REVIEW ARTICLE

EXPLORATION AND CONSERVATION OF FOREST GENETIC RESOURCES

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Abstract: Increased use of forest resources and a shrinking forest land base threaten the sustainability of forest genetic resources and highlight the importance of conservation and sustainable management of these resources. Conservation of forest genetic resources could be defined as a set of activities and strategies that are being implemented with the aim of ensuring the continued existence, evolution and availability of these resources for present and future generations. As forest trees are normally the keystone species of forest ecosystems, their continued existence is essential for many floral and faunal associations of these ecosystems. Major opportunities for conservation of forest genetic resources include: use of molecular genetic markers and adaptive traits for developing conservation strategies; *in situ* conservation through natural reserves, protected areas, and sustainable forest management practices; *ex situ* conservation through germplasm banks, common garden archives, seed banks, DNA banks, and tissue culture and cryopreservation; incorporation of disease, pest, and stress tolerance traits and ecological restoration of rare or declining tree species. Forest genetic resource conservation and resource use should be considered complementary rather than contradictory to each other. Therefore, the aim of genetic resources management is to improve conditions for the continuous evolution of the species, which represents the defense mechanism of organisms in suppression the environmental changes.

Keywords: Forest genetic resources, In situ gene conservation, Ex situ gene conservation

INTRODUCTION

Forest genetic resources (FGR) refer to the inherited materials maintained within and among tree and other woody plant species that are of actual or potential economic, environmental, scientific or societal value. Conserving forest genetic resources is fundamental, as FGR constitute a unique and irreplaceable resource for the future including for sustainable economic growth and progress and environmental adaption. Efficient use of existing genetic resources can only be achieved if sufficient information is available on their extent, structure and composition (Brazier *et al*., 1976). For a large number of tree species, especially for species growing in the tropics, there is a great lack of knowledge on the ecology and biology, as well as on their potential as plantation species and the potential use of non-wood products derived from them. Even for species of proven value, their variation throughout their natural range has often been explored inadequately. Exploration is the act of searching for the information of discovery of resources. For practical purposes, the field activities in the fundamental step of exploration can be divided into two types

1. **Botanical exploration**: It includes the correct taxonomic identification of species and knowledge of the limits of their distribution, with particular reference to isolated occurrences. Such type of exploration logically leads to species trials.

2. **Gynecological exploration**: In Gynecological exploration, patterns of ecological and phenotypic variation within the natural range of species are studied. Such type of exploration leads to provenance seed collections and provenance evaluation. Hence, provenance trials help in the exploration of tree gene resources.

Conservation of forest gene resources

Conservation of forest genetic resources can be defined as the policies and management actions taken to assure the continued availability and existence of forest genetic resources. Conservation when used in its proper sense, embraces both preservation and utilization. It is in fact, an aspect of resource management which ensures that utilization of the resource is sustainable, at the same time safeguarding genetic diversity essential for its maintenance.

Need for conservation of tree gene resources

Erosion of genetic resources and extinction of species occur on a large scale throughout the world. These developments have given rise to gene conservation as a distinct discipline within the plant sciences.

1. **Human influences**: In many regions of the world, uncontrolled forest exploitation diminishes genetic resources. The selective logging of high-quality individual trees reduces population size, may increase inbreeding and random drift and exposes some species to extinction. Deforestation is even more serious in which entire forests are cleared of trees. The destruction of forests on a large scale leads to the loss of entire regional populations and perhaps

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species. Environmental deterioration is another threat to genetic resources and it has accelerated substantially.

2. **Climate change**: The accumulation of CO_2 in the atmosphere has accelerated during the last few decades and is predicted to increase further in the upcoming years. Also unprecedented is the expected effect of increasing temperature on climate viz., solar radiation, droughts, wildfires etc. but to some extent, rainfall will still be controlled mainly by latitude, but northern lands will experience milder winters with shorter days and warmer summers with longer days. If climatic changes take place as predicted, the established pattern of forest ecosystem distribution and genetic variation will be affected. A warmer climate will lead to an expansion of grasslands and a northward shift of forests. The belt of boreal forests will become narrower and some species growing in higher elevations may disappear. Many southern or low-elevation populations may become smaller or disappear. Natural or artificial regeneration of forests with local seed may no longer be possible but introduced provenances may be viable. Climate change can also give way to new insect and disease problems. Hence, gene conservation efforts must be accelerated to prepare for the many issues arising from climatic change.

3. **Domestication:** Plant evolution is influenced not only by human activities that reduce the range of a species and total population size but also by cultivation and breeding i.e., domestication (forest trees = wild). Domestication can influence genetic diversity in two ways. Indirectly by the method of seed collection, extraction, storage and by nursery and plantation culture and directly by conscious artificial selection to increase the frequency of desirable physical attributes such as straight stems, narrow crowns, finer branches and more rapid growth than in wild trees. The selection process requires a restricted population size and this can reduce genetic diversity in the long term. Control of some persistent insect or fungal diseases can be achieved by seeking genes for resistance in wild ancestors of present crops. Resistant individuals (wild ancestors) can then be brought into the breeding programmes. Thus, the conservation of native wild populations will always be desirable.

Strategies for conservation of gene resources: As per Burley and Styles (1976), the strategy for conservation of gene resources is three fold: Conservation of ecosystems, Preservation of rare and threatened species and Prevention of genetic erosion i.e. depletion of genetic variability

METHODS OF GENE CONSERVATION

In situ gene conservation

In situ gene conservation means preserving tree populations in their natural habitats with their common associated plants, animals and microorganisms and their continuing evolution in these habitats. This method allows whole ecosystems to be maintained if the areas included are large enough and can be coordinated with the reservation of forest lands for ecological reserves, national and provincial parks and wildlife sanctuaries. In situ gene conservation may be the only conservation method available for species that are not planted and for which there is no knowledge of seed storage requirements, nursery culture and plantation establishment (e.g., many tropical species). The principal advantage of this method is its compatibility with forest management. This type of conservation of FGRs is often considered to be the core activity of FGR conservation and management as it maintains the existing natural pool of genetic variability while at the same time permitting natural selection processes to operate. It is therefore most desirable method of conserving forest genetic resources, provided the area can be granted full protection and genetic material conserved is made available for use both within and outside the country of origin (FAO, 1975). It also maintains the typically wide range of variation required for effective selection for breeding and genetic improvement of trees with high commercial or service value. This method of gene conservation ensures that in the absence of catastrophe and genetic bottlenecks, the genetic variability contained in the target species is maintained at a high level and serves as the foundation on which selection pressures can direct adaptation to new conditions. In situ conservation therefore allows for the genetic variation contained in a population or species to change over time and hence in European countries it is often referred to as dynamic gene conservation.

In situ conservation can be carried out by several methods:

1) Biosphere reserves

For nearly 200 years, most of the forested ecosystems have undergone scientific forestry management. The purpose of formation of biosphere reserves is to conserve in situ all forms of life along with its support system in its totality so that it could serve as a referral system for monitoring and evaluating changes in natural ecosystems. Biosphere reserves can thus indirectly serve as a method of in situ tree gene conservation.

2) Seed stands & seed production areas

The best natural stands or plantations that are nearly full stocked are used for the development of seed production areas. Seed stands refer to the conservation of species or provenances as parts of a viable existing ecosystem. Zoble and Talbert (1984) have recommended 125 trees per hectare in a seed stand, depending on tree size and selection intensity. The seed stands are selected for a particular area with regard to its ecological and environmental conditions to fulfill the seed demand of particular species. They are, therefore certainly working as an aid for in situ gene conservation.

3) Protected areas

In order to protect critical ecosystems and to preserve the genetic resources of un-quantifiable commercial and non-commercial values, national parks, wildlife sanctuaries and Tiger reserves have been created in India over the years. The prime objective for the protected areas is to preserve them as samples of the interdependent combinations of ecological gene pools and as gene bank capital.

4) Plus trees

Plus tree is a phenotypically superior tree. Plus tree selection is one of the methods to conserve diversity of trees at species level. In forestry, it is easy to see that trees of a particular species vary in growth, form and wood character. In some cases, much of the variation may be genetic and in others environmental.

5) Other field repositories of tree genetic resources

In addition to biosphere reserves and protected areas, a variety of field repositories of genetic resources have been developed within the framework of selection and tree improvement programmes to increase the productivity of forest (Katwal *et al*., 2003). These include other protected areas, private and publicly owned forests, plantations, trees outside forests (TOFs) managed in agro forestry systems or growing on homesteads, along rivers and roads, field trials and live collections etc.

Ex situ gene conservation

Ex situ gene conservation refers to the conservation of genetic material or biological diversity outside of the natural habitats of the species. Ex situ conservation has always been concerned primarily with ensuring the survival of genetic resources that would otherwise have been extinguished. In general, conservation takes place in facilities that allow either storage or the continuity of situations that are suitable for maintaining the viability and genetic composition of genetic material or diversity. Ex situ gene conservation includes the storage of seed, pollen, scions or tissue cultures and the establishment of trees in arboreta, seed orchards, plantations etc. Many recently developed technologies have possible applications in ex situ conservation. This type of conservation may play a special role where native species are endangered by industrial pollution or climatic change. Evacuation to safer areas with suitable climates may become a viable solution.

Ex situ conservation can be carried out by several methods

1) Clonal repositories or archives:

Clones that have been vegetatively propagated can be planted and preserved in clonal archives. Whereas if grafting were effective, the grafted or rooted trees can usually grow to a considerable age. Of course, the trees would need to be re-grafted on fresh rootstock at some time in the future. It's necessary to keep the archives in good condition in the early years to avoid rootstock shoots or sprouts taking over the grafted scion material. Weeding and tending must have been done on a regular basis, and proper labelling and mapping are required. For the conservation of FGRs, most organizations are focused with perennial or vegetatively propagated domesticated plants maintain clonal field repositories. ICFRE has established field repositories in the form of Vegetative Multiplication Gardens (VMGs) and clonal banks at its regional institutes in Dehradun, Jabalpur, Coimbatore, Jorhat, Bangalore, Ranchi and Shimla. For their usage, such repositories have also been developed under the control of state forest departments. Similarly, at various regional stations in Shimla, Bhowali, Jodhpur, Thrissur, Akola, Amravati, and Hyderabad, the National Bureau of Plant Genetic Resources (NBPGR) has established field repositories of select perennial and tree species.

2. **Provenance trails:**

Ex situ conservation is another term for provenance trials. These traits are important in gene conservation since these provenances are collected from variety of geographical, ecological, and environmental conditions (Emmanual *et al*., 1990). More than 90 species provenance trials have been set up throughout India to find the best provenances for producing new plantations with higher productivity. The first provenance trials for two important native species, *Tectona grandis* (teak) and *Pinus roxburghii* (chir pine), were initiated by M.L. Laurie and Harry Champion at Forest Research Institute in Dehradun. Teak provenance trials were established at a range of areas across India between 1928 and 1930. In collaboration with the Danida Forest Seed Centre, international provenance trials of *Tectona grandis* and *Gmelina arborea* have been established in several states. ICFRE has conducted provenance trials on *Tectona grandis, Pinus roxburghii*, and *Bombax ceiba* at the national level. International provenance testing of eucalypts, particularly *Eucalyptus tereticornis, E. camaldulensis*, and *E. grandis*, has also been done by ICFRE. Acacias and tropical pines such as *Pinus oocarpa*, *Pinus caribaea*, and *Pinus kesiya* have also been studied.

3. **Seed orchards:**

A seed orchard is an area where seeds are mass-produced in order to achieve the greatest genetic gain in the shortest period and for the least amount of money. Seed orchards play an important role in the development of high-quality planting stock for the species in the area. As a result, these are plantations that have been developed mainly for the production of high-quality seed. Clonal seed orchards and seedling seed orchards are the two types of seed orchards. These orchards fall under the category of selective conservation, which is one of the objectives of ex situ conservation. The establishment of seed orchards is seen as a part of a long-term conservation management program and also a long-term breeding technique.

4. **Botanical gardens & Arboreta:**

More than 100 botanical gardens exist in India, each with its own management system and geographical area. In general, an arboretum is a place dedicated to the preservation of tree species. An arboretum of 130 forest tree species and a bambusetum with 53 species has been established at the Forest Research Institute in Dehradun. Botanical gardens and arboreta provide an useful method for preserving tree genetic resources for distinctive phenotypes and genotypes. The establishment of arboreta for tropical species whose seed storage behaviour is largely unknown or poorly understood might be the first step toward their conservation.

5. **Gene banks:**

The use of conservation stands in the field is enhanced by ex situ conservation of forest genetic resources in gene banks. A gene bank is a collection and storage center for seeds and other plant reproductive material that signify a species' possible gene pool. Gene bank technology for genetic conservation has been developed and documented since early 1970s to a large extent under the auspices of IBPGR and FAO. Gene banks are of six types viz., seed bank, pollen bank, tissue culture bank, cryogene bank, field gene bank and DNA bank.

a) Seed storage through seed banks

Storage of orthodox seeds is the most widely practised method of ex situ conservation of plant genetic resources. About 90% of the 6.1 million accessions stored in genebanks are in fact maintained as seed. The techniques involve drying seeds to low moisture content (3–7% fresh weight basis, depending on the species) and storing them in sealed containers at low temperatures. Seeds of many orthodox species may be conserved in this way for several decades and possibly centuries. An attempt has been made to develop a 'low-input' alternative to the conventional cold storage of seed. The technique is called ultra-dry storage and allows preservation at room temperature. It is considered a useful low-cost option when no adequate refrigeration can be provided. Conventional seed storage is believed to be a safe, effective and inexpensive method of conservation for seed-propagated species. Large numbers of trees and woody species can be conserved long term in seed banks. A major international example of this conservation strategy for trees and woody species is the Kew Millennium Seed Bank Partnership. The Millennium Seed Bank Partnership, based in the United Kingdom is the largest ex situ conservation project based on seed storage in the world. The project has banked 10 percent of the world's wild plant species, including many woody species and aims to conserve 25 percent of wild plant species by 2020.

b) In vitro conservation through Tissue culture banks

Invitro conservation involves the maintenance of explants in a sterile, pathogen-free environment and is widely used for the conservation of species which produce recalcitrant seeds or no seeds at all or for material which is propagated vegetatively to maintain particular genotypes (Engelmann, 1997). In vitro techniques can be effectively used for collection, multiplication and storage particularly with problematic species. Plant material with high multiplication rates can be propagated in an aseptic environment through tissue culture systems such as micro propagation and somatic embryogenesis. It is also used in cases where the target species would not have seeds to be collected or when bud wood would quickly lose viability or is highly contaminated. The establishment of aseptic cultures in the field will facilitate collecting and improve its efficiency (Engelmann and Engels, 2002).

c) Pollen storage through pollen banks

This technique is comparable to seed storage, since most pollen can be dried to less than 5% moisture content on a dry weight basis and stored below 0ºC. Some species however produce pollen with recalcitrant-type storage characteristics. There is limited experience on the survival and fertilizing capacity of cryopreserved pollen more than 5 years old (Towill, 1985). However, pollen has a relatively short life compared to seeds (although this varies significantly among species), and viability testing may be time-consuming. For these reasons, despite being a useful technique for species which produce recalcitrant seed, pollen storage has been used only to a limited extent in germplasm conservation (Hoekstra, 1995). Other disadvantages of pollen storage technique are the small amount of pollen produced by many species, the lack of transmission of organelle genomes via pollen, the loss of sex linked genes in dioecious species and the lack of plant regeneration capacity, although there are indications that pollen can be regrown into whole plants (Hoekstra, 1995). On the other hand, pollen transfer of pests and diseases is rare with the exception of some viral diseases, thus allowing the safe movement and exchange of germplasm.

d) Cryopreservation through cryogene bank

Cryopreservation is the storage of biological material at ultra-low temperatures, usually that of liquid nitrogen (–196ºC). At this temperature, all cellular divisions and metabolic processes are stopped and therefore, the material can be stored without alteration or modification for a longer period of time. Coupled with in vitro culture, this technique often represents the only safe and cost-effective option for storage of non-orthodox species. Seeds of mahogany (*Swietenia macrophylla*) or neem (*Azadirachta indica*) are relatively small and tolerant to desiccation and can thus be cryopreserved directly after partial desiccation (Berjak and Dumet, 1996). In cases when seeds are not amenable to cryopreservation, excised embryos or embryonic axes are used. In this case, selecting embryos at the right developmental stage is critical for the success of cryopreservation. The disadvantage of cryopreservation is the overall difficulty of regeneration of whole plants.

e) Field gene banks

Field gene banks are often referred to as living collections. The term living collections as opposed to other types of gene bank collections is somewhat misleading, as the reproductive material in gene banks is also alive. The so-called living collections consist of plants, typically growing in the nursery or in the field. Ex situfield gene banks maintain sources of variation of functional traits for direct use in production through propagation and breeding programmes. Field gene banks are costly to maintain and their existence can only be justified when gene bank material can be accessed by users and meets their needs (Dawson *et al*., 2013).The two examples of ex situ conservation in planted field gene banks both from South Australia, include ex situ plantings and grafted trees of *Eucalyptus globulus* established by the Southern Tree Breeding Association at the National Genetic Resources Centre at Mount Gambier and the Currency Creek Arboretum, a largely self-funded arboretum specifically for eucalyptus which has the largest global ex situ collection of living Eucalyptus species (over 900 species, subspecies and varieties) and over 8,000 individual plants of Eucalyptus established on a single site.

f) DNA storage through DNA bank

The DNA storage method is rapidly increasing in terms of importance. DNA from the nucleus, mitochondrion and chloroplasts is now routinely extracted and immobilized into nitrocellulose sheets where it can be probed with numerous cloned genes. With the development of the polymerase chain reaction (PCR), it is now routinely possible to amplify specific oligonucleotides or genes from the entire mixture of genomic DNA. These advances have led to the formation of an international network of DNA repositories for the storage of genomic DNA (Adams, 1997). The advantage of this technique is that it is efficient and takes up little space. The main disadvantages, besides demanding requirements for capacity and equipment lie in problems with subsequent gene isolation, cloning and transfer. The obvious problem is that it does not allow the regeneration of entire plants (Maxted *et al*., 1997).

Some latest emerging concepts in conservation of genetic resources of trees

i. Genecological zonation using GIS:

When there is a lack of genetic information on population structure, assessing the ecogeographic variance in the distribution becomes a focus. Genecological zonation is a term that is commonly used to describe the method. A genecological zone is an area with plenty of similar ecological characteristics for phenotypic or genetic factors to be inherited within a species. This form of zonation also implies that significant gene flow is limited, which might contradict local adaptations to local environmental and ecological selection forces. Natural vegetation, topography, temperature, and soil, as well as challenges to pollen and seed dispersion, are often considered for zonation. The stratification method was one of many ways that ecological zonation may be developed to assist cover genetic differences that may exist without having a clear understanding of genetic variability in the species under conservation. A more comprehensive technique is now being developed to account for the changing and dynamic structure of in situ reserves, as well as new genetic information and climate change. The adoption of more modern computer and mapping technologies (GIS) will provide a more dynamic management system that will be able to handle changes in conservation goals. These objectives may vary as more knowledge regarding patterns of genetic variability in particular species, as climatic, social, and economic needs (such as breeding collection and management or ex situ management) change. As a result, ecological stratification principles mixed with modern GIS capabilities can be a beneficial tool for planning gene conservation or breeding units in a range of species.

ii. Use of molecular markers for conservation of FGRs

A molecular marker is a DNA fragment that is associated with a particular region in the genome. In molecular biology and biotechnology, molecular markers are used to identify the exact DNA sequence in a pool of unknown DNA. Scientists have been able to better understand the impact of silvicultural techniques on the long-term evolution of forest tree genetic variation, rapid evolution of such technologies (e.g. molecular markers) for analysing the genetic variability of forest trees (Carnus *et al*., 2006).Allozyme markers are valuable in the preservation of forest tree genetic diversity, for example, in developing conservation surveys, measuring genetic variability within and within populations, and monitoring changes(Millar and Westfall, 1992).Thus, molecular markers will help in tree conservation and permit national programmes to adopt biodiversity patterns into their own management techniques. Molecular approaches can also help in the identification of variations between local and non-local provenances and genotypes, as well as recognizing where diversity is being lost and enabling the introduction of new variability for integrated conservation and breeding programmes. It might also help for intra-specific genetic variation management (Karp, 2000).

Through identifying the origin of accessions and monitoring genetic changes in collections, molecular genetic methods, especially using genetic markers, may help in some of the management tasks for ex-situ populations. The identification of potentially helpful genes in gene bank accessions can be enhanced by molecular techniques like all those being used in genomic research. In a short, molecular markers can assist to genetic conservation in two ways. For beginning, they can also be used to study the genetic background of ex situ plantations and in situ sites for any forest tree species that are developed using conventional silvicultural techniques. Second, they can be used to examine the situation of genetic resources of species for which conservation plots have yet to be developed but are anticipated to be included in conservation strategies.

CONCLUSION

We need to ensure the accessibility and availability of tree species knowledge and information. To preserve genetic variety, enhance FGR in situ and ex situ conservation. It is useful to evaluate whether existing in situ reserves adequately protect tree species and are sufficient for future conservation needs, as well as to strengthen policies and institutional capacity to address long-term planning for long-term sustainable use, management, and conservation of FGRs.

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