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Review

Biotechnology, genetic conservation and sustainable use of bioresources

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The loss of Africa's forests and bioresources is occurring at an alarming rate, a consequence of increasing population pressure, agricultural land degradation, urbanization and neglect. There is a growing recognition worldwide that conservation and sustainable management of bioresources are pressing priorities in the world today. The choice of conservation methods and techniques depend on the objectives of the particular conservation effort, the breeding system and behaviour of the species in question as well as the available resources including funds, trained personnel, infrastructure and technologies. The use of biotechnological tools and "bioprospecting" will open new vistas in medicine, agriculture, silviculture, horticulture, environment and other important issues. This paper reviews some biotechnological tools that could be harnessed in promoting conservation and sustainable use of bioresources.

Key words: Bioresources, genetic conservation, biotechnology.

INTRODUCTION

Bioresources refers to the total biological variation manifested as individual plants, animals or their genes, which could be taken by man for use as drugs, food, livestock feed, construction materials for shelter, environmental protection, etc or in the development of improved crops and animals for higher yield and tolerance to biotic and abiotic stresses (Eneobong, 1997). Man depends on these bioresources for his continued existence and, therefore, he must use and preserve them for future generations yet unborn. The concept of sustainable development indicates that economic and environmental protection are inextricably linked and that the quality of present and future life rests in meeting basic human needs without destroying the environment on which life depends.

The rapid diminishing rate of Africa's forests and bioresources have been variously attributed to civil war, conversion of land for agriculture, wild fires, poor management of available land, uncontrolled search for food, fuel wood, medicine, construction timber, overgrazing by cattle, displacement and loss of landraces, lower yielding varieties, pests and diseases, pollution (e.g. acid rain) and incomplete knowledge of the biology of many plants especially the propagation genetics aspect and adaptability of many forest plants (Eneobong, 1997).

Deforestation and forest degradation are large-scale problems in developing countries. In Nigeria, the situation is made particularly pathetic with the frequent increases in the prices of petroleum products, which have made a lot of

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people to resort to the use of the cheaper and more steady fuelwood. Between 1990 and 1995, the World Wildlife Fund (WWF) estimated that Africa lost 3.7 million hectares of forests every year, a deforestation rate of 0.7%, which is more than twice the world average of 0.3% and about half of the world's original forest cover has now disappeared. The FAO (2001) also reported that global forest cover was shrinking at a rate of around 9 million hectares per year. These views are supported further by recent reports from the World Resources Institute that while deforestation may have increased in tropical Africa, it remained constant in Central America and declined only slightly in tropical Asia and South America.

Since we cannot do without exploiting the available bioresources to our advantage, there has to be a balance between uses of resources and their conservation. In this way, we would preserve an ecosystem, which although altered would still be rich in bioresources and at the same time would provide food and other needs as well as perform vital environmental functions on a long term basis.

It is necessary to conserve these naturally endowed resources which apart from their direct usage by man, also serve several ecological functions such as the control of flood, soil erosion, landslides and hurricanes, maintenance of water quality, climate amelioration and checking desertification (Okoro, 1994). A 1982 El Salvador landslide that killed more than 1000 people was attributed to deforestation. The hurricane Mitch that occurred in Central America in 1998 was said to be compounded by about 30% deforestation. The endangered species and threatened crops deserve urgent and special attention. For example, the world's only remaining wild Orang -utangs would be gone for good if Indonesia's remaining natural forest is destroyed. The Afi River Forest Reserve (Cross River State, Nigeria) is located in one of Africa's diversity 'hotspots'. This area is also one of West Africa's three 'deforestation hotspots', recognized by the Tropical Ecosystem Environment Observations by Satellite program (TREES, European Commission). The Afi reserve is home to the newly recognized *vellerosus* subspecies of chimpanzee and the Cross River Gorilla, *Gorilla gorilla diehli*, recognized as a distinct and critically endangered subspecies by the Primate Specialist group of the International Union for the Conservation of Nature (IUCN) Species Survival Commission in February 2000. Several species of birds and monkeys in Africa would have become extinct by now if not for the intervention of wildlife conservationists, as there is now greater international willingness to 'pay' for the conservation of biodiversity.

Biotechnology can be defined as any technique that uses living organisms or parts thereof to make or modify a product, improve plants or animals or develop microorganisms for specific uses (Alhassan, 2001). It is invaluable in research on conservation of bioresources. Although modern biotechnology is a newly introduced science (less than 50 years old) its impact has greatly excited the imagination and provoked the concern of almost every part of the society worldwide (Eneobong, 2003). Developments such as the tomato that can be frozen and the cassava and other agricultural crops that have been genetically engineered for insect and virus resistance and which are in or near commercial release are simply spectacular by any standard. The richness of plant and animal diversity in developing countries is a major asset in agricultural development and, therefore, the conservation of such resources is fundamental to the progress and usefulness of biotechnology.

CONSERVATION OF GENETIC RESOURCES

Plant Genetic Resources

Efficient conservation of plant genetic resources can best be achieved through an appropriate combination of *in situ* (in natural or original areas) and *ex situ* (in artificial habitat or habitat different from the original one) methods (IPGRI, 2001). The choice of conservation methods and techniques will depend on the objectives of the particular conservation effort, the breeding system and seed behaviour of the species in question as well as on the available resources including funds, infrastructure and technologies (Perrino, 1990; Eneobong, 1997). Generally plants with orthodox seeds (high tolerance of low temperature storage conditions) are best preserved *ex situ,* under medium or long term conditions as comparatively dry seeds stored at low temperatures (Ng, 1991). Plants that produce recalcitrant seeds (intolerant of desiccation and low temperatures) could be preserved as *ex situ* live-gene banks (or gene libraries) or by *in vitro* conservation methods of enforced reduced growth storage. Plant resources are also routinely preserved *in situ* in parks, reserve areas and rangelands.

This section of the paper will discuss *in vitro* methods for storage as well as the cryopreservation of embryos, seeds, protoplasts and other materials in long-term liquid nitrogen base-storage systems. Many plants, especially forest plants, are extremely difficult to propagate through conventional means since they are frequently polyploids and aneuploids or produce seeds with little or defective endosperms. In Nigeria, very little is known about the biology of some of these "orphan" plants. There is therefore the risk of losing some of these plants due to industrialization and urbanization, characterized by rapid deforestation, uncontrolled logging, burning and uncontrolled search for food and other non -timber forest products. Many of these plants constitute an important component of the diet in many West African countries (e.g. *Gnetum africanum, Treculia africana* (breadfruit), *Irvingia gabonensis*) and costly timbers (e.g. *Diospyros mespiliformis* (iroko), *Entandrophragma cylindrica* (mahogany) and *Chlorophora excelsa*). Plant tissue culture provides a method for the mass clonal

propagation of such materials, as well as serving as a tool for their germplasm conservation.

Micropropagation: This refers to *in vitro* mass production of plant propagules from any plant part or cell. Such propagules are used to raise whole plants. The principal approaches are:

(a) Axillary budding: The induction of adventitious buds on non-meristematic tissue (that is, inducing a shoot where one should normally not exist).
(b) Somatic embryogenesis: Where individual cultured cells or small groups of cells undergo development resembling that of the zygotic embryo. The embryoids produced can be used to produce whole plants.

The attraction of micropropagation, as an alternative to other propagation methods, lies in its ability to multiply elite clonal material very rapidly. More than 1000 plant species have been micropropagated, including more than 100 forest tree species (FAO, 2001). Work done with some crop species indicates the possibility of encapsulating somatic embryos to form artificial seeds, which can then be handled like conventional seeds. Such propagules may be used in forest plantation establishments.

Micropropagation exploits the "totipotency" nature of plant cells and tissues. The explants are made to form callus under appropriate nutrient environments. Numerous clonal plants can be obtained from sub-cultured callus or from embryoids, which are then hardened and transferred into potted soil in nurseries. In this way planting propagules can be provided for rare or threatened plant species as well as for plants with inviable or difficult-to-germinate seeds. Furthermore, tissue culture techniques can serve as an enhancing tool in plant breeding for the rescue of defective hybrid embryos, caused by post-zygotic incompatibility during crossing (Enebong and Okonkwo, 1994). Nutrients provided in the culture medium will perform the function of the malformed endosperm. Embryo cultures also adopt the nutritional and physical requirements for embyonic development to bypass seed dormancy (thus shortening the breeding cycle) and seed sterility and to provide micro-cloning material (Hu and Wang, 1986).

***In vitro* conservation:** Germplasm conservation of vegetatively propagated crops (e.g. banana, plantain, yam, cassava etc) forest species especially those with recalcitrant (hard to store) seeds (e.g. mango, cocoa, *Symphonia globulifera* , *Irvingia gabonensis*) in live gene-banks in fields poses tremendous problems in terms of required land space and labour input during annual or perennial replanting, testing and documentation. Such collections are also exposed to threats by biotic and abiotic stress agents. Consequently, *in vitro* conservation is recommended, at least as a supplement to field collections, as long as an adequate protocol for micropropagation has been worked out for the species.

The advantage of *in vitro* or reduced growth storage include little space necessary in growth rooms for maintaining thousands of genotypes and the absence of diseases and pest attack in culture vessels. Furthermore, *in vitro* storage eliminates the need for long and frustrating quarantine procedures during movement and exchange of germplasm.

Virtually any part of the plant could be used as explant in establishing cultures for storage, although the best results have been obtained using apical meristems, axillary buds, embryos and gametes. Normal *in vitro* cultures use media like Murashige and Skoog and Arnold and Eriksson. Frequent subcultures into fresh media are necessary. Excised plant tissues, organs, or cells are usually cultured on these media. The culture medium could be liquid or solidified using agar. Under these conditions the cultures rarely last longer than a few months, requiring transfers into fresh media to maintain optimal growth.

During *in vitro* storage the growth of the culture is slowed down through one or a combination of several methods namely

(a) Reducing the concentrations of the minerals or by using media with lower salt concentrations (Ng and Ng, 1991).
(b) Using low incubation temperatures (Dale, 1980; Henshaw et al., 1985; Ruredzo and Hanson, 1991).
(c) By the addition of osmotica (Henshaw et al., 1980, Ng and Ng, 1991; Ruredzo and Hanson, 1991).
(d) Reduction of the gas pressure in culture vessels (Bridgen and Staby, 1981).
(e) By varying the light regime (Mullin and Schelegel, 1976).

Cryopreservation: Collected plants are normally stored either in active gene banks containing material that is kept ready for distribution, evaluation or exchange; or as base collections containing duplicates that are kept for future use (long term) or "emergency" material in case of loss from the active gene banks. Base collections can be maintained under very cold conditions in high tech ultra low temperature freezers. Cryopreservation is an attractive alternative for the storage of base collections and involves the freezing of plant material, usually to the temperature of liquid nitrogen (-196°C), at which point cell division and consequently growth and all other biological activities are completely arrested. This must be done in a manner that viability of the stored material is retained and biological functions and growth can be reactivated after thawing (Towill, 1991; De Smet, 1995). Liquid nitrogen storage is useful for the preservation of various types of plant material including whole seeds, embryos, suspension cells, callus, protoplast cultures, gametes and meristems.

Although the techniques of freeze-preservation in liquid nitrogen have been modified in several ways to minimize

freeze damage, there are three major steps involved.

(i). The material to be frozen needs to be cryoprotected from freeze-damage by treatment with cryoprotectants. The commonly used chemicals for this purpose include dimethyl sulphoxide (DMSO), ethylene glycol, glycerol and proline, used at concentrations usually less than 10% (w/v), to enhance the survival of hydrated tissues or cells during freezing. These chemicals cause changes in cell permeability, freezing point and responses to stresses of freezing and thawing. Cryoprotectants can be added before freezing or even at lower temperatures during the freezing process. For the former, incubation for a few hours may be necessary before initiating the freezing process. Some pretreatment methods, which have been found to be successful in some species when used with cryoprotectants, include

(a) cold treatment for callus or cell suspension cultures (Chen et al., 1984),
(b) preculture of shoot tips with 0.25-0.75 M sucrose and 5% DMSO (Dereuddre et al., 1987), and
(c) cooling under osmotic stress induced with mannitol or sorbitol (Towill, 1991).

(ii). A two-step process of cooling is recommended for most plant material, since continuous slow cooling below –33°C to –40°C leads to considerable loss of viability in many plants (Towill, 1991). In practice, the initial cooling down to –40°C may be fast (several hundred ^{o}C/min) or slow (0.5-2°C/min) depending on plant genotype, before the storage in liquid nitrogen (Ng, 1991). Freezing may be initiated by the induction of a nucleation with a small ice crystal achieved by briefly touching the outside of the cryotube with LN-cooled forceps (Towill, 1991) or, as in bananas and plantains by plunging the cryotube for a few seconds in liquid nitrogen at temperatures near zero and then slow cooling to -40°C (De Smet, 1995).

(iii). Rapid warming is necessary during the thawing process and the tube containing the cryopreserved material can be dipped directly into warm water (30-40°C) for a few seconds to melt the ice. In the cases of cultured cells, callus and meristems, the samples are then diluted with liquid culture medium to remove the cryoprotectants and the viability of the cryopreserved material can be tested through regeneration or germination in the case of seeds.

Animal genetic resources

Animal genetic resources, like plant genetic resources need to be conserved for future generations. The use of artificial reproduction is a very useful tool in conservation of endangered species. It should, however, be made to complement the conventional methods of breeding. Some of the biotechnological methods used for production and conservation of animal genetic resources are summarized below:

(i) Cryopreservation

The procedure is similar to what has been described already for plants. Materials such as cells, tissues, gametes, oocytes, DNA samples etc are stored in a genetic databank for future use.

(ii) *In vitro* production embryos

Methods used in the production of embryos *in vitro* include splitting and cloning of embryos, marker- assisted selection, sexing of embryos and transfer of new genes into an embryo (First, 1992). Cloning in animals is enhanced by nuclear transplantation, a method used to produce a large number of viable identical embryos and offspring of desirable genotype in cattle, sheep, rabbits and swine. The procedure involves the separation and transfer of nuclei of a valuable embryo at a multicellular stage into enucleated oocytes at metaphase II followed by serial cloning (First, 1992).

(iii) Embryo Culture and Transfer

This technique is used to introduce fertilized embryos into surrogate mothers. Sometimes closely related species can be used to produce the offspring of an endangered species. The great majority of commercial embryo transfer is done with cattle for strictly economic reasons since the economic value of production per head is much higher for cattle (and buffaloes) than for other farm animal species (Serdel and Serdel, 1992).

(iv) Artificial insemination

This technique is useful in livestock farming. Cryopreserved sperm from selected males are thawed and introduced into ovulating females.

(v) Intracytoplasmic sperm injection

Here sperm from selected males are microinjected directly into the oocyte.

These biotechnological methods offer many advantages to conventional captive breeding procedures. Firstly, less stress is experienced since the animals do not have to be moved around. Secondly, the problem of space for keeping the animals is also solved since samples can be taken in the wild. Thirdly storage of genetic resources will help to preserve biodiversity and counter the effect of genetic drift on small populations. Fourthly, even if an animal dies, its genes will still be available for future breeding work. Also gametes can be extracted from animals that have been dead for up to 24 h and cryopreserved for future use. The main disadvantage is that sometimes preserving only the DNA samples may not be enough to conserve the entire animals as many animals need to learn behaviour (which may not be in the genes) in order to survive. Moreover, the use of

biotechnological tools for endangered species is still at a very early stage and is very expensive.

SUSTAINABLE USE OF BIORESOURCES

Wrong utilization or exploitation of bioresources has led to genetic erosion, desertification and a general threat to the survival of man. Sustainable use of bioresources demands that while utilizing the resources so generously placed by nature at our disposal, we should try not to be cruel to the environment and our children yet unborn. Biotechnology provides methods through which a balance between the economic exploitation of bioresources and their conservation for the future can be achieved. Some of the techniques of biotechnology and their applications for the sustainable use of bioresources are summarized below:

(a) Plant Cell Tissue Culture
This refers to the culture of explants usually embryos, seeds, cells (virtually any part of the plant) on specific media composed of major and minor mineral salts, iron, vitamin and a carbohydrate source (usually sucrose) and subsequently regeneration of whole plants therefrom. It has found applications in:

- (i) Mass clonal propagation; disease elimination (mainly viral); germplasm exchange; *in vitro* conservation and cryopreservation of seeds, embryos, suspension cells, meristerms and other suitable plant parts. It is especially useful for threatened plants, and crops with recalcitrant seeds and seedless polyploids.
- (ii) Embryo culture for overcoming postzygotic incompatibilities.
- (iii) Anther/pollen and ovary cultures for fast production of homozygous plants through embryogenesis and chromosome doubling. Haploids could be useful for isolation of desirable recessives.
- (iv) *In vitro* production of plant secondary metabolites.
- (v) Generation of variability in somaclones
- (vi) Somatic embryogenesis

(b) Protoplast isolation, fusion and culture. This is useful in overcoming prezygotic incompatibilities in crossing.

(c) Biological Nitrogen fixation. Used for development of biofertilizers; improvement of the capability of free-living N-fixing bacteria and development of farming systems using green algae and Azolla.

(d) Use of Molecular Markers:
Plant and animal breeders use markers to aid selection for desirable/beneficial genotypes. These molecular markers are based on DNA variation and can be grouped into two:

(i). Those based on restriction and hybridization techniques and include restriction fragment length polymorphism (RFLP), which is costly, cumbersome and use isotopes in blotting and is thus avoided by many laboratories (Eneobong 2003).

(ii). Those based on the polymerase chain reaction (PCR) is used for gene amplification and include:

- Random amplified polymorphic DNA (RAPD), otherwise called Arbitrary Primed PCR (AP-PCR). Here, a pair of DNA primers are designed to hybridize to opposite strands of the genomic DNA, acting as primers for the *in vitro* synthesis of the intercalated DNA sequence (Mignouna et al., 1999; Eneobong, 2003).
- DNA Amplification finger printing (DAF). It uses DNA primers to generate amplified products through the PCR. Such products can be stained in mercury during gel electrophoresis. The method is useful in germplasm and phylogenetic studies (Eneobong, 2003).
- Amplified fragment Length Polymorphism (AFLP). This is a genetic fingerprinting technique based on detection of selected genome restriction fragment by PCR amplification. The method is useful for detection of genetic variation *in vitro* (Ubi et al., 2003).

(e) Gene Transfers/Genetic Transformation:
The modern techniques for gene transfer are based on the natural process of transformation. They are mainly recombinant DNA technology plus tissue culture, aided by several molecular biology tools such as gene isolation, cloning and vector construction. The technique is used for production of transgenic organisms. Examples include:

- (i) Agrobacterium-mediated transfer which is quite successful for dicots but not monocots (Eneobong, 2003).
- (ii) Direct DNA uptake. This has found application more in animals than in plants. The first attempt to transfer foreign DNA in animals was done in mice by microinjection. The first transgenic sheep and pigs were reported in 1985 when a mouse metallothionin growth hormone (mMThGH) fusion gene was transferred into sheep. Since then, many transgenic farm animals have been produced on a routine basis. Examples are transgenic fish that grows 2-3 times faster than normal and is cold-tolerant was produced by microinjection of desired DNA into oocytes, transgenic cattle, sheep, swine and rabbits produced by microinjection of desirable gene into zygote to produce faster-growing animals with better meat quality, transgenic goats and sheep which produce human milk because of the transfer of human genes into such animals, and

transgenic chicken which grow faster and are tolerant to viral diseases because of the transfer of growth hormone gene as well as a gene that increases viral resistance based on interference (Bazer, 1992; Fox, 1992; Forano and Flind, 2000).

(iii) Particle mediated gene transfer, using gene gun.

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