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Review Article

Molecular Characterization of Livestock Breeds in Ethiopia: A Review

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ABSTRACT

This paper is aimed to review and discuss the methods and criteria that are currently available from research and past experiences concerning the molecular characterization of livestock breeds in Ethiopia. Molecular characterization is defined as the complementary procedures used to unravel the genetic basis of phenotypes, their patterns of inheritance from one generation to the next, within-breed genetic structure and levels of variability, and relationships between breeds. It is characterized at the molecular level without any effect of environment or development or physiological state of the organism. That's why DNA-based markers are called molecular markers. Because of the low level of polymorphism observed in proteins, and hence limited applicability in diversity studies, DNA-level polymorphisms are the choice of molecular genetic characterization. Characterization of animal genetic resources encompasses all activities associated with the identification, quantitative and qualitative description, and documentation of breed populations. Its objective is to increase knowledge of Animal Genetic Resources (AnGR), their abundance, and their potential for future uses, in wider environments. The importance of molecular characterization of animal genetic resources and its relevance is only slowly being accepted by policymakers in Ethiopia. The presence of economic crisis, fiscal constraints, rapid social change, and frequent political instabilities associated with major policy changes are the major contributing factors. The most severe issues that have limited molecular genetics advancement and conservation of AnGRs are poverty

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and lack of expertise or deficiency in specialized animal breeders. Thus, possible solutions and recommendations for such challenges are monetary support from the government, global initiatives, capacity-building, communication, public awareness, livestock-related policy, and motivating the role of public and private sectors.

Keywords: Animal genetic resource, DNA-level polymorphism, Molecular characterization

INTRODUCTION

Ethiopia has served as a gateway to domestic animals from Asia to Africa and its diverse ecology favored the diversification of these resources. There are many indigenous breeds of livestock in Africa adapted to a wide range of ecological conditions, and Ethiopia stands first in Africa and 10th in the world in terms of livestock population. However, maintaining such resources requires the conservation of diversity among indigenous livestock, to cope with unpredictable future genetic reserves that are capable of readily responding to directional forces imposed by a broad spectrum of environments. Maintaining genetic diversity is an insurance against future adverse conditions and thus, can serve as a genetic pool for the selection of suitable strains and lines (FAO, 2013). Thus, there is a need to characterize the livestock species and breeds, their population sizes, geographic distribution, and importantly their genetic diversity, which is generally undertaken as a preliminary step in any national program for the management of livestock genetic resources for food and agriculture. The primary purpose of characterization is to document the current state of knowledge in terms of a population's ability to survive, reproduce, produce, and provide services to farmers. Characterization, therefore, provides the baseline information as well as the criteria that will be used to establish and update the inventory or records. Characterization provides data on present and potential future uses of the Livestock genetic resources under consideration and establishes their current state as distinct breed populations and their risk status. As the use and management of livestock genetic resources are dynamic processes, monitoring the status of a population has to be done regularly. Thus, risk status indicators for use during the monitoring process need to be defined following the characterization steps (Phenotypic, Morphological, cytogenetic, and molecular genetic characterization).

Genetic improvement of livestock depends on access to genetic variation and effective methods of exploiting this variation. Genetic diversity constitutes a buffer against changes in the environment and is key in selection and breeding for adaptation and production in a range of environments. Recent advances in molecular biology, principally in the development of polymerase chain reaction (PCR) for amplifying (DNA), DNA sequencing, and data analysis, have resulted in powerful techniques which are used for the screening, characterization, and evaluation of genetic diversity. The extensive number of research information describing the use of these techniques

on a wide range of animal species and diversity problems is a testimony to their increasing impact in this field. The increasing availability of molecular tools for genomic studies had improved genetic information on livestock species for improved utilization and management (FAO, 2015). Thus, this paper is intended to review and discuss the methods and criteria that are currently available, from research and past experiences regarding the molecular characterization of livestock breeds in Ethiopia.

LTRATURE REVIEW

Characterizations of Livestock and Its Importance

Characterization is the primary assessment or baseline survey, which should include the collection of data on population size and structure, geographical distribution, production systems in which the breed is found, phenotypic attributes (physical features, performance levels, and any unique features), the historical development of the breed through exchange, upgrading, and selection, and the genetic connectedness of populations when these are found in more than one country. The within-population genetic diversity is measured both at the phenotypic level (phenotypic breed diversity) and a molecular level; the two are complementary. All these data are needed to inform decisions on the utilization, improvement, and conservation of the population (FAO, 2015). The importance of Characterization in Animal Genetic Resources (AnGRs)is when a breed/population/species is found to be at risk, active conservation strategies have to be implemented or the potential loss of the breed must be accepted or known.
On the other hand, if characterization is not achieved, information about the particular breed population could not be available, whether it is self-sustainable or is at risk. It also causes a missing a strategic and coherent approach to the identification, description, and documentation of breed populations.

Classification of Genetic Characterization

Phenotypic Characterization is used to identify and document diversity within and between distinct breeds based on their observable attributes. The measurement of genetic relationships between breeds and genetic heterozygosity within breeds is the task of molecular characterization. Phenotypic characterization of AnGR is the process of identifying distinct breed populations and describing their external and production characteristics in a given environment and under given management, taking into account the social and economic factors that affect them. The information provided by characterization studies is essential for planning the management of AnGR at local, national, regional, and global levels. The term “phenotypic characterization of AnGR” generally refers to the process of identifying distinct breed populations and describing their external and production characteristics within a given production environment (FAO, 2012).

The term "production environment" is here taken to include not only the "natural" environment but also management practices and the uses to which the animals are put, as well as social and economic factors such as market orientation, niche-marketing opportunities, and gender issues. Recording the geographical distribution of breed populations is here considered to be an integral part of phenotypic characterization (FAO, 2015). Complementary procedures used to unravel the genetic basis of phenotypes and their patterns of inheritance from one generation to the next, and to establish relationships between breeds, are referred to as molecular genetic characterization.

Morphological Characterization (Morphological Marker) refers to external animal characteristics which can be obtained by direct visual observation and measurement and used in the identification, classification, and characterization of genetic evaluation of different species or populations (Hailu and Getu, 2015). The morphological description is an essential component of breed characterization that can be used to physically identify, describe, and recognize a breed, and also to classify livestock breeds into broad categories. Dossa *et al*. (2007) reported that morphological measurements such as heart girth, height at withers, and body length can be used for the rapid selection of large-size individuals in the field to enable the establishment of elite flocks or herds.

Biochemical Characterization (Biochemical Marker) these are related to variations in proteins and amino acid banding patterns and are known as biochemical markers. A gene-encoded protein that can be extracted and observed; for example, isozymes and storage proteins. Biochemical characterization is the characterization of the biochemical state of the organism, which is affected by environment, development as well as physiological state. So, in my opinion, biochemical characterization is giving us a picture of the interaction of the molecular state with the other states (environmental, developmental, physiological) while molecular characterization is solely giving us the sight of the molecular state (Priyanka Siwach, 2014).

Cytogenetic characterization (Cytological marker) cytogenetics is the study of chromosomes and the related disease states caused by abnormal chromosome number and/or structure. Cytogenetic characterization studies are highly useful in genetic characterization and for the effective conservation of the specie seriously at risk of extinction. Research strategies involving cytogenetics hold the promise of yielding insight into the mechanisms underlying chromosome instability in embryos and the impact of the in vitro environment on the chromosome makeup of embryos (Alok *et al.,* 2017). In addition, cytogenetic characterization of animals will help to identify the animals with congenital and acquired chromosome abnormalities whose elimination will help to maintain the herds cytogenetically clean (Alok *et al*., 2017). As a tool, cytogenetics has an equally important contribution to make to the various disciplines

involved in the study of ways to enhance the more economical production of animal products for human consumption.

Molecular Characterization can be defined as the complementary procedures used to unravel the genetic basis of phenotypes, their patterns of inheritance from one generation to the next, within-breed genetic structure and levels of variability, and relationships between breeds (FAO, 2015; Gamaniel and Gwaza, 2017). It is characterized at the molecular level without any effect of environment or development or physiological state of the organism. That is why DNA-based markers are called molecular markers and characterization (PriyankaSiwach, 2014). Molecular genetic characterization explores polymorphism in selected protein molecules and DNA markers to measure genetic variation at the population level. Because of the low level of polymorphism observed in proteins, and hence limited applicability in diversity studies, DNA-level polymorphisms are the markers of choice for molecular genetic characterization.

The molecular characterization should ideally be done along with phenotypic characterization. Most molecular works was based on the use of neutral genetic marker data, which served as a proxy or estimate of the likelihood of important functional genetic variation within breeds or breed groups. Ideally, molecular characterization should be undertaken as part of a comprehensive national program for the management of AnGR. For maximum efficiency, molecular characterization of AnGR should be done in concert with phenotypic characterization. Outcomes of morphological or phenotypic characterization need to be complemented by genetic characterization (Gizaw *et al.,* 2011). Molecular or Genetic characterization involves the description of breeds in terms of the relative allelic frequencies, and degree of polymorphism using a set of neutral reference markers, and classifying livestock breeds using genetic distances between populations/breeds.

Molecular genetics characterization comprises field sampling of biological material (often blood or hair root samples), laboratory extraction of DNA from the samples, DNA storage, laboratory assaying (e.g., genotyping or sequencing), data analysis, report writing, and maintenance of a molecular genetic information database. Sampling for molecular analysis may be combined with surveying and/or monitoring, as molecular information on its own cannot be used for utilization and conservation decisions (FAO, 2012). The advantages of molecular genetic technologies are: it measures the genetic constitution of a breed/population of a species at a molecular level; assess the genetic uniformity, admixture or subdivisions, inbreeding, or introgression in the population.

In developing countries, the most important resource limitation is the lack of funds to set up facilities to support molecular research. Even in the existing research facilities, adequate funding for maintenance is not there. Other limitations include the inadequacy of scientific equipment to support research and technical manpower.

Molecular genetics research is highly sophisticated and also required skilled manpower as well as technologist. Without an established scientific culture, it is almost impossible to engage in and keep up with developments in molecular biology research.

Molecular Markers Available for Characterization

Molecular markers are identifiable physical locations on a chromosome whose inheritance can be monitored. These are a set of DNA-based genetic markers that can detect DNA polymorphism both at the level of specific loci and at the whole genome level. 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. According to the review in Guo-Liang (2012) reported that, depending on the application and species involved, ideal DNA markers for efficient use in marker-assisted breeding should meet the following criteria: High level of polymorphism. Even distribution across the whole genome (not clustered in certain regions). Co-dominance in expression (so that heterozygotes can be distinguished from homozygotes), clear distinct allelic features (so that the different alleles can be easily identified), Single copy, and no pleiotropic effect. Low cost to use (or cost-efficient marker development and genotyping). There are different types of molecular markers. Some of these markers are RFLPs, AFLPs, RAPDs, ISSR, ISSCP, Microsatellites, or SSRs, and SNPs.

Restriction Fragment Length Polymorphisms (RFLPs)

A molecular method of genetic analysis that allows individuals to be identified based on unique patterns of restriction enzymes cutting in specific regions of DNA. It is an application of the Southern Hybridization Procedure. RFLP is Genomic DNA digested with Restriction Enzymes, DNA fragments separated via electrophoresis and transfer to a nylon membrane, Membranes exposed to probes labeled with P32 via southern hybridization, and Film exposed to X-Ray (Paras et al., 2015). They are co-dominant and can be mapped using linkage analysis, measuring variation at the level of DNA sequence, not protein sequence, RFLP loci are very large so even very small segments of chromosomes can be mapped and also study phylogenetic relationships, very reliable for linkage analysis and for detecting coupling phase of DNA molecules. It requires a relatively very large amount of DNA, requirement of a radioactive probe makes the analysis expensive and hazardous, they are not useful for detecting single base change or point mutations. It is time-consuming, laborious, and expensive and the level of polymorphism is low.

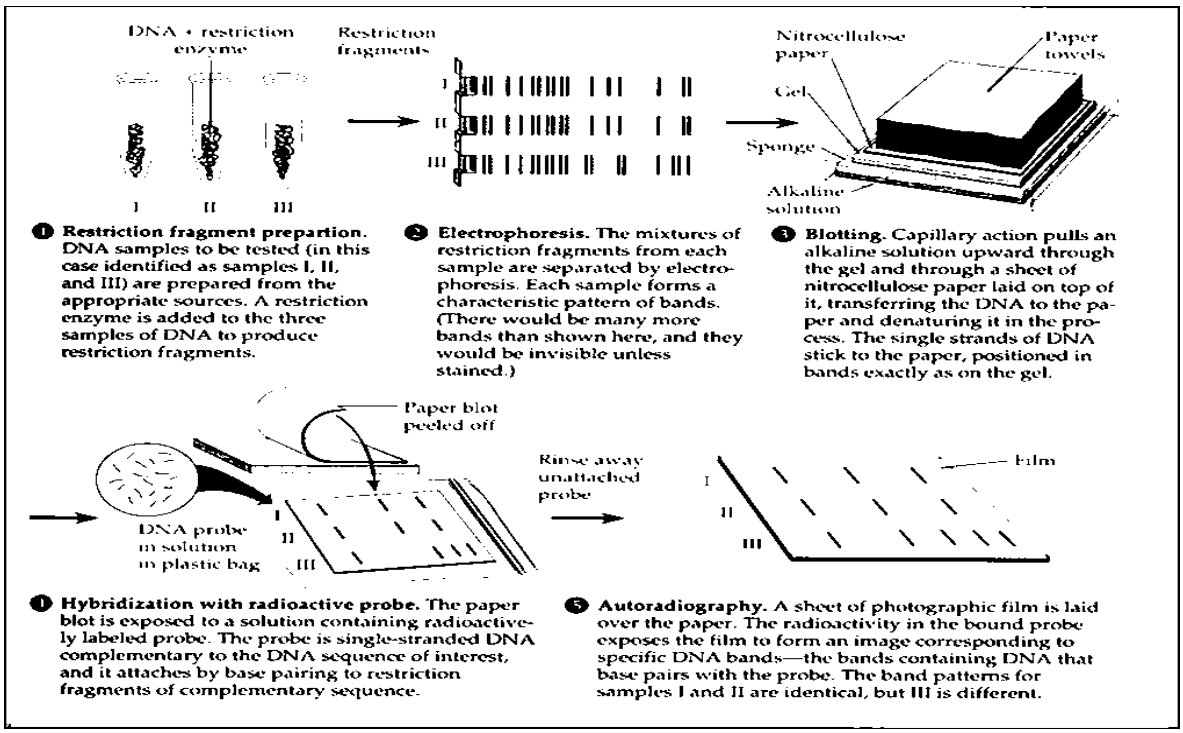

Fig.1: Basic techniques of Restriction Fragment Length Polymorphisms (Amit Kumar, 2018)

Amplified Fragment Length Polymorphism (AFLP) Marker

AFLP is a PCR-based tool developed by Voset al. (1995), used in genetics research and fingerprinting. It is a multi-locus technique resembling RAPD, and is highly popular. The technique is based on PCR amplification of restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of a few nucleotide bases. The amplified products are radioactively labeled and separated on a polyacrylamide gel. A subset of the restriction fragments is then selected to be amplified. Their main strengths include high specificity and reproducibility owing to the restricted digestion of DNA, selective neutrality, specific adaptors, and high annealing temperatures for selective amplification and no prior sequence information or probe (Sabir *et al*., 2014).

Randomly Amplified Polymorphic DNA (RAPD)

RAPD is a PCR-based molecular marker developed independently by two different laboratories (Salisu *et al*., 2018). RAPD is a type of PCR reaction, but the segments of DNA that are amplified are random. It creates several short primers (8–12 nucleotides) and then proceeds with the PCR using a large template of genomic DNA, the fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be assembled from an RAPD reaction. It is a PCR-based technique for identifying genetic variation. It involves the use of a single arbitrary primer in a PCR reaction,

resulting in the amplification of much discrete DNA. RAPD technology provides a quick and efficient screen for DNA sequence-based polymorphism at a very large number of loci (Amber Hassan, 2017).

Inter Simple Sequence Repeat (ISSR)

Inter simple sequence repeat (ISSR)-PCR is a technique, which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. It is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD). ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping, and evolutionary biology (Reddy *et al.,* 2002).

Single-Strand Conformation Polymorphism (SSCP)

The SSCP technique was employed to screen individual PCR products for variation. Most researchers use SSCP to reduce the amount of sequencing necessary to detect new alleles at loci of interest or to better estimate allele frequencies of populations. Additionally, SSCP may be used to screen PCR products for genes intended to be sequenced for phylogenetic analysis. This SSCP screening allows researchers to determine if the gene in question contains sufficient polymorphism, which portion of the gene is most polymorphic, what level of intra-specific variation exists, and if there is a polymorphism among multi-copy genes within individuals. SSCP is an efficient technique for obtaining information about levels of polymorphism within anonymous nuclear loci as compared to the restriction enzyme protocol used (Sabir *et al*., 2014).

Microsatellites (SSR)

The term microsatellite was invented by (Litt&Luty, 1989; reviewed by Yang *et al*., 2013) and to characterize simple sequence repeats. Microsatellites, Short Tandem Repeats (STR), or Simple Sequence Repeats (SSR) are the DNA sequences of 1 to 6-nucleotide length tandem repeats and are widely distributed throughout the genome. The change in the number of repeats caused by unequal crossing over between homologous tandem repeats or due to gain or loss of repeat units at a particular locus represents a variety of alleles with different numbers of tandem repeats. Therefore, in the population, these loci have a variable numbers of tandem repeats (VNTR) and hence are also called VNTR loci. Microsatellites are the marker of choice in livestock genetic characterization studies, and population genetic analysis and are used in the evaluation of genetic resources (Paras, et al., 2015, Anim Kumar, 2017).

Single-nucleotide Polymorphism (SNP)

SNP is a substitution of a single nucleotide that occurs at a specific position in the genome, where each variation is present at a level of more than 1% in the population (For example, at a specific base position in the human genome, the Cytosine (C) nucleotide may appear in most individuals, but in a minority of individuals, the position is occupied by an Adenine (A). This means that there is an SNP at this specific position, and the two possible nucleotide variations, Cytosine or Adenine are said to be the alleles for this specific position. A single-nucleotide variant (SNV) is a variation in a single nucleotide without any limitations of frequency and may arise in somatic cells. A somatic single-nucleotide variation (e.g., caused by cancer) may also be called a single-nucleotide alteration.DNA sequencing has allowed the discovery of SNPs and is the most recent contribution to the study of DNA sequence variation. Genomic selection using the SNP markers is a powerful new tool for genetic selection breeding (Seidel, 2009) and population studies.

Molecular Characterization in Livestock Species and Its Challenges

FAO estimates that there are more than 6,300 breeds of livestock in the world belonging to 30 domesticated species (FAO, 2015). These breeds were developed following domestication and natural and human selection over the past 12,000 years. Different livestock populations have evolved unique characteristics for adaptation to their production systems and agroecological environments. Their genetic diversity has provided the material for the very successful breeding program in the 19 and 20th centuries, these livestock breeds represent a unique resource to respond to the present and future needs of livestock production, both in developed and developing countries However, livestock diversity is shrinking rapidly. Loss of biological diversity is a serious problem or potential problem in many places of the world.

Progress in sequencing techniques and the opportunities offered by the development of high-density marker arrays have considerably improved the availability of DNA information over the last ten years, both in terms of the number of markers and in the cost of genotyping (FAO, 2015). Several kinds of research have been conducted concerning the genetic characterization of various livestock populations /breeds using molecular markers in developed and developing countries.

Molecular characterization of animal genetic resources through efficient in developed countries, its applications in developing countries are hindered due to mainly shortage of well-trained personnel, inadequate high-throughput capacity, poor phenotyping infrastructure, lack of information systems or adapted analysis tools, or simply resource-limited breeding programs. The magnitude of these challenges is exacerbated where there is an imperative to breed for biotic (pests and diseases) and abiotic stress (drought, heat, cold, and salinity), making accurate phenotyping challenging.

The importance of molecular characterization of animal genetic resources and its relevance is only slowly being accepted by policymakers in Ethiopia. In the presence of economic crisis, fiscal constraints, rapid social change, and constant political instabilities, the difficulty associated with major policy changes in Ethiopia is enormous.

Ethiopia is endowed with most of the African domestic animal diversity-landraces, strains, or breeds. Some livestock breeds in it are not characterized, their genetic resources are unknown such that others are endangered, some had extricated, and others that survived are either highly threatened or endangered through indiscriminate crossbreeding or destruction of the environment that enhanced their continued survival. The importance of indigenous livestock breeds lies in their adaptation to local biotic and abiotic stress and to traditional husbandry systems. However, most of these animal genetic resources are still not characterized and boundaries between distinct populations are unclear. Major causes threatening the diversity of genetic resources in Ethiopia include poorly designed and managed introduction of exotic genetic materials, droughts and consequences of drought-associated indiscriminate restocking schemes, political instability and associated civil unrest, and weak development interventions (Nigatu *et al.,* 2004). The effects of the misguided and uncontrolled introduction of exotic genes and that of interbreeding among indigenous breeds might require the application of molecular genetics for purposes of precision. In extreme scenarios, however, it could have a drastic effect leading to the extinction of a breed within a few generations.

Due to a lack of resources and political instability during most of the last century in Ethiopia, there is very little existing genetic knowledge about the indigenous cattle resources, and the molecular characterization based on paternal lineages.

Measurement of Genetic Variation

Biochemical markers involve the characterization of blood groups and allozyme systems (allelic variants of enzymes encoded by structural genes) of livestock. However, the level of polymorphisms observed in proteins is low; this has reduced the applicability of protein typing in genetic diversity studies. Furthermore, allozymes are phenotypic markers, hence, they may be affected by environmental conditions. DNA-level polymorphisms are the markers of choice for molecular genetics characterization (FAO, 2015). Because they are based on differences in the DNA sequence, DNA markers are not environmentally influenced, which means that the same banding profiles can be expected at all times for the same genotype. A variety of DNA markers are used for the study of genetic variation at the DNA level. The most widely used are (RFLP, SSRs, RAPD, AFLP, and SNPs). These markers provide the means to examine directly nucleotide sequence differences in the DNA and to determine the amount of genetic variation present in the population's Variation at the DNA level is

identified by gel electrophoresis. Among the DNA markers, microsatellites are very useful for the study of genetic variation within and between closely related populations such as livestock breeds or strains.

Measuring Genetic Diversity within a Population

Within-population, diversity is measured by allelic diversity, which is the average number of alleles at a locus across all loci analyzed. Another measure of within-population genetic diversity is observed heterozygosity, which is the total number of heterozygotes divided by the total number of animals that have been analyzed.

Measuring Genetic Diversity Between Populations

Genetic variation between populations (species, breeds, or strains) is measured by assessing the genetic uniqueness of the breeds. To know the uniqueness of a breed or strain, one must study the genetic variation in a set of breeds. The genetic uniqueness of breeds or strains is measured by the relative genetic distances of such breeds or strains from each other (FAO, 2015). The tools of molecular genetics are likely to have a considerable impact in the future. For example, DNA-based tests for genes or markers affecting traits that are difficult to measure currently, such as meat quality and disease resistance, will be particularly useful (Leakey, 2009). Genomic selection should be able to at least double the rate of genetic gain in the dairy industry (Heins *et al*., 2009), as it enables selection decisions to be based on genomic breeding values, which can ultimately be calculated from genetic marker information alone, rather than from pedigree and phenotypic information. Advances in genomics and bioinformatics have allowed the identification of genomic similarities/differences among livestock breeds (Simoni Gouveia *et al*., 2014). Some of these genomic signatures may contribute to explaining the phenotypic uniqueness of breeds (Huson *et al*., 2014; Somavilla *et al.,* 2014) and facilitate prioritization and the use of genomic breeding tools to preserve these important traits. A further option is the landscape genomics approach, whereby the association between alleles and geographic locations and/or climatic variables is targeted and assumed to be suggestive of signatures of adaptation, giving information on the environmental forces acting on the genome (Joost *et al*., 2013).

According to (IBC, 2004; Houle *et al.,* 2010) explained the various factors that have limited molecular genetics advancement and conservation of AnGRs, for example, poverty, which is the most severe issue that restricts the genetic advancement and sustainable utilization of farm AnGR. Lack of human resource capacity, i.e., deficiency in technical know-how and expertise is another challenge confronting better utilization of molecular genetics. Besides, there are very few animal breeders, or specialists in new reproductive technology and molecular genetics. The Ethiopian government is prepared and devoted to offering necessary support in this area.

Nonetheless, the nation is faced with a lack of budgetary assets. Therefore, monetary support is essential from national, regional, and global initiatives, and the international community.

Problems with Economic Sustainability

Molecular genetics improvement programs require significant investments. Although well-designed molecular breeding can be expected to eventually provide positive returns on investments, in local breeds the costs will often be relatively high on a per-animal basis. The breeding strategy and system that maximize genetic response may not be optimal from an economic standpoint. Recording of performance and pedigrees may not be economically sustainable, even if restricted to a portion of the population (e.g., the nucleus). Lack of infrastructure (such as molecular genetics lab) in marginal areas where local breeds are often found may impair the introduction of genetic improvement programs, and the development of these infrastructures may be costly. Therefore,cost–benefit analysis should be conducted before implementing molecular techniques to determine the optimal approach. In genetic improvement programs, economic returns should be evaluated in the long term, given generation intervals and genetic cumulative effects. Additionally, organizational and infrastructural shortcomings are often associated to local breeds: these could be circumvented by taking advantage of existing organizations and infrastructures developed by larger breeding organizations.

Molecular Characterization of Livestock Species in Ethiopia, their Objectives, and Work Done So Far

Cattle: There have been some attempts to characterize populations at the genome level (Zerabruk *et al*., 2011; Edea *et al*., 2017;). African cattle are believed to have developed a wide range of adaptations to tropical environments. Hailu *et al.,* (2008), evaluated the genetic diversity, population structure, and degree of admixture of 10 Ethiopian cattle populations using 30 microsatellite markers. The main target was to find out if the current uncontrolled mating practices resulted in a high risk of becoming genetically homogeneous. The study revealed that the various levels of admixture and high genetic diversity make Ethiopian cattle populations suitable for future genetic improvement, and utilization under a wide range of agroecologies in Ethiopia. The genetic variability and extent of population substructures in five indigenous cattle breeds of North-Western Ethiopia were also studied using 22 microsatellite markers.

Controlling gene flow between breeds by adopting effective breeding and management practices to maintain variability and overcome within-breed substructures is suggested to facilitate the conservation and utilization of each breed (Zewdu *et al.,* 2010). Zerabruk *et al.,* (2011), considered microsatellite variation to

determine genetic diversity, population structure, and admixture of seven North Ethiopian cattle breeds by combining multiple microsatellite data sets from other cattle populations abroad (Cited in Tadelle *et al.,* 2019). Overall, North Ethiopian cattle showed a high level of within-population genetic variation and indicated their potential for future breeding applications. Meseret *et al*., (2020) conducted genetic diversity and population structure of six Ethiopian cattle breeds from different geographical regions using high-density single nucleotide polymorphisms. A genome-wide SS analysis revealed 816 loci ($p < 0.01$) associated with candidate genes to adaptation (Edea *et al*., 2014). The candidate genes are involved in metabolism, hypoxia response, and heat stress. Noyes *et al.,* (2011) detected the ARHGAP15 gene for trypanotolerance in Sheko cattle.

Camel: Camel is an important animal in arid areas of the country. DNA sequences from the mitochondrial cytochrome-b gene and genotyping of 6 nuclear microsatellite loci were examined to assess the genetic diversity and phylogenetic relationship of Ethiopian camels (Yosef *et al*., 2019).

Donkey: genetic diversity and matrilineal genetic signature of native Ethiopian donkeys (Equusasinus) inferred from mitochondrial DNA (mtDNA) sequence polymorphism was conducted (Kefena *et al*., 2014). In the study, mtDNA sequence polymorphisms of six morphologically diverse domestic donkeys populations in Ethiopia were investigated. The result suggested that Ethiopia could be one of the centers of diversities for domestic donkeys in the Horn of Africa. This paper also overrides some previous reports that claimed donkeys were solely an Egyptian domesticates.

Sheep: The genetic and morphological diversity and population structure of 14 traditional sheep populations originating from four ecological zones in Ethiopia (subalpine, wet highland, sub-humid lowland, and arid lowland) were studied by Gizaw *et al*. (2007). The study showed a strong indication of adaptive divergence in morphological characters, with patterns of morphological variation being highly associated with ecology. The genetic diversity and population structure of Ethiopian sheep populations were characterized using high-density SNP markers to reveal their genetic diversity for improving breeding strategies and mapping quantitative trait loci associated with productivity (Zewdu *et al*., 2017). The high-density SNP data generated in the study can be used to identify genes and pathways relevant to physiological adaptation to extreme environments and variation in phenotypic traits (Zewdu *et al.,* 2017).

Goats: The genetic diversity within and among 11 indigenous Ethiopian goat populations/types was investigated using microsatellite markers (Tesfaye, 2004). Solomon (2014) studied the molecular genetic diversity and homozygous segments of two goat breeds of Ethiopia using 47K genome-wide SNPs markers to understand the within and between breed diversity for future breed improvement and conservation

planning. Getnet *et al.,* (2017) also used mtDNA markers to characterize the genetic diversity and population structure of Ethiopian goats. Another study by Getnet *et al.* (2017), identified genetic variants associated with fecundity traits in some Ethiopian goat populations, and this could be used in Marker Assisted Selection.

Chickens: Genetic improvement of indigenous chicken exercises in Ethiopia, as in other developing countries, has been towards the use of exotic chicken strains to improve the local chicken. Extensive crossbreeding has been common over the 5 decades of poultry research in Ethiopia. An exception is a single selective breeding program in indigenous chicken in Ethiopia with the application of quantitative genetics approaches. The earlier research on genetic characterization was by Tadelle (2003) who characterized five chicken ecotypes of Ethiopia using microsatellite markers. The result has led to the discovery of some unique alleles that are believed to be involved in production traits. Later, Halima (2007), (Cited in Tadelle *et al.,* 2019) characterized some indigenous ecotypes from Northwestern parts of Ethiopia using microsatellite markers to reveal the between- and within-population genetic variations. A genome-wide association study (GWAS) was conducted by Psifidi *et al.* (2016) using single nucleotide polymorphism (SNP) markers to reveal the association of markers with phenotypic traits. SNPs significantly associated with the immune system, disease resistance, and production traits in indigenous village chickens were identified.

Suggested Solutions and Recommendations to Overcome the Problems

The tools of molecular genetics are likely to have a considerable impact in the future. For example, DNA-based tests for genes or markers affecting traits that are difficult to measure currently, such as meat quality and disease resistance, will be particularly useful (Leakey, 2009). Genomic selection should be able to at least double the rate of genetic gain in the dairy industry (Heins *et al*, 2009), as it enables selection decisions to be based on genomic breeding values, which can ultimately be calculated from genetic marker information alone, rather than from pedigree and phenotypic information. Key factors hampering the use of molecular technologies in most developing countries include poor infrastructure; inadequate capacity and operational support; and lack of an enabling policy, statutory and regulatory framework at the country level, which in turn affects research institutions. Ethiopia has faced the challenge of rapidly increasing agricultural productivity to help feed its growing population without depleting the natural resource base. Marker identification will help to enhance the selection of superior genotypes for breeding to improve important traits. Molecular characterization of animal genetic resources, though efficient in developed countries, its applications in developing countries are hindered due to mainly shortage of well-trained personnel, inadequate high-throughput capacity, poor phenotyping infrastructure, lack of information systems or adapted analysis tools, or

simply resource-limited breeding programs. The importance of molecular characterization of animal genetic resources and its relevance is only slowly being accepted by policy makers in Ethiopia. In the presence of economic crisis, fiscal constraints, rapid social change, and constant political instabilities, the difficulty associated with major policy changes in Ethiopia is enormous.

The importance of indigenous livestock breeds lies in their adaptation to local biotic and abiotic stress and to traditional husbandry systems. However, most of these animal genetic resources are still not characterized and boundaries between distinct populations are unclear. Major causes threatening the diversity of genetic resources in Ethiopia include poorly designed and managed introduction of exotic genetic materials, droughts and consequences of drought-associated indiscriminate restocking schemes, political instability and associated civil unrest, and weak development interventions. The effects of uncontrolled introduction of exotic genes and its interbreeding with indigenous breeds might require the application of molecular genetics for purposes of precision. However, due to the lack of resources and political instability during most of the last century in Ethiopia, there is very little existing genetic knowledge about the indigenous cattle resources, and the molecular characterization of paternal lineages.

Thus, despite the largest livestock population and diversity in Africa, most of the livestock breeds in Ethiopia are not characterized and their genetic resources are not well documented.

ch as tolerance to diseases and resistance to environmental stresses. Thus, developing nations, like Ethiopia, must take the advantage of utilizing molecular biology for the better advancement of livestock productivity. To this end, the following recommendations are suggested to overcome the challenges of effective utilization of molecular technology:

Capacity-building

There is a serious need for capacity-building, in developing countries, if biotechnology is to be successfully applied to improve the management of animal genetic resources, for the benefit of farmers and consumers. This capacity building needs to be at all levels. There is a need to strengthen competence in the areas of science and technology, but also for regulatory issues and policy analysis.

Communication and Public Awareness

There is a need for Government to inform the public as to the benefits and risks of new biotechnologies and their potential role in the management of animal genetic resources. In building national development strategies and Global Strategy in general, it, therefore, appears crucial to address from the beginning the question of public awareness, education, and information.

Encourage the Role of Public and Private Sectors

The new biotechnologies have targeted improvement in livestock production in industrial countries and have increasingly been developed in the private sector. Such research will necessarily concentrate on species and breeds that can associate with public and private sector research and generate a near-term profit, to the exclusion of less profitable species or traits. At the same time, there has been limited public investment in animal biotechnology in most developing countries and only modest support from or conventional livestock research and development to improve the productivity, nutrition, and health of farm animals. There is a need for additional public sector investments in developing and applying biotechnologies in the characterization, sustainable use, and conservation of AnGR, where local capital is unavailable and where private sector investments are unlikely to be commercially attractive in the medium term. Governments need to consider how best to support private-public sector collaborations in animal biotechnology research.

Livestock-related Policy and Regulations

Lack of appropriate livestock policies has been identified as one of the increasing key factors causing threats to farm animals' genetic resources (FAnGR) in the developing. At present, there is no legal framework in Ethiopia to regulate crossbreeding or to regulate the importation and distribution of exotic genetic materials (ESAP, 2007). In an increasingly globalized market, the absence of breeding policies and regulations, as well as the absence of a gene banks for animal genetic resource conservation, could put indigenous breeds at risk and endanger the future generations of livestock in Ethiopia (Desalegn, 2008). With the present increasing trend for high-out animals, unorganized crossbreeding programs and the absence of crossbreeding policies would put a threat to the FAnGR of Ethiopia in the future (ESAP, 2009).

Establish Regulatory Systems and Safety

A key role for governments is to ensure that an open, transparent, and effective regulatory system is in place, that permits harmonious development of animal production, particularly in the light of the ongoing livestock revolution, to maximize production while minimizing ecological risks.

Intellectual Property Management

The use of new and frequently proprietary, biotechnologies in the management of animal genetic resources will require developing countries like Ethiopia, more systematically consider their relevant intellectual property policies and legislation, to

provide enabling environments for the conservation and utilization of animal genetic resources product development.

CONCLUSION

The tools of molecular genetics are likely to have a considerable impact in the future. DNA-based tests for genes or markers affecting traits that are difficult to measure currently, such as meat quality and disease resistance, will be particularly useful. Marker identification will help to enhance the selection of superior genotypes for breeding to improve important traits.

Molecular characterization of animal genetic resources, though efficient in developed countries, its applications in developing countries are hindered due to mainly shortage of well-trained personnel, inadequate high-throughput capacity, poor phenotyping infrastructure, lack of information systems. Key factors hampering the use of molecular technologies in most developing countries include poor infrastructure; inadequate capacity and operational support; and lack of an enabling policy, statutory and regulatory framework at the country level, which in turn affects research institutions. The importance of molecular characterization of animal genetic resources and its relevance is only slowly being accepted by policy makers in Ethiopia. In the presence of economic crisis, fiscal constraints, rapid social change, and constant political instabilities, the difficulty associated with major policy changes in Ethiopia is enormous.

The importance of indigenous livestock breeds lies in their adaptation to local biotic and abiotic stress and to traditional husbandry systems. However, most of these animal genetic resources are still not characterized and boundaries between distinct populations are unclear. Major causes threatening the diversity of genetic resources in Ethiopia include poorly designed and managed introduction of exotic genetic materials, droughts and consequences of drought-associated indiscriminate restocking schemes, political instability and associated civil unrest, and weak development interventions. The effects of uncontrolled introduction of exotic genes and its interbreeding with indigenous breeds might require the application of molecular genetics for purposes of precision. However, due to the lack of resources and political instability during most of the last century in Ethiopia, there is very little existing genetic knowledge about the indigenous cattle resources, and the molecular characterization of paternal lineages.

Thus, despite the largest livestock population and diversity in Africa, most of the livestock breeds in Ethiopia are not characterized and their genetic resources are not well documented

AUTHOR'S CONTRIBUTION

The authors equally contributed to the data collection, reviewed information, and write up the manuscript. The authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that no conflict of interest concerning the research, authorship, or publications of this article.

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