

# Assess Molecular Marker Applications for Genetic Variety Analysis In Biodiversity Conservation Status

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## Abstract

As study conducted biodiversity is vital for human wellbeing and its conservation is a critical issue at this time throughout the world. In fact, analysis of genetic diversity, identification of individual organisms, monitoring genetic integrity and characterization and evaluation of biological resources are initial requirements for biodiversity conservation and its management.

Genetic diversity is the variety of genetic information contained in all of the individual plants, animals and microorganisms within populations of species. Genetic diversity analysis is the most important to assess genetic variation among individuals and help us in determining what to conserve as well as where to conserve. Characterization is an activity of assessing genetic diversity within collections in the context of the total available genetic diversity for each species. Types of markers are Classical Marker Classification and Molecular markers. These markers are used in most biodiversity conservation activities such as identification and genetic diversity analysis, characterization of biological diversity and genetic integrity monitoring and illegal wildlife trafficking. Molecular markers are important for biodiversity conservation such as identification of organisms, genetic diversity study (analysis), characterization of diversity, genetic integrity monitoring for conserved seeds in gene-bank, and illegal wildlife trafficking. As concluding this study were discuss the principal application, classification, and types of molecular marker in biodiversity and the main molecular marker types such as AFLP, RFLP, RAPD and SSR are discussed as well as their specific application in biodiversity conservation and management such as genetic diversity analysis, and characterization of germplasm

**Keywords:** Molecular Marker. Molecular marker application. Biodiversity. Conservation. Genetic Variation analysis. Types of Markers.

## Introduction

As stated in the Convention on Biological Diversity, encompasses "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (CBD 1992). Biodiversity also includes genetic differences within each species - for example, between varieties of crops and breeds of livestock. Chromosomes, genes, and DNA-the building blocks of life-determine the uniqueness of each individual and each species [1]. Biodiversity have tremendous importance for human wellbeing such as promoting the aesthetic value of the natural environment, contribution to our material well-being through utilitarian values, maintaining the integrity of the environment by maintaining CO<sub>2</sub>/O<sub>2</sub> balance, regulation of biochemical cycles, absorption and breakdown of pollutants and waste materials through decomposition, determination and regulation of the natural world climate, protective services through acting as windbreaks and acting as indicators of environmental changes [2]. Hence, it's vital to conserve each component of biological diversity.

Biodiversity conservation means the methods of saving life varieties on the Earth in all its forms and keeping natural ecosystems functioning and healthy. This integrates the preservation, maintenance, sustainable use, recovery and enhancement of the components of biological diversity. The conservation of biological diversity requires the investigation and assessments of each component that makes it. Several ways to quantify biological resource developed such as studying genetic variation through genetic diversity analysis, identification of each organism, population structure and characterization and evaluation of biodiversity and its component (IPGR, 2003) but also enables to identify best traits contained in biological resource which will be used by breeder for further improvement to meet the demand of the increasing world

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population. Therefore such study is also not only important to biodiversity conservation and planning its management and sustainable benefit sharing.

Analysis of genetic diversity, identification of individual organisms, and characterization and evaluation of biological resources requires molecular markers and its knowledge. Molecular markers are specifically DNA-based markers which can provide a good estimation of genetic diversity and biodiversity conservation. A molecular marker is a DNA sequence with a known location on the chromosome and whose inheritance of biodiversity structure that can be monitored [3]. The development of molecular markers depends on polymorphisms found in DNA, and the information obtained in biological diversity can be used to measure the relationships between organisms and other genetic diversity studies Therefore, molecular markers have vital importance in biodiversity conservation [4]. Therefore, this study intended to discuss the classification of various molecular markers was deliberated as well as the application of the main molecular marker in biodiversity conservation, ways of genetic diversity analysis, composition, status of risky, management and sustainable use.

## Methods of Biodiversity Analysis

### Biodiversity and its conservation

The biodiversity is considered to incorporate variation at all levels within and among ecosystems, communities, species, and populations. It is now common practice to define biodiversity in terms of genes (intra-population diversity or genetic diversity), species and ecosystems conforming to three fundamental and hierarchically related levels of biological organization. We are directly or indirectly dependent on Biodiversity, So Biodiversity is vital application in our life hood safely on the earth. Currently, the main threat to biological resource are: habitat alteration and destruction by the land use change, over exploitation of biological resources, climate change, pollution and invasive species as well as the spread of the very improved varieties whose existence depends on this diversity [5-7]. Establishing schemes in maintaining and/or conserving biodiversity is a means of securing these resources in the light of such pressing concerns [8]. Generally Biodiversity can be considered at 3 major levels [9].

**Genetic diversity:** This is the variety of genetic information contained in all of the individual plants, animals and microorganisms occurring within

populations of species. Simply it is the variation of genes within species and populations.

**Species diversity:** This is the variety of species or the living organisms. It is measured in terms of- Species Richness - This refers to the total count of species in a defined area. Species Abundance - This refers to the

**Ecosystem diversity:** This relates to the variety of habitats, biotic communities and ecological processes in the biosphere.

## Genetic Variation Analysis

The term "Genetic variation" refers to all forms of genetic variation within a species, including neutral and functional sequence variations and variation in gene expression [10]. More strictly, though, the term refers specifically to the variability and variety within species, among species and among the ecological processes that connect them and it's also called polymorphism [11-13]. 'Genetic diversity' refers to the variation of genes within species, that is to the mix of genes contained within individuals and distinct populations of the same species or genetic variation within a population. Ultimately, genetic diversity resides in changes in the sequence of the four base-pairs of the DNA that constitutes the genetic code [14]. Variety means the existence of differences and Variation is the measure of variety, the extent or range of difference, whereas Variability means the ability to vary, or the capacity to change.

Generally genetic differences are found between individuals within a population, and differences are also found in allelic frequencies between populations. In all, the relative amount of variation depends on the species, history and environment. Some of this variation influences morphological, physiological, and other types of functional genetic variation that affect the performance of individuals and populations. Therefore, this "functional (non- neutral) genetic variation" has important consequences for population dynamics, species interactions, and ecosystem function. Variations of biological diversity were occurred at the DNA level [15].

- At Single nucleotide polymorphisms (SNPs)
- At Insertions or deletions (Indels)
- At Variable number of tandem repeats (VNTRs)

## Source of Genetic Variation In Biodiversity

Genetic diversity arises primarily as variants in the linear sequence of nucleotides in DNA. Mutations can happen in the coding region of genes, or the spacer regions within and between genes, in the number of copies of genes, the linkage relation between several genes or indeed in whole chromosomes [16] as well as sexual recombination [17]. According to the report conducted by [18] there are three methods to detect the polymorphism of biological diversity.

1. Southern blotting, a nucleic acid hybridization technique,
2. PCR, a polymerase chain reaction technique as well as
3. Microarray chip techniques use DNA hybridization combined with labeled nucleotides, and new sequencing techniques detect polymorphism by sequencing.

## Importance Of Genetic Variation For Biodiversity

- The inherited differences between individual plants or groups of plants are the basis of all improvements sought by plant breeders [19].
- Genetic diversity allows populations to adapt to changing environmental conditions and developed traits that resisted change conditions [20].
- To developed a valuable natural resources and important modified traits to plays a key role for future breeding and conservation [21].
- Variation are used as a resource for tree breeding and improvement programs to developed and well-adapted tree species varieties and to enhance the genetic gain for a multitude of useful traits [22].
- Diversity in plant genetic resources (PGR) provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits (yield potential

and large seed) and breeders preferred traits (pest and disease resistance and photosensitivity) [23].

## Genetic Diversity Analysis Of Biodiversity

Genetic diversity analysis is the most important to assess genetic variation among individuals. The presence of variants (polymorphism) in a sample of biodiversity may be assessed by its genotypes, alleles, haplotypes or nucleotides [24]. Samples biodiversity can be hierarchically divided at the level of the species population or within the population and sampling size should be sufficient when the goal is to study genetic variation. Generally genetic diversity analysis is vital to biodiversity conservation. A better understanding of genetic diversity and its distribution is essential for its conservation and use. And also genetic diversity analysis will help us in determining what to conserve as well as where to conserve, how will be improved our understanding of the taxonomy, origin and evolution of plant species of interest [25,26]. In order to manage conserved germplasm of biodiversity there is also a need to understand the genetic diversity that is present in collections.

Information of the genetic diversity of the threatened tree species in any region of the world may contribute to the creation of effective strategies for their preservation and future use [27]. Nowadays, molecular markers have proven to be invaluable tools for assessing genetic resources of biodiversity by improving understanding of the users with regards to the distribution and the extent of genetic variation within and among species.

Genetic markers such as morphological, cytological, biochemical and molecular markers can be used in genetic diversity analysis. Initially, morphological markers were used for diversity analysis and are still in use [28]. These were naturally occurring variants of a particular plant species. Later, cytological and biochemical differences occurring in the genotypes of a species started to be used in genetic diversity assessment. With the advent of genomic tools, molecular markers became the method of choice for genetic diversity assessment.

Molecular marker can be used for genetic analysis of different biological diversity components including forest, plants and animals, aquatic and marine animals and plants the report conducted by [29-34]. Molecular markers can also be used in genetic diversity analysis of microorganisms [35]. Genetic diversity analysis has been conducted on different biological diversity components using different kinds of marker Table 1 summarizing some of biological components analysed using molecular markers.

## Importance of Genetic Diversity Analysis

Generally studying the genetic diversity analysis has significant contribution to biological diversity analysis throughout the world including:

- determining what to conserve as well as where to conserve the considering biodiversity and plant species [36,37].
- A comprehensive study of the molecular genetic variation present in germplasm would be useful for determining whether morphologically based taxonomic classifications reveal patterns of genomic differentiation and provide molecular analysis [38].
- Information on genetic diversity is essential in optimizing both conservation and utilization strategies for genetic resources sustainably.

## Characterization of Germplasm

Characterization is an activity of assessing genetic diversity within collections in the context of the total available genetic diversity for each species and existing passport data document the geographic location where each accession was acquired [39]. However, Molecular markers may extend and complement characterization based on morphological or biochemical descriptions, providing more accurate and detailed information than classical phenotypic data. For the genetic approach to succeed, the genetic variation provided by nature and currently conserved in seed banks must be harnessed. The initiation of PCR-based molecular markers, such as simple sequence repeats (SSRs), has created an opportunity for fine-scale genetic characterization of germplasm collections [40]. The ex situ crop germplasm

**Table 1:** Some biological diversity components studied using application of molecular marker.

Biodiversity components	Genetic diversity studied Species	Types Molecular Marker analysis	Citation
Aquatic and aquaculture	Crayfish	RAPD	Gouin et al, 2001
	Shrimp	-	Fietas et al, 2007
Plants	Barley ( <i>Hordeum Vulgare</i> )	SRR	Maniruzzaman et al,2014
	Banana ( <i>Musca cvs</i> )	AFLP	Opara et al, 2014
	Lentils ( <i>Lens culinaris</i> )	AFLP	Alghamdi et al, 2014
	Fenugreek( <i>Trigonella-foenum graecum</i> )	RAPD	Haliem and Al-Haqail, 2014
	Palm germplasm	SSR	Elmeer and Mattat, 2015
	<i>Saccharum spp and Erianthus spp</i>	SRR	Ali A et al, 2019
Animals	Harpy eagle ( <i>Harpia harpyja</i> )	SSR	Banhos et al, 2008
	Butterfly	AFLP	Takami et al, 2004
	Old-field-Mice ( <i>Peromyscus polionatus</i> )	AFLP	Dasmahapatra et al, 2008
	Snake ( <i>Trimeresarus albolabris</i> )	AFLP	Giannasi et al, 2001
	Ostrich	-	Kawaka et al, 2007

maintenance the molecular marker tools may contribute to the sampling, management and development of 'core' collections as well as the utilization of genetic diversity of biodiversity and the in situ and 'on farm' preservation strategies of genetic resources, molecular markers also help in the recognition of the most representative populations within the 'gene pool' of a landrace and the identification of the most suitable strategies for their managing and use [41,42]. Generally the Molecular markers may be used in four types of measurements needed for effective ex situ conservation, all of which are useful in resolving the numerous operational, logistical, and biological questions that face gene banks managers [43].

**Identity:** the determination of whether an accession or individual is catalogued correctly is true to type, maintained properly, and whether genetic change or erosion has occurred in an accession or population over time;

**Similarity:** the degree of similarity among individuals in an accession or between accessions within a collection.

**Structure:** the partitioning of variation among individuals, accessions, populations, and species. Genetic structure is influenced by in situ demographic factors such as population size, reproductive biology and migration.

**Detection:** the presence of particular allele or nucleotide sequence in a taxon, genbank accession, in situ population, individual, chromosome or cloned DNA segment.

## Molecular Marker Types and its Applications

### Classification of genetic marker

Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene [44]. Genetic markers used in genetics can be classified into two categories: classical markers (pre-genomic era) and DNA markers (genomic era).

### Classical Marker Classification

#### Morphological marker

Morphological markers are based on visually accessible traits such as flower color, seed shape, growth habits, and pigmentation, and it does not require expensive technology but large tracts of land are often required for these field experiments, making it possibly more expensive than molecular assessment in western (developed) countries and equally expensive in Asian and Middle East (developing) countries considering the labor cost and availability [45,46].

#### Cytological Marker

The other type of classical marker is cytological marker. In this type of marker, the structural features of chromosomes can be shown by chromosome karyotype and bands. The banding patterns, displayed in color, width,

order and position, reveal the difference in distributions of euchromatin and heterochromatin. For instance, Q bands are produced by quinacrine hydrochloride, G bands are produced by Giemsa stain, and R bands are the reversed G bands. These chromosome landmarks are used not only for characterization of normal chromosomes and detection of chromosome mutation, but also widely used in physical mapping and linkage group identification. The physical maps based on morphological and cytological markers lay a foundation for genetic linkage mapping with the aid of molecular techniques

### Biochemical Marker: Allozymes (Isozyme)

Second type of genetic marker is called biochemical markers, allelic variants of enzymes called isozymes that are detected by electrophoresis and specific staining. Isozyme markers are codominant in nature. They detect diversity at functional gene level and have simple inheritance. It requires only small amounts of plant material for its detection. However, only a limited number of enzyme markers are available and these enzymes are not alone but it has complex structural and special problems; thus, the resolution of genetic diversity is limited to explore.

### Molecular Marker Classification (DNA Markers)

A molecular marker is a DNA sequence in the genome which can be located and identified. As a result of genetic alterations (mutations, insertions, deletions), the base composition at a particular location of the genome may be different in different plants. These differences, collectively called as polymorphisms can be mapped and identified.

DNA marker systems, which were introduced to genetic analysis in the 1980s, have many advantages over the traditional morphological and protein markers that are used in genetic and ecological analyses of plant populations:

1. An unlimited number of DNA markers can be generated;
2. DNA marker profiles are not affected by the environment and
3. DNA markers, unlike isozyme markers, are not constrained by tissue or developmental stage specificity.

### Classification Of Molecular Marker

Several molecular markers have been developed and each of them has different application. The most commonly used molecular marker for biodiversity conservation are: RFLP, AFLP, RAPD, SSR, SNP, mtDNA, and microsatellite .however there are two developments in molecular biology have had unprecedented significance for conservation biology: the PCR (polymerase chain reaction) process and the discovery of microsatellites. The most commonly used molecular markers in biodiversity in Figure 1 conservation are AFLP, RAPD, RFLP and Microsatellite (SSR). These markers are used in most biodiversity conservation activities such as identification and genetic diversity analysis, characterization of biological diversity and genetic integrity monitoring and illegal wildlife trafficking. Hence, as part of the review, their basic principle and application considered.

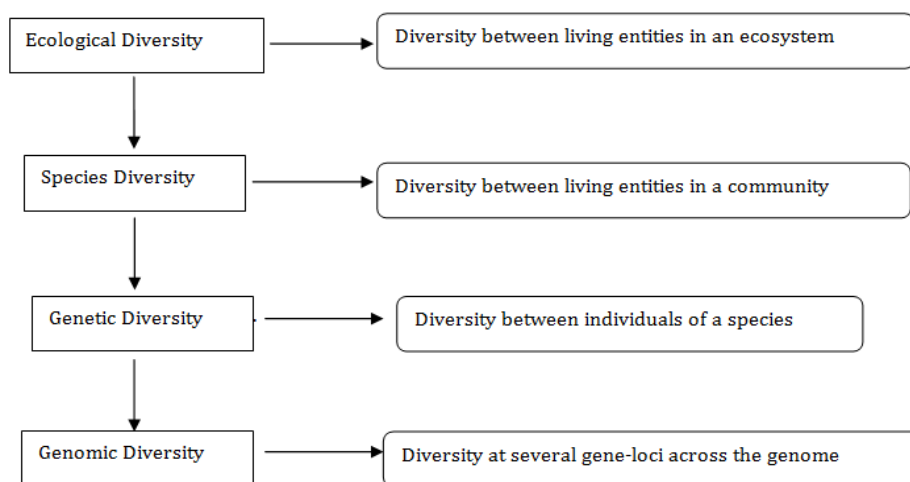


Figure 1. Hierarchical level of biodiversity.

Feature and description	RFLP	RAPD	AFLP	SSR	SNP
Genomic abundance	High	High	High	Moderate to high	Very high
Genomic coverage	Low copy coding region	Whole genome	Whole genome	Whole genome	Whole genome
Expression/inheritance	Co-dominant	Dominant	Dominant / co-dominant	Co-dominant	Co-dominant

Figure 2. The Comparison of some molecular marker systems in plants (Source: Jiang, 2013).

### Application Of Molecular Markers In Biodiversity Conservation

Molecular markers are important for biodiversity conservation such as identification of organisms, genetic diversity study (analysis), characterization of diversity, genetic integrity monitoring for conserved seeds in gene-bank, and illegal wildlife trafficking. In generally the uses of molecular genetic techniques in conservation biodiversity in Figure 2 and have proven to be invaluable tools with the following applications are include;

- genetic conservation efforts by identification of genetic diversity hotspots,
- the monitoring and characterization of population dynamics and gene flow,
- the proper delineation of species taxonomy for management issues associated with conservation
- identification of individuals, species, populations and conservation units;
- detection of hybrid zones and admixed populations;
- quantification of dispersal and gene flow;
- estimation of current and historical population size;
- Assessment of parentage, relatedness, reproductive success, mating systems and social organization.
- Detection of relationships among different germplasm in seed banks,
- search for promising heterotic groups for hybrid breeding,
- identification of duplicates in seed banks, and
- Assessment of the level of genetic diversity present in germplasm pools and its flux over time.

### Conclusion

Biodiversity is vital for human beings and its conservation is critical at this time from local to worldwide. The first and primary activity/operation/steps for

biodiversity conservation is to know biological diversity that exists for better conservation of the representative samples. This is done by assessing genetic diversity analysis within the population followed by characterization. This analysis can be done either morphological or molecular analysis but the former does not fully exploit the variation that exists particularly at the genetic level and is subject to plasticity. Therefore, morphological characterization supported by molecular characterization is sounding for conservation activities to be done fruitfully.

This review concluded that the major molecular marker has been developed to replace the more problematic phenotypic markers in biodiversity analysis used at the infancy of genetic diversity studies. While we touched on their most important applications and showed how broad such applications have been focused specifically on biological genetic diversity studies. We emphasize that integrative approaches using future climate modeling have been very successful in uncovering potential threats of declines of genetic diversity and the distribution of forest tree species. Finally, we stress the value of integrating knowledge on adaptive complex traits as a companion to molecular markers for making informative management and conservation decisions.

Based on the review the following recommendations were forwarded;

- The use of molecular markers in biodiversity conservation is hindered due to capacity man power coupled with lack of groundwork to perform it. So, capacity building for experts of the field and infrastructure accessibility is necessary.
- The effective manipulation of the genetic diversity found in large gene bank collections consumes considerable time, energy and resources. Therefore, molecular marker technologies used to create representative subsets of either the entire or a core collection. And phylogenetic characterization had better be done for the whole collection, not just for the core or subset.
- To evaluate the status of genetic resources as the criteria for ex situ and in situ conservation from genetic information provided.
- To exploit the management of gene conservation by combining adaptive traits and genetic survey for both in ex situ and in situ conservation.

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