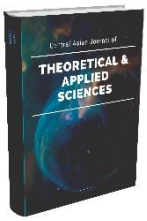

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Role of Biotechnology in the Conservation of Plant Biodiversity

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Abstract: *Biological diversity provides the variety of life on the Earth and can be defined as the variability among and between the living organisms and species of surrounding ecosystems and ecological complexes of their life support. It has been estimated that one third of the global plant species are threatened in different level according to the International Union of Conservation of Nature (IUCN).The major threat to rapid loss and extinction of genetic diversity due to habitat destruction, pollution, climate change, invasion of exotic species, human population pressure, ever increasing agricultural pressure and practices, life style change etc. are well-known. Biodiversity conservation is a global concern. All member states of the Convention on Biological Diversity (CBD) took measure to preserve both native and agricultural biodiversity. The global concern of biodiversity conservation initiated either by in situ or ex situ methods. In situ methods protect both plants and their natural habitat. On the other hand, ex situ methods involves preservation and maintenance of plant species or plant parts (such as seeds, cuttings, rhizomes, tubers etc.) outside their natural habitat for the purpose of developing seed banks or more preciously gene banks following classical / advanced methods of plant propagation. Classical methods of plant propagations have certain limitations in terms of rapid production of plants or plant propagules and their long term conservation. So, the biotechnological methods such as plant tissue culture, plant cell culture, anther culture, embryo culture etc. are quite applicable and useful techniques for ex situ conservation. On the other hand, the production of superior quality seeds has enhanced by the application of plant biotechnology. So, plant biotechnology offers new means of improving biodiversity conservation rather than threatening biodiversity in various ways.*

Keywords: *biotechnology, biodiversity, conservation, plant, nature, convention, seeds, earth, global, pollution.*

Introduction

Biodiversity conservation is the protection, restoration and sustainable management of wildlife and natural resources such as forests, water and the biological diversity within it. Biotechnology is a set of techniques by which human beings modify living things or use them as tools. In its modern form, biotechnology uses molecular biology techniques to understand and manipulate the basic building blocks of living things. [1,2] Biotechnology is the art of utilizing living organisms and their products for the production of food, drink, medicine or for other benefits to the human being, or other animal species. Biotechnology plays an immense role in biodiversity conservation such as vegetative multiplication of many species, allows the production of large numbers of plants from small pieces of the stock plant in

relatively short period of time and in some cases for recovery of virus-free plants. Biotechnology also has potential application in production of somatic hybrids, organelle and cytoplasm transfer, genetic transformation and germplasm storage through freeze-preservation (Cryopreservation). Apart from its uses there are also some concerns or worries with modern biotechnologies such as the Terminator technology and Genetically Modified Organisms (GMO) which are developed through genetic engineering, which may cause "Genetic pollution" and "Genetic contamination" and they may have social consequences which needs economic, ethical and environmental considerations. [3,4] Even though, biotechnology has so many advantages and different technologies which can complement conservation of biodiversity; as that of other new technologies the introduction and spread of new biotechnologies generally have social consequences with winners and losers. For biotechnology, this has led to intense public debate across many different aspects such as ethical, economic, legal and environmental issues. Modern biotechnologies such as terminator technology and GMO can have a negative effect on biodiversity. Genetic pollution" and "genetic contamination" are among the environmental concerns which need to be cleared or ascertained before releasing new technologies which can pollute the biodiversity and the natural/ wild genetic pool and may cause irreversible damage.[5,6] Since many countries have banned the use of technologies such as GMO and terminator technology; for the country like Ethiopia, it is important to learn from others, rather than endangering or risking the genetic resources from genetic pollution and genetic contamination which may occur. On the other hand, the use of safe and known technologies should be encouraged and used to augment the biodiversity conservation efforts and other development efforts of the country.[7,8]

At present, more than 50 000 plant species are used in phytotherapy and medicine. About 2/3 of them are harvested from nature leading to local extinction of many species or degradation of their habitats. Biotechnological methods offer possibilities not only for faster cloning and conservation of the genotype of the plants but for modification of their gene information, regulation, and expression for production of valuable substances in higher amounts or with better properties. *Rhodiola rosea* is an endangered medicinal species with limited distribution. It has outstanding importance for pharmaceutical industry for prevention and cure of cancer, heart and nervous system diseases, and so forth. Despite the great interest in golden root and the wide investigations in the area of phytochemistry, plant biotechnology remained less endeavoured and exploited. The paper presents research on initiation of *in vitro* cultures in *Rhodiola rosea* and some other *Rhodiola species*. Achievements in induction of organogenic and callus cultures, regeneration, and micropropagation varied but were a good basis for alternative *in vitro* synthesis of the desired metabolites and for the development of efficient systems for micropropagation for conservation of the species.[9,10]

The continuous loss of biodiversity has posed a serious threat to the survival of mankind. Worldwide one third of the plant species are threatened due to several reasons. As the conservation of biodiversity is a global concern, several strategies have been adopted in understanding and conserving plant diversity throughout the world. Both ex situ and in situ methods of biodiversity conservation are equally important. Though it is generally believed that biotechnology has adverse effects on biodiversity, but in fact, biotechnology offers new means of improving biodiversity. It is now well recognized that an appropriate conservation strategy for a particular genotype requires combining approach of ex situ and in situ techniques according to the need of the program. As it is known fact that genetic variability is the main perquisite of the survival of any plant species in their natural habitat, so study of genetic diversity in conserved germplasm is important and application of different biotechnological process playing a promising role. In vitro plant propagation is a helpful technique in the conservation of genetic diversity of all types of plants (including rare, threatened and endangered plants) in a rapid and reliable way by maintaining the same clone or stock of plant material.[11,12]

Advances in plant biotechnology provide new options for collection, multiplication and short- to long-term conservation of plant biodiversity, using in vitro culture techniques. Significant progress has been made for conserving endangered, rare, crop ornamental, medicinal and forest species, especially for non-orthodox seed and vegetatively propagated plants of temperate and tropical origin. Cell and tissue culture techniques ensure the rapid multiplication and production of plant material under aseptic conditions. Medium-term conservation by means of in vitro slow growth storage allows extending subcultures from several months to several years, depending on the species. Cryopreservation (liquid nitrogen, −196 °C) is the only technique ensuring the safe and cost-effective long-term conservation of a wide range of plant species. Cryopreservation of shoot tips is also being applied to eradicate systemic plant pathogens, a process termed cryotherapy. Slow growth storage is routinely used in many laboratories for medium-conservation of numerous plant species. Today, the large-scale, routine application of cryopreservation is still restricted to a limited number of cases. However, the number of plant species for which cryopreservation techniques are established and validated on a large range of genetically diverse accessions is increasing steadily[13,14]

Biological diversity provides the variety of life on the earth and agricultural diversity forms a part of it. Agricultural biodiversity refers to the variability among and between all forms of life relevant to food and agriculture. The Indian subcontinent is recognized as one of the important centres of origin of crop plants. There are around 811 plant species under cultivation in India with more than 900 wild relatives which are distributed over different biogeographic regions of the country. However, rapid genetic erosion of the species and surrounding ecosystem due to various factors is a big threat to this diversity. Biodiversity conservation is a global concern and all the member states of the Convention on Biological Diversity are taking measures to preserve both native and agricultural biodiversity. Biotechnological tools are proving valuable for effective and efficient conservation efforts of agricultural plant genetic resources. Among the various applications available, tissue culture, molecular marker technology, establishment of DNA banks and genotypic database for various crops and accessions have been delineated [15]

Discussion

In vitro techniques are very useful for conserving plant biodiversity, including (a) genetic resources of recalcitrant seed and vegetatively propagated species, (b) rare and endangered plant species and (c) biotechnology products such as elite genotypes and genetically engineered material. Explants from recalcitrant seed and vegetatively propagated species can be efficiently collected under field conditions using *in vitro* techniques. *In vitro* culture techniques ensure the production and rapid multiplication of disease-free material. Medium-term conservation is achieved by reducing growth of plant material, thus increasing intervals between subcultures. For long-term conservation, cryopreservation (liquid nitrogen, −196°C) allows storing plant material without modification or alteration for extended periods, protected from contaminations and with limited maintenance. Slow growth storage protocols are routinely employed for a large number of species, including numerous endangered plants, from temperate and tropical origin. Cryopreservation is well advanced for vegetatively propagated species,[16,17] and techniques are ready for large-scale experimentation in an increasing number of cases. Research is much less advanced for recalcitrant species due to their seed characteristics, viz., very high sensitivity to desiccation, structural complexity and heterogeneity in terms of developmental stage and water content at maturity. However, various technical approaches should be explored to develop cryopreservation techniques for a larger number of recalcitrant seed species. A range of analytical techniques are available, which allow understanding physical and biological processes taking place in explants during cryopreservation. These techniques are extremely useful to assist in the development of cryopreservation protocols. In comparison with crop species, only limited research has been performed on cryopreservation of rare and endangered species. Even though routine use of cryopreservation is still limited, an increasing

number of examples where cryopreservation is used on a large scale can be found both in genebanks for crops and in botanical gardens for endangered species.[18]

Biological diversity provides the variety of life on the Earth and can be defined as the variability among and between the living organisms and species of surrounding ecosystems and ecological complexes of their life support. It has been estimated that one third of the global plant species are threatened in different level according to the International Union of Conservation of Nature (IUCN). The major threat to rapid loss and extinction of genetic diversity due to habitat destruction, pollution, climate change, invasion of exotic species, human population pressure, ever increasing agricultural pressure and practices, life style change etc. are well-known. Biodiversity conservation is a global concern. All member states of the Convention on Biological Diversity (CBD) took measure to preserve both native and agricultural biodiversity. The global concern of biodiversity conservation initiated either by *in situ* or *ex situ* methods. *In situ* methods protect both plants and their natural habitat. On the other hand, *ex situ* methods involves preservation and maintenance of plant species or plant parts (such as seeds, cuttings, rhizomes, tubers etc.) outside their natural habitat for the purpose of developing seed banks or more preciously gene banks following classical/advanced methods of plant propagation. Classical methods of plant propagations have certain limitations in terms of rapid production of plants or plant propagules and their long term conservation. So, the biotechnological methods such as plant tissue culture, plant cell culture, anther culture, embryo culture etc. are quite applicable and useful techniques for *ex situ* conservation. On the other hand, the production of superior quality seeds has enhanced by the application of plant biotechnology. So, plant biotechnology offers new means of improving biodiversity conservation rather than threatening biodiversity in various ways.[19,20]

Genetic variation is believed to be a prerequisite for the short-and long-term survival of the plant species in their natural habitat. It depends on many environmental factors which determine the number of alleles on various loci in the genome. Therefore, it is important to understand the genetic composition and structure of the rare and endangered plant species from their natural habitat to develop successful management strategies for their conservation. However, rare and endangered plant species have low genetic diversity due to which their survival rate is decreasing in the wilds. The evaluation of genetic diversity of such species is very important for their conservation and gene manipulation. However, plant species can be conserved by *in situ* and *in vitro* methods and each has advantages and disadvantages. DNA banking can be considered as a means of complimentary method for the conservation of plant species by preserving their genomic DNA at low temperatures. Such approach of preservation of biological information provides opportunity for researchers to search novel genes and its products.

Experts attending the ongoing United Nations Biodiversity Conference in Egypt say that adopting smart agronomic practices can play a significant role in conserving the environment.

Those practices include agricultural biotechnology, which can protect diversity by making crops more efficient, reducing pesticide use and helping to prevent species extinction, they said in exclusive sideline interviews with the Alliance for Science.

Dr. Margaret Karembu, director of ISAAA AfriCenter Kenya, identified three ways that biotechnology can help conserve biodiversity: “By increasing productivity per unit of land. This means reducing the amount of land you open up for crops and so you’re able to sustain biodiversity in these regions. Secondly, when you use very selective techniques that only kill the harmful pests for your crops it means this reduces the amount of harmful chemicals you pump into the environment —especially those that are broad spectrum, killing many of the other non-target pests that help in pollination. Thirdly, when you select tools that help you grow crops in the areas that will give you the highest yield. For example, in drought then you don’t open up semi-arid land, and that also conserves very unique biodiversity.”[21,22]

Biological diversity is the foundation for livelihoods and sustainable development, supplying basic needs such as food, energy, medicine, recreational and cultural benefits. However, population growth, industrialization and urbanization have accelerated the decline and extinction of species as well as the degradation of ecosystems. The losses are due to a range of pressures driven by socio-economic factors, including climate change, ecosystem degradation, illegal trade and unsustainable use.

To halt biodiversity loss, the international community developed the Convention on Biological Diversity (CBD) prior to the Rio Earth Summit in 1992. The CBD has become one of the world's most important multilateral environmental agreements and a key tool for sustainable development. More than 196 member-states and over 6,000 participants have been meeting for more than 25 years to deliberate and come up with policies to protect biodiversity.

Dr. Manuela Campa, a biotechnologist in the genetics department at Stellenbosch University in South Africa, opined that agricultural biotechnology portends no danger to the environment and instead helps to improve crops and conserve the environment.

He noted that biotechnology can help prevent crop deterioration during storage, thus reducing food waste. "So that means that you can use less land because you don't have a loss. This is even true for crops that can be implemented for better yield or resistance to pathogens or abiotic stresses. This means less loss during agriculture. So you can use less land and that piece of land can be more efficient and you can leave the other land for the natural environment."

Both Karembu and Campa pointed out that biotechnology depends on biodiversity, which provides the genes and traits that support human innovation.

"Obviously, there are a lot of different [plant] varieties and it is very important to preserve these because they are actually the source of information for improvement," Campa added. "Because if you go back there might be a cultivar that's very resistant to a particular pathogen or that is adapted to a particular soil and you can use that information for a cultivar that is good for production."

Prof. Mahammad Ishiyaku, a plant breeder at the Institute of Agricultural Research (IAR) at Ahmadu Bello University (ABU) in Nigeria, said that biotechnology can help protect some species from going extinct. Harmful chemicals are often used to clear lands for agriculture, unintentionally killing other plants and animals. Biotechnology could help ensure that only targeted species are controlled.

"So in short, there is very close relationship between biodiversity and biotechnology," he said. "The essence of having a convention on biodiversity and then a whole protocol popularly known as the Cartagena Protocol on Biosafety was developed because of this very germane reason that the world community appreciated the potential positive impact of biotechnology in the conservation of biodiversity. But to do that, any perceived risk should be mitigated using scientifically designed means. In other words, if the relationship does not exist the protocol would have not been developed."[23]

The plant breeder urged parties to the convention in Sharm El-Sheikh to look at science in a natural way, contending that scientific breakthroughs should not be impeded out of fear of non-existent risks.

"Science is based on things you can see and measure," he said. "It is not based on emotions or some imagined non-existent things. The laws that should be agreed upon by the world community should be such that would facilitate the evaluation and monitoring of products of this science from time to time on its own merits. Then products of any of the technologies should be made available to those societies that require them to solve their developmental problems.

"I am completely opposed to some of those people who think that we shouldn't try anything. If we had lived like that there wouldn't have been aeroplanes for us to fly around, there wouldn't have been tractors

where 200, 300 hectares of land can be cultivated in a day and so on. So, science should be exploited in a responsible and safe manner to develop technologies that would save our individual countries," he emphasized.

Results

Biodiversity conservation is of global concern which requires a holistic approach. Recognizing the need of conservation of biodiversity, the United Nations prepared Convention on Biodiversity (CBD) and adopted it in 1992. This was the first time that a large majority of States agreed to a legally binding instrument for biodiversity conservation and the sustainable use of biological resources. The CBD is a comprehensive approach to biodiversity conservation of both wild and domesticated species. It aims at conservation at the genetic, species and ecosystem levels. There are various methods to conserve biodiversity. Both in situ and ex situ methods of biodiversity conservation are equally important. It is now recognized that ex situ techniques can be efficiently used to complement in situ methods, and they may represent the only option for conserving certain highly endangered and rare species (Ramsay et al., 2000). The choice of one or the other technique, or a combination of both, depends on the particular case. The maintenance and protection of natural habitats are the in situ methods of conservation. Preserving the habitat is the most important issue in the conservation of biodiversity. Destruction of habitat should be immediately checked and steps should be taken to restore the habitat of animals and plant species. Since chemical pesticides are responsible for a large number of animal deaths occurring every year, minimizing the use of chemical pesticides is another technique for the survival of biodiversity. Conservation of biodiversity through establishment of protected areas like National Park, Wild life sanctuary, Biosphere Reserves, Marine Reserves etc. are very effective in controlling the loss of biodiversity. Special care should be taken for the species which are threatened and at the verge of extinction. Efforts should be made to protect the indigenous genetic diversity. There is an urgent need to check the unsustainable exploitation of the biological diversity. This should be improved through appropriate legal and institutional system. Preserving diversity through gene bank, seed bank and in vitro storage are effective methods for biodiversity conservation. In gene banks, the plant and animal materials are conserved and are available for breeding, reintroduction, research and other purposes. This method is useful for long living perennial trees and shrubs.

Seed banks are the most efficient and effective method for sexually reproducing seeds under long term storage. There are a number of seed banks in the world with specialization in the nature of the collection, geographical area, taxonomic groups, wild plants, forestry trees etc.There are a large number of species for which seed banking cannot be used as a method of conservation. Some plants such as banana do not produce seeds and are propagated vegetatively. Some species such as potato or sugarcane include both sterile genotypes and genotypes which produce orthodox seeds. However, these seeds are generally highly heterozygous and are thus of limited interest for the conservation of particular genotypes. Similarly, some fruit and forest tree species produce recalcitrant seeds, i.e. seeds that cannot be dried to sufficiently low moisture level to allow their storage at low temperature. For such plants other methods of conservation are used. Restriction on the introduction of exotic species is another approach in biodiversity conservation. There should be restriction on introduction of exotic species without proper investigation.

Plant cell methods and techniques were initially used in fundamental scientific investigations at the beginning of their development in the early 60-ties of the last century. Plant biotechnology is based on the totypotence of the plant cell .This process of *de novo* reconstruction of an organism from a cell in differentiated stage is highly linked to the process of dedifferentiation when the cell is returning back to its early embryogenic/meristematic stage. In this stage cells undergo division and may form nondifferentiated callus tissue or may redifferentiate to form new tissue, organs and an entire organism. Morphogenesis *in vitro* is realized via two major pathways: (i) organogenesis when a group of cells is

involved for *de novo* formation of organs and (ii) somatic embryogenesis when the new organism is initiated from a single cell.[24]

Micropropagation is a vegetative propagation of the plants in vitro conditions (in glass vessels under controlled conditions) leading to development of numerous plants from the excised tissue and reproducing the genetic potential of the initial donor plant.

Usually tissues containing meristematic cells are used for induction of axilary or adventitious shoots but induction of somatic embryos can be achieved from differentiated cells as well.

Micropropagation is used routinely for many species to obtain a large number of plants with high quality. It is widely applied to agricultural plants, vegetable and ornamental species, and in some less extent to plantation crops. One of the substantial advantages of micropropagation over traditional clonal propagation is the potential of combining rapid large-scale propagation of new genotypes, the use of small amounts of original germplasm (particularly at the early breeding and/or transformation stage, when only a few plants are available), and the generation of pathogen-free propagules. . Compared to the other spheres of in vitro technologies clonal propagation has proved the greatest economical and market importance in industry including pharmaceutical industry which needs for raw material from the medicinal plants is increasing constantly. It offers faster and alternative way for production of raw material and from another side overcoming the problems arising from the limited natural resources.

At present, there is a long list of research groups worldwide investigating hundreds of medicinal species. Various success procedures and recipes for many of these species have been developed. However, there is not a universal protocol applicable to each species, ecotype, and explant tissue. From another side all these continuous tedious studies on the standardization of explant sources, media composition and physical state, environmental conditions and acclimatization of in vitro plants have accumulated information, continuously enriched, which is a good basis for elaboration of successful protocols for more species. Wider practical application of micropropagation depends on reduction of costs so that it can become compatative with seed production or traditional vegetative propagation methods (e.g., cuttings, tubers and bulbs, grafting)

Conclusions

Species that are on the brink of extinction because of the rapid loss of genetic diversity and habitat come mainly from resource-poor areas of the world and from global biodiversity hotspots and island countries. These species are unique because they are endemic, and only a few small populations or sometimes only a few individuals remain in the wild. Therefore, the challenges to support conservation by in vitro measures are many and varied. The editors of this invited issue solicited papers from experts from Asia, Africa, Europe, Australia, and North and South America. This compilation of articles describes the efforts in these diverse regions toward saving plants from extinction, and details the direct application of in vitro and cryopreservation methods. In addition, these contributions provide guidance on propagation of rare plants, including techniques for large-scale propagation, storage, and reintroduction. The in vitro techniques for conserving plant biodiversity include shoot apical or axillary-meristem-based micropropagation, somatic embryogenesis, cell culture technologies and embryo rescue techniques, as well as a range of in vitro cold storage and cryopreservation protocols are utilized as various biotechnological approaches for conserving plant biodiversity.[25]

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