Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Review Article **DOI: 10.26479/2018.0405.25**

STRATEGIES FOR SUSTAINING PLANT GERMPLASM EVALUATION AND CONSERVATION - A REVIEW

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ABSTRACT: In the last decade there has been an alarming increase in the number of disappearing species from the spontaneous flora due to the pressures put upon the environment (land clearing, drainage, pollution of the soil etc.) affecting their vitality, determining their number to fall under their biological possibilities of regeneration. The disappearance of species is from 100 to 1000 more alert since the invention of the anthropic factor in the environment, one in eight species in threatened by extinction. It is estimated that in the last 50 years, more than 300.000 species have become extinct. The partial destruction and the degradation of the natural habitat, the destabilization of the ecosystems due to the climatic modification, pollution, the increase of the number of invasive species and the implication of the human factor can be several of the causes of the biodiversities' decline. The danger of extinction of more and more species of flora in our country is acknowledged, which has determined the experts to manifest an interest in the preservation of rare and endangered elements. As a beneficial result, the forms of conservation, of whatever nature they may be, begin to be viewed and analyzed very closely. Because of this, the necessity of finding solutions for the disappearance of these species has determined the intensification of conservation actions of the plant germoplasm, the actualization of the lists and of the red book of plants for bringing to the attention of the experts in this field - researchers and those who are actively involved in the protection of the environment.

KEYWORDS: Anthropic factors, invasive species, germoplasm, conservation.

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1.INTRODUCTION

The sum total of all the genes present in a crop and its related species constitutes its germplasm. It is ordinarily represented by a collection of various strains and species. Germplasm provides the raw .materials (= genes). The breeder uses these to develop commercial crop varieties. Conservation of genetic diversity in the face of rapidly depleting natural resources has considerable significance and worldwide importance for food security and agro-biodiversity. Indiscriminate clearing of forests and agricultural land has led to the drastic loss of plant genetic resources. The partial destruction and the degradation of the natural habitat, the destabilization of the ecosystems due to the climatic modification, pollution, the increase of the number of invasive species and the implication of the human factor can be several of the causes of the biodiversities' decline. In the last decade there has been an alarming increase in the number of disappearing species from the spontaneous flora due to the pressures put upon the environment affecting their vitality, determining their number to fall under their biological possibilities of regeneration. In the present there are 60.000 vascular species on the verge of extinction on Earth. Many plant species are now in danger of becoming extinct [1]. More than fifteen million hectares of tropical forests are vanished each year [2]. Their preservation is essential for plant breeding programs. Biodiversity provides a source of compounds to the medical, food and crop protection industries [1]. Genetically uniform modern varieties are being replaced with highly diverse local cultivars and landraces of traditional agro-ecosystems. Deforestation, urbanization, pollution, habitat destruction, fragmentation and degradation, spread of invasive alien species, climate change, changing life styles, globalization, market economies, over-grazing and changes in land-use pattern are contributing indirectly to the loss of diversity [2,3]. The genetic erosion of natural populations is a growing phenomenon, due to the pressure of different factors on natural ecosystems. Conservation actions, [4] represent a priority and measures must be taken for the conservation of the genetic variability of natural populations. The incidence is more conspicuous in the tropical and sub-tropical regions where the richest and most important genetic resources on the earth exist. Therefore, immediate efforts are required to safeguard these germplasms, to ensure their continued availability for present and future use. This is also India's national obligation following ratification of legal binding 'Convention on Biological Diversity'. Moreover, the prevalence of genetic diversity provides great opportunity for crop improvement today and in distant future, when confronting situations would demand reconstruction of new cultivars and hybrids for sustaining higher production. Medicinal plants growing at high altitudes have slow growth and poor seedling establishment due to harsh environmental conditions. Conventional methods of propagation are not sufficient and especially for endangered species, attempts for conservation using both in situ and ex situ methods are immediately required. It is therefore, imperative to recognize the problem and to develop strategies for the conservation and rational exploitation of Himalayan herbs [5]. Advances in biotechnology, especially in the area of in vitro culture techniques and

molecular biology provide some important tools for improved conservation and management of plant genetic resources [6,7].

Methods of Germplasm Conservation

Conservation of plant genetic resources can be carried out either in the natural habitats (in situ) or outside (ex situ). The in- situ is based on the 1992 Convention on Biological Diversity, its role being to protect and monitor natural populations, proposing to remove or maintain a certain level of causes that lead to the destruction of the species. Ex situ conservation is generally used to safeguard populations, in danger of destruction, replacement or deterioration. An approach to ex situ conservation includes methods like seed storage in seed banks, field gene banks, botanical gardens, DNA and pollen storage [2]. Among these, seed storage is the most convenient method of long-term conservation for plant genetic resources. This involves desiccation of seeds to low moisture contents and storage at low temperatures. Seeds of some species, especially a large number of important tropical and sub-tropical tree species, are recalcitrant or intermediate, i.e. they cannot stand desiccation below a relatively high critical water content value (10-12% or 20% of fresh weight) [8,9] and cold storage without losing viability [10]. In situ conservation has practical limitations associated with shrinking of natural habitats, urbanization, industrialization and changing government policies. Ex situ conservation of crop germplasm bearing orthodox seeds is conventionally carried out in seed gene banks by way of reducing moisture content and storing at sub-zero temperature (usually at -20°C). However, many economically important plant species produce recalcitrant seeds (desiccation and freezing sensitive) or these are predominantly vegetative propagated. Conservation of germplasm of these groups thus posses' serious problems. Due to high moisture content in plant prop gules, conservation of these problem species, under the conditions of seed gene banks is not possible. However, maintenance of germplasm in field gene banks/clone repositories is labor intensive, space oriented and prone to loss of germplasm due to pest/pathogen attacks and natural calamities. Biotechnological approaches, including in vitro conservation, have been proposed recently, as an adjunct to field gene bank for these problem species because it can help in conservation and exchange of disease free germplasm. In vitro conservation strategies can be divided into two categories like in vitro conservation under slow growth (IVAG- in vitro active gene bank) and cryopreservation (IVBG- in vitro base gene bank). In vitro slow growth has been used at various national and international research centers (CIP-International Potato Center, IITA-International Institute of Tropical Agriculture, NBPGR- National Bureau of Plant Genetic Resources) for conservation of vegetative propagated germplasm. This method can satisfy only short to medium term conservation strategy but management of large collections through this method is problematic. Moreover, collections maintained under in vitro slow growth are prone to losses due to contamination and genetic instability. In vitro collecting poses contamination challenges beyond those of normal tissue culture. Work is done in the field, often in the open air thereby increasing the

chances of contamination.

Factors Affecting Contamination of In vitro Collected Cultures

1. Age: Older plants tissue taken later in the growing season are often more infected than younger plants tissue [11]

2. Position: Underground tissues, such as roots, rhizomes and corms, generally have high levels of endogenous contaminants and can be extremely difficult to clean [12].

3. Complex tissue: Vegetative and floral buds often habour contaminats in complex tissue which can protect even external microorganism from surface sterillant [13].

4. Environment: Contamination may also be affected by environment. Explants taken from plants in a moist tropical site has higher rate of contamination than those from a temperate site. On the other hand desert species appear to have less surface contamination by bacteria and fungi and are more easily disinfected than tissue from moister areas [14]

Since the last decade cryopreservation has been used successfully to various crops, more recently, for conservation of plant germplasm [15]. Potentially valuable techniques are now available on cryopreservation of cultured plant tissues for a few species [16]. However, majority of crops that were worked out for cryopreservation belong to the temperate region, which has inherent capacity to tolerate low temperature. Cryopreservation methods are relatively less investigated with tropical species [17], though rich diversity of crop germplasm is predominant in this region. Yams (*Dioscorea* spp.) belong to the same group of tropical plant species and are one of the most important tubers crops used for both food and / medicine (as they are commercially used for extraction of Diosgenin which is the precursor of steroids). Hence development of protocol for cryopreservation is important [18]. In vitro culture is a feasible alternative for genetic conservation of plants where the seed banking is not possible [19]. In vitro culture not only provides a method for clone propagation and safe exchange of plant material but also used for medium-term germplasm conservation [2, 20]. Slow growth methods allow plant material to be held for a few years under tissue culture conditions with periodic sub culturing. In the other word, in vitro culture includes some techniques involving the growth under sterile conditions and constant environmental factors of plant germplasm on artificial culture media. Explants are mostly shoot, leaf, flower pieces, immature embryos, hypocotyls fragments or cotyledons [21]. Problems with the in vitro techniques include high costs for maintaining a large number of stocks, space requirements, and risks of contamination and soma clone variation over time. Undifferentiated callus cultures are more susceptible to somaclonal variation than organized tissue systems, such as shoot cultures. Only organized cultures are recommended for slow-growth storage. This technique of long-term root, tuber, or shoot tissue culture storage is well developed for some crops such as banana [22]. Cryopreservation is an alternative choice for a long-term conservation of germplasm. Cryopreservation at -196ºC in liquid nitrogen (LN) has been considered to be an ideal tool which

offers long-term storage capability, maximal stability of phenotypic and genotypic behavior of stored germplasm, and minimal storage space and maintenance requirements [23]. Pretreatments were crucial for the survival and regeneration of plant tissues after cryopreservation. Since at present, cryoprotectants alone cannot provide enough protection to untreated cells or tissues for high rates of survival, pretreatment techniques are needed to condition the cells to withstand the stresses imposed by freezing at ultralow temperatures [24]. Micro propagation and cryopreservation are tools with multiple applications and benefits within an integrated plant conservation research program. Biotechnological tools like in vitro culture, cryopreservation, and molecular markers offer a valuable alternative to plant diversity studies, management of genetic resources and ultimately conservation [25]. Cryopreservation is the storage of viable biological material at ultra-low temperatures, which provides a means for the long-term stable storage of plant germplasm. Cryopreservation is a safe and cost-effective technique for preservation of germplasm and management of in vitro produced material for biotechnological application [25]. To ensure reproducible results and continuity in research and biomedical process, scientist faced the task of genetically stabilized living cells. Serial sub culturing is time consuming and lead to contamination or genetic drift. The genetic stability of cryopreserved plants can be assessed by analyzing them at phenotypic and molecular level analysis with a range of techniques. DNA-based markers have been routinely used for monitoring genetic stability of these species on cryopreservation [25]. Safe and long term preservation of the production source is essential for any commercial applications as it secures the investments for the producer of raw material; it secures the investment for product development for the dealer of a specific brand and it is an essential suggestive requirement of regulatory aspects for approval and patent protection. Cryopreservation is well established for vegetative propagated species. However, it is much less advanced for recalcitrant seed species in order to some of their characteristics, including their very high sensitivity to desiccation, structural complexity and heterogeneity. However, two new approaches to cryopreservation may lead to more widespread applications for genetic conservation. They focus on reducing cell damage from ice crystal formation. One approach is through vitrification of cellular water by a cryoprotectant mixture and the other involves encapsulation of specimens within an alginate gel that is then dehydrated. For vitrification the specimen is infused with a cryoprotectant mixture that promotes the conversion of much of the cellular water into a non crystalline, vitreous solid when rapidly cooled [26]. For encapsulation the specimen, such as a shoot tip or somatic embryo, is encased in an alginate gel to form an artificial seed. This artificial seed is then dehydrated in her air before cooling [27]. The enveloping gel appears to minimize deleterious effect from dehydration and also protects the specimen from physical damage, being larger and more robust than an isolated shoot tip or embryo.

2.CONCLUSION

Germplasm collecting involves application of both the theoretical knowledge on population sampling and practical knowhow in overall understanding of plant diversity and environment including the socio-economic and cultural aspects of the farming societies. Tactics, logistics, preparations and procedures have been elaborately dealt with by Bennett [28, 29, 30, 31, 32, 33].

Biotechnology has created significant contributions to improved conservation and use of plant genetic resources. The rapid progress made in in vitro culture technology, cryopreservation and molecular markers has helped in improving the plant germplasm conservation and offer a valuable alternative to plant diversity studies, and management of genetic resources. [34,35] Cryopreservation has proven to be an efficient long-term conservation method for genetic resources. Nowadays, vitrification method is a standardized method, although more studies should be performed. Adjustments of the methods to the gene bank would be necessary to exploit all the advantages of cryopreservation. The two most important factors that need to be optimized are the preparation phase of tissues towards dehydration (especially by sugar and cold treatments) and the length of explants treatment with the vitrification solution. Researches should move toward standardizing and simplifying the methods.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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