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Strategies for Improvement of Indigenous Ethiopian Cattle Breeds: Breeding Plans and Sequence Polymorphisms of Selected Candidate Genes (BoLA-DRB3 and Leptin) as a Potential Molecular Markers

PhD Dissertation

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Addis Ababa, Ethiopia

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A Thesis Submitted to the Institute of Biotechnology, School of Graduate Studies of the Addis Ababa University

for Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biotechnology

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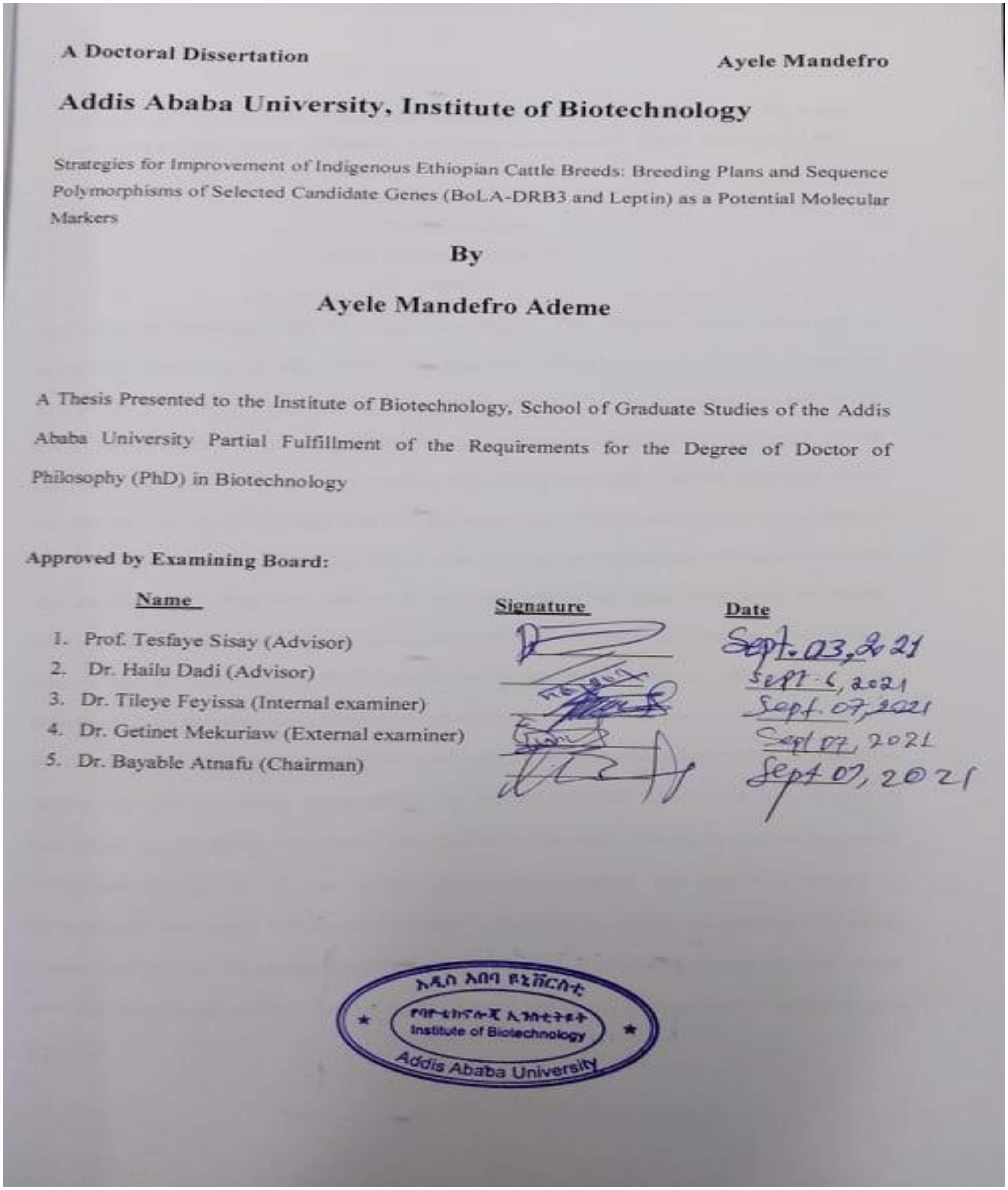

A Doctoral Dissertation **Ayele Mandefro**

Addis Ababa University, Institute of Biotechnology

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By

Ayele Mandefro Ademe

A Thesis Presented to the Institute of Biotechnology, School of Graduate Studies of the Addis Ababa University Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (PhD) in Biotechnology

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Abstract

Agriculture is the major pillar of Ethiopia's economy. The livestock sector is one part of agriculture, accounting for about 45% of the total value of agricultural production. Simulation method and genomic selection approaches were implemented to select superior animals for the aim of enhancing production and productivity. Two indigenous cattle breeds of Ethiopia (Boran and Horro) were simulated with the objective of evolving applicable breeding programs. Boran was evaluated for beef, milk, and both beef and meat (dual purpose) while Horro was evaluated for milk and dual purposes. Yearling weight, milk yield, age at first calving, calving interval, and calf survival to yearling were defined as selection criteria for milk production. Moreover, yearling weight, age at first calving, calving interval, and calf survival to yearling were used to improve beef production in the Boran breed. For all goal traits examined, more genetic gains were found from bull selection groups than cow selection groups. The genetic polymorphisms of the BoLA-DRB3 and leptin gene in Ethiopian and Asian zebu and taurine cattle breeds were evaluated by sequence-based typing (SBT). For the BoLA-DRB3 exon2 locus we detected 59 total alleles and 16 alleles were newly identified from this study. The Hardy Weinberg genetic equilibrium *(*HWE) of all the breeds investigated revealed non-significant excess of heterozygosis that could show over-dominant selection. The results of pairwise *FST* values directed low genetic differentiation of the Ethiopian breeds with Asian Zebu and *taurine*, rooted from the evolutionary history of cattle breeds. Exon2 and exon3 were evaluated for leptin gene polymorphism. Five SNPs at positions 73 (C>T), 143 (C>A), 399 (T>C), 411 (T>C), and 495 (C>T) were identified and only the second SNP (C143A) was new to this study. Mutations detected on exon2 region were missense that causes a change of amino acids from arginine to cysteine (R25C) and from threonine to lysine (T48K). The evolutionary divergence showed that large-sized cattle breeds were closely clustered together. Simulation study could be used to improve the performance of cattle production. Also, selection of superior animals based on selected candidate genes (BoLA-DRB3 and leptin) could be important to improve cattle genetics.

Key words: PCR-SBT, SNP, Leptin, BoLA-DRB3, Cattle

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Entirely ensued with the Willpower of almighty God!

Dedication

In **memory** of the 21st century technology that overlooks the planet to connect and advocates to tear down the geographical and administrative barrier; who sacrificed their lives in Ethiopia (Metekel, Wollega, Ataye, Guraferda *etc*.) during 2020/21: just because of their ethnicity, my father; Mandefro Ademe (1942-1998), and my mother; Shashe Mitiku. Also, my devotion is to my dear wife; W/ro Wogayehu Getaneh, my Kids; Minilik Ayele and Kirubel Ayele.

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List of Acronyms

AI	Artificial insemination
AnGR	Animal genetic resource
ART	Assisted reproductive technology
BLUP	Best linear unbiased prediction
BLV	Bovine leukemia virus
BoLA-DRB3	Bovine leucocyte antigen DRB3 gene
BTA	*Bos taurus* autosome
eQTL	Expression quantitative trait loci
GEBU	Genomic estimated breeding values
IPD	Immuno-polymorphism data base
MAF	Major allele frequency
MAS	Marker assisted selection
MEGA	Molecular evolutionary genetic analysis
MHC	Major Histocompatibility complex
MJN	Median joining network
Ob	Obesity gene
PBR	Peptide binding antigens
PCR-SBT	Sequence based typing polymerase chain reaction
PIC	Polymorphic information content
SNPs	Single nucleotide polymorphisms

Chapter 1

1. Introduction

1.1.General Introduction

The United Nations proposed 17 sustainable development goals (SDG) to amend the planet by 2030. Among these, the second goal has been designed to end hunger, attain food security, and enhanced nutrition, and encourage sustainable agriculture. Doubling agriculture, pledging viable food fabrication systems and appliance resilient agricultural practices, and keeping genetic diversity have mentioned as a tool to realize the aforementioned objective (UNDP, 2015). The population of Ethiopia will grow from 110 million to 190 million within the upcoming three decades (2050) and the demands for agricultural products become higher (FAO, 2019). Consequently, the objectives described by the United Nations to end hunger never be achieved by traditional agricultural practices. These objectives should be supported by modern technological principles and techniques to achieve the aforementioned goal. The backbones of the economy of Ethiopia (agriculture) contributes 35% to gross domestic product (GDP) and 80-85% of employment, and 90% of export value (FAO, 2019). The livestock sector is one part of agriculture, accounting for about 45% of the entire value of agricultural production and supporting the livelihoods of an enormous number of the population. Quite 14 million households (70 percent) of the country’s population keep livestock, including many poor. The national herd comprises about 57 million cattle, 30 million sheep, and 23 million goats, and 57 million chicken (FAO, 2019).

Many developing countries including Ethiopia are found in the tropics and posses huge biodiversity. However, these biodiversity can’t satisfay the demand of agricultural products in the tropical environment. Thus, the challenge of tropical agriculture remains unresolved and looking solutions from the scientific world. The sector of cattle agriculture in the tropics faces challenging factors of growing productivity and profitability, comparative to the size of the human population. Adaptation of cattle with the corresponding environment is an immense issue that many scholars suggested so far (Barendse, 2017). Not only the environment but also technical, economical, and institutional constraints, livestock productivity is usually low. Of these, the entire production of milk (5.6 billion litres), meat (1.1 million tons), and 419 million eggs per annum are produced (FAO, 2019). After observing these low agricultural products, scientists aimed to improve the efficiency of production or to enhance the standard, quantity, and also profitability of livestock. Bearing in mind this panorama, it's time to improve the implementation of reproductive biotechnologies and modern biotechnological toolkits in cattle to enhance their impact on future successes, with attention on Ethiopia.

Reproductive biotechnologies (artificial insemination, multiple ovulation, and embryo transfer, in vitro fertilization also as transgenesis) aim to be used regularly to truncate generation interludes and to disseminate genetic material among breeding of animal population (Rodriguez-Martinez, 2012). The system of selecting superior animals within breeds (open nucleus breeding approach) is the leading suitable cattle genetic improvement option. Genetic improvement programs supported the choice within local populations are recommended during a low-input animal production system where the animal environment is stressful, feed resources are scarce, disease prevalence is high and animals are well adapted. It’s simultaneously an appropriate strategy for the sanctuary of the local genetic resource. The genetic improvement program was enhanced by using advanced animal breeding technologies. Meanwhile, the exploitation of molecular information to form selection decisions within the breeding program was envisaged decades ago. The recent availability in dense panels of single nucleotides polymorphisms (SNPs) markers has offered new opportunities to undertake marker-assisted selection. The principle of the candidate gene approach plays a task within the molecular breeding of livestock vis-à-vis marker-assisted selection (MAS) to enhance their productivity and production environment.

In this study, two important candidate genes that have a possible impact on economically important traits (MHC class II DRB3 gene and leptin gene) were selected. Among genes related to reduce mastitis and foot and mouth disease (FMD) virus occurrence, precise consideration is being given to the MHC BoLA-DRB3 gene thanks to the role this gene plays within the system. Also, production, growth, and reproductive traits are highly influenced by the leptin gene in livestock. MHC is an assembly of closely associated molecule that forms an important genetic constituent of the immunity of mammals and plays a vital role in pathogen recognition (Paracha *et al*., 2015). Bovine MHC isolation and characterization including sequence analysis and genomic hybridization of the genes were determined long years ago (Andersson *et al*., 1986; Groenen *et al*., 1989; Groenen *et al*., 1990). However, scientists around the globe got to study the genetic component of the MHC molecule in bovines. Thanks to the roles of those genes related to economically important traits, the MHC molecule is the principal segment of adaptive immunity in livestock especially in bovines and also offers an alternative to discourse evolutionary principles in cattle.

Hence, the roles of MHC molecules within the immunological recognition of foreign peptide antigens infer that the explanation for balanced selection is said to the consequences of MHC polymorphism on host defenses against pathogens. The physical location (map) of MHC molecule in cattle indicated on the bovine autosome 23 (BTA 23). The MHC of cattle is termed bovine leukocyte antigen (BoLA), which is exteremly variable and useful within the immunity of animals. The role of BoLA in cattle is to admit pathogens via the immune systems and is mainly used as a disease marker in breeding programs (Phillips *et al*., 2017; Bohórquez *et al*., 2020). Within the region of the BoLA class II molecule two genes, DR and DQ are encoded which bind to the peptides and present to CD+4 T-cells. Likewise, the region of BoLA-DQ embraces DQA and DQB loci by which the amount often varies counting on the haplotype (Takeshima and Aida, 2006; Miyasaka *et al*., 2011). The gene BoLA-DR comprises BoLA-DRA which is monomorphic by nature and additionally three DRB loci. Hence, the unique fully functional locus is BoLA-DRB3 which has been investigated by many scholars (Bohórquez *et al*., 2020; Takeshima and Aida, 2006).

The BoLA-DRB3 locus is the most studied and polymorphic region among the rest of MHC molecule in bovines (Wieczorek *et al*., 2017). The allele variety and polymorphisms of the BoLA-DRB3 gene from cattle breeds are reported recently using sequence-based typing (Takeshima *et al*., 2018). To date, the amount of alleles reported within the immuno-polymorphism database (IPD-data base) is 144, by which such inspiring variabilities are located on the β1 domains of the peptide-binding regions (PBR) (Sigurdardottir *et al*., 1991; Maccari *et al*., 2017, Suprovych *et al*., 2021). The character of polymorphisms of the BoLA-DRB3 gene and the connotations of the gene with various diseases were reported by scholars. For instance, Lei *et al*. (2012) researched to look at the association between polymorphism of the BoLA-DRB3 gene with foot and mouth disease (FMD). Among genes related to abridged mastitis occurrence, specific consideration is given to the BoLA-DRB3 gene thanks to the role of the gene in adaptive immunity (Hameed *et al*., 2006). Among the six exons of the BoLA-DRB3 gene the second exonic region has previously been described to be the foremost polymorphic and potentially disturb many features of the immune response, vegetative cell count, and mastitis frequency (Hameed *et al*., 2006). Furthermore, current investigations stated that the BoLA

DRB3 gene has been related to susceptibility and resistance to mastitis (Welderufael *et al*., 2018; Suprovych *et al*., 2017). This region encodes the antigen adhesion side of MHC molecules and plays an important role in the immune response to pathogens. In the past decades, most studies intended toward the documentation of molecular markers associated to bovine diseases and immunological traits are limited to commercially selected *taurine* cattle breeds (Takeshima *et al*., 2002; Lee *et al*., 2012; Takeshima *et al*., 2015). The improvement of the livestock industry has been focused for several years on traits linked with milk production and meat quality. However, traits linked with disease-resistant/susceptibility are neglected. This powerful selection for production traits has directed to an increase in the occurrence of diseases like mastitis in dairy cattle, the foremost important disease in dairy cattle breeding programs due to its high prevalence and association with economic loss (Owen *et al*., 2000). Production inclinations are related to a decrease in milk yield and variations within the main milk compositions like fat, protein, lactose, anions, and cations. These fluctuations harm the dairy industry due to the loss of processed milk and its byproducts (Nascimento *et al*., 2006). Improvement of cattle production and reproduction approaches have been supported by selection which is currently assisted by the applications of molecular markers (Singh *et al*., 2014). Many of these molecular markers are associated with the variation of immune reactions and can disturb all features of livestock such as production and reproduction performances (Nascimento *et al*., 2006; Zambrano *et al*., 2014; Morales *et al*., 2020).

Through the arrival of sequencing technologies and reduced sequencing costs, it is now possible to discover genome-wide molecular markers associated with disease resistance and susceptibility in locally adapted breeds. Ethiopia anchorages various cattle populations adapted to a diverse harsh agro-ecologies. Sheko cattle are adapted to the humid environments of southwestern

Ethiopia characterized by high tsetse infestation (Mekuriaw and Kebede, 2015). Contrariwise, cattle populations such as Boran, Afar, and Ogaden have long developed in the arid and semi-arid parts of eastern and southern Ethiopia where the incidence of trypanosomiasis is moderately low. Although some alterations in their susceptibility/resistance to local pathoges, no research has been shown to examine the MHC BoLA DRB3.2 gene in Ethiopian cattle breeds, which are crucial for forthcoming breeding platforms.

Leptin is a hormone produced by the obesity gene (ob), identified first in mice and humans, and later in bovines. It’s secreted by adipocytes, and it's been related to feeding consumption and energy balance in mice and humans. In bovine, the leptin gene is found on chromosome 4 related to feed intake, fat content, metabolism, meat quality, and growth traits (Dubey *et al.*, 2007). Leptin may be a peptide hormone primarily produced by white fat, and to a smaller level within the placenta and striated muscle (Liefers *et al*. 2005). This peptide hormone plays a task in the synthesis of glycogen and glucose transport and is especially active within the brain tissue within the region of the hypothalamus. This part of the brain takes part in regulating eating behavior, and therefore, the presence of leptin decreases hunger and depresses willingness for food. When fat grows larger correspondingly larger amounts of leptin are produced (Alberts *et al*., 2002). A major candidate for an endocrine regulation of leptin expression is insulin. Insulin appears to extend leptin secretion; a possible explanation for action might be that increasing levels of insulin cause an increased metabolism and glucose transport (Margetic *et al*., 2002). Documentation of candidate genes affecting the assembly and reproduction of cattle is a beautiful part of researches within the area of recent animal breeding. Leptin gene polymorphism is related to key economic traits that directly or indirectly influence the assembly and reproduction of livestock (Wylie, 2011; Symonds *et al*., 2016; Fernandes *et al*., 2020). Considering rich resources for livestock in Ethiopia, scientists trying a couple of assays to spot a gene that controls economicaly important traits. Identifying the candidate genes particularly in cattle breeds can greatly help to enhance the long-term breeding plans of the country. The cytological and molecular mapping of the leptin gene indicated that the gene is found on the fourth chromosome (BTA4q32) and transcribed into 167 amino acid peptides (Corva *et al*., 2009). Buchanan *et al* (2002) investigated that the missense mutation within leptin gene might be the cause to match cattle breeds by the differences of fat deposition. The idea stated allele differences of leptin gene are related to the differences in fat content in cattle in support of the finding of Buchanan *et al* (2002). The applications of genetic markers (leptin gene) might be of use in beef production (Shin and Chung, 2007; He *et al*., 2020; Chung *et al*., 2018). Numerous SNPs are previously reported within the leptin gene and studies revealed that polymorphisms were related to lean and fat cattle. The molecular information for the leptin gene is effective for molecular breeding to spot high-quality meat through marker-assisted selection (MAS). Although it's been reported that genetic variability of candidate genes like mitochondrial DNA is associated with growth traits in cattle, polymorphism information of leptin in cattle remains unclear (Yoon *et al*., 2005). The information on the Leptin gene in Ethiopian indigenous cattle remains a niche that scientists should justify the function, and therefore, the polymorphism of this gene. Characterization of such candidate genes has the potential application to be used in molecular breeding in cattle. Thus, this work characterized polymorphism of the leptin gene for selected local breeds of Ethiopia and Korean Hanwoo.

In general, this study mainly designed to evaluate the breeding plans of selected cattle breeds and to charachterize the selected potential candidate genes (BoL-DRB3 and leptin) responsible for economic traits.

1.2 Objectives

1.2.1 General objective

The overall objective of this study was designed to define the breeding scheme of selected Ethiopian indigenous cattle breeds and characterize the selected candidate genes (BoLA-DRB3 and leptin) in order to introduce the base line information about marker assisted selection (MAS) based on these selected candidate genes

1.2.2 Specific objectives

- To simulate the breeding plan of selected Ethiopian indigenous cattle breeds
- To assess sequence polymorphism of MHC II BoLA-DRB3 gene and genotype frequencies among Ethiopian cattle breeds compared with Asian breeds
- To assess sequence polymorphism of Leptin gene and genotype frequencies among Ethiopian cattle breeds compared with Korean Hanwoo breed
- To analyze the genetic relationship between Ethiopian cattle breeds with Asian breeds based on the selected candidate genes

Chapter 2

2. Literature review

2.1. Background and Domestications of Cattle

2.1.1 Classification of Cattle

Although the main breeds of the globally distributed cattle population are divided into two groups (*taurine* and *indicine*), their taxonomic classification is still mysterious. Based on the presence or absence of a hump, these groups of cattle are formally classified as *Bos taurus* and *Bos indicus* species (Loftus and Cunningham, 2000). Nonetheless, these species can produce fertile offspring when interbred. Sequencing technology and advancements of genomic toolkits influence the taxonomic classification both in animals and plants; Phylogenetic studies of regulatory region sequences of extant *Bos taurus* and *Bos indicus* compared with archeological specimens of the extinct wild aurochs (*Bos primigenius*) reveal four major maternal lineages. The new haplotype (P haplotype) of *Bos primigenius* was not grouped among domestic cattle. However, the new haplotypes of *Bos primingenius* representing the new lineage were almost equidistant with the groups of *Bos indicus*, indicating domestications from numerous isolated populations of *Bos primingenius*, a subspecies known for the taurine (*Bos primingenius taurus*) and zebuine (*Bos primingenius indicus*) cattle (Hiendleder *et al*., 2008).

Domestications of numerous Bovine species were known; however, taurine (*Bos tuarus*) and zebu (*Bos indicus*) cattle groups represented almost all cattle around the globe (Felius *et al*., 2014). Taurine (*Bos taurus*) and zebu (*Bos indicus*) cattle groups descended from wild aurochs (*Bos primigenius*). The two subspecies B.p. primigenius (South West Asia) and B.p. namadicus (India) were the descendants of taurine and zebu cattle respectively (Mapiye *et al*., 2019).

Genetic studies to evaluate the origin of livestock species have been revealed a reasonable set of novel questions concerning the origin and expansion of domestic animals for the last two decades. Investigation on domestications (single versus multiple), breeding of wild and domestic animals (inter-breeding versus intra-breeding), and tradition and faunal connections is changing present outlooks on the domestication routs and nature of biodiversity (Larson *et al*., 2014; Marshall *et al*., 2014; Pérez-Pardal *et al*., 2018). Research results of cattle domestication based on genetic criterion justified that *Bos taurus* and *Bos indicus* cattle breeds (modern cattle groups) descended from two genetically discrete auroch population of *Bos primigenius* and *Bos nomadicus* respectively (Bradley *et al*., 1998; Chen *et al*., 2010; Pérez-Pardal *et al*., 2018).

2.1.2 History of Cattle Domestication

Archeologists and historians puzzled more than a century to know the origin of domestic cattle. Based on archeological pieces of evidence, the domestication of zebu cattle was dated from 8,000-9,000 years ago and migrated throughout northwestern South Asia by 6,000 years before present (Meadow, 1996; Patel, 2009). Moreover, South India has been considered as one of the domestication centers for cattle (Fuller, 2006; Pérez-Pardal *et al*., 2018). The lack of primary faunal material and the broad range of phenotype diversification expressed by the existing cattle population have perplexed the subject to be highly argumentative (Caramelli, 2006). Scholars suggested diverse theories which clarified different distinct and sequential models for the origin and blowout of domesticated cattle. Origin of domestication (single/multiple) for cattle was one point of argument; nevertheless, most specialists' well-thought-out that the first center of domestication for cattle was directed in Asia (southwest) and that domesticated cattle arrived Europe with livestock keepers migrating from this part of the continent. The domesticated Taurine cattle breeds migrated to Africa from southwest Asia, whereas African domesticated zebu cattle breeds were migrated from the Arab and Indian sub-continent later than Taurine cattle (Caramelli, 2006).

2.2. Cattle Genetic Resources in Africa

2.2.1 Bos taurus Cattle Breeds

Bos taurus (humpless) cattle breeds are among the earliest cattle breeds of Africa trailed by the advent of humped indicines (Gebrehiwot *et al*., 2020). The group of *Bos taurus* cattle in Africa comprises humpless shorthorns and longhorns. These groups of cattle mainly populated in Western and Central Africa by which the size for both groups is small, as well as the productivity, is relatively lower than most of the zebu cattle breeds (Rege, 1999; Mwai *et al*., 2015). The groups have a distinctive evolutionary adaptation to the harsh environmental conditions and various pathogenic diseases (Mwai *et al*., 2015). In general, African zebu (*Bos indicus*) cattle breeds are more adaptive than European *taurine* (*Bos taurus*) for thick-born disease (Mattioli *et al*., 2000). We can justify Sheko breed from Ethiopia as a representative *Bos taurus*. Sheko is first reported in 1929, inhabited in South-west Ethiopia, and later in 1982 (Albero and Haile-Mariam, 1982) with the name of Gimira (Kuri-humpless longhorn) cattle breeds of Ethiopia that was declared in 1929 initially by Encyclopedia Britannica (Mekuriaw and Kebede, 2015). The present Sheko breed is inhabited merely in the secluded corner of the Southwestern part of Ethiopia especially at the clammy Sheko and Bench Maj zone maintained by smallholder farmers who reared them for thousands of years due to their nature of resistance to various disease specifically trypanosomiasis (Mwai *et al*., 2015). Sheko cattle breed signifies the earlier remnants of Africa’s unique *Bos taurus* (humpless Shorthorn) cattle which were possibly the principal breed domesticated in Eastern continent of Africa (ILRI, 2007). According to Dadi *et al* (2008), phylogenetic tree analysis based on the genetic distance indicated that Sheko cattle breed is out grouped to Sanga cattle breeds of Ethiopia. Sheko breed can be characterized as having a thinner abdomen and hindquarters, and smaller horns or absence of horns that made them greatly simple to manage. Also, the breed has good feed conversion efficiency, longevity, fertility, and great mothering capacity to other cattle breeds in the contiguous zones (Takele *et al*., 2009). The selected morphologies (fast growth rate and ownership of large teats) are selected morphological traits measured as important to improve milk production in Sheko breed (Takele *et al*., 2009). Great emphasis had been given by Rege (1999) and Sheko cattle breed is purposefully advanced for milk production. Due to the introgression of zebu cattle breeds in the area, nowadays some Sheko cattle inherited obvious small humps (Tatek and Abegaz, 2013; Mekuriaw and Kebede, 2015).

2.2.2 *Bos indicus* Cattle Breeds

Bos indicus cattle is further classified into zebu, sanga, and zenga. Humped Zebu and Sanga cattle breeds of Africa have traditionally been categorized as *Bos indicus* and accommodate the common of cattle types (Frisch *et al*., 1997; Mwai *et al*., 2015). Their shoulders have a fatty thoracic hump and large dewlap best articulate zebu cattle breeds (Mwai *et al*., 2015). According to Mattioli *et al* (2000), zebu cattle breeds are highly preferred to adapt to the harsh environmental conditions and more resilient for thick borne-diseases relative to *Bos taurus* cattle breeds. The Western and Eastern parts of Africa are the main habitats of zebu cattle. Phenotypically the body size is large, the production level in trypanosome-free areas is high, and they have been selected by the local farmers; and consequently somewhat elucidate their richness and wide dissemination in Africa (Mwai *et al*., 2015). Sanga is the cross of *Bos taurus* with *Bos indicus* cattle breeds which are an intermediate type of humped (cervico-thoracic not thoracic) cattle breeds. They live mainly in the eastern and southern parts of Africa and are recognized to be well amended to variable harsh environmental conditions (Okello and Sabiiti, 2006). Hanotte et al. (2002) justified sanga cattle breeds are introduced by the hybridization of taurine and zebu cattle nearby 700 AD. Crossbreeding between sanga cattle, and newly hosted zebu headed to a new cattle type called Zenga (Rege, 1999). The habitat of Zenga cattle breeds are eastern Africa.

Hence, Arsi breed of Ethiopia grouped under large East African Zebu (howbeit, small in size) and characterized as poor milkers, extremely active and aggressive (Rege and Tawah, 1999; Mwai *et al*, 2015). They are compact animals and mainly kept for draught with diminutive length. Highly variable coat colour like red, black, roan, white, and grey expressed well for this breed (Rege and Tawah, 1999).

Table 2.1 Geographic distribution and classifications of Ethiopian Cattle Breeds

Category	Type of cattle	Distribution in Ethiopia	References
Large East African zebu	Arsi	Oromia (Arsi, Bale, E. Shoa)	Albero and Haile-Mariam, 1982 ; Dadi, 2014
	Boran	Borana lowlands of Bale, part of Somali	Albero and Haile-Mariam, 1982
	Begait	Western Tigray	Zerabruk *et al*., 2007; Abraham and Abebe, 2018
	Barca	North West Tigray	Albero and Haile-Mariam, 1982
Humpless Shorthorn	Sheko	SNNP in Bench Maj Zone	Taye, 2005; Albero and Haile-Mariam, 1982
Small East African zebu	12 zebu cattle types	All over the country	Albero and Haile-Mariam, 1982; Dadi, 2003
Senga	Anuak	Region of Gambella	Albero and Haile-Mariam, 1982
	Afar/Danakl/Kereyu	North East lowlands of Afar, Rift Valley	Albero and Haile-Mariam, 1982; Shiferaw *et al*., 2006
	Raya-Azebo	South Tigray, North Wollo	Albero and Haile-Mariam, 1982
Zenga	Arado	North Highlands of Tigray	Albero and Haile-Mariam, 1982
	Fogera	Around Lake Tana, Gondor	Albero and Haile-Mariam, 1982
	Horro	East Wollega, W.Shoa, Keffa, Illubabur	Dadi, 2003

According to DAGRIS (2007), Horro cattle breed is one of the ecotypes identified in Ethiopia and experienced in the highland agro-ecological regions of the country (Mekennen *et al*., 2012). Horro cattle breed belongs to the group of Zenga which is the result of the cross of Zebu and Sanga (Rege, 1999; Edea *et al*., 2012). The main habitat of Horro cattle breed is in the Horro Gudru zone of Eastern Wollega and bordering parts of Western Showa and Illubabur. The breed is mainly characterized by calm disposition and variable milk production in a mixed crop-livestock system (Edea *et al*, 2012; Mwai *et al*., 2015). Fogera cattle breed is found in the North-west highlands of Ethiopia in the surrounding area of Lake Tana and mainly reared for drought and dairy production. The breed belongs to the Zenga group which is the cross of Sanga and Zebu (Rege, 1999; Zerabruk *et al.*, 2007). Docile temperament can best describe the Fogera cattle breed (Mwai *et al*., 2015). The breed is good for fattening and the feed intake capacity is higher.

Begait cattle are recently registered as one of the indigenous cattle of Ethiopia (IBC, 2004). The breed is grouped under large East African Zebu classification (Zerabruk *et al*., 2007; Abraham and Abebe, 2018; Mekonnen and Meseret, 2020). According to the report of DAGRIS (2014), Begait cattle assembled to the North Sudan Zebu group and tended for milk and beef purpose (Mekonnen and Meseret, 2020). On the basis of body frame Begait breed is comparable to Boran and Ogaden breeds (Rege, 1999; Mekuriaw *et al*., 2009). Moreover, the breed is relatively superior to other Ethiopian breeds, which survives in trypanosome prevalent areas of the country (Zegeye *et al*., 2021).

Boran breeds are mainly inhabited in the Borana plateau of southern Ethiopia, extending from the Liban plateau to the extreme southern part of the country. The breed is mainly reared for beef; however, they are milked by the pastoralists (Rege and Tawah, 1999). Boran breed is categorized under large East African Zebu and the program to improve the Boran cattle breed developed in Kenya including the foundation of breed society (Rege and Tawah, 1999). Also, Ethiopia undertook some evaluation studies to improve the genetics and productivity of the Boran breed.

The improvement of cattle genetics requires major skills that the cattle owners can advance the production and profit of their herds. Moreover, genetic improvement embraces choice of superior animals from a herd to generate healthier yields in the future generations (Nwogwugwu *et al*., 2020).

Many diverse cattle genetic resources have been adopted and dispersed in various agro-ecological regions of Ethiopia. Hence, cattle production can be considered as one of the core constituents of agricultural activities almost in every part of the country (Terefe *et al*, 2012; Terefe *et al*., 2015). To date, many scholars conducted different research to identify the available cattle genetic resources and justify the phenotypic and genetic diversities of cattle (Terefe *et al*., 2015). The dwindling number of indigenous populations capable of surviving in harsh environments poses a threat to the poor's food and livelihood survival, as well as their ability to survive in remote areas. There is an embedded belief that imported exotic breeds are superior in all aspects. There is a very limited awareness on the problems of animal genetic resources erosion and on the value and relative advantage of the indigenous breeds. Several breeds are reported to be extincted and many are on the verge of extinction in Ethiopia. For example, the Gimira or Kuri cattle from South-western parts of the country, the Arsi-Sanga from the area of present day Arsi and Bale highlands as well as Baria zebu from North Ethiopia have faced extinction. The Sheko, Mahbere-Slassie, Qocherie, Wegera and Semien are some of the endangered cattle populations while the risk status on many populations is unknown (DAGRIS, 2007). Among these, the endangered Sheko cattle symbolizes the only remnants of the original African taurine cattle in East Africa. The breed represents a unique component of the global domestic animal genetic resources that deserve conservation.

The unimproved Boran cattle of southern Ethiopia are facing genetic erosion from introgression of highland cattle bloods, ecological changes and recurrent drought. Sustainable use, breed development and conservation of Borana cattle are vital to agriculture, food production, rural development and the environment. There is therefore a pressing need to addresses conservation and genetic improvement of indigenous Boran cattle in Ethiopia. Borana cattle genetic improvement programme has been suggested, and there is on-going activity to realize this issue at Oromia regional state. However, there are several technical problems to undertake a meaningful Borana cattle genetic improvement programme.

2.3. Cattle Production Systems in Ethiopia

To date, the estimated cattle population of Ethiopia is 59.5 million, of which 55.5 % and 44.5 % are female and male cattle, respectively. Thus, the cattle population showing 98.2 % indigenous, 1.62 % cross, and 0.18 % exotic breeds (CSA, 2017). The majority of cattle production system in Ethiopia depends on low producing indigenous breeds of cattle. Production systems of Ethiopian cattle belong mainly to the following four livestock production systems: pastoral and agro-pastoral production, mixed crop-livestock production, specialized commercial production urban, and peri-urban smallholder production systems.

The crop-livestock production system in the Ethiopian highlands has a high potential for cattle development and is the leading of its kind in sub-Saharan Africa (Tedla *et al.*, 1999). This production system mainly exists in the highland agro-ecological zone, where the climate favors both crop cultivation and livestock rearing as harmonizing creativities and major types of farm animal species. The milk production in this production system depends on a high amount of indigenous breeds and a small number of crossbreeds of cattle. In this farming system, the entire feed requirement is driven from native pasture and a balance arises from crop residues and stub grazing.

Pastoral and agro-pastoral cattle production system exercised is in the major lowland areas of the country where the climate is arid and semiarid agro-ecology. This system is branded by sparsely populated pastoral rangelands, where livelihood is mainly based on livestock and livestock products except in agro-pastoral areas, where some crops are produced for both livelihood and marketing. Pastoralists exercised this system grazed large herds of cattle on the common and public land. Because of the seasonal scarcity of feed and water, cattle trek over long distances. Pastoralists are unable to settle and take advantage of available production technology. Goats, cattle, sheep, and camels are the major livestock husbandry in this system. Mainly milk serves as a source of food and pastoralists have a habit to keep a large number of herds to secure food in the form of milk supply (IBC, 2004).

The fourth cattle production system located in the cities and/or towns (Urban cattle production systems) mainly for production and sale of milk, with little or no land resources, using the accessible human and capital resources commonly for specialized cattle production under compartmental feeding circumstances (Azage *et al*., 2013). By advantage of their location, urban manufacturers are not likely to have access to pasture land, as the procedure takes place within cities and as a result they are forced to buy feed (Zegeye, 2003).

2.4. Genetic Improvement Options of Tropical Cattle

The most valuable species in the livestock sector is cattle delivering many services for the farmers and also contribute to the national economy of many developing countries. In the dairy industry, minimizing the production costs, improving the profitability from the sale of milk, reducing feed costs, and economically exploiting male calves are essential (Hernández-Castellano *et al*., 2019). In cattle breeds that males are feasible, breeding is mainly preferred for beef purposes over those completely applied for dairy purposes. The dual-purpose system of cattle production (milk and beef) illustrating a flexible and gorgeous option for tropical cattle breeding strategies (Rojo-Rubio *et al*., 2009). In many tropical countries like Ethiopia, dual-purpose cows are very vital because they offer the major sources of animal protein such as milk and meat (Mao *et al*., 2016; Ruíz-Guevara *et al*., 2018). For breeds applicable for dual-purpose, particular attention being paid to appreciate the association between milk yield and growth traits to appraise hostile features that motivate the improved traits over joint selection (Brito *et al*., 2020).

Reproductive traits should be included in the selection scenario because these traits are considered to be essential in recent times due to the decreased fertility and reproductive potential of tropical zebu cattle breeds in the tropics (Santana *et al*., 2015). Thus, the primary goal is to enhance sexual maturity over the selection of age at first caving (AFC), among one of the major relevant and common traits related to reproductive efficacy and female fertility (Brito *et al*., 2020).

The foremost aims of breeding platforms have been designed to improve meat and milk recital of *taurine* and Zebu cattle breeds as well as introducing disease tolerance of *taurine* cattle breeds. Sometimes, “closed nucleus” breeding schemes (CNBS) have been verified as limited and therefore have over-jumped into “open nucleus” breeding schemes (ONBS). Currently, Community based breeding approaches have a promising insight to the dairy producers. The main difficulty of breeding packages leftovers defining truthful breeding objectives and safeguarding the association of stakeholders (Ouédraogo *et al*., 2021). Entirely the approaches that this research project addressed may be very helpful for the design for proper breeding objectives to the selected cattle breeds in Ethiopia.

2.4.1 Cattle Breeding Schemes and Design of Breeding Objectives

The main objective of breeding livestock is to produce genetically improved generation in terms of their performance compared to their parental generation and one or more number of traits could be focused to achieve the objective. Multifunctional group of livestock (cattle) provide service like traction, milk production, income generation, manure, reproduction and meat production (Edea *et al.*, 2006). The main breeding objective traits in cattle was gaining better milk yield, and moreover, enhancing milk yield, earning of good breeding bull, and draft, good mothering ability and shortening of calving intervals were also designed as breeding objectives (Godadawu *et al.*, 2015) and the other traits significant for obtaining breeding objectives were drought power, coat color, ability of mothering, butter yield and attaining marketable animals for better improvement and production.

Open nucleus breeding scheme is a hierarchical breeding system in which animals are transferred between levels in both directions. The simplest open nucleus breeding system has two tiers, a nucleus consisting of small number of elite breeding animals or “Upper tier” and participating herds consisting of large number of animals or “Lower tier”. In general, there may be more than two tiers, and there may be several animals in one tier. Usually, the lower tier is kept for subsistence or commercial production and the nucleus to breed superior sires. Nucleus breeding schemes would be a good strategy for Boran cattle genetic improvement where there is no adequate money, expertise and structures for operation of an effective improvement programme based on artificial insemination (AI) and recording in the whole population. Closed nucleus breeding schemes can be used for the breeding design of cattle and had a higher disadvantage of preventing inbreeding and smaller genetic gain compared to open nucleus breeding schemes, which should be predicted due to the huge number of breeding animals in open schemes (Meuwissen and Woolliams, 1994).

Cunningham (1989) also stressed that open nucleus breeding scheme is more appropriate for subsistence production system in developing countries. Detailed recording procedures would be required only in the nucleus herd and expensive infrastructures may not be required. Such breeding programme integrates farmer resources and reduces overhead costs of genetic improvement programme. Open nucleus breeding schemes have been suggested to overcome some of the technical constraints linked to implementation of a breeding scheme for low-input extensive production systems (Yapi-Gnoare, 2000). Generally, the advantages of Open Nucleus Breeding Scheme include the following:

- Open-nucleus breeding schemes can be distinguished from closed-nucleus schemes by the possibility of introducing non-nucleus animals into the nucleus herd, which allows to -reduce substantially the rate of inbreeding in the nucleus.
- It maximizes genetic improvement because there are more animals which are potential candidate for selection and reduce total cost of recording in participants herds.
- The environment automatically reflects the production environments; hence there is low genotype by environment interaction.
- It integrates farmers/pastoralists resources, reduce overhead costs and encourage cattle owners participation in the programme and develop sense of ownership.
- The scheme is simple enough to allow breed improvement programs to be launched without many resources.

The nucleus herd which is at the top of the pyramid (**Figure 2.1**) represents the main breeding unit. Open nucleus breeding scheme has the following key features:

(i) The nucleus is the tier that where tremendous selection is the main activity (embryo transfer, artificial insemination will be implemented), it is the tier that builds genetic gain. After accurate data development genetic markers will be developed and evaluated.

(ii) There is transfer of bulls from the nucleus herd to sire progeny in the participating herds. It is difficult to organize any kind of breeding intervention other than the provision of bulls.

(iii) There is introduction of dams born in the participating herds to the nucleus, dams are selected subjectively based on easily and cheaply measured traits from participating herds.

(iv) The bulls born in the nucleus are used to produce cows and to sire bull calves that are kept for use to mate a proportion of the cows in the participating herds. The dams in the participating herds are used to produce both bulls and cows in this sector.

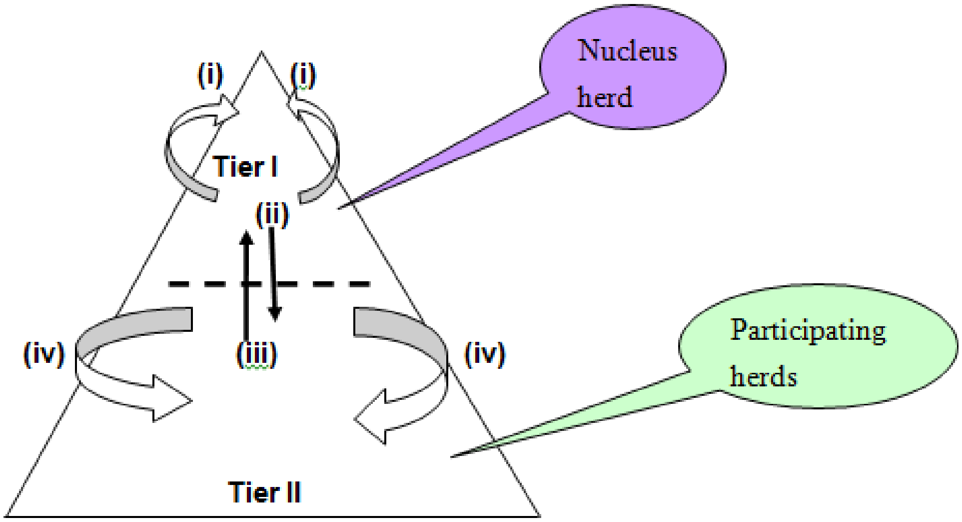


Figure 2.1 Open nucleus breeding scheme proposed for genetic improvement of indigenous cattle

Approximately, with an annual human population growth rate of 2.4%, the present about 77.4 million Ethiopia's human population will grow to about 190 million by the year 2050 (FAO, 2019). The rural to urban ratio will also continue to change and is expected to increase in favor of urban population in the coming 25 years. To meet the ever-increasing demand for milk, milk products and beef production and thus contribute to poverty alleviation in Ethiopia, genetic improvement of indigenous cattle is proposed. It should be recalled that even European breeds were as unproductive as the tropical breeds before the application of genetic improvement programmers and it is the application of planned breeding programmers that has brought the advance that we see today. Since, the techniques for genetic improvement are even more developed today than when they were first used on European cattle, it is possible that similar achievements could be made in our local Boran cattle population by combing conventional selection method, reproductive technologies and genomic information (Thomasen *et al*., 2016).

2.4.2 Reproductive Biotechnologies: Improvement Options in Livestock

Livestock is the major sector of agriculture which subsidizes major revenue for dairy farmers (small holder farmers at large) in developing countries to alleviate hunger. Animal genetic improvement nowadays becomes a primary alarm for the researchers in the globe. Reproductive performance and management systems are the main influancial factors in all livestock production systems such as milk and meat. In latest times, there has been growing challenges for enhancing productivity and inhibition of diseases with changing the climate. These aims can be achieved by traditional reproduction techniques. The advent and application of modern reproductive technologies have opened many paths to study, treat and control the reproductive phenomenon both *in vitro* and *in vivo* to improve reproductive performance in various domestic species of livestock (Choudhary *et al*., 2016). The major objectives of applying reproductive biotechnologies in livestock sectors are to boost production, reproductive efficiency and rates of genetic improvement (Andualem, 2016). Some potentially applicable methods of reproductive biotechnology to improve livestock genetics are listed below.

2.4.2.1 Artificial Insemination

AI is the method by which semen is manually collected from superior male animals and placement of collected semen in the reproductive tract of the superior female by breaking the rule of natural mating. This is the only major important traditional technique applied for cattle genetic improvement (Sugulle *et al*., 2006). It is one of a group of technologies commonly known as "assisted reproduction technologies" (ART), whereby offsprings are generated by facilitating the meeting of gametes (Souza *et al*., 2015).

AI (the first generation biotechnology) permits genetically selected supercilious bulls to produce large numbers of progeny in a short time period with selected superior females. Cattle genetics can be improved up to 50% by the aid of AI; using either extended semen that has been preserved in liquid form, or deep-frozen. Global advancement and application of AI with well-kept (chilled or frozen) semen have been increased exponentially through the past 50 years. The number of produced semen doses is >250 million worldwide, using standardized methods for extension, cooling, freezing and thawing basically all over the world, with only fine differences between *Bostaurus*, *Bosindicus*, *Bubalusbubalis* or *Bosjavanicus* (Rodriguez-Martinez, 2012). Many possible benefits of AI have been justified as compared to natural service. Thus, cost efficiency, disease management, safe breeding, flexibility, and fertility management are the main encouraged factors to promote AI as a means of genetic improvement (Geberemedhin, 2005; Holm *et al.*, 2008). AI also plays significant role to enhance the yielding capability of cows and is the suitable and inexpensive way of genetic improvement. The available AI equipment with proper inputs are not well managed and increased cost of production, storage, and transport of semen was considered as a limiting factors of AI (Pope, 2000; Desalegn, 2008).

2.4.2.2 Multiple Ovulation and Embryo Transfer

Smith (1988) announced the perception of multiple ovulation and embryo transfer (MOET) and established how cell-designed multiple ovulations and embryo transfer programs could lead to increase selection intensity and reduced generation intervals resulting in improvement of genetic gain. MOET is a complex type of reproductive biotechnology and includes super ovulation, estrus synchronization among recipient, AI of donor, embryo recovery from donor, short-term *in vitro* culture of embryos, embryo transfer into recipients. There is still substantial possibility for development and proper exploration on all these component parts. Nucleus schemes of MOET include creating a nucleus herd of elite males and females, exhaustive selection and testing in the herd, selecting elite males and females at early age based on family pedigree data i.e. Performance of their sisters and half-sisters denoted to as sibling test in the case of pedigree selection, thus the generation interval is considerably reduced (Rajesh and Subh, 2015).

Furthermore, MOET international movement of materials and reduces the risk of transmitting specific diseases, provided the embryos are free from contamination. Although MOET would be imagined beneficial as an approach for genetic advancement, and up to 80% of embryos have been commercially transported, the technology has not well optimized due to the different responses of the ovarian response to the super ovulatory gonadotrophin treatment used so far (Wu and Zan, 2011).

Embryo transfer allows heritably superior cows to equally produce a huge number of progeny in a short period of time through transfer of their embryos into recipient females. Despite its superiority respect to *Bos taurus* under tropical or sub-tropical environments, where stressors like high temperature, high humidity, parasitic pressure, and low quality pastures are less likely to disturb *Bos indicus*, the latter presents smaller pre-ovulatory follicles, a lower LH secretion capacity and a shorter oestrous duration. Oestrus detection responses to oestrus synchronization and management of follicle development in super-ovulation are highly variable (Bo *et al.*, 2014). Regarding with the manipulation of ovulation scientists concerned asynchrony between time of AI and rate of ovulation, as well as the reduced sperm transport registered after a super-ovulation treatment, both of which lead to low fertilization rates in cattle. As a technique, ET fundamentally wants synchronization of the donor and the recipient females so that the embryos are recovered and transferred in synchrony in order to warrant a proper embryo elongation and the recognition of pregnancy by the recipient cow (Rodriguez-Martinez, 2013).

2.4.2.3 *In vitro* Fertilization (IVF)

The concept of *in vitro* fertilization (IVF) was introduced by Thibault (1954) after the birth of offspring accomplished in the rabbit. Unfertilized eggs are impregnated in the laboratory and cultivated for a few days until they have developed in to an early embryo. These are then transplanted by using special long syringe in to the uterus of the receiver cows that are at the correct open stage of the oestrus cycle. Obviously semen and eggs are possibly collected from high quality parents based on their family history (Chakravarthi and Balaji, 2010).

2.4.2.4 Cloning and Transgenesis

It is one of the reproductive technologies that has been designed to produce identical offspring and introduced for several decades. Developing embryos divided to produce identical offspring and there have been commercial endeavors to apply this technology in the livestock industry. The major latest reproductive technology in livestock was the removal of somatic (non-germ) cells from adult animal and generating a clone. Cloning, consequently, permits to produce a genetic copy of an already genetically verified animal. Transgenesis includes placing a particular gene or genes (play a role for economically important traits) into the genome of an animal and thus the capability to transmit this gene in future generations. After having the cloned animal that already contained candidate genes, breeders allow incorporation of genes through crossbreeding of animals. Transgenesis thus offers a more rapid method for introducing new and desirable genes into a specific genome (Laibl and Wells, 2006).

2.4.3 Markers and their types

2.4.3.1 Morphological markers

Characteristics of animals detected externally by visual outlook and direct measurement like coat pigment, body shape, skin assembly, and anatomical physiognomies are the main features of morphological markers (Van Wezel and Rodgers, 1996; Gizaw *et al.*, 2007). They function mainly for identification, taxonomy, and description of genetic evolution of various species (populations). However, the phenotype of animals is mainly resulted by the experience of genetic inheritance and the environment (i.e. GxE). The accuracy of assessment of farm animal genetic resources (AnGR) over morphological markers is low due to subjective rulings and images. Moreover, the capacity and sympathy of animal morphological traits ordinarily needs a long time, and it is not easy to regret the possessions of environmental factors. Therefore, the presentation of morphological markers is restricted in the appraisal of quantitative traits capable of mapping quantitative trait loci (QTL). Conversely, morphological markers are an effective method for the assessment of qualitative traits so far, for which it is simple to characterize phenotypic variances between individuals through straight surveillance and measurement (Yang *et al*., 2013).

2.4.3.2 Cytological markers

Based on chromosome number and morphology, scientists reported that cytological markers are functionally applicable to justify farm animal genetic resources (Nadler *et al*., 1973 and Popescu *et al*., 1976). Cytological markers comprise the philosophy of karyotype, bandings, repeats, deletions, translocations, and inversions. Chromosomes are the carriers of genetic material and chromosome mutations are crucial sources of genetic variation, we can use these mutations as markers to determine the specific location of a gene on the chromosome, and its position relative to other genes. For example, researchers can suggest the origins and evolutionary history of livestock, and assess the genetic multiplicity of domesticated animals by associating the chromosome number and organization between domesticated animals and their wild descendants (Hailu and Getu, 2015).

2.4.3.3 Biochemical markers

Blood types and isozymes have been used as markers (biochemical markers); analysis can be carried out based on the protein electrophoresis. According to Buvanendran *et al* (1967) the genetic variability within species and phylogenetic investigations that the genetic variation within species and phylogenetic associations between species by variances in the amino acid

(AA) component of isozymes and solvable proteins (biochemical markers mainly dependent on gene products) (Buvanendran and Finney, 1967; Teneva and Petrović, 2010). However, neither proteins nor isozymes are genetic apparatus but the products of gene expression and they are susceptible to ecological influences and individual growth inconsistencies, controlling the extensiveness of their use. Protein electrophoresis is a fast, economic, and direct forward technique and delivers a more comprehensive illustration of polymorphisms than morphological or cytological markers; thus, it is still widely used in clarifying the origin and taxonomy of species (Jonker *et al*., 1982; Drinkwater and Hetzel, 1991; Yang *et al*., 2013).

2.4.3.4 DNA based Molecular Markers

Traditional selective breeding in livestock aims at improving the genetics of local populations of animals to allow them to survive and thrive in the prevailing environment, thus providing food and a source of traction for the indigenous communities (Singh *et al*., 2014). Now we are in the era of genomics. Hence, identifying economically important traits and characterizations of responsible genes for the economic traits could be possible at the gene level. Genomic selection techniques focus on the incorporation of molecular information in the breeding programs of livestock (Singh *et al*., 2014). The markers interpreting variabilities at the DNA level are referred to as the molecular markers. The advancement of livestock genetics became possible with the advent of molecular markers. Molecular markers are detectable DNA sequences, originated at specific positions of the genome, and conveyed from generation to generation (Wakchaure *et al*., 2015). The use of gene marker tests allowed breeders to improve livestock using the MAS as part of their overall improvement programs. Among the molecular markers, DNA-based markers are preferred to study polymorphism and diversity of livestock populations. Even if, hybridization-based markers and PCR-based markers play their great role in the study of polymorphism and gene characterization sequence-based DNA markers exceed the applications of molecular information in the livestock sector (Deb *et al*., 2012).

MAS is an advanced new technology that can harmonize traditional breeding methods for rapid genetic gains (Moniruzzaman *et al*., 2014). The classification of molecular markers is mainly rooted in the origin of methodologies used for the detection of polymorphism (Viryanski, 2019). In the studies of animal inheritances, molecular markers illuminating variability at the DNA level has a fantastic role. Smart breeding which can be defined as marker-supported breeding approaches is very important to improve the livestock sector (Al-Samarai and Al-Kazaz, 2015).

In the sector of livestock, protein variability was the first molecular marker identified. A large number of researches, predominantly in the 1970s, renowned the characterization of blood group and allozyme systems of livestock (Manwell and Baker, 1980). Nevertheless, the degree of genetic differences visualized in proteins is commonly low which has abridged the general applicability of protein typing in diversity studies. DNA-based markers are the primary choice for molecular-based analyses of genetic variability in both plants and the livestock sectors due to the advent of PCR and sequencing techniques associated with automatic and/or semi-automatic large-scale screening systems. The history of animal domestication achieved a considerable part in the existence of up-to-date life and urbaneness. The narrative of genetic origin and phylogenetic associations within and between animals is vital for the understanding of cultural adjustments in these civilizations, and ultimately has community and scientific appeal (Anthony *et al*., 1986; Levine *et al*., 1999). The descriptions of animal genetic origin have been justified based on three genetic resources (mitochondrial genetic apparatus, Y chromosome, and nuclear genetic apparatus) (Meadows *et al*., 2006). The mitochondrial genome (mtDNA) habitually functions to elaborate the definition of species genetic variability of domestic animals, frequently involved in species definition and genetic differentiation of domestic animals, for the reason of their unique features like stable gene conformation, higher copies than the nuclear genome, lack of introns, high polymorphic rate, lack of recombination, and inherited by the maternal fashion (Han *et al*., 2010; Cai *et al*., 2011; Ünal *et al*., 2020). More principally, mtDNA sequences will be used to recognize the putative wild ancestors, the number of maternal ancestries and their terrestrial origins (Hanotte *et al*., 2000). From the history of different male generations the DNA sequence of each Y chromosome conserves a specific record of mutation occasions due to their uniparental transmission and absence of recombination. Researches of genetic polymorphisms in the regions of the Y chromosome (no recombination) exemplify simple and fast way to identify and enumerate male mediated genetic admixture, and many scholars suggested for the identification of male mediated immigrations actions, renovating paternal history and trace individual founder lines or relatives (Malaspina *et al*., 1990; King and Jobling, 2009; Lorenzo *et al*., 2016). Active population size is regularly bargain additional by the comparatively higher polymorphism of male propagative achievement. Subsequently, current demographic occasions (bottlenecks, extension of population, and founder effects) have been sensitively indicated by the Y chromosome *(*Edwards *et al*., 2011).

2.4.3.4.1 Microsatellite Markers

The development of DNA-level genetic markers; specifically, DNA microsatellites, have made the detection of QTL in all major livestock species. Thus, unlike poultry or majority of plant species, dairy cattle breeding programs are dependent on selection within the commercial herd, rather than selection within specific herds controlled by breeding enterprises. Most modern dairy cattle breeding programs are based on the “progeny test” scheme which has several major weaknesses like the need for a large population with recording on production traits and pedigree and inaccurate recording can significantly reduce rates of genetic gain (Weller, 2017).

Short nucleotide repeats (2-6) are distributed in the genome for microsatellite markers, which are highly polymorphic. In mammals, the most common dinucleotide motif is (CA)n, where n represents the number of repeats. Microsatellites are the most recommended elements in research to study genetic diversity, parentage verification, and genome mapping programs. Microsatellites are thought to have a high mutation rate, and there are always a large number of alleles of different sizes at a single locus (Beuzen *et al*., 2000). For different organisms, including humans, rodents, fruit flies, and farm animals, a large number of microsatellite markers have been mapped (cattle, sheep, pigs and chickens at large). Microsatellites are DNA segments that are a few nucleotides long (2 – 6 bp) and are replicated several times in the genome (Yadave *et al*., 2017). Many microsatellite markers have been identified and recommended based on their amplification condition in PCR technique, markers information and amplification reactions (Barendse and Armitage, 1994; Bishop and Kappes, 1994). Microsatellites were the most common and effective technique for genetic diversity investigation prior to the use of SNP markers, not only in livestock but also in humans (Goddard and Hayes, 2009).

2.4.3.4.2 Single Nucleotide Polymorphisms

Recently, single nucleotide polymorphism (SNP) has become the most common sequence-based technology for studying DNA sequence variation. Variable nucleotides appear at the same location in the DNA sequence, resulting in an SNP. These markers are prevalent in the genome's coding and non-coding areas. Between 1.6 and 3.2 million SNPs are predicted to exist when two human DNA sequences are compared (Sabir *et al*., 2014). They're bi-allelic markers, meaning they only display a polymorphism in two alleles of a population. SNP in coding regions can be directly linked to protein function, and since the inheritance pattern is more stable, they are better candidates for long-term selection (Beuzen *et al*., 2000).

In animal genomes, SNPs are the most common types of sequence variations (Jiang *et al*., 2016). SNPs are points in a DNA sequence where more than one nucleotide form is present in a population. SNPs are base substitutions within nucleotide sequences in a strict molecular context, and their high density in the genomes of eukaryotes, including animals, has been of great importance in population genomics studies (Goddard and Hayes, 2009).

Despite the fact that SNPs are bi-allelic (sometimes tri-allelic or quadri-allelic) co-dominant molecular markers, their high density allows for more quantitative knowledge on genome dynamics to be elucidated within a sample population than any other technique. They also have more detail about linkage disequilibrium and haplotype diversity, pedigree information, and historical demographic events like bottlenecks within a target population than microsatellites. These characteristics, combined with the fact that SNP markers have a low error rate, are allowing SNP markers to be used in a broader range of applications in livestock genetics. These characteristics, combined with a low error rate, are allowing SNP markers to be used in a broader range of applications in livestock genetic architecture, such as precise identification of genomic regions that regulate economically and survival-related traits, and ultimately genomic selection (Yaro *et al*., 2016). The establishment of improved livestock production systems will be aided by advances in genetic marker application for use in population genetic studies, which will also make it easier to develop effective conservation strategies.

The crucial need to genotype thousands of SNPs instantaneously on large numbers of individual samples has accelerated the development of new SNP genotyping platforms, such as oligonucleotide arrays (Affymetrix) and BeadArray microarrays (Illumina). Affymetrix currently sells genotyping arrays for livestock and aquaculture species (buffalo, cattle, chicken, pig, salmon, and trout) as well as biomedical and model organisms (human, dog, mouse, and *Arabidopsis thaliana*), while Illumina sells whole genome genotyping BeadArrays for human and non-human species including cattle, dog, corn, pig, and sheep (Jiang *et al*., 2016).

SNP analysis, more than any other molecular marker currently available, is better suited for high throughput genotyping that is needed to elucidate greater molecular insights such as historical signatures of selection, phenotypic variations within livestock breeds, and linkage disequilibrium over short physical distances, according to researchers. The task is now to apply these new insights and understanding of molecular methods to the development of breed-specific identification tools that can be applied to livestock populations in various environments. Identification and characterization of unique phylogenomic SNPs in next generation sequenced pooled-genomic DNA from a minimum of 25 unrelated pure breed individuals can be used to establish such a breed-specific method. Any phylogenomic SNPs that are discovered can then be used to establish multiplexed SNP assays for the simple and accurate identification of purebred members from mixed populations of breeds in different ecosystems. These tools will not only make it easier to diagnose the conservation status of livestock breeds in a timely manner, but they will also allow for frequent monitoring of endangered breed populations, which is especially important in developing countries where a lack of technical and financial ability is cited as a major impediment. The data may also be used to calculate effective population size, past effective population size, and homozygosity runs. This demonstrates that SNP chips can be used to assess population relationships and levels of inbreeding (Jiang *et al*., 2016).

2.5 Genomics and Genomics toolkits in Livestock

A promising improvement of the livestock industry associated to the biological estimation of various genotypic traits has been proven after the advent of genomics and related advanced genomic toolkits. Great Britain, United States, France, Australia, Germany, New Zealand, Netherlands, Canada, Ireland, and Scandinavian countries are the foremost dairy producing countries and are applied genomic assessments in their breeding schemes, model to substantial advancements in the global livestock industry (Weller *et al*., 2017 and Gutierrez-Reinoso *et al*., 2021).

During a consecutive order, genomics became the pioneering tools we recognize today thanks to its evolution from genome sequencing, and genome wide selections of complex traits (Weller *et al*., 2017; Kadarmideen, 2014). Within the early 1990s, the first genomic assessment recognized QTL via microsatellite-distribution markers simultaneously with the variabilities of quantitative traits within the phenotypic characters of a population (Boichard *et al*., 2012).

Genomic sequencing methods can be applied for the forward selection of heritable evaluations of gene expression whereas Bayesian model approach is a statistical background for genomic evaluations. High density genomics is very vital for the investigation of the genetic variability determining population structure, computing high-density genetic maps, and providing genotypes for genome wide association analysis. Genomic inbreeding footprint for a particular sub-population is determined by runs of homozygosity via evaluating the individual autozygosity

(McGuire *et al*., 2020 and Gutierrez-Reinoso *et al*., 2021). The detail overview of the genomics under each dairy science topic is indicated (**Figure 2.2**).

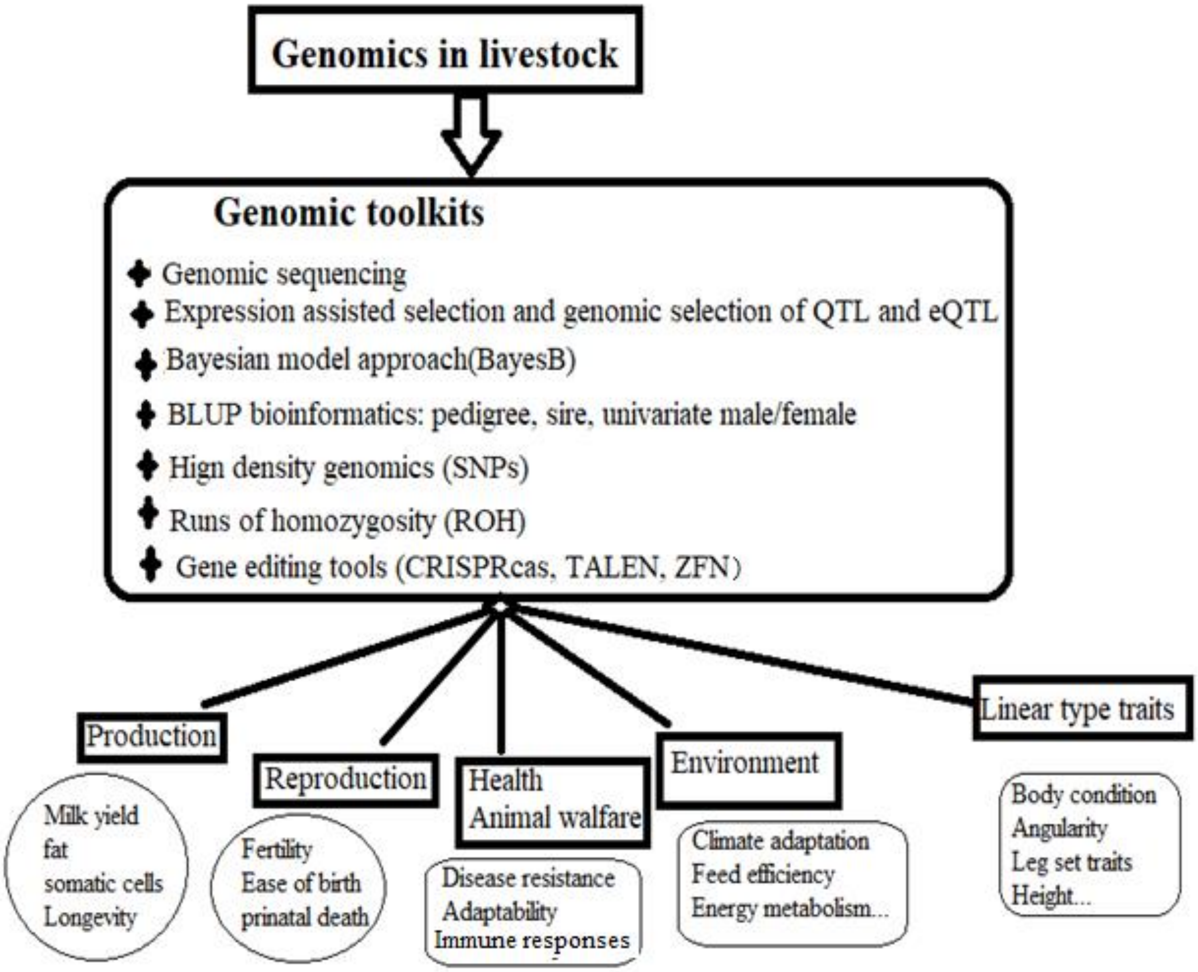


Figure 2.2 summary of the power of genomics on numerous dairy science themes **(**Gutierrez-Reinoso *et al*., 2021).

2.5.1 Livestock Genomics in Africa

The number and nature of diversity of livestock breeds in Africa have been evaluated and preferable to adapt the harsh environmental conditions that they live in. They have been serving to deliver livelihoods and guarantee of food security over centuries. The rural poor (pastoralists) are the most responsible group of a society to keep popular of the livestock breeds, wherever they aid for various purposes like food safety, revenue, living divergence, inputs to crop manufacturing, transportation, several uses of hides and skin, permitting families to profit from shared stuff assets (Marshall *et al*., 2014; Marshall *et al*., 2018; ILRI, 2019).

Research packages focusing on livestock breeding and genetics are motivating to improve vigorous animals that can resist to local environmental conditions and propagate at finest levels under embarrassed environments. Expounding the linked association between production atmospheres and the inheritances of animals, with the aim of launching selection significances and emerging appropriate improvement policies, is vital. Beforehand, livestock improvement policies have failed to attain predictable economic gains due to the deficiency of recital data, pedigree records and funding. The field of livestock genomics initiated to generate sufficient data in genetics via the arrival of whole genome sequencing and genome wide SNP genotyping (Muchadeyi *et al*., 2019). However, the opportunities of genomic technologies in developing countries are not well addressed. This dissertation tried to put the baseline information about genomics of cattle by evaluating the two selected candidate genes in Ethiopian indigenous cattle breeds.

2.5.2 Genomic selection and Genes Employed for Selections

On the subject of livestock genomes, genomic selection and evolution are expected to play a major role in the establishment of best livestock breeding approaches aimed at developing economically important traits (Mastrangelo *et al*., 2018). A genomic assessment for improved heat tolerance in Australian dairy cattle, for example, was recently created (Nguyen *et al*., 2016). Diverse techniques may be used to test the genome for areas of homozygosity, in addition, to evaluate variations within the frequency of alleles or haplotypes among divergent populations or maybe generations inside a population (Boschiero *et al*., 2018; Ceballos *et al*., 2018).

Association analysis of many candidate genes showed that DNA polymorphisms have been linked with milk production traits and these genes include prolactin, leptin, diacylglycerol acyltransferase (DGAT1), stearoyl-CoA desaturase, bovine leukocytic antigen (BoLA)-DRB3, growth hormone receptor gene, casein α s1 (CSN1S1), ATP-binding cassette subfamily G member 2 (ABCG2) gene, protease inhibitor gene, osteopontin gene, proliferator-activated receptor gamma, coactivator (CoA) 1α gene, growth hormone (GH) gene, signal transducer and activator of transcription (STAT), oxidized low-density lipoprotein receptor 1, cytochrome P450, subfamily XI B polypeptide 1, fatty acid synthase, caspase recruitment domain-containing protein 15, bovine K-casein gene CSN3, stearoyl CoA desaturase, thyroglobulin gene, POU class 1 homeobox 1, b-lactoglobulin gene, and STAT5A (Singh *et al*., 2014).

2.6 Major Histocompatibility Complex

2.6.1 History and Descovery of MHC

At the time when researchers were exploring the biological background of tissue graft rejection,a major histocompatibility complex (MHC) was invented. Many dominant Mendelian factors subjected the resistance of mice to tumor grafts, proposing a genetic origin was liable for transplant rejection for the first time (Little and Tyzzer 1916). Furthermore, scientists in 1927 reinforced this hypothesis and showed transplants between identical twins weren't rejected, and a patient with critical kidney failure successfully recovered after kidney transplantation from his monozygotic twin (Hume *et al*., 1955). Not as far away from the above-mentioned period, scientists discovered the properties of the human MHC, and researches in mice were signifying a similar genetic system controlled tissue transplantation in rodents. The primary MHC was identified in 1936 by a young pathologist working in Lister Institute for Preventative Medicine in

London while researching transplantation in mice (Gorer 1936). Moreover, other scientists identified the MHC of mouse when studying transplantation by using strains of congenic mice (Snell 1948; Snell and Higgins 1951). The MHC was mentioned as "Antigen II" by Gorer whereas Snell named as "histocompatibility (H locus)" and H-2 nomenclature remains applied for mouse MHC (Ellis *et al*., 2006).

2.6.2 Major Genomic Organization of MHC BoLA-DRB3

MHC class I and class II molecules of cattle exhibit an extraordinarily high degree of genetic polymorphism compared to many other vertebrate species. MHC class I genes are very variable and translate expressed cell surface glycoproteins responsible in antigen activation or inhibition and presentation of natural killer (NK) cell responses (Trowsdale, 2001). Additional non-classical class I genes have been reported on MHC molecule. These heterogeneous genes have different characteristics; however, expression is non-ubiquitous, less polymorphism, and encoded heavy chains with shortest cytoplasmic domains could be the character. It seems that non-classical MHC class I genes have unique but vitally important especially in association with pathogen envision strategies, management of NK stimulation and successful reproduction (Wang *et al*., 2002; Birch *et al*., 2008). Sometimes the expression fashion and role have not been well studied. In species except primates and rodents, few reports are available about non-classical MHC class I genes (Birch *et al*., 2008). This heterodimer of MHC molecule comprised from 45 kDa of heavy alpha chain and 12 kDa light chain of β2 microglobulin. The heavy chain whch is membrane-spanning contains a transmembrane domain, cytoplasmic domain, and three extracellular domains, α1, α2, and α3, with which the β2 microglobulin chain is covalently linked (Flutter and Gao, 2004). The Bovine MHC in cattle (bovine leukocyte antigen-BoLA) is situated on chromosome 23 (BTA23) and has the complete assembly describing other mammals'

MHC (Takeshima and Aida, 2006). Two genes (DR and DQ) encoded with the MHC BoLA class II region that attach the peptides which will be offered to T-lymphocytes, signifying the major class II restriction elements for CD4+ T- cells. Again the BoLA-DQ regions include DQA and DQB loci, that may differ in number based on the haplotypes; this allows extra diversity by the mechanisms of interhaplotype and intrahaplotype combination of DQA and DQB molecules (Glass *et al*., 2000). BoLA-DR involves the monomorphic *BoLA-DRA* locus and three DRB loci, which enables the *BoLA-DRB3* gene as the only component of MHC in bovines known to be fully functional (Burke *et al*., 1991).

MHC is a cluster of closely linked genes which introduces an imperative genetic constituent of the mammalian immune system and plays a role in the recognition of foreign peptide antigens (Paracha *et al*., 2015). The MHC components particularly class I and class II are the fundamental gears of the MHC region and naturally (selectively) the foremost polymorphic regions of the genome within the majority of the mammalian genome (Bohórquez *et al*., 2020). MHC class I molecules are expressed by the assistance of all nucleated cells and peptide presentation from intracellular proteins to CD8+ T-cells while professional antigen-presenting cells (B cells, dendritic cells, and macrophages) promote the expression of MHC II molecules and peptide presentation from intracellular proteins to CD+4 T-cells, thus both molecules play a pivotal role to combat pathogens (Neefjes *et al*., 2011; Bohórquez *et al*., 2020). Predominantly, the MHC polymorphism occurs at residues that participated in peptide binding domains, and there's resounding evidence that the polymorphism is maintained by the principles of adaptive introgression of variants into the species (Dudek *et al*., 2019).

The bovine leukocyte antigen (BoLA) system is the major histocompatibility complex (MHC) of cattle. There is one predominant class II DRB locus in cattle, namely, bovine MHC (BoLA)-

DRB3 and this locus is also the most polymorphic class II locus in cattle. The genes located in the MHC class II region encodes glycoproteins that are composed of α- and β-chains and are expressed on the surface of antigen-presenting cells (Mohammadi *et al.*, 2009). Mapping and molecular organization of MHC molecule in different vertebrate groups are justified hereunder (**Figure 2.3**).

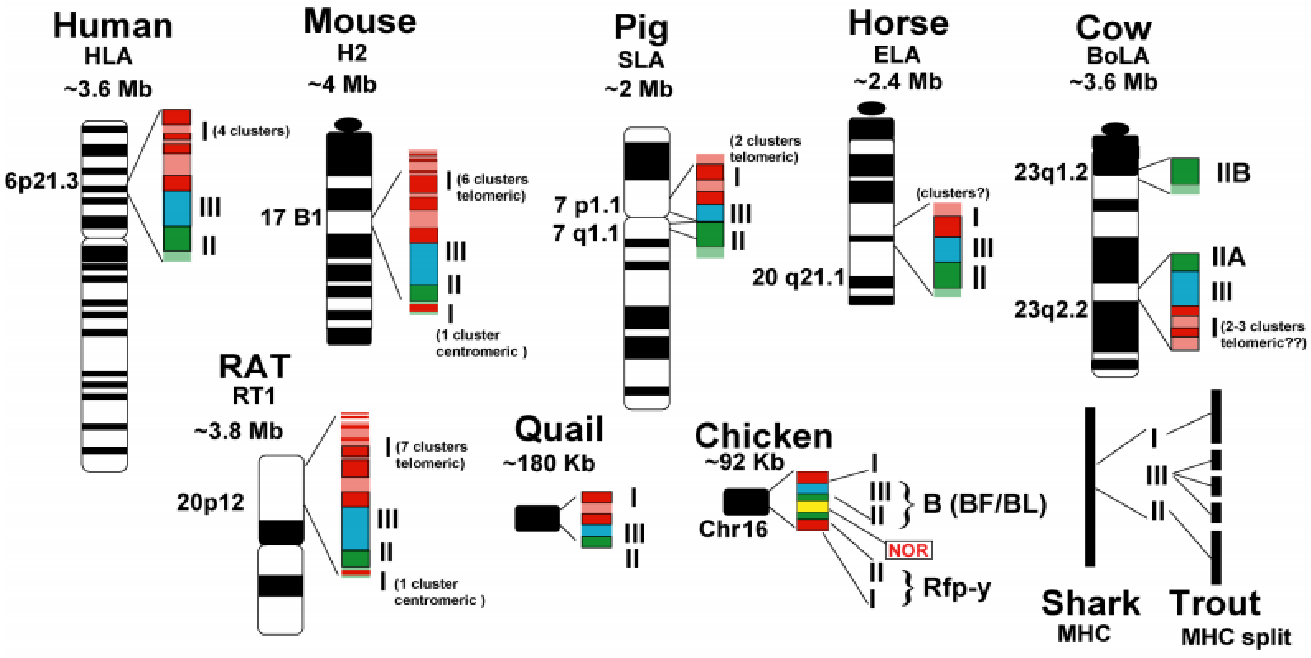


Figure 2.3 Organization of the MHC molecule in several vertebrate species (Ramlachan, 2004).

In bovines and jawed vertebrates the overall speculation of the MHC class I, II and III regions are conserved. When known, the number of clusters of class I sequences are designated as red and conserved framework of the genes in class I molecule is indicated in pink (**Figure 2.3**). The class III region (blue) is conserved in terms of gene content and location across most species. The class II region is in dark green, with the light green representing the extended class II's framework genes. Note the centromere splitting the SLA and the nucleolar organizing region (NOR) separating the chicken MHC into two regions BF/BL and Rfp-Y. Moreover, chromosomal translocation and inversion within the bovine MHC was identified and dispersed MHC in trout with the three regions on different chromosomes are mapped (Ramlachan, 2004).

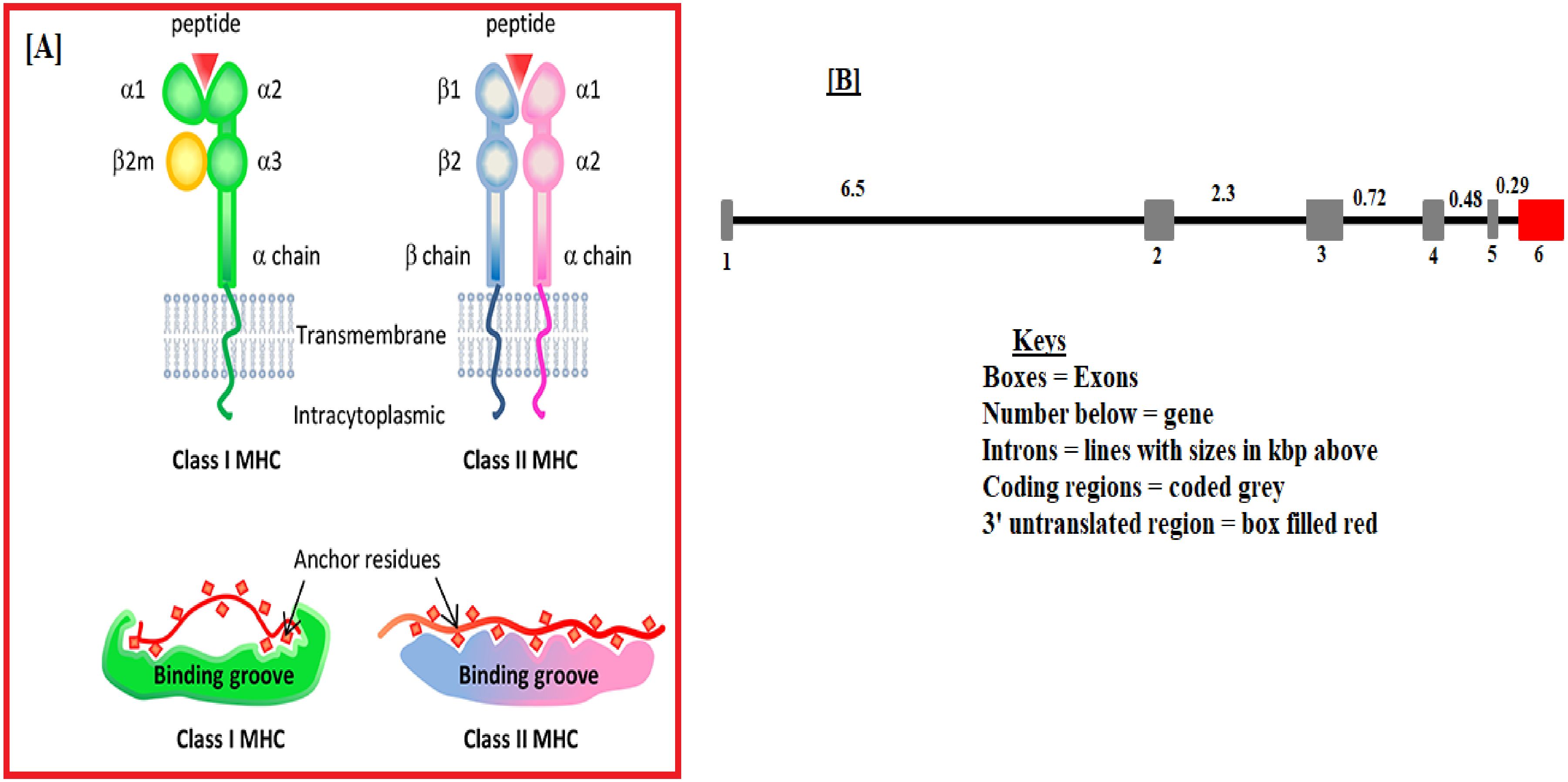


Figure 2.4 Structure of MHC classes I and II molecules [A]**, t**he organization of the *DRB3* gene **[B]**.

Adopted from (Ramlachan, 2004; Russel *et al*., 2004)

2.6.1.2 Allele Functions and Polymorphisms of MHC BoLA-DRB3 gene

Researchers in the field of cattle proposed many genetic markers associated with economically important traits (Singh *et al*., 2014). The identification of variability of genes (alleles) that associate significantly to economic traits such as disease resistant/susceptibility, productivity, fertility, and production one can state that genetic conditioned association of allele traits or availability of viable genetic markers. From all the genetic markers identified in cattle, the BoLA-DRB3 gene is distinctive. The polymorphism of MHC BoLA-DRB3 gene is associated with immunity reactions of organisms to bacterial and viral pathogens, responsible for the examination of challenges of resistance to disease. Due to this and many reasons scientists consider the use of BoLA-DRB3 genes as a marker. Evolutionary advancements in MHC have established various exceptional peculiarities of BoLA-DRB3 exon2. This region is the highest polymorphic region of the gene among all the reported loci of MHC. To date, there are pronounced 144 BoLA-DRB3 alleles for cattle, and 303 sub-types are registered in the Immuno-Polymorphism Database designed for livestock species (IPD-MHC database: www.ebi.ac.uk/ipd/mhc/group/BoLA) (Suprovych *et al*., 2021). The variability of MHC molecule showed mirror images of evolutionary and adaptive procedures within and between the populations and very essential for researching a huge domain of open queries in conservation and evolutionary biology (Sommer, 2005). Tremendously higher level of genetic variability in BoLA-DB3 genes authorizes it to be applied as a vastly informative marker for investigating genetic variation and biodiversity of cattle genetic resources. The allele's polymorphisms of this gene are important for vivacity of populations and species suffering sustained and disastrous decreases in numbers. The level of biodiversity and population differentiation of dozen numbers of cattle breeds were investigated depending on the sequence information of BoLA-DRB3 gene.

The research teams organized by Takeshima studied more about BoLA-DRB3 gene and reported allele distributions and associations to many diseases. Hence, more than 10 breeds were analyzed depending on the genetic polymorphism of BoLA-DRB3 gene. Predominantly, Japanese Shorthorn and Jersey, Japanese black and Holstein cattle in Japan, Philippine native cattle, South American Holstein cattle populations, Chilean cattle, South American Zebu cattle etc. were the breeds investigated by the leadership and participations of Takeshima (Takeshima *et al*., 2003; Miyasaka *et al*., 2011; Miyasaka *et al*., 2012; Takeshima *et al*., 2014; Takeshima *et al*., 2018).

Many of the researchers have been looking for BoLA-DRB3 gene to analyze the genetic variability of native cattle breeds in different continents. Thus, Kankrej Indian cattle, Sahiwal, Rathi and Hariana Indian breeds, Mongolian, Kalmyk and Yakut cattle breeds, Korean native cattle, Latin American creole cattle breeds, Mexico creole cattle, Ukrainian black-pied (black-and-white) dairy cattle, Myanmar cattle, native Sudanese cattle from Taurine and Zebu breeds, Kostroma and Yaroslavl indigenous Russian cattle breeds, and Ukrainian Gray cattle breeds were reported. (Behl *et al*., 2007; Behl *et al*., 2009; Ruzina *et al*., 2010; Lee *et al*., 2012; Giovambattista *et al*., 2013; Fernández *et al*., 2015; Suprovych *et al*., 2017; Giovambattista *et al*., 2020; Salim *et al*., 2020; Lazebnaya *et al*., 2020; Suprovych *et al*., 2021). Another important direction to researchers about the BoLA-DRB3 gene is their link to dairy productivity which is one of the economic traits of interest in the area (Gladyr *et al*., 2012).

Many of the researchers spent their time beyond characterization of MHC II BoLa DRB3 gene in different breeds such as disease association. Lei *et al*. (2012) investigated the association between polymorphism of BoLA-DRB3 gene and foot and mouth disease (FMD) resistance/susceptibility of Wanbei cattle challenged with foot and mouth disease virus (FMDV). Investigations for identifying marker(s) for bovine diseases and immunological traits have been the great challenge in the last decades. Now with the advent of DNA sequencing the problem has become easier. Marker identification approach requires identification and documentation of the allelic diversity of BoLA among different animals across the world (Vandre *et al*., 2014).

BoLA-DRB3 allele diversity and population tree analysis (phylogenetics) of Philippine native cattle breeds have been reported by PCR-sequence-based typing (Takeshima *et al*., 2014). Recently, scientists conducted a genome-wide association study to identify SNPs associated with bovine leukemia virus (BLV), which is an exogenous C- type oncovirus in the Retroviridae family) proviral load in Japanese black cattle. Three SNPs displayed a substantial association with proviral load. Two SNPs (which were not in linkage disequilibrium) were detected in the bovine MHC region on chromosome 23. These outcomes propose that polymorphisms in the MHC region affect proviral load. Such type of report is vital for the understanding of host factors and may provide important clues for controlling the spread of BLV in cattle (Takeshima *et al*., 2017).

Among the genes linked with reduce mastitis frequency specific consideration is being paid to the BoLA-DRB3 gene due to the role this gene plays in the immune system. According to the previous report, exon 2 *BoLA-DRB3* locus is highly polymorphic. This region encodes the antigen adhesion side of MHC molecules and plays a crucial role in regulation of the immune response to pathogens. Several studies have demonstrated that the *BoLA-DRB3.2*24* allele tended to be associated with mastitis susceptibility and *BoLA-DRB3.2*3* tended to be associated with mastitis resistance (Sender *et al*., 2013).

2.6.2 Leptin gene

2.6.2.1 History and Biology of Leptin

Leptin is the hormone produced by the obesity gene (ob) identified first in mice and humans and later in bovines. It is secreted by adipocytes, and it has been allied with energy balance and feed consumption in mice and humans. In bovine the leptin gene is located on chromosome 4 associated with feed intake capacity, metabolism, fat content, meat quality, and growth traits (Dubey *et al.*, 2007).

Leptin is a peptide hormone primarily produced by white adipose tissue and in lesser extent in the placenta and skeletal muscle. This peptide hormone plays a role in the synthesis of glycogen and the glucose transport and is particularly active in the brain tissue in the region of the hypothalamus. This area of the brain takes part in regulating eating behaviour, and the presence of leptin decreases hunger and depresses willingness for food. When adipose tissue grows larger correspondingly larger amounts of leptin are produced (Alberts *et al.*, 2002). A prime candidate for an endocrine regulation of the leptin expression is insulin. Insulin appears to increase the leptin secretion; a possible cause of action could be that increasing levels of insulin cause an increased metabolism and glucose transport. If the reverse is true, i.e. if increasing levels of leptin also cause increasing levels of insulin still remains uncertain. The hypothesis has been tested in several different studies but with conflicting results, as reviewed by Margetic *et al.* (2002).

2.6.2.2 Molecular Organization of Leptin Gene

The genes identified in animals targeting to affect energy balance, milk yield, and feed intake capacity is an interesting area of researches in animal breeding scheme. The polymorphism identified on leptin exonic regions are associated with many economic traits. Considering rich resources for animals, in our country, accomplishing a few assays to identify a gene that controls their traits with molecular genetics, and identifying the candidate genes in cattle breeds using DNA test can greatly help to improve breeding program. The leptin gene is located on bovine chromosome 4 (BTA4) and transcribed into a 167 amino acid peptides. The sequence includes a 21 amino acid long signal sequence, which is cleaved off during translocation of the leptin into microsomes. Therefore the final protein circulating in the blood contains only 146 amino acid residues (Margetic *et al*., 2002). The protein circulates in the serum bound to other proteins. These "transporting proteins" may impact on the half life and the biological activity of leptin (Houseknecht *et al.*, 1998). A genetic marker was identified in the sequence and in the promoter region of the bovine gene. Buchanan *et al*. (2002) proposed that the missense mutation in the gene sequence could be considered the causative mutation in differences of fat deposits in cattle. Barendse *et al*. (1997) indicated that there is no association between genetic markers in the leptin gene with fat in cattle; however, additional report have rebuilt the theory that variable alleles of the gene are associated with different fat content in cattle. The application of leptin gene as a genetic marker could be important in beef producing industries.

Since the finding of the bovine leptin gene on BTA4, several SNPs have been formerly identified in both introns and exons of LEP gene among diverse breeds of cattle. Several studies have revealed that polymorphisms were associated with lean and fat cattle. The molecular information for leptin gene is valuable for molecular breeding in order to identify high quality meat through MAS. Although it has been reported that genetic variants of candidate genes such as mitochondrial DNA is related to growth traits in cattle, polymorphism information of leptin in cattle is still obscure (Yoon *et al.*, 2005).

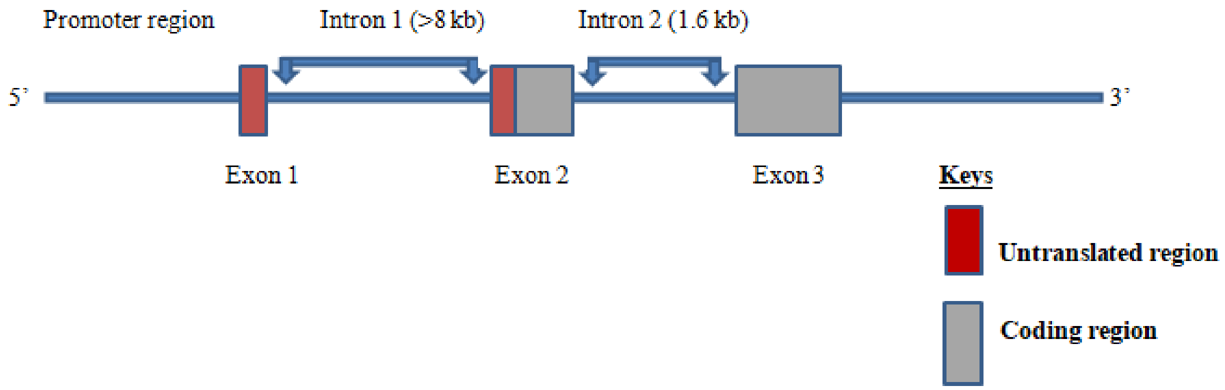


Figure 2.5 Molecular organization of LEP gene in cattle [Liefers, 2004]

The information of Leptin gene in Ethiopian indigenous cattle is still a gap that any scientist can justify the function and the polymorphism of this gene. Characterization of such candidate genes can be the potential pre-requisite for the background of molecular breeding in cattle.

2.6.2.3 Leptin Polymorphism and Role in Cattle Production Traits

Some hereditary circumstances are measured by a single gene which calls them monogenic or qualitative whereas many of others are managed by many genes named polygenic or quantitative traits. According to Healy (1996) many qualitative traits in cattle inherited recessively (87%). Don't be surprised of the specificity of genetic makeup of breeds, supposing cattle breeds were well known in a comparative genetic isolation and independent of each other (Healy, 1996). Before the advent of modern molecular biology, scientists faced a challenge to get a technology to identify genes linked with quantitative traits and the variations within a gene that affected partially its expression with a major negative or positive implication on animal productivity (Casas and Kehrli, 2016). Now a days genome-wide association studies (GWAS) are plausible due to the convenience of technological advancement that allows high-throughput genotyping, expression of economically important traits are genetically decoding (Casas and Kehrli, 2016).

Animal database mining publicized about 344 QTL linked with milk yield and 71 with mastitis associated traits in cattle (Ogorevc *et al*., 2009). Some of the milk proteins such as kappa casein, beta casein, leptin (LEP), DGAT1, and prolactin (PRL) are confirmed to be linked with milk production in dairy cattle (Deb *et al*., 2020). LEP is known to be an influential biomolecule for supplementing productivity in livestock. Because of its vital function in lactogenesis, colostrum secretion, galactopoiesis, and immunity to mastitis, LEP has become a substantial candidate gene for genetic association with dairy production (Singh *et al*., 2012). Several SNPs have been identified on the exonic as well as in the promoter regions of the LEP gene and detected to be highly allied with milk production traits (Liefers *et al*., 2005).

Many scholars justified the different regions of the leptin gene (LEP) in cattle from different breeds and explained the association of this gene with milk yield traits. For instance, De Matteis et al (2012) stated that variability detected in the promoter region of the LEP gene was linked with the level of milk fat in Holstein Friesian cows, whereas polymorphisms in the second intron region identified in Iranian Holstein and Slovak Simmental cattle have been linked with milk yield (Moussavi *et al*., 2006; Trakovicka *et al*., 2015). Variations detected on the third exonic region of the LEP gene (c.239C/T) substitution that would influence the substitution of an amino acid (p.Ala80Val) and the presence/absence of restriction endonuclease cleavage site (Sau3A I) in the second intron region (Maletić *et al*., 2019). Maletić et al (2019) analyzed Busha cattle revealed two genotypes of SAUAI (AA and AB); BB genotype was not detected in Busha cattle. The prevalence of the genotype differs from breed to breed. Cattle groups having the genotype AB introduced higher milk yield compared to the AA genotype. The reduced percentage of protein in milk has been also reported in New Zealand Holstein-Friesian × Jersey-cross dairy cows proficient with AB genotype (Haruna *et al*., 2020). The variability of LEP genotype (p.

Ala80Val) identified from Jersey cows showed alteration in milk yield, protein, and fat yield, that cows having TT genotype produced reduced milk with reduced components (protein and fat) than CC and CT cows (Kulig *et al*., 2009). Many scholars investigated that the LEP allelic variants are associated with many production traits and has to be the focus of research in different cattle breeds (Wang *et al*., 2020; Kulibaba *et al*., 2021).

In addition to production traits, the allele variability of LEP gene has been investigated to evaluate veterinary aspects and somatic cell count in milk from different cattle breeds, can be the promising candidate gene in mastitis research (Kiyici *et al*., 2019). Moreover, the researches were examined on other livestock species beyond cattle. Therefore, investigations on sheep, goat, and pig recommended LEP gene as the most important candidate gene for the aim of implementing MAS in livestock (Saleem *et al*., 2018; Avondo *et al*., 2019; Snegin *et al*., 2020).

The current hot topic that many researchers looking in developing countries like Ethiopia is improvement of livestock production. Improving breed can deliver a pivotal role to increase the reproduction performance of cattle population. Nevertheless, there are trends for genetic improvement platforms to emphasize on single, market oriented traits like milk or meat production in isolation of production systems in developing countries. This hypothetically directs the genotypes that are not appropriate to adapt the production systems and not proficient of delivering multifunctional roles expected in developing countries. A comprehensible and broad breeding program suitable to the prevailing production systems is essential to support producers/cooperatives in the viable administration of animal genetic resources. Community based breeding program (CBBP) which mainly focused on the indigenous stock including farmers' interest, opinions, judgments, and active involvement from inception through to implementation have been identified as a platform of choice in developing countries. Hence, simulation methods interpreted by ZPLAN computer package to define the appropriate breeding program and selection of superior animals via genomic approaches can introduce the best direction of breed improvement in Ethiopia. Leptin gene associated with many other genes identified in animals targeting to affect energy balance, milk yield, and feed intake capacity is an interesting area of researches in animal breeding scheme. Also, the genes linked with reduce mastitis and production of cattle special attention is being paid to the BoLA-DRB3 gene. Unlike Bos taurus breeds, lack of information about the aforementioned genes (BoLA-DRB3 and leptin) in Ethiopian breeds initiate the researcher to conduct this study.

Chapter 3

3. Evaluation of alternative breeding plans for two indigenous cattle breeds of Ethiopia

Abstract

The highest number of cattle genetic resources in different agroecologies of Ethiopia have been reported. However, the production of cattle genetic resources are low due to lack of appropait breeding programs. Different breeding approaches targeting two indigenous cattle breeds of Ethiopia (Boran and Horro) were evaluated with the aim of advancing appropriate breeding programs that would improve the production and productivity of pasture-raised breeds using a deterministic approach. The Boran breed was evaluated for beef, milk and both beef and meat (dual purpose), while the Horro breed was evaluated for milk and dual purpose. Two units, breeding unit and production unit, were delineated for both breeds, each with six selection groups. Yearling weight, milk yield, age at first calving, calving interval and survival of calf to yearling were used as selection criteria for milk yield and dual purpose program. While age at first calving, calving interval and survival of calf to yearling were considered as selection criteria for milk production program. In addition, yearling weight, age at first calving, calving interval and calf survival to yearling were used as selection criteria for increasing beef production in the breed Ethiopian Boran. Three different levels of breeding bull selection percentages (5%, 10% and 15%) were used. There were more genetic gains in the bull selection groups than in the cow selection groups for all target traits studied. The bull selection groups also had the shortest generation interval compared to the cow groups (4.07 vs. 6.42 years). Of the estimated annual genetic gains for each target trait, all traits had favorable genetic gains except age at first calving. The highest genetic gain was recorded per year for milk yield followed by live weight. For the other traits, genetic gain was close to zero. Nearly comparable annual genetic gains were estimated for the two cattle breeds, Boran and Horro, adopted for the milk improvement program under different selection proportions. The estimated genetic improvements of 291.96, 268.63 and 252.73 litters of milk were simulated for Boran under selection proportions of 5%, 10% and 15% of breeding bulls, respectively, and the corresponding improvements for Horro were 291.55, 268.31 and 252.43 liters. However, gains were higher for Boran when the two cattle breeds are adopted for the dual purpose program (85.92 to 98.64 liters for Boran vs. 78.14 to 90.26 liters for Horro). Estimated genetic improvements for live weight ranged from 38.96 to 45.11 kg for Boran and 34.17 to 39.46 kg for Horro when both cattle breeds were studied for the dual purpose program. The current study lacks information on economic values for each target trait. Therefore, further work is required. With the exception of age at first calving, the genetic gain per year in yearling weight, milk yield, calving interval, and survival to yearling achieved by the various options is satisfactory and may lead to reasonable genetic improvements in these cattle breeds.

Key words: Borana breed; breeding plans; deterministic approach; Horro breed; genetic gain

3.1. Introduction

Ethiopia is home to a diversie animal genetic resources leading by cattle populations. The population of these cattle breeds has been estimated at about 57 million head (FAO, 2019). Despite the presence of large and diverse cattle populations and the critical role that cattle play in the country's economy, their productivity remains low. For instance, the potential milk production of Boran cow is 6 litres whereas 2.6 litres per day in Arsi highland cows (FAO, 2019). The reported per capita consumption of milk and meat is 16 litres and 13.9 kg/year, respectively, which is lower than the African and world per capita averages, which are 27 and 100 kg/year, respectively (Yilma *et al*., 2009). The Ethiopian Economic Association (EEA, 2005) also reported that per capita consumption of meat and milk in Ethiopia is below the average reported for Sub-Saharan Africa. Although their productivity is low, indigenous breeds have acquired through millennia of natural selection the merits of adapting to various harsh environmental conditions (e.g., heat tolerance, resistance to disease, and ability to withstand long periods of feed and water shortages) (Baker and Rege, 1994; Baker and Gray, 2004), and such conditions vary widely among locations and production systems.

According to the previous reports, both production and reproductive capabilities within breed variations were higher for indigenous Ethiopian cattle breeds (Banjaw and Haile-Mariam, 1994; Haile-Mariam and Kassa-Mersha, 1995; Negussie *et al*., 1998). Recent genetic descriptive analyzes (Li *et al*., 2007; Zerabruk *et al*., 2007; Dadi *et al*., 2008, Dadi *et al*., 2009) also discovered that Ethiopian cattle populations have maintained high levels of genetic variation within a population. In general, the research results of both phenotypic and genetic evaluation indicate that improvement within the breed is possible. Appropriate utilization of indigenous breeds/ecotypes will ensure reduced external inputs such as feeding and health care and hence higher profit margin for smallholder farmers and pastoralists, an asset for the breeder. Consequently, these will help to improve food security and reduce pressure on the environment. Such a method would also enable *in situ* conservation of genetic traits of livestock, the only feasible and practical conservation method in less developed countries compared to *ex situ* or cryopreservation approaches.

In Ethiopia, there have been no effective programs for genetic improvement of livestock species due to various constraints such as lack of technical skills, small herd sizes by households and poor infrastructure. Therefore, there have been no efforts to improve the productivity of indigenous cattle breeds through systematic selection, with the exception of crossbreeding programs using indigenous animals as dams. In fact, some activities on government ranches and research centers were literally called "cattle breeding and genetic improvement" (e.g., Boran Cattle Breeding and Improvement Ranches in Abernosa and DidaTiyura; Horro Cattle Breeding and Improvement Ranch in Horro Guduru and Arsi Cattle Breeding and Improvement Ranch in Gobe). Most of these centers did not have proper recording practices, let alone a well- planned breeding program, and focused on improvement through cross-breeding programs.

Therefore, the objective of this study was to simulate alternative breeding programs that enhance the contribution of the livestock sector to rural livelihood improvement through the use of indigenous cattle breeds.

3.2. Materials and Methods

3.2.1. Cattle breeds

Boran cattle breed is a group of large sized zebu and predominantly distributed in the semi-arid and arid areas of Southern Ethiopia, Northern Kenya, and South Western Somalia and are maintained by pastoralists (Rege, 1999). However, due to the recurrent drought in Southern Ethiopia, the estimated population might have declined. There are two sub-types of the Ethiopian Boran cattle: the large framed Qorti and the smaller framed Ayuna/Geleba (Reda, 2000; Zander and John, 2004; Edea *et al.*, 2006). Crossbreeding or replacement with highland cattle, changes in ecology and lack of breed development programs were some of the major reasons for the genetic erosion or dilution (Zander and Drucker, 2008). The Boran breed is known for its high degree of heat tolerance, resistance to ticks, feed and water shortage, and has good meat quality (Ojango *et al.*, 2006).

Horro cattle breed is extensively dispersed in South Western and Western Oromia National Regional State, West Ethiopia. The name of the breed is derived from a place called Horro Gudru from where it spread widely to different areas of west Oromia. Horro cattle breed is one of the Sanga x Zebu intermediate types and is suited for milk, draft power, and meat. The hump is small to medium in size. Body conformation is good with a deep chest and barrel, well-sprung ribs, and a straight top line with a slight sloping rump. The legs are straight and slender. The breed has a well-proportioned small udder with good teat placement (Rege and Tawah, 1999). The males are of darker color being almost black on the humps and withers (Albero and Haile-Mariam, 1982). For simulation purpose we used 1500 breeding cows and 453 breeding bulls as a population parameter for both breeds.

3.2.2. Location

Simulated breeding plans target two state owned cattle breeding and improvement ranches: Dida Tiyura and Horro Guduru within their respective environment. They are located within areas supposed to be home tracts for the Boran and Horro cattle breeds, respectively. Dida Tiyura is found in Borana zone, Oromia National Regional State, at a distance of about 570km from Addis Ababa to the Kenya border. The area has an arid to semi-arid climate; with an average annual rainfall ranging from 110 to 600mm. Drought is common with interval of 5 to 20 years. The mean annual temperatures vary from 15ºC to 24°C and show little seasonal variation. The vegetation consists predominantly of savannah but presently bush encroachment becomes a problem for the community (Coppock, 1994; Aynalem Haile *et al*., 2011). According to Desta (2000), of the total population of the area which is estimated to be 0.42 million ha, 89% was pastoral; the remaining being crop cultivators. The area is occupied by the major Oromo ethnic group, the Borana.

Horro Guduru Cattle Breeding and Improvement Ranch is situated in Horro Guduru zone, Oromia National Regional State, about 300 km west of Addis Ababa, with geographical coordinates of 09º29´N and 37º26´E, and at an altitude of approximately 2296 meters above sea level (m.a.sl). Mixed crop-livestock agricultural production system is the main traditional practice in the area. The area has one long rainy season extending from March to mid-October with annual rainfall ranging from 1000 – 2400 mm (Tefera, 2006). The monthly mean temperature observed in the area was 14.9 ℃ to 17.5 ℃.

3.2.3. Statistical Analysis

Some features of the environment can be concluded by herd size, production level, region, or weather conditions, management system, and many other factors can be thought of. A statistical measure of the environment is the fixed effect of herd, herd-year or herd-year-season estimated with a sire or animal model. To quantify the environment by the mean performance of a population in the given environment is an approach that has verified to be useful when a reasonable number of genotypes and environments can be measured (studies of GxE interaction be possible). The mean performance may, but does not have to, measure similar traits as the trait being evaluated. The major benefit of this approach is that the environment can simply be scored as more or less promising, and a continuous gradient is produced (Kolmodin, 2003).

3.2.4 Determination of Objective Traits

Five and four goal traits were selected to simulate alternative breeding plans to improve Boran and Horro cattle breeds for dual purpose (beef and milk – both breeds) and beef (Boran breed) or milk (both breeds) production programs, respectively. The breeding goal traits were aimed at increasing production and productivity of the breeds maintained on pastures without or with minimum supplementation. The selection criteria set to be used for each breeding goal traits are indicated **(Table 3.1)**.

Table 3.1 Determined objective traits and selection criteria

Objective trait	**Selection criteria**
Body size	Yearling weight ((kg)
Lactation	Milk yield (kg)
Age at First Calving	Age at First Calving (year)
Calving Interval	Calving Interval (year)
Calf survival to yearling	Survival to yearling

3.2.5 Simulation methods

The computer program ZPLAN (Willam *et al*., 2008) was used to model the alternative breeding programs. This computer program is based on a comprehensive evaluation of both genetic and economic efficiency of breeding strategies considering a selection cycle. Important outputs of ZPLAN are the annual monetary genetic gain for the overall genotype, the annual genetic gain for individual traits, the discounted rate of return, and the discounted profit for a given investment period. ZPLAN was developed to optimize breeding strategies in livestock through deterministic calculations. The gene flow method (Hill, 1974; McClintock and Cunningham, 1974) and the selection index method form the core of the program. For the selection index part, the information available to score an individual candidate must be defined by the number and type of relatives contributing to an animal's index and records of its own performance (Willam *et al*., 2002). ZPLAN cannot account for reduced genetic variance due to selection (Bulmer effect) and inbreeding. Although the program ignores reduced genetic variance due to selection and inbreeding, it is able to calculate selection indices for breeding animals within a selection round and apply order statistics to obtain adjusted selection intensities for populations of finite size, assuming that parameters and selection strategies remain unchanged during the investment period. Inbreeding rates per generation (ΔF) can be estimated using the formula $\Delta F = (1/(8Nm) + (1/(8Nf))$, where Nm and Nf denote the number of breeding males and females, respectively, relative to the effective population size (Falconer and MacKay, 1996).

Three selection groups; breeding bulls (BB), breeding cows (CB) and production cows (CP), consisting of six selection routes were defined for both breeds. The generation and transmission of genetic gain occurs in the breeding and production units, respectively. The defined selection pathways are: 1) bulls for production of breeding bulls (BB>BB), where bull selection is done to improve the bulls used in the breeding unit, 2) bulls for production of breeding cows (BB> CB), where bull selection is done to improve the cows used in the breeding unit, 3) cows for production of breeding bulls (CB> BB), where cow selection is done to improve the bulls used in the breeding unit, 4) cows for the production of breeding cows (CB> CB), where cow selection is done to improve the cows used in the breeding unit, 5) bulls for the production of breeding cows (BB> CP), where bull selection is done to improve the cows used in the production unit, and 6) cows for the production of breeding cows (CP> CP), where cow selection is done to improve the cows used in the production unit. The transfer of genetic gain to the production unit is only through the selection group (BB> CP). Genetic gain is generally expected from the selection groups originating from the breeding unit where selection decisions are made and breeding costs are incurred (Nitter *et al.*, 1994). The contribution of the other selection groups in terms of genetic gain is very small only due to the 5% replacement heifers.

Table 3.2 Selection groups and the gene flow pathways

		Genes from		
		Bulls in BU* (BB)	Cows in BU (CB)	Cows in PU** (CP)
Genes to	Bulls in BU (BB)	BB>BB	CB>BB	-
	Cows in BU (CB)	BB>CB	CB>CB	-
	Cows in PU (CP)	BB>CP	-	CP>CP

*BU = breeding unit; **PU = production unit

Key input parameters for ZPLAN are given in **Table 3.3** and the phenotypic and genetic parameters used are listed in **Table 3.4**. Information on the biological parameters considered was obtained from previous work on the two breeds and other tropical breeds (Kebede *et al.*, 1991; Banjaw and Haile-Mariam, 1994; Haile-Mariam and Kassa-Mersha, 1998; Rewe *et al.*, 2009). Three selection percentages were used for the breeding bulls: 5%, 10% and 15%.

Information on economic values is completely lacking in this country. The relative economic weight for each trait was based on the program for which the breeding plan was designed and was calculated by standardizing the values with the additive genetic standard deviation (σA) according to FAO (2010) guidelines. However, it should be noted that these values are only an approximation, but can still serve as fair economic estimates when information is completely lacking, as in the present case.

Table 3.3 Input parameters for simulation of alternative breeding plans

Parameters	**Boran**	**Horro**
Population parameters		
Population size (breeding cows)	1500	1500
Number of bulls tested/year	453	453
Proportion of bulls selected	0.05;0.10; 0.15	0.05;0.10; 0.15
Biological parameters		
Breeding cows in use (years)	5	5
Breeding bulls in use (years)	3	3
Mean age of bulls at birth of first offspring (years)	3	3
Mean age of cows at birth of first offspring (years)	3.5	3.5
Calving rate	0.80	0.80
Calving interval (years)	1.20	1.20
Number of calves/cow/tu‡	0.84	0.84
Survival to yearling (%)	0.90	0.90
Bulls survival between subsequent time units	0.95	0.95
Cows survival between subsequent time units	0.90	0.90
Cost parameters		
Interest rate return (%)	0.03	0.03
Interest rate costs (%)	0.075	0.075
Investment period (years)	25	25

‡=time unit

Table 3.4 Phenotypic (upper triangle), genetic (lower triangle) correlations and heritabilities (diagonal, in bold) of the traits

Variables	YWt	MiY	AFC	CI	SURV
YWt	**0.30**	0.20	0.00	0.00	0.09
MiY	0.10	**0.27**	0.01	0.07	0.04
AFC	0.00	0.05	**0.06**	-0.21	0.00
CI	0.00	-0.11	0.09	**0.04**	0.00
SURV	0.04	0.15	0.00	0.00	**0.09**

YWt= yearling weight; MiY= Milk yield; AFC = Age at first calving; CI=Calving interval; SURV= Calves' survival to yearling

Table 3.5 shows selection criteria used in calculating the selection index and the economic values as well as phenotypic and genetic standard deviations. The estimates of genetic and phenotypic parameters were from available literatures on the two breeds (Banjaw and Haile-Mariam, 1994; Haile-Mariam and Kassa-Mersha, 1995; Rewe *et al*., 2009). The selection criteria used in the simulation included those parameters recorded routinely in breeding unit. The information sources are individual own record and maternal.

Table 3.5 Selection criteria and their relative economic weights, phenotypic (σ_P) and genetic (σ_A) standard deviations

Objective traits	**Selection criteria†**	**Unit**	**σ_P**	**σ_A**	**Economic weight**	**Economic Value (Econ. Weight/ σ_A)**
Boran Beef						
Body size	Ywt	Kg	32.913	18.027	50	2.774
Age at first calving	AFC	Month	4.547	1.113	20	17.958
Calving interval	CI	Month	4.310	0.862	20	23.202
Survival to yearling	SURV	%	1.860	0.558	10	17.921
Boran Milk						
Milk yield	MiY	Kg	85.335	44.341	50	1.128
Age at first calving	AFC	Month	4.547	1.113	25	22.448
Calving interval	CI	Month	4.310	0.862	15	17.401
Survival to yearling	SURV	%	1.860	0.558	10	17.921
Boran Dual						
Body size	Ywt	Kg	32.913	18.027	25	1.387
Milk yield	MiY	Kg	85.335	44.341	30	0.677
Age at first calving	AFC	Month	4.547	1.113	20	17.958
Calving interval	CI	Month	4.310	0.862	15	17.401
Survival to yearling	SURV	%	1.860	0.558	10	17.921
Horro Dual						
Body size	Ywt	Kg	27.920	15.292	25	1.635
Milk yield	MiY	Kg	85.335	44.341	30	0.677
Age at first calving	AFC	Month	5.933	1.453	20	13.761
Calving interval	CI	Month	2.900	0.580	15	25.862
Survival to yearling	SURV	%	1.860	0.558	10	17.921
Horro Milk						
Milk yield	MiY	Kg	85.335	44.341	50	1.128
Age at first calving	AFC	Month	5.933	1.453	25	17.201
Calving interval	CI	Month	2.900	0.580	15	25.862
Survival to yearling	SURV	%	1.860	0.558	10	17.921

†YWt = yearling weight; MiY = Milk yield; AFC = Age at first calving; CI = Calving interval; SURV = Calves' survival to yearling

3.3. Results and Discussions

The different selection proportions, selection criteria, selection intensity, selection accuracy and monetary genetic gain for each target trait studied under the different production purposes. In general, selection accuracies were similar under the different selection proportions because the sources of information used to generate the indices were the same. For all target traits studied, more genetic gains were obtained in the bull selection groups than in the cow selection groups. The bull selection group also had the shortest generation interval compared to the cow group (4.07 vs. 6.42 years). Considering the more stringent selection intensity and short generation intervals, more genetic gains were obtained in the bull selection group. The estimated selection intensities for bulls ranged from 2.05 to 2.45, while that of cows was 0.55 (**Table 3.5**). Kahi and Hirooka (2005) reported that for faster genetic progress, it would be desirable to put more emphasis on breeding bull selection. Borrowing or lending of breeding bulls is common in Ethiopia. But breeding cows are given only to very close relatives based on some commitments or preconditions. Therefore, most of the expected genetic progress for the improvement of Boran and Horro cattle will be due to selection of breeding bulls.

Of the estimated annual genetic gains for the individual target trait, all traits had favorable genetic gains except age at first calving (**Table 3.6**). Almost comparable annual genetic gains were estimated for both Boran and Horro cattle breeds adopted for the milk improvement program under the selection percentages of 5%, 10% and 15%. However, gains were higher for Boran (**Table 3.5**) when the two cattle breeds were adopted for dual purpose (milk and meat) program. According to Yilma *et al* (2006), milk yield could influence the trait of mothering ability in cows since mothering ability depends mainly on their milk production potential. However, since higher milk production leads to higher feed intake by cows, only minimal manipulation of milk yield is advisable in areas where animals depend on grazing such as Boran cattle (Rewe *et al*., 2009).

Table 3.6 Genetic gain year^{-1} for the breeding objective traits under the different options

Breeds, programs and objective traits	**Proportion of selection**		
	5%	10%	15%
Boran Beef ($r_{IH=0.076; L=5.243}$)			
Ywt	0.0091	0.0084	0.0079
AFC	0.0791	0.0728	0.0685
CI	0.0157	0.0144	0.0136
SURV	0.0070	0.0065	0.0061
ΔmG/year	1.936	2.214	1.676
Selection intensity	2.453	1.781	2.050
Boran Milk ($r_{IH=0.494}$)			
MiY	6.5831	6.0583	5.6997
AFC	0.0390	0.0359	0.0338
CI	-0.0125	-0.0115	-0.0108
SURV	0.0138	0.0127	0.0120
ΔmG/year	8.331	7.7667	7.213
Selection intensity	2.453	2.214	2.050
Boran Dual ($r_{IH=0.390}$)			
Ywt	2.5025	2.2971	2.1611
MiY	2.2246	2.0596	1.9377
AFC	0.0089	0.0082	0.0077
CI	-0.0032	-0.0030	-0.0029
SURV	0.0078	0.0072	0.0068
ΔmG/year	5.198	4.804	4.520
Selection intensity	2.453	2.214	2.050
Horro Dual ($r_{IH=0.414; L=5.243}$)			
Ywt	2.5806	2.3872	2.2343
MiY	2.0355	1.8402	1.7623
AFC	0.0049	0.0045	0.0042
CI	-0.0017	-0.0015	-0.0015
SURV	0.0101	0.0068	0.0088
ΔmG/year	5.803	5.293	5.024
Selection intensity	2.453	2.214	2.050
Horro Milk ($r_{IH=0.488}$)			
MiY	6.5752	6.0510	5.6929
AFC	0.0115	0.0106	0.0100
CI	-0.0124	-0.0114	-0.0107
SURV	0.0140	0.0128	0.0121
ΔmG/year	7.544	6.943	6.532
Selection intensity	2.453	2.214	2.050

ΔmG/year =monetary genetic gain per year; YWt = yearling weight; MiY = Milk yield; AFC = Age at first calving; CI = Calving interval; SURV = Calves' survival to yearling

Genetic gain was highest for yearling weight followed by milk yield in simulated breeding plans for dual purpose animals in both breeds. However, when genetic gains were altered in natural units, the gain (genetic gain/generation) was higher for milk production (85 to 98.64 litter for Boran and 78.14 to 90.26 litter for Horro) than for yearling weight (38.96 to 45.11 kg for Boran and 34.17 to 39.46 for Horro). A positive increment, which is detrimental, was expected for age at first calving (AFC) in both breeds in all options studied. Nevertheless, the results are not different from zero. With respect to the current simulation for Boran beef, milk and dual purpose programs, heifers will delay age at first calving by about 0.08, 0.04 and 0.01 days, respectively. For Horro heifers, about 0.01 and 0.02 days more will be required to reach age at first calving for dual purpose and milk production, respectively. The probable explanation could be that the information assumed as phenotypic standard deviation for the trait is not large enough. Although the magnitude is small, the positive gain for age at first calving is not appropriate because a higher age in the trait implies a lower probability of having more calves per cow per lifetime. In addition, the negative gains estimated for calving interval (CI) are desirable because this indicates a bargain of time between successive calvings. A cow with a short calving interval will have a higher probability of having more offspring during her lifetime. Survival to yearling (SURV) had a lower level of genetic gain but with beneficial direction. Survival traits in selection criteria could be particularly valuable in protecting against a likely increase in negative trends in production or loss of adaptability (Rewe *et al*., 2009).

As expected, annual genetic gain was highest at a selection percentage of 5% and lowest at 15% for all selection criteria considered. This means that the annual genetic gain is highest when the

5% best bulls are selected and used for breeding purposes. The predicted association between aggregate genotype and selection index (precision of breeding value estimation) was low when simulated for the Boran beef program. Conversely, modest correlations were obtained for the other programs simulated for both breeds. The low precisions of breeding value estimates found for Boran cattle and expected for the beef production program was due to the lack or complete absence of evidence for genetic and phenotypic parameters for beef traits. Studying the genetic and phenotypic association between traits could allow finding ideal, sound selection criteria to combine the desired breeding objectives and achieve higher selection precision (Wasike *et al.*, 2007).

The application of the higher genetic gains achieved by the bull selection group offered researchers a greater emphasis on breeding bull selection for faster rates of genetic improvement. Consequently, a candidate bull is nominated based on the age of the dam at the birth of her first offspring, the time interval between two consecutive calvings of the dam (calving interval of the dam), and the ability of the dam to rear calves/calves for all programs (meat production program, dual purpose program, and milk production program), indicating that bull selection is done only from cows of two or more parities. Yearling weight (deadweight) of candidate bulls is one of the selection criteria measured for the dual purpose and meat production programs in addition to the above selection criteria. The other selection standard for the milk production and dual purpose program is maternal lactation milk yield. Natural mating can be practiced to allow identification of progeny by exposing cows to a new bull when introduced into cow herds kept in groups of 100 to 150 on ranches after approximately one to two weeks of rest. Alternatively, semen from the selected bulls could be used for ranch herds. For herds of participating pastoralists (Borana zone) and producers in the mixed cropping-livestock system (Horro Guduru -zone), both natural mating and artificial insemination could be used depending on the situation on the ground. The establishment of semen processing facilities at the two ranches ensures that few superior bulls are used over a large number of breeding cows in both the nucleus and the production unit. The bulls are used for three time units (3 years), after which they are disposed of. The replacement heifers for the nucleus herd come from our own herd, but about 5% are supplied from the herds of participating farmers/pastoralists. The selected animal (from both breeding and production units) must meet breed standards for observable phenotypic parameters such as conformation, horn and colour.

According to Rewe *et al* (2009), the other important consideration is the management of the animals, which should meet the minimum requirements for feeding, health and other routine management practices that support the conservation of the breed under the management of the owners. Performance recording, genetic estimation and planned mating are the major platforms for structured breeding programs (Rewe *et al*., 2009). To carry out the above activities and to distribute the genes of the selection group in the population over a period of time, producer organizations (small farmers/pastoralists) such as cooperatives and breeding bull clusters are essential. Membership in such groups/cooperatives provides access to micro-credit after acquiring legal credit so that methodological and market problems of individual cattle farmers can be eliminated. The organization of breeders into cooperatives or associations is considered critical for breed improvement involving communities (Koehler-Rollefson, 2000). In addition, a strong convention of borrowing or lending breeding bulls for a period of time is common in communities. Accordingly, for the operational application of the breeding program, the existing traditional collaboration and mechanisms of borrowing and lending of breeding bulls need to be strengthened. In addition, strong linkages between the various stakeholders need to be established. However, the role of stakeholders in breeding programs needs to be carefully recognized to regulate available supports for defensible breed development. Strong acquaintanceships among stakeholders are important to coordinate joint actions and to fruitfully operate the designed breeding program. Two questions need to be answered for effective operation of a breeding program: 1) when to do what (synchronization of timing of planned phases); and 2) who does what (which person/organization is responsible for which task(s) to carry out the planned breeding program)?

Chapter 4

4. Sequence Polymorphisms of BoLA-DRB3 Polymorphisms by Comparing Bangladesh, Ethiopian, and Korean Cattle

Abstract

Because of their important function in pathogen recognition, the use of bovine leukocyte antigens (BoLA) as disease markers in immunological traits of cattle is well established. However, there are few reports of BoLA gene polymorphism in zebu cattle breeds using high-resolution typing methods. Therefore, we used a polymerase chain reaction sequence-based typing (PCR-SBT) method to sequence exon 2 of the BoLA class II DRB3 gene from 100 animals (Boran, n = 13; Sheko, n = 20; Fogera, n = 16; Horro, n = 19), Hanwoo cattle (n = 18), and Bangladesh Red Chittagong zebu (n = 14). We also included data from the public database (NCBI) of 102 Holstein Freisian samples in the analysis. Of the 59 alleles identified, 43 were previously deposited at Immuno Polymorphism Database for the major histocompatibility complex (IPD-MHC database), while 16 were unique to this study. Evaluation of the level of genetic variability at the population and sequence levels with genetic distance in the breeds considered in this study showed a higher gene diversity score, a nucleotide variability score, and a mean number of pairwise differences, which was very similar to the values reported in other cattle breeds. With respect to the neutrality tests analyzed, excess number of alleles was observed in all breeds except Hanwoo, which could be due to recent population expansion or genetic hitchhiking. However, the observed heterozygosity was not considerably higher than the expected heterozygosity ($p< 0.05$). The Hardy-Weinberg equilibrium analysis (HWE) showed a non-significant excess of heterozygous animals, suggesting plausible overdominant selection.

Pairwise FST values indicated low genetic variation among all breeds (FST = 0.056; $p < 0.05$), in addition to rooting in the evolutionary or domestication history of cattle. No separate clade was observed when examining the evolutionary divergence of the BoLA-DRB3 gene inferred from the phylogenetic tree based on the maximum likelihood model. The investigation in the present work suggests that the historical divergence between the cattle breeds *Bos taurous* and *Bos indicus* is a consequence of origin, selection and adaptation, which could explain the marked differences in BoLA-DRB3 gene variability between Ethiopian and Asian cattle breeds.

Key Words: BoLA-DRB3, Cattle breeds, Sequence based typing (SBT), genetic diversity, Alleles

4.1 Introduction

Major histocompatibility complex (MHC) is an assembly of closely associated molecules that introduces a unique genetic factor of the immune system in mammals. Bovine MHC isolation and characterization were determined long years ago (Andersson *et al*., 1986; Groenen *et al*., 1989; Groenen *et al*., 1990). MHC molecule is the principal constituent of adaptive immune system in bovines and MHC class I and II regions are the main gears of MHC molecule; usually the most variable regions the gene in the majority of the mammalian genome (Bohórquez *et al*., 2020). The cytological investigation of MHC molecule indicated that the molecule is physically mapped on the bovine autosome 23 (BTA 23). The scientific nomenclature of MHC in cattle nominated as bovine leukocyte antigen (BoLA), which is strongly polymorphic and applicable in the immunity related traits. The role of BoLA in cattle is to recognize pathogens via the immune systems and mainly used as a disease marker in cattle breeding programs (Bohórquez *et al*., 2020; Phillips *et al*., 2017).

In bovine genome BoLA-DRB3 locus is the broadly researched and variable region. The allele variability and polymorphisms of DRB3 gene in cattle breeds have recently been reported by sequence-based typing (Takeshima *et al*., 2018; Suprovych *et al*., 2021). According to the report available in immuno-polymorphism database (IPD-data base) 144 alleles were deposited so far; by which such impressive polymorphisms have resided on the β1 domains of the peptide-binding regions (PBR) (Takeshima *et al*., 2018; Maccari *et al*., 2017; Suprovych *et al*., 2021). Among the six exons of BoLA-DRB3 gene, exon 2 has previously been reported to be the most polymorphic and potentially affect many immunities, somatic cell count and mastitis occurrence (Hameed *et al*., 2006). Besides, current research investigation indicated that the resistance/scusceptibility of livetstock to mastitis disease has been associated with BoLA-DRB3 gene (Welderufael *et al*., 2018; Suprovych *et al*., 2018).

Polymorphisms of BoLA-DRB3 exon2 in Korean cattle and Holstein cattle were characterized using a next generation sequencer (Lee *et al*., 2012) and aimed at the documentation of molecular markers connected to bovine pathogenes and immunological traits by comparing other unique cattle breeds from developing countries like Bangladesh and Ethiopia.

Ethiopia is a port to huge and variety of cattle populations experienced to an assortment of harsh ecological conditions. Sheko cattle are soundly adjusted to humid atmospheres of south-western Ethiopia characterized by high tsetse fly infestation (Mekuriaw and Kebede, 2015). Contrariwise to Sheko, cattle populations like Boran, Ogaden, and Afar have long been grown under arid and semi-arid regions of eastern and southern Ethiopia where the threat of trypanosomiasis is comparatively low. Despite certain variances in their reaction to local pathogens (viral and bacterial parasites), to the best of our understanding no research has been made to address the MHC BoLA DRB3 exon2 gene in Ethiopian native cattle breeds which are critically important for future breeding programs. The aim of this study was therefore, to characterize the genetic variability of DRB3 exon2 in the Ethiopian and Asian zebu and taurine cattle breeds.

4.2 Materials and Methods

4.2.1 Study Cattle Breeds and DNA Extraction

The study considered four indeginious Ethiopian cattle breeds (Boran, n= 13; Sheko, n = 20; Fogera, n = 16; Horro, n = 19), Korean Hanwoo (n = 18), and Bangladesh Red Chitagong (n=14). Thus, totaly 100 animals were included targeting different geographical environments and production systems. The study was designed to collect samples randomly from multiple herds and 102 additional Bos taurus sequences were downloaded from public databases (National Center for Biotechnology Information (NCBI) to strength the analysis.

Table 4.1 Descriptions of cattle breeds considered in this study

Population	**Sample size**	**Number of farms**	**Breed**	**Type**	**Country of origin**	**Sampling country**
Red Chittagong	14	Randomly collected	Red Chittagong	Zebu		Bangladesh
Boran	13	1	Boran	Zebu		Ethiopia
Fogera	16	1	Fogera	Zebu	Ethiopia	Ethiopia
Hanwoo	18	Randomly collected	Hanwoo	Zebu x Taurine	South Korea	Korea
Horro	19	Randomly collected	Horro	Zebu	Ethiopia	Ethiopia
Sheko	20	Randomly collected	Sheko	Zebu x Taurine	Ethiopia	Ethiopia
Total	**100**					

Nasal swabs were collected using Animal Swabs Collector (BlueGene Life Science, Cheongju, Korea) and Performagene LIVESTOCK's nasal swabs (DNA Genotek Inc., Kanata, On, Canada). Sampling countries and all informations of considered breeds indicated above (**Table 4.1**). DNA was isolated from nasal swabs following the DNA Genotek manufacturer's instructions (http://www.dnagenotek.com).

The quality and concentration of DNA were measured by NanoDrop1000 and gel electrophoresis in 0.8 % agarose gels. DNA samples that fulfilled the standard in terms of quality and quantity were further considered for amplification and sequencing.

4.2.2 PCR amplification of BoLA-DRB3 Exon2 region

To amplify exon2 of the BoLA-DRB3 gene, we used primers reported in the previous study (Van Eijk *et al*., 1992). Polymerase chain reaction (PCR) technique amplified 284 bp product at the second exonic region. The reactions were carried out in a total volume of 30 µL containing genomic DNA (2 µL of 50 ng/µL), 21.1 µL distilled water, 10 × PCR buffer (3 µL), 10 mM dNTPs (0.6 µL), forward and reverse primers (1.5 µL of 10 pM for each), and 0.3 µL of *Taq* DNA polymerase (Promega, San Diego, CA). Reactions were performed; at 35 cycles of 94 ^{0}C for 30 s, annealing at 60 ^{0}C for 30 s, and 72 ^{0}C for 40 s preceded by initial denaturation at 94 ^{0}C for 10 min and a final extension at 72 ^{0}C for 5 min. Finally, the PCR products were visualized by gel electrophoresis on 2% agarose gels with acetate EDTA (TAE) buffer followed by ethidium bromide staining. Sequencing was accomplished by Macrogen (Seoul, Republic of Korea).

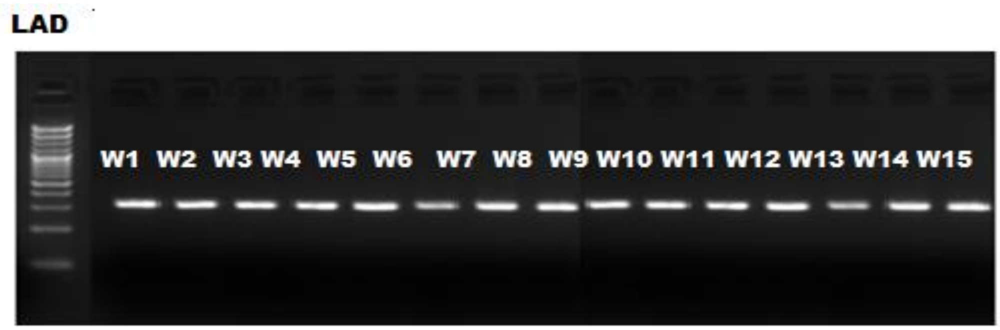


Figure 4.1 PCR amplification product of BoLA-DRB3 exon2 region

Table 4.2 Primers for MHC class II BoLA DRB3.2 gene (F= Forward and R= Reverse)

Types	**Name**	**5'-Sequences-3'**	**Annealing temperature (^{0}C)**	**Fragment size**	**Source**
F	HL-030	ATCCTCTCTCTGCAGCACATTTCCT	60 ^{0}C	284 bp	Van Ejik et al., 1992
R	HL-032	TCGCCGCTGCACAGTGAAACTCTC			

4.2.3 Sequence Based-Typing and Analysis of BoLA-DRB3 Exon2

We conducted PCR sequence based typing (PCR-SBT) technique to evaluate the genetic variability of BoLA-DRB3 exon2 gene. The study was pioneer for characterization of BoLA DRB3.2 region in the Ethiopian cattle breeds. Sequence polymorphism of the BoLA-DRB3 exon2 region was evaluated in four Ethiopian indigenious cattle breeds (Boran, Fogera, Horro, and Sheko), Korean Hanwoo, and Bangladesh red Chittagong. The DNA sequences were edited using Bio-edit version 7.0.5.3 and aligned by clustalX2 software package (Larkin *et al.*, 2007). This research mainly focused on six parameters (number of polymorphic sites, nucleotide diversity, the total number of mutations, number of haplotypes, the average number of nucleotide differences, and haplotype diversity) to examine population structure based on the DNA sequence polymorphisms of MHC II BoLA-DRB3 exon2 gene applying ARLEQUIN software version 3.5.2.2 (Excoffier and Lischer, 2010). Also, the allele frequencies and number of alleles were estimated by the same software. The deviations from Hardy Weinberg equilibrium (HWE) were computed by F-statistics (Weir and Cockerham, 1984). Moreover, observed and unbiased expected heterozygosity was calculated by ARLEQUIN and GENPOP4.0 software (Rousset, 2008). Pattern of sequence variability, synonymous and non-synonymous substitutions were computed by DnaSP version 6.12.03 software (Librado and Rozas, 2009). Nucleotide similarities of the newly identified alleles with the reference sequence (BoLA-DRB3:016:01) were generated by sequence demarcation tool (Muhire *et al.*, 2014). The average haplotype count per individual (AHC) was calculated by dividing the number of haplotypes by the number of animals considered (Peters *et al.*, 2018).

4.2.4 Population diversity and neutrality tests

Population differentiation due to population genetic structure was also assessed from sequence data, population pairwise Wright's F_{ST} (Wright, 1965; Weir and Cockerham, 1984) values were calculated by using ARLEQUIN software version 3.5.2.2 applying 1000 replication values (Excoffier and Lischer, 2010). Pairwise F_{ST} graph was displayed by Rcmd (console version of the R statistical package) installed on the computer integrated with ARLEQUIN (http://www.r-project.org/). Moreover, population genetic differentiations based on the BoLA-DRB3 gene of the breeds were evaluated by Ne's genetic distance (G_{ST}) by DnaSP software. Molecular diversity indices statistics, number of transitions, and the numbers of transversions were computed by ARLEQUIN software version 3.5.2.2 (Excoffier and Lischer, 2010).

Also, population structure was examined by neutrality tests (Tajima's *D* and Fu's *Fs*) implemented by the infinite site model using ARLEQUIN software. These neutrality tests (Tajima's *D* and Fu's *Fs*) were assessed depending on the mean number of pairwise differences between haplotypes and possibility of observing the average number of nucleotides (number of alleles), correspondingly (Tajima, 1989; Fu, 1997).

4.2.5 Phylogenetic inferences and Median joining network

Phylogenetic investigation of a population was accomplished by using the Maximum Likelihood scheme based on the Kimura 2-parameter model (Kimura, 1980) via MEGAX v.10.1 software (Kumar *et al.*, 2018) through implementing1000 bootstrap values (Nei and Kumar, 2000). We excluded the loci comprising gaps or missing data and identical sequences from the analysis. The Median-joining network (MJN) tree was constructed using the NETWORK software (version 10.0.0) (Bandelt *et al.*, 1999). To estimate the median network, the DNA sequences were first altered into binary data, whereas sequences having identical sites were excluded from the analysis. Each split was automated as a binary character, satiating the values of 0 and 1. Binary vector represent the haplotypes in this approach. The median vectors were estimated for each triplet of vectors until the construction of the median network was achieved (Bandelt *et al*., 1999).

4.3. Results

4.3.1 Alleles distribution of MHC class II BoLA-DRB3 Exon2

Investigations of BoLA-DRB3 exon2 by PCR-SBT resulted in the detection of 59 BoLA-DRB3 exon2 total alleles by which 16 alleles (BoLA-DRB3*164:01, BoLA-DRB3*165:01, BoLA-DRB3*166:01, BoLA-DRB3*167:01, BoLA-DRB3*168:01, BoLA-DRB3*169:01, BoLA-DRB3*170:01, BoLA-DRB3*171:01, BoLA-DRB3*172:01, BoLA-DRB3*173:01, BoLA-DRB3*174:01, BoLA-DRB3*175:01, BoLA-DRB3*176:01, BoLA-DRB3*177:01, BoLA-DRB3*178:01 and BoLA-DRB3*179:01) were reported for the first time and were not found in the Immuno Polymorphism Database for major histocompatibility complex (IPD-MHC). All sequences were deposited in the NCBI gene bank data base with accession numbers MT919537-MT919549 (Bangladesh), MT919550-MT919562 (Boran), MT919563-MT919578 (Fogera), MT919579-MT919596 (Hanwoo), MT919597-MT919615 (Horro), and MT919616-MT919637 (Sheko). From the total of 59 different alleles reported in this study, Sheko breed had more number of alleles (18) and Korean Hanwoo breed had small number of alleles (8). Whereas, 11, 12, 13, add 14 alleles were detected in Bangladesh Red Chittagong, Ethiopian Boran, Fogera and Horro, respectively. Alleles were named temporarily based on the principles of IPD-MHC data base. The two Ethiopian breeds (Boran and Horro) shared the allele BoLA-DRB3*145:01 with the Bangladesh red Chittagong. Three Ethiopian indigenous cattle breed Boran, Fogera, and Horro shared the allele BoLA-DRB3*100:01. Moreover, three Ethiopian cattle breeds (Fogera, Horro, and Sheko) shared the allele BoLA-DRB3*021:01 with the Korean Hanwoo breed. But, no alleles identified in this study that shared between Hanwoo and Red Chittagong. Regarding the allele frequencies, the highest allele frequency was recorded 0.444 (BoLA-DRB3*002:01) and the lowest allele frequency was 0.050 (observed in many alleles) (**Table 4.3**).

Table 4.3 Allele distributions and frequencies of BoLA-DRB3 exon2 sequences

Alleles	Allele numbers	RC	BO	FO	HN	HO	SH
BoLA-DRB3*145:01	6	0.071	0.077	-	-	0.053	-
BoLA-DRB3*120:01	1	0.071	-	-	-	-	-
BoLA-DRB3*157:01	3	0.071	-	-	-	-	-
BoLA-DRB3*112:01	1	0.286	-	-	-	-	0.050
BoLA-DRB3*164:01	**1**	**0.143**	**-**	**-**	**-**	**-**	**-**
BoLA-DRB3*032:01	4	0.071	-	-	-	-	0.050
BoLA-DRB3*165:01	**1**	**0.071**	**-**	**-**	**-**	**-**	**-**
BoLA-DRB3*166:01	**1**	**0.071**	**-**	**-**	**-**	**-**	**-**
BoLA-DRB3*167:01	**2**	**0.071**	**-**	**-**	**-**	**-**	**-**
BoLA-DRB3*018:01	1	0.071	-	-	-	-	-
BoLA-DRB3*020:12	1	-	0.077	-	-	-	-
BoLA-DRB3*168:01	**1.**	**-**	**0.077**	**-**	**-**	**-**	**-**
BoLA-DRB3*169:01	**1**	**-**	**0.077**	**-**	**-**	**-**	**-**
BoLA-DRB3*135:01	1	-	0.077	-	-	-	-
BoLA-DRB3*012:01	1	-	0.077	-	-	-	-
BoLA-DRB3*170:01	**2**	**-**	**0.154**	**-**	**-**	**-**	**-**
BoLA-DRB3*020:08	1	-	0.077	-	0.056	-	-
BoLA-DRB3*100:01	2	-	0.077	0.063	-	0.158	-
BoLA-DRB3*007:01	1	-	0.077	-	-	-	-
BoLA-DRB3*011:01	2	-	0.077	-	0.056	-	-
BoLA-DRB3*171:01	**1**	**-**	**0.077**	**-**	**-**	**-**	**-**
BoLA-DRB3*087:03	3	-	-	0.188	-	-	-
BoLA-DRB3*021:01	1	-	-	0.062	0.111	0.053	0.150
BoLA-DRB3*172:01	**3**	**-**	**-**	**0.062**	**-**	**-**	**-**
BoLA-DRB3*093:01	4	-	-	0.062	-	-	-
BoLA-DRB3*145:01	1	-	-	0.062	-	-	-
BoLA-DRB3*137:01	1	-	-	0.062	-	-	-

*Alleles written in **bold** indicated that the new alleles identified in the current study.

Table 4.3 Continued

Alleles	Allele numbers	RC	BO	FO	HN	HO	SH
BoLA-DRB3*130:01	1	-	-	0.062	0.056	-	-
BoLA-DRB3*008:02	1	-	-	0.062	-	0.053	-
BoLA-DRB3*044:01	1	-	-	0.062	-	-	-
BoLA-DRB3*007:03	3	-	-	0.062	-	-	0.050
BoLA-DRB3*173:01	**1**	**-**	**-**	**0.062**	**-**	**-**	**-**
BoLA-DRB3*015:04	3	-	-	0.062	-	-	-
BoLA-DRB3*122:01	1	-	-	0.062	-	-	-
BoLA-DRB3*009:02	1	-	-	-	0.111	-	-
BoLA-DRB3*002:01	2	-	-	-	0.444	-	-
BoLA-DRB3*020:08	9	-	-	-	0.056	-	-
BoLA-DRB3*013:03	1	-	-	-	0.056	-	-
BoLA-DRB3*174:01	**1**	**-**	**-**	**-**	**0.056**	**-**	**-**
BoLA-DRB3*024:12	1	-	-	-	-	0.105	-
BoLA-DRB3*032:03	2	-	-	-	-	0.105	0.100
BoLA-DRB3*123:01	1	-	-	-	-	0.105	-
BoLA-DRB3*086:04	1	-	-	-	-	0.053	-
BoLA-DRB3*100:02	1	-	-	-	-	0.053	-
BoLA-DRB3*094:02	2	-	-	-	-	0.053	-
BoLA-DRB3*107:03	1	-	-	-	-	0.053	0.100
BoLA-DRB3*104:01	1	-	-	-	-	0.053	-
BoLA-DRB3*028:03	2	-	-	-	-	0.053	-
BoLA-DRB3*175:01	**2**	**-**	**-**	**-**	**-**	**-**	**0.050**
BoLA-DRB3*141:01	1	-	-	-	-	-	0.050
BoLA-DRB3*007:02	1	-	-	-	-	-	0.050
BoLA-DRB3*010:03	1	-	-	-	-	-	0.050
BoLA-DRB3*176:01	**1**	**-**	**-**	**-**	**-**	**-**	**0.050**
BoLA-DRB3*177:01	**2**	**-**	**-**	**-**	**-**	**-**	**0.050**

*Alleles written in **bold** indicated that the new alleles identified in the current study.

Table 4.3 Continued

Alleles	Allele numbers	RC	BO	FO	HN	HO	SH
BoLA-DRB3*178:01	**1**	**-**	**-**	**-**	**-**	**-**	**0.050**
BoLA-DRB3*107:01	2	-	-	-	-	-	0.050
BoLA-DRB3*013:04	1	-	-	-	-	-	0.050
BoLA-DRB3*027:08	2	-	-	-	-	-	0.050
BoLA-DRB3*179:01	**2**	**-**	**-**	**-**	**-**	**0.053**	**-**

*Alleles written in **bold** indicated that the new alleles identified in the current study and not reported in IPD-MCH database.

4.3.2 Genetic Variability Analysis of BoLA-DRB3 Exon2 Gene

The novel alleles detected in this study were computed to each other to examine the degree of variability. Moreover, reference sequence (BoLA-DRB3*106:01) downloaded from public database (IPD-MHC database) compared with the newly identified alleles and greater variabilities were observed (**Figure 4.2**).

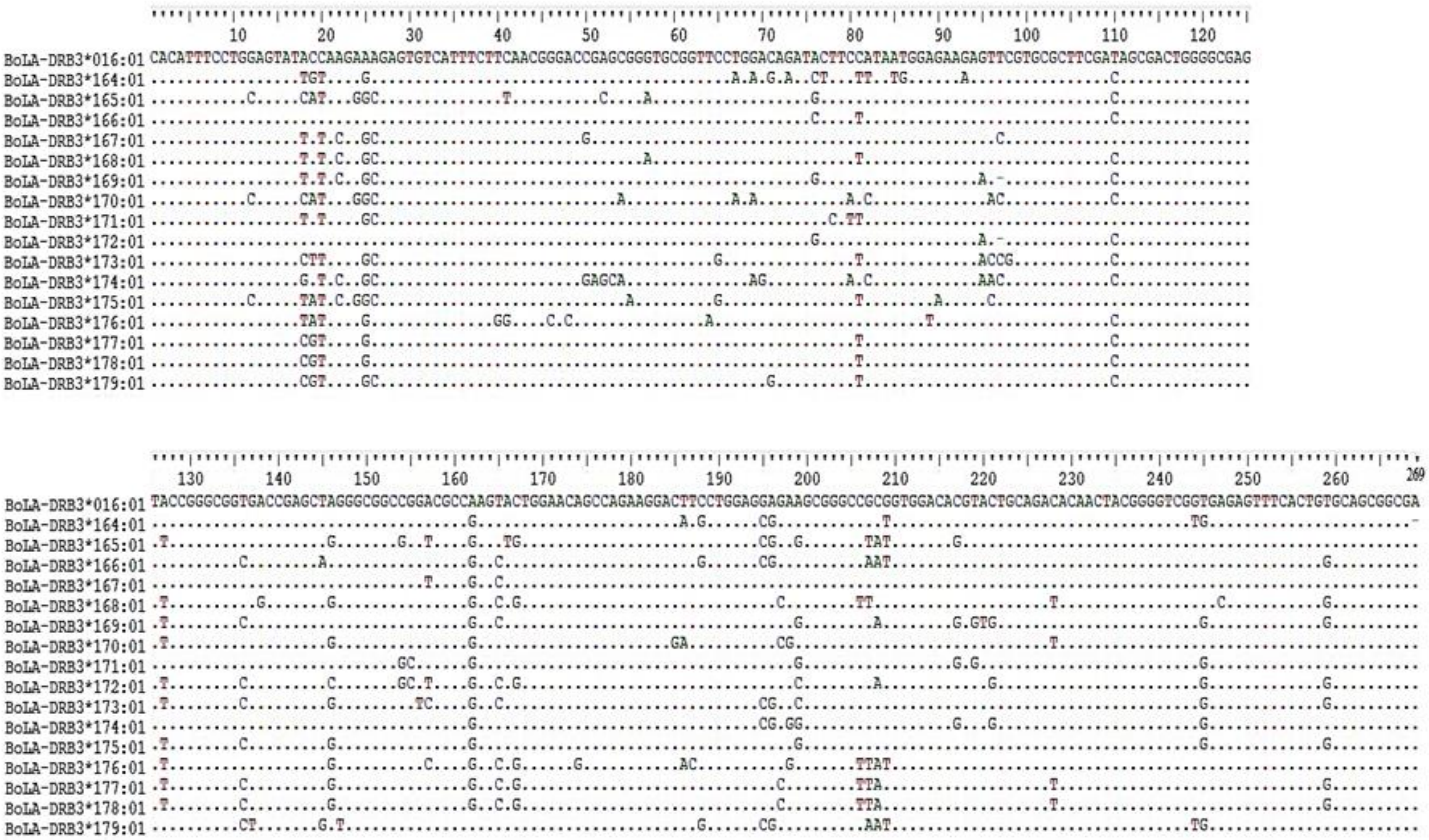


Figure 4.2 New alleles detected in this study aligned with reference sequence (BoLA DRB3*016:01)

The nucleotide similarities of the new alleles identified from this study compared to the *Bos taurus* reference sequence downloaded from IPD-MHC data base showed 86-100% similarity computed by sequence demarcation tool (**Figure 4.3**).

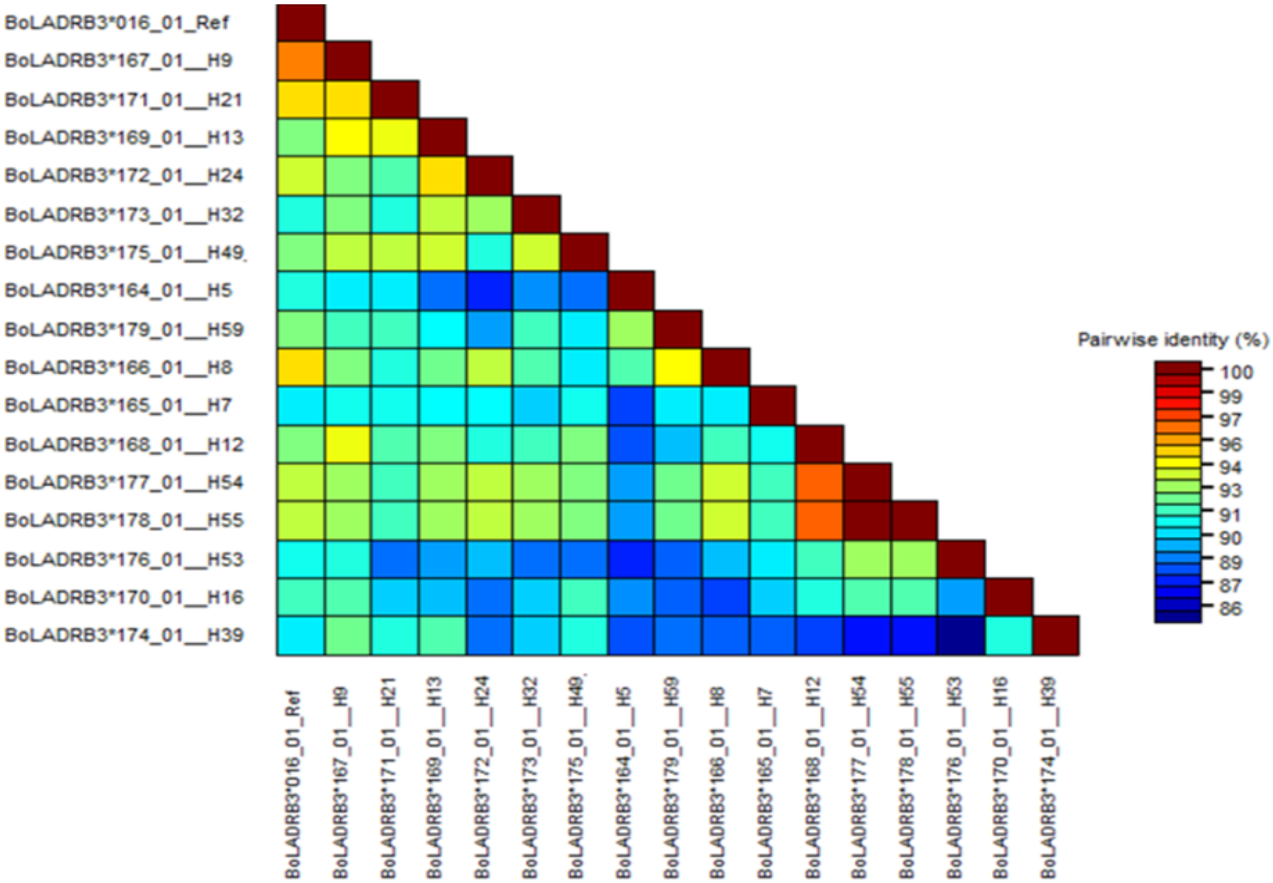


Figure 4.3 The pairwise identity graph of the newly reported alleles with reference sequence (BoLA-DRB3*016:01)

The polymorphic regions of the BoLA-DRB3 exon2 gene named polymorphic sites (S) that measure the usable loci detecting more than one allele per locus were investigated. The recorded values of S were banded from 65 (Sheko) to 47 (Hanwoo). One Ethiopian cattle breed (Fogera) showed similar polymorphic sites with Bangladesh Red Chittagong (53). The nucleotide diversities (Π) were relatively highest in Boran breeds (0.072) whereas in the Korean Hanwoo breeds a relatively lowest number of Π (0.056) was observed. For all breeds, synonymous substitutions were less as compared to the non-synonymous substitutions. The numbers of haplotypes (H) were computed for each breed and 59 total numbers of haplotypes were identified. Compared to all analyzed breeds, the values of haplotype number were higher in Sheko (18) and lower in Korean Hanwoo (8). Lower haplotype diversity (Hd) of the Korean Hanwoo breed was examined compared to all Ethiopian breeds and Bangladesh Red Chittagong. Thus, the haplotype diversity for Korean Hanwoo was 0.751 which was the lowest as compared to the rest of the breeds. Haplotype count per animal was surveyed and recorded higher values from Ethiopian Boran (0.846) and lower values from Korean Hanwoo (0.500). Sheko breed had a moderately higher recorded value of AHC (0.800) next to Boran. Also, the highest values of average numbers of nucleotide differences (NPD) were recorded in Boran (18.307) and the lowest values recorded in Hanwoo (14.339). The parameter regarding nucleotide substitution was investigated and the proportion of non-synonymous substitution (NSs) to synonymous substitution (Ss) was highest in Sheko (3.300) and lowest in Korean Hanwoo with an estimated value of 1.500 (**Table 4.5**).

Table 4.4 DNA polymorphism of BoLA-DRB3 exon2 showed several genetic parameters with estimated ratios for considered breeds.

Breeds	**Π**	**NPD**	**S**	**H**	**Hd**	**AHC**	**SS**	**NSS**	**NSs/Ss ratio**
Red Chittagong	0.063	16.186	53	11	0.934	0.714	13	24	1.850
Boran	0.072	18.307	54	12	0.987	0.846	11	27	2.450
Fogera	0.068	17.292	53	13	0.966	0.625	13	21	1.610
Hanwoo	0.056	14.339	47	8	0.751	0.500	16	24	1.500
Horro	0.064	16.380	58	14	0.964	0.736	11	23	2.090
Sheko	0.065	16.631	65	18	0.989	0.800	10	33	3.300

Thus, π → nucleotide diversity, NPD→ mean number of nucleotide differences, S→ number of polymorphic sites, H→Haplotype number, Hd→ haplotype diversity (Hd), AHC → Average haplotype count, SS→ synonymous, and NSS→ non-synonymous substitutions.

4.3.3 Gene Diversity, HWE and Neutrality Tests of BoLA-DRB3 Exon2 Gene

To understand genetic diversity within a population, the mean number of alleles per locus (Na), the average expected (He) and observed heterozygosity (Ho) values, HWE in terms of FIS coefficient and neutrality tests were estimated. Hereafter, in Korean Hanwoo the number of alleles was lower (8) whereas in Sheko higher number of alleles was identified (18). The observed heterozygosity (Ho) ranged between 0.750 in Fogera to 0.980 in Ethiopian Boran. Korean Hanwoo breed showed a lower value of expected heterozygosity (HE=0.804) and Ethiopian Boran showed a higher value (HE=0.987). Across the survey we found the following HE values 0.923, 0.975, 0.965, and 0.979 respectively of Bangladesh Red Chittagong, Fogera, Horro, and Sheko breeds.

The Tajima's D value obtained was negative in four breeds (Bangladesh, Horro, and Sheko), but Tajima's D value in Boran, Fogera, and Hanwoo was 0.233, 0.350, and 0.203, respectively. Except for the Korean Hanwoo breed which had Fu's Fs recorded value of 4.594 all remaining breeds had a negative estimated value of Fs. Fu's Fs P-values were highest in Hanwoo (0.959) breed and lowest in Sheko (0.052). According to the computed values of Hardy-Weinberg Equilibrium (HWE), all the breeds considered in this study revealed a significant deviation from the theoretical assumption (we assessed significant excess of homozygotes) (**Table 4.6**).

Table 4.5 Sequence and allele numbers (N and Na), observed and expected heterozygosities (Ho and He), neutrality test statistics (Fu's *Fs* and Tajima's *D*) and Hardy Weinberg equilibrium (HWE) in terms of F_{IS} coefficient.

Breed	N	Na	Ho	He	F_{IS}- p value	Fu's Fs	Fs P-value	Tajima's D	Tajima's P-value
Red Chittagong	14	11	0.920	0.923	0.082	-0.116	0.439	-0.126	0.458
Boran	13	12	0.980	0.987	0.053	-1.699	0.164	0.233	0.630
Fogera	16	13	0.750	0.975	0.066	-0.841	0.318	0.350	0.695
Hanwoo	18	8	0.800	0.804	0.081	4.594	0.959	0.203	0.612
Horro	19	14	0.960	0.965	0.055	-0.478	0.424	-0.052	0.516
Sheko	20	18	0.970	0.979	0.055	-4.219	0.052	-0.374	0.376

4.3.4 Genetic structure and population differentiation of BoLA-DRB3 gene

We estimated two main parameters (F_{ST} index and exact G test) for testing the genetic structure of the population and the level of differentiation between cattle breeds. F_{ST} parameters showed significant differences across all cattle breeds (FST = 0.056; p value ≤ 0.0001); pairwise comparisons ranged from -0.003 (between Boran and Sheko) to 0.164 (Hanwoo with Horro and Sheko). The calculated FST values between populations pairs were also plotted graphically using the R statistic (**Figure 4.4**). The negative FST values recorded between Sheko and Boran and between Sheko and Fogera indicated that there was no genetic subdivision between these populations. The exact G test was compared between cattle breeds and the highest G_{ST} value was observed between Bangladesh Red Chittagong and Sheko (0.110). The lowest G_{ST} value (0.005) was found between Horro and Boran (**Table 4.6**).

Table 4.6 Genetic distances between pairs of populations based on Wright's F-statistics

F_{ST} below the diagonal and Nei's genetic distance G_{ST} above the diagonal estimated.

Breed	HF	RC	BO	FO	HN	HO	SH
HF	-	0.019	0.010	0.010	0.050	0.008	0.006
RC	0.029	-	0.017	0.023	0.086	0.024	0.110
BO	0.012	0.015	-	0.007	0.064	0.005	0.007
FO	0.036	0.020	0.020	-	0.074	0.008	0.005
HN	0.084	0.137	0.092	0.151	-	0.074	0.067
HO	0.029	0.019	0.005	0.036	0.164	-	0.008
SH	0.029	0.007	-0.003	-0.015	0.164	0.001	-

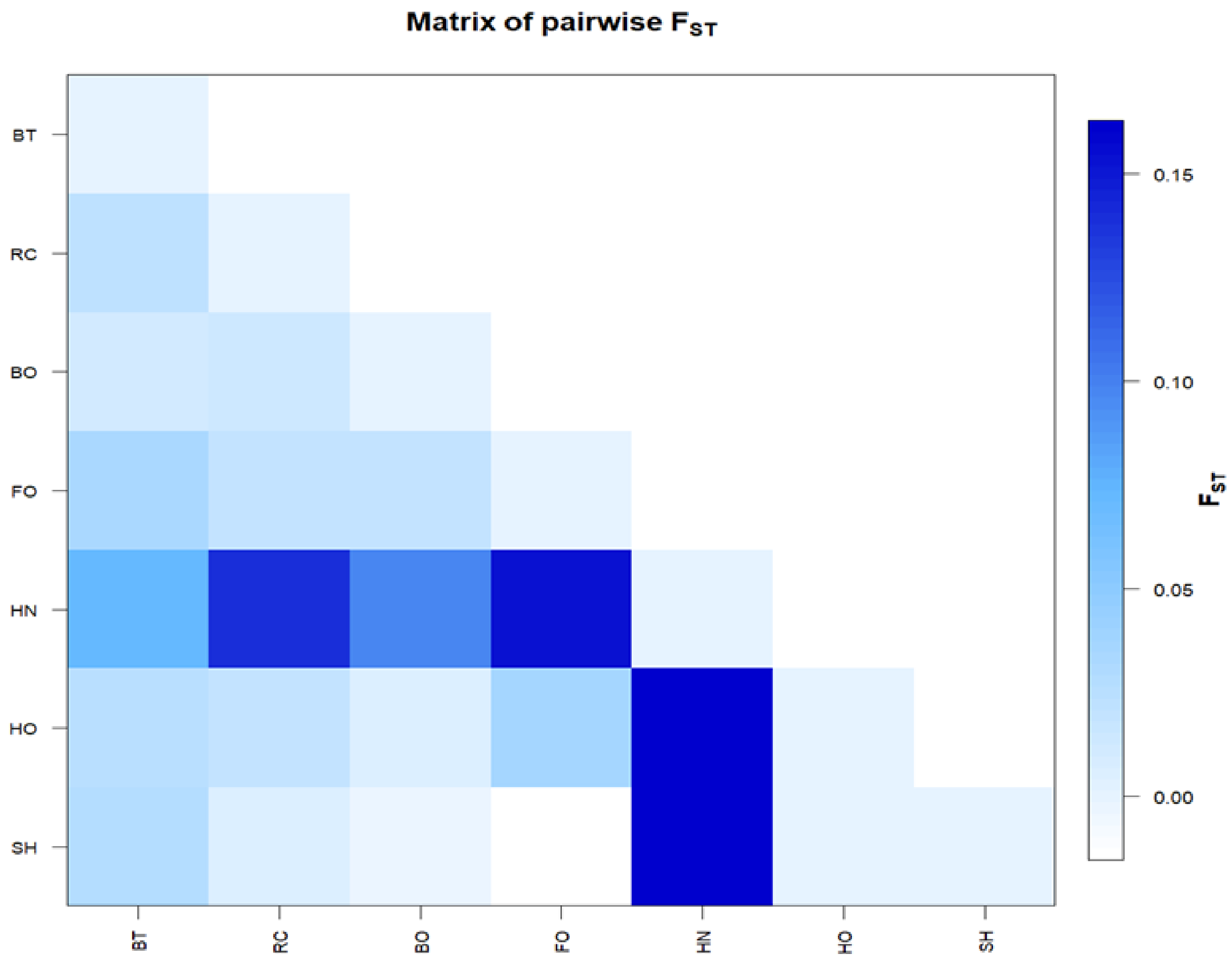


Figure 4.4 Illustration of computed F_{ST} values between pairs of populations graph generated by R- function: pair F_{ST} matrix.r. Hence, (RC= Red Chittagong, BO= Boran, FO= Fogera, HN= Hanwoo, HO= Horro, SH= Sheko, and BT= Bos taurus

4.3.5 Phylogenetic inferences and Median Joining network

The maximum likelihood model depending on Kimura-2 parameter approach through 1000 bootstrap replication values was applied to investigate the evolutionary divergence and population structure of cattle breeds. The evolutionary tree generated from nucleotide sequences of DRB3.2 locus are indicated hereunder (**Figure 4.5)**. There was no clearly separated cluster among the cattle breeds considered in this study and they shared a common node from the constructed tree. It looks that DRB3 alleles might have evolved over multiple lineages.

To further demonstrate the relationships among the haplotypes found in Ethiopian cattle breeds, Korean Hanwoo and Bangladesh Red Chittagong, reduced median-joining networks were constructed. Each circle denotes a unique sequence in which the diameter is proportional to the number of sequences represented. We counted 59 haplotypes for exon2 of the BoLA DRB3 gene and constructed a median joining-network tree. Therefore, we found that H-23 and H-36 were haplotypes with higher frequencies and expected to be the ancestral haplotypes. The majority of the haplotypes were represented by one or two individuals. Medium Joining network identified from haplotype data indicated that most of the haplotypes were condensed around a centric area (**Figure 4.6**).

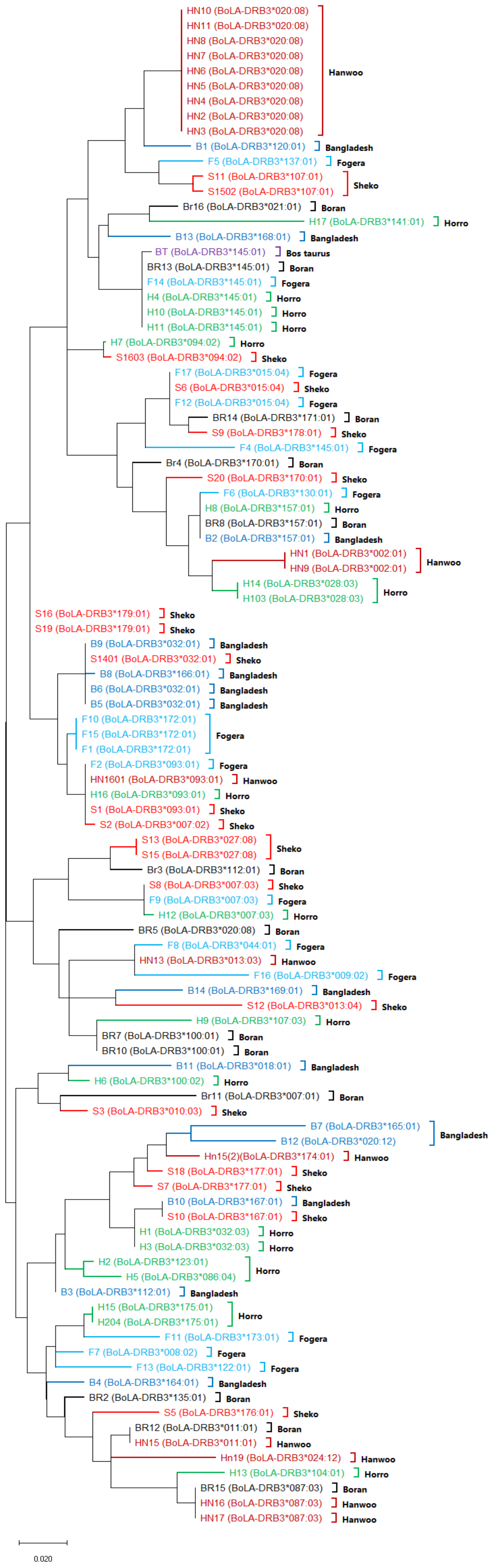


Figure 4.5 Phylogenetic tree (evolutionary history) of BoLA-DR3.2 gene inferred by using maximum likelihood method based on Kimura-2 parameter model.

(B=Bangladesh (Red Chittagong), Br=Boran, F= Fogera, Hn= Hanwoo, H= Horro, S=Sheko, and BT= *Bos taurus)*.

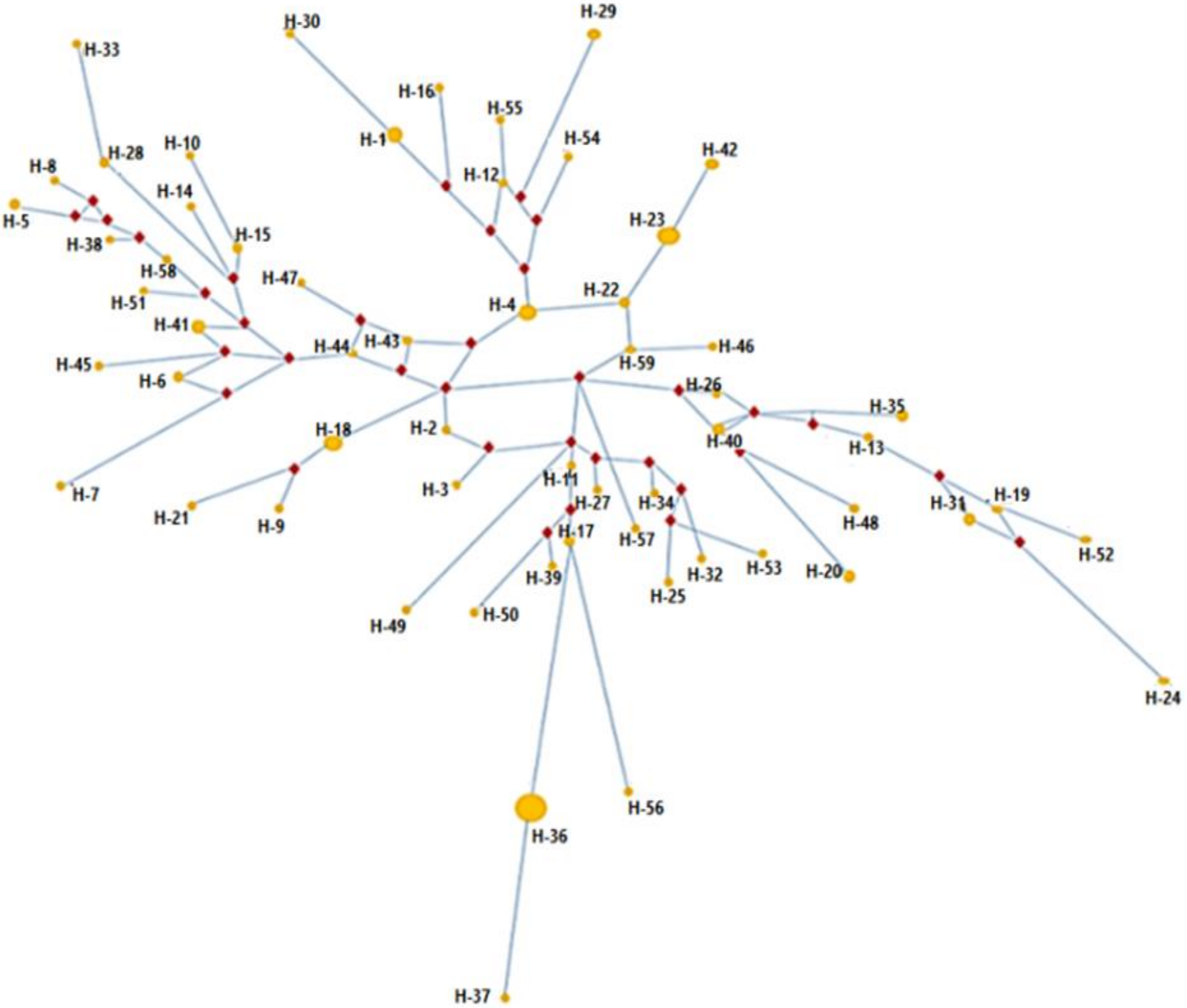


Figure 4.6 Median joining network tree for the sequences of DRB3 exon2.

The Yellow circles represent the individual sequences and have sizes proportional to frequency and the red diamond represents the median vector (A hypothesized ancestral sequences required to connect the existing sequences within the network). The branch sizes are comparative to the number of mutations.

4.4 Discussion

Investigating the MHC gene can generate information about domestic species levels of overall genetic diversity and mechanisms of pathogen defense in the immunological traits of all vertebrate groups. BoLA-DRB3 gene in bovines was reported to be functional with higher variability. Hence, recently scientists reported that the BoLA-DRB3 gene is one of the most variable regions in cattle genome and the second exon is the most extensively polymorphic region of the gene where 144 alleles have been identified sofar (Takeshima *et al*., 2015; Takeshima *et al*., 2018; Peters *et al*., 2018; Lubambe *et al*., 2018; Bohórquez *et al*., 2020).

The new alleleles identified from the sequences of BoLA-DRB3 exon2 locus across the analyzed cattle breeds could function as additional evidence for specific *Bos indicus* and *Bos taurus* genotypes. All the identified alleles were variable and similar with other reports elsewhere in the world (Lubambe *et al*., 2018; Takeshima *et al*., 2002; Nassiry *et al*., 2005; Behl *et al*., 2009). The ability of a population responding well to pathogens and parasitic infection is strongly dependent on the level of genetic diversity at the MHC loci (Hedrick and Kim, 2000; Ojong *et al*., 2016). The alleles BoLA-DRB3*002:01 and BoLA-DRB3*009:02 identified in this study were associated with the *BLV* as confirmed by other study (Takeshima *et al*., 2019). Allele BoLA-DRB3*009:02 was intensively associated with *BLV* and breeds gained this allele were reported to be resistant to BLV (Takeshima *et al*., 2019). Also, allele BoLA-DRB3*002:01 was reported to be resistant for BLV with lower impact as compared to allele BoLA-DRB3:009:02. On the contrary, susceptible allele (BoLA-DRB3*012:01) was reported by the same group of scientists and was detected in the Boran breed of the current study. We found the highest polymorphic sites and the total number of mutations of the BoLA- DRB3 gene in Sheko breed that directed the researchers as the BoLA-DRB3 gene could be responsible candidate gene for the future breeding plan in the area of tsetse verminous regions.

Sequence evaluation of the MHC II BoLA-DRB3 exon2 gene for base substitutions revealed a higher frequency of nonsynonymous substitutions (NSS) computed with synonymous substitutions (SS). The proportion of (NSS/SS) was evaluated and the recorded values were greater than one in all the considered breeds. This outcome suggests that the deviation at the antigen binding site (ABS) is subject to positive selection which could be for the recognition of a wide range of pathogens (Hughes and Yeager, 1998; Radwan *et al.*, 2020).

The higher variability of the BoLA-DRB3 gene is strongly related to the regions of peptide binding sites (PBS) where amino acid residues networked directly with antigens. Consequently, molecules coded by diverse alleles have diverse antigen-binding profiles and also disturb susceptibility to certain pathogens (Radwan *et al.*, 2020). Therefore, the BoLA-DRB3 gene raises the capability of populations existing in pathogenic areas and thus has a positive influence on animal breeding. The broadly described researches specified that zebu cattle have high disease tolerance to tropical infectious diseases such as ticks and intestinal parasites (Porto Neto *et al.*, 2011; Magee *et al.*, 2014). Then, the outcome of this research delivered abundant awareness and a call to the scientific community about the BoLA-DRB3 gene, exclusively for livestock breeders and vaccine designers. Exploring a population in terms of genetic diversity, scholars recycled nucleotide and haplotype diversities as communal directories (Adefnwa *et al.*, 2013). A higher level of gene and haplotype diversity was detected in all the breeds we considered. Thus, gene and haplotype diversities increased the fitness of a population and directly proportional to the productive and reproductive traits of cattle (Peters *et al.*, 2018). The higher values of heterozygosity found in all the breeds contradicted the lower values reported previously from Tanzania for Shorthorn Zebu, Boran, and Holstein Friesian breeds (Msalya *et al*., 2017). Another investigation in Tanzanian short horn Zebu reported the higher values of heterozygosity (Lubambe *et al*., 2018). The heterozygosity in Japanese cattle breeds was higher as reported by previous studies (Takeshima *et al*., 2003) with corresponding values of Ho ranged from 0.905 to 0.921 and He ranged from 0.887 to 0.914. Likewise, Jolanta et al. (2012) reported higher values of He (ranged from 0.920 to 0.927) in Poland. The estimated Ho in our breeds ranged from 0.980 (Boran) to 0.750 (Fogera) and the higher values of heterozygosities in the breeds could be the direction to suggest increased adaptation to pathogens in our breeds. We found the positive values of FIS in all analyzed breeds which were similar to the other scientific reports (Takeshima *et al*., 2015; Takeshima *et al*., 2018) indicated the heterozygote deficiency. Thus, heterozygote deficiency observed in breeds suggested that; other evolutionary forces like inbreeding and/or bottlenecks could have been acting as a deriving force for the evolution of the BoLA-DRB3 gene (Bohórquez *et al*., 2020). In this study, the observed heterozygosity was not considerably higher than the expected heterozygosity *($p<0.05$)*. Therefore, HWE of all the breeds examined exhibited non-significant excess of heterozygotes animals that could divulge over-dominant selection. The result of this study was supported by the recent reports accompanied to study South American Zebu breeds (Takeshima *et al*., 2018).

The low genetic differenciation identified in this study in terms of pairwise F_{ST} indicated that Ethiopian breeds and Asian breeds shared a common ancestery and supported by recent reports investigated the relationship among Asian, African and American breeds (Peters *et al*., 2018). The negative F_{ST} values recorded between Sheko and Boran as into the bargain between Sheko and Fogera, proposing no population sub-division at the DRB3.2 locus. Furthermore, the genetic differentiation of all the breeds based on Nei genetic distance (G_{ST}) was small and supported by the previous studies (Peters *et al*., 2018; Takeshima *et al*., 2018; Lubambe *et al*., 2018). The little genetic differences inferred from the breeds could be due to the evolutionary or domestication history of cattle breeds (Bradley *et al*., 1998).

The neutrality test conducted due the principle of Fu's (Fu, 1997) indicated *FS* identity of all the breeds were neutral (*p*>0.02). The result of this study suggests that indigenous Ethiopian cattle breeds and Asian breeds are not considerably different vis-à-vis of the genetic diversity of the BoLA-DRB3 gene. The examined negative values of Fu's *FS* designate an excessive number of alleles and could be predictable from recent population expansion or genetic hitching. In contrast, the positive values of *FS* indicate a defficiency of alleles, as would be expected from a latest population exposed for genetic bottleneck. We found anegative *FS* values for all the breeds except Korean Hanwoo breed. Sheko breed distributed in the Southern parts of Ethiopia specifically in the Bench Maji zone, described to be trypanotolerant equated to zebu breeds. Due to their nature of resistance to trypanosomiasis disease, Ethiopian Sheko cattle are kept in tsetse infected regions (Bahbahani *et al*., 2018). Cattle breeds retained in pathogenic ecological zones are challenged by the philosophies of natural selection to develop adaptive immunity at large and diversification of immune related genes could occur. Therefore, we identified the higher variable regions and higher number of total mutations rate in Sheko breed which implied that the variability of BoLA-DRB3 gene may be due to the consequence of their exposure to pathogenes.

To find the phylogenetic relationship, it was hypothesized that the BoLA-DRB3 alleles originated from related species, which is supported by the trance species theory, and survived the speciation process, and this hypothesis is also supported by the phylogenetic analysis of DRB gene sequence of Bovidae (Sena *et al*., 2003; Mishra *et al*., 2016). We detected adjacent genetic association between Ethiopian cattle breeds and Asian breeds from the phylogenetic tree and median joining network tree constructed via BoLA-DRB3 exon2 gene sequences. The close genetic linkage between Ethiopian cattle breeds and Bangladesh Red Chittagong is reliable with the evidence that all Ethiopian cattle breeds rooted a higher proportion of Asian Zebu ancestry (Edea *et al.*, 2013). The phylogenetic tree showed similarity with previous studies that indicated no distinct clade in different cattle breeds (Takeshima *et al*., 2001; Jeong *et al*., 2007).

The phenomenon of trans-species polymorphism, i.e., maintenance of old allelic lineages, remained and remains a perplexing observation. However, scientists reported the more likely ancient lineages in cattle. Thus, shared parasites between species may cause convergent evolution of MHC alleles that could provide patterns of similar trans-species polymorphism. Similarities between and within species may arise from the retention of ancestral traits (shared ancestral traits) and the gain of traits in the common ancestor of the allele lineage (shared derived traits) (Dudek *et al.*, 2019). Scientists reported that some specific ancient allelic lineages were conserved and most major lineages in the phylogenetic tree of a gene diverged in some major animal species well before family-level diversification. Therefore, DRB3 alleles may have evolved across multiple lineages (Russell *et al.*, 2000).

This research work concluded that the MHC II DRB3.2 locus is enormously essential and may deliver a baseline information for the understanding and exploitation of MHC gene variation in Ethiopian cattle breeds exposed to a wide range of pathogens. The alleles we identified in the present populations require further validation for disease susceptibility/resistance association. In addition, the sequence polymorphism of BoLA-DRB3 exon2 identified in our study should be confirmed by sequencing additional samples.

Chapter 5

5. Single Nucleotide Polymorphisms of Leptin Gene in Five Ethiopian Indigenous Cattle Breeds and the Korean Hanwoo Breed

Abstract

Discerning the intensifying number of scientific booms on the association between the cattle leptin gene (LEP) and diverse physiological traits, this research was designed towards detecting single nucleotide polymorphisms (SNPs) in the LEP gene among five native cattle breeds of Ethiopia. DNA samples were extracted from the nasal swabs of the Ethiopian indigenous cattle breeds: *Arsi* (n=18), *Horro* (n= 20), *Begait* (n= 21), *Boran* (n = 19) and *Fogera (*n= 17) and the Korean Hanwoo (a demonstrative *taurine* breed) (n = 20), surveyed by PCR amplification of two exonic regions (exon 2 and exon 3) of the LEP gene and sequence investigation of the PCR products. Five SNPs, two (generating missense mutations) on exon2, and three (generating silent mutations) on exon3 regions were explicated in this study. The allelic and genotypic frequency distributions concerning the identified SNPs were documented for the investigated cattle breeds, moreover, positive F_{IS} index were disclosed for all the markers excluding SNP2, descriptive of heterozygote deficiency. The evolutionary divergence examination of the breeds depending on the SNP data through MEGA-7 software integrated with power marker generating phylogenetic tree indicated that large sized breeds; Hanwoo, Begait, Boran, and Fogera were more closely grouped together compared to the small-sized Arsi breed. From all the SNPs reported from the analyzed breeds, sequence investigation was allusive of haplotypes 1 and 2 to be ancestral haplotypes for the LEP gene. This research is envisioned to enhance molecular breeding programs for the genetic improvement of the Ethiopian cattle breeds.

Midst to the seven haplotypes recognized from the different breeds considered in this study, sequence investigation was indicative of haplotypes 1 and 2 to be ancestral haplotypes for the leptin gene. This study is envisaged to accelerate molecular breeding programs for the genetic improvement of the Ethiopian cattle breeds.

Keywords: Ethiopia, Cattle population, Genetic diversity, SNP, Leptin gene, haplotype

5.1. Introduction

The pertinence of the cattle population in an agrarian society as of Ethiopia is easily understandable. With 56.7 million heads of cattle, Ethiopia shelters the biggest livestock population in Africa (UNDP, 2017; Mandefro *et al*., 2017). The biodiversity of indigenous cattle herds is projected as a critical dictate in ensuring sustainable growth and maintenance of the farming societies, particularly those with minimal agricultural production inputs. Over decades, the horn of Africa has been proved to the introduction of many livestock (example, Indian and Arabian zebu (*B. indicus)* further the Near Eastern *B. taurus*) on one hand while events for breed identification and description has led to the cataloguing of the Ethiopian cattle genetic resources into five major groups; Large East African Zebu, Small East African Zebu, Senga, Zenga, and Taurine (hump-less short horn) breeds on the other (Rege 1999; Hanotte *et al*., 2002; Terefe *et al*., 2015).

However, it has been previously pointed out that many breeds, earlier documented in the continent, mostly in sub-Saharan Africa have either become extinct or are at risk of extinction in the background of breed substitution, indiscriminate crossbreeding, and the widespread climate changes and ecological modifications (Edea *et al*., 2013). This is guaranteed to have a negative consequence in the context of wearing away of the indigenous genetic biodiversity, thereby, limiting the opportunities and potentials to handle and adapt to the changes in the cattle-production rudiments in diverse environments. In this setting, a profound vision into the genetic diversity of the farm animals is visualized to support the objectives of breeding and continual genetic enhancement besides, accelerating coping with varying environmental circumstances. In this framework, though, reports do exist on the manipulation of low-density microsatellite as well as mitochondrial or Y-chromosome markers to investigate the genetic diversity of Ethiopian cattle populations (Li *et al*., 2007; Dadi *et al*. 2008; Dadi *et al*., 2009; Zerabruk *et al*., 2011), howbeit, current activities on hitching single nucleotide polymorphism (SNP) markers (with the hallmarks of genetic stability, abundance in genome and acquiescence to high-throughput programmed assessment) for diversity investigation and genome-wide studies (GWS) merit special mention (McKay *et al*., 2008; Lin *et al*., 2010; Edea *et al*., 2013; Edea *et al*., 2017).

Having stated this, it is pertinent to mention that identification of candidate genes affecting production and reproduction of cattle populations has been a prime research thrust in the realm of animal breeding. Amongst others, dictated by the objective of identifying high quality meat producers through marker-assisted selection (MAS), harnessing molecular information about the leptin gene seems to have garnered commendable research-impetus in the niche of molecular breeding. The leptin gene, located on the fourth chromosome (BTA4q32), transcribes a 167 amino acid peptide (Corva *et al*., 2009) while the final peptide hormone with influence on multiple physiological functions (including energy-economics and immunity-related attributes) (Jayaprakash *et al*., 2016) contains 146 amino acid residues, generated post scissoring of 21 amino acids- long signal sequence during its translocation into microsomes (Margetic *et al*., 2002). Over the years, numerous SNPs have been described in introns and exons of LEP gene among diverse breeds of cattle. While there are complementary reports on the effect of mutations in the LEP gene sequence (ensuing in altered amino acid profiles) on the fat content in cattle

(Buchanan *et al.*, 2002; Barendse *et al.*, 1997), growing evidences are suggestive of the prospective applications of the genetic markers, identified in the LEP gene in selecting superior animals for beef production and better meat quality (Symonds *et al.*, 2016; Wang *et al.*, 2020; Geng *et al.*, 2020; Fernandes *et al.*, 2020).

At this juncture, it is worthwhile to mention that more than half a decade ago, we had resorted to the use of nearly 4235 autosomal genome-wide SNPs to trace the genetic diversity status, population structure, and the relationships among Ethiopian cattle populations and the Hanwoo breed (Edea *et al*., 2013). Extending this study, we have dealt with specifically identifying the SNPs of the LEP gene among five different Ethiopian cattle breeds (Arsi, Horro, Begait, Boran, and Fogera) (**Table 5.1**) in this current delving. The Korean Hanwoo cattle breed, as a representative of the *taurine*, assisted as the reference in the study. This study is envisaged to open up new opportunities in molecular breeding programs and assist the genetic improvement of cattle breeds in the country.

5.2 MATERIALS AND METHODS

5.2.1 Study-samples and DNA extraction

The overall of 115 experimental animals, randomly appraised from multiple herds of the five Ethiopian native cattle breeds (Arsi, n=18, Horro, n= 20, Begait, n= 21, Boran, n = 19 and Fogera, n= 17) and Korean Hanwoo breed (n = 20) were considered for the study (**Table 5.1** and **Figure 5.1**). 1). Arsi, Boran, and Horro breeds were sampled from southern areas of Ethiopia, namely, Arsi, Borana, and Horro Gudru zones of Oromia region respectively. The Begait and Fogera breeds were sampled from the northern part of Ethiopia: Kafta Humera district of Tgrai region and Metekel zone of Amhara region respectively. Nasal swabs were collected from the animals applying Animal Swabs Collector (BlueGene Life Science, Cheongju, Korea) and

Performagene™ LIVESTOCK’s nasal swabs (DNA Genotek Inc., Kanata, Ontario, Canada). The technique concerning sample collection monitored the recommendation of the instruction 2010/63/EU. DNA was isolated from the collected nasal swabs, following the manufacturer’s instructions (http://www.dnagenotek.com). The quality of the DNA and its concentration were assessed via Nano Drop1000 and electrophoresis in 0.8% agarose gels respectively.

Table 5.1 Cattle breeds with corresponding major features

Breed Name	Major features (Reference)	Sample-size (n)
Arsi (Ethiopian)	• Grouped under large East African Zebu (but small in size) • Characterized as poor milkers, extremely active and aggressive. • Compact animals with diminutive length, mainly kept for draught. • Highly variable coat colour like red, black, roan, white, and grey expressed well for this breed (Rege and Tawah, 1999; Mwai *et al*., 2015)	18
Begait (Ethiopian)	• Recently registered as one of the indigenous cattle of Ethiopia (IBC, 2004). • Grouped under large East African Zebu classification. • Reared for milk and beef • Based on body frame, this breed is comparable to Boran and Ogaden breeds • Relatively superior to the other Ethiopian breeds, which survives in trypanosome prevalent area of the country (Rege 1999; Mekuriaw *et al*., 2009; Zerabruk *et al*., 2007; Mekonnen and Meseret 2020).	20
Boran (Ethiopian)	• Mainly inhabit the Borana plateau of southern Ethiopia, extending from the Liban plateau to the extreme southern part of the country. • Reared mainly for beef, although, they are also milked by the pastoralists • Categorized under large East African Zebu (Rege and Tawah, 1999).	21
Fogera (Ethiopian)	• Inhabit the North-west highlands of Ethiopia in the surrounding area of Lake Tana • Mainly reared for drought and dairy production. • Belongs to the Zenga group (cross between Sanga and Zebu) (Moderate to large in size, docile temperament • The high feed intake capacity (Mwai *et al*., 2015; Rege 1999; Zerabruk *et al*. 2007).	19
Horro (Ethiopian)	• Represents an ecotype, experienced in the highland agro-ecological regions of the country with the main habitat in the Horro Gudru area of Eastern Wollega Zone as well as neighboring parts of Western Showa and Illubabur. • Belongs to the group of Zenga. • Mainly characterized by calm disposition and variable milk production in a mixed crop-livestock system (Rege 1999; Edea *et al*., 2012; Mekonnen *et al*., 2012; Mwai *et al*., 2015).	17
Hanwoo (Korean)	• Small cattle, native to Korea • Low milk-producers, mainly reared for meat (although, formerly used as drought animal) • SNP analysis suggested that it forms a distinct group with the Yanbian breed of China, separate from European taurine breeds and distant from the indicine group (Chung *et al*., 2018)	20

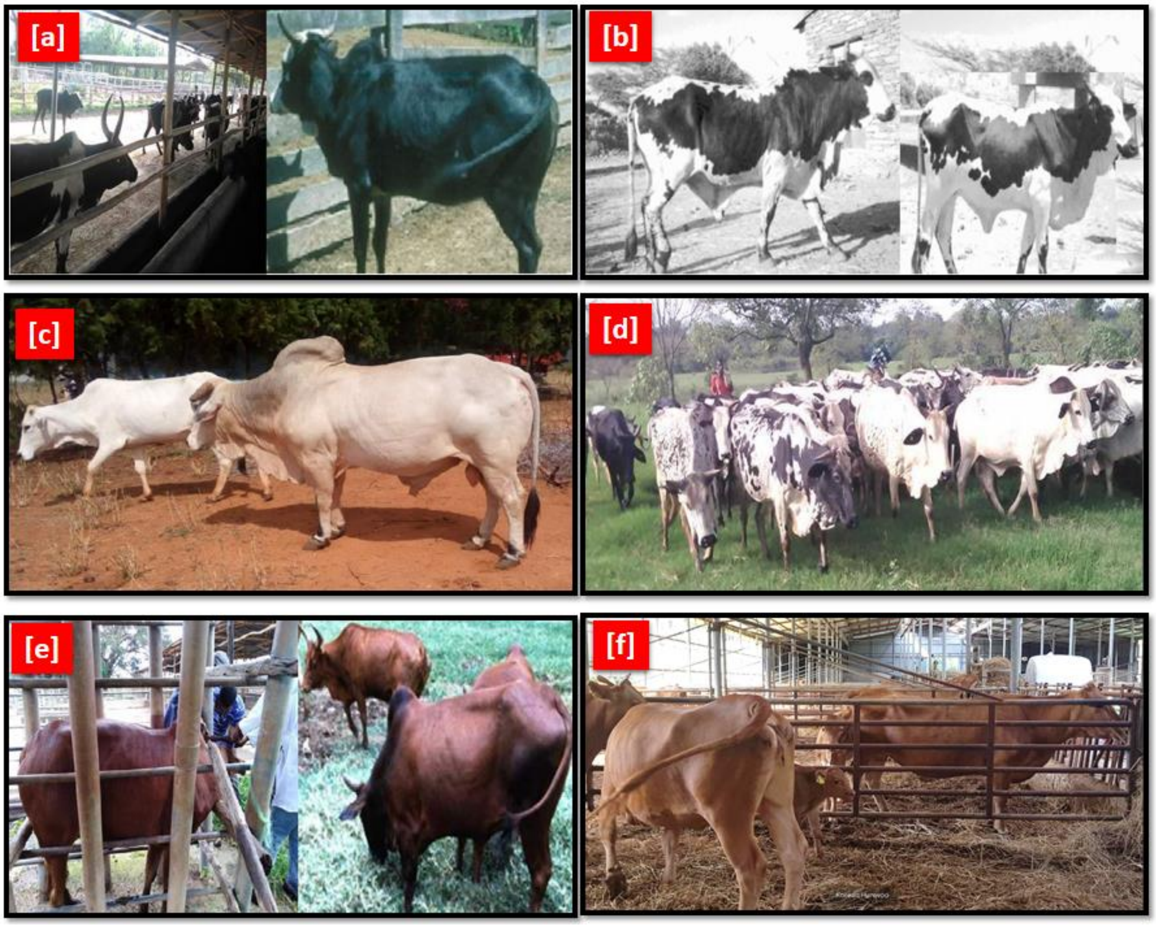


Figure 5.1 Sampled cattle breeds [a] Arsi, [b] Begait, [c] Boran, [d] Fogera, [e] Horro, and [f] Hanwoo

5.2.2 PCR amplification of selected exon

Amplifications of exon2 and exon3 of leptin gene were conducted post modification of previously reported protocol (Liefers *et al*., 2003). Polymerase chain reaction (PCR) (Prime Thermal Cycler, TECHNE, UK) was used to amplify exon 2 (582 bp, spanning part of intron2, exon2 and intron3) and exon 3 (680 bp, spanning intron3, exon3 and intron4). To amplify leptin exon2 region, previously published forward and reverse primer sequences (Liefers *et al*., 2003) were used (**Table 5.2**). However, for amplification of exon3, we resorted to Primer3Plus online software package to design the corresponding primers based on the reference *Bos taurus* sequence (accession number: U50365.1, NCBI database) (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi).

Table 5.2 **Primers used to amplify the two exons of leptin gene**

Exons	**Types**	**Primer Name**	**Sequence (5´ -3´)**	**PCR product size**	**Source**
Exon2	Forward	Leppr2A	GTGGGGGATACAGGGGGAGTTTT	582 bp	Liefers *et al.*, 2003
	Reverse	Leppr2B	ACGGGATGGCCACGGTTCTAC		
Exon3	Forward	$FLpE3_1$	AAACATGGTGGTCACGTGGG	680 bp	This study
	Reverse	$RLpE3_1$	CGCAGTTTTCTGCCTTTGGA		

The reactions were carried out in a total volume of 30 µL containing genomic DNA (2 µL of 50 ng/µL), 21.1 µL distilled water, 10× PCR buffer (3 µL), 10 m*M* deoxynucleoside triphosphate (0.6 µL), 1.5 µL of 10 pM forward and reverse primers, and 0.3 µL of *Taq* DNA polymerase (Promega, San Diego, CA). The reactions cycling conditions encompassed initial denaturation at 94 °C for 10 min; 35 cycles at 94 °C for 30 s, annealing at 60 °C for 30 s, and 72 °C for 40 s and a final extension at 72 °C for 5 min. Finally, the PCR products were visualized post electrophoresis on 2% agarose gel with acetate EDTA (TAE) buffer followed by ethidium bromide staining (**Figure 5.2**). The PCR products were sequenced at Macrogene (Seoul, Korea).

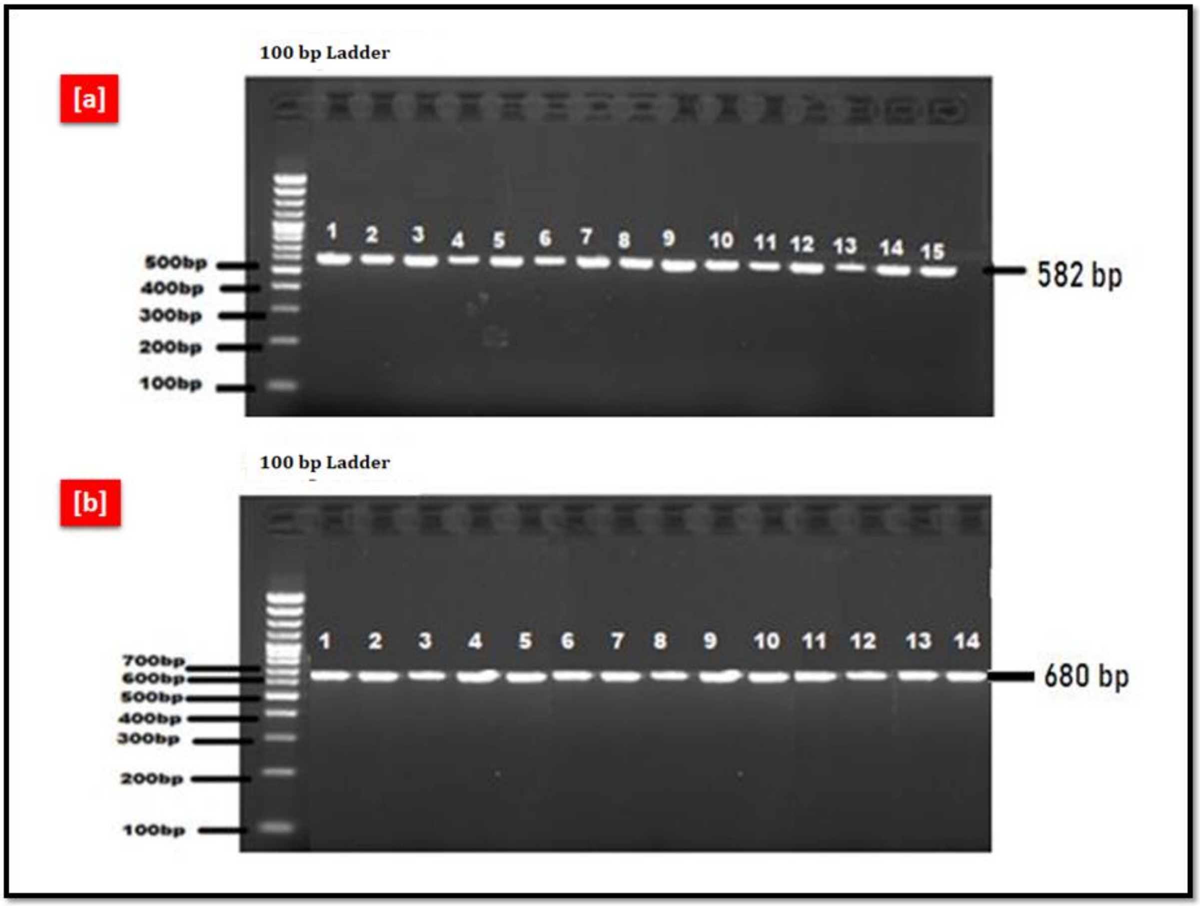


Figure 5.2 PCR amplification product of leptin gene; [a] exon2 and [b] exon3.

5.2.3 Sequence-data analysis

PCR- sequence based typing was employed for the leptin gene typing. As noted above, exon2 and exon3 of the leptin gene were considered in this study. The *Bos taurus* reference sequence of the LEP gene was downloaded from the NCBI database. The SNPs identified on both exonic regions were mined by using Sequencher (version 5.4.6) software at the Department of Animal Sciences, Chungbuk National University, Cheongju, South Korea. The sequenced nucleotides were aligned, translated, and compared, using BioEdit and Clustal W (Larkin *et al*., 2007). Phylogenetic analysis was carried out in accordance with the maximum likelihood method based on the Kimura 2-parameter model (Kimura, 1980) using MEGA-X software (version10.1)

(Kumar *et al*. 2018) via implementing 1000 bootstrap values (Nei and Kumar, 2000). The number of haplotypes and population F_{ST} statistics were computed using ARLEQUIN (version 3.5.2.2) (Excoffier and Lischer, 2010). F_{ST} values were calculated using 1000 bootstrap replication values. Meanwhile, the pairwise F_{ST} graph and the average number of pairwise differences were computed using the R package (https://cran.rproject.org/bin/windows/base/). The values for the observed heterozygosity (H_o), expected heterozygosity (H_E), polymorphic information content (PIC), major allele frequencies (MAF), and the deviation from Hardy Weinberg equilibrium were calculated using Power Marker (version 3.25) (Liu and Muse, 2005). Un-weighted pair group method with arithmetic average (UPGMA) tree was constructed using the same software. Median-joining network (MJN) tree was constructed using the NETWORK software (version 10.0.0) (Bandelt *et al*., 1999). To estimate the median joining network, the nucleotide sequences were first changed into binary data, while similar sites were omitted from the analysis. Each split was automated as a binary character, sustaining the values of 0 and 1. The haplotypes were represented as a binary vector in this approach. The median vectors were estimated for each triplet of vectors until the construction of the median network was achieved (Bandelt *et al*., 1999).

5.3 RESULTS

The first phase of the investigation was the enumeration and distribution assessment of the allele frequencies of the five SNPs identified within the coding region of the leptin gene for the different cattle breeds (**Table 5.3**). The prime highlights of the observations for the allele frequencies are enlisted: For SNP1 (LEP-73) (substitution of cytosine, C with thymine, T), the allele-frequencies for C and T were 0.610 and 0.390 respectively. The C allele-frequency was the highest in Arsi breed (0.806) compared to that in the other counterparts. The frequency of T allele was bounded between 0.194 (Arsi) and 0.525 (Horro). For SNP2 (LEP-143), the frequencies for the alleles A and C were 0.830 and 0.170 respectively. Allele A-frequency was higher for all the breeds, ranging from 0.618 (Arsi) to 0.921 (Hanwoo) while the frequency distribution of C allele was higher in Arsi (0.382). For SNP3 (LEP-399), T allele-frequency was relatively higher in Horro breed (0.875) in comparison to that in the rest of the breeds. Likewise, for SNP4 (LEP-411), a higher T allele-frequency was documented for all the breeds, with the values ranging from 0.694 (Arsi) to 0.952 (Begait). For SNP5 (LEP-495) (cytosine, C substituted by thymine, T), the allele frequencies for C and T were 0.820 and 0.180, respectively. Pertinently, the (breed) distributions of allele C ranged from 0.639 for Arsi to 1.000 for Hanwoo.

Table 5.3 Allele frequencies and breed distribution of identified SNPs.

SNP	Allele	Allele frequencies	Breed distribution of alleles					
			AR	**BG**	**BO**	**FO**	**HN**	**HO**
SNP1(LEP-73)	C	0.610	0.806	0.571	0.632	0.559	0.605	0.475
	T	0.390	0.194	0.429	0.368	0.441	0.395	0.525
SNP2(LEP-143)	A	0.830	0.618	0.881	0.842	0.794	0.921	0.875
	C	0.170	0.382	0.119	0.158	0.206	0.079	0.125
SNP3(LEP-399)	C	0.420	0.361	0.476	0.605	0.529	0.421	0.125
	T	0.580	0.639	0.524	0.395	0.471	0.579	0.875
SNP4(LEP-411)	C	0.120	0.306	0.048	0.053	0.206	0.105	0.050
	T	0.880	0.694	0.952	0.947	0.794	0.895	0.950
SNP5(LEP-495)	C	0.820	0.639	0.762	0.816	0.824	1.000	0.900
	T	0.180	0.361	0.238	0.184	0.1765	0.000	0.100

Table 5.4 Details of genotypic frequency distributions among the breeds

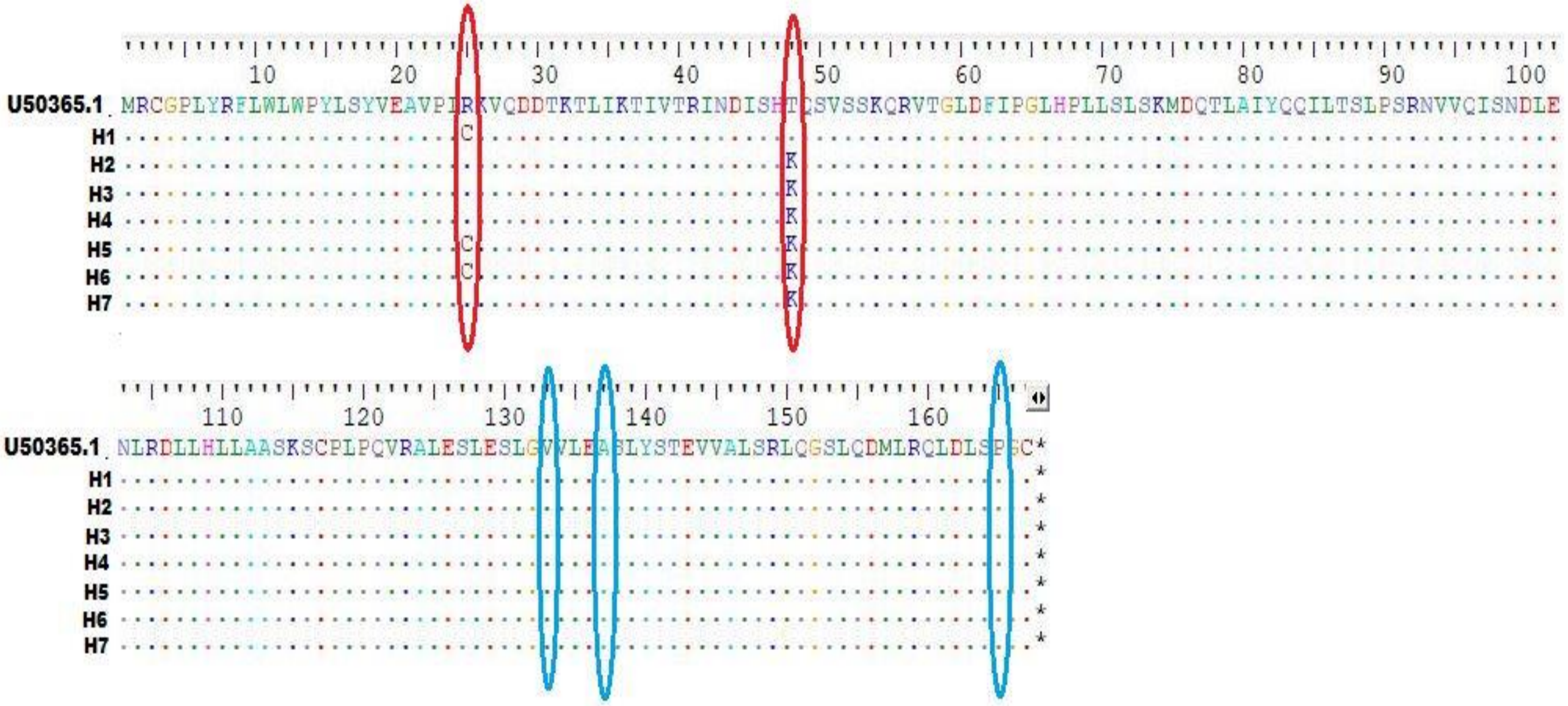
SNP	Region	Genotype	Genotype frequency	Cattle breed						MAF*	HWE**
				AR	BG	BO	FO	HN	HO		
LEP-73	Exon2	CC	0.450	0.722	0.381	0.474	0.295	0.474	0.350	0.390	0.081
		CT	0.310	0.167	0.381	0.316	0.529	0.263	0.250		
		TT	0.240	0.111	0.238	0.210	0.176	0.263	0.400		
LEP-143	Exon2	AA	0.670	0. 353	0. 762	0.684	0.588	0.842	0.550	0.170	-0.012
		AC	0.310	0.529	0.238	0.316	0.412	0.158	0.450		
		CC	0.020	0. 118	0.000	0.000	0.000	0.000	0.000		
LEP-399	Exon3	CC	0.270	0.222	0.333	0.421	0.353	0.316	0.000	0.420	0.098
		CT	0.290	0.278	0.286	0.368	0.353	0.211	0.250		
		TT	0.440	0.500	0.381	0.211	0.294	0.473	0.750		
LEP-411	Exon3	CC	0.050	0.222	0.000	0.000	0.118	0.000	0.000	0.120	0.038
		CT	0.140	0.167	0.095	0.105	0.176	0.211	0.100		
		TT	0.810	0.611	0.905	0.895	0.706	0.789	0.900		
LEP-495	Exon3	CC	0.770	0.555	0.715	0.737	0.764	1.000	0.850		
		CT	0.110	0.167	0.095	0.158	0.118	0.000	0.100		
		TT	0.120	0.278	0.19	0.105	0.118	0.000	0.050	0.180	0.092

Pertaining to SNP1 (LEP-C73T), the frequencies for CC, CT and TT genotypes were 0.450, 0.310 and 0.240 respectively. The incidence of homozygous CC genotype was the highest for Arsi (0.722) compared to the rest of cattle breeds. Comparatively low frequencies for the heterozygous CT genotype (0.167) and the homozygous TT genotype (0.111) were noted in Arsi. On a contrasting note, relative highest CT and TT genotype frequencies were observed in the remaining cattle breeds. For SNP2 (LEP-C143A), the frequencies of 0.670, 0.310 and 0.020 were documented for AA, AC and CC respectively. The AA genotype frequency was maximum in Hanwoo (0.842) and the lowest in Arsi (0.353). Similarly, the AC genotype frequency was the highest in Arsi (0.529) and the lowest in Hanwoo (0.158). The genotype CC (with a very low frequency of 0.118) was detected only in Arsi. Corresponding to the SNP3 (LEP-T399C), the frequencies of TT, CT and CC genotypes were 0.440, 0.290 and 0.270 respectively. The CC genotype exhibited the highest frequency in Boran (0.421) while it was not detected in Horro. Similarly, the frequency of CT was the highest in Boran (0.368) and lowest in Hanwoo (0.211).

Contrastingly, the frequency of TT was the lowest in Boran (0.211) and highest in Horro (0.750). As far as SNP4 (LEP-T411C) is concerned, the genotype frequencies of 0.050, 0.140 and 0.810 were registered for CC CT, and TT respectively. The frequency of homozygous TT genotype was ranged from 0.611 (Arsi) to 0.900 (Horro) whereas, the heterozygous CT genotypic incidence scaled from 0.095 (Begait) to 0.211 (Korean Hanwoo). The homozygous CC genotype was detected only in Arsi and Fogera with the corresponding frequencies of 0.222, and 0.118). For SNP5 (LEP-C495T), the genotype incidences for CC, CT and TT were verified as 0.770, 0.110, and 0.120 respectively. The incidence of CC genotype scaled from 0.555 (Arsi) to 1.000 (Hanwoo). The genotypic frequency of CT, and TT revealed higher distribution (0.167, and 0.278) respectively in Arsi breed whereas not applicable in Hanwoo. The minor allele frequency

(MAF) varied between 0.120 (LEP-411) and 0.420 (LEP-399). Except for SNP2 (LEP-143), all others segregated to the sample population and emerged non-deviated ($p<0.05$) from Hardy Weinberg equilibrium (**Table 5.4**).

Thus, compared to *Bos taurus* reference sequence, two mutations ((C73T) and (C143A)) in exon2 and three mutations ((T399C), (T411C) and (C495T)) in exon3 were identified (**Figure 5.3**). Moreover, base-deletion at the positions 172-180 (covering both exons) was distinguished in the Arsi breed. The missense mutations identified on the second exonic region directed to amino acid-changes from arginine to cysteine (R25C), and from threonine to lysine (T48K) correspondingly. These SNPs were apparent in all the cattle breeds, considered in this study. The SNPs, identified on the third exonic region generated silent mutations (LEP-T399C, LEP-T411C, and LEP-C495T) paralleled to syntsesis of valine (V133V), alanine (A137A), and proline (P165P) respectively. SNP3 (LEP-T399C) was not detected in Korean Hanwoo and Ethiopian Horro breeds while the SNP4 (LEP- T411C) was identified only in Arsi and Fogera breeds. Albeit, absent in Korean Hanwoo breed, the SNP5 (LEP-C495T) was common for all the Ethiopian breeds.



```
                 10        20        30        40        50        60        70        80        90       100
U50365.1 MRCGPLYRFLWLWPYLSYVEAVPIRKVQDDTKTLIKTIVTRINDISHTQSVSSKQRVTGLDFIPGLHPLLSLSKMDQTLAIYQQILTSLPSRNVVQISNDLE
H1       ........................C...........................................................................
H2       ...............................................K....................................................
H3       ...............................................K....................................................
H4       ...............................................K....................................................
H5       ........................C......................K....................................................
H6       ........................C......................K....................................................
H7       ...............................................K....................................................

                110       120       130       140       150       160
U50365.1 NLRDLLHLLAASKSCPLPQVRALESLESLGVVLEASLYSTEVVALSRLQGSLQDMLRQLDLSPGC*
H1       ..................................................................*
H2       ..................................................................*
H3       ..................................................................*
H4       ..................................................................*
H5       ..................................................................*
H6       ..................................................................*
H7       ..................................................................*
```

Figure 5.3 SNP$_{LEP}$ detected at exon2 corresponding amino acid changes circled by red colour, silent mutations identified at exon3 circled with blue, dot represents similar amino acids with reference sequence (U50365.1), and H = haplotypes.

Generally, a total of seven haplotypes (H) were distinguished among all the analyzed cattle breeds. Although documented for all breeds studied haplotype 1 (H-1) was most common in the breed Korean Hanwoo (0.450) and lowest in Ethiopian Boran (0.105), while the incidence of haplotype 2 (H-2) was comparatively high in Horro (0.556) and low in Fogera (0.235). The distribution of Haplotype 3 (H-3) was recorded only in two Ethiopian breeds (Arsi and Fogera) with frequencies of 0.278, and 0.058, correspondingly. Haplotype 4 (H-4) was detected in Begait and Fogera with corresponding frequencies of 0.190 and 0.058, unlike haplotype 6 (H-6) which was observed in Boran and Horro with haplotype frequencies of 0.105 and 0.056, respectively. Haplotype 5 (H-5) was observed only in Boran (frequency of 0.263), while haplotype 7 (H-7) was observed only in Fogera breed (frequency of 0.058) **(**Table 5.5**).**

Table 5.5 Haplotype distribution of leptin gene in the study cattle breeds

Haplotypes	**AR**	**BG**	**BO**	**FO**	**HN**	**HO**
H-1	0.278	0.429	0.105	0.388	0.450	0.389
H-2	0.444	0.381	0.526	0.235	0.550	0.556
H-3	0.278	-	-	0.058	-	-
H-4	-	0.190	-	0.058	-	-
H-5	-	-	0.263	-	-	-
H-6	-	-	0.105	-	-	0.056
H-7	-	-	-	0.058	-	-

In population and ecological genetics, F-statistics are implemented to explain the statistically expected degree of population-heterozygosity; more accurately, the general expected amount of decrease in heterozygosity regarding the Hardy–Weinberg equilibrium (HWE). The calculated F_{ST} values between the reference *Bos taurus* and individual cattle breeds, deliberated in this study, ranged from 0.000 (*Bos taurus* vs. Fogera) to 0.252 (*Bos taurus* vs, Hanwoo). Compared to the Ethiopian breeds, the calculated F_{ST} value was moderately high (F_{ST} = 0.130) recorded between Arsi and Horro, and low (F_{ST}=0.003) recorded between Boran and Begait. By the same nominee, higher F_{ST} value (0.160) was evaluated between Arsi and Hanwoo, opposite to a low recorded value between Hanwoo and Boran (0.025). Above and beyond, interspecies computation of F_{ST} values was examined provided that higher genetic differences (F_{ST}>0.900 distances) detected in all species (**Table 5.6**).

Table 5.6 Pairwise distances of leptin gene within and across species.

	BT	**AR**	**BG**	**BO**	**FO**	**HN**	**HO**	**CH**	**EC**	**OA**	**SS**
BT	0.000										
AR	0.161	0.000									
BG	0.080	0.033	0.000								
BO	0.053	0.033	0.003	0.000							
FO	0.000	0.020	0.009	0.007	0.000						
HN	0.252	0.160	0.028	0.025	0.035	0.000					
HO	0.200	0.130	0.020	0.032	0.017	0.025	0.000				
CH	1.000	0.987	0.992	0.992	0.991	0.996	0.995	0.000			
EC	1.000	0.988	0.992	0.992	0.991	0.996	0.995	1.000	0.000		
OA	1.000	0.987	0.991	0.991	0.991	0.996	0.995	1.000	1.000	0.000	
SS	1.000	0.988	0.992	0.992	0.991	0.996	0.995	1.000	1.000	1.000	0.000

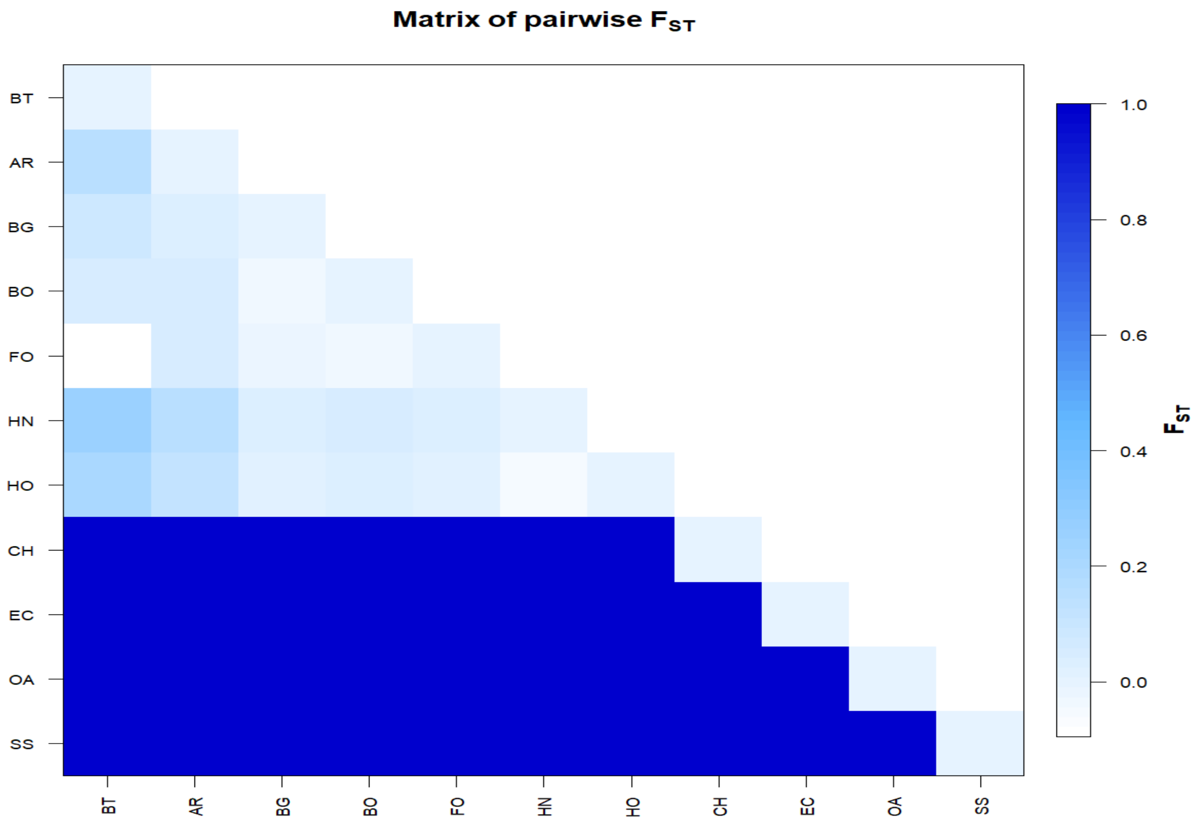


Figure 5.4 Demonstration of computed F_{ST} values between pairs of populations graph generated by R- function: pair F_{ST} matrix.r.

(BT= *Bostaurus*, AR= Arsi, BG = Begait, FO= Fogera, HN= Hanwoo, HO= Horro, CH= *Capra hircus*, EC= *Equuscaballus*, OA= *Ovis-aries* and SS= *Susscrofa*)

All identified SNPs showed greater expected heterozygosity (H_E) compared with observed heterozygosity (H_O). Howbeit, H_O was greater than H_E for SNP2 identified on the second exonic region. The degree of heterozygosity was low for all the SNPs identified in this study. Another interpretation would be the degree of homozygosis was greater for LEP gene of both exons. The H_E and H_O values (x and y) equivalent to SNP1 (LEP-73), SNP2 (LEP-143), SNP3 (LEP-399), SNP4 (LEP-411), and SNP5 (LEP-495) were (0.478 and 0.316), (0.286 and 0.310), (0.486 and 0.290), (0.216 and 0.140), and (0.289, 0.105) with the respective order. According to Botstein *et al*., 1980) the measured polymorphic information content (PIC) were classified in to three main groups; low (PIC< 0.250), moderate (PIC<0.500), and high (PIC >0.500). We identified low PIC for SNP2 (PIC= 0.245), SNP4 (PIC= 0.192), and SNP5 (PIC= 0.248) whereas moderate PIC was distinguished for SNP1 (PIC= 0.364), and SNP3 (PIC= 0.370). Thus, no high PIC detected in this study about LEP gene. The coefficient of inbreeding (F_{IS}) was recorded low for SNP2 (0.080) and the values of F_{IS} for SNP1, SNP3, SNP4, and SNP5 were 0.343, 0.408, 0.352, and 0.639 respectively (**Table 5.7**).

Table 5.7 Expected and observed heterozygosities (H_E and H_O), polymorphic information content (PIC) and coefficient of inbreeding (F_{IS}) for leptin SNPs.

Marker	H_E	H_O	PIC	F_{IS}
SNP1(LEP-73)	0.478	0.316	0.364	0.343
SNP2(LEP-143)	0.286	0.310	0.245	-0.080
SNP3(LEP-399)	0.486	0.290	0.370	0.408
SNP4(LEP-411)	0.216	0.140	0.192	0.352
SNP5(LEP-495)	0.289	0.105	0.248	0.639

Depending on the evidences concerning to 115 nucleotide sequences with an entire of 504 positions in the absolute dataset, evolutionary analysis was accompanied by MEGA-X version 10.1 (Kumar *et al*., 2018), as illustrated formerly. Tree produced by power marker designated that large sized breeds (Boran, Begait, Fogera, and Hanwoo) were persistently grouped as compared to the small sized breeds (Arsi and Horro). Relative to Arsi, Horro was grouped closely to Korean Hanwoo. Thus, Arsi was outgrouped from the remaining cattle breeds considered in this study (**Figure 5.5 [a]**). The haplotypes, mined from this work was compared to the reference sequences from *Bos taurus, Sus scrofa, Equus caballus, Ovis aries* and *Capra hircus* to trace the evolutionary history of the LEP gene (**Figure 5.5 [b]**). The reference *Bos taurus* sequence was assembled together with the haplotypes identified in the current study.

LEP gene sequences of cattle were distantly grouped vis-à-vis the other species considered in this study for comparison. The horse LEP gene sequence was relatively closure to that in sheep, and goat paralleled to the wild pig. Interestingly, the wild pig DNA sequences were comparatively closer to the cattle DNA sequences compared to that of horse, sheep, and goat.

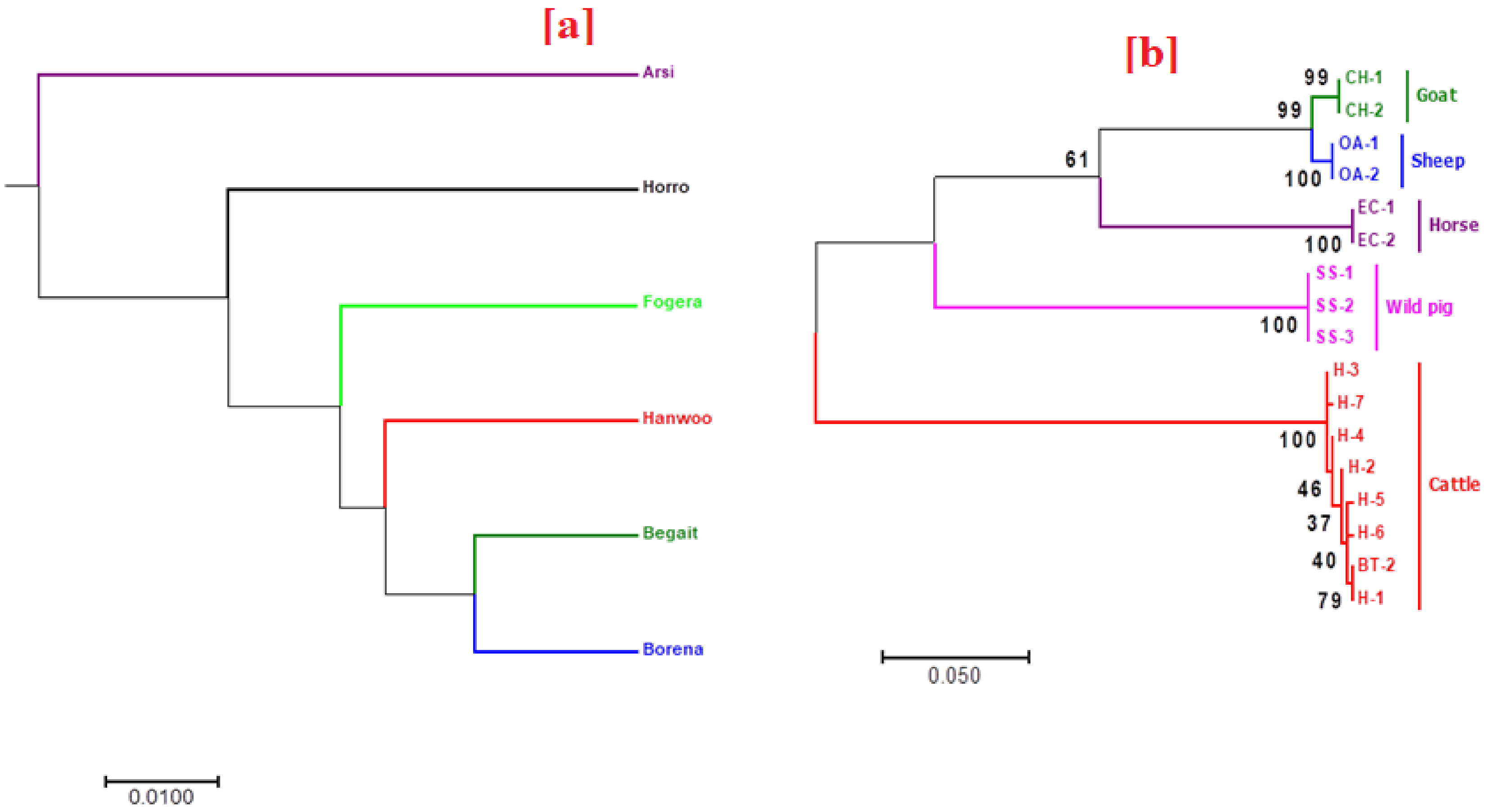


Figure 5.5 [a] UPGMA tree generated by power marker v 3.25 based on SNP_{LEP} data with friendly linked software MEGA7 **[b]** Molecular Phylogenetic analysis by Maximum Likelihood method that we compared haplotypes of cattle breeds with other species (H= Haplotype, BT= *Bos Taurus*, SS= *Susscrofa*, EC= *Equuscaballus*, OA= *Ovisaries* and CH= *Capra hircus*).

Furthermore, median joining network tree was constructed based on the haplotypes (**Figure 5.6**). The number of individual sequences in H-1, H-2, H-3, H-4, H-5, H-6 and H-7 were respectively 43, 51, 6, 5, 5, 3 and 1.

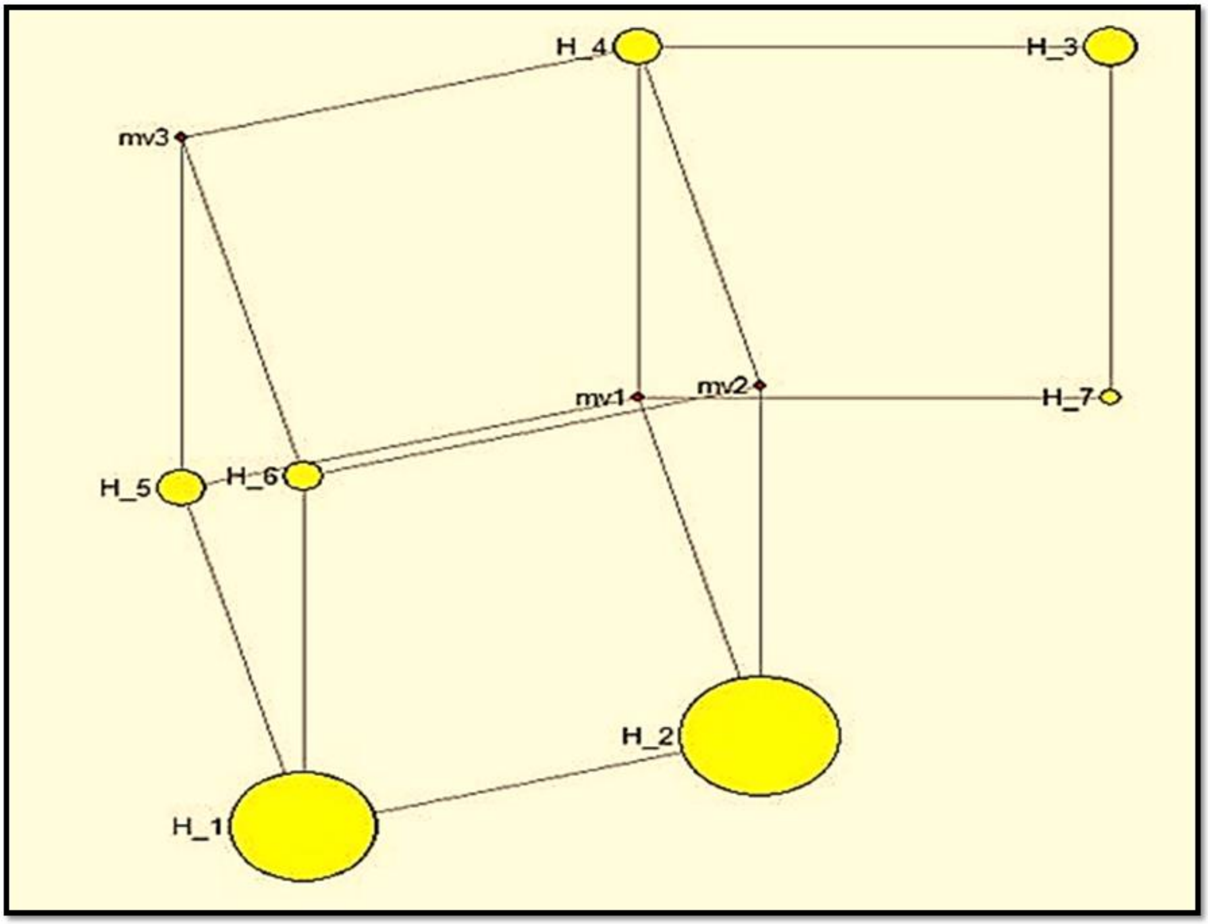


Figure 5.6 Median joining network constructed by network software version 10.0.0 and yellow circles represent the number of sequences that have sizes proportional to the frequencies. The branch length proportional to the mutation rate of the haplotypes whereas the red diamond represents the median vector.

5.4 Discussion

Numerous heritable markers have been extensively dominated to select cattle breeds of superior quality across the globe (Felius *et al*., 2015; Kiyici *et al*., 2018). Together with others, the LEP gene, managing the production traits and other economically useful characteristics of livestock has been advocated as an appropriate candidate gene (Yoon *et al*., 2005; Wylie, 2011; Symonds *et al.,* 2016; Fernandes *et al.,* 2020). In this background, this research was undertaken to investigate SNP in LEP exons 2 and 3 regions in five Ethiopian cattle breeds *vis-à-vis* the Korean Hanwoo breed.

In this study, five SNPs, two on exon2 region and three on exon3 region, were revealed. The two SNPs on exon2 (C73T and C143A), were grouped together with missense mutations of arginine to cysteine (R25C) and threonine to lysine (T48K), respectively. For C73T, allele occurrence of C was higher with record of an opposite fashion for T allele frequency in the Arsi breed paralleled to that in Korean Hanwoo and the remaining breeds of Ethiopian. The frequency of CC genotype was reported to be highest in Arsi and lowest in Fogera, whereas the opposite holds true for the frequency of the heterozygous CT genotype (highest in Fogera and lowest in Arsi). The frequency of TT genotype was higher in Horro and lower in Arsi. The occurrence of TT genotype at this locus was associated with higher carcass weight and intramuscular fat content (Kononoff *et al*., 2017). Formerly, TT genotype positively associated with sub-cutaneous fat content and marbling score compared to both the heterozygous CT and homozygous CC genotype (Nkrumah *et al*., 2005). Moreover, reports indicated that homozygous CC genotype was positively associated with sub-cutaneous fat content and marbling value in Korean Hanwoo (Shin and Chung, 2007).Thus, the low genotypic incidence of homozygous TT in Arsi might be related to their small body size and poor milking ability. In contrast, the other breeds are classified in the large groups and reared for meat and milk (**Table 5.5[a]**). In contrast, Pannier et al. (2009) found no relationship between intramuscular fat content and leptin SNP in Irish crossbred cattle. Likewise, Banos et al. (2008) described lack of association of the LEP gene with body energy balance traits. Howbeit, current booms demonstrate that the poisonous mutation of exon2 (R25C) determines the LEP gene function in the physiological activities of numerous mammalian species (Anugratama and Hartatik, 2020). In addition to the connotation of LEP gene with rate of growth, live weight, metabolic process, feed efficiency, and feeding interval, booms also validate its association with pathogene related traits, inflammation, calf mortality and longevity (Nkrumah *et al*., 2004; Leifers *et al*., 2005; Chebel *et al*., 2008; Brickel *et al*., 2010; Oikonomou *et al*., 2009; Szyda *et al*., 2011). However, the SNP (C143A) connected with the alteration from threonine to lysine (T48K) was detected only in this study on exon2 and no valid trait association has been detected so far. However, it is striking that the genotype incidence of AA was smaller in Arsi and higher in Hanwoo, Begait, Boran, Fogera and Horro (in chronological order). In the light of this observation, an association of meat quality, body size, and milk yield in cattle with genotype AA seem plausible; this necessitates extra research. In the jurisdiction of population genetics, Wright's fixation index, FIS denotes the inbreeding coefficient of an individual concerning the local sub-population while the FST describes the average inbreeding coefficient of sub-populations compared to the total population (Moravčíková *et al*., 2019). In the current research, positive FIS index values were verified for all the markers except SNP2 (C172A), evocative of deficient heterozygotes (Norezzine *et al*., 2019). Substantially enormous relationships between the analyzed cattle breeds, demonstrated by the FST values and low genetic differentiation were expressive of the origin of the population from a shared ancestor (Moravčíková *et al*., 2019). Sequence homology showed disentanglement of the mammalian LEP gene from cloning in fish and amphibians, visualization of LEP genes across virtually in all major vertebrate classes, catalyzed by the thought of gene synteny protection (Friedman *et al*., 2011).

The current efforts to realize to functional genomics modeling many genes such as leptin(LEP), endospanine (LEPROT), leptin receptor (LEPR) from both vertebrates and invertebrates value distinct references in the background of evolutionary findings of the LEP gene (Londraville *et al*., 2017). In agreement with previous results (Dubey *et al*., 2012), phylogenetic tree analysis showed that the cattle breeds considered in our study were relatively distant from goat, sheep, horse and wild pigs. At this point, the number of alleles identified at the LEP locus from different species overlooks special mention. Alleles identified in one species are more closely related to alleles in closely related species than to other alleles in the same species. Concepts of gene conversion and balancing selection can be invoked to justify the observations mentioned in the results section (Hiwatashi *et al*., 2011; Akumbugu and Zanwa, 2017). As presented in **Table 5.5 [a]**, Arsi breed is small in size compared to the other breeds considered in this study. Earlier research outputs on the evolutionary history of cattle LEP genes are evocative of autonomous assembling in ruminant species (Akumbugu and Zanwa 2017). Exertions to disclose the connection among the six cattle breeds via phylogenetic tree examination discovered that the large-sized breeds, Hanwoo, Begait, Boran, and Fogera, were more closely grouped compared to the small-sized Arsi breed. Based on the analysis of the median-joining network tree, haplotypes H-1 and H-2 with the utmost number of individual sequences could be supposed to be the ancestral haplotypes for the LEP gene. A superior in-depth evolutionary discrepancy revision would surely support molecular breeding programs and simplify the improvement of genetics to the indigenous cattle breeds.

6. General conclusion and future direction

In this paper, the breeding scheme of selected Ethiopian cattle breeds was evaluated and the selected candidate genes responsible for important economic traits were characterized. Different breeding options were simulated for two indigenous cattle breeds from Ethiopia considering a limited number of production and reproductive traits. The overall objective was to improve the production and reproductive performance of the breeds. With the exception of age at first calving, the genetic gain per year in yearling weight, milk yield, calving interval and survival to yearling resulting from the various options is satisfactory and may lead to reasonable genetic improvements in these cattle breeds. However, the realization of these predictions largely depends on the three platforms of a structured breeding program: performance recording, genetic evaluation and planned mating. Related management issues such as health and feed are also worth considering.

On the other hand, the sequence polymorphism of BoLA-DRB3 and leptin genes in cattle was studied to provide information for breeding programs. Thus, the population structure of selected Ethiopian cattle breeds was analyzed in comparison with Asian breeds with these selected genes. The diversity and polymorphism information of selected Ethiopian cattle breeds with Asian breeds were reported for the first time for MHC II BoLa-DRB3 gene. We prove that the divergence of DRB3 locus and the type of diversity and polymorphism could be very informative for population level related to immunity related traits in cattle. The current work confirms that the MHC II DRB3 gene is very crucial and highly polymorphically influenced for positive selection in cattle. Therefore, it is very important to improve our knowledge about the diversity and polymorphisms of BoLA-DRB3 gene by analyzing more Ethiopian breeds adapted to harsh environmental conditions. Consideration of genetic architecture and further validation of SNPs for associations with diseases should be considered for future to reveal the association of this locus with specific diseases in cattle. Therefore, this study will be very important for the next research interests in the field of MHC II molecule to design a breeding program in cattle.

Single nucleotide polymorphisms of the leptin gene indicated that a missense mutation identified on exon2 could be used as a marker to improve cattle production and productivity. This groundbreaking finding could be a guide for future scientists to consider the association between the LEP gene and economic traits of interest to human welbeing in the context of Ethiopian breeds. Further investigation of the association of the discovered SNPs at LEP loci with body weight, growth traits and meat traits, and milk yield in promising Ethiopian native cattle breeds will be carried out in the future by genotyping a larger number of animals.

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Appendices

Appendices I. List of Articles published under reputable journals

1. **Ayele Mandefro**, Gemeda Duguma, Tadele Mirkena and Hailu Dadi (2017). Evaluation of alternative breeding plans for two indigenous cattle breeds of Ethiopia. *Livestock science* (**205**); 122-128. https://doi.org/10.1016/j.livsci.2017.09.022
2. **Ayele M.**, Tesfaye S., Kim K-S., Zewdu E., Rocktotpal K., Hailu D. (2021). Single nucleotide polymorphisms of leptin gene in five Ethiopian indigenous cattle breeds and the Korean Hanwoo breed. Tropical Animal Health and Production. 53:202 https://doi.org/10.1007/s11250-021-02642-1.
3. **Ayele M.**, Tesfaye S., Zewdu E., Md. Rasel U., Kim K-S., Hailu D. (2021). Genetic assessment of BoLA-DRB3 polymorphisms by comparing Bangladesh, Ethiopian, and Korean Cattle. J Anim Sci Technol 63(2): https://doi.org/10.5187/jast.2021.e37

Appendices II. Considered Cattle Breeds in this study

[A] Arsi

[B] Ethiopian Boran

[C] Begait

[D] Fogera

[E] Horro

[F] Bangladesh Red Chittagong

[G] Korean Hanwoo

[H] Sheko

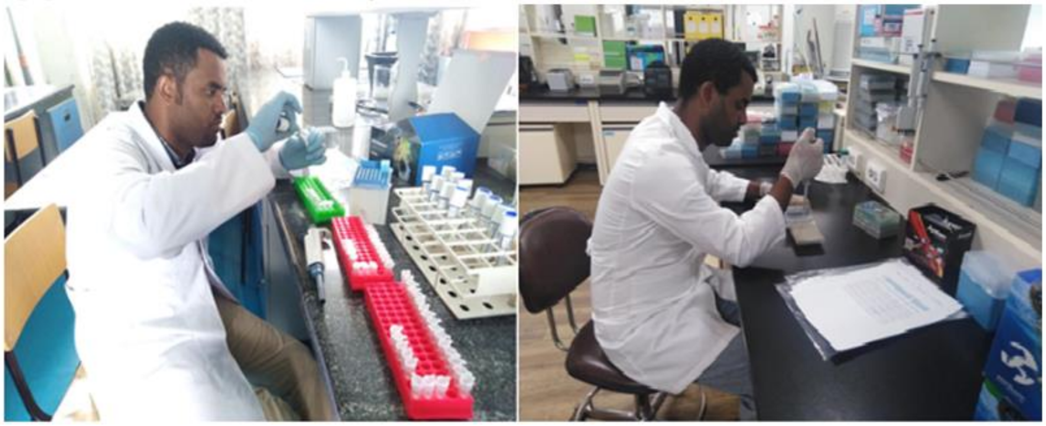
Appendices III. DNA extraction protocol from collected Nasal swabs by using Animal Swabs Collector (BlueGene Life Science, DNA Genotek Inc., Kanata, On, Canada)

1. The collected nasal swabs were vortexed gently for 3-5 minutes.
2. Nasal swab (450 µl) transferred to a sterile 1.5 ml micro-centrifuge tube and centrifuged at 13000 rpm for 3 minutes. The supernatant (400 µl) transferred into the fresh micro-centrifuge.
3. We added 300 µl isopropanol and vortexed for 20-30 seconds. The supernatant was transferred into the spin columns and centrifuged for 3 minutes at 13000 rpm. The liquid from the bottom cap was discarded and the spin columns recombined.
4. We added 700 µl washing buffer I and centrifuged at 13000 rpm for 2 minutes. Again carefully we discarded the liquid from the bottom cap and recombined the spin column.
5. We added 400 µl washing buffer II in to the spin column and heated for 10 minutes at 60 °C in water bath. After heated, the spin columns were centrifuged for 1 minute at 13000 rpm. The alcohols were completely removed before elution of DNA samples. The lower cap of the spin column was removed properly and the newly prepared sterile 1.5 ml micro-centrifuge was combined with the upper part of the spin column. Then 50 µl of elution buffer were added and centrifuged for 1 minute at 10000 rpm.

Appendices V. Field and Laboratory Activities

[A] Collection of Swab Samples

[B] DNA Extraction and Amplification



Declaration

I, the undersigned, announce that this PhD dissertation is my inventive work and has been submitted in partial fulfillment of the requirement for a PhD degree of Biotechnology at Addis Ababa University, Ethiopia. This thesis has not been submitted to other Universities anywhere for the reward of any degree. All sources of materials and supported institutions for this dissertation have been appropriately acknowledged.

Name: **Ayele Mandefro Ademe (ID No. GSR/7623/09)**

Place: Institute of Biotechnology, Addis Ababa University, Addis Ababa.

Signature: ________

Date: ______________

This thesis has been submitted for examination with my approval as a thesis advisor

Tesfaye Sisay (DVM, MSc, PhD, Assoc. prof.) _____________ _____________

Thesis Advisor Signature Date

Hailu Dadi (PhD, Assist. Prof.) __________________ ____________

Thesis advisor Signature Date