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To cite this article: S Merlyn Keziah and C Subathra Devi 2017 *IOP Conf. Ser.: Mater. Sci. Eng.* **263** 022047

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Essentials of Conservation Biotechnology: A mini review

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Abstract. Equilibrium of biodiversity is essential for the maintenance of the ecosystem as they are interdependent on each other. The decline in biodiversity is a global problem and an inevitable threat to the mankind. Major threats include unsustainable exploitation, habitat destruction, fragmentation, transformation, genetic pollution, invasive exotic species and degradation. This review covers the management strategies of biotechnology which include *sin situ*, *ex situ* conservation, computerized taxonomic analysis through construction of phylogenetic trees, calculating genetic distance, prioritizing the group for conservation, digital preservation of biodiversities within the coding and decoding keys, molecular approaches to assess biodiversity like polymerase chain reaction, real time, randomly amplified polymorphic DNA, restriction fragment length polymorphism, amplified fragment length polymorphism, single sequence repeats, DNA finger printing, single nucleotide polymorphism, cryopreservation and vitrification.

1. Introduction

Biodiversity refers to various living organisms that live within the ecosystem. It includes variety of terrestrial, marine freshwater plants, animals and microbes which are important resources to be considered. They provide the basic needs of mankind, adds on aesthetic value and most of all maintains the ecosystem supporting our existence. Biotechnology approaches through value addition, conservation of plant genotypes which are of medicinal importance, possibilities of cloning, induction of organogenic, callus cultures, transgenic plants and animals which are having both pros and cons. Agriculture has been in practice since 10, 000 years by cultivating the crops and domesticating the livestock species. With the emergence of biotechnology new techniques have been applied to provide desirable products. Biotechnology also paves way for evaluation, conservation of biodiversity. Globalization has brought about decline in biodiversity in a rapid pace. A substantial loss of biodiversity has occurred since 1960s due to deforestation of the tropical forests, a terrestrial habitat of most plant and animal species. The increase in population may be one of the reasons for forest exploitation. Hence, the destruction continued extending towards the temperate and boreal forests converting them to lands of mining, agricultural and fossil fuel extraction. The encouragement of corruption and misuse of forest resources due to institutional failures, industrial failures and policy failures, leads to lack of reach of incentives for forest degradation to forest dependent communities. It results in secure resource access rights, lack of transparency in forest resource pricing and allocation.

2. Habitat destruction:



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Habitat destruction not only concerns with the land exploitation but also habitat fragmentation and habitat transformation. Extinction rate of freshwater frogs, fishes, crocodiles and turtles are much higher than the marine organisms. In 1999, Hanski predicted that the isolated populations are more likely to go extinct with a meta population model. [1].

2.1. Habitat fragmentation

Assessment of habitat fragmentation also includes the invertebrate populations like bees, beetles wasps, butterflies and moths. The minimum requirements for habitat must include larval food plants and nectar of flowers including trees, fruits and wastes as food resources as well as adult food resources. For some taxa, such as lycaenid butterflies, habitat will also include the presence of mutualists such as ants. Because the ants milk the nectar secreted by the larvae and build a thatch to protect the symbiont from its predators Thomas and Elmer (1998). To meet out the food needs of the growing population all the intense woody forests and grasslands were converted to cultivable lands.

Habitat fragmentation due to urbanization has significantly contributed to the declines of the California red-legged frog *Ranadraytonii*. Organophosphorus malathion, aquatic borne insecticide used for pest control in the fields affects the amphibians like frogs to absorb moisture through their skin. The survival rate of Bull frog (*Ranacatesbeiana*) decreased with higher concentrations of malathion with reported deformities in their developmental stages inhibiting cholinesterase affecting nerve function Fordham et al. (2001). Turtles and tortoises are another important freshwater habitats of Niger delta reported to be at the verge of extinction due to anthropogenic disturbances like pipeline constructions, oil spills, seismic exploration, dredging, drilling and annual practice of bush burning [2].

2.2. Habitat transformation

On the other hand, the secondary effect of habitat destruction is habitat transformation. Construction of Inland dams, irrigation wells curb the fresh water runoff and cut off the fish migration routes. This increases the salinity of coastal waters. When the free flowing rivers are impounded with the construction of dams and irrigation wells from shallow streams to deep standing water bodies the native fishes are extirpated to lentic fishes [3]. It was reported that constrained swimming performance and body shape and predictable divergence in morphological traits [4] and changes in maneuverability to escape from the predators were observed [5].

3. Threats to biodiversity

3.1. Natural calamities

Natural calamities like tsunamies, hurricanes, typhoons and storms cause massive habitat loss occurs in the oceans. Wetlands are dredged to fill in the urban population. Factory effluents and run off cause destruction to the coral reefs, phytoplanktons, birds and fishes. Habitats in and around the sea is destroyed by unethical and destructive fishing techniques like bottom trawling, dynamite poisoning. Added on to this, all the earth's extra heat is absorbed by the ocean waters leading to the increase in temperature which has far-reaching effects on the underwater dwellers.

3.2. Land encroachments

The fast growing industries and change in life style of the present generation have started replacing even the cultivable lands into multinational companies, franchise industries and apartments for furnished abode of the human population.

3.3. Invasive plant species

The presence on non native exotic plant species is a threat to the native plant species. It competes with the native ones for food and water resources eventually driving them to extinction. One such is pernicious Kuduz plant, much renowned as preventer of soil erosion, was originally imported from Japan during the 20th century by the United States [6]. It is believed that a volatile compound, isoprene emitted by this plant in the presence of sunlight combines with nitric oxide and produces sizable amounts of ozone thus exposing other plants in the vicinity.

3.4. Genetic pollution

Genetic pollution occurs in a mixed gene structure acquired from unrelated species may include bacteria, virus, other animals and even some foreign plants. The foreign genes inserted into other organisms itself are a contamination in the gene pool. The oilseed has tremendous capability of cross breeding with other similar species which is quite lethal as it could alter the original gene structure of the native plant. These cases are quite common in Europe. This oil seed can form hybrid with wild radish, beet root, wild cabbage and hoary mustard [7,8]. If a disease occurs as a result of complete loss of function of a gene or if a disease occurs due to over expression of a particular gene, the gene can be knocked out or targeted on inserting a transgene. The transgenes induce genetic diversity as the insertion site experiences deletions and rearrangements in the DNA segment. Transposable elements excise and reinserted into the genome rapidly. The introgressive hybrids thus displace the native genepool due to repeated backcrossing.

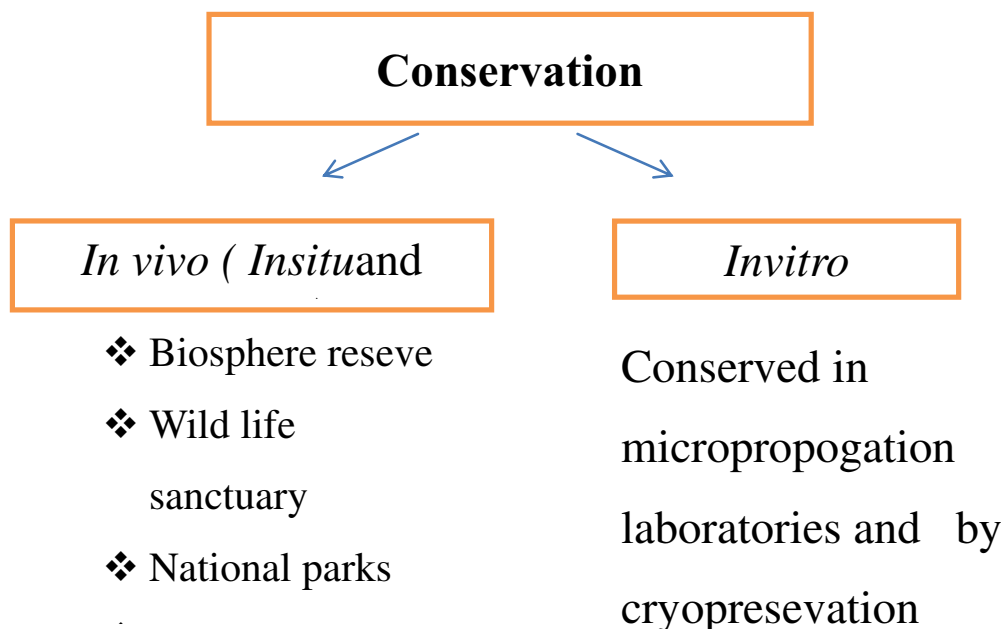


Figure 1: Conservation methods

4. Conservation trends in biotechnology

4.1. *In situ* conservation

The landscapes are destroyed with industrial effluents; bioremediation is an inevitable contribution of biotechnology to bring about land reclamations with the help of microbes. Methanotrophic bacteria are methaneoxidizers with the carbon substrates, known for chlorinated solvent degradation. It can reduce halogenated aliphatic compounds such as Trichloroethylene (TCE) [9]. These organisms were reported to be isolated from peat bogs [10,11] sea water[12,13] plant rhizospheres[14], salt reservoirs[15,16]. *In situ* conservation provides the relative stability of species diversity within an adapted community [17] and is essential for subsistence agriculture. The Landrace method of cultivation is followed traditionally for *insitu* conservation. Ethiopia is well known for its diverse gardening standing one amongst the eight Vavilovian gene center, notably barley (*Hordeumvulgare*) and wheat (*Triticum* spp.) is concentrated and where several important crop plants including sorghum (*Sorghum bicolor*), sesame (*Sesamumindicum*), coffee (*Coffeaarabica*) and minor millets became domesticated [18]. Conservation of wild life in nation parks and conservation reserves, sanctuaries are also termed as *insitu* conservation.

4.2. *Exsitu* conservation

In *exsitu* conservation of germplasm is done by seed gene banks and frequently checked for viability, collection of living plants and recalcitrant seed plants are conserved in plant gene banks. Aseptically propagated germplasms are conserved and propagated through horticulture in the form of meristems. In 1958 Maheswari and Rangaswamy reported regeneration of embryos *invitro* from the cell nucleus. Somatic embryos have been regenerated from nuclei excised from abortive and unfertilized ovaries.[19,20]. *Saturejaavromanica* Maroofi is a new perennial herb with violet flowers covered by papilla, yellowish sessile glands and long braced with geographic distribution exclusively restricted to Kurdistan Mountains, West of Iran [21].

4.3. Ordering of conservation group: computer analysis.

Phylogenetic analyses are principal analysis involved in searches of random taxon addition and construction of pedigree charts for analysis of the phylogenetic relationships. Some of the reliable methods used are minimum and maximum parsimony methods, neighbour- joining method. Some of the basic software used to construct phylogenetic trees were Clustal W, Clustal Omega, MEGA (Latest Version7). On construction of the tree genetic phylogenies are examined for instance. For instance, When a cattle bit by tsetse fly experiences sore, muscle ache, bloody urine and swelling of lymph gland, it is probably affected with trypanosomiasis, and the disease transmission is was by tsetse fly. The African humpless breeds have natural habitual resistance to the tsetse fly and hence those evolved with them is suitable for the susceptible disease spreading regions. The tripano tolerant trait attributed entirely to the indigenous West African taurine leads to the need for gene conservation in animals [22].

The nuclear DNA markers are biparently inherited it can be used to assess population differentiation, identifying phylogenetic relationships using pedigree analysis. Informations on genetic variation and differentiation of a species into distinct populations were generated with the help of these DNA markers. Outcrossing and inbreeding would critically influence the inherent genetic load of deleterious gene thereby the threshold level of the population size pushing the population into the imminent of danger resulting in loss of genetic diversity. Inbreeding depression is decline in biological fitness due to unmasking and partially masking of the recessive alleles and genetic purging as a result of repeated selfing. Reduction in generation interval increased the number of inbreeding per year, affected the lactation performance and reproductive ability of the breed.

4.4. Molecular markers

The evolution relies on physical and biological forces such as migration, selection, genetic drift, and geographic barriers. Molecular marker technology elucidates the population structure and gene distribution patterns in the ecosystem provide information which can be used to support *in situ* conservation programs. Some of the molecular markers used. With the invention of Polymerase chain reaction in 1985, the encryptions of the genetic makeup have been decoded. The heat stable *Taq polymerase* enzyme was isolated from *Thermusaquaticus* a heat labile bacterium isolated from hot springs. The *taq* polymerase was resistant to high temperatures and hence was able to denature the DNA templates [23]. This is later termed as DNA polymerase.

Three main steps involved in making copies of DNA are (i) Denaturation; (ii) Annealing and (iii) Synthesis of DNA.

(i) Denaturation

The Double stranded DNA is denatured on increasing the temperature of about 95°C breaking the hydrogen bonds and exposing the single stranded nucleotides for the primers to bind.

(ii) Annealing

The temperature is lowered in this step where the added primers were able to form complementary hydrogen bonds and get attached to their complementary bases.

(iii) Synthesis of DNA.

Here the temperature is once again increased to 72°C for wherein the *taq polymerase* binds at the gaps synthesizes complementary nucleotides for the template and extends the strands connecting the bases. Thus synthesized DNA repeats are amplified with the thermocycler.

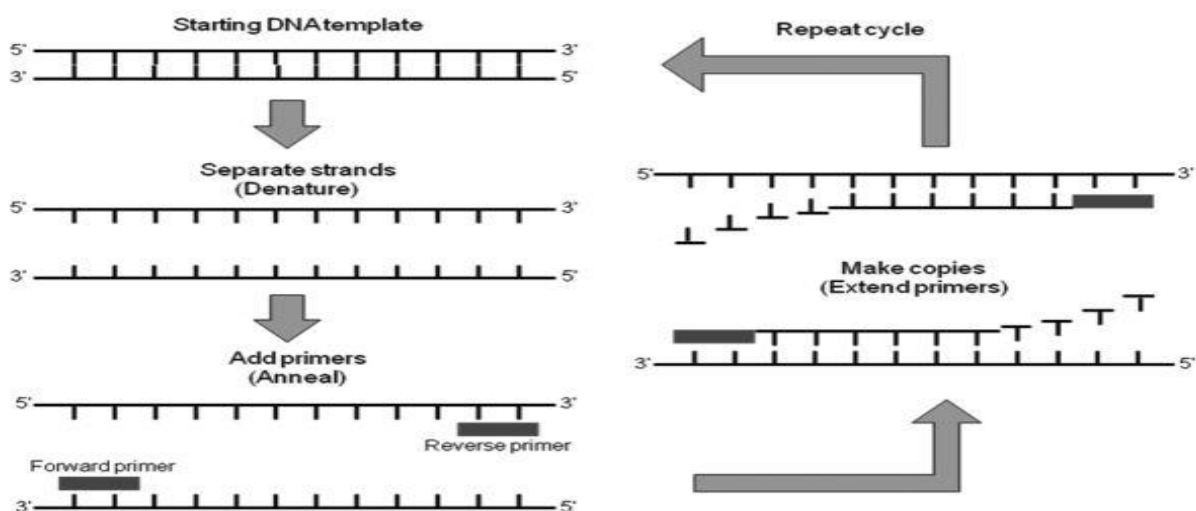


Figure 2. Polymerase chain reaction amplification (Hashiyada M. DNA biometrics. In Biometrics 2011. In Tech.)

To assess genetic variations microsattelites, minisattelites ,mitochondrial control region, cytochrome b and MHC loci serve as markers. Satellites consist of units of several thousand base pairs, repeated thousands or millions of times. The Marker Assisted Selection (MAS) with the molecular markers aids phenotypic selection examining the genetic linkage and traits among the generations. This selects the parents of the next generation by determining the nucleotide sequence through genotyping. The markers located near the gene of interest are called linked markers. The marker which itself is a part of gene of interest is known as direct markers. Gene variability in organisms were first identified using restriction fragment length polymorphisms. The DNA fragments digested with restriction enzyme when cloned with differential hybridization Restriction Fragment Length Polymorphism is based on the differential hybridization of cloned DNA to DNA fragments in a sample of restriction enzyme digested DNA. It is specific to a single clone restriction enzyme combination. A large number of RFLPs were recorded in regenerated maize, rice, wheat, barley, triticale, potato [24] and some tree species including populus, eucalyptus and coffee for studying the variation and genetic fidelity during micropropagation [25]. Amplified Fragment Length Polymorphism; a molecular marker generated by a combination of restriction digestion and PCR amplification. Higher levels of polymorphisms can be identified in AFLP when compared to RFLP. The nucleotide sequences neighbouring the restriction sites is also detected. Minisatellites consist of DNA sequences of some 9–100 bp in length that is repeated from 2 to several 100 times at a locus. Minisatellites discovered in human insulin gene loci with repeat unit lengths between 10 and 64 bp were also referred to as “variable number of tandem repeats”. The detection of polymorphism were made possible with the advent of Polymerase chain reaction technique in 1985, the DNA segments were amplified using thermocyclers [26]. The genetic diversity of the endangered hawksbill turtle (*Eretmochelys imbricate*), was identified on isolating twelve novel polymorphic microsattelites. Eight of 12 markers were used to study genetic diversity of two sea turtle species: *E. imbricate* and green sea turtle (*C. mydas*) amplified specific and polymorphic PCR products in other six turtle species [27]. The molecular marker Randomly Amplified Polymorphic DNA is based on the differential PCR amplification of a sample of DNA from short oligonucleotide sequences. Isozyme-a molecular marker system, based on the staining of proteins, with identical function, but different electrophoretic mobility.

Single Nucleotide Polymorphisms are found out by high density genotyping and genome wide association analysis. The SNPs are physically linked to the ecologically important traits controlled by major loci. It has facilitated the field of molecular forensics feasible to trace out the phenotypic mutations in traits responsible for skin, eye and hair colors. The single nucleotide polymorphism gives information of phenotype of the organisms on determining the genetic loci. The Genome Wide Association Analysis (GWAAS) helps to link SNPs that are responsible to locate important traits. The adaption of the location for instance the pelage color of the old field mice (*Peromyscus polionotous*). The allele specific primers are used for allele specific amplification of the varied sequence [28]. The quantitative trait locus is a statistical analysis (QTL) where in a section of DNA the variation of the gene loci, they map using these SNPs, single interval mapping, multiple interval mapping and composite interval mapping. Simple sequence repeats and restriction length polymorphisms. The nocturnal pigmy hippopotamus (*Choeropsis liberiensis*) endemic to Nigeria, West Africa is hunted for its meat and is one of the greatest threat of endangered species. They are also killed by leopards, crocodiles and pythons. They have been facilitated for captive breeding. As reported in 2014, there were only 2000-3000 left out. A high confidence candidate single nucleotide polymorphisms (SNPs) were generated to analyse the genetic population with restriction-site associated DNA sequencing from five pygmy hippo samples [29]. Using the mitochondrial control region and cytochrome b protein region differences between the *Kogia* species within the ocean basins, were figured out. The phylogenies were generated using maximum parsimony and maximum likelihood and neighbor joining algorithms. The tree length and consistency indices revealed that the tree length for cytochrome b was much shorter, conservative and reliable for reconstructed the phylogenetic relationships of *Kogia* sp. [30]

The hawksbill sea turtle (*Eretmochelysimbricata*) is described as a highly endangered species, commonly poached for its ornate shell. The hawksbills are distributed globally, though they have genetic differences it is difficult to determine their origin. In a recent report, confiscated tortoiseshell items were obtained from the U.S. fish and wildlife service, and DNA from 56 of them was analyzed. The mitochondrial DNA was isolated amplified and multiple mitochondrial halo types were identified. This encourages conservation [31].

MHC genes are polymorphic genes in vertebrates, plays a crucial role in immune function via immune-recognition and -surveillance and host-parasite interaction. Therefore measuring levels of polymorphism at these genes can bring success in planning and executing the conservation management. It has gained pathogen resistance and mate choice. The viral burdens vary between animals, Caprine arthritis and encephalitis infection caused by lentivirus in Sheep. It causes encephalomyelitis in kids, polyarthritis in adults which resembles the rheumatoid arthritis of human. In addition it also causes in durative mastitis resulting in decreased milk production. This CAEV infection is similar to the MVV infection that occurs in goat [32].

4.5. Tissue culture for conservation

A plant tissue culture technique involves culture initiation, culture maintenance and multiplication, and storage. Slow growth strategies are applied. Flasks of nutrient medium are heat sterilized under pressure to keep the medium free from bacterial and fungal contaminations. And inoculation of the explants must be carried out in a laminar air flow hood. The medium consist of nutrients such as sugars, inorganic salts, plant hormones and gelling agents. The setup is then placed under lights in controlled conditions and monitored for contamination. After stipulated time period the explants showed signs of growth of callus and development of roots, leaves and other plant parts.

In tissue culture, the plant propagation is done under *invitro* controlled conditions in a conical flask, jar, and test tube. These plants would be the exact copies or clones of the parent plants. The clones are produced in multiples, hundreds and thousands. When seeds are used in plant tissue culture each individual plant is genetically different from its parent plant. Micro propagation is a distinctive technique to conserve the plant species. It involves production of plant using shoot tip and nodal culture, the newly formed shoots and roots served as explants for repeated proliferation of plants. This would increase the pharmacological value of the plant. [33].

4.6. Cryopreservation

Recalcitrant seeds are cryopreserved in liquid nitrogen; some of the rare plant species which is authentically known for its medicinal properties are conserved. Regeneration process increases by 20 % when cryopreservation method is followed when compared to other conservation methods. Cryopreservation is done with liquid nitrogen with -132°C [34]. The protoplasts and callus can also be preserved by cryopreservation. The embryo genic cultures also dehydrated and encapsulated with the polymers like polyethylene glycol and sodium alginate beads. They can also be vitrified using vitrification solution [35].

5. Conclusion

Some of the cons of biotechnology techniques is induction of genetic pollution, and some ethical controversies have raised for transgenic plants with the production on frost resistant strawberries (*Fragariaananassa*) with a gene of arctic flounder (*Liopsettaglacialis*) is considered against nature and unethical by many including vegetarians. Nevertheless the advancements of biotechnology had brought about preserving the human umbilical cord for future need in case of trauma disease or disorder to produce an organ (organogenesis) or even a whole clone. Thus biotechnology would bring revolution by

bringing up new trends in conservation with respect to traditional ones. Thus contributing to the rejuvenation of the wreak havoc ecosystem.

Acknowledgement

The authors are very much thankful to VIT University for providing necessary facilities.

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