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Comprehensive Overview of Plant Germplasm Augmentation: Strategies, Techniques and Applications

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Review Article

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ABSTRACT

Plant genetic resources (PGR) are fundamental for crop improvement and conservation, encompassing a wide range of genetic materials including land races, modern varieties, and wild relatives. Effective germplasm exploration is essential for advancing plant genetic resources and ensuring agricultural sustainability. Traditional germplasm collection faces challenges such as remote locations, limited material availability, and transport issues. This review explores various strategies to address these challenges, including coarse-grid and fine-grid survey methods, multi-

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species versus species-specific collecting missions, and the implications of breeding systems on germplasm collection. It highlights the benefits of Geographic Information Systems (GIS) in managing PGR, from incentivizing mapping strategies to optimizing conservation and evaluation processes. Additionally, the review emphasizes the role of in vitro techniques in overcoming limitations of traditional methods, detailing field and laboratory procedures for successful in vitro collection. By integrating these approaches, the review aims to enhance the efficiency and effectiveness of PGR management, ensuring the preservation of genetic diversity for future generations.

Keywords: PGR; germplasm augmentation; GIS; conservation strategies; field exploration; genetic diversity.

1. INTRODUCTION

Exploration is to look for some genetic material with specific intentions to utilise them for future. Plant genetic resources (PGR) are the genetic material of plants having value as a resource for present and future generations. PGR include land races and primitive cultivars, modern varieties, parental lines of hybrids, genetic stocks with know traits and wild and weedy relatives of crop plants. Being the building blocks of improved crop varieties, the availability of PGR is pivotal requirement in cultivar development programmes [1-2]. Earlier there was sufficiently wide food basket that ensured balanced nutrition of human beings. As civilization progressed, the ever-increasing population and changes in lifestyle necessitated research programmes for productivity enhancement [3]. Commendable achievements of these programmes in some crops led to overdependence on wheat, rice, maize, potato and cassava which meet 75% caloric requirement of our society. The narrowed food basket resulted in nutritional deficiencies. The spread of improved varieties also narrowed on-farm diversity accentuating the damage due to insect-pests and diseases [4]. Recently, the climate change and volatility have added further dimensions. For addressing these problems, the focus has been greatly enhanced on search, development and use of PGR for traits like tolerance to temperature and moisture extremes, high protein, zinc and iron concentration, and even traits like low glycemic index and low gluten concentration to tackle the increasing incidence of diabetes and gluten intolerance [5].

Germplasm and herbarium collection are crucial activities for organizations involved in the management of plant genetic resources (PGR). These activities are essential for utilizing valuable resources in crop improvement and analysing temporal changes [6]. The need for PGR, whether general or specific, is often

unpredictable and dynamic. In general, 'using germplasm' involves incorporating it into crop breeding or plant introduction and selection programs. While plant breeders typically maintain their own active collections of carefully selected genotypes, there is an ongoing demand for new and specific traits and trait combinations. This necessity supports the introduction, selection, domestication, and improvement programs, helping to address emerging problems and meet new demands [7].

2. PURPOSE OF AUGMENTATION OF PLANT GERMPLASM

According to Engels *et al.* [8] the rationale for collecting germplasm is multifaceted:

- **1. Genetic Erosion Risk**: Germplasm may be at risk of genetic erosion or extinction.
- User Demand: There may be a clear need for it expressed by users at both national and international levels.
- Diversity Gaps: It may represent diversity that is either missing from or inadequately represented in existing ex situ germplasm collections.
- **4. Knowledge Expansion**: More information may be needed about the germplasm.
- 5. Rescue Collecting: If genetic diversity is critically threatened in an area and in situ conservation methods are inadequate, germplasm collecting may be necessary, as exemplified by the Sardar Sarovar dam project.
- Immediate Use: Local communities often collect germplasm for immediate practical use.
- Future Use: Collecting may address immediate needs, but material that seems less useful now might become crucial in the future.
- **8.** Research: Building a comprehensive knowledge base of the target gene pool is

- a significant motivation for germplasm collection.
- 9. Opportunistic Collecting: Germplasm may also be collected opportunistically during missions focused on other species, traits, or ecological conditions, particularly if it exhibits striking phenotypic features, occurs in unusual situations, or has novel or interesting local uses.

3. TYPES OF COLLECTION MISSION

3.1 Coarse Grid Survey

A preliminary survey visit could also involve collecting material for genetic diversity analysis, the results of which could then be used to formulate a more efficient germplasm sampling strategy. The material collected could be leaf fragments to be used in isozvme deoxyribonucleic acid (DNA) variation studies or germplasm to be characterized for morphological characters. Such survey work will tend to be of the sort described as coarse-grid by Frankel and Bennett [9] as well as Hawkes [10]. This involves collecting systematically (typically every certain number of kilometres) on a broad scale, covering all major environments. It is followed up with finegrid collecting in specific areas of interest as revealed by analysis of the material collected.

3.2 Fine Grid Survey

In practice, the most common procedure will be a combination of course-grid and fine-grid sampling, with the team staying for a certain period in one locality, using this as a temporary base (with a fair degree of intensive sampling within a readily accessible distance), and then moving on to another target locality. There would be further collecting, on a systematic basis (with the interval depending on environmental heterogeneity), when travelling between the localities. Advantage could be taken of stops at such temporary bases to dry material (seed samples and herbarium specimens) and arrange for the dispatch of samples to the gene bank if necessary.

3.3 Multi-Species Vs. Species-Specific Collecting

In a multi-species collecting mission, a region is targeted and an attempt is made to sample as much as possible of the diversity of as many species as possible. Such missions may be described as being area-driven. Usually, they are planned when no systematic collecting in the area has been conducted before and/or when the area is difficult to reach and future visits are therefore unlikely. The motives for multi-species missions have frequently more to do with conservation than immediate use an extreme example being emergency rescue collecting in areas imminently threatened by genetic erosion. Some problems with this kind of collecting should be noted. The most important is the relatively restricted knowledge the collectors are bound to have of many of the species they will be dealing with. This means that it will not be possible to follow an optimal sampling strategy for all the species, interesting and perhaps unique material which an expert would have recognized will be missed and the information on each sample will not be as complete as it might have been. It will therefore be all the more important to tap the large store of indigenous knowledge about plants and the environment maintained by local communities. Furthermore, it may not be possible to collect many potentially interesting species or landraces within the course of the mission because of differences in maturation time. Finally, different kinds of species may require radically different collecting techniques and even equipment. For all these reasons, a multi-species collecting mission will sometimes need to be focused at least to some extent, usually on a 'plant category'. Examples might include collecting Andean root and tuber crops in Ecuador or forages in the semiarid regions of Kenya.

3.4 Species-Specific (or Gene-Pool-Specific) Missions

Species-specific (or gene-pool-specific) missions, in contrast, tend to be driven by the eventual users of the germplasm, typically breeders and plant introduction people. The targets of such missions may be relatively broad (e.g. wild Phaseolus spp. in southern Mexico) or very specific indeed (e.g. the high-lysine gene in barley in the highlands of Ethiopia), in which case specialists like pathologists, entomologists and microbiologists may also be involved. Generally, species-specific missions are less complicated to plan than multi-species missions. The ecogeographic distribution of the target material will be known in more detail, including the specific habitats (or even precise localities) in the case of wild species, and the maturation period will be better documented and more restricted. In addition, the team members will have detailed knowledge of the gene pool and will probably be familiar with material collected during previous missions.

4. BREEDING SYSTEM AND GERMPLASM COLLECTION STRATEGY

4.1 Sampling Method (Self, cross pollinated and vegetatively propagated material)

- When collecting seeds, the explorer should ensure to gather enough material for longterm conservation, typically between 2,000 and 4,000 seeds for self-pollinated and cross-pollinated crops, respectively, while also meeting the needs for characterization, evaluation, and other related studies.
- The ideal sample size per site should be sufficient to achieve, with 95 percent certainty, the inclusion of all alleles at a random locus presents in the target population with a frequency greater than 0.05 [10-11].
- For species with very small seeds, low seed set, asynchronous maturity, and low seed viability, it is important to collect a sufficiently large sample to ensure adequate representation.
- For highly variable populations, either collect larger samples (bulking) or take separate sub-samples of interesting variants, each assigned distinct collecting numbers.
- To obtain a representative and adequate sample, random sampling should involve collecting a single spike, panicle, fruit, berry, or pod from at least 50 plants along multiple transects throughout the field, avoiding plants at the field's border [11-12].
- 6. When encountering wild populations with few individuals, it is advisable to collect from all available plants to ensure a representative sample from that site. For wild and semi-domesticated species found in small, scattered populations with specific uses or traits, seeds should be bulked. However, care should be taken not to deplete farmers' planting stocks or wild species, nor to remove significant genetic variation [13,14].
- 7. For large tubers, collect only a portion such as the head or proximal ends in yams, or the crown or tuber in taro and other aroids. Since vegetative propagules deteriorate quickly after harvest and can be damaged

- during transport, handle them with care during both sampling and transportation.
- 8. For scion collection intended for budding and grafting, ensure that the sample size is based on the number of available rootstocks, with a minimum of ten samples to ensure at least eight successful grafts. For cuttings and rooted suckers (e.g., grapes, ornamentals, passion fruits, black pepper, beetle vine, banana, cardamom), collecting 15-20 cuttings is typically sufficient.

4.2 Back at Base

Upon returning to the base camp, collectors must complete several key tasks to finalize a mission: sorting and preparing germplasm samples, reference specimens, and ancillary materials; collating and editing collecting data; and distributing the samples and data. Adequate planning is essential, including allocating time and resources for sample sorting, ambient and active drying, and data documentation. Specialized equipment, such as for drying seeds or documenting data, should be available, and arrangements for sample distribution must be made.

4.3 Post Collection Handling

Seed extraction and cleaning should ideally be completed on the same day or immediately after the expedition, followed by drying under shade, sun, or controlled conditions. Seeds with short longevity should be processed promptly, and care should be taken during threshing and cleaning to prevent damage. If processing is delayed, measures should be taken to preserve viability. Observations on variability parameters of fruit, pods, or seeds should be documented for reporting, record-keeping, and publication. Cleaned and dried material should be placed in envelopes with labels indicating the botanical name and collector number. One set, along with passport data, should be sent for accessioning and conservation (LTS/MTS), while another set should be sent to a collaborating institute for initial seed increase, maintenance, characterization, and evaluation, if required. Vegetatively propagated material should be transferred for establishment or maintenance at a field genebank or a suitable site. Material intended for in vitro or cryo-genebanks should be handed over to the relevant curators. Elite material, if present, should be thoroughly studied to generate supporting information and validate

known traits for registration with the appropriate agency.

4.4 Report Writing and Publication

After completing a germplasm collection mission, it is essential to compile a comprehensive report to meet the mission's objectives. This report aids in follow-up collections, informs users about the availability of the germplasm, and supports publication efforts. Information on the collected samples should be entered into a database to facilitate user access.

5. KEY FACTORS FOR SUCCESSFUL GERMPLASM COLLECTING

Successful field germplasm collecting hinges on several key factors. First, thorough research and meticulous planning are crucial for ensuring the effectiveness of the mission. Involving local communities not only fulfils ethical and legal obligations but also enhances the efficiency of collecting, particularly for crops. Flexibility is essential, especially for wild species with short seed-shedding periods, as good timing is critical. Developing a search image helps in quickly locating populations of target species. Selecting appropriate collecting and processing techniques tailored to each species and sample is vital. Scrupulous documentation. includina comprehensive passport data, enhances the utility of the collected germplasm. Maintaining high sample quality and preventing deterioration through regular inspection is crucial. Ensuring safety throughout the process is necessary for the longevity and success of collecting programs. Lastly, follow-up actions are essential complete the mission and ensure ongoing success.

6. THE APPLICATIONS OF GIS FOR PGR MANAGEMENT

6.1 GIS Technology

Geography examines Earth's features and their spatial and temporal variations. Many aspects of plant genetic resources (PGR) are inherently geographic, as their availability is influenced by spatial and temporal climatic conditions. Managing PGR involves accessing extensive multidimensional geographical data, including information on weather, soils, topography, water resources, and socio-economic factors [15]. The term "geographic information system" (GIS) was

introduced by Roger Tomlinson in 1968. GIS is a framework that facilitates the capture, analysis. and visualization of spatial and geographic data. It integrates hardware, software, and data to manage and display geographically referenced information. GIS enables users to view, understand, and interpret data in various forms maps, reports. and charts—revealing relationships, patterns, and trends. By mapping collected diversity, predicting areas rich in diversity, and identifying suitable sites using climate and crop models. GIS technology can be seamlessly incorporated into enterprise information systems [16]. The applications of Geographic Information System (GIS) for Plant Genetic Resources (PGR) management.

Incentivization and Mapping Strategies: GIS facilitates the surveying and inventorying of plant genetic resources by integrating geo-referenced data with various attributes. Software such as ArcGIS, FloraMap, and Quantum GIS supports the mapping of biodiversity and the spatial distribution of plant species, aiding in the assessment of high-diversity areas and the planning of effective conservation strategies. This mapping includes ecogeographic surveying, which helps in understanding the ecological and geographic factors that influence plant genetic resource distribution.

Collecting Strategies: GIS assists in preparing distribution maps, locating collection sites, and analyzing pockets of high diversity. By linking passport databases with geographical layers, GIS identifies explored areas and gaps in germplasm collection. The technology also supports field exploration by providing spatial data to plan and execute missions effectively. Additionally, remote sensing data aids in impact assessments and the identification of new collection sites.

Conservation Strategies: GIS plays a crucial role in identifying gaps in both ex-situ and in-situ conservation efforts. It helps design and manage conservation sites and predicts the impact of climate change on plant genetic resources. This includes designing, managing, and monitoring in situ reserves through spatial information. GIS also forecasts the spatial distribution of crop landraces and threatened species, supporting targeted conservation planning and management decisions.

PGR Characterization and Evaluation: GIS manages large datasets and identifies suitable

locations for germplasm evaluation. It integrates morphological and genetic data with environmental attributes to aid in the selection of useful germplasm accessions. By linking geographic and environmental data, GIS enhances the effectiveness of germplasm evaluation processes.

Crop Expansion Strategies: GIS supports the identification of suitable locations for the multiplication and evaluation of germplasm from other countries. It helps in optimizing the introduction and cultivation of new varieties by linking genetic and morphological data with environmental factors. This strategic approach ensures that the potential of germplasm for crop expansion is maximized.

PGR Documentation: GIS aids in managing genetic resource databases and generating agro-ecological models. This facilitates thorough documentation and prediction of the agronomic potential of germplasm accessions. ensuring that comprehensive records are maintained for research and breeding purposes.

Plant Quarantine Strategies: GIS is used for pest monitoring, detection, and risk pathway analysis. It helps in determining high-risk areas for pest introduction and supports proactive pest management strategies. GIS also contributes to quality control and decision-making in plant quarantine, ensuring the protection of agricultural

resources. These GIS applications enhance various aspects of plant genetic resources management, including surveying, field exploration, conservation, evaluation, and documentation, ultimately optimizing the management and utilization of genetic resources. Examples of GIS and grid mapping, georeferencing and GPS.

6.2 GIS Software for Plant Genetic Resources Management

Several Geographic Information System (GIS) software tools are employed for mapping and analyzing plant genetic resources, each offering distinct functionalities to support diverse tasks. These tools assist in data integration, spatial analysis, and predictive modeling, enhancing various aspects of GIS applications in plant genetic resources management (Table 1).

6.3 Georeferencing

Georeferencing is the process of assigning positional information of earth features/germplasm collection sites on the map by linking geo-coordinate/geocoding (latitude and longitude) values of locations to a geographic frame of reference. In this case, it is the assigning of geocordinates to the plant germplasm (seed samples) from where it has been collected and displaying its location on map for further analysis of collected diversity [15].

Table 1. Key GIS Software for Plant Genetic Resources Management [17]

1	ArcGIS	A comprehensive GIS platform for mapping, spatial analysis, and data management.
2	FloraMap	Specialized software for mapping plant species distribution and diversity.
3	DIVA-GIS	A free tool for mapping and analyzing spatial data, particularly useful for biodiversity studies.
4	Quantum GIS (QGIS)	An open-source GIS application for visualizing and analyzing spatial data.
5	GARP	Genetic Algorithm for Rule-set Production (GARP) Software for species distribution modeling using genetic algorithms.
6	ECOSIM	A tool for ecological modeling and simulation.
7	MaxEnt	A software for species distribution modeling using maximum entropy methods.
8	ECOGEO	A tool for ecological and geographical data analysis.
9	SPADE	A tool for spatial and ecological analysis of species data.
10	HyperNiche	A tool for modeling ecological niches and species distributions.

7. ECO-GEOGRAPHIC SURVEYS IN EXPLORATION AND PGR MANAGEMENT

Eco-geographic surveys are essential tools in the conservation and management of plant genetic resources (PGR). Defined as the process of collecting, collating, and analyzing diverse data types related to a specific taxon within a designated region [18], these surveys provide foundational knowledge crucial for developing effective conservation strategies. The data gathered in eco-geographic surveys-validated and refined through subsequent fieldwork—help establish a comprehensive understanding of a species' taxonomy, genetic diversity, geographic ecological adaptations. distribution. ethnobotanical uses, alongside insights into the geography. ecology, climate, and human communities within the target area [19].

Such detailed analysis is invaluable for addressing several key conservation questions, including:

Timing and Location of Germplasm Collection: Determining the optimal time and place for collecting plant genetic material.

Placement and Management of Genetic Reserves: Identifying the most suitable sites for genetic reserves and developing effective management and monitoring protocols.

Integration of Conservation Strategies: Assessing the roles of both ex situ (outside natural habitats) and in situ (within natural habitats) conservation methods to create a balanced and effective conservation strategy.

Originally developed to support the conservation of wild plant gene pools, eco-geographic surveys have primarily focused on data gathered from herbaria, where specimens and their associated data provide crucial insights into plant diversity and distribution. Although this approach has been extensively applied to wild species, it has not been widely adopted for crops. However, Maxted *et al.* [18] emphasized that ecogeographic surveys are equally applicable to cultivated species, underscoring their broader relevance in PGR conservation.

7.1 The Importance of Eco-geographic Surveys

Eco-geographic surveys play a crucial role in optimizing the use of resources for plant genetic conservation by providing precise information

about target taxa and their specific locations. This ensures that conservation efforts are both efficient and effective, focusing resources on areas with the greatest need or potential for success. Additionally, these surveys guide plant collectors by offering detailed knowledge of the geographic distribution, ecology, phenology, and diversity of plant species. This information allows collectors to locate and gather necessary plant materials more effectively, ensuring are well-targeted collection missions successful. Furthermore, comprehensive ecogeographic data help prevent redundant efforts by avoiding unnecessary or poorly timed collecting missions, thus conserving resources and maximizing the impact of conservation initiatives.

8. In vitro METHODS FOR GERMPLASM COLLECTION

Traditional germplasm collection often faces challenges due to remote collection sites, adverse weather, and limited viable plant material. In vitro methods provide an alternative by allowing collection and preservation of plant genetic resources under controlled conditions. This section discusses the reasons for using in vitro techniques, their benefits, and the procedures involved.

8.1 Reasons for In vitro Collections

In vitro germplasm collection addresses several challenges of traditional methods:

Extended Exploration Duration: Collection missions may cover remote areas with harsh conditions, limiting viable material availability.

Insufficient Material Availability: Poor growing conditions, scarcity, pest damage, or immaturity may result in inadequate collections.

Transport Challenges: Large vegetative propagules and recalcitrant seeds can be difficult to transport and may spoil before reaching a gene bank.

In vitro methods mitigate these challenges by providing a controlled environment for collection and preservation.

8.2 Advantages of In vitro Techniques

In vitro techniques significantly enhance germplasm collection by offering several

advantages over traditional methods. These techniques allow for the collection of larger sample sizes in a more compact form, effectively reducing bulk and making transportation easier. Additionally, in vitro methods increase germplasm diversity by enabling the collection of various plant materials, including vegetative propagules and unripe seeds, which might not survive in standard conditions [20]. They also the risk of loss by minimizing deterioration during collection and transport. ensuring the viability of the germplasm. Furthermore, in vitro techniques simplify logistics by easing quarantine restrictions and facilitating the safe and efficient transfer of genetic materials across regions.

8.3 Methods of In vitro Collection

Effective in vitro collection requires careful field and laboratory procedures:

Field Procedure: Surface sterilization in in vitro germplasm collection involves using low-toxicity, portable sterilant that ensure the safety of plant tissues while effectively minimizing microbial contamination. To facilitate ease of handling in the field, lightweight plastic containers are preferred for use as culture vessels. Additionally, the culture medium should be formulated with low sucrose levels and may include antibiotics to further reduce the risk of contamination, ensuring the viability and integrity of the collected samples during the collection and transport process.

Laboratory Procedure: Explant preparation for in vitro germplasm collection involves selecting suitable plant tissues, carefully sizing them, and thoroughly cleaning and sterilizing them to remove any contaminants. Once prepared, the explants are placed on a sterile culture medium to promote growth and are then incubated under controlled environmental conditions. This process helps ensure the successful establishment and growth of the explants, maximizing their viability and potential for conservation.

Handling at Recipient Laboratory: Different explants may require tailored handling procedures, including re-sterilization, dissection, or direct transfer to soil, to ensure their optimal survival and growth. Each type of explant has unique needs that must be addressed to maximize the chances of successful establishment and development.

In vitro collection serves as a valuable supplement to traditional germplasm collection methods, addressing challenges such as bulkiness, limited availability, and preservation of plant materials. With careful preparation, planning, and species-specific knowledge, this technique significantly enhances the efficiency and effectiveness of plant genetic resource conservation, ensuring better preservation of genetic diversity.

9. INTERNATIONAL CODE OF CONDUCT FOR PLANT GERMPLASM COLLECTING AND TRANSFER

Adopted by the FAO Conference in November 1993, the International Code of Conduct for Plant Germplasm Collecting and Transfer aims to ensure the rational and sustainable use of genetic resources, prevent genetic erosion, and protect the interests of both germplasm donors and collectors. Developed by the FAO and negotiated by its Member Nations through the Commission on Plant Genetic Resources, the Code is a voluntary framework. It upholds the principle of national sovereignty over plant genetic resources and establishes standards and principles for adherence by participating countries and institutions [21].

The Code outlines procedures for requesting and issuing licenses for collecting missions, offers auidelines for collectors. and extends responsibilities to mission sponsors, genebank genetic curators. and material users. It emphasizes the inclusion of farmers and local institutions in collecting missions and advocates for sharing the benefits derived from plant genetic resources with host countries and their farmers.

Objectives of the Code:

- To promote the conservation, collection, and use of plant genetic resources in a way that respects the environment and local traditions.
- To encourage the active participation of farmers, scientists, and organizations in conservation and use programs.
- To prevent genetic erosion and loss of resources due to uncontrolled collection.
- To facilitate the safe exchange of plant genetic resources, related information, and technologies.

- To ensure that germplasm collection adheres to national laws, local customs, and regulations.
- To establish conduct standards and define collector obligations.
- To promote benefit-sharing between donors and users of germplasm and related resources, considering conservation and development costs.
- To recognize the rights and needs of local communities and farmers and promote mechanisms for compensating them and protecting their benefits from plant genetic resources.

Nature of the Code:

- The Code is voluntary.
- It acknowledges national sovereignty over plant genetic resources while emphasizing that access should not be unduly restricted and that conservation is a common concern.

Scope: The Code outlines responsibilities of collectors, donors, sponsors, curators, and users to maximize benefits and minimize adverse effects. It calls for cooperation and reciprocity and encourages governments to support adherence to the Code.

Relationship with Other Legal Instruments

The Code should be implemented in harmony with:

- The Convention on Biological Diversity and related agreements.
- The International Plant Protection Convention and pest control agreements.
- National laws of the host country.
- Agreements between collectors, host countries, sponsors, and gene banks.

Collectors' Permits:

- States have the right to establish national policies for plant genetic resource conservation and to issue permits to collectors.
- Governments should designate competent authorities for permit issuance and provide information on rules, regulations, and approval processes.

10. CONCLUSION

The exploration and augmentation of plant are essential germplasm for sustaining agricultural diversity and ensuring food security in the face of evolving challenges. This review highlights the multifaceted strategies innovative techniques employed to enhance the collection and conservation of plant genetic resources. By integrating traditional methods with advanced technologies such as Geographic Information Systems (GIS) and in vitro techniques, researchers and practitioners can overcome the limitations of conventional germplasm collection and address critical issues related to genetic erosion, climate change, and nutritional deficiencies. Effective germplasm augmentation requires careful planning. comprehensive fieldwork, and ongoina management to maintain genetic diversity and adaptability. As the demand for resilient and highquality crops continues to grow, the continued development and application of these strategies will be crucial for the future of global agriculture and the preservation of plant genetic resources for future generations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative Al technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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