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Phenotypic Characterization of Coffee (*Coffea Arabica* L.) Germplasm, in Ethiopia

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Abstract: Identification and characterization of coffee accessions in the base population is important for a successful conservation and utilization of genetic resources. The study was conducted at Metu Agricultural Research Sub Center to characterize extent of genetic variability of coffee accessions. Sixty four Coffee collections were used for this study. The experiment was superimposed during 2018 cropping seasons on six years old coffee trees, which were laid down in 8x8 simple lattice designs. The orchard was managed as per the coffee agronomic production practices. Data on 12 qualitative traits were recorded from four representative trees per row for each accession. Estimates of frequency distribution and Shannon and Weaver diversity index using qualitative traits revealed the presence of genetic variability between coffee geremplasm. The maximum diversity index (H') (highly polymorphic) was found for fruit color (1.22) followed by young leaf tip color (1.08), stipule shape (1.06), leaf shape (1.04), angle of insertion on primary branches (0.97), fruit shape (0.91), growth habit (0.90) and branching habit (0.73), whereas low diversity (lowest polymorphic) was observed in fruit ribs (H`=0.50) and stem habit (H`=0.35). Cluster analysis Grouped 64 coffee accessions in to five clusters. Maximum numbers of accessions were included in cluster-II (29) followed by cluster-I (27), cluster-III (6) and cluster-IV (1). Thus, there is a chance to develop hybrid vigor through crossing diverged parents found in different cluster. Therefore, current study substantiated the existence of sufficient genetic variability in Yayu coffee germplasm for various morphological traits, which can be employed for successful conservation and utilization of genetic resources, as well to identify possible duplicates.

Keywords: Accessions, *Coffee*, Shannon Index, Cluster

1. Introduction

Coffee (*Coffea Arabica* L.) is understory shrub or small woody perennial that differs greatly in morphology, size and ecological adaptations and it may reach a size of 4 to 5 meters. The plant has a dimorphic habit of branching in which vertical (orthotropic) branches from horizontal (plagiotropic) branches, which bear the flowers and fruits in cluster. Arabica coffee (2n=4x=44) is commonly known as the only allopolyploidy and self-infertile species [18, 26]. Flowers of *C. arabica* with short corolla, long style and exerted stamen are typical of the genus *Coffea*. Such floral morphology would permit natural cross-pollination; however, *C. arabica* is an autogamous which sets fruit after self-pollination [6]. Most diploid species have proved to be highly self-incompatible and are allogamous (out crossing) [18, 16].

Characterization of genetic resources refers to the process by which accessions are identified, differentiated or distinguished according to their character or traits [21]. Characterization provides information on diversity, within and between crop collections. This enables the identification of unique accessions essential for curators of gene banks [25]. Moreover, information obtained on genetic relatedness among genetic resources of crop plants is useful, both for breeding and for the purpose of geremplasm conservation [5]. Such knowledge and visualization can be achieved through the study of morphological, structural and functional attributes of germplasm as the carrier of all hereditary characteristics of any given species [15].

One of the key questions for plant genetic resources workers at the time and at present is the dilemma of the length of descriptors list for the characterization of germplasm collections. Such descriptors are mainly taxonomic in kind and of limited use for plant breeders, whereas the actual needs of

the plant breeders is detailed agronomic information. The later is generally not well covered by descriptor lists meant for characterization of geremplasm accessions. Whereas taxonomic or botanical characters are, in general highly heritable, the agronomic characters are not and there, need special ecological and technical conditions to generate reliable and reproductive data 9]. Effective of geremplasm collection and *ex situ* conservation, especially in expensive and vulnerable field genebanks, will only be achieved if such collections are adequately used. The later depends among other things, on the availability for the individual accessions, including passport, characterization and evaluation. In order to facilitate and standardize the data recording, descriptor lists for individual crop species or genepools have been developed [9].

Since Ethiopia is origin and center of genetic diversity for Arabica coffee species, the entire genetic diversity of indigenous (wild) Arabica is confined mainly in the Afromontane rain forest located in the West and East of Great Rift Valley [16, 30]. Starting from 1973 considerable coffee germplasm collection have been made during the national coffee collection program to capture the available coffee genetic variability for the purpose of selecting and developing adaptable coffee varieties. Hence, a total of about 12,452 indigenous and exotic coffee germplasm were collected and *ex-situ* conserved at the Institute of Biodiversity Conservation (5731 accessions) [31] and Jimma Agricultural Research Center (6721 accessions) field gene banks [28]. Most of the collected geremplasm are having been characterized and evaluated mainly through quantitative traits. phenotypic markers in coffee are vital to distinguish variation based on external observation differences, such as size and shape of leaf, color of the shoot tip, the characteristics of the fruit, angle of branching and the length of internodes and the like [8].

Yayu forest is one of the remained parts of Afromontane rainforest in southwestern highlands of Ethiopian which is designated as UNESCO Biosphere Reserves for its primary purpose as a gene reserve for *in situ* conservation of wild *Coffea arabica* [4]. Some diversity assessment studies at population were previously done in Yayu coffee gene pool using quantitative traits [12, 29, 10, and 17]. However, diversity study using qualitative traits have not yet done in this gene pool, so facilitating individual accessions through this pr-breeding activities using heritable traits is helpful to access the material more easily for the breeder. Consequently the current study was undertaken to estimate the extent of genetic variability of Yayu coffee collections with respect to qualitative traits.

2. Materials and Methods

2.1. Description of the Experimental Site

The experiment was conducted at Metu Agricultural Research Sub Center during 2018 cropping season. The sub-center is located 600 km away from Addis Ababa in Illubabor zone of the Oromia Regional State (Figure 1). M Metu is located on latitude 8°19' 0" N longitude 35°35' 0"E at an altitude of 1558 m.a.s.l. The mean annual temperature ranges from 12.7 and 28.9°C with annual rainfall of 1829 mm/annum. The major soil type is Nitosols with pH of 5.24 and phosphorus level of 9.36 ppm [23].

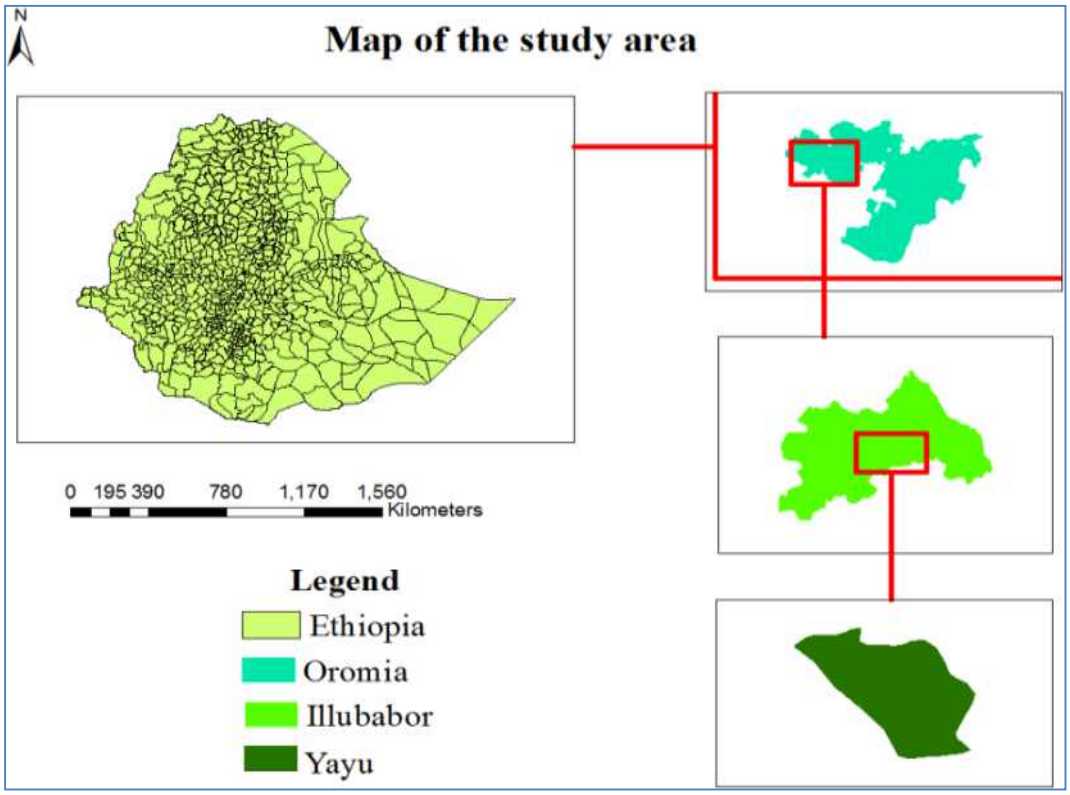

Figure 1. *Map of Arabica coffee germplasm collection site.*

2.2. Experimental Materials, Design and Field Management

Sixty-two *Coffea arabica* L. germplasm which have been collected in the year, 2012 from Yayu woreda of Illubabor zone and two commercially grown standard check varieties were used for this study (Table 1). The study was superimposed during the 2018 cropping seasons on six years old coffee trees. Experiment was laid down in an 8X8 simple lattice design with eight accessions per each incomplete block. Each accession was planted in a single row of six trees using spacing of 2m by 2m. Accessions were established under uniform *Sesbania sesban* temporary shade trees and all other management practices were also uniformly applied for the orchard as per the coffee agronomic production practices.

Figure 2. *Pictorial illustration of the experiment.*

2.3. Data Collection

According to the International Plant Genetic Resources Institute [14] coffee descriptor, data of 12 qualitative traits were recorded from each accessions as described below table 2. The accuracy of data was given priority. Whenever, possible reference material was used either in the form of a color chart, or drawings or the shapes etc. of the various descriptors sates.

Table 1. *Description of Coffea Arabica L. germplasm used in the study.*

Accessions	District	peasant association	Specific collection site	Accessions	District	Specific collection site
Y63	Yayu	Gechi	Dogi	Y95	Yayu	Geri geba
Y64	Yayu	Gechi	Dogi	Y96	Yayu	Geri geba
Y65	Yayu	Gechi	Dogi	Y97	Yayu	Geri geba
Y66	Yayu	Gechi	Dogi	Y98	Yayu	Geri geba
Y67	Yayu	Gechi	Dogi	Y99	Yayu	Geri geba
Y68	Yayu	Achebo	Sembo	Y100	Yayu	Geri geba
Y69	Yayu	Achebo	Sembo	Y101	Yayu	Geri geba
Y70	Yayu	Achebo	Sembo	Y102	Yayu	Geri geba
Y71	Yayu	Achebo	Sembo	Y103	Yayu	Geri geba
Y72	Yayu	Achebo	Sembo	Y104	Yayu	Geri geba
Y73	Yayu	Achebo	Sembo	Y105	Yayu	Gordeya
Y74	Yayu	Achebo	Sembo	Y106	Yayu	Gordeya
Y75	Yayu	Achebo	Sembo	Y107	Yayu	Gordeya
Y76	Yayu	Achebo	Sembo	Y108	Yayu	Gordeya
Y77	Yayu	Achebo	Sembo	Y109	Yayu	Gordeya
Y78	Yayu	Achebo	Sembo	Y110	Yayu	Gordeya
Y79	Yayu	Achebo	Sembo	Y111	Yayu	Gordeya
Y80	Yayu	Achebo	Sembo	Y112	Yayu	Gordeya
Y81	Yayu	Achebo	Geba	Y113	Yayu	Degitu
Y82	Yayu	Achebo	Geba	Y114	Yayu	Degitu
Y83	Yayu	Achebo	Geba	Y115	Yayu	Degitu
Y84	Yayu	Achebo	Geba	Y116	Yayu	Degitu
Y85	Yayu	Achebo	Geba	Y117	Yayu	Degitu
Y86	Yayu	Achebo	Geba	Y118	Yayu	Degitu
Y87	Yayu	Yayu	Achebo	Y119	Yayu	Degitu
Y88	Yayu	Yayu	Achebo	Y120	Yayu	Degitu
Y89	Yayu	Yayu	Achebo	Y121	Yayu	Degitu
Y90	Yayu	Yayu	Achebo	Y122	Yayu	Degitu
Y91	Yayu	Yayu	Achebo	Y123	Yayu	Degitu
Y92	Yayu	Yayu	Achebo	Y124	Yayu	Degitu
Y93	Yayu	Yayu	Achebo	74110	Metu	Bishari
Y94	Yayu	Yayu	Achebo	74112	Metu	Bishari

Table 2. *Qualitative traits of Coffea Arabica L. germplasm, Coding of qualitative triats.*

Sr/n	Qualitative traits	Phenotypic class with code
1	Growth habit	1 (Open), 2 (Intermediate), 3 (Compact)
2	Stem habit	1 (Stiff), 2 (Flexible)
3	Branching habit	1 (Very few Primary branches), 2 (Many primaries with few secondary branches), 3 (Many primary with many secondary branches), 4 (Many primary with many Secondary and Tertiary branches)
4	Angle of insertion on the main stem	1 (Drooping), 2 (Horizontal spreading, 3 (Semi- erect)
5	Young leaf tip color	1 (Greenish), 2 (Green), 3 (Brownish), 4 (Reddish brown), 5 (Bronzy)
6	Leaf shape	1 (Obovate), 2 (Ovate), 3 (Elliptic), 4 (Lanceolate), 5 (Other)
7	Leaf apex shape	1 (Round), 2 (Obtuse), 3 (Acute), 4 (Acuminate), 5 (Apiculate), 6 (Spatulate)
8	Stipule shape)	1 (Round), 2 (Ovate), 3 (Triangular), 4 (Deltate), 5 (Trapezium)
9	Fruit shape	1 (Round), 2 (Obovate), 3 (Ovate), 4 (Elliptic), 5 (Obolong)
10	Fruit color	1 (Light red), 2 (Red), 3 (Dark red), 4 (Yellow)
11	Calyx-limb persistence	0 (Not persistent), 1 (Persistent)
12	Fruit ribs	0 (Absent), 1 (Present)

2.4. Data Analysis

Shannon Index (H') was used to analyze the phenotypic diversity of coffee germplasm depending on the 12 qualitative traits of 64 coffee accessions. The type of diversity used here is α diversity which is the diversity of species within a community or habitat. The diversity index was calculated by using the Shannon and Weaver [27].

$$H' = -\sum_{i=1}^{S} P_i \ln P_i$$

Where: Pi is the relative proportion of the total number of entries (N) in the i^{th} class, which means Pi = S / N, Where S = number of individuals of one species, N = total number of all individuals in the sample, ln = logarithm to base e. The analysis was done with Microsoft excel.

In biological communities, Shannon-Wiener diversity index varies from 0 to 5. According to this index, values less than 1 characterize low diversity, values in the range of 1 to 3 are characteristics of moderate diversity, while above 3 indicates highest diversity [20].

Cluster analysis was used to group the accessions in to homogeneous forms based on qualtitative traits. Hierarchical clustering was employed using the similarity coefficients among the 64 coffee accessions. The analysis was performed using SAS software by employing the method of average linkage clustering strategy of the observation [24]. The numbers of clusters were determined by looking into three statistics approach namely Pseudo-F, Pseudo-t^2 and cubic clustering criteria which is suggested by Copper and Miligan [7]. Accordingly, the number was decided where local peaks of Pseudo-F statistics and cubic clustering criteria combined with small values of Pseudo-t^2 statistics followed by a larger Pseudo-t^2 statistics for the next cluster fusion.

3. Results and Discussion

3.1. Estimates of Phenotypic Frequency and Diversity Index

Analysis of phenotypic diversity of 12 qualitative traits is presented in table 3. The result of frequency distribution of the phenotypic classes showed that the coffee accessions were grouped based on 12 qualitative traits. According to percent of frequency distribution, the predominated traits shared among the majority of coffee germplasm were stiff stem (89.06%), without fruit ribs (80.00%), without calyx limb persistence (73.00%), many primary with many secondary branches (61.00%), intermediate growth habit (60.94%), horizontal spreading angle of insertion on primary branches (58%), round fruit shape (56.25), green young leaf tip color (55%), acuminate leaf apex shape (52.00%), round stipule shape (50.00%), red fruit color (28.00%) and lanceolate leaf shape (26.56%). The result of this distribution pattern was comparable to those observed by Getachew *et al*. [11]. The similar result obtained may be due to qualitative traits are highly heritable and less influenced by environmental factors.

The expression of growth habit at Metu could be attributed to the high moisture and cool temperatures that led to high branching, dense foliage and prolonged vegetative phase. Accordingly, about 28.13% and 60.94% of the coffee germplasm were open and intermediate growth habit, respectively, whereas 10.94% were compact. Similarly, 89.06% of the germplasm were stiff stem, while 10.94% had flexible stem. In case of branching habits, many primaries with many secondary branches (61.00%) were predominant traits followed by many primaries with few secondary branches (38%) and very few primary branches (20%). The variation in branching habit may be due to the genotypes had the ability of exhibiting different growth habit, which is possibly due to natural selection or adaptation mechanism.

Angle of insertion is another important qualitative trait which groups the germplasm in to horizontal spreading (58.00%), semi- erect (22.00%) and drooping (20%) types. The predominance of the intermediate growth habit, stiff stem and horizontal spreading angle of insertion are the manifestation of suitability of such traits for ease of management practice, and permits uniform exposure and better interception of sunlight for all leaves and other vegetative parts. It will also create less favorable environment for disease development, as compared to compact types and drooping growth habits. Moreover, chance of the fruit to contact the ground is less, as compared to

drooping types, which at the end increases fruit quality and yield. The current observation is in line with the report of Getachew *et al*. [11] who found intermediate (61.22%) and open (38.72%) growth habit, stiff (51.10%) and flexible (48.9%) stem habit, and drooping (38.78%), horizontal spreading (36.73%) and semi erect (24.48%) angle of insertion among 49 Lemu coffee accessions. This is further in harmony with Manyasa *et al*. [19] who observed 70% of indeterminate and 26% of determinate growth habit in Ugandan pigeon pea landraces. Muluken *et al.* [22] also noted that almost all okra genotypes had densely branched base growth habits.

The diversity index (H') values ranged from 0.35 for stem habit (lowest polymorphic) up to 1.22 for fruit color (highly polymorphic). Relatively, the highest diversity was found for fruit color (1.22) followed by young leaf tip color (1.08), stipule shape (1.06), leaf shape (1.04), angle of insertion on primary branches (0.97), fruit shape (0.91), growth habit (0.90) and branching habit (0.73) which is might be due to oligogenic nature of gene action and slight environmental interaction. Relatively low diversity was observed in fruit ribs (H`=0.50) and stem habit (H`=0.35). This low diversity value implies that majority of the population tends to fall within the same state, signifying the possibility of close association between coffee genotypes for these two traits. Further, Manyasa *et al*. [19] also found the diversity within flower streak pattern (H`=0.472), growth habit (H`=0.459), flowering pattern (H`=0.518), pod form (H`=0.470) pod hairiness (H`=0.474) and seed eye color (H`=0.532) in Ugandan pigeon pea landraces.

3.2. Cluster Analysis of Coffee Germplasm

The distribution pattern revealed that, maximum numbers of coffee accessions were grouped in cluster-II (29) followed by cluster-I (27), cluster-III (6) and cluster-IV (1) (table 4). In cluster analysis, if the classification is successful, individuals within or intra cluster (homogenous) shall be closer and inter clusters (heterogeneous) shall be farther apart [13].

Cluster-II comprised of 29 (45%) of the accession, which are varied from other clusters by having compact growth habit, round fruit shape and yellow fruit color. Cluster-I which consisted of 27 (42%) is differing from other by having elliptic and oblong fruit shape. Cluster 3 comprised of 6 (9%) coffee accession, differed from other clusters by having in its deltate stipule shape and. Cluster–IV and V which consisted of one accession each (Y7 4& Y67) are differing from others by having bronzy leaf tip color. Therefore, most of the accessions were grouped together both from their outsource and in source area of collection, which indicated that, qualitative traits were highly heritable and less environmental affected. Therefore, geographic diversity is not always related to the genetic diversity [2]. Thus, there is a chance to develop hybrid vigor through crossing diverged parents found in different cluster. This is more underlined by Bayeta [3], who suggested as morphological variation is more considerable than geographical origin as an indicator of genetic diversity in coffee.

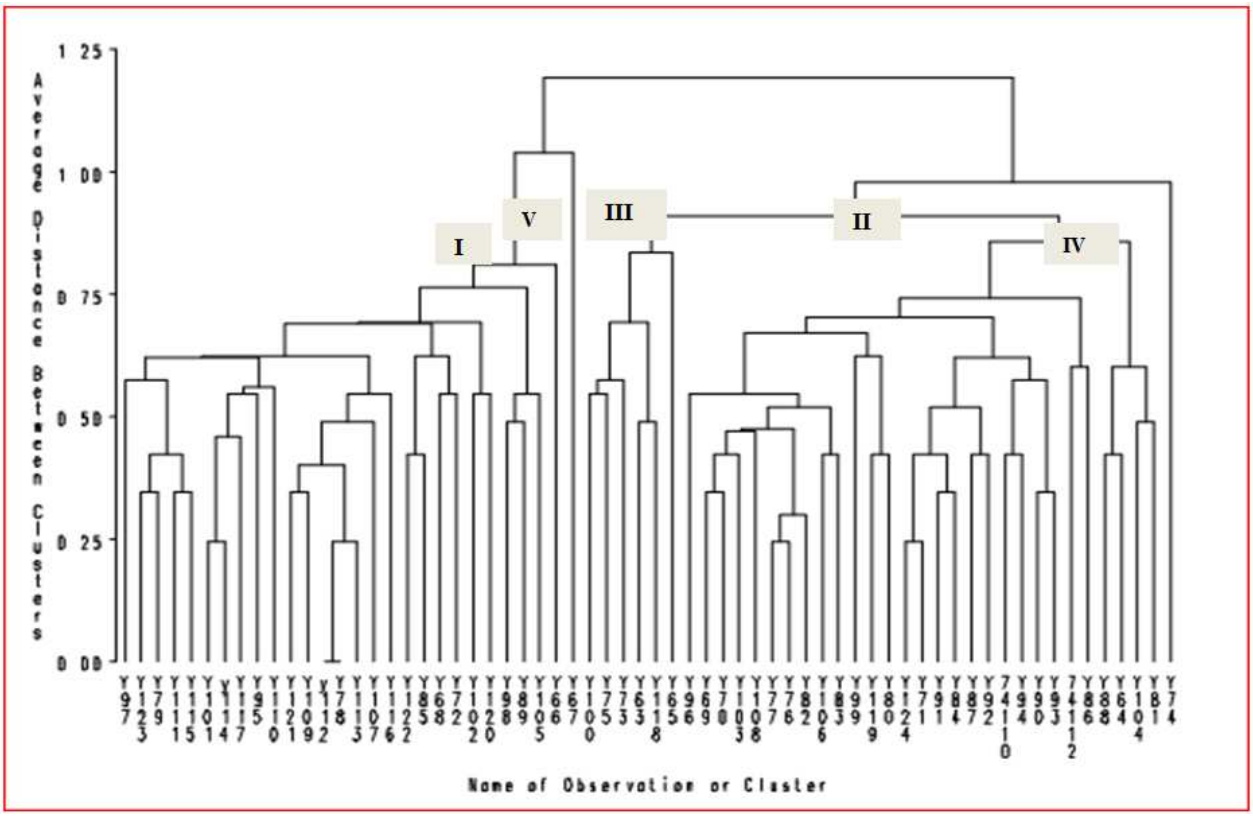

Figure 3. *Dendrogram showing the clusters of 64 coffee accessions.*

Table 3. *Phenotypic classes and diversity for qualitative traits in 64 coffee germplam studied at Metu (2016/2017).*

Traits	code	phenotypic classes	Frequency distribution		Diversity Index (H')
			Number of accessions	Percent (%)	
Growth habit	1	Open	18	28.13	0.90
	2	Intermediate	39	60.94	
	3	Compact	7	10.94	
Stem habit	1	Stiff	57	89.06	0.35
	2	Flexible	7	10.94	
Branching habit	1	Very few primary branches	1	20.00	0.73
	2	Many primary with few Secondary branches	24	38.00	
	3	Many primary with many Secondary branches	39	61.00	
Angle of insertion on primaries	1	Drooping	13	20.00	0.97
	2	Horizontal spreading	37	58.00	
	3	Semi- erect	14	22.00	
Young leaf tip color	1	Greenish	17	27.00	1.08
	2	Green	35	55.00	
	3	Brownish	10	16.00	
	5	Bronzy	2	3.00	
Leaf shape	2	Ovate	15	23.44	1.04
	3	Elliptic	32	50.00	
	4	Lanceolate	17	26.56	
Leaf apex shape	4	Acuminate	33	52.00	0.69
	5	Apiculate	31	48.00	
Stipule shape	1	Round	32	50.00	1.06
	2	Ovate	24	37.50	
	3	Triangular	4	6.25	
	4	Deltate	4	6.25	
Fruit shape	1	Round	36	56.25	0.91
	4	Elliptic	6	9.38	
	5	Obolong	22	34.38	
Fruit color	1	Light Red	24	38.00	1.22
	2	Red	24	38.00	
	3	Dark Red	12	19.00	
	4	Yellow	4	6.00	
Calyx limb persistence	0	not persistent	47	73.00	0.58
	1	Persistent	17	27.00	
Apex fruit ribs	0	Absent	51	80.00	0.50
	1	Present	13	20.00	

Table 4. *The distribution of 64 coffee accessions in to five clusters tested at Metu (2018).*

Cluster No.	No. acc.	Percent (%)	STDEV	Accessions
I	27	42	2.8	y112, y78, y101, y114, y113, y111, y115, y121, y109, y123, y79, y122, y85, y117, y98, y89, y107, y95, y102, y120, y116, y105, y68, y72, y110, y97, y66
II	29	45	3.2	y124, y71, y76, y82, y90, y93, y69, y70, y84, y87, y92, 74110, y94, y88, y64, y103, y119, y80, y106, y83, y108, y118, y104, y81, y96, y100, y77, y91, 74112, y86, y99
III	6	9	1.4	Y118, Y63, Y75, y100, y73, y65
IV	1	2	2.3	Y74
V	1	2	2.3	Y67

4. Conclusions

The results of this study have confirmed the existence of enormous genetic variability among the 64 *Coffea arabica* accessions for most of the qualitative traits considered. Analysis of frequency distribution and diversity Index using qualitative traits showed the presence of genetic variability between coffee accessions. The maximum Shannon index (H') was found for fruit color, followed by young leaf tip color, stipule shape and leaf shape. Cluster analysis Grouped 64 coffee accessions in to five clusters. Maximum numbers of accessions were included in cluster-II (29) followed by cluster-I (27), cluster-III (6) and cluster-IV (1). Based on the study of overall similarities between accessions, it could be concluded that phenotypically smellier accessions also possess genetic similarities. Thus, there is a chance to develop hybrid vigor through crossing diverged parents found in different cluster. These taxonomic studies are of direct interest to the plant breeder and can be successfully employed in field gene-banks for initial identification unknown accessions, as well as to identify possible duplicates. These are essential steps toward rational coffee germplasm conservation and use, thus showing the importance of systematic data collection for individual accessions. Therefore, current study substantiated the existence of sufficient genetic variability within Yayu coffee germplasm for various morphological traits. Hence there is an opportunity to exploit these traits in order to develop genotypes that perform better than the existing varieties for the future coffee improvement program.

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