

Ethiopian Coffee (*Coffea arabica* L.) Germplasm Genetic Diversity: Implication in current research achievement and Breeding Program

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Abstract

Coffea arabica L. is the dominant coffee species in the world coffee production contributing over 60% of total production. Ethiopian 30% of foreign exchange income earned from Arabica coffee. Arabica coffee has unique aroma, flavor and low caffeine that make it highly demanding coffee species in coffee producing and consuming countries. As Ethiopia is home land for Arabica coffee very enormous genetic variability expected among coffee germplasm which is vital for further improvement to response the existing world's demand on Arabica coffee. Thus, this review conducted with the intention to assess the genetic diversity among Ethiopian Coffee (*Coffea arabica* L.) germplasm for the next breeding program and in the current research results achievement. Up to date, about 7,067 coffee accessions were collected by Jimma Agricultural Research Center; but, 15.33% were died due to enormous environmental factors. Those collected from different coffee growing areas are under utilization as germplasm in order to search for desirable traits and genetic improvement. Different methods like Morphological marker and molecular markers which include DNA markers and RNA markers are being under use for genetic diversity studies. From studies conducted in Ethiopia on coffee germplasm collected from forest coffee, semi forest and garden coffee production system across region, clearly indicated that the availability of coffee genetic diversity which is the primarily required for coffee genetic improvement. The current reviewed scholars authenticated that the existence of wider genetic diversity in Arabica coffee collection in terms of yield and other agronomical traits, diseases and insect pest resistance potential. Hence, up to date 7 hybrid and 35 pureline varieties, totally 42 coffee varieties released in Ethiopia for different coffee growing regions. The highest yield potential of pure line and hybrid variety is 2380Kg ha⁻¹ and 2680 Kg ha⁻¹ respectively; still, the average national coffee yield is 646Kg ha⁻¹; thus, further extension work and improvement for yield and disease resistance are lacking. The achievement of 60%-120% heterosis in yield, implies that the availability of great opportunity and experts to be confident in *Coffea arabica* L. genetic potential improvement for economic traits, diseases resistance and insect pest tolerance in Ethiopia specifically and in the World generally.

Key words: - Coffee arabica L., Diversity, Germplasm, Molecular marker and Morphological marker

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Introduction

Coffee is the second principal commodity in world mark after petroleum. It belongs to genus *coffea* which is Rubiaceae families, one of the largest flowering plant consisting 500 genera and 6000 species (Thiago *et al.*, 2019). Currently, around 140 coffee species belongs *coffea* (Couturon *et al.*, 2016). However, *Coffea arabica* L. and *Coffea canephora* Pierre ex A. (Robusta) are the only two dominant species providing over 60% and less than 40% of world's production respectively (Farah and dos Santos, 2015). Arabica coffee is a predominantly self-pollinating species and its home land is in southwestern highlands Ethiopia (Sérgio and Tumoru, 2012). *Coffea canephora* P. is a cross pollinating diploid species and is more widely dispersed in tropical Africa (Ky *et al.*, 2001; Leroy *et al.*, 2006).

Coffea arabica L. is the most important cash crop and it generates up to US\$ 14 billion annually for the producing countries. More than 80 countries, including Ethiopia cultivate coffee, which is exported as raw, roasted, or soluble product to more than 165 countries worldwide providing a livelihood for some 125 million people around the world (ICO, 2016). Ethiopia is the largest in Africa and the fifth largest in the world in Arabica coffee production.

The agriculture based Ethiopian economy is highly reliant on Arabica coffee. Up to date, it has been contributing more than 30 percent of the country's foreign exchange earnings. It also provides significant employment

opportunities in rural areas for one million house hold of people in Ethiopia (Davis *et al.*, 2012). Thus, in addition to being an important export crop, coffee plays a fundamental role in both cultural and socio-economic life of the country (Workafes and Kassu, 2000). *Coffea arabica* L. is characterized by its lower bitterness, lower caffeine and better flavor, sweeter taste with an aromatic fragrance than Robusta. Hence, it is more appreciated by consumers and is sold at distinctly higher price than Robusta (Leroy *et al.*, 2006).

Ethiopia is considered as the primary center of origin and diversification for coffee (*Coffea arabica* L) and high genetic variability is expected to exist for yield and components of yield, diseases and pest resistance and in addition to other agronomic traits (Melaku, 1982; Mesfin, 1980). In agreement with this Labouisse and Bellachew (2008) reported as Ethiopia holds a unique position in the world as *Coffea arabica* L. has its primary centre of diversity in the south-western highlands of the country. This fact is strongly authenticated by observations and publications of travelers and scientists (Vavilov, 1935; Meyer 1965) and, more recently, by several studies using DNA-based genetic markers, it is realized that Arabica coffee is highly diversified in Ethiopia (Anthony *et al.*, 2002; Tesfaye *et al.*, 2007).

Additionally, several authors like Tewolde (1990) reported that some truly wild coffee populations can still be found in a few remote pockets of mountain rainforest, mainly in the southwestern highlands, near Tepi, Gore (Illubabor), along Upper Didessa River (Wollega), and possibly in the Harenn Forest (Bale) in the south-east of the country (Tadesse and Nigatu 1996; Aga *et al.* 2003). These enormous proportions of coffee diversity existing in Ethiopia is being exploited as germplasm by gathering or picking, in more or less managed forests, or grown in highly diversified cropping systems spread over different types of environments. These collections have been used to assess the diversity of the Ethiopian coffee genepool by the analysis of phenotypic characters (Montagnon and Bouharmont, 1996; Dawit, 2018; Masreshaw, 2018) and using DNA-genetic markers (Silvestrini *et al.*, 2007), to search for traits of agronomic interest, and to improve yield and quality by hybridization with different cultivars (Anzueto *et al.*, 2001). Also, Dawit *et al.* (2020) reported the availability of genetic diversity among twenty six genotypes of wollega coffee landrace in western Ethiopia using organoleptic traits.

The existence of a varied range of Ethiopian origin coffees on the world market reflects that the existence of diversity (Labouisse and Bellachew, 2008). For several decades, importers in industrialized countries have applied a green coffee classification based on geographical provenance and quality control prior to export from Ethiopia. Nine different areas are recognized in international trade; namely Limu, Jimma, Gimbi, Sidamo, Yirgacheffe, Illubabor, Harar, Tepi and Bebeke (Jobin 1992).

Despite the vast area of cultivation, wealth of tremendous genetic diversity and importance to the national economy, the productivity of coffee per unit area remained very low with the average national yield 646 kg ha⁻¹ clean coffee (CSA, 2019). The major contributing factors for such low yield include the limited availability and adoption of improved coffee cultivars and lack of well characterized and distinctly variable breeding materials that are eagerly available for breeding program in addition to lack of high yielder hybrid varieties in most coffee producing areas except south western Ethiopia.

All genetic resource conservation activities require characterization of the diversity present in both the gene pools and the gene banks (Karp *et al.*, 1997). Thus assessment of the genetic diversity in *Coffea Arabica* L. is crucial for developing conservation strategies for this economically important crop species. For this reason, collection, evaluation of the genetic diversity and available desirable traits within the genus *Coffea* is an important step in current research results achievement and for the next coffee breeding program (Cubry *et al.*, 2008). Therefore, the intention of this Review is to understand the existence of genetic diversity among Ethiopian Coffee (*Coffea arabica* L.) germplasm for the next breeding program and its role in the current research results achievements.

General Concepts of Genetic Diversity

Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. It is distinguished from genetic variability which describes the tendency of genetic characteristics to vary. Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals <http://www.wow.com/wiki/>.

Crop diversity is the variance in genetic and phenotypic characteristics of plants used in agriculture. In addition, diversity of a harvested plant can be the result of genetic differences which collectively determine a crop's overall characteristics and their future potential. Diversity within a crop includes genetically-influenced attributes such as seed size, branching pattern (growth habit like very open, open, mid-compact and compact),

height, flower color, fruiting time, and flavor from which current plant breeders develop new crop varieties to meet specific condition like response to climatic condition, high yielding, more disease and insect pest resistant and better quality than the varieties from which they were bred <https://en.wikipedia.org/wiki/>. Specifically when we consider coffee genetic diversity, World Arabica coffee production is largely based on using a very small number of cultivars which resulted from few introduced coffee trees from center of origin to different parts of world coffee producing countries. This shows that low availability of coffee genetic diversity observed within those cultivars which make this crop particularly vulnerable to biotic and climatic hazards. However; the largest proportion of coffee diversity existing in Ethiopia is being exploited by gathering or picking, in more or less managed forests, or grown in highly diversified cropping systems spread over different types of environments (Labouisse *et al.*, 2008). In the traditional production system Ethiopian coffee tree exhibit large phenotypic diversity as it has been observed by different Travelers and scientists. Recently different experimental results indicates that the presence of Coffee genetic diversity in this country. Thus; within Ethiopian planting material, differences in level of genetic diversity are found according to the cropping systems from which the coffee plants were collected (Anthony *et al.*, 2001).

Center of Origin and Distribution of *Coffea arabica* L.

There are numerous evidences for the belief that Ethiopia is the original home of *Coffea arabica* L., which is confirmed by the fact that within small areas four or more genetically different types of coffee trees can be found. Thus, *Coffea ethiopica* would have been a more correct name than *Coffea arabica* (Strengé, 1956). However, there are some believe that enable to give the name *Coffea arabica* than *Coffea ethiopica*. The first belief is that this plant was of Arabic origin was due to the fact that the first knowledge of the beverage and the tree was obtained from Arabia; hence the scientific name given by Linnaeus (Sylvain, 1958). Even though Yemen was almost the sole source of coffee germplasm over most of the recorded history of Arabica coffee, yet there is no evidence to suggest that it is native to Yemen (Esayas, 2005). The time when coffee was introduced into Yemen from Ethiopia is mysterious. Until now except Ethiopia no other countries of the world seems to be associated with the history of the Arabica coffee plant although the closest relatives of *Coffea arabica* L. are absolutely tropical African (Meyer *et al.*, 1968). The *Coffea arabica* L. plant is a rain forest species with a tolerance to grow in a wide range of climatic and ecological conditions. It has been cultivated in Yemen and spread to South East Asia and Brazil and to Latin America from Amsterdam and Paris in 18th century (Harer, 1956; Carvalho *et al.*, 1969.)

All botanists who had explored the forests in southwestern highlands of Ethiopia agreed in their observation that this area is the center of diversity of Arabica coffee (Strengé, 1956; Meyer, 1965). On the other hand, Thomas (1942) found wild populations of Arabica coffee in secondary forest on the Boma plateau in southeastern Sudan. Berthaud & Charrier (1988) also reported the presence of *Coffea arabica* L. populations on Mount Imantong in Sudan and Mount Marsabit in Kenya. It is not clear whether these Arabica coffee trees are really wild or in earlier times taken from Ethiopia by man.

Cultivation of Coffee (*Coffea arabica* L.) in Ethiopia

Coffea arabica L. is the only the genus *Coffea* originated in Ethiopia and it grows in wide ecological ranges (500m-2200m) which provide its existence in diverse forms and wide scope for its improvement in term of genetic plus ecological adaptation. Arabica coffee grows in many parts of Ethiopia. However; main cultivation is limited to the southern, southwestern and western regions. Coffee is generally grown on deep reddish-brown clay soils and slightly acidic with pH values from 4.5 to 6 (Van der Graaf, 1981). In Ethiopia, coffee production under four different systems: 1) Forest coffee which is sometimes referred to as "wild" coffee. This accounts for 5% of coffee production in which self sown seedlings have been transplanted erratically in the forest. 2) Semi forest is coffee plants in which seedlings raised in nurseries are planted more or less regularly in thinned forest. It accounts about 35% of total coffee production. 3) Garden coffee accounts for 50% of coffee production, and is plots of varying sizes around private residence. 4) Plantation coffee is established on previously cleared land in which seedlings are raised in nurseries and regularly planted together with shade plants .This accounts 10% of total coffee production (Petty *et al.*, 2004; Labouisse *et al.*, 2008).

Generally, the coffee production per hector increase as we go from forest coffee production system to plantation production system. Hence, the average yields 200kg ha⁻¹ for forest and 400kg ha⁻¹ semi-forest. For garden coffee range from 200 –700kg ha⁻¹, it is highly variable depending up on agricultural practice. Range from 450-750 kg ha⁻¹ for plantation but, it is reach up to 1,700kg ha⁻¹ for selected lines under optimum coffee growing condition (Woldetsadik and Kebede, 2000; Bellachew and Labouisse, 2007; Labouisse *et al.*, 2008) . Thus, the average yield obtained per hector from plantation production system is extremely better than other production system, but in genetic diversity encompass forest production system is followed by semi-forest, garden and plantation production system respectively.

Major Coffee Species

Around 140 species of the genus *Coffea* have been identified up to date, but commercial production relies only on two species, *Coffea arabica* L. and *Coffea canephora*, which represent about over 60% and less 40% of the total coffee market, respectively (Bridson & Verdcourt, 1988; Farah and dos Santos, 2015). Arabica coffee is the only allotetraploid ($2n = 4x = 44$), formed from natural hybridization between *C. canephora* and *C. eugenoides* (Lashermes *et al.*, 1999). In line with this, Coulibaly *et al.* (2002) reported that *C. arabica* is the only allopolyploid ($2n = 4x = 44$) coffee species and self-fertile at approximately 90%; also its out cross capability can extended up to 40%. The other *Coffea* species are diploid ($2n = 2x = 22$) and self-sterile except for *C. heterocalyx* and *C. Moloundou*, which are diploid but self-fertile. The allotetraploid nature and self-fertilization of *C. arabica* probably contribute to its relatively low genetic diversity compared to diploid *Coffea* species (Lashermes *et al.*, 2000).

Coffea arabica L. do best at higher altitude where the slower growing process concentrates their flavor. As it is susceptible to disease, frost, and drought, it requires very careful cultivation with the right climatic conditions (Lashermes *et al.*, 1993). In contrast, *C. canephora* grows at low elevation and warmer condition. Its cup quality is generally regarded as inferior when compared with that of *C. arabica*. However, *C. canephora* is more resistant to adverse conditions than *C. arabica*, particularly to several diseases and insect pests.

Another diploid coffee species originating from Mozambique, *C. racemosa*, is characterized as having low caffeine content, high drought tolerance and resistance to coffee leaf-miner (*Leucoptera coffeella*) (Clarke *et al.*, 1983), and has been used in breeding programs for introgression of important agronomic traits to *C. Arabica* (Guerreiro Filho *et al.*, 1991). *Coffea Liberica* (from Western Africa) is the third commercial species but has no great importance in coffee trade.

Coffee Genetic Biodiversity

The spread of coffee all over the world was based on seeds from a single tree or a few trees introduced to Yemen, thus, cultivated coffee varieties have a very narrow genetic base. In line with this Mendes and Guimarães (1998) and Bayisa (2015) indicated that many cultivars of *C. arabica* have been developed for Yemen and Brazil depend up on the narrow genetic basis of the species and phenotype differences among them are mainly due to gene mutations. The best hope for crop improvement lies in the progenitors or wild relatives of the cultivated plants that harbor rich genetic resources for tolerance against abiotic (drought, cold, heat, salt and solar radiation), and biotic (pathogens, parasites and competitors) stresses (Nevo, 1998; Schoen and Brown, 1993). In line with this, the Ethiopian Arabica coffee gene pool represents the most important and diversified gene pool of this species found in different countries. The natural genetic diversity or gene pool of economic plants has three distinct categories, namely: a) the primitive cultivars or landraces of traditional agriculture, b) the advanced cultivars produced by plant breeders in the last 100 years, and c) the wild or weedy species related to domesticated cultivars (Teketay and Tigneh, 1994).

Ethiopia is well noted as centre of origin and diversity of many domesticated crops including Arabica coffee. It possesses all three categories of the gene pool for *C. arabica* (Tewolde, 1990). Seyoum *et al.* (2004) reported the existence of a great variation among the coffee plants in Ethiopia; also Dawit (2018) reported the availability of genetic diversity among some coffee accessions collected from western Ethiopia.

Diversity in Wild Coffee

Wild coffee which was sometimes referred to as "forest" coffee accounts for 60% of coffee production in which self sown seedlings have been transplanted irregularly in the forest (Van der Graaff, 1981). In opposite to this, Petty *et al.* (2004) reported that in Ethiopia, from four major coffee production systems: forest account about 5%, plantation about 10% and semi-forest and garden account about 35 % and 50% respectively. This indicates that forest coffee production system decrease from time to time due to land covered by forest used for different purpose. The variability in natural populations of *C. arabica* has been clear to most botanists and geneticists, who visited and explored the southwestern highlands of Ethiopia. Morphological variability exists within locations (Dawit, 2018) and between regions. For instance, the ratio of trees with different leaf tip colour (green or bronze) varies between locations (Esayas, 2005); also higher level of genetic variability with molecular markers was observed among spontaneous and sub spontaneous accessions of this species collected from Ethiopia (Lashermes *et al.*, 1996; Anthony *et al.*, 2001).

The wild populations of *C. arabica* in the mountain rainforests are the most important gene pool of the crop. Tesfaye (2006) reported high genetic variability within and between different wild populations in Ethiopia. He further noted that wild coffee plants are genetically distinct. Wild Arabica coffee is a unique potential source of genetic diversity for selection and breeding for enhance arabica cultivars, including varieties with low caffeine content, increase yields, or increase resistance to pests and pathogens such as coffee berry disease (CBD, caused

by *Collectotrichum Kahawae*), coffee rust (caused by *Hemileia vastatrix*), *Meloidogyne* root nematodes and the coffee berry borer (*Hypothenemus hampei*) (Hein and Gatzweiler, 2006; Silvestrini *et al.*, 2007; Dessalegn *et al.*, 2008; Boisseau *et al.*, 2009).

Diversity in Cultivated Coffee

Surveys in major coffee growing regions of the country showed that there is a high diversity of coffee landraces. In garden coffee systems and other cultivated coffee production systems, farmers choose the coffee types of their preferences and often produce mixing more than one landrace. Some farmers plant up to five landraces in their garden. Each has its own compensation. Some are high yielder, some have good aroma and flavor and some are resistant to diseases and insect pest (Tadesse, 2015). Farmers identify their traditional coffee landraces by color of leaves, gross morphology of trees, weight and shape of fruits and beans, presence or absence of aroma during roasting of beans, etc. They give names to the landraces based on the different attributes of the landrace (Teketay and Tigneh, 1994; Gole *et al.*, 2001).

In Ethiopia, there are different coffee types recognized by their origin and quality, and used as trade names. These include Bebek which has medium-to-bold bean and known for its fruity taste, Harar has mocha flavor, Jimma/limmu has heavy bodied cup with winy taste, Lekempti/Wollega known for its large bean size, and fruity flavor after taste, Sidama and Yirgacheffe has spice characteristics, Tepi Low acidity but better body than Bebek (Boot, 2011; Desse, 2008)

Methods of Detecting Genetic Diversity

Morphological Marker

Morphological characteristics are among the earliest genetic markers used for assessment of variation and still have great importance. Usually, these characters are inexpensive and straightforward to score. The sharing of physical features is also often accepted as an indication of relatedness (Esayas, 2005). There are several sets of physical character assessment for different crops at different developmental stages such as seed, juvenile, adult vegetative, flower and fruit. Similarly, Morphological characteristic are a conventional method to distinguish variation based on the observation of the external morphological differences such as the size and shape of the leaf and plant form, the color of the shoot tip, the characteristics of the fruit, the angle of branching and the length of the internodes (De Vienne *et al.*, 2003).

Several authors substantiated the possibility to determine genetic diversity using morphological characteristics. Kebede and Bellachew (2008) reported the existence of genetic diversity among 49 hararge coffee germplasm accessions by studying on 14 quantitative characteristics. In agree with this Seyoum *et al.* (2004) reported the presence of genetic diversity by studying on 12 quantitative characteristics of 81 coffee accession collected from coffee growing regions of Kulo, Sidamo, Wollo, Harar, Maji, Wollega, Illubabour, Kafa and Gambella at seedling stage. Olika *et al.* (2011) and Dawit (2018) reported similar results using 22 and 23 quantitative characteristics of 49 limmu and 26 Wollega coffee accessions respectively at productive age.

Molecular Markers

Molecular markers have been using for precise than traditional morphological and agronomic characterization, since they are virtually unlimited, cover the whole genome, are not influenced by the environment, and less time consuming (Esayas, 2005). Therefore, application of molecular marker to diversity questions need to take into account whether or not data derived from a technique provide the right type of information for answering the question being addressed through morphological marker (Karp *et al.*, 1997). This in turn depends on the taxonomic levels of the material being studied (different species, subspecies, populations, cultivars and individuals). The closer the relationship of the materials to be studied, the more necessary it may be to consider highly discriminatory techniques. Thus; molecular marker used to cluster the coffee genotypes according to their similarity in the same group and classify in different clusters based on their genetic dissimilarity (Figure 1). Cluster-I consists of the Welega and Ilubabor populations, which were further grouped into two sub-clusters and an outlier (Welega-II population) at 74 % resemblance. Cluster-II encompasses of the Jima and Bale populations, which were further differentiated on the basis of their zone of sample collection sites at 75 % similarity. In agreement with this, Alemayehu *et al.* (2010) grouped arabica coffee accessions in to two clear cluster indicating wide genetic variation in Ethiopian accessions; genetically distant from each other and from their cultivated relatives.

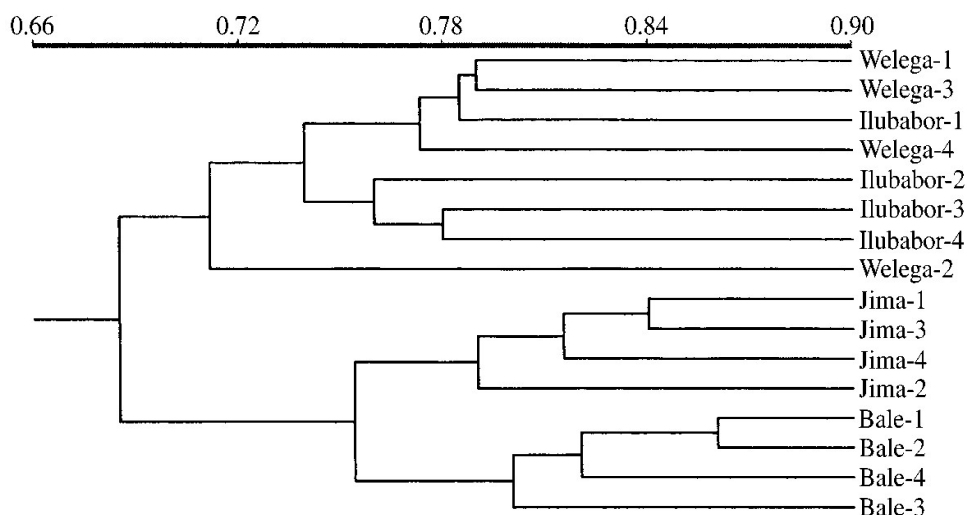


Figure 1. Dendrogram generated by UPGMA cluster analysis on genetic diversity of the 16 forest Arabica coffee populations (Aga *et al.*, 2003)

When all populations were considered together (H_{sp}), variation 0.46 ± 0.04 was recorded (Table 1). The genetic variation within and between populations revealed that within population variation accounted for 65% and the remaining 35% occurred between populations. Also, 80% of variation revealed among population in the same zone which is greater when compared with the existing variation between populations (20%) of different zones. This pointed that it is not obligatory to go across different coffee growing regions to find genetically and morphology divergent coffee accessions in Ethiopia. Similarly, Meyer *et al.* (1995) and Anthony *et al.* (2001) reported analogous level of polymorphisms diversity among coffee (*Coffea arabica* L.) accessions derived from spontaneous and sub spontaneous population of coffee in Ethiopia. Also, in line with the current results, Ayana *et al.* (2000) reported that higher values of genetic variation within zones of sample collection sites than between zones conducted experiment on self pollinated crop of both cultivated sorghum (*Sorghum bicolor* (L.) and wild sorghum (*Sorghum bicolor ssp. eriticilliforum* (L.)).

Table1. The genetic dissimilarity within and between populations and within and between zones of sample collection (Aga *et al.*, 2003)

Categories	Parameter	Mean	S.E	S.D
Population	Hpop	0.30	0.04	0.12
	Hsp	0.46	0.04	0.13
	Hpop/Hsp	0.65	0.04	0.13
	(Hsp-Hpop)/Hsp	0.35	0.04	0.13
Zone	Hzone	0.37	0.04	0.12
	Hzone/Hsp	0.80	0.04	0.12
	(Hsp-Hzone)/Hsp	0.20	0.04	0.12

H_{pop} and H_{zone} = Mean genetic variation for the populations and zones, respectively. H_{sp} = Mean for genetic variation computed from the entire data, when individuals of all populations were considered together. H_{pop}/H_{sp} and H_{zone}/H_{sp} = Proportion of genetic variation within populations and zones, respectively. $(H_{sp} - H_{pop})/H_{sp}$ and $(H_{sp} - H_{zone})/H_{sp}$ = Proportion of genetic variation between populations and zones, respectively.

DNA-based Molecular Markers

DNA-based markers have been used for studying genetic diversity in many plant species. This type of marker, besides make possible the analysis of variation present in DNA itself, can also be used for variety identification. In addition, they are environmentally independent, and may be detected in any type of tissue and developmental phase of the plant (Arens *et al.*, 1995; Ferreira and Grattapaglia, 1998).

Plants DNA polymorphisms assay are powerful tools for distinguishing and examining germplasm resources and genetic relatedness (Powell *et al.*, 1996). These include sequencing of a known region of a genome; using non-PCR-based DNA markers such as restriction fragment length polymorphisms (RFLP) and PCR based DNA markers. In coffee, DNA-based molecular marker technology has previously been implemented in germplasm characterization and management, detecting genetically divergent breeding subpopulations (for example to predict hybrid vigour), establishing gene introgression from related species and molecular marker-assisted selection (Lashermes *et al.*, 1996).

In agreement with this, Esayas(2005) studied on coffee germplasm collected from southwest regions (Welega, Ilubabor, Jimma, Kafa and Bench Maji) and southeast (Bale) region of Ethiopia taking DNA sample from each genotype at seedling stage and able to corroborate the distribution of genetic variation in the populations, using Nei's (1973) gene diversity statistics(Table 2). The results indicated that most of the variation is found among populations. This observation corresponds well to the genetic structure of principally self-pollinating populations of a species, which are characterized by a relatively high value of total gene diversity (H_T), a low value of gene diversity within populations (H_S), high value of gene diversity among populations (D_{ST}) and a high value of the coefficient of gene differentiation (G_{ST}). Self-pollinating species maintain high genetic diversity at their polymorphic loci, and most of this variation is found among populations. The results of this study confirmed with the hypothesis that *C. arabica* is mainly a self-pollinating species with most of its variation exist between populations. Comparable pattern of genetic differentiation were reported for other self-pollinating species (*Elymus fibrosis*) (Díaz *et al.*, 2000). Additionally, Alemayehu *et al.* (2010) conducted experiment on 133 coffee accessions collected from Wollega, Ilubabor, Kaffa and Jimma areas using 32 microsatellite (SSRs) markers and authenticated the existence genetic variability among these accessions.

Table 2. Results of the Nei's (1973) genetic diversity statistics of forest coffee samples analysed with the four different markers.

Marker type	H_T	H_S	D_{ST}	G_{ST}
RAPD	0.28	0.12	0.16	0.57
ISTR	0.31	0.14	0.17	0.55
ISSR	0.29	0.09	0.19	0.68
SSR	0.25	0.08	0.17	0.68

H_T = total gene diversity; H_S = gene diversity within populations; D_{ST} = gene diversity among populations; and G_{ST} = coefficient of gene differentiation.

Source: - Esayas, 2005

Coffee Genetic Resource Collection in Africa

The interest of coffee genetic resource collection increased during the second half of 20th century as breeders became conscious of coffee natural habitat deforestation which causes coffee genetic erosion. It was anticipated that the high forest in Ethiopia had declined to only 18% by 1997, which represent a loss of 60% in less than 30 years (Gole *et al.* 2002). Considering the socio-economic momentous of *C. arabica* cultivation, two large survey were conducted in Ethiopia by FAO from 1964-1966 (Fernie *et al.*, 1968) and ORSTOM (now IRD) in 1966 (Guillaumet and Halle, 1978). Hence, 690 coffee accessions (*C. arabica*) were collected during 1964-1966 from different coffee growing areas of south western Ethiopia. Also, in collaboration with other collecting missions like IPGRI (International Plant Genetic Resource Institute), IBPGR (International Board for Plant Genetic Resources) and CIRAD (Centre de coopération internationale en recherche agronomique pour le développement) the organizations had collected totally more than 11,753 coffee accessions (different coffee species) up to 1989 from seven different countries of African coffee growing and some from Yemen. Generally, the State of the World's Plant Genetic Resources for Food and Agriculture (FAO, 1998) reported 21, 087 coffee accessions conserved worldwide. Recently, Dulloo *et al.* (2009) reported 41,915 coffee accessions in field gene bank collections worldwide. Field gene banks that hold significant *C. arabica* collections are located in Africa (Cameroon, Cote d'Ivoire, Ethiopia, Kenya, and Tanzania), Madagascar, India, and the Americas (Brazil, Colombia, and Costa Rica) (Engelmann *et al.*, 2007).

Coffee Genetic Enhancement Method Applied in Ethiopia

In addition to broadening the genetic base of established crops, genetic enhancement and plant breeding in the sense of final cultivar development can be used to develop new variety from uncultivated species and to change old cultivar into new variety. Among kinds of genetic enhancement methods employed Hybridization, selection, back crossing and new biotechnology techniques are popularly known (Duvick, 1990). Hence, there are many methods under use for coffee genetic enhancement like other crop. However, selection and hybridization are the

most widely used in Ethiopia. Hybridization method carried out especially for yield potential improvement using parental lines having major disease resistance gene background including acceptable quality traits and other agronomic characteristics. Coffee collection has been implemented for yield, other agronomic traits, quality, disease and insect pest resistance genetic enhancement.

Coffee Germplasm Conservation in Ethiopia and Its role in next breeding program

Ethiopia holds a distinctive position in the world as it is a primary center of origin for *Coffea arabica* L. that are found plenty in the south-western highlands of the country. The largest proportion of coffee diversity existing in Ethiopia is being exploited by collection, in more or less managed forests, or grown in highly diversified cropping systems spread over different coffee ecologies (Labouisse *et al.*, 2008). To solve world low genetic base of arabica coffee production, collections were undertaken in Ethiopia from the beginning of the 20th century (Sylvain, 1958; Meyer, 1965), which led to the establishment of valuable gene banks at several international research centers in Africa (Cameroon, Côte d'Ivoire, Ethiopia, Kenya, Madagascar, and Tanzania), America (Brazil, Costa Rica, and Colombia), and Asia (India and Indonesia) (Anthony *et al.* 2007). The first collection of Ethiopian coffee germplasm started by Sylvain from 1954-1956 to solve the narrow genetic base problem in *C. arabica* var. *typica* and *C. arabica* var. *bourbon* which were produced widely in the world especially during the early coffee production (Sylvain, 1955), but the collection was not conserved. The most widely documented collections were those carried out under the guidance of the FAO in 1964–1965 (Meyer *et al.*, 1968) and by ORSTOM2 in 1966 (Guillaumet and Halle, 1967). These collections have been used to assess the diversity of the Ethiopian coffee gene pool by the analysis of phenotypic characters (Charrier, 1978) or using molecular breeding method like DNA –genetic marker (Anthony *et al.*, 2001) to search for desirable agronomic traits and to improve yield and quality as per customers interest. However, the collection carried out from 1964-1965 were left without planting in the field in Ethiopia due to the absence of the Institute or Organization like JARC which can take over the mandate for ex situ and/or ex situ conservation during that time, but this problem had been solved after the establishment of JARC in 1966.

In Ethiopia, conservation of coffee genetic resources mandate is given to the Institute of Biodiversity Conservation (IBC) and Jimma Agricultural Research Centre (JARC) which latter being responsible for coordinating coffee research within the Ethiopian Institute of Agricultural Research (EIAR). In Ethiopia 5,196 coffee accessions conserved by IBC (Gole *et al.*, 2002), and including collection carried out at international and national level 2,691 accessions were conserved by JARC from 1966 to 1990 means over 20 years ((Labouisse *et al.*, 2008); from 1994 to 2019 around 4,376 indigenous accessions. Hence, in five decades a total of 7,067 coffee accessions had been collected and conserved by JARC at Melko and different implementing centers and sub-centers (Table 3). From time of coffee germplasm collection very large indigenous coffee accessions collected from 1994-2019 collection years. However, out of total coffee accessions collected by JARC 15.33% were died by different environmental factors. Thus, In Ethiopia over 12,263 coffee accessions had collected and 11,192 conserved up to date.

The huge coffee accessions conserved at JARC and its implementing sub-centers possess different desirable traits. They possess different in yield ability, quality traits (bean size, organoleptic and biochemical traits, leaf color and other quality traits), growth habit (very open, open, mid-open, very compact, compact and mid-compact), desirable agronomic traits, disease reaction, insect pest and abiotic factors tolerance. These important germplasm conserved in ex situ for genetic improvement of economically valued traits in which breeder is interested. Generally, the diverse coffee germplasm conserved are our momentous assets that play imperative role in every coffee genetic improvement in the future coffee breeding program.

Table 3. Summary of indigenous and exotic coffee collections

Type of Collection	Year of Collection	No of Collected accession (Original)	No. of Alive accession	Number of Lost accession (%)	Remark
National	1966-1990	1633	1431	12.37	Indigenous collection
Exotic Coffee Collection	1968-1984	190	78	58.95	Exotic collection
CBD Resistant selection program	1973-1987	868	825	4.95	Indigenous collection
Local Landrace Collection program	1994-2019	4376	3662	16.32	Indigenous collection
Total		7067	5996	15.15	

Current Coffee Research Achievement in Ethiopia

Variety Development via Selection

JARC Established four sub centers and two trial sites staff in different coffee growing regions to conduct strong coffee research and develop coffee varieties for each coffee growing region in order to avoid quality blending effect. Hence, as a consequence of long time research effort accomplished in collaboration with these sub centers and trial sites, JARC able to released 35 pure line varieties via selection for diverse coffee growing regions from its establishment in 1967 to 2019 using long term, modified long term and crash variety development programs (Table 4).

Table 4. Lists of released pure line coffee varieties yield potential and recommended Altitude for production in Ethiopia

No.	Varieties	Yield potential (Kg/ha ⁻¹)	Recommended alt. m a.s.l	Remark
1	741	1220	1550-2100	Released for south western Ethiopian coffee growing areas. Some varieties like Desu, 7440, 7454 and 74110 are under production across coffee growing regions; hence, known for their wider ecologies adaptation.
2	744	1660	1550-2100	
3	7440	1620	1000-1550	
4	7454	1830	1000-1550	
5	7487	2380	1550-2100	
6	74110	1910	1550-2100	
7	74112	1810	1550-2100	
8	74140	1970	1550-2100	
9	74148	1800	1550-2100	
10	74158	1910	1550-2100	
11	74165	1730	1550-2100	
12	754	1480	1550-2100	
13	75227	1790	1550-2100	
14	Desu	1820	1540-1750	
15	Catimo J-19	1660	1000-1750	
16	Catimor J-21	1940	1000-1550	
17	Mioftu (F-35)	2140	1550-1750	
18	Gesha	2340	1000-1550	
19	Merda chareko	1540	1750-2100	
20	Bino washi	2350	1750-2100	
21	Yachi(7576)	1900	1750-2100	
22	Wushwush(7514)	1640	1750-2100	
23	Angafa(1377)	2040	1550-1750	
24	Limu-01	19	150-170	Recently released for Limu SW Ethiopia
25	Haru-1	1570	1550-1950	Released for Wollega coffee growing areas (Western Ethiopia)
26	Chala	1560	1550-1950	
27	Sinde	1610	1200-1550	
28	Mena sibu	1640	1200-1550	
29	Harus	1600	1550-1750	Released for Hara coffee growing areas (Eastern Ethiopia)
30	Moka	1350	1550-1750	
31	Mechar-1	1190	1650-1850	
32	Bultum	1700	1650-1850	
33	Feyate	1950	1500-1750	Released for Sidama coffee growing areas (Southern Ethiopia)
34	Odicha	2020	1750-1950	
35	Koti	2120	1750-1950	

Hybrid Variety Development

In Ethiopia coffee hybridization program was started in 1978 by crossing coffee line with the objective of increasing coffee yield (Mesfin and Bayetta, 1983). The diversity in *C. arabica* result important heterosis in yield and other desirable traits; heterosis calculated on the basis of the best parent was evaluated from crosses between

different gene pools. The accessibility of high level of heterosis in crosses among elite indigenous coffee (*Coffea arabica* L.) cultivars has been well determined in Ethiopia as well. This was noted from different set of crosses that exhibited better parent heterosis ranging from 60% to 120% for yield (Mesfin and Bayetta, 1983; Bayetta, 2001). As a result of series efforts on hybridization investigational work have been conducted, yet 7 high yielder having acceptable quality and disease resistance reaction hybrids coffee (*Coffea arabica* L.) varieties was successfully released by JARC in Ethiopia (Table 5). Those released hybrids coffee varieties yield potential range from 2300-2680Kg ha⁻¹ clean coffee (Bayetta *et al.*, 1998 and Ashenafi *et al.*, 219). These are released for low land, mid land and highland of south western Ethiopian coffee growing areas. However, the adoptability trail of these released hybrid variety for wider Ethiopian coffee growing regions such as Western, Easter, and Northern without coffee quality blending effect is lacking.

Table 5. Released hybrid coffee varieties clean yield potential (Kg ha⁻¹) and recommended Altitude in Ethiopian coffee growing areas.

No.	Varieties	Yield (Kg ha ⁻¹)	Released year	Recommended Alt. m.a.s.l
1	Gawe	2610	2002	1550-1750
2	Aba buna	2380	1998	1000-1750
3	Melko-CH2	2400	1998	1000-1750
4	EIAR50/CH	2680	2016	1200-1750
5	Melko-Ibsitu	2400	2016	1200-1750
6	TepiHC5	2340	2016	1200-1750
7	Gera CH1	2300	2018	>1750 (high land)

Source: Ashenafi *et al.*, 2019 and EIAR/JARC, 2004

Conclusion and Future Directions.

Coffee (*Coffea arabica* L.) is the most important cash crop and its beverages enjoyed throughout the world. Many countries including Ethiopia produce coffee and export it to different countries in the world. The livelihoods of one million house hold in Ethiopia depend on this crop production. Accordingly, Ethiopian economy more than 30% depends on coffee production.

Different authors are substantiated that Ethiopia is the primary center of origin and diversification for Arabica coffee (*Coffea arabica* L) and later distribute to different countries. High genetic variability in yield and components of yield, diseases and pest resistance and other traits are elucidated using variability analysis methods. Also, Ethiopia holds a unique position in the world as *Coffea arabica* L. has acceptable aroma, flavor and typical quality that identified by their growing areas in the World market like Hara, Wollega, Limu, Jimma and Yirgachefe.

The study of coffee genetic diversity is essential for breeding programs and for the conservation of genetic resources for further research program. There are different methods of studying the presences of genetic diversity such as morphological marker, protein based molecular marker and DNA based molecular marker. Using these methods many authors authenticated that the existence of genetic diversity among coffee collection in Ethiopia. Hence, there is great opportunity to improve coffee for economic traits (yield); also coffee genetic potential enhancement is possible for drought tolerance, insect pest and disease resistance in Ethiopia. Up to date, 7,067 accessions collected by Jimma agricultural research center, and 7 hybrids and 35 pure-lines, totally, 42 varieties that are high yielder, disease resistance and acceptable in quality had been released in Ethiopia.

The high yielder released pureline and hybrid varieties yield potential is 2380Kg ha⁻¹ and 2680 Kg ha⁻¹ respectively. However, the average national coffee yield is 646Kg ha⁻¹. This implies that coffee technology distribution with well organized extension work is required. Current status of Coffee genetic diversity affected by many factors like biotic and Abiotic factors is burning issues that need to be addressed especially for its home land Ethiopia. Abiotic factors that influence coffee genetic diversity are ecosystem properties including climate, geography, and soil or sediment type. From biotic factors insect pests, diseases and economically important insect like pollinators are important one. However, pollinators' vital role in the current Coffee genetic diversity is constrained by different factors. Generally; both Abiotic and biotic factors affect coffee genetic diversity by assassination those species that are susceptible and cause Coffee genetic erosion. Therefore, the impacts of abiotic and biotic factor in the current coffee diversity have to be clearly assessed in Ethiopia.

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