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Review Article

Review on Genetic Diversity Analysis of Ethiopian Coffee Accessions Collected from Limmu Coffee Growing Areas

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Abstract: Coffee is the world's most widely traded tropical agricultural commodity next to oil and is the backbone of the Ethiopian economy. It greatly contributes to the economy of Ethiopia. Limmu is one of the major coffee-producing woredas of Jimma Zone and coffee from this area is well known by the name of 'Limmu Coffee' and fetches very high prices on the world market for its peculiar winy flavor. Having known this fact, Jimma Agricultural Research Center collected 220 coffee accessions from this area to improve productivity and quality. Apart from collection, studies on different batches of Limmu coffee collection have also been conducted as information on genetic variability for morphological and organoleptic traits is a prerequisite for further improvement of the yield and quality of coffee. However, the results of the studies have not been summarized in a way to address the readers. Thus, the objective of this review paper is to summarize the findings of different studies conducted on the genetic diversity of Limmu coffee collections. The results of the studies showed that there is sufficient variability among the accessions for quantitative traits and cup quality traits to improve the yield and the quality of Limmu coffee. In conclusion, the observed variability for quantitative and cup quality attributes should be utilized to improve the productivity as well as the quality of coffee through selection and hybridization. However, as most of the diversity studies were conducted using conventional methods, the contemporary diversity analysis method should be employed to supplement the conventional method. Besides, the organoleptic quality analysis should also be supported with biochemical characterization.

Keywords: Coffee, Contemporary Diversity Analysis, Limmu, Organoleptic Traits

1. Introduction

Ethiopia stands in respect not only as the origin of *Coffea arabica*, an important and leading coffee producer and exporter but it is also the highest coffee consumer in Africa [1]. Among the major crops grown in Ethiopia, coffee is the leading commodity with wide genetic variability and great economic and social importance in the country. Besides, Ethiopia has immense potential and opportunity for growing Arabica coffee because of favorable agro-climatic conditions. Coffee is the major cash crop of the Jimma Zone, which is produced in the eight woredas namely, Gomma, Manna, Gera, Limmu Kossa, Limmu Seka, Seka Chokorsa, Kersa, and Dedo, which serves as a major means of cash income for the livelihood of coffee farming families [2]. According to the report from the same source, 30-45 % of the people in Jimma Zone directly or indirectly benefited from the coffee industry.

Genetic diversity is defined as the total genetic differences present among different individuals, genotypes, strains, clones, or populations of a species [3]. It is expressed in differences in morphological characteristics such as flower color or plant form, in biochemical characteristics (e.g. in protein structure or isoenzyme properties), in physiological properties (e.g. abiotic stress resistance or growth rate), and the DNA sequence. Although mutation and recombination are the ultimate sources of genetic diversity, natural selection, genetic drift, and gene flow are the evolutionary mechanisms that cause changes in allele frequencies over time [4, 5]. Genetic diversity is an essential raw material for evolution, enabling populations of the species to survive, adapt, and evolve into new genetic variants that meet long-term changes in the environment [6, 7]. When one or more evolutionary forces are acting in a population, the population violates the Hardy-Weinberg assumptions, and eventually, evolution occurs [8]. Besides, genetic diversity is also the raw material for improving important agronomic traits of any crop through selection and hybridization.

However, despite the availability of coffee genetic diversity in the country, coffee genetic resources (CGR) are under serious threats of extinction, mainly due to deforestation, replacement of traditionally grown landrace by improved varieties, environmental degradation, change in land use, and climate change [9]. To this end, the Jimma Agricultural Research Center has collected more than 7000 coffee accessions from different coffee-growing areas of the country and conserved them in the *ex-situ* field gene bank of different coffee research centers. In addition to ex-situ conservation by JARC, the Ethiopian Biodiversity Institute (EBI) has also conserved 5,196 randomly chosen accessions in the field gene bank of Choche [10]. Hence, detecting genetic diversity using different methods of analysis is vital for the cost-effective conservation of germplasm and the improvement of the crop through selection and hybridization.

Limmu is one of the major coffee-producing woredas of Jimma Zone and Coffee from this area is well known by the name of 'Limmu Coffee' and fetches very high prices on the world market for its peculiar winy flavor [11]. Thus, it is essential to improve the yield the quality of coffee from these areas by studying the variability and association among different characters on different of batches Limmu coffee collections. Despite a few genetic diversity studies conducted on Limmu coffee, the results are not organized in a way that can reach to the users. Therefore, the objective of this review paper is to summarize research findings on conventional diversity analyses of Limmu accessions.

2. Research Findings on Genetic Diversity of Different Batches of Limmu Coffee Collections

Jimma Agricultural Research Center has been conserving coffee germplasm accessions from different coffee-growing regions of the country. During the initial phase of the coffee breeding program, the major emphasis was given to the development of improved varieties with wide ecological adaptation and resistance to coffee berry disease (CBD). However, since indigenous cultivars were largely location-specific in their adaptation [12], the approach was not successful. Ethiopia is ecologically very diverse and coffees grown under these environments are different in quality, disease resistance, yield potential, and many other traits. Therefore, the development of a breeding strategy that fits these conditions is of paramount importance to exploit all the available advantages. A new breeding strategy, known as the 'Local Coffee Landrace Development Program', was designed to alleviate these problems. The new approach is aimed at the development of varieties for each specific agro-ecology using the respective local landraces and this is elaborated in the text. Cognizant of this fact, the conservation program was designed to maintain the germplasm collections according to their area of origin and adaptation to minimize the risk of genetic erosion that may occur due to natural selection. After redesigning the breeding strategy as a local landrace development program in 1994 [13], Agaro, Haru, Awada, Mechara research centers were selected to improve the productivity and the quality of Limmu, Wollega, Sidama, and Harrargae coffee through collection, characterization and evaluation local land races. Under this program, more than 220 Limmu coffee accessions have been collected and conserved at Agaro research center under four batches of collections. Since then, different diversity studies have been conducted for morphological and quality attributes. Thus, the results of genetic diversity studies conducted on Limmu coffee genotypes using morphological characters and cup quality attributes are summarized and discussed in the following sections.

2.1. Coffee Genetic Diversity Analysis of Limmu Coffee Base on Morphological Traits

Morphological markers generally represent genetic polymorphisms that are visible as differences in appearance, such as the relative difference in plant height and color, distinct differences in response to abiotic and biotic stresses, and the presence/absence of other specific morphological characteristics [14, 15]. Morphological traits were the earliest genetic markers used in scientific studies. Since these traits are scorable by the naked eye, they are also termed naked eye polymorphisms and these traits represent the actual phenotypes of plants that are relevant to plant breeders [16]. Morphological characters have been used to classify coffee germplasm collected from major coffee growing areas in Ethiopia. The findings [17-23] proved the possibility of measuring genetic diversity using morphological markers. When it comes specifically to the Limmu coffee collections, Olika et al. [19] conducted diversity analysis on 49 coffee accessions collected from Limmu-kossa woreda using morphological markers and reported that there was sufficient diversity among the accessions for quantitative traits. The authors also reported that cluster analysis grouped 49 accessions into four divergent groups (Table 1).

Table 1. *Distribution of 49 coffee accessions collected from Limmu- Kossa woreda (SET II) over four clusters using quantitative traits.*

Cluster I		Cluster II	Cluster III	Cluster IV
744	L32/2003	F-59	L01/2003	L17/2003
L02/2003	L33/2003	L15/2003	L03/2003	1 (2.04)
L04/2003	L34/2003	L20/2003	L07/2003	
L05/2003	L35/2003	L42/2003	L08/2003	
L06/2003	L36/2003	L43/2003	L11/2003	
L09/2003	L37/2003	L47/2003	L12/2003	
L10/2003	L38/2003	L48/2003	L13/2003	
L14/2003	L40/2003	7 (14.29%)	L28/2003	

Cluster I		Cluster II	Cluster III	Cluster IV
L16/2003	L41/2003		L29/2003	
L22/2003	L49/2003		L30/2003	
L23/2003	L50/2003		L39/2003	
L24/2003	L27/2003		L44/2003	
L25/2003	L26/2003		L45/2003	
26 (53%)			L46/2003	
			L51/2003	
			15 (30.61%)	

Source: Olika et al. (2011)

In a similar other experiment conducted on another batch of Limmu coffee collection (SET IV), Getachew et al. [18] reported that there was considerable variation among the 49 coffee accessions for quantitative traits and the coffee accessions were grouped into five clusters with significant genetic distance, suggesting the possibility of getting suitable accessions for hybridization program among the tested materials (Table 2). According to the authors, PCA analysis showed the variation in the first two principal components, which explained the lion's share of the observed variation, (42%), was mainly due to internode length of the main stem and primary branches, leaf length, hundred bean weight, plant height, number of main stem nodes, number of primary branches, length of primary branches, number of secondary branches and canopy diameter.

Similarly, Ermias [23] also conducted a variability study on 64 coffee accessions collected from Limmu woreda and reported that the existence of wide ranges of genetic variation for all studied traits, which creates a great opportunity for genetic gain through selection or hybridization. They have also reported that a high amount of heritability with higher values of genetic advance was observed for the number of main stems which provides evidence that these attribute might be under the control of additive genetic effects and selection can be beneficial for the improvement of coffee accessions (Table 3).

Table 2. *The distribution of germplasm accessions into five clusters based on D^2 analysis for 49 coffee germplasm accessions collected from Gomma woreda (SET IV) based on morphological characters and tested at Agaro.*

Cluster No.	No. acc.	Percent (%)	Accessions
1	19	39	L23/05 L08/05, L11/05, L40/05, L19/05, L15/05, L32/05, L31/05 L25/05, L36/05, L48 /05, L17/05, L35/05, L20 /05, L12/05, L37/05, L39/05, L42/05 and F-59
2	19	39	L07/05, L33/05, L43/05, L44/05, L26/05, L45/05, L18/05, L47/05, L41/05, L05/05, L03/05, L13/05, L28/05, L34/05, L14/05, L09/05, L16/05, and L27/05 and 744
3	5	10	L10/05, L06/05, L01/05,, L21/05 and L46/05
4	5	10	L24/05, L04/05, L30/ 05 L38/05 and L29/05
5	1	2	L02/05

Source: Getachew et al. (2013)

Table 3. *Broad-sense heritability and genetic advance as percent of the mean for nine traits of 64 Limmu coffee accession collected from Limmu- Kossa woreda (SET I).*

Traits	Range	H^2b	GA	GAM
Number of primary branches	63.15-32.00	50.00	6.72	12.9
Canopy diameter	194.93-136.08	38.37	11.32	6.79
Internodes length	7.78-5.37	54.99	0.62	9.66
Number of main stem nodes	33.15-16.50	73.21	5.98	35.43
Length of the first primary branch	108.85-63.85	30.84	6.77	7.88
Height up to the first primary branch	45.35-25.20	43.33	4.27	12.45
Stem diameter	4.96-2.65	53.28	0.46	11.64
Total plant height	256.50-145.15	27.65	13.35	6.46
Yield	2624.9-1108.2 1	27.65	13.35	19.05

Note: H^2b= Broad sense heritability, GA=Genetic advance, GAM= Genetic advance as percent of mean
Source: Lemi and Ashenafi (2016)

Moreover, the diversity analysis conducted on 49 coffee accessions collected from Limmu kossa woreda revealed that there was sufficient variability among the tested accessions and the cluster analysis grouped the accessions in three divergent groups [24] (Table 4).

Table 4. *Diversity classes for 49 coffee germplasm accessions (SET III) studied for nine quantitative traits at Agaro.*

Cluster No.	No. acc.	Percent (%)	Accessions
1	21	42.86	L27/04, L47/04, L11/05, L44/04, L16/04, L18/04, L10/04, L11/04 L13/04, L40/04, L31 /04, L32/04, L25/04, L26/04, L39/04, L07/04, L14/04, L46/04, L41/04, L45/04, L35/04and L24/04
2	25	51.02	L36/04, L37/04, L28/04, L05/04, L38/04, L12/04, L29/04, L03/04, L02/04, L33/04, 744, F-59, L34/04, L09/04 and L01/04
3	3	6.12	L10/05, L06/05, L01/05,, L21/05 and L46/05
Total	49	100	

Source: Lemi et al. (2021)

In general, the variability studies conducted on four batches of Limmu coffee collections revealed that there is sufficient diversity for morphological characters to improve Limmu coffee through selection and hybridization based on the breeding interest. Besides, as the present studies revealed the presence of variability, collection program should also designed to address the unaddressed areas of Limmu and properly conserve the collected germplasm accessions to be utilized for future coffee breeding programs targeting to improve Limmu coffee.

2.2. Coffee Genetic Diversity Analysis Based on Quality Attributes

Production and supply of the best coffee quality to the world market seem more critical than ever before for coffee-exporting countries. Consequently, some countries consider the evaluation of coffee quality as important as disease resistance and productivity in their coffee variety development program [26]. Coffee quality assessment is done organoleptically by panels of experienced coffee tasters [27]. For this purpose, standardized procedures are followed during both roasting and brewing [28]. Coffee quality in Ethiopia is evaluated by a group of cuppers using both bean physical (shape and make, bean color, and bean odor) and organoleptic characteristics (aromatic intensity, aromatic quality, astringency, bitterness, acidity, body, flavor, and overall quality) which accounted for 40 and 60%, of the total evaluation, respectively [29]. Among the terms used to describe the characteristics of coffee the most commonly used include. Aroma: This is a fragrance or odor perceived by the nose. Some coffees are more fragrant than others and badly prepared and treated coffee may have an undesirable aroma. Most people have ideas as to what is a good coffee flavor which differs even between areas of the same country. Astringency: It is described as a complex sensation accompanied by shrinking, drawing, or puckering mucosal surface in the mouth, produced by tannins and sloe tannins. Bitterness: is the perception of coffee brew on the tongue. It is the opposite of sweetness. Acidity: It is the dryness sense that the coffee brew produces under the edges of the tongue and on the back of the palate. Body: The body is the feeling of coffee in our mouth. It is the viscosity, heaviness, thickness, or richness that is perceived on the tongue. Flavor: This is a combination of aroma and taste. Overall quality: It can be recorded based on the combination of all the liquor quality attributes (aromatic intensity, aromatic quality, acidity, astringency, body, bitterness, and flavor [29]. Thus, few researchers conducted variability studies among Limmu coffee collections based on the above criteria. In line with this, Olika et al. [20] reported that there was significant variation among Limmu accessions for most coffee quality attributes and 49 coffee accessions were grouped into three divergent groups (Table 5).

Similarly, from the experiment conducted to see the variability among 49 coffee accessions collected from Gomma woreda, it was reported that all quality attributes showed significant variation among the accessions [30]. According to the same authors, cluster analysis based on organoleptic traits grouped the accessions into three groups which make them also moderately divergent (Table 6). Moreover, in an experiment conducted on 64 Limmu coffee accessions for three consecutive years in two locations (Agaro and Gera), it was reported that there was considerable variation both for green bean physical and cup quality characteristics [31]. They have also indicated that there is a great opportunity to select coffee accession with desirable coffee quality characteristics to improve the quality of Limmu coffee.

Table 5. *Average inter-cluster divergence (D^2) values obtained based on organoleptic quality attributes for 49 coffee germplasm accessions collected from Limmu-Kossa woreda (SET II) and tested at Agaro.*

	Cluster I	Cluster II	Cluster III
Cluster I		4.66ns	38.82**
Cluster II			23.21**

Note: **= Highly Significant at P=0.01 (χ 2) = 20.09, ns= non-significant at P=0.05 (χ 2)= 13.36

Source: Olika et al. (2011)

Table 6. *Average inter- cluster divergence (D^2) values obtained based on organoleptic quality attributes for 49 coffee germplasm accessions collected from Gomma woreda (SET IV) and tested at Agaro.*

	Cluster I	Cluster II	Cluster III
Cluster I		20.03*	50.80**
Cluster II			122.09**

**= Highly Significant at P=0.01 (χ 2) = 20.09, *= Significant at P=0.05 (χ 2)= 13.36

Source: Getachew et al. (2015)

From the studies conducted to see the variability among the Limmu coffee accessions, the results showed that there was considerable variation among the coffee accessions of different batches of Limmu coffee collection in terms of organoleptic attributes and this is an important and pre-request to improve the quality of Limmu coffee using selection and hybridization. There is, therefore, an opportunity to select accessions with desirable cup quality. Hence, coffee germplasm collection, conservation, and evaluation attempts should focus on Limmu areas, especially in the unaddressed areas.

2.3. Correlation Between Quantitative Traits of Limmu Coffee Accessions

The association of characters among yield, its components, and other economic traits is important for making a selection in the breeding program and combining several desirable attributes. It suggests the advantage of a scheme of selection for more than one character at a time [32]. Correlation coefficient is the measure of the degree of linear association between two variables Thus, to facilitate selection in breeding for high yield, it is logical to examine various components and give more attention to those having the greatest influence on yield.

In line with this, Olika et al. [19] conducted correlation analysis among 22 morphological characters of 49 Limmu coffee accessions and reported that yield per plant showed significant and positive phenotypic correlation with a percentage of bearing primary branches (r=0.53) which it revealed significant positive genotypic correlation with bean width (r=0.47), fruit length (r=0.61), hundred bean weight (r=0.59), plant height (r=0.28), canopy diameter (r=0.29), leaf length (r=0.30) and percent of bearing primary branches (r=0.62). Similarly, the same authors also did correlation analysis among different morphological characters and coffee bean yield per plant and reported that coffee yield showed positive and significant correlations with average internode length of the main stem diameter, angle of primary branches and fruit length, width and thickness. Moreover, characteristics like, the number of primary branches, canopy diameter; number of main stem nodes, and main stem diameter had positive and significant correlations with yield, which indicates the selection of these characteristics would give a better response in yield. However, the height up to the first primary branch alone showed negative and non-significant significant genotypic and phenotypic correlation with yield. This means simultaneous selection for the character might negatively affect the improvement of coffee yield [24].

Therefore, based on the results of different experiments conducted on Limmu coffee, one can vividly understood that coffee breeders should give major emphasis to a few number of component characteristics such as canopy diameter, stem diameter, number of primary branches and some fruit and bean characteristics to carryout selections for improvement of coffee bean yield.

3. Summary and Conclusion

The results of many experiments conducted using morphological characters such as plant height, number of main stem nodes, stem girth, average internode length, number of primary branches, number of secondary branches, and clean coffee per hectare revealed that most of these characteristics can differentiate among different coffee accessions and can also successfully used to estimate the genetic variability of coffee to use such genetic information in a coffee breeding program. The significant inter-cluster distances between clusters observed almost in all experiments indicated that there is a high opportunity for obtaining transgressive segregates and maximizing heterosis by crossing germplasm accessions belonging to these divergent clusters. Therefore, the grouping of accessions by multivariate methods could be of considerable practical value to the coffee breeders so that representative accessions could be chosen from such clusters for hybridization programs. However, since morphological markers are highly affected by the environment, it should be complemented with molecular diversity analysis to get reliable information on the variability among the accessions.

Moreover, few studies conducted to estimate variation among Limmu coffee accessions for quality attributes confirmed that there is sufficient variability among coffee accessions to use for future coffee breeding programs to improve the quality of this population. However, as the quality is also affected by the biochemical constituents of green coffee bean, the organoleptic analysis should also be supported with biochemical characterization to be able to have reliable information.

Therefore, based on the results of different experiments, it is vividly understood that coffee breeders should give major emphasis to a few component characters to carry out selections for improvement of coffee bean yield. This can help to minimize the time required to search for more component characters and improve the efficiency of the breeding program.

Conflicts of Interest

The authors declare no conflicts of interest

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