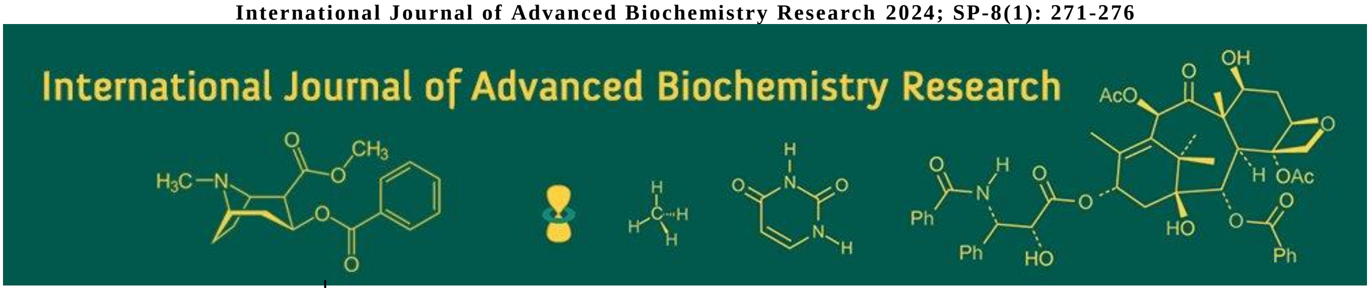

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(1): 271-276
www.biochemjournal.com
Received: 15-11-2023
Accepted: 28-12-2023

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Germplasm conservation, evaluation, characterization and improvement of genetic resources of fruit crops in India: Preserving of future fruit crops for the future

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DOI: https://doi.org/10.33545/26174693.2024.v8.i1Se.346

Abstract
India boasts a diverse and abundant biodiversity legacy that spans a broad range of environments, including alpine vegetation, tropical rain forests, temperate forests, and coastal wetlands. Four of the world's eighteen biodiversity hotspots - the Western Ghats, the Eastern Himalaya, the Western Himalaya, and the Nicobar Islands - are located in India. Four of the world's eighteen biodiversity hotspots - the Western Ghats, the Eastern Himalaya, the Western Himalaya, and the Nicobar Islands—are located in India. Our legacy, biodiversity, which represents variation among genetic resources at the gene, population, species, and ecosystem levels, is essential to both sustainable development and well-being. While several institutes also do conservation in field gene banks, NBPGR is the main organization in India responsible for managing germplasm on fruits. In addition to field gene banks, cryobanks and in vitro tissue culture are methods used to preserve temperate fruit germplasm. International breeding efforts to create novel cultivars or rootstocks, mainly for banana, citrus, and mango, have also made use of Indian material. Classical examples of the global usage of Indian fruit germplasm include the creation of Floridian mangoes in the United States, the use of Indian citrus rootstocks globally, and the use of Indian banana genotypes. In the past, NBPGR introduced 5,687 accessions of 104 fruit crop species comprising temperate, tropical and sub-tropical fruit plants during 1976 to 2023. However, In order to support the preservation of cultural traditions and to make it easier to conduct research into the history of food and novel food sources, it is necessary to gather and share this knowledge. The detailed information on Germplasm conservation, genetic improvement and evaluation of genetic resources for development of improved varieties in fruit crops are presented in this chapter.

Keywords: Germplasm, Cryobanks, NBPGR, Ecosystem, Genebank

Introduction
Nowadays, fewer plant species are necessary for both global food security and economic expansion. Between 40 and 100,000 plant species have been consistently utilized by humans throughout history for food, fiber, housing, industry, culture, and medicine. (Magbagbeola *et al.*, 2010) [3]. Biodiversity refers to the variation in life in all forms. Crop diversity refers to the variety of genes and genotypes found in a particular crop species. This diversity may occur within species, genera or ecosystem. Biodiversity as our heritage makes a key contribution to well-being and sustainable development. India is divided into three biomass types: tropical humid forests, tropical dry/deciduous forests, and warm deserts/semi-deserts. The two main realms are the Palaearctic and the Indo-Malayan. The biodiversity of India has a high endemism. Approximately one-third of the nation's documented plant life is indigenous to the region, primarily found in the North-East, Western Ghats, North-West Himalaya, and the Andaman and Nicobar Islands. Historically, many of these varieties were preserved by the monarchs and rulers in the grounds surrounding their palaces or in the areas surrounding temples. Documented evidences indicate that the orchard in Lakhbagh in Darbangha established by Mughal Emperor Akbar consisted of one lakh mango trees, proving eloquently the interest the Mughal emperors had in making selections for quality. The Mughal gardens in Kashmir, Punjab, Delhi and Uttar Pradesh are testimony to their contributions to floriculture. The conservation of diversity and plants of different species was

started by Britishes, Mughals and Portuguese through introductions such as pineapple through Philippines, papaya, guava, cashew, chili and tomatoes were the introductions. Grapes were an introduction by Mughals in 1300. Around 1832, Christian missionaries brought grape to Tamil Nadu's Salem and Madurai areas in the southern part of the country. Plant genetic resources are a storehouse of genes and gene complexes that serve as the foundation for horticultural crop improvement. (Singh, 2010) [4]. The plant breeders require reservoir of genetic variation (gene pools) for crop improvement. The likelihood of discovering specific traits, such as genes for worm, pest, and disease resistance or for adaptation to broader ecological amplitudes and stress conditions, increases with the size of the reservoir of variation. (Chandel and Pandey, 1991) [1]. "Vast diversity in tropical and temperate fruits cultivated and wild -109 species several wild, endangered and endemic species"

You can find biodiversity in your backyard as well as in the wild. Many tropical fruit species exhibit variety that can be found in their natural habitats, where they continue to grow. Examples of this include the presence of *Mangifera sylvatica* in northeastern India and the M *andamanica* and M *Nicobariaca* species in the Andaman group of islands. Diversity was created in the wild throughout time, mostly as a result of spontaneous mutations, seed distribution, and the population of seedlings. As seen in the cases of fruits like mango and jackfruit, seedling populations have been the source of variation in backyard ecosystems. Natural seed distribution has led to the emergence of diversity in some areas, like as the Western Ghat regions and the Appemidi mango pickle variety in Karnataka's Uttara Kannada district.

Characteristic features of fruit tree diversity

The primary factors contributing to India's tropical fruit diversity, whether it be in the form of cultivated or wild varieties, which includes

1. The presence of high heterozygosity.
2. Cross pollination.
3. Seed propagation.
4. Absence of vegetative propagation in the earlier days.
5. Indiscriminate multiplication.

The more difficult issue with tropical fruit species is managing diversity, as opposed to other crops where variability must be created. In fact, varietal diversity in crops like mangoes is seen to be a barrier to their advancement. (Naik *et al*., 1958) [10].

Genetic resources constitute the foundation upon which horticulture is based, which includes

1. **Wild-forms:** These are important source of resistance to biotic and abiotic stresses. These can cross easily with cultivated species.
2. **Landraces:** These are the primitive or traditional cultivars which are the product of selection carried out by farmers continuously over many generations. They provides high degree of resistance to biotic and abiotic stresses.
3. **Obsolete cultivars:** Improved varieties of recent past are known as obsolete cultivars.
4. **Modern cultivars:** Presently cultivated high yielding varieties are known modern cultivars. These varieties have high yield potential and uniformity as compared to obsolete varieties and land races.
5. **Advanced breeding lines:** Pre-released plants which have been developed by plant breeders for use in modern scientific plant breeding are known as advanced breeding lines.

Gene pool

It refers to whole library of different alleles of a species or sum total of genes. It includes all cultivars, wild species and its relatives containing all the genes for breeding use.

Classification of Gene pool

1. **Primary gene pool (GP1):** Here crossing is easy and leads to production of fertile hybrids. It includes plants that belong to same species or closely related species.
2. **Secondary gene pool (GP2):** Consists of all biological species that can be crossed with a crop but where the hybrids are sterile, gene transfer is difficult but not impossible.
3. **Tertiary gene pool (GP3):** Consists of distantly related species and gene transfer is possible with advance techniques.

Biodiversity hotspots

Earth's biological richest places, with high number of species found nowhere else. These represent highest diversity of crop plants. There are presently 34 biodiversity hotspots in world out of which two namely Himalayas and Western Ghats are located in India. (http://www.conservation.org).

Centre of diversity

It refers to the area where the crop plants show maximum diversity. Centre of diversity is classified into two types *viz*., primary centre of diversity and secondary centre of diversity.

1. **Primary centre of diversity:** It refers to the geographical area where crop plants have originated. Such diversity has maximum number of dominant genes and normally wild traits.
2. **Secondary centre of diversity:** It refers to area where the crop plants show considerable diversity but they were not originated there.

Vavilov in 1951 gave 8 centres of origin which are given below in table:

1. The Chinese Centre	Peach, Apricot, Cherry, litchi, kiwi, plum, loquat, sweet orange, persimmon
2. The Hindustan Centre	Mango, orange, tangerine, coconut, tamarind, banana, phalsa, jackfruit
3. The Central Asiatic centre	Pear, Apple, Pistachio, Almond, grape, walnut
4. The Asia Minor Centre	Apricot, pistachio nut, almond, pomegranate, quince, date palm
5. The Mediterranean Centre	Olive
6. Abyssinian Centre	Coffee
7. The central American Centre	Cocoa, papaya, guava
8. The South American Centre	Pineapple, cashew

Why to manage biodiversity

1. **Genetic erosion:** Genetic erosion is the term used to describe the loss of genetic variety brought on by either natural or artificial mechanisms. It is brought on by industrial agriculture, farming in natural habitats, clean cultivation, contemporary cultivars replacing land races, and developmental activities.

2. **Climate change:** In some fruit-growing regions such as the Central Valley of California, the percent of the landscape suitable to apple production has decreased from 50 percent in 1950, to less than 4 percent today, and will likely be entirely lost by 2050. In order to sustain temperate fruit production, with decrease in cool hours will soon necessitate a change in varietals. The production and preservation of a wide variety of fruits are already being hampered by the expansion of recently introduced illnesses and pests, the occurrence of more frequent tropical storms, and severe droughts. (Nabhan, 2010) [6].

3. **Continuous destruction of habitat and extinction of wild relatives:** Humans are currently destroying natural ecosystems for a variety of reasons, such as construction and other developmental works, as a result of global population growth and urbanization. This has led to the extinction of many wild species and important genes.

Management of germplasm

It involves following steps:

1. Germplasm introduction.
2. Germplasm collection.
3. Germplasm characterization.
4. Germplasm conservation.
5. Germplasm evaluation.
6. Germplasm utilization.

1. Germplasm introduction

Introduced crops are either directly used as varieties without further improvent into the previously non cultivated area (primary introduction) or used in breeding for improving quality, productivity and imparting resistance (Secondary introduction). The NBPGR has been instrumental in introducing many new varieties like Red Delicious, Bartlett pear, Elberta peach, Santa Rosa, Loose Perlett, Thompson Seedless, Kinnow, etc. (Singh, 2010) [4]. The Fruit science division of Sher-e- Kashmir University of Agricultural Sciences and Technology Kashmir have introduced some crop cultivars.

2. Germplasm exploration/ Collection

Collection of germplasm of fruit crops is very important and should be carried in the areas having high diversity. Germplasm collection is undertaken by NBPGR in India and they have collected germplasm from different parts of country. There is a large germplasm collection maintained in The Fruit science division of Sher-e-Kashmir University of Agricultural Sciences and Technology. The National collection of apples was assembled and is maintained by the Plant Genetic Resources Unit (PGRU) located on the campus of Cornell University's New York State Agricultural Experiment Station. The Central Institute of Temperate Horticulture is the regional station of NBPGR in Jammu and Kashmir has various collections of pome, stone and nuts fruits.

3. Germplasm characterization

It refers to the observation, quantifying and documentation of heritable plant traits in a collection. However, Inadequate comprehensive examination has been done of the response to biotic and abiotic stressors, nutritionally significant components, or processing features. The expression of highly heritable characteristics, such as morphological or agronomical traits, seed proteins, or molecular markers, is determined by the description of plant germplasm.

a. **Morphological characterization:** It is imperative to characterize germplasm in order to furnish end users with information regarding the characteristics of accessions that guarantee optimal utilization of the germplasm collection. Phenotype discrimination is made simple and quick by the recording and compilation of data on significant traits that differentiate accessions within a species. It makes it easier to verify that homogeneous sample true-to-type, enabling the discovery of misidentifications or duplicates and pointing out potential mistakes made during other gene bank processes. As long as there are enough seeds or plant materials to sample, it can be done at any point during the conservation process. To increase the value of any collection, it should be completed as soon as feasible. However, because it is exceedingly costly and time-consuming, many gene banks either delay it or perform it during regeneration in order to save cost.

b. **Molecular characterization of germplasm:** The molecular characterization is achieved through DNA finger printing Technique to make the characterization more effective. The DNA fragments, resembling barcodes, are unique to the individual and hence can be used in much the same way as conventional fingerprints- to identify individuals with absolute certainty. Importance of DNA fingerprinting is that it helps to form primary core collection which can be conserved and utilized and helps in removal of duplicates.

Genome wide association studies (GWAS)

It could be able to get around a number of the drawbacks of traditional linkage mapping and offer a potent supplementary method for breaking down complex features. Compared to QTL analysis, GWAS uses previous recombination in a variety of association panels to more precisely identify genes associated with phenotypic variables. Because GWAS allows for the unrestricted access to a wide range of genetic resources for marker trait association without compromising marker availability, it has emerged as a potent technique for QTL mapping in plants.

Genomic selection

In order to predict the genomic estimated breeding values (GEBVs) for each individual, a better approach known as genomic selection (GS) simultaneously uses large genotypic data (genome wide) that exceeds phenotypic data, phenotypic data, and modeling using statistical tools. (Crossa *et al.*, 2017) [2]. In genomic selection, a training population - a representative subset of the breeding population - is used to create a statistical model. After that, this model is used to determine the allelic effects of each marker locus, or genomic assisted breeding values, which can be used to preselect genotypes specific to traits without the need for phenotypic data. Emphasized that pre-breeding can be accelerated by combining genome-wide data with genomic selection, which offers excellent specificity and predictability. By reducing the length of the selection cycle, GS can be used to quickly improve complicated traits by producing phenotypes that are dependable. The breeding cycle of pasture grass Lolium perenne was shortened by four years as a result of GS application.

4. Germplasm conservation

Conservation refers to the preservation of germplasm. The conservation of germplasm is achieved by two methods.

1. *In-situ* conservation.
2. *Ex-situ* conservation.

***In- situ* conservation**

This type of conservation allows the crop species to grow in their natural habitat. As this is done in natural habitats it allows evolutionary processes to continue, which are base of genetic diversity and plant adaptability. It can be carried out in the form of biosphere reserves, habitats, wildlife parks, gene sanctuaries and national parks (Chaudhary, 2000) [7]. India has 18 biosphere reserves, many national parks and gene sanctuaries (http:// www.wikipedia.com). Highest diversity of fruits in India is in North eastern region followed by Western Peninsular tract and Western Himalayas. First *In situ* gene sanctuary in India was established for citrus in Tura hill range of Garo hills in Meghalaya (Singh, 2010) [4].

***Ex-situ* conservation**

In this method conservation of germplasm is carried outside their native habitat in the form of seed, embryo, tissue, etc. Objective of ex-situ conservation is to maintain the accessions without any change in genetic constitution. It involves conservation through:

a) Seed gene bank
b) Field gene bank
c) *In-vitro* gene bank
d) Pollen storage
e) DNA storage

Seed gene bank

It involves conservation of genetic resources for long-term through seed storage. Seeds with 3-5% moisture content are stored at – 20 °C. It is mainly used for orthodox seeds e.g., temperate fruits at NBPGR, New Delhi [NBPGR, 2011 [11]; http://www.nbpgr. ernet.in]. On the basis of temperature regime, the storage is classified into:

a) Medium term storage for active collections at 0-4°C for a time period of 10-15 years.
b) Long term storage for base collections at - 20 °C for a period of 50-100 years.
c) Storage of seeds at 5-10 °C for working collection for a period of 3-5 year (Gupta, 2010) [9].

Field gene bank

The region where the growing plants are conserved is the field gene bank. Because most fruit crops grow vegetatively and are therefore kept in field gene banks, their genetic resources are either difficult or impossible to conserve as seeds. Main fruit germplasm is kept in FGBs by horticultural institutes, NRCs, SAUs, and NAGS.

Crop based national active germplasm sites for HGR

Crop	Institute	Field bank
Arid fruits	Central institute on Arid Horticulture, Bikaner	1229
Banana, Plantain	NRC on Banana, Tiruchirapalli	907
Cashew	NRC for Cashew, Puttur	519
Citrus species	NRC on Citrus, Nagpur	150
Grapes	NRC for grapes, Pune	600
Litchi, Bael, Aonla & Jackfruit	NRC on Litchi, Muzaffarpur	2426
Mango, Guvava	CISH, Lucknow	848
Sub-tropical fruits	AICRP on Sub-tropical Fruits, CISTH, Luknow	
Temperate Horticultural crops	CITH, Srinagar and CITH, NBPGR RS, Shimla	780 &908
Tropical fruits	IIHR, Banglore	1983
Tropical fruits	AICRP on Tropical Fruits, Banglore	1754

***In-vitro* gene bank**

It involves conservation of horticultural genetic resources (HGR) using tissue culture techniques. The high rate of multiplication, aseptic plant growth, comparatively small space requirements, decreased genetic erosion, readily available material for distribution, and minimal quarantine restrictions for material exchange are only a few benefits of using in vitro procedures. (Gupta, 2010) [9]. It is achieved through:

- **Slow growth:** To reduce requirement of frequent sub-culturing by low temperature incubation of 0-4 °C and by Use of Growth Regulator like ABA, phosphon D, maleic hydrazide.
- **Cryopreservation:** Conservation of various crops for long term storage at -196 °C under liquid nitrogen or vapour phase use of like dimethyl sulphoxide (DMSO), glycerol or sorbitol are given to tolerate cryoinjury.

Pollen storage: Involves conservation of germplasm through pollen cryopreservation.

DNA storage: Involves storage of total genomic information in the form of DNA libraries.

5. Germplasm evaluation

The main aim of evaluation is to identify gene sources for resistance to biotic and abiotic stresses, earliness, dwarfness, and quality characters and to know the significance of individual germplasm line. The germplasm evaluation is done by NBPGR.

Registration of plant germplasm

ICAR entrusted NBPGR as nodal agency for implementation of plant germplasm registration. As a component of the Indian National Agricultural Research

System (NARS), the Central Sub-Committees on Crop Standards, Notification and Release of Varieties of Agricultural Crops (CVRC) fulfill the need to provide acknowledgment to the developers of new enhanced varieties. Further, The Protection of Plant Varieties and Farmers' Rights Act (PPV&FRA) 2001, which was passed, safeguards the farmers' and plant breeders' intellectual property rights when they develop new plant varieties. The procedure for crop variety evaluation, identification, and recommendation for release is run by the Indian Council of Agricultural Research (ICAR). In accordance with Section 5 of the Seed Act, 1966, the Ministry of Agriculture guarantees that seeds produced and sold meet the Minimum Seed Certification Standards and outlines the process for notifying released varieties through CVRC.

Germplasm Registration Information System

A well-structured digital information system offers equitable and just access to information for everyone. Information systems provide improved utilization and effective PGR administration. The Plant Germplasm Registration Committee's decision-making process, expert evaluation, and application submission for germplasm registration are all streamlined and expedited through the use of the Germplasm Registration Information System. It is anticipated that the system will give policy makers a trustworthy source of information as well as practical tools for managing the germplasm registration process for genebank managers, breeders, and plant researchers. It is anticipated that the introduction of this technology will simplify, expedite, and make the entire germplasm registration procedure transparent.

6. Germplasm utilization

Germplasm is utilized in two ways:

Cultivated germplasm: Used as a variety or as a parent in hybridization.

Wild germplasm: Transfer of resistant genes to biotic and abiotic stresses and quality characters.

GIS tools and preparation of distribution maps

GIS, or geographic information system, is extensively utilized in natural resource management. Currently, several groups are using GIS extensively to map biodiversity. Geographic Information System (GIS) is a database management system designed to handle geographical data, such as latitude and longitude. Numerous GIS software have been created for specialized management or commercial uses, such as GRID, FloraMap, DIVA, and Atlas for biodiversity mapping, and MapInfo for Windows, Arc/Info, and other commercial applications. Software like FloraMap and DIVA, which were created for research purposes by the International Potato Centre (CIP) and International Centre for Tropical Agriculture (CIAT), is used for mapping biodiversity and evaluating it for tropical fruit tree species. There are two types of software for GIS: raster-based and vector-based systems. Geographical data is stored as points in the rector-based system and as grid cells in the raster-based system. A prominent method for mapping genetic diversity is the rector-based system. Maps have been created for fruit species such as Citrus and Mangifera using DIVA.

Organizations associated with plant genetic resources

(a) IBPGR: Established in 1974

(b) IPGRI: Established by CGIAR (Consultative Group on International Agricultural Research) in 1994, situated in Rome, Italy at Food and Agriculture Organization of United Nations. It conducts research and promotes International network of Plant Genetic Resources activities.

(c) NBPGR: NBPGR was established by the Indian Council of Agricultural Research (ICAR) in 1976 with its main campus at New Delhi. Acts as nodal institute at national level for acquisition and management of indigenous and exotic plant genetic resources (PGR) for food and agriculture and carry out related research.

Important developments in plant genetic resources

- Convention of Biological Diversity (CBD): 1993.
- The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA): 2001.
- The Protection of Plant Varieties and Farmers Rights Act (PPVFRA): 2001.
- The Biological Diversity Act (BDA): 2002.

Conclusion

India is eager to add new crops and genotypes to its fruit basket in order to increase diversity and contribute to the country's goal of achieving food and nutritional security. Given the current state of climate change and the depletion of natural resources, feeding the world's expanding population is becoming increasingly difficult. As a result, great effort is needed to explore previously uncharted territory in order to gather valuable horticultural biodiversity for future use and conservation for the benefit of all people. Fruit crop genetic variety is important because it serves as the basis for breeding efforts and provides a storehouse of qualities needed to develop the crop. In order to address the new challenges, genetic resource gains must be maintained. Additionally, additional new gene collections must be explored and exploited for gains where a robust base of horticultural plant biodiversity must be the primary driver of a gene revolution.

Challenges in germplasm conservation

- Financial constraints stand out as a significant hurdle, as establishing and maintaining germplasm conservation facilities for fruit crops require substantial funding.
- the awareness gap among farmers and policymakers regarding the importance of preserving genetic diversity in fruit crops poses a substantial challenge.
- Lack of understanding can hinder the adoption of conservation practices, potentially leading to the neglect of traditional or locally adapted fruit varieties that contribute to genetic resilience.
- Ethical considerations, including issues related to access and benefit-sharing, can complicate collaborative efforts, especially when germplasm from indigenous or local communities is involved.
- The rapid expansion of monoculture and the encroachment of urbanization further exacerbate the risk of losing traditional fruit varieties and their unique genetic traits.
- Climate change introduces an additional layer of complexity, as shifting environmental conditions

necessitate the conservation of genetic resources that confer resilience to new challenges.

- Furthermore, legal frameworks for germplasm exchange and biodiversity conservation differ across regions, impeding international collaboration.

Future thrusts

- Use of GIS for geo-referencing/gap analysis and prediction and distribution of species using environment variables to plan future explorations.
- Use of GIS for mapping of trait-specific germplasm with respect to bioactive compounds.
- Use of biotechnological tools like *in-vitro* storage/ cryopreservation including pollen preservation to strengthen the conservation of germplasm.
- Use of molecular marker tools like SSR/SNP/ GWAS) improve the understanding of extent, nature and distribution of diversity and develop the varieties with high yield and quality for sustainable production.
- Priority for collection of wild relatives and under exploited genetic resources.
- Introduction of targeted germplasm for crop improvement
- Evaluation of germplasm for yield, quality, shelflife, and resistance to biotic and abiotic stresses.
- Registration of germplasm, breeding lines and parental lines
- Awareness generation related to patenting, farmers right and benefit sharing.

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