Biotechnology: Lifeline for Endangered Biodiversity

*Coker O.M., and Oko, P.A.
Department of Wildlife and Ecotourism management, University of Ibadan

*liftingup2003@gmail.com

Abstract
Recent threats to species and ecosystems are the greatest recorded in recent history. In the face of our ever-changing environment, the threshold for the conservation of biodiversity must be raised. A possible viable method is the use of biotechnology in the conservation efforts. This review therefore highlights the use of biotechnology to conserve, maintain and enhance biodiversity. Biotechnology is any technique that uses living organisms, or substances to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses. Biotechnology has been used to improve and enhance animal and crop productivity, as well as to conserve, evaluate and utilize various aspects of biodiversity. Several biotechnological approaches are important to conserve, analyze and detect genetic diversity of rare and endangered plants and animals. Cloning, plant tissue culture, transgenesis and cryopreservation are quite advantageous and a useful technique that has served to conserve biodiversity. Biotechnological approach, though expensive, can be helpful in the conservation of biodiversity, including threatened, endangered and critically endangered species.

Keywords: Biotechnology, biodiversity conservation, threatened species, gene bank

Introduction
According to Snyder (1990), the extinction of species is an irreversible loss and it must be rigorously and intelligently resisted . Biodiversity refers to the comprehensive umbrella term for the degree of variety or variation within the natural system; both in number and frequency (Mutia, 2009). In general, it refers to the variety of all forms of life on earth. The different plants, animals, micro-organisms, the genes they contain and the ecosystem they form. Biodiversity exists at three different levels; genes, species, and ecosystems. Each of the components has its own composition, structure and function (Noss, 2005; Redford and Richter, 2001). So, preservation of biodiversity or animal genetic diversity are both historical (conservation of our natural heritage) and economical (food security and recycle capacity) (Oliehoek *et al.,* 2006). Biodiversity provides the basis for ecosystems and their services, upon which all people fundamentally depended (Cardinale *et al.,* 2012). Unfortunately, biodiversity is declining. The major causes of the loss of biodiversity is due to human activities such as habitat destruction, pollution, climate change, introduction of exotic species, human population pressure and agricultural practices (Opdam and Wascher, 2004). Currently there are more than 82,900 species on the IUCN Red List, and more than 23,900 are threatened with extinction, including of amphibians (41%), conifers (34%), coral reef (33%), mammals (25%) and birds (13%) (IUCN, 2018). Hence, biodiversity loss is one of the worlds most pressing crises. According to the United Nations Food and Agricultural Organization (FAO), global population will approach 9.1 billion by 2050 and there is a need of 70% increase in food production (FAO, 2014). Therefore, biodiversity loss threatens the very basis of more sustainable development and the quality of life. To ensure substance, FAO advised to conserve animals and plants genetic biodiversity as it is essential for future food security (FAO, 2014).

Conservation of biodiversity incorporates the preservation, maintenance, sustainable use, recovery and enhancement of the components of biological diversity (Mutia, 2009). Also, it is the protection of valuable natural resources for future generations as well as well-being of eco-system function. This increasing awareness and great concern about global biodiversity conservation, has prompted the United Nations (UN) to declare the current decade (2011-2020) as the Decade of Biodiversity and has set 2020 as the target for restoring at least 15% of degraded ecosystems as well as conserving 17% of terrestrial and 10% of inland water, marine and coastal areas (CBD, 2014; Tscharntke *et al.,* 2012). With this target at hand, biotechnology approach seems to be a viable alternative to saving threatened species. The origin of biotechnology is deep rooted in the human history from the starting of domestication of wild plants and animals till recent time (Wolfe, 2000; Brink *et al.,* 1999). Genetic manipulation by classical methods of plant breeding and selection of superior and new varieties started since prehistoric time with animal coming up more recently (Pathak and Abido, 2014). Similarly, biotechnology has been used to improve and enhance animal and crop productivity, as well as to conserve, evaluate and utilize various aspects of biodiversity. Several biotechnological approaches are important to conserve, analyze and detect genetic diversity of rare and endangered plants and animals (Khan *et al.,* 2012). Biotechnological methods are reliable and can provide continuously safe, higher quality natural products for food, pharmaceuticals and cosmetic industries. They are also applicable in preserving biodiversity in several ways (Julsing *et al.,* 2007; Nalawade *et al.,* 2003). The aim of this review is to highlight the use of biotechnology to conserve, maintain and enhance biodiversity.

Biotechnology and Biodiversity Conservation

Human population growth and the consequent consumption of resources had been identified as important drivers of global environmental change, resulting in environmental degradation, and loss of biodiversity (Van-Alphen and Bertola, 2013). Climate change and global warming affect the habitats of plants, animals, microorganisms and may lead to the alteration at genetic makeup of individual cell (Mutia, 2009). It has been suggested that, extensive and rapid land use due to human activities and climate change are the main actors of increasing species threats (Mutia, 2009). Conservation biology provides perhaps the most difficult and important questions ever faced by science (Pimm *et al.,* 2001). The problems are enormous, difficult and complex. It threaten the continued existence of human species and the future of the biosphere itself. The solutions to these problems require a major overhaul of our political and social systems. An urgent paradigm shift in human behaviour towards the environment is also required. A possible viable method is the use of biotechnology in the conservation efforts. According to Pathak and Abido, (2014), biotechnology is any technique that uses living organisms, or substances to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses. It consists of a gradient of technologies, ranging from the long-established and widely used traditional techniques to novel and advanced bio techniques of tissue-culture and transgenic methods .

According to the guidelines of International Union for Conservation of Nature, both in situ and ex situ methods are well known and applied for the conservation of Red listed organisms (IUCN, 2013). The choice of any of the methods, or a combination of both, depend on the particular case and both are complementary to each other based on the adoption of conservation policy. In situ conservation maintains and manages existing genetic diversity and viable populations of wild taxa in order to maintain biological interactions, ecological processes and functions under natural conditions (Ashmore, 1997). Being a naturally adopted process of conservation, in situ conservation is faced with some challenges such as habitat fragmentation, climate change, attack of pathogenic organisms and invasive species. Ex situ method can offer a complimentary effort, so far it is governed

with rules, regulation and adopted methodology in artificial way (Reed *et al.,* 2004). The ex situ method follows the propagation of animals and plants species, varieties and specific clones either by classical method using seed, stems, rhizomes, corms or by using biotechnological methods. Such methods include, cell, tissue or organ culture; micropropagation techniques; cryopreservation; germplasm banking; gene banking; and applied advanced research to conserve and introduce genetic modifications in the existing populations (Schemske *et al.,* 1994).

Genetic Engineering and Biodiversity Conservation

Genetic engineering is the name of a group of techniques used for direct genetic modification of organisms or populations of organisms using recombination of DNA (Montaldo, 2006). These procedures are used to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms (Izquierdo, 2001; Karp, 2002). Most techniques are related to the direct manipulation of DNA oriented to the expression of particular genes. In a broader sense, genetic engineering involves the incorporation of DNA markers for selection (marker-assisted selection, MAS), to increase the efficiency of the so called 'traditional' methods of breeding based on phenotypic information (Montaldo, 2006). The most accepted purpose of genetic engineering is focused on the direct manipulation of DNA sequences. These techniques involve the capacity to isolate, cut and transfer specific DNA pieces, corresponding to specific genes (Lewin, 1999; Klug and Cummings, 2002). The mammalian genome is large and has a more complex organization than in viruses, bacteria and plants (Montaldo, 2006). Consequently, genetic modification of animals, using molecular genetics and recombinant DNA technology is more difficult and costly than in simpler organisms. In mammals, techniques for reproductive manipulation of gametes and embryos such as obtaining of a complete new organism from adult differentiated cells (cloning), and procedures for artificial reproduction such as in vitro fertilization, embryo transfer and artificial insemination, are frequently an important part of these processes (Murray *et al.*, 1999; Izquierdo, 2001).

Transgenesis

Transgenesis is a mode of experimentation involving insertion of foreign gene into the genome of an organism, followed by germ-line transmission of the gene and analysis of the resulting phenotype in the progeny (Renaud and Soares, 2011). The first microinjection of genetic material in mammals was reported in 1966, but it took 15 years until Gordon *et al.*, (1980) reported the first transgenic mice by microinjection. Two years later, Palmiter *et al.,* (1982) produced the first transgenic mice growing twice than normal, and three years later the first transgenic livestock was reported (Hammer *et al.,* 1985). This created enormous prospect about what could be done with transgenic animals, particularly in the field of animal production. In the last 25 years predictions about the future of transgenesis and genetic engineering have been invariably optimistic. Few successes of transgenic animals are now available: myo-inositol hexakisphosphate (M. phytase) transgenic pigs (Golovan *et al.,* 2001) digest phytase better; lysostaphin transgenic cows (Wall *et al.,* 2005) have better resistance to mastitis; bovine κ- and β-casein transgenic cows (Brophy *et al.,* 2003) possess better milk composition; -lactalbumin transgenic pigs (Noble *et al.,* 2002) have better litter weight at weaning; lysozyme transgenic goats (Maga *et al.,* 2006) have healthier udder. Some other few transgenic animals have also been reported; sheep with more/clear fleece (Damak *et al.,* 1996), leaner pigs (Pursel *et al.,* 1999; Lai *et al.,* 2006). Transgenic livestock and plants/crops can be produced for conservation, food security, better yield and resistance to the effect climate change. Also, food security reduces the pressure on the wild species as alternative sources of protein, thereby conserving biodiversity.

Cloning

According to the National Human Genome Institute, NHGRI (2017), cloning is a number of different processes used to produce

genetically identical copies of a biological entity. The copied material, which has the genetic makeup as the original, is referred to as the clone. Cloning from somatic cells can be used to repeat the genotype of valuable animals (Blasco, 2008). Since transgenic organisms usually have reproductive problems, cloning may help in transferring and multiplying the results of transgenesis, thereby saving biodiversity (Blasco, 2008). Cloning and the reconstruction of functional DNA from extinct species has been a dream for decades. Possible implications of this were dramatized in the 1984 novel Carnosaur and the 1990 novel Jurassic Park (Ehrenfeld, 2006; Holt *et al.,* 2004). The best current cloning techniques have an average success rate of 9.4 to 25 percent (Ono *et al.,* 2010; Wakayama *et al.,* 2013) when working with familiar species, while cloning wild animals is usually less than 1 percent successful (Jabr, 2013). Several tissue banks have come into existence, including the "Frozen Zoo" at the San Diego Zoo, to store frozen tissue from the world's rarest and most endangered species (Pence, 2005; Holt *et al.,* 2004). The modern cloning techniques involving nuclear transfer have been successfully performed on several species including Northern leopard frogs, sheep, endangered Asian ox, rat, horse, water buffalo, extinct Pyrenean ibex (Sinha, 2009; Gray and Dobson, 2009). Tissue samples, frozen from the last Pyrenean ibex (*Capra pyrenaica pyrenaica*) which died in the year 2000 was cloned in year 2009 (Gray and Dobson, 2009). Using polymerase chain reaction (PCR), geneticists at the Australian Museum replicated the DNA of the extinct Tasmanian tiger (Holloway, 2002).

Many of the cloned species had health and life span issues. But some Japanese researchers created 25 generations of healthy cloned mice with normal lifespans, showing that clones are not necessarily intrinsically shorter-lived than naturally born animals (Wakayama *et al.,* 2013). Other sources have noted that the offspring of clones tend to be healthier than the original clones and indistinguishable from animals produced naturally (Carey, 2012).

Plant Tissue Culture

Plant tissue culture (PTC) is a quick, season independent and efficient in-vitro technique to propagate plants under sterile micro environment. It is very a effective method of cloning of plant material and to develop disease free clean plant stock. There are different types of culture methods using different organs (Chawla, 2009). The technique of different types of culture is applied with several objectives, the most important one is the enhancement of plant production rate by quick regeneration of plants in the absence of seed, or otherwise by using the seeds which have very low chances of germination capability (Abo, 1997; Ellis, 1991;). Different techniques in PTC may offer certain advantages over traditional methods of propagation for assembly, proliferation, preservation and storage of plant genetic resources (Bunn *et al.,* 2007). The success of plant tissue culture depends on the success of shoot regeneration in a rapid and reproducible way. It has great importance in the crop improvement program which is facing the increasing depletion of natural resources. Moreover, tissue culture techniques can be applied in germplasm conservation of medicinally important plants. It can be applied for regenerating different clean disease free stock of plants in the field of agriculture, horticulture, floriculture and pharmaceutical industry (Fischer *et al.,* 2004). Tissue culture is a useful technique to preserve somatic embryos which can be applied in the medium and long-term conservation process. The cultivation and conservation of the new germplasms in the changed environmental situation can be able to add some specific impact in changing environmental situations. Rapid and mass propagation of plant species and their long term germplasm storage can be achieved in a small space within short period, with no damage to the existing population using PTC techniques. Plant material can be produced throughout the year without any seasonal limitation. Large numbers of uniform and disease-free, virus free plants can be produced from very small portions of the mother plant

due to the aseptic nature of tissue culture technique. The sterile nature of in vitro cultures facilitates the exchange of germplasm or plant materials even at international level (Sharma and Sharma, 2013). Genetic resources of recalcitrant seeds which are difficult to germinate, vegetatively propagated plants, rare and threatened plant species, elite crop varieties and some genetically modified plant materials can be efficiently multiplied and stored on long term basis by using in vitro techniques (Lidder and Sonnino, 2011).

Cryopreservation

Cryopreservation is one of the biotechnological methods of ex situ plant conservation and it is applicable for long term storage of plant genetic material. Cryopreservation is an extremely helpful method to conserve rare, endangered, threatened plant species (Paunescu, 2009; Zhao *et al.,* 2008;). It is another genome conservation technique in which living tissues are conserved at very low temperatures (196°C) in liquid nitrogen to arrest mitotic and metabolic activities (Dulloo *et al.*, 2010). It is now realized that cryopreservation method can offer greater security for long-term, cost effective conservation of plant genetic resources, including orthodox seeds (Dulloo *et al.*, 2010). The storage in liquid nitrogen clearly prolonged shelf life of lettuce seeds with half-lives projected as 500 and 3400 years for fresh lettuce seeds stored in the vapor and liquid phases of liquid nitrogen, respectively (Dulloo *et al.*, 2010). Maintenance of cryogenic cultures in liquid nitrogen at -196C or in the vapor phase at -135C is in such a way that the viability of stored tissues is retained following re-warming. The cryopreserved tissue is considered as safe, clean, disease free and genetically stable and suitable for international exchange (Liu *et al.,* 2008; Feng *et al.,* 2011).

In-Vitro Gene Bank

The maximum possible genetic diversity of a particular genetic stock can be maintained using in vitro gene bank technique. It involves culturing of different parts of the plant (meristem, tissues, and cells) into pathogen-free sterile culture in a synthetic medium with growth retardants, which has been cited as a good way of complementing and providing backup to field collections (Dulloo *et al.*, 2010). There are about 7.4 million Plant Genetic Resources for Food and Agriculture, PGRFA accessions conserved in over 1750 gene banks (Dulloo *et al.,* 2010). The Frozen Ark database holds details of 28,060 frozen DNA samples. Among these 6,997 are from species listed in the IUCN Red List (Reid *et al.*, 2013). The principal aim of gene bank conservation is to maintain genetic diversity alive as long as possible and to reduce the frequency of regeneration that may cause the loss of genetic diversity (Dulloo *et al.*, 2010).

With the rapid development in the field of molecular genetics and genomics, DNA is becoming more and more in demand for molecular studies and is one of the most requested materials from gene banks. Some efforts have been made to establish DNA banks for endangered animals (Ryder *et al.*, 2000), and a few plant DNA banks in different parts of the world such as the Missouri Botanic Garden, Kew Royal Botanic Garden and Australian Plant DNA Bank. Many research groups are already developing their own archives of extracted genomic DNA. The Global Biodiversity Information Facility in Germany has established a DNA bank network, which provides DNA samples of microorganisms, protists, plants, algae, fungi, and animals (Dulloo *et al.*, 2010).

Seeds are usually the most convenient and easiest material to collect and to maintain in a viable state for long periods of time and that makes it preferred for conservation in gene banks (Brutting *et al.*, 2013). Seed banking techniques rely on the storage of dried seeds of threatened plants or other plants at low temperatures. (Brutting *et al.*, 2013; Dickie *et al.*, 1990) Seeds are typically conserved at moisture content between 3 and 7 percent and stored at 4 degrees Celsius for short-term conservation, and between -18 and -20 degrees celsius for long-term conservation (Dulloo *et al.*, 2010).

The maximum possible genetic diversity of a particular genetic stock can be maintained using in vitro gene bank technique.

Several international organisations are engaged potentially to conserve disease free, clean and elite class of genetic stock and using this process mainly following slow growth, in vitro techniques, cryopreservation of several stocks together with routine analysis of genetic diversity. The International Plant Genetic Resources Institute (IPGRI), International Institute of Tropical Agriculture (IITA), Consultative Group on International Agricultural Research (CGIAR) and International Center for Agricultural Research in the Dry Areas (ICARDA) are involved in the conservation of rare and endangered plant species by maintaining in vitro gene bank (Reed *et al.,* 2004; IITA, 2011; ICARDA, 2014). Determination of storage conditions, provision of inventory, evaluation of viability and verification of genetic stability are very important components of gene bank structure (Khan *et al.,* 2012).

Challenges of Biotechnology in the Conservation of Biodiversity

Inadequate Expertise

Biotechnology is a technology that has a wide application across several sectors of development. Various disciplines, among which are biochemistry, the engineering sciences, genetics, informatics, molecular biology, microbiology, the neurosciences and nanotechnology amongst others, need biotechnology in one way or the other. Deficiency in professionality, and national legal instruments concerning patents and intellectual property rights, and of financial support widen the gap in biotechnology development between the industrialized and developing countries (Dasilva *et al.,* 2002). Hence there is a distinct need for education and capacity-building important elements in the use of biotechnology for development. Other challenges faced include shortage of staff.

High Cost and Inadequate Funding

Biotechnology developments need high inputs of finance for sophisticated equipment and relevant infrastructure, which are in short supply in most developing nations. Life spans of research capacities in sub-Saharan African countries in plant and animal biotechnology are limited in scope and are donor-dependent (Dasilva *et al.,* 2002). Many research institutions and Universities are usually inadequately funded. This indeed, hampers their efficiency. More so, the majority of institutions could not go on the routine exploration and collection of germplasms to boost their biodiversity collections, because of a shortage of funds (Borokini, 2013).

Ethical Issues in Biotechnology Techniques

The development and regulation of biotechnology has brought ethical concerns and issues to the foreground (OECD, 2008). Diverging views have been expressed, as representations of our "natural" world were being challenged. It is true that biotechnology has the potential for enormous contributions to science, medicine and biodiversity conservation. What remains uncertain is whether engineering new life and modifying existing forms is ethical in modern society. There is a need to balance between the risks and benefits involved (Macer, 2004).

The advantages of using transgenic animals can be divided into three broad categories: medical, scientific, and food benefits. Medical advantages are seen with disease models. Scientific benefits are seen in some animals engineered to over-express a specific protein to help elicit a newly discovered proteins function. Food benefits are seen in super fish that grow faster and larger than regular fish (Camara *et al.,* 2008). Additionally, disease resistance and faster production times offer even more incentive to progress. The potential to successfully utilize transgenic animals is huge, as is the possibility of exploiting them. One fact to keep in mind when discussing the tampering with genes and genomes is that, humans have been doing it for centuries via selective breeding and crossbreeding. Selective breeding has given us the broiler chicken which grows to approximately 2 kg in about 40 days, half the time it took 30 years ago (Camara *et al.,* 2008). What is really germane is a balance between the possible risks and benefits.

Conclusion

Biodiversity is the very basis of human survival and economic development. Ever increasing loss of biodiversity poses a serious threat to the

survival of mankind. Worldwide, vast numbers of biodiversity are threatened. Since the conservation of biodiversity is a global concern, several strategies has been adopted in understanding and conserving biodiversity throughout the world. It is now well recognized that an appropriate conservation strategy for a particular genotype requires combining approach of ex-situ and in-situ techniques. Biotechnological approach, though expensive, can be helpful in the conservation of threatened, endangered and critically endangered biodiversity.

References

Abo E. M. (1997). Role of plant tissue culture propagation in the rehabilitation of desert environment. In:Alawadhi, N., Bbalba, M. T., Kamizawa, C. (Eds.) Restoration and rehabilitation of the desert environment, Elsevier, Amsterdam, pp. 197-205.259

Ashmore, S. E. (1997). Status report on the development and application of in vitro techniques for the conservation and use of plant genetic resources. IPGRI, Rome, Italy, pp. 67-68.

Blasco, A. (2008). The role of genetic engineering in livestock production. *Livest Sci* 113:191201.

Borokini, T. I. (2013). The State of Ex-Situ Conservation in Nigeria. *Int J Conserv Sci* 4 (2): 197-212. ISSN: 2067-533X

Brink, J. A., Prior, B., and Da Silva, E. J. (1999). Developing biotechnology around the world. *Nature Biotechnology* 17: 434-437.

Brophy, B., Smolenski, G., Wheeler, T., Wells, D., L'huillier, P. and Laible, G. (2003). Cloned transgenic cattle produce milk with higher levels of beta-casein and kappa-casein. *Nature Biotechnology* 21(2) 157-162.

Brutting, C., Hensen, I. and Wesche, K. (2013). Ex situ cultivation affects genetic structure and diversity in arable plants. *Plant Biology* 15: 505513.

Bunn, E., Turner, S. R., Panaia, M., Dixon, K. W. (2007). The contribution of in vitro technology and cryogenic storage to conservation of indigenous plants. *Australian Journal of Botany* 55: 443-450.

Carey, N. (2012). *The Epigenetics Revolution*. London, UK: Icon Books Ltd. pp. 149 150. ISBN 978-184831-347-7.

Cardinale, B. J., Duffy, E., Gonzalez, A., Hooper, D., Perrings, C., Venail, P., Narwani, A., Mace, G., Tilman, D., Wardle, D., Kinzig, A., Daily, G., Loreau, M., Grace, J., Larigauderie, A., Srivastava, D., and Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature* 486: 59-67.

Camara, D., Dimitrova, I. R., Doynova, M., Jachacz, L., Kachakova, D., Kepka, M., Ould-Isselmou, CB., Vorniere, J. P., Yungarva, T.S. (2008). Transgenic and cloned animals: Ethical Problems? Bio thique en science de la vie et de lenvironnement Pp. 1-20. http://bioethics.agrocampusouest.eu/infoglueDeliverLive/digitalAssets/74558_transgenic_r.pdf.

Chawla, H. S. (2009). Germplasm storage and cryopreservation of genetic material. In: Chowla HS (Ed) Introduction to biotechnology. Oxford and IBH Publishing Company Pvt. Limited, 3rdEdition, New Delhi, India, pp. 245-258.

Damak, S., Su, H., Jay, N. P., and Bullock, D. W. (1996). Improved wool production in transgenic sheep expressing insulin- like factor I. *Biotechnology* 14: 185-188.

Dasilva, E J., Baydoun, E. and Badran, A. (2002). Biotechnology and the

developing world. *EJB Electronic Journal of Biotechnology*. 5 (1). Available from: DOI: 10.2225/vol5/-issues1-fulltext-1 ISSN 0717-3458.

Dickie, J.B., Ellis, R. H., Kraak, H. L., Ryder, K. and Tompsett, P. B. (1990). Temperature and seed storage longevity, *Annals of Botany* 65(2): 197204.

Dulloo, M. E., Hunter, D. and Borelli, T. (2010). Ex situ and in situ conservation of agricultural biodiversity: major advances and research needs, *Notulae Botanicae Horti Agrobotanici ClujNapoca*.38(2) 123135.

Ehrenfeld, D. (2006). "Transgenics and Vertebrate Cloning as Tools for Species Conservation". *Conservation Biology*. 20 (3): 723732. doi:10.1111/j.1523-1739.2006.00399.x. PMID 16909565.

Ellis, R. H. (1991). The Longevity of Seeds. *Horticultural Science* 26: 1119-1125.

FAO, Food and Agricultural Organization, (2014). The International Treaty on Plant Genetic Resources for Food and Agriculture available on http://www.planttreaty.org/content/texts-treaty-officialversions access on March 25, 2014.

Feng, C., Yin, Z., Ma, Y., Zhang, Z., Chen, L., Wang, B., Li, B., Huang, Y., and Wang, Q. (2011). Cryopreservation of sweet potato (Ipomoea batatas) and its pathogen eradication by cryotherapy. *Biotechnology Advance* 29: 84-93.

Fischer, R., Stoger, E., Schillberg, S., Christou, P., Twyman, R. M. (2004). Plant-based production of biopharmaceuticals. *Current Opinion Plant Biology* 7: 152-158.

Golovan, S. P. Meidinger, R. G. Ajakaiye, A., Cottril, M. Wiederkehr, M. Z., Barney, D., Plante, C. Pollard, J. Fan, M. Z., Hayes, M. A., Laursen, J. Hjorth, J. P, Hacker, R. R., Philips, J. P. and Forsberg, C. W. (2001). Pigs expressing salivary phytase produce low phosphorus manure. *Nat. Biotechnol* 19: 741-745.

Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A., and Ruddle, F. H. (1980). Genetic transformation of mouse embryos by microinjection of purified DNA. Proceedings of the National Academy of Sciences of the United States of America, December 1980, vol. 77, no. 12, p. 7380-7384.

Gray, R. and Dobson, R. (2009). Extinct ibex is resurrected by cloning. The Telegraph. London. http://www.telegraph.co.uk/news/science/science-news/4409958/Extinct-ibex-is-resurrected-by-cloning.html. Retrieved June 28, 2017.

Hammer, R. E. Pursel, V. G., Rexroad. C. E. Wall, R. J. Bolt, D. J. Ebert, K. M., Palmiter, R. D., and Brinster, R. L. (1985). Production of transgenic rabbits, sheep and pigs by microinjection. *Nature* 315: 680-683.

Holloway, G. (2002). "Cloning to revive extinct species". Cable News Network. http://edition.cnn.com/2002/WORLD/asiapcf/auspac/05/28/aust.thylacines

Holt, W. V., Pickard, A. R., and Prather, R. S. (2004). Wildlife conservation and reproductive cloning. *Reproduction*, 127: 317 -324.

ICARDA, Genebank.International Center for Agricultural Research in the Dry Areas (2014). Genebank.International Center for Agricultural Research in the Dry Areas. Available on http://www.icarda.cgiar.org/research -sub/biodiversity-and-its utilization.

IITA, International Institute of Tropical Agriculture. (2011). Germplasm collections,conservation health documentation and distribution. https://www.slideshare.net/IITA-CO/75-agrobiodiversity-dumetreview. Retrieved June 17, 2017.

IUCN, (2018). A campaign to support the IUCN Red List. http://support.iucnredlist.org/about. Retrieved January 14, 2018.

IUCN, International Union of Conservation of Nature. (2013). www.iucn.organization access on May 25, 2014.

Izquierdo, R. M. (2001). Ingeniera gen tica y transferencia g nica. 2nd ed. Madrid, Ediciones Pir mide, 2001. 344 p. ISBN 84-368-1563-7.

Jabr, F. (2013). Will cloning ever saved endangered species? Scientific American https://www.scientificamerican.com/article/cloning-endangered-animals. Retrieved January 16, 2018.

Julsing, K., Matthys, K., Wim, J. Q., and Kayser, O. (2007). The engineering of medicinal plants: prospects and limitations of medicinal plant biotechnology. In: Kayser, O. and Wim, J. Q., (Eds) Medicinal Plant Biotechnology: From Basic Research to Industrial Application. WILEY-VCH Verlag GmbH &Co.KGaA-Weinheim, Germany, pp. 3-8.

Khan, S., Al-Qurainy, F., and Mohammad, N. (2012). Biotechnological approaches for conservation and improvement of rare and endangered plants of Saudi Arabia. *Saudi Journal of Biological Sciences* 19: 1 -11.

Klug, W. S. and Cummings, M. R. (2002). *Concepts of Genetics*. 7th ed. New Jersey, Prentice Hall, 2002. p. 800. ISBN 0130929980.

Lai, L. X., Kang, J. X., Li, R. F., Wang, J. D., Witt, W. T. Yong, H. Y., Hao, Y. H., Wax, D. M., Murphy, C. N., Ricke, A., Samuel, M., Linvelle, M. L., Korte, S. W., Evans, R. W., Starzl, T. E., Prather, R. S., and Dai, Y. F. (2006). Generation of cloned transgenic pigs rich in omega-3 fatty acids. *Nat. Biotechnol*. 24: 435-436.

Lewin, B. (1999). *Genes VII*. Oxford, Oxford University Press, 1999. p. 990. ISBN 01-987-9276-X.

Lidder, P., and Sonnino, A. (2011). Current status of biotechnologies for the management of crop genetic resources. In: Lidder. P., Sonnino, A. (Eds.) Biotechnologies for the management of genetic resources for food and agriculture, FAO, pp. 1-153.

Liu, Y. G., Liu, L. X., Wang, L., Gao, A. Y. (2008). Determination of genetic stability in surviving apple shoots following cryopreservation by vitrification. *CryoLetters* 29: 7

Maga, E. A., Cullor, J. S., Smith, W., Anderson, G.B., and Murray, J. D. (2006). Human lysozyme expressed in the mammary gland of transgenic dairy goats can inhibit the growth of bacteria that cause mastitis and the cold-spoilage of milk. Foodborne Pathogens Disease 3:384-392.

Macer, D. R. J., (2004). Bioethics education for informed citizens across cultures. *School Science* 86: 315

Montaldo, H. H. (2006). Genetic engineering applications in animal breeding. *Electronic Journal of Biotechnology*. Vol 9 (2) : 1-6.

Murray, J. D., Oberbauer, A. M. and Mcgloughlin, M. M. (1999). *Transgenic animals in Agriculture*. Davis, CABI Publishing, 1999. p. 304.

Mutia T. M. (2009). Biodiversity Conservation: Presented at Short Course IV on Exploration for Geothermal Resources, Naivasha, Kenya. Pp. 1 9.

Nalawade, S. M., Sagare, A. P., Lee, C. Y., Kao, C. L., and Tsay, H. S. (2003). Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. *Botanical Bulletin of Academia Sinica* 44: 79-98.

National Human Genome Institute, NHGRI (2017). Cloning Fact Sheet.

https://www.genome.gov/25020028/cloning-fact-sheet. Retrieved March 21, 2017.

Noble, M. S., Rodriguez, Z. S. Cook, J. B., Bleck, G. T., Hurley, W. L., and Wheeler, M. B. (2002). Lactational performance of first- parity transgenic gilts expressing bovine alpa lactalbumin in their milk. *J. Amin Sci* 80: 1090-1096.

Noss, R. F. (2005). Indicators for Monitoring Biodiversity: A Hierarchical Approach. *Conservation Biology* 4: 355-364.

Oliehoek, P. A., Windig, J. J., van Arendonk , J. A. M.; and Bijma, P. (2006). Estimating Relatedness Between Individuals in General Populations with a Focus on Their Use in Conservation Programs. *Genetics*, 173 (1): 483-496.

Organisation for Economic Co-operation and Development, OECD, (2008). Biotechnology: Ethical and social debates. https://www.oecd.org/futures/long-termtechnologicalsocietalchallenges/40926844.pdf. Retrieved, February 19, 2018.

Ono, T., Li, C., Mizutani, E., Terashita, Y., Yamagata, K. and Wakayama, T. (2010). "Inhibition of class IIb histone deacetylase significantly improves cloning efficiency in mice". *Biol Reprod*. 83 (6): 929 37.

Opdam, P. and Wascher, D. (2004). Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation* 117: 285297.

Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E.; Rosendfeld, M. G., Birnberg, N. C. and Evans, R. M. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* 300: 611-615.

Pathak, M. R. and Abido, M. S. (2014). The Role of Biotechnology in the Conservation of Biodiversity. *Journal of Experimental Biology and Agricultural Sciences*. 2(4): 352-363.

Paunescu, A. (2009). Biotechnology for Endangered Plant Conservation: A Critical Overview. *Romanian Biotechnological Letters* 14: 4095-4103.

Pence, G. E. (2005). *Cloning After Dolly: Who's Still Afraid*? Rowman and Littlefield. ISBN 0-7425-3408-1.

Pimm, S. L., Ayres, M., Balmford, A., Branch, G., Brandon, K., Brooks, T., Bustamante, R, Costanza, R., Cowling, R., Curran, L. M., Dobson, A., Farber, S., Da-Fonseca, G. A. B., Gascon, C., Kitching, R., McNeely, J., Lovejoy, T., Mittermeier, R. A., Myers, N., Patz, J. A., Raffle, B., Rapport, D., Raven, P., Roberts, C., Rodriguez, J. P., Rylands, A. B., Tucker, C., Safina, C., Samper, C. Stiassny, M. L. J., Supriatna, J., Wall, D. H., and Wilcove, D. (2001). Environment can we defy natures end? *Science* 293: 2207 2208.

Pursel, V. G., Wall, R. J., Michell, A. D., Elsasser, T. H., Solomon, M. B., Coleman, M. E., DeMayo, F. and Scwartz, R. J. (1999). Transgenic animals in Agriculture. In: Expression of insulin-like growth factor I transgene. *Anim Biotechnol* 15: 33-45.

Redford, K. H., Richter, B. D. (2001). Conservation of Biodiversity in a World of Use. *Conservation Biology* 13: 1246-1256.

Reed, B. M., Engelmann, F., Dulloo, M. E., and Engels, J. M. M., (2004) Technical guidelines for the management of field and in vitro germplasm collection. Handbook for Genebanks No.7, IPGRI/SGRP, Rome.

Reid, G. M., Macbeath, T. C. and Csatadi, K. (2013). Global challenges in freshwater-fish conservation related to

public aquariums and the aquarium industry . *International Zoo Yearbook* .47: 645.

Renaud, J. S. and Soares, M. J. (2011). *Strategies for investigating hemochorial placentation: Reproductive and Developmental Toxicology*. Academic Press pp. 10291038.

Ryder, O. A., McLaren, A., Brenner, S., Zhang, Y. P., and Benirschke, K. (2000). DNA banks for endangered animal species, *Science* 288, no. 5464, pp. 275277.

Schemske, D. W., Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M., and Bishop, J. G. (1994). Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75: 584 -606

Convention on Biological Diversity, CBD. (2014). Aichi Biodiversity Targets. Available on http://www.cbd.int/sp/targets/ access on May 12, 2014.

Sharma, D. K., and Sharma, T. (2013). Biotechnological approaches for biodiversity conservation. *Indian Journal of Scientific Research* 4: 183-186.

Sinha, K. (2009). "India clones world's first buffalo". The Times of India. https://timesofindia.indiatimes.com/india/India-clones-worlds-first-buffalo/articleshow/4120044.cms?referral=PM Retrieved August 4, 2010.

Snyder, G. 1990. *The Practice of the Wild*. North Point Press, San Francisco.

Tscharntke, T., Bat ry, P., Clough, Y., Kleijn, D., Scherber, C., Thies, C., Wanger, T. C., and Westphal, C. (2012). Combining biodiversity conservation with agricultural intensification. In: Lindenmayer, D., Cunningham, S., and Young, A. (Eds.) Land use intensification -Effects on agriculture, biodiversity and ecological processes. CSIRO Publishing, Collingwood, Australia, pp. 7-15.

Van-Alphen, J., and Bertola, L. (2013). Conservation Biology and Wildlife Genetics lectures presented at Kathmandu University, Dhulikhel, Nepal. pp. 15 19.

Wakayama, S. I., Kohda, T., Obokata, H., Tokoro, M., Li, C., Terashita, Y/, Mizutani, E., Nguyen, V.T., Kishigami, S., Ishino, F., and Wakayama, T. (2013). "Successful serial recloning in the mouse over multiple generations". *Cell Stem Cell*. 12 (3): 2937.

Wall, R. J., Powell, A. M., Paape, M. J., Kerr, D. E., Bannnerman, D. D., Pursel, V. G. Wells, K., Talbot, N. and Hawk, H. W. (2005). Genetically enhanced cows resist intramammary Staphylococcus aureus infection. *Nat. Biotechnol* 23: 445-451.Wolfe, M. S. (2000). Crop strength through diversity. *Nature* 406: 681 682.

Zhao, Y., Wu, Y., Chang, Y., and Reed, B. M. (2008). Cryopreservation of fruit and ornamental trees. Plant conservation: a practical guide pp 387-420.