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Chapter

Germplasm Conservation

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Abstract

With the increase in risk of extinction of various plants, the trend has been shifted to employment of many biotechnological techniques for preservation of genetic resources of plant and is the area of research which needs to be revolutionized after a specific time period because it allows the production and selection of crop varieties with desirable characteristics during breeding process such as improved fuel, food and health facilities. Having an immense research in conservation of non-threatened species, there is a small collection of knowledge available for conservation of endangered ones. This chapter aims to highlight the various techniques in germplasm conservation of endangered or the species which are at extent of extinction and also the future directions in this field. In developing countries where most of agriculture depends upon food crops, the maintenance of genetic variation is of immense importance. On farm conservation provides the best example of preservation and evolution based on genetic variability which can occur ex-situ and in- situ environment in farms or gene bank. So, it presents the best option for conservation or maintenance of ecosystem and biodiversity which ensures survival of endangered species via germplasm. The most point to consider is that germplasm or genes have to be conserved instead of genotype. In situ conservation involves preservation of plant crops in the field condition in ecosystem where plant is adopted to grow in order to maintain self –sustaining process in natural ecosystem. Similarly ex-situ involve the collections of seed banks of genes collected from plant under natural conditions to produce desirable varieties or from tissue culture in laboratory also referred as in-vitro methodology. In –vitro techniques include cryopreservation which include freezing at much lower temperature than that of freezing point i.e. -196 °C in liquid nitrogen for preserving species which are near to extent of endangerment. Cold storage and storing at lower temperature provides best opportunity for protection against damage caused by rapid freezing. Germplasm exchange has become now a usual practice ensuring exchange of varieties between cultivated and wild types as for example in potatoes specie etc. DNA as well as gene or seed banks provide molecular sources for conservation at biotechnological level. The techniques of introgression and incorporation are basic approaches for germplasm conservation. So there is need to revolutionize and practice germplasm conservation for fulfilling future needs being aimed at conserving endangered or threatened species from conservation hotspots.

Keywords: germplasm, threatened species, gene resources, cryopreservation, introgression, incorporation, endangered species, tissue culture technique, seed bank, gene bank, gene pool, and breeding technology

1. Introduction

Conservation of plant genetics is one of the main areas which is to be refined and revolutionized again and again with knowledge. The phenomenon of conservation is helpful in the maintenance of the genetic basis being needed for breeding. This allows the production and selection of varieties with desirable characteristics in crops, which later can be used for purpose of feeding, fuel, and health sectors [1]. Germplasm is the plant's genetic resources such as tissues or cells which are being preserved for purpose of obtaining desired breeding characteristics. These resources are obtained from gene banks, plants grown in nurseries, and laboratory culture. The collection of germplasm usually ranges from wild species to genes which are supposed to capture traits of plants as a result of natural selection [2].

A germ is defined as the collection of genetic resources for an organism. In the case of plants, the germplasm is stored or preserved in form of seeds or trees in the nursery. So, it is the living tissue from which new varieties of plants can be grown i.e., it can be the seed from which the whole plant can be grown because it contains all the genetic makeup or information required for resources of the diversity of plants. Plant germplasm is a spice of generic materials needed by breeders to develop new varieties. This includes seeds, leaves, stems, pollen, and cultured cells. So it provides the necessary raw material to develop the commercially valuable varieties of plants [3].

It is of prime importance in the maintenance of diversity in the biological system and the security of food. Conservation of plant resources is of great importance because most of the plant species are getting endangered with time. Genetic resources are a potential sustainable source of agricultural products i.e. efficient production of crops used as food, for the reduction of poverty and maintenance of economic conditions of the population [4]. For example, in countries like Nigeria, the major source of food is from crops sowed by simple farmers which maintain them by their efforts utilizing their resources. This involves the conservation of gene resources which preserve them for storage and usage systemically at both national and international levels [5]. Hence maintaining these species for purpose of variation in genetics is therefore of immense importance especially in the case of poor farmers who are participating in the agriculture of the country at much lower input conditions in marginal land [6].

On-farm conservation provides the best example of preservation as it is helpful in the maintenance of evolution responsible for genetic variability. Variations in genes are observed in both ex-situ i.e. in a natural environment and ex-situ in the form of gene banks obtained from laboratory culture. A huge collection of the most value able crop fields are being reserved in the gene bank and are placed in modern aseptic conditions in gene bank facilities. The variety includes collection from national and international worldwide programs i.e., NAGRAB, OSTROM, and IITTA. It also includes varieties obtained from plant genetic programmers in collaboration with a national action plan [7]. It has been revealed from a scientific investigation that about 3 lac plant species of higher plants exist in the world but only 1% of them are being utilized in the world today. About 80% of food is provided by only 8–10 crops ranging from wheat, rice species to millet, and rye. Most advancements in the field of agriculture in the present day world is based on a wide range of genetic resources possessing two types of values [8]. The plant genes and various genotypes are considered for many characteristics such as insect and pest resistance, bearing the conditions of drought, plant structure, function, and color acting as an immediate source of plant genotype conservation for desired properties. Secondly, diversity in genes or genetics ensures future requirements. Hence in turn contribute to the farming system at both local or small level and national levels [9].

Moreover, variations in genetics have also resulted in losing information in an already present generation which makes the preservation of these genes much important. Because if genes for variations are not preserved, it would lead to endangerment of plant species. International board [7] as a bank for further next generations [10]. The conservation involves

- Preservation of breeding lines
- Conservation of commercially important species
- Stock for genetics
- For direct or indirect usage of wild species which include either in form of crops or stocks of roots.

2. Need for germplasm conservation of endangered plants

There are several reasons why breeders use this technique which is as follows;

- Loss of genetic diversity among plant species.
- Humans and animals are dependent on plants for their food which means they require plants for basic food crops such as wheat, maize, etc.
- Humans also utilize plants for their social activities such as buildings construction, obtaining waxes, and perfumes, resume fibers, and therapeutics.
- Deforestation has led to the endangerment of many valuable varieties of plants which present an utmost need to preserve them [11].
- To keep the stability of the ecosystem, genetic diversity provides food prints that can be maintained via germplasm.
- It provides an esthetic value to the natural ecosystem and bio-diversity of plants [12].

3. Classification of germplasm

Based on its need, germplasm has undergone an evolution over a certain period of years in response to particular requirement including

1. Base collections
2. Backup collections
3. Active collections
4. Breeder collections
5. Working collections

To a certain extent, these collections are artificial to a much extent because some of the classes or classifications are useful for more than one reason. Hence an active number of collections were previously breeder such as for formal breeding purposes. So, the following discussion is required to explain the classes of the collection which must serve.

3.1 Base collection

It presents the method of long-term preservation of genetic variability by storing in presence of optimum conditions. For base collection, the materials are not used for distribution except with the need for replacement of material that has been lost either from active or backup collection types. It includes the most explanatory sampling method being employed for checking out variability within the species group. They are most stable in the sense that they can store the variation which arises in the natural condition. But they are also dynamic in the sense that they have some novel collected materials, some collections being produced via plant breeding and population involving genetic materials are added as they are available. In this way, the storing for many decades can be possible so the loss of variability occurs during the processes of regeneration and storing present within the acceptable limits. It is the collection under a low level of humidity at the temperature of subfreezing which must be below −150 °C to 190 °C. But some difficulties are present, which include that they cannot bear the chilling or drying temperature. So, an alternative and long-term methodology are required which includes cryopreservation within the in-vitro cultures. A huge collection at a global level is initiated with a proper guidance and help of Food and agriculture organization by designing specific agencies which serve as the base as well as back up collections for principal species in case of principle crop plants. But most agencies also vary in their ability to fulfill all the responsibilities regarding its designation [13].

3.2 Back-up collection

It supplements base collection at another location or another level. For example, laboratory at US national seed stores holds the collection of some of the duplicate backup samples of maize for the improvement of these crops. Similarly, the international research Centre of rice is abbreviated as IRR present in the Philippines for the collection of rice. So it holds collection as well as insurance for loss in primary CIMMYT and IRRI collections of crops [14].

3.3 Active collection

Active as well as base collection mostly includes the same type of materials. So, it provides the seeds and other raw materials for purpose of distribution as well as for other uses. So, it has been found that a certain collection of material is conserved for maintenance of sufficient collections of plants of each type in active collection particularly when it is required in a huge collection or amount. All the materials in this type of collection are maintained under a shorter half-life and in the response to more standards of variability. So, grow outs or techniques for replacing seed supplies in form of the active collection as compared to that available in the form of the base collection. Hence replacing the active collet ions being necessary at regular intervals is being necessary in the case of the base collection so puts the genetic association at the risk [15].

3.4 Breeders and working collection

Breeders as well as the working collection include materials being used in breeding programs and are used for the short term in nature. Breeders get knowledge from their experience such as superior performance in their local region has resulted in the favorable combinations of different alleles at an almost different genetic locus. Hence attempts for the introduction of alleles from exotic resources into adapted materials are determined to the performance in the short-term way. So, the breeder's collection includes the advanced cell lines developed in their programs in addition to professional cultivars, advanced breeding lines, and finally genetically enhanced as obtained from a breeding population in the presence of a similar type of ecological variable conditions. It is suggested from breeders that dependence on the already available stock has resulted in slow advancement towards the new technology. But modern breeders turn into exotic materials for utilization of variability in the active collections. But they obtain the variability in exotic alleles or genes from the genetically enhanced population or breeding stocks in both of them the most useful and desirable alleles have been introduced. So breeder's collection has turned ultimately to increase in proportion for genetically enhanced stock which can possess the useful alleles in the genetic backgrounds too [16].

4. Classification of the gene pool

Gene pool includes almost all the cultivars which can be obsolete or current, or wild species and their relatives which in turn contain genes available for utilization in true-breeding lines. Based on their relationship, the gene pool can be classified into three major classes;

- Primary gene pool
- Secondary gene pool
- Tertiary gene pool

4.1 Primary gene pool

It is abbreviated as GP1 and it is the form of gene pool where the crossing of two species is much easier which ultimately leads to the production of sexually fertile organisms. It includes plants or other species which upon mating produce a very closely related species which is fertile via its reproductive means. In the gene pools, the genes can be exchanged in between the two reproductive lines via arranging simple crosses or hybridization patterns. So, it is also known as Gene pool one and is of prime importance in breeding lines.

4.2 Secondary gene pools

This type of genetic material can lead to a partial type of fertility upon crossing with GP1 being referred to as the secondary gene pool. It can ultimately cross with the primary generic pool but the hybrids obtained after the process of hybridization usually produce offspring which are fertile to some extent which means that some of them are fertile while others are sterile. Transferring such genes to the primary form of the gene pool is a much difficult and laborious task and such type of genetic pool is also known as Gene pool two (GP2) [17].

4.3 Tertiary gene pool

It includes the type of genetic material which produces the ultimate sterile type of hybrids while crossing with the primary types and hence the name tertiary is given to them being abbreviated as GP3. It owns the material that can be easily crossed with the primary type of gene pool but the offspring after hybridization will produce a sterile organism. So transferring such materials to the primary gene pool is only possible in the presence of specific biotechnological techniques.

5. Activities of germplasm

There are six types of activities being related to gene resource which include;

- Collection or exploration of germplasm
- Conservation of germplasm
- Evaluation
- Documentation
- Distribution
- Utilization of germplasm

5.1 Collection of germplasm

Exploration refers to the collection of germplasm or in other words collecting the variable genetic resources from different sources and placing them at one place which is a highly scientific procedure. Collection can be done from five sources i.e., from diversity centers, gene Banks or sanctuaries, companies for seed collection, and finally through fields. Secondly, germplasm collection is done based on endangerment i.e., the species or crops which are more at the extent of extinction are preferred more as compared to others. The method of collection is done in presence of agricultural universities in collaboration with the National Bureau of genetic resources of the plant in New Delhi. For collection at the global level, it is done at the global level by International plant genetic resources being abbreviated as IPGRI with Rome and Italy [18]. The collection is done based on migration to areas of more genetic diversity, by visiting the gene bank by yourself, and finally via the exchange of genetic material. Similarly, there exist two methods for the exchange of germplasm which include random sampling involving the collection of genetic traits for both the biotic or abiotic stresses while abiotic involves collecting the different phenotypically traits. Hence both the random as well as non-random sampling methodologies are employed for collecting germplasm. Sampling size should be such that it can collect about 96% of diversity occurring in genetic traits [19]. Hence it involves the collection of 55% crop plant species of seeds per plant. Also, a wider range of habitats is sampled for obtaining maximum diversity accordingly. But there are certain drawbacks of exploration or collection such as reduction of genetic diversity due to occurrence of genetic erosion, collection from other countries or sites leads to disease condition leading to spread of weeds or pests. Moreover, it is a tedious job that requires drilling, lodging, and transport. Lastly collection from

huge resources promotes problems in the collection as well as transportation. While some of the merits include the discovery of new species while exploration and also help in the preservation of certain genotypes that have become either extinct or at the extent of extinction [15].

5.2 Conservation

Conservation involves the protection of the genetic diversity of plant crops from genetic erosion which can be either ex-situ or in situ. In situ refers to conservation under natural habitat requiring establishing resources of biosphere or ecosystem for the preservation of endangered crops or plants for future usage. Following this method both wilds, as well as natural biospheres, are conserved presenting the disadvantage of covering a very small area of genotype in the case of single species, it is a much expensive methodology and also requires a proper management system. An ex-situ conservation germplasm is conserved in form of a gene bank which is a most practical application being employed under laboratory conditions. This methodology enables the preservation of whole genetic diversity in one place. Moreover, the method is in-expensive and easy to operate [20].

5.3 Evaluation

Evaluation involves the investigation or examination of genetic resources of plants based on their phenotypically, genetics, economic, biological, and chemical characteristics. It is essential for the identification of resources for the resistance, production, yield, and other quantitative characteristics. It provides all the necessary information regarding the classification of germplasm and their characterization of each of the individual germplasm attributes. It involves the requirement of a team of specialists from physiology, biotechnology, biochemistry, and entomology. For all the characters evolution is done separately and experts from IPGR, Italy. Evolution is either done infield, in the laboratory, or greenhouse. Observation is done on basis of morphological characteristics and is recorded via specific instrumentation. The characters of resistance and biotic or abiotic stresses are screened in the greenhouse. While the evaluation of biochemical characters is done on basis of conditions under laboratory. Both visual as well as instrumentation is done accordingly [21].

5.4 Documentation

Documentation involves storage, analysis, and dimension. In-plant genetic resources includes the collection, evolution, storage, and conservation of information. But now it is termed as an information system. A large collection of information is available for major crops such as maize, sorghum, wheat, and rice, etc. Till now about 7.6 million germplasm are available for the conservation of about 300 or more species. Handling of the huge collection is done via the involvement of electronic computers. For uniformity of characteristics, it involves standard characters and further descriptors for comparison in IPRGI. The information is stored in the memory of a computer and must be available at the time of need when required.

5.5 Distribution

Distributions are the most important activity for genetic resource centers. During this process, specific germplasm is supplied to the users for improvement of genetic traits and is responsible for the maintenance of conserved germplasm and its supply

at a time for utilization. Distribution is the responsibility of the gene bank center's where they are maintained and being stored and to those who are engaged in specific research activities of a particular crop. The amount transferred as a sample is very small and depends upon the type of material available in raw form and also several other factors. A proper recording system is maintained and checked after the report by the user which tells the most important characteristics of association to the distributor. Germplasm is usually distributed after collection for at least two crop seasons because it is helpful in the adoption and purification of plant material [22].

5.6 Utilization

Utilization involves the employment of conserved germplasm in research and improvement programs and can be utilized in various paths mainly in three forms such as;

- As a new variety of crop
- As a sample in the hybridization of plants
- As a genetic variant allele in crop improvement.

Some of the crop varieties are made available instantaneously after their testing because in this case performance of these exotic gene lines are found to be better than that of local varieties so it will be available for usage at the commercial level. In another case, new varieties are developed based on selection done from the already present collection. In either case, some of the germplasm is not usable at all but possesses certain characteristics such as resistance, economic or wider adaptability. Transfer of such germplasm is easy because it can show cross-compatibility. The similarly wild form of germplasm is used for providing resistance to biotic or abiotic stresses and other characteristics such as strength in cotton. But it will present some further problems which include; the inability of the hybrid to survive for a large period. Sometimes the hybrid plant is unable to produce its offspring's and desirable characters get linked to undesirable ones. Hence the utilization of germplasm is a difficult task and requires special attention [23].

6. Involvement of organizations in germplasm conservation

Two of the organizations on both national as well as international level are available having an association with preservation or conservation of germplasm of plants. Thus, providing the facility of their abrupt usage when necessary by them. These include the international plant genetic resources institute located in Rome, Italy operating at the global level. Various types of institutes work and deal with the germplasm of concerned and most important crop plants. However, in India National Buraeu of Plant Genetic Resources abbreviated as NBPGR deals with a huge collection of both horticulture and agriculture crops. In addition to them, Forest research institutes deal with species living in forests and lastly Botanical survey of India located in Kolkata deals with the remaining plant species [24].

6.1 Role of IPGRI

IPGRI old IBPGR is an international scientific organization whose work in addition to other institutions is analyzed by CGIAR which is a consultative group on

international agricultural research. Its main role is to conduct and organize research and also to promote and ensure collection, documentation, and utilization of these plant germplasm and it will be helpful in the collection and exchange of plant materials. It also possesses an advisory committee which helps in the collection, evaluation, and utilization of germplasm of crop plants. So it promotes global collection and conservation of all the genetic resources of plant species. It was changed from IBPGR to IPGRI in 1993 while its predecessor was established in 1974.

6.2 Role of NBPGR

This institute was established by the Indian Council of Agriculture research Centre (ICAR) in New Delhi in 1976. In India, the introduction of the plant was done in 1946 in the Division of Botany and a separate introduction was done by Dr.H.B. Singh who made a well-known achievement in the fields of the introduction of plants in India. He also arranged a large collection of germplasm of various species of plants and systemized the research in this field. In 1976, this decision of introduction of plants was revolutionized to an independent agency named NBPGR. The basic function is that it is helpful in the import and export of genetic resources of plants hence facilitating the exchange of germplasm. Also, it promotes activities of germplasm like collection, conservation, documentation, and utilization. It also organizes short term courses of collection, conservation, evaluation, and utilization of genetic resources of crops. Besides, it also guides the development of storage of plants at cold temperatures and short-term conservation of germplasm. It's also a decision about the setting of the gene for endangered species of plants [25].

7. Genetic erosion

It is also known as genetic depletion in which a limited number of genes of species that are endangered get more reduced where reproductive individuals die before reproduction with others in their low population. In a more detailed way, it is described as a loss of some alleles or genes while referring to further loss of the whole phenotypic trait or genotype. It occurs because each individual has a unique set of genes that get lost when they die before they breed. A low level of genetic diversity leads to further reduction of the genetic pool thus breeds a combination and also weakens the immune system taking the species to the level of eventual extinction. Genetic erosion is greatly observed in endangered species. Most crop species get benefits from most of the human-associated programs to keep the production viable. So in this way avoids extinction for a large time frame [26]. A small collection of populations are more vulnerable to erosion than that of a larger one. The level of erosion gets worse and is being accelerated with time-based on the loss of habitat and fragmentation of habitat which also forms the firm basis of barriers inflow of genes between two or more than two types of populations. A genetic pool is a complete set of all alleles investigated by the genetic material of all members of living species or a set of populations. A large pool indicates a greater level of diversity occurring in genetics that is associated with populations that survive as a result of selection phenomenon similarly low level of diversity leads to a reduced level of fitness and increase in chances of extinction of any species [27].

7.1 Process of genetic erosion

Bottlenecks of population results in the creation of genetic pools that possess very few mating partners which are fertile too. Reduction in the number of breed

Plants by unique genes will be similar to the situation where dealers operate with similar five cards again and again. Hence producing very few numbers of limited hands. As the sample inbreeds, it's both physical as well as reproductive effects appear to have existence much often. The most common and wide effects are on the Immune system which becomes weaker with time, presenting less resistance to diseases and in turn increases the count of bacteria, virus, parasites resulting in threats of diseases. So even if any endangered species in the genetic pool or bottleneck can bear with human development or growth. So, it faces the threat of epidemic which proves to be dangerous to the whole set of population [28].

7.2 Agricultural or crop plant hazardous loss from genetic erosion

Erosion in genes has resulted in the loss of a particular gene or gene which has undergone a recombination process i.e., complex set of genes that are either produced locally by the racers of land for domestic plants and animal species that adapt to natural conditions where these species grow. The major force behind the genetic erosion is the clearing of land, over the employment of species, deforestation, and degradation and finally grazing to a large extent. The major factors are the replacement of local varieties with varieties that are found to be non-locale. When commercial species overcome the traditional species and are introduced into the traditional farm system, it will also result in a reduction of a huge collection of varieties however the major problem is that it results in a reduction in tendency for uniformity in both genetic as well as economic factors in the development of a modern form of agriculture. So, if any endangered species can tolerate the process of human development and are adopted at a place much away from their natural habitat. It will still result in facing the danger of a serious threat to the whole population. With the advancement in science and technology, several techniques have been checked for checking defects of genetic erosion which result in the extinction of species that are at the extent of endangerment. But many techniques are very expensive for using them at a practical level. So, the best way is to preserve them by the protection of their natural habitat and to allow them to live in natural conditions as long as possible [29].

8. Endangered plant species and medicinal uses

Medicinal plants grow in. A natural environment around us and with the advent of technology, humans have gained the knowledge of how these can be utilized in fighting an illness or for maintenance of human health. The capability to use wild species in the improvement of health is not dependent on humans alone. The ability is affected by various factors such as pests, diseases, climate, environment, and other biotic or abiotic factors. According to society in America in 1999 the capability for maintainability of crop production depends on the compounds or genes being extracted from wild species of medicinal plants as depicted in **Table 1**. Because of their extensive use in commercial as well as the scientific environment, there has been increased pressure on wild species from which all these medicinal plants have been extracted. Over-harvest action, as well as commercial exploitation, has resulted in the unavailability of traditional medicine where the people utilize them. For all these reasons there is an urgent need to conserve these plant species [31].

Some of the examples of species include slippery elm which involves the use of gummy lining for used in North America as a therapeutic agent for cough and cold, gastrointestinal diseases, and allergy to skin epidermis. But this medicine being

Name of crop	Medicinal usage	Origin
Aloe Vera	For curing burns and wounds	South Africa
Aspirin	Pain killer, health rate normalization, and blood thinning	Europe
Bloodroot	Treatment of skin cancer	US
Camphor	As a pain-relieving agent	Asia
Digitalis	For the treatment of heart failure	Europe
Quinine	Malaria	South America.

Table 1.
Some medicinal plants which are at extent of endangerment [30].

used by local people is demanded by Millions of people now. They are not used commercially so the trees are separated from bark and are left to die. So, for about 50 pounds, 15 trees are sacrificed every year and now the species is identified as at risk by the US.

Another example is yew belonging to Texas specie that is used for the production of cancer drugs such as taxol. It is also identified as endangered based on its over-harvesting. Similarly, black cohosh is used for the cure of a large number of ailments such as colds, pains, and largely menopause and also found to be a list of endangerment due to degradation of habitat as well as over-harvesting. Moreover, goldenseal has numerous uses being used as a toner treatment of diseases such as hemorrhoids. It is already threatened, endangered, and vanished in many states.

9. Methodologies for the conservation of germplasm

There are various methods for the preservation of genetic resources or traits for crop plants. The easiest and most economical method is the storage of seeds of crop species. But not all the plant species can be stored easily in this condition because some seeds have a shorter span of viability and several species do not produce seed vegetative. Still there exist many methods that depend on storage conditions, storage vassals, the extent of conservation, and finally on the facilities available for proper storage of plants [32]. The most efficient way is to store biological crops in the environment where they were produced or developed earlier i.e., in situ farmlands as shown in **Figure 1**. This type of method can be employed when the natural environment is balanced and there are no chances of off balance. The most important point which must be considered is that genes should be conserved instead of the genotype of plant species [33].

9.1 In-situ conservation

This method employs the conservation of resources totally in a natural habitat. It involves the maintenance of plant species in which it grows and also in the habitat to which it is adopted for a long time in the past. The objective behind this methodology of preservation is to maintain the self-sustaining species in a natural ecosystem [34]. A huge collection of plant and animal species can be conserved by this mechanism. But along with it, there exists a limitation that it is impossible to sustain and preserve the genes of crops without conservation of the ecosystem of which it is adopted by nature [35]. It allows the conservancy of naturally occurring

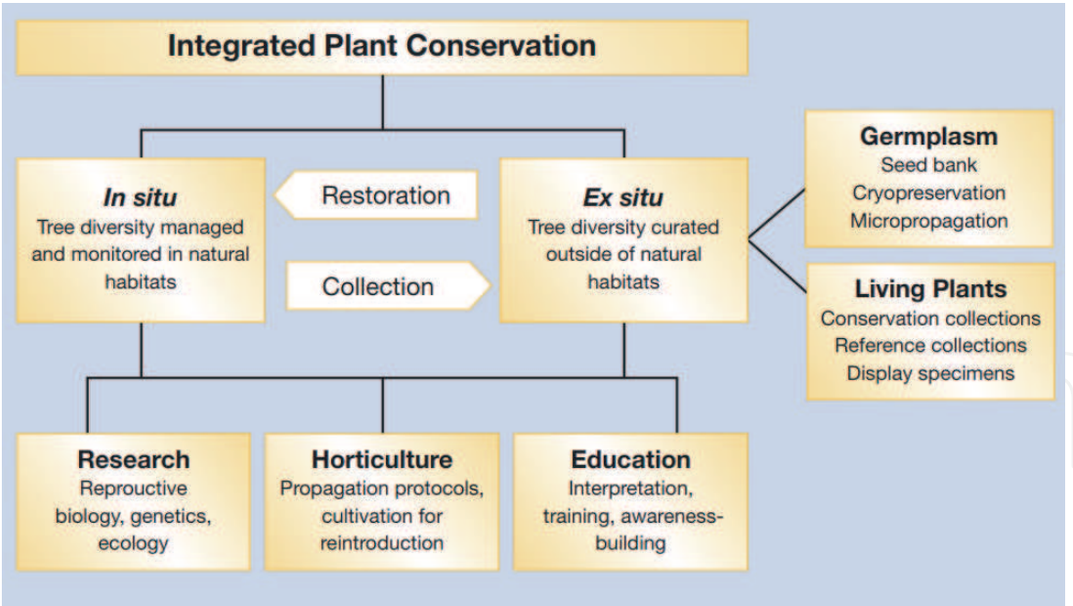

Figure 1.
Elaboration of integrated plant conservation involving both in-situ as well as ex-situ methodology. In-situ conservation allows the conservation of germplasm of and living parts of endangered plant species while ex-situ conservation involves the plant material available for research purpose, horticulture and reintroduction of materials preventing materials from getting extinct.

beneficial species in a condition where it continues to undergo evolution with time. Moreover, it also allows the conservation of both wild and cultivated genotypes without having much expenditure on the area. The major benefit of this process is that species selected by nature continue to evolve timely which results in the production of new recombinant forms of a living being. In the case of reluctant seeds that grow either in tropical or perennial regions, it serves as the best phenomenon of conservation within the in vitro environment [36]. Although in situ conservation is the best method but, its operation in any country or ecosystem is possible only when it is ensured by people who are in continual conflict with national plans and when its continuity is also confirmed in any environment. Usually, if continuous and control monitoring does not operate, its survival rates get much lower. This results in loss of naturally occurring habitat and also replacement of old generation of crops with new varities which occur as a normal part of the crop growing system [37].

In situ conservation is, therefore, can be achieved by the protection of naturally occurring wild species in their natural or adopted habitat via cultivation in fields. Such areas or regions are being discovered in natural parks and recreational areas under government territory. Practices such as horticulture and floriculture present an efficient way of conservation in the naturally existing ecosystem [38]. Horticulture is a phenomenon in agriculture whereby plants are preserved for the purpose of feed but mainly for comfort and decoration purposes. It employed the use of knowledge and skills to grow plants for use in both food and non-food areas and also for social requirements. It includes both tropical and perennial species, vegetable varieties, tasty fruits in addition to decorative indoors, and other Landscape plant varieties [39]. Floriculture is also a subtype of horticulture which is mainly concerned with cultivating flower varieties of ornamental plants for use in the floral industry, gardens, and orchids. Development and growth of varieties via breeding techniques to a novel variety of species is a major point of focus in floriculture which allows the transfer of desirable characteristics to next-generation resulting in maintenance of specific genetic traits [40]. This methodology of preservation has some advantages which include;

- Each preserved area will contain a small portion of whole diversity i.e., Small portion of total diversity. So, it requires the preservation of a large number of areas for the conservation of the whole genetic pool.

- The maintenance and management of all these areas also require labor and present problems.

- This is the most expensive method for the conservation of germplasm.

9.2 Ex-situ conservation

Another methodology involving the collection of plant gardens and banks of seeds where the plants are grown under natural conditions [41]. Seed banks are maintained and produced by research institutions and universities produced via the technique of tissue culture and utilization of much lower temperature in the environment for its operation [42]. It also ensures that the plant materials are easily available, characterization is done efficiently and well documented and its exposure to the outer environment is safe i.e., it should not possess any threat to the natural ecosystem of humans as well as animals. However, it provides the best alternative to naturally existing methods which in addition to providing an opportunity to wild species having desirable traits to continue undergoing the evolutionary process in a naturally existing environment. This method has the advantage of safeguarding the germplasm while it is in its natural environment resulting in the genetic variation in naturally occurring verities and is readily available for use. Examples of plant undergoing this subtype of preservation under biotechnological area are sugarcane, cocoa, and maize, etc. [43].

This is also referred to as offsite conservation which employs the conservation of species outside their natural habitat or system. In this method, the genetic information of the plant is preserved in form of banks which may be either seed or gene bank or in the form of cultures to increase their half-life so that they can be used for a long period inefficiently [44]. The class of preservation technique results in the formation of collection or bank of genes, DNA, seeds, and germplasm forming a genetic library in the form of gardens. This will lead to the creation of a good option for the conservation of species that are thought to be endangered or near the extent of it, which are primitive and in turn, are much valuable for use in industry for commercial purposes. It includes certain techniques such as cryopreservation and other genetic transfer approaches for the eradication of diseases, pest and stress control, and lastly conservation of endangered species in the long run [45]. It is almost similar to that of in-vitro methodological practice. Other disadvantages include loss of viability of seed structure, destruction of the crop by pest or insect, poor germination of a seed plant, and lastly, it is a much expensive procedure [46]. On the other hand, major advantages can be summarized as,

- Small areas can store a large collection of materials

- It protects all other environmental-based methods [47].

- It is the cheapest method and preservation of germplasm is much easier.

- It is possible to store the whole genetic material in a single place.

The most advanced form of preservation of genetic resources is to maintain them in laboratory conditions. This is the conservation technique which employs

the use of test tubes or laboratory apparatus which is sealed in one or other way for maintenance of resources [48]. The genetic resources such as tissue cells or callus are placed in the sealed tubes which operate on the fact that plant parts can be kept alive under controlled laboratory conditions which proves the fact that plants are totipotent. This means that every part of a plant can develops into a whole organism. This phenomenon has made this fact clear that disease-free plants and species can be transferred to the next generation within the laboratory controlled conditions [49]. Or in other words, engineered species provide a viable means for the transfer of pest and insect-free species from one generation to another. The source of such genotypes is from the culture of laboratories or having origins from international seed banks [50].

In vitro conservation of plants was first done in the mid-1970s. Although whole 'is not can be regenerated from any part of the plant because of its totipotency but due to the involvement of unorganized culture there exist some risks of a generation of somatic mutation and mutants. In comparison to it, the cultures containing somatic meristem culture are much more stable in their transformation mode but also it can propagate more frequently as these areas do not have to recover after differentiation [49]. Most efficient storing systems are usually not much expensive, are easy to maintain, and reduce the work labor and load in germplasm working bank. Scheduled monitoring of the cells along with viability and contamination assessment is not that necessary. The exploitation of in Vito technique of genetic conservation is hindered if any species is unable to prorogate to the next generation from tissue or cell culture [51]. For example, a proper technique for the prorogation of coconut does not exist yet unlike other crops of this class which can be propagated inefficient way via callus differentiation. But in this case, the leaves or plantlets can only be produced from a zygote or embryo. Each embryo in this case will produce a new plant which represents no further division of genetic material. In the same manner, the effect of in-vitro culturing is much less for woody plants as compared to other species as it can result in difficulty in culturing and regeneration of new species [52]. In these cases, less research has been done for the development of an appropriate cultural technique in vitro. But a thorough examination of the problem occurring in the handicap pathway of procedure for wood culture can solve this problem, presenting a suitable solution to the development of plant and conservation of their genetic resources efficiently [53].

The most important drawback of this phenomenon is that it requires the utilization of modern technology and labor force under the controlled conditions of an aseptic environment. Also, it requires proper laboratory skills with excessive usage of electricity which makes this procedure much labor-intensive and expensive [54]. This process is helpful in the production of disease-free varities of plants that are also pest-free and these species include sugarcane etc. The produced genetic resources are used in several ways such as genetic improvement, maintenance of biodiversity, mechanism-based research of ecosystems, classification according to taxonomy, monitoring of environmental characteristics, epidemiological, and forensic based studies. One of the main strategic reasons behind germplasm conservation is that it maintains biological diversity and provides germplasm which is validated in both genotypic and phenotypic aspects [55]. Germplasm is either conserved in the form of seed or meristem form.

10. Gene bank

Gene banks are the type of repository in biology for the preservation of genetic resources. In the case of plants, it is done by storing in laboratory conditions,

freezing cuts from the plant materials, or maintaining stocks of seed. Accession is the term provided to each sample in a gene bank like the species or variety. In plants, it is easy to unfreeze the materials for their propagation and usage.

Gene banks are also classified as both in vivo that is within the body and in-vitro which involve sustaining of characters in proper laboratory conditions. The type of gene bank where traits or alleles are stored by employing conventional methodologies are termed in-vivo. For example in the form of seeds and vegetative collections [56]. While the subtypes where the characteristic resources are stored in form of non-conventional methods in form of cellular structure and tissues are referred to as in vitro. Both techniques are of prime importance in the development of valuable trait crops for breeders to develop both new and improved varieties [57]. This involves using DNA as a source of DNA in terms of germplasm being employed in breeding technologies. When these are properly identified and after that efficiently characterized, it will result in the production of the transgenic organism which can express these genes. Genetic disruption can be avoided by the phenomenon of transformation which also involves sexual hybridization. It is not limited by compatibility from the sexual life cycle and can be evolved from other forms of life in the short run. The transgenic genes are helpful in the production of plants which in addition to herbicide-resistant are also pest resistant and are conveniently preserved as a transgenic or cloned form of genetic material. The process is limited by the identification of potable genes which will result in the production of higher yield along with greater stability of Transformants in host genera. Such genes have been produced successfully for conservation as well as patent purposes so that they can be employed at a commercial level. Economically or technically, is not worthy in the future that this synthesis of the gene will store these genetic traits in the form of physical germplasm i.e., in form of seeds, tissue, or the whole plant, etc. Conservation of DNA molecules and similarly the assembly of these molecules in the form of DNA data sequence is not the best alternative to conventional methods for germplasm because genes are not coordinated in them in a small similar fashion.

Recently with the discovery of artificial chromosomes in yeast has raised the fact that coordinated assemblies of genes can be made and therefore can be conserved which will allow further morphological or phenotypical changes to be engineered in the laboratory efficiently. To use them practically, it is important to conserve the host organism but the genetic information in them is not yet fully discovered. But gene liberates, sequence data, and gene banks cannot be employed to reproduce a whole organism but have a significant role in preserving genetic resources of crop plants which are either on the extent of danger or found to be endangered. The conservation of plant or genetic diversity involves the collection of small parts of plants such as tissues, cells, shoots, etc. A tissue sample from all the plants and species will be collected in liquid nitrogen as described in cryopreservation at a much lower temperature of freezing point. In theory, these samples are not indefinite and DNA extraction is not performed until recommended. So, at that moment, DNA can be identified, immobilized in a membrane to act as a source of a specific gene or sequence of DNA molecules. The technique is helpful in the conservation of both undefined and undescribed species of plants whose seeds cannot be stored directly and is used for diverting those whose seed values have been found earlier and observed in germplasm banks already. DNA sequencing is carried out in almost all laboratories throughout the world because they can be compared to novel sequences with those which are properly and characterized considerably. Comparisons also highlight the unrevealed homologies and suspected functional properties between the organisms which are unrelated. Most organizations support coordinated DNA sequencing and storage. The most famous banks are the European molecular biology laboratory located in the US, GenBank operated by the United States laboratory.

The rapidly increasing data of sequence raises voice on important problems of storing and facilitating rapid comparison regarding new information on data sequence of the gene. Gene banks are of three types which include;

10.1 Seed bank

It stores the seed at very low temperatures after they have been dried efficiently. Spores, as well as meristems, are stored by this method in seed banks but the vegetative plants which do not possess seed are not concerned by this technique. The largest bank for storing seeds is Millenium seed bank which is located at WTMB near London.

10.2 Tissue bank

By this technique buds, roots and meristem are stored in this way by utilizing light, the temperature in presence of aseptic conditions, and nutrient media containing all the essentials such as carbon, nitrogen, etc. Hence the technique is based on the preservation of seedless plants and which reproduce by sexual reproduction.

10.3 Cryobank

By this technique, a seed or embryo cell is stored at a much lower temperature or temperature much lower than freezing temperature in liquid nitrogen at a temperature of about −196 °C. So, it is helpful for the conservation of species that are at the extent of extinction or have become endangered. But mostly it is used for the cryopreservation of genetic resources of animals. But in the case of plants, conservation of pollen grains is done by storing at a much lower temperature of −196 °C. Hence this methodology is used for cross breeding and also in the production of plants having a set of chromosomes.

10.4 Field gene bank

The method is used for the conservation of genes of planting plants where the ecosystem is created artificially. By this method, the different plant species can be compared so that they can be studied in detail. It needs more artificial requirements such as soil, water, and weather, etc. The germplasm of most crop plants is conserved by this process. For example, 43000 rice varieties are conserved in Orissa at a central research institute.

11. Cryopreservation

The word is derived from two Greek words. i.e., Kryos means frosting while preservation means storage for a long time or increasing half-life in one way or another. Following these techniques cells and tissues are stored at a much lower or frozen temperature either using carbon dioxide at −79 °C or nitrogen gas at −160 °C in the form of vapors in deep freezers. In the c as e of liquid nitrogen, the limit of temperature would be from 170 °C to 197 °C. The technique involves four stages involving freezing, thawing, and re-culturing, etc. [58]. Thus, freezing temperature inactivates the cells and tissues so that it can be preserved for a longer period. Any of the tissues of the plant can be preserved under proper conditions for example meristem, stem, ovules, anther, embryos, endosperm, cells, and leaves, etc. [59].

The process of cryopreservation being followed by regeneration of the whole plant invoke the following steps **Figure 2**;

- Isolation and development of sterile tissue culture
- Addition of cryoprotectants
- Pretreatment
- Freezing of plant material
- Storing of plants parts
- Thawing followed by culturing
- Assessment of viability of cells based on their rate of survival
- Regeneration of plants

11.1 Isolation of sterile tissue or cell

Physiological and structural conditions of plant effects ultimately the survival of the plant during cryopreservation. Tissues to be used in preservation must be healthy, small, young, having rich cytoplasm, and highly vacuolated. In either case, callus acts as the best source of tissues as it is more resistant to damage caused by freezing [60]. So, a callus after 1 to 2 weeks of subculturing is selected for the

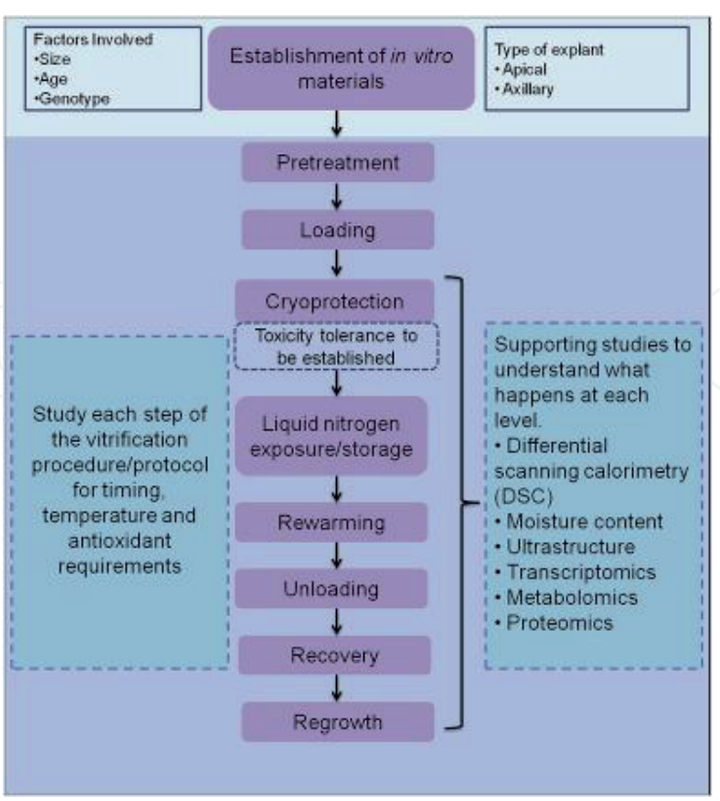

Figure 2.
Schematic flow sheet representation of steps involved in cryopreservation of plant materials in biotechnology for genetic resource(germplasm) conservation of endangered plant species such as golden paintbrush from natural environment to cryobank in form of seeds, tissues, roots, meristem and shoots etc.

cryopreservation process. But old and black areas should be avoided and organized structures are preferred more.

11.2 Addition of cryoprotectants

To prevent the damage resulting from abrupt freezing or thawing, the chemicals such as glycerol, alcohol, dimethyl sulfate, glycerol, praline, etc. are being added for the purpose of conservation. This protectant referred to as cryoprotectants are added to protect freeze cells or low freezing temperatures etc. But the limitation of the procedure is that only a few biological materials can be frozen below the minus temperature in presence of gas without affecting the viability of cell structures. Liquid nitrogen is used because of the following reasons;

- It is inert in chemical form.
- It is a less expensive process posing less burden on the economy.
- It is non-toxic posing no side effects on the environment.
- It is a non-flammable and most readily available method so far.

11.3 Vitrification

However, two more practical approaches in biotechnology may lead to widespread applications of conservation of germplasm of plants to reduce damage from abrupt cooling. This includes vitrification by using cryoprotectants mixture and another is an encapsulation of a sample with gel which is dehydrated later on as described above. For the process of vitrification, the sample is submerged in a cryoprotectants mixture which results in the promotion of conservation of cellular water into non-crystal-like solids which later cool rapidly. In the case of encapsulation, the sample material such as root or shoot tip is dipped in a gel to form an artificial seed-like structure which is then dehydrated before cooling. The gel performs the function of protection against physical damage and is more robust than shoot tip or embryo culture. Despite the presence of optimistic methodologies using plant tissues, further research is required to find the development of preservation to that available for animal and human embryos. But there exist many barriers that prevent the utilization of technology in one or other way.

11.4 Pretreatment

The process involves regrowth which involves the application of additives to enhance growth e.g. abscisic acid etc. [61]. On the other hand, cryoprotectants act as an anti-freeze, increases viscosity, and prevents damage which resulted wither due to the formation of ice crystals during cryopreservation or due to an increase in intracellular concentration of solutes before or during the process of freezing as a result of dehydration.

11.5 Dehydration

Vitrification is a process of conversion of liquid into solid in the absence of crystallization. When the cells have properly undergone the process of slow freezing, it will result in vitrification where ice formation does not take place because here the aqueous solution is much concentrated which results in permitting the formation

of ice cubes. Instead, the water gets solidified into a glassy clear state. Dehydration during this process is achieved by the high concentration of osmotically active compounds like sugars, polyols performed in the sterile cabinet over silica gel [62]. In the process of dehydration, a reduction in the amount of water followed by the formation of ice and an increase in osmotic pressure occurs which ultimately depress the freezing point [63].

11.6 Rapid freezing

Then plant material is placed in liquid nitrogen at a much lower temperature ranging from −300 to −1000 °C. Dry ice can also be similarly used in the process. The more quickly freezing is done the less will be the intracellular state of crystal formation. The methodology is simpler and easy to handle and can also be used for tips of potatoes and strawberry species. Dry ice can also be utilized for this purpose.

11.7 Stepwise freezing method

In this method, the temperature is lowered to about −30 °C for at least a period of 30 minutes and then abrupt cooling is done via using liquid nitrogen at the much lower temperature of −196 °C. Slow freezing increases dehydration while abrupt freezing promotes crystal formation. It gives excellent results in the preservation of strawberries in the suspension culture.

11.8 Storage

Storing at the correct temperature is as important as that of freezing. For storage, the temperature is left to almost −70 °C to 197 °C because this temperature is sufficiently low for the preservation of cells without metabolic damage to them. Long term storage is mostly done at about −197 °C.

11.9 Thawing

It involves the rapid thawing of the ampule containing the sample in a water bath at about 40 °C. They are plunged into warm water with swirling for rapid mixing just to the point where ice gets disappears and is important for the survival of tissues that the sample must be removed from the water bath after melting of ice. Tissues being thawed at a much lower temperature are then abruptly thawed following this step.

11.10 Determination of rate of survival

Regrowth of stored tissue is the best indication of the survival of plant tissues. For this purpose, many viability tests are used which involve fluorescein diacetate staining, measurement of growth by cell number, and finally by calculating the dry and fresh weight. The two most popular methods are m

- Triphenyl tetrazolium chloride
- Evans blue staining

It provides the best opportunity for the conservation of endangered species being used for medicinal purposes. It also provides an ideal approach for the suppression of cell division to avoid further need for sub culturing. Pathogen free cultures and subcultures can be frozen and stirred when required and also provide

a much suitable material for the selection of cold-resistant strains of mutant cell lines which later get differentiated into frost resistant cells or plants. The seeds may be stored for food crops or to protect biodiversity or reason for storage also varied which involve first drying of moisture to less than 5% and then stored at the much lower temperature of −18 °C or below it [64].

12. Work done by the vavolian center of biotechnology

For the last 20 years, advancement in tissue culture technologies has led to the development of the micro propagation method which is a novel technology, providing young and fresh plants for horticulture, agricultural, and agriculture purposes. One of the main consequences is the rapid growth of in-Vito exchange as a viable means for the transfer of germplasm between different laboratories [65]. The International Board for genetic research has elucidated that more than 140 plant germplasm has been exchanged from 1980 to 1986s. Out of all attempts, about 97% were found to be successful. Now almost every agriculture research center is attributed to the exchange of germplasm in-vitro. For example, the exchange of germplasm of potato culture is now a routine procedure. Shoot cultures are incubated for about 3 weeks after inoculation to induce roots and any contamination resulting from the microbial mass [66]. Transferring them to a fresh medium compensates this problem and increases rates of survival. Now the new era has replaced the cultures with small test tubes produced under in-vitro conditions [67]. These are more robust and rapid methods, and the produced plants or germplasm can be stored for months or even years. The recipient can place them in nursery beds without the involvement of further culture step. In the modern process of potato breeding, the in-vitro cultures provide another species that is disease free followed during the process of field testing required to select the most desirable form of clone as in **Table 2** [69].

The successful application and conservation of genetic resources in every country for the purpose of food and agriculture depend upon the collaboration of the government. Policymakers, germplasm scientists, rural populations, and

Species/crops	Applications
Rice	Food, fodder, and beverage
Sorghum	fodder
Cowpea	Food, fodder
Maize	Food, industry, fodder
Soya bean	Industry, food, fodder
Sesame	fodder
Cassava	fodder
Millet	Medicinal, fodder, industry
Hungry rice	Food, industrial, fodder
Yarn	Food
Sugarcane	Food, beverage, industry
Groundnut	food

Table 2.
Utilization of plant species/crops in various aspects after their conservation in the form of germplasm which are thought to be endangered in future [68].

breeders or farmers. Usually plant genetic resources are conserved because they are ultimately used in food and agriculture and sustainable agriculture depends upon their usage. Farmers in modern agriculture use their plants or crops for purpose of not only food but for medicine and fodder also as shown in **Table 3**. Deployment of genetic resources in a better idea paves the way for the reduction of vulnerability of crops or plants to that of insects, pests, and other fertilizers making them herbicide, pesticide, and insecticide resistant. In national research institutes of every country about 13% rice, 7% soybean, and 8% of sugarcane species are conserved in various breeding technologies as illustrated in **Table 1**. With the abrupt increase in population and reduction in land available for agricultural purposes, an increment in the production of food, as well as its distribution across the globe, is much necessary. There is an utmost need in every country to use their genetic resources for better purposes utilizing the breeding techniques effectively. The involvement of genetic resources in the techniques has resulted in almost the compensation of food required by the increasing population of the world. So the stress of poverty alleviation in developing countries has been reduced effectively and is also involved in food security depending on the availability and utilization of species that produce a higher amount of crops or plants with desirable characters especially in rural areas where most families rely on farming for their survival. So utilizing a small collection of gene bank resources can lead to greater benefits as elaborated in breeding programs. However, less usage of them can lead to fewer benefits in both the social and economic sectors. The constraints involved in the low level of germplasm conservation include lack of ability to characterize and evaluate gene data banks, insufficient knowledge, inappropriate documentation and poor relationship between users of gene banks and germplasm. Currently, in sugarcane industry much data is available on characterization and evaluation of data on sugarcanes for utilization by stakeholders.

Name of crop	Type of conservation	Improvements resulted
Soybeans	Gene banks, bottles, and tissue culturing	Reducing days required for maturation. Transferring smut resistance from wild to cultivated type.
Sugarcane	Gene banks	Transfer of smut resistance from wild to cultivated type. Introduction of sucrose and improved protein content.
Rice	Storing bottles and tissue culturing	Hybridization of both wild and cultivated species. Development of short duration techniques Development of iron resistant specie.
Sorghum	Field gene banks, tissue culture	Production of short duration variety. Mildew resistant varieties.
Maize	Storing bottles and tissue culturing	Production of varieties which are high in lysine content
Cowpea	Storing bottles and tissue culturing	High yield of product. High protein species.
Sesame	Storing bottles and tissue culturing	Early maturing species. Development of verities that are resistant to black specks. High oil containing verities.

Table 3.
Improvement in crops by germplasm conservation of plants (conservation type) being used in everyday life which are thought to get endangered in future [70].

13. Conclusion

The development of various successful methodologies for preservation of genetic resources enables the establishment of basal collections of endangered species with the representative diversity. The collections include many of species which have been threatened for loss of habitat. Collections related to critically endangered species are maintained in simple media. These threatened species could also be maintained at lower temperature after acquiring specific laboratory conditions. From the whole discussion, the fact has become clear that there is a need to conserve germplasm in a variety of ways. It is also elucidated that the effectiveness of conservation technology depends upon the maintainability of the collection of genetic resources in a much cost-effective manner. So, there is an abrupt need to emphasize evaluation of the efficiency of conservation procedure by realizing efforts in an in-expensive way. Because the future will depend upon the presence and utilization of conserved germplasm. Thus, there is an urgent need to conserve the most important crops such as maize, rice, sorghum, wheat, etc. The variation in them is leading to genetic erosion which will ultimately degrade them and their availability in the future will be affected. So on the whole, involvement of germplasm in its preserved form and plant breeders in growth and development of these crops for improved and better varieties. Moreover there is a large collection of endangered species in under developing countries and creation of botanical gardens for in vitro and in vivo conservation allows establishment of culture facilities which in turn results in rescue as well as regrowth of endangered species. Additionally networking in exchange of information or materials and dissemination of useful protocols are important steps in continuous development and exchange of mechanisms for germplasm conservation. So at this point scientists and conservationists need to work together in order to develop better programs for germplasm conservation of plant species which are at risk of extinction.

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