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Plant Biodiversity Conservation using Biotechnological Tools

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Abstract—*Biological diversity refers to the variation in living organisms amongst and within the species and ecosystem. Since time immemorial, plant genetic diversity has always been a boon for agricultural progress as the expanded gene pool offers better resistance against various biotic or abiotic stresses. Over the last century, three-quarters of entire genetic diversity in agricultural crops has been lost due to the burgeoning population, climate change, deforestation and soil erosion (FAO, 2020). Additionally, domestication, selection of high yielding traits, followed by creation of new uniform varieties have also contributed to this rapid loss leading to extinction or endangerment of species. With limited gene pool, the conventional breeding strategies are facing difficulty to improve varieties, necessitating the advent of technologies that would offer a promising future. Biotechnology covers a wide spectrum of applications and techniques which can alter living organisms making them better resilient to face the changing environment. Presently for economically important crops, conservation, plant evaluation and biodiversity utilization are few areas where biotechnology is utilized. Depending on the type of plant, different approaches of in-situ or ex-situ conservation or their combinations are used. Though former is still considered to be the most appropriate tool, but due to plant extinction, latter methods are forced to intervene like invention of DNA banks, genotypic databases, plant tissue culture technologies, direct gene transfer, molecular marker techniques. In spite of their benefits, the Genetically modified organisms (GMO) and terminator technology meets a lot of opposition at ethical, social, economic and environmental levels. In today's times, when biotechnology is still seen with a hint of doubt, this field of science has enhanced knowledge of plant science which when mixed with traditional approaches will benefit greatly. The present work gives an overview of current scenario of various biotechnological tools that are used for conservation and few risks involved therein.*

1. INTRODUCTION

Biodiversity refers to the variety of all forms of life present, be it different species of animals, plants and microorganisms or their interdependence upon each other for survival; inclusive of species' richness and abundance [1]. In fact, for any organism, it exists at three levels; genes, species, and ecosystems. Each of them has configuration, structure and purpose, of its own [2-3]. People depend upon ecosystem and the services which is provided by biodiversity [4].

Biodiversity is very essential for humankind. It provides abundant economic benefits as it is the raw material for all types of livelihoods, be it that of a farmer or a fisher. Moreover, it provides operative ecosystems supplying essentials like oxygen, fresh air and water, and many other ecosystem services. The tourism industry is totally dependent upon biodiversity, providing an essential source of recreation. Even the aesthetic value of home and surrounding locality is highly benefitted from variety of flora around. Finally, it represents a wealth of systematic ecological data. To understand the origin and evolution of life, in depth knowledge of biodiversity is the first step. But unfortunately, due to lack of complete discovery, tree of life remains incomplete as many missing links are yet to be connected. Even though there are huge economic, social and commercial benefits of biodiversity, still the behaviour of human beings has always led to huge loss of the same [5].

The Living Planet Report 2020, published by WWF, has reported that a catastrophic decline has been seen in global species; accounting to almost 68 percent in less than 50 years [6]. From 1970 to 2016, the total number of mammals, birds, fish, plants and insects have fallen more than two thirds of their earlier value. In spite of being extremely essential for human survival, anthropogenic involvement has resulted into a steep decline of biodiversity. Since industrial revolution, various human activities have led to loss of important ecosystems like forest, grasslands and wetlands threatening the wellbeing of humankind. The report states, that 75% of the ice-free land surface has already been changed by humans, more than 85% of wetlands have been lost and most of the oceans are already polluted causing the rapid decline in the variety of forms of life [7]. Director general of WWF International, stated that COVID 19 is a manifestation of human intervention in nature and in fact, it is being considered to be a result of the broken relationship between the two [6].

India is considered to be very rich in biodiversity owing to presence of four out of thirty-six hotspots of the world. It has almost 45,000 species of plants in only 2.4 percent of the world's land area [8]. In addition to the rich biodiversity, it is also considered to be the centre of origin for many cultivated plants of today's world. Also, it is thought to be the centre of biological diversity of many important plants as well, such as rice, millets, many pulses, vegetables and fruits. Due to this, conservation has always been of prime importance in India and it has been successfully achieved by creation of Wildlife Sanctuaries, Biosphere Reserves and National Parks [9].

In 2002, to meet the obligations of Convention on Biological Diversity (CBD), the Biological Diversity Act was passed by the Parliament of India for preservation of biological diversity in India; India being an active member. It was followed by the establishment of National Biodiversity Authority in 2003 to implement the Biodiversity Act of 2002 [10]. This body is helped by statutory bodies at state level like the State Biodiversity Board (SBB). These bodies are responsible for regulatory functions related to any Indian commercial utilization of biological resources. But still we are spiralling fast towards big loss of biodiversity as can be seen from the trends of last few years. The living planet report, 2020 suggests bending the curve initiative as the only possibility of saving the earth. According to this initiative, the world needs transformational changes and more ambitious conservation efforts, if earth needs to be stabilized by the year 2030 [6]. Protection, restoration and promotion of sustainable management of varied ecosystems, reversing the land degradation and halting biodiversity loss is goal number 15 of the 2030 agenda for sustainable development as demarcated by the United Nations, sustainable management of world's resources and conservation of biodiversity also being major targets.

2. PLANT BIODIVERSITY AND IT'S LOSS

In agriculture, since time immemorial, biodiversity is considered as a foundation providing the stock of gene pool for all the domestication, be it of crops or animals [11]. But often with time and demand, domestication and selection of plants has led to creation of varieties which are only beneficial to mankind as far as the yield, taste and looks are concerned. At the same time, it may have also led to loss of some useful attribute or inherent traits of plants, for example, resistance to biotic and abiotic stress, like harsh climatic conditions, pests, soil conditions etc.

Currently, the speed at which we are witnessing the extinction or endangerment of species, is quite alarming. Increasing population, urbanisation, habitat destruction etc., has hit the plant biodiversity very badly leading to a loss of thousands of species globally. According to FAO, since the start of agriculture, we have lost almost 70% of our genetic diversity of plants due to cultivation and commercialisation [12]. Modern agriculture expects us to produce more of uniform, commercial varieties replacing our old ones which is leading to loss of plants in their place of origin, gene pool erosion and thereby, change in ecosystem [13].

Agriculture is very crucial for both developing and developed nation as meeting demands of the global food security is always the most important challenge for any nation. It is also essential that we create enough nutritious food for our increasing population. Moreover, in addition to dietary requirements, plants have always been used for various other reasons by human beings. So, it would be futile to stop the attempt of man to create better and improved varieties of plants in order to become self-dependent. In that scenario, there is a need of supplementing other methods by which we can conserve the biodiversity of plants without affecting the needs of humankind. We will need help offered by modern technologies like biotechnology along with computational knowledge for conserving the plant biodiversity. According to Article 2 of the UN Convention of Biological Diversity, biotechnology refers to the use of biological system for any application of technology [14]. In this paper, attempt has been made to discuss how the diverse tools of biotechnology like molecular biology, tissue culture, genomics and other methods which biodiversity of plants can be conserved.

3. *IN SITU* AND *EX SITU* CONSERVATION

There are two methods for conserving plant biodiversity: *in situ* and *ex situ*. Former refers to conservation under normal and natural survival conditions of the species. While latter means preserving the plants outside the natural survival conditions of the species offering a wide scope of using biotechnological tools for conservational purposes. *In situ* conservation faces several limitations due to restricted habitat, pathogenic and animal attack, climate variations and invasive species. In comparison, *ex situ* conservation is more effective and flexible because the methodology is adopted in an artificial but better manner following several rules and regulations; although it may hamper the process of evolution [15]. Any conservation method targets conserving maximum genetic diversity of a plant for future usage. In certain scenario, *in situ* conservation has an advantage as, in a natural habitat, environmental factors cause maximum genetic diversity. *In situ* conservation maintains the available biodiversity and wild taxa populations under natural conditions, ecological processes and functions and biological interactions amongst each other [16]. The *ex situ* method uses conventional method of propagation of plant species by cuttings or various biotechnological tools such as organ culture, cell culture, tissue culture, micropropagation, gene banking, cryopreservation and various genomic tools.

4. *IN VITRO* TECHNIQUES

In vitro techniques like tissue culture play a crucial role in conserving plant biodiversity. It involves culture initiation, stock maintenance, propagation and storage of infection free plants [17]. For medium term storage usually, slow growth strategies are applied while for undefined periods, or for very long-term conservation, cryopreservation is used. Cryopreservation is storage of tissues at a very low temperature, to slow down growth of plants allowing for only 20% regeneration. Conservation of rare and endangered plants has been feasible owing to tissue culture and cryopreservation techniques worldwide.

5. PLANT TISSUE CULTURE

It's a method to culture the cells, tissues, organs and other parts of the plant *in- vitro* under a controlled well-defined environment with appropriate nutrients, pH, photoperiod and temperature conditions and also taking advantage of the innate totipotent property of plant cells [18]. The culture medium is composed of an array of artificial nutrients supplemented with various ions, organic nutrients, a source of fixed carbon and under a specific pH. The media used for nurturing the explants can be either a solid culture medium having a gelling agent or a liquid medium.

The various strategies and roles of plant tissue culture which aid in biodiversity conservation are:

6. MICROPROPAGATION

The process of growth and multiplication of a small explant derived from plant tissues or seed in aseptic, artificial growth medium under favorable conditions [19]. The growth of explants rests entirely on cell totipotency and cell plasticity.

7. SOMATIC EMBRYOGENESIS

A process by which a non-zygotic embryo is produced from vegetative plant cell or tissue which can develop into an entire plant. The formation of somatic embryo is succeeded by direct or indirect organogenesis wherein either direct plantlet or an intervening callus is formed, respectively. If callogenesis is induced, it is followed by culturing of callus onto rich auxin medium and then transferring the cultured calluses into medium with differing levels of growth hormones like increasing cytokinin levels and decreasing auxin levels, finally resulting into the formation of plant [20].

8. SLOW GROWTH CURVE

A tissue culture technique which is used for storage of *in vitro* plants by restricting growth of *in vitro* materials and increasing the intervals between subcultures [21].

9. CRYOPRESERVATION

It is a method for long term conservation in which the vital material is fully inhibited of growth using frozen conditions, thereby slowing down or stopping the metabolic process in them. Usually, this can be achieved by many methods like freezing at -20 ^{0}C, -79 ^{0}C in solid CO_2, -80 ^{0}C in freezers or -196 ^{0}C of liquid nitrogen. Cryopreservation or freezing in -196 ^{0}C, allows conservation of dedifferentiated cells as well as isolated organs [22]. Usually, cryopreservation or cryoconservation permits maintenance of plant material for infinite time period, thereby making this an essential conservation tool. The limitation of this technique is that a small organ or tissue can only be preserved. Also, a lot of parameters like intrinsic resistance to cryoprotectants, must be considered before going for cryopreservation. It can be considered to be a very high potential conservation technique if one can overlook the costly equipment and other limitations.

10. SECONDARY METABOLITES

Compounds majorly produced by the plant as a part of defense mechanism. Unlike primary metabolites they do not play any essential role in living of the plant and hence they are termed as secondary metabolites.

With rapid increase in world population and extreme pressure on the cultivable land accompanied by fast disappearances of natural habitats, use of tissue culture technology for enhanced secondary metabolite production is an ideal alternative to acquire plant derived compounds. In vitro tissue culture offers an effective and potential alternative of production of bioactive compound because the amounts of secondary metabolites produced in the technique is enormously higher than in the parent plant.

11. MOLECULAR MARKERS FOR CHARACTERIZATION

Ex situ and *in situ* conservationists always want to ensure that maximum genetic diversity is conserved for a species. This is possible, only with genetic information of the germplasm. Thereby, molecular markers, which give maximum genetic information of a germplasm, is used for the same.

Plant germplasm has been maintained since long for the purpose of conservation, in form of seed banks, nurseries, gene banks etc. But their utilization for the purpose of breeding has not been that successful due to of lack of data for their characterization and evaluation. Characterization and evaluation of germplasm means, observing, measuring and documenting data of heritable traits in a germplasm.

Characterization is usually done using morphological characters and molecular markers [23]. Molecular markers are far more stable and are not influenced by environmental factors, unlike other markers. Molecular markers are small segments of nucleic acid segment which are usually present either in the flanking region of gene or within the gene. They act as flags to identify whether a gene is present or absent. Thus, they are very useful for marker assisted breeding or selection in plants [24].

For *ex situ* conservation, molecular markers are used for identification, particularly in determining whether a species is maintained properly and true to its type or not. It also, helps in depicting the similarities between members of same species. Since, a good amount of genetic variation arises because of migration, population size and reproductive biology, molecular markers help in identifying variations amongst population, accessions and species. Even the presence or absence of a particular allele can be detected using molecular markers, thereby making them essential biotechnological tool for biodiversity conservation and maintenance.

Even *in situ* mode conservationists are benefitted with the use of molecular markers. It's essential that they identify appropriate populations, which can survive and evolve with time. Hence, knowledge of molecular level is important for all species. Management plans must be developed for *in situ* conserved species to ensure their long survival and maintenance. Genetic information of all these germplasms is important since *in situ* conserved plants are highly beneficial to many communities which depend upon them.

Molecular markers can be broadly categorised into three types: 1. **non-PCR based methods** which includes markers like Restriction Fragment Length Polymorphism (RFLP) and Variable Number of Tandem Repeats (VNTR). 2. **PCR based methods** which includes molecular markers like Multiple arbitrarily primed PCR (MAAP) techniques. Under this category lies many markers like Random Amplified Polymorphic DNA (RAPD) and its variations like Arbitrary primed PCR (AP-PCR) and (DNA Amplification Fingerprinting (DAF). Amplified Fragment Length Polymorphism (AFLP) is also a popular PCR based molecular marker. 3. **site-targeted PCR** which includes markers like Sequence-tagged microsatellites (STMS), Single Primer Amplification Reaction (SPARs), Inter-simple sequence repeat amplification (ISSR) and Single nucleotide polymorphism (SNP) [25].

12. DNA BANK

Earlier, seed banks were considered to be an important aspect for conservation of plant biodiversity. But with time, scientists have realized that it is not only space consuming, labour and cost intensive but also not a very good choice for long term conservation. With the extraction of high molecular weight molecules, like DNA and RNA, their conservation in form of banks have been an important aspect in long term conservation of plant biodiversity [26]. The sample size is small and the molecules are stable in nature. Also, it can be easily shared between different DNA banks worldwide. Major world plant DNA Banks are: Australian Plant DNA Bank, Lismore, Australia; Missouri Botanical Garden, Missouri; Royal Botanic Garden, Kew, Great Britain; South African National Biodiversity Institute DNA Bank, Kirstenbosch, South Africa; National Institute for Agro biological Sciences, Japan and many others.

These techniques although efficient have their own conservation problems chief being – Cryptic lineage identification, delimitation of conservation sites, optimization of *ex situ* conservation, detection of invasive species, establishment of baselines for restoration, assessment of adaptive and acclimation potential and lastly inversion of extinction. This has led us to sail the fleet towards genomics which provide solution to these problems.

13. CONSERVATION GENOMICS:

Genomics is the study of entire set of DNA in an organism. Every cell of that organism contains the whole set of the genome. All the information required for the organism's development and growth is present in it's genome. Interaction of the genes amongst each other and with the environment can be understood with the detailed study of genome. A comparison of genomes in related species helps in finding the relationship between them by studying the conserved or homologous regions.

With passage of time, the cost of sequencing has reduced a lot. Moreover, the loopholes of other conservation techniques can be easily taken care of by using different genomic techniques like metabarcoding, next generation sequencing (NGS), Reduced representation sequencing (RRS), RADseq, LDNA sequencing and others, leading to coining of new term "conservation genomics."

Reduced representation sequencing (RRS) is a technique in which representative subset of genome is targeted which leads to great reduction of costing per sample, thereby, increasing in-depth coverage per locus [27]. Presently, RADseq (restriction site- associated DNA sequencing), is very prevalent in conservation genomics. In this sequencing is done of fragments adjacent to restriction cut sites. It does not require any prior information.

The next generation sequencing (NGS), is a technique used for rapid and cheap sequencing of DNA or RNA; appropriately called the second-generation sequencing or high throughput sequencing. It takes very less time to study the genome of plants and this can be considered better than Sanger sequencing. NGS platform can not only be used for DNA sequencing but also for RNA sequencing (transcriptomics) and protein sequencing (proteomics). It has proved to be helpful in identifying methylated DNA (epigenetic modification).

Metabarcoding is one more important conservation genomics technique which has found substantial importance in present scenario. It is a technique which uses primers from common barcoding regions and the NGS technique. This allows evaluating the mixture of bulk taxa which can not be assessed using the traditional techniques [28]. The data helps in conservation strategies, planning of land use, environmental change monitoring amongst others. Genomics has not only been helpful in finding and conserving new potential taxa but also, it plays a significant role in finding genetic changes in old museum and herbarium specimen. This kind of DNA is degraded and is known as LDNA (low-quality DNA). Sequencing of the LDNA has helped conservationists to identify genetic changes and also take appropriate decisions on conservation intervention [29].

Beyond genomics there is transcriptomics, epigenomics and various other fields of biotechnology which can turn out to be a boon in conservation. New techniques like gene editing tools of CRISPR/CAS9 can be used in conserving after editing only those genes which have led to extinction of species [30]. A new field of biotechnology "Synthetic Biology" that designs and construct new biological parts, devices and system can be a highly impactful in the process of conservation in future. We can use the idea of bringing back the extinct species to life. Even better, the nucleus of a somatic cell of a recently extinct species can be transferred into the denucleated egg of the close living relative, and this will then be used as a surrogate mother [29].

14. CONCLUSION

Even though in present day scenario, biotechnology and modern tools have its limitations of high cost and maintenance, but with the amalgamation of traditional knowledge, we can get multiple options to conserve, study and use plant biodiversity. It is very crucial for the increasing human population, climate change and with decreasing gene pool, that the scientists use these tools to conserve plant biodiversity which will ensure a safe and happy future for humankind.

India is proud to have many scientific institutes like National Bureau of Plant genetic Resources, NBPGR, where there have been huge investments done by Government of India to establish seed bank, DNA bank, cryopreservation and tissue culture units for conserving and maintaining the Indian plant germplasm at national level.

But with passage of time, new biotechnological tools especially conservation genomics must be roped in to become saucerful in "bending the curve" initiative.

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