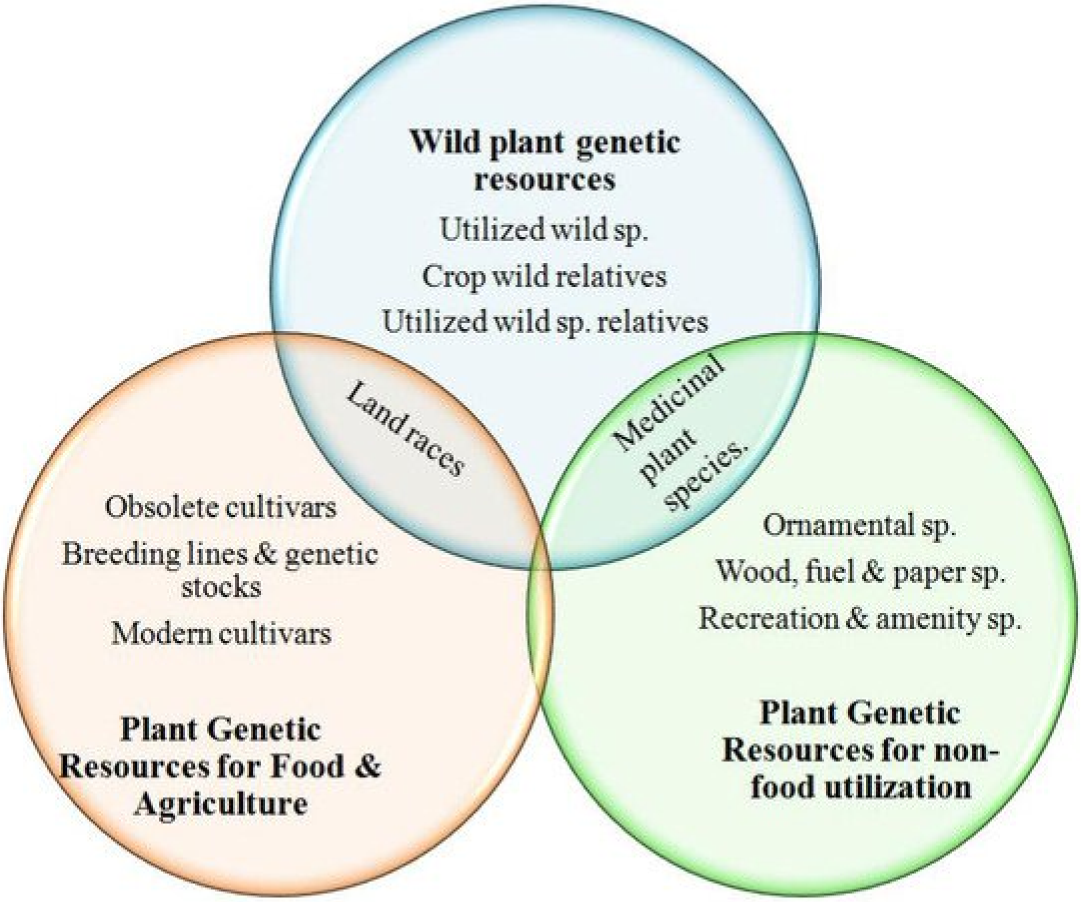

Figure 1. Overall representation of global plant genetic diversity.

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.

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 [16]. 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 [17][18][19] led to the conclusion that cultivated crops have become less varied after domestication, due to selection pressures and dispersal bottlenecks [20]. 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” [21]. 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 [22]. 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 [23]. It has been reported that about 12.5% (34,000 species) of vascular plants worldwide have been at threat (Table 1).

Table 1. International Union for Conservation of Nature (IUCN) Red List for the year 2019–2020 [24].

Category	Status
EX—Extinct	122
EW—Extinct in the wild	42

Category	Status
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

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 [25]. 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 [26].

4. Methods of Germplasm Storage and Conservation

Accessions are generally stored as different kinds of collections. Core collections [27] serve as the 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 [28]. 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 [29]. 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 [30][31]. The two fundamental storage approaches; ex situ and in situ conservation [32], employed for germplasm storage are explained in Figure 2. 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 [33]. 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.

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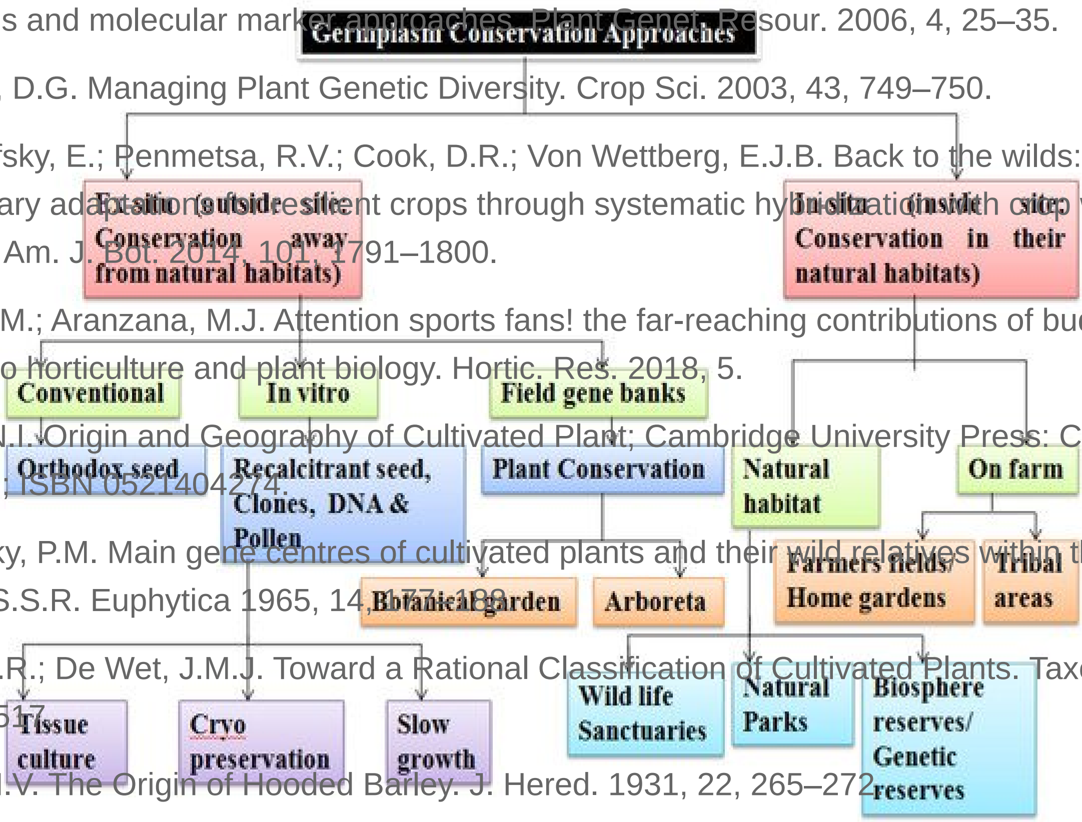

Figure 2. Schematic outline of germplasm conservation approaches.

5. Status of Germplasm Conservation

By the end of 2019, gene banks worldwide held 5.43 million accessions [34] and only 5.8% of these accessions are retained as living field collections; the rest are cryopreserved and deposited as DNA [35]. 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)) [36].

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 [37][38] while woody perennials are less represented [39].

The USDA collections at Geneva, New York, Davis, Central America, and Riverside hold 78% of all accessions, including economically important crops like apple, grape, kiwifruit, walnut, pomegranate, mandarin, almond, and other related plants [39]. 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)) [40], 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)) [41], and the Foreign Centre for Research in Agronomy (http://www.vir.nw.ru/unu-kollekts (accessed on 15 March 2021)) [42][43]. The Crop Trust's CGIAR Gene

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bank Platform allows CGIAR gene banks to meet their obligations under the International Treaty on PGRFA to sustain and provide more accessions of crops and trees [44]. 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 [34] (Table 3) and the overall conservation trend depicted in Figure 3.

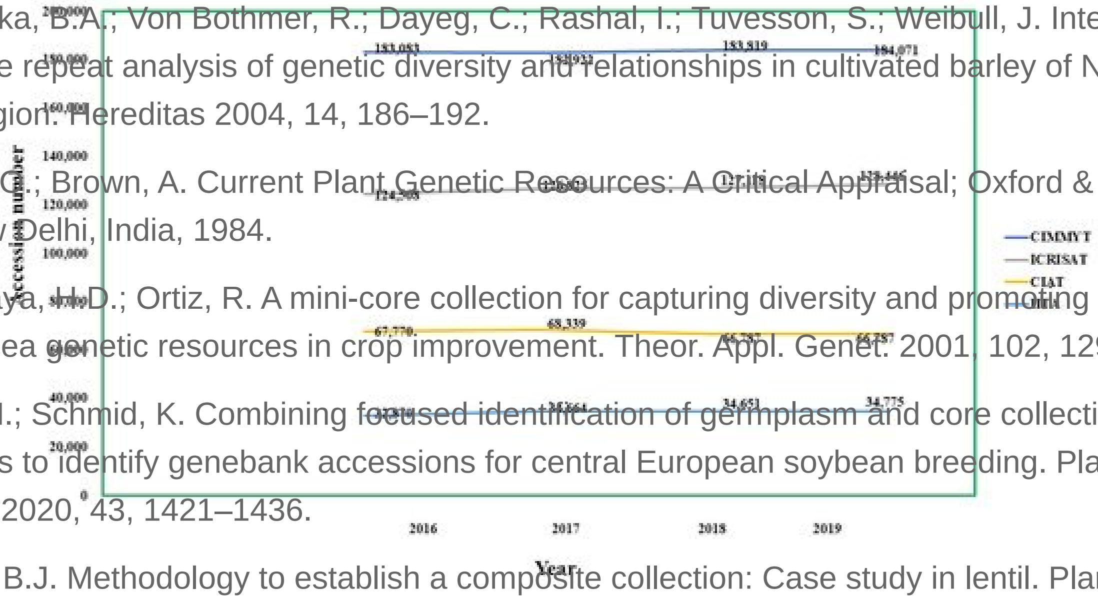

Figure 3. Representation of total conserved accessions among various gene banks since 2016–2019, where years are represented by x-axis and accession numbers by the y-axis.

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

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)) [45]	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)) [46]	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)) [47]	Maize-28746, Wheat-155325
CIP- International Potato Centre, Lima, Peru (https://www.genebanks.org/genebanks/international-potato-centre/	Andean roots and tubers-2526, Potato-7224, Sweet potato-

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International Institutes	Number of Accessions under Corresponding Crops as Per 2019–2020
(accessed on 15 March 2021)) [48]	8080
ICARDA- International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria (https://www.genebanks.org/genebanks/icarda/(accessed on 15 March 2021) [49]	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)) [50]	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)) [51]	Rice- 21300
Bioversity International, Rome, Italy (https://www.genebanks.org/genebanks/biodiversity-international/(accessed on 15 March 2021)) [52]	Musa-1617
ICRAF- World Agro forestry, Nairobi, Kenya (https://www.genebanks.org/genebanks/icraf/(accessed on 15 March 2021)) [53]	Fruits-8246, Multipurpose trees-6456
ILRI- International Livestock Research Institute, Nairobi, Kenya (https://www.genebanks.org/genebanks/ilri/(accessed on 15 March 2021)) [54]	Forage grasses and legumes-18662
IRRI- International Rice Research Institute, Los Baños, Philippines (https://www.genebanks.org/genebanks/irri/(accessed on 15 March 2021)) [55]	Rice-132661

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