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Cluster and Principal Component Analysis of Coffee (Coffea Arabica L.) Diversity Using Quantitative Traits in the South Western Ethiopian

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ABSTRACT

Coffee (Coffea arabica) is widely used economically important crop in the world. Four replicated standard check varieties and a total of 133 accessions of coffee from the Bale and West Arsi zones were assessed in the Gera agricultural research sub center. The study was done to determine the level of genetic diversity among clusters and to identify major traits that contribute to overall genetic variability for future coffee breeding works. The experimental design was set out using augmented design with three block of a single row, with six trees per plot. A study was conducted on four-year-old coffee trees that were planted in July 2015 during the 2018–19 crop seasons. In the univariate analyses of variance, the germplasm accessions varied significantly for each of the 10 morphological agronomic traits demonstrating existence of variability. A cluster means analysis for quantitative attributes showed significant variation. Cluster distances between the majorities of these clusters were highly significant at (P ‹ 0.01); as a result, the distance between clusters II and VI (142.82) was found to be the greatest, followed by cluster I and VI (100.94). Among the 137 variables, the first four main components accounted for 70.55 percent of the total variation. However, the variance as a whole was more strongly influenced by the first two principal components (PC1 and PC2). However, additional traits of interest should be studied over year and locations including physiological, quality, disease and biochemical analysis with the support of advanced molecular techniques.

Keywords: *Coffea arabica, Genetic divergent Cluster analysis, Principal component analysis.*

INTRODUCTION

Coffee (Coffea arabica) is widely used brewed drink (Shukla, S.K. and Kumar, V., 2013) and, economically important crop in the world. Coffee belongs to the genus Coffea of the Rubiaceae family, mostly grown in the tropical and subtropical regions (Berthaud and Charrier, 1988). It is an important commodity crop in Ethiopia (WeldeMichael el al., 2016). The genus Coffea L. comprises 124 species (Davis et al., 2011). However, only C. arabica (Arabica coffee), C. Canephora (Robusta coffee); and C. liberica (Liberian or Liberica coffee, or excels coffee) are the economically important species of the genus (Davis et al., 2006). Coffea arabica the only allopolyploid (2n = 4x = 44) coffee species and self-fertile (Silvarolla et al., 2004). Arabica coffee has its primary center of origin and genetic diversity in the high lands of Southwestern, Ethiopia (Sylvain, 1955). Over the past 50 years, both production and consumption of coffee have risen considerably. Approximately 60 percent of the world's coffee production comes from Arabica, while the remaining 40 percent is contributed by Robusta; the former considered a superior quality and fetches a higher price (Moat et al., 2017).

Globally, total coffee production is estimated to be 169. 634 million of 60-kg bags, of which Arabica coffee production is about 103.60 million of 60-kg bags, while Robusta coffee production was estimated to be 65.46 million 60-kg bags (ICO, 2021). Economically, coffee is the second most exported commodity after oil, and employs over 100 million people worldwide (Gray et al., 2013). Coffee is not only one of the highly preferred international beverages, but also one of the important agricultural commodities in the world.

According to, ICO (2019) Ethiopia is Africa's largest coffee producer and the world's fifth largest exporter of Arabica coffee. The total

cultivated coffee area in Ethiopia is estimated around 758.523.3 ha (CSA, 2021). The annual estimate of national production of coffee is about 7.38 million of 60-kg bags (ICO, 2021) and national average yield is low (636 kg/ha) (CSA, 2021). Coffee is Ethiopia's main export commodity, contributing to the livelihoods of more than 15 million smallholder farmers and other actors in the coffee sector (USAD, 2022). It represents the major agricultural export crop, providing 25% of the foreign exchange earnings (USDA, 2019).

As indicated by Krug and Carvalho, (1951) World arabica coffee production is largely based on using a very small number of cultivars: C. arabica var. typica Cramer, C. arabica var. bourbon. The low genetic diversity observed makes this crop, vulnerable to biotic and climatic hazards. However, Ethiopia holds a unique position in the world as Coffea arabica the primary center of origin and diversity. The major coffee growing area and diversity is found in Oromia Region, of South West Ethiopia (Wollega, Illubabor, Jimma-Limu, Tepi, Kaffa and Bench-Maji). Southern Nations Nationalities Region is the second coffee producing region. Whereas, Modest coffee production in Amhara region and minor output in Benishangul-Gumuz region (Moat et al., 2017).

Different research findings illustrate the importance and existence of natural Arabica coffee genetic variability in Ethiopian, to get highly productivity and disease resistance coffee genetic materials (Adugna, 2005; Labouisse et al., 2008). To make use of this genetic potential and seek a solutions to pre-existing production constraints, Jima agricultural research center (JARC) conserved about 6923 coffee accessions from different coffee producing areas of the country to its Center and its' Sub-centers over the last five decades and ultimately released about 44 improved varieties (35 pure lines and nine were hybrids) for different localities. It is important to plan coffee variability assessment for yield and its component characters before doing any breeding improvement program.

As continued breeding efforts, JARC have collected about 133 coffee germplasm from Bale and West Arsi Zones of Oromia Regional State. However, these materials have not been characterized and their genetic potential for disease resistance and yielding potential is not well known. Hence, it is relevant to characterize and conserve these coffee accessions to reduce the loss of coffee genetic resources and use in a breeding program to improve the productivity of the crop by developing high yielding and disease resistant coffee varieties. The study was conducted based on objectives to determine the level of genetic diversity among clusters and to identify major traits that contribute to overall genetic variability for future coffee breeding works.

Materials And Methods

Experimental Material, Design and Management

The experiment was done in South West of Ethiopia using One hundred thirty-three C. arabica along with four standard check varieties at Gera agricultural research center. The center is located at 425 km. southwest of Addis Ababa capital city of Ethiopia. It is located at 7046 N latitude and 360 26' E longitudes, at an altitude of 1974 meters above sea level. The mean annual rainfall of the area is 1880 mm with average maximum and minimum air temperatures of 24.5oC and 10.4oC, respectively. The center has contained Acrisols and Nitoso soil with PH of 5-6 and medium to high exchangeable cation (Paulos, 1994; Paulos and Tesfaye, 2000). The experiment was established in an augmented design with three replications. A plot is laid out in a single row with six trees. Spacing both, between rows and plants were 2m x 2m (plot area of 4m2) and Spacing between block was 4 meter. All management practices were applied as per recommendation.

Data Collection

Data on 25 Quantitative traits were collected on tree basis from three sample trees per row in each accession using standard coffee descriptor of IPGRI (1996). The measured accessions for morphological characters is namely: Plant height, stem diameter, number of primary branch (no), number of node on main stem (no), canopy diameter(m),average inter node length of main stem (cm), fruit length (mm), fruit thickness (mm), fruit width (mm), bean length (mm), bean thickness (mm), bean width (mm), leaf length (cm), leaf width (cm), leaf size (mm), petiole length (cm), length of first primary branch (cm), hundred bean weight (gr), yield (kg/ha), number of secondary branch (no.), number of node on primary branch (no), height up to first primary branch (cm), percentage of bearing primary branch. Other traits Coffee berry disease severity and coffee leaf rust severity were recorded through visual estimation per three trees.

Statistical Analysis

The statistical analyses of variance were computed using SAS soft version 9.2 (SAS, 2010) based on augmented design for 25 quantitative morphological

characters and two years yield data. In this study sets of 25 quantitative morphological data were subjected to cluster analysis to determine the variability among the accessions. However, ten morphological characters that showed statistically significant variations among the accessions were used for principal component analysis. Principal component with Eigen values greater than one were considered to explain observable variability. Cluster analysis is a process of identification and categorization of subsets of objects and a multivariate technique whose primary purpose is to group individuals or objects based on the characteristics they possess. Hierarchical clustering was employed using the similarity coefficients among the 137 coffee accessions. Clustering was performed using the proc cluster procedure of SAS version 9.2 (SAS institute, 2010). The dendrogram constructed based on the average linkage and Euclidean distance was used as a measure of dissimilarity. The number of cluster was determined by following the approach suggested by Copper and Miligan (1988) by looking in to statistics namely Pseudo F, Pseudo t2 and cubic clustering criteria. Genetic divergence between and within clusters were calculated using the generalized Mahalanobis's D2 statistics (Mahalanobis, 1936) using the equation:

$$D^2p = (Xi - Xj)\ S^{-1}\ (Xi - Xj)$$

Where, D2 P= the distance between any two groups i and j, Xi and Xj = the p mean vectors of accessions i and j, respectively. S-1 = the inverse of the pooled covariance matrix. The D2 values obtained for pairs of clusters were tested for significance at 0.05 level of significance against the tabulated values of x2, for 'P' degrees of freedom, where p is the number of variables considered (Singh and Chaudhary, 1987).

Result and Discussion

The analyzed mean square results for most of measured quantitative traits were significant difference at ($P<0.05$) for bean yield, fruit thickness, canopy diameter, fruit length, fruit width, coffee berry disease (CBD), Coffee leaf rust (CLR), number of secondary branches, percent (%) of bearing primary branch and height up to first primary branch (Table 1). Thus, it is a good chance to improve the accessions through selection and breeding using these traits.

This study result agrees with the findings of Olika et al., (2011) who found that significant variations among 49 accession for 22 characters. (Atinafu and Mohammed, 2017, and Abdulfeta (2018) and Desalegn (2018) also found a substantial amount of variability for different traits among tested genotypes of arabica coffee, which shows the possibility to bring improvement through selection. Bayetta (1997) also reported high genetic variability within the Arabica coffee population for yield, growth characters and coffee berry disease resistance. Moreover, Mesfin and Bayyeta (2008) reported the mean square of treatment showing that significant difference among 100 Hararge coffee accession for 14 quantitative characters. In the present studied traits, checks versus accessions were significant for all characters except stem diameter, number of primary branches, number of nodes on main stem, bean length, bean thickness, number of nodes on a primary branch (Table 3). This also showed that existence of variation among collected accessions and control check varieties.

Cluster Analysis Based On Quantitative Characters

Six clusters were identified using a cluster analysis of 137 coffee accessions (Table 9). The four clusters I, II, III, and IV contained the greatest number of accessions. Smaller accession numbers, however, were grouped in clusters V and VI. Thus, cluster-II was the largest and had 41 accessions (30%). It was followed by cluster-I, which had 37 accessions (27%), cluster-IV, which had 29 accessions (21%), cluster-III, which had 25 accessions (18%), cluster-V, which had 4 accessions (3%) and cluster-VI, which had 1 accession (1%). 74165 and 74148, perennial self-pollinated standard check varieties, were classified in cluster I, whereas 74110 and 75227 were grouped in clusters III and IV, respectively.

Coffee collections were made in Bale and West Arsi zones, from three districts: Nensebo, Gololcha and Ginir, which was grouped into different clusters. In this study coffee accessions collected from the West Arsi zone: Rafisa kebele was grouped in a cluster I, III and IV. Whereas, accessions from the same district, Kore kebele grouped in cluster I, II, III and IV. This revealed that existence of higher genetic diversity in accession collected within same districts. Variation of accessions revealed that due to admixture of different coffee genotypes through moving by human or other wild animals' from place to place.

These findings agree with Getachew *et al*., (2013) who reported that accessions collected from different kebeles clustered together, while accessions collected from same kebeles were

clustered into different clusters. Seyoum (2003) also reported that, rather than geographical region morphological variation is more important because it shows variation in coffee. Generally, accessions collected from the same place clustered in to different group, whereas accession collected from different places grouped into the same clustered.

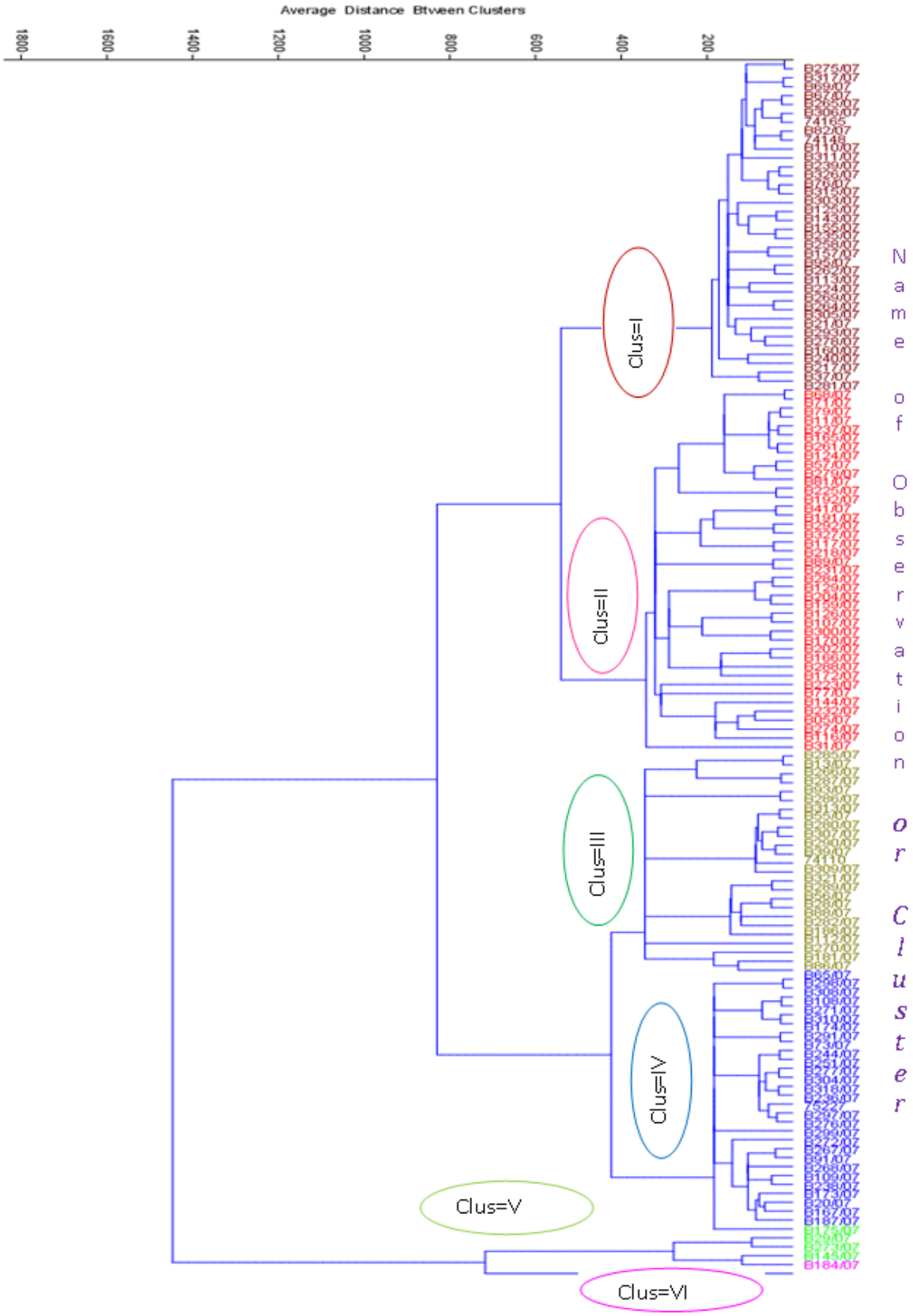

Figure1. *Tree diagram of 137 accessions using 10 quantitative traits.*

Table1. *Distribution of Bale and West Arsi coffee accessions clustered based on D^2 analysis.*

Clus No.	No. ace	Percent (%)	Name of accessions						
I	37	27	B275/07	B317/07	B69/07	B67/07	B265/07	B306/07	74165
			B82/07	74148	B110/07	B311/07	B239/07	B326/07	B76/07
			B315/07	B303/07	B125/07	B143/07	B155/07	B235/07	B258/07
			B157/07	B95/07	B262/07	B113/07	B224/07	B269/07	B264/07
			B305/07	B21/07	B293/07	B278/07	B160/07	B240/07	B217/07
			B37/07	B281/07					
II	41	30	B68/07	B71/07	B79/07	B11/07	B237/07	B165/07	B261/07
			B124/07	B57/07	B279/07	B81/07	B225/07	B192/07	B41/07
			B191/07	B292/07	B327/07	B117/07	B218/07	B89/07	B231/07
			B284/07	B129/07	B204/07	B159/07	B126/07	B107/07	B300/07
			B170/07	B202/07	B166/07	B288/07	B172/07	B223/07	B77/07
			B144/07	B232/07	B05/07	B274/07	B116/07	B31/07	
III	25	18	B285/07	B13/07	B266/07	B287/07	B93/07	B286/07	B313/07
			B55/07	B280/07	B307/07	B290/07	B39/07	74110	B309/07
			B321/07	B289/07	B56/07	B28/07	B88/07	B282/07	B186/07
			B112/07	B270/07	B181/07	B86/07			
IV	29	21	B65/07	B298/07	B308/07	B108/07	B271/07	B310/07	B174/07
			B291/07	B73/07	B244/07	B251/07	B277/07	B304/07	B318/07
			B236/07	75227	B297/07	B276/07	B299/07	B272/07	B267/07
			B91/07	B268/07	B109/07	B238/07	B173/07	B20/07	B167/07
			B187/07						
V	4	3	B175/07	B29/07	B273/07	B145/07			
VI	1	1	B184/07						

Inter Cluster Distance (D^2) Analysis Based On Quantitative Traits

The D2 chi-square test for the 6 clusters revealed that there were highly significant differences (P < 0.01 X2 =21.67) between the clusters except cluster I and II, I and III, I and IV, IV and II, IV and III, and III and V (Table 2), indicating little genetic diversity between these clusters. This suggests that, crossing of genotypes from these clusters might not give a higher heterotic value in F1 and a narrow range of variability in the segregating F2 population.

Clusters II and VI had the highest Inter cluster distance (142.82), while clusters I and IV had the smallest (2.337), followed by clusters VI and IV (76.06), II and V (73.89), III and VI (53.76), I and V (43.76), IV and V (27.71), and clusters I and VI (100.94). On other hand, Clusters I and IV had the smallest inter-cluster distance (2.337), while clusters II and VI had the greatest (142.82), followed by clusters I and VI (100.94), VI and IV (76.06), II and V (73.89), III and VI (53.76), I and V (43.76), IV and V (27.71), and II and III (26.24), indicating the presence of genetic variability between groups of tested genotypes.

Different genotypes with distant clusters could be used in the hybridization program to obtain a higher heterotic response in the hybrids and a wide range of variation among the sergeants. Since, the maximum inter-cluster distance was observed between clusters II and VI (142.82), followed by clusters I and VI (100.94), which helps to get a superior hybrid or recombinant by crossing between desirable lines of these clusters. However, the selection of parents should consider the special advantages of each cluster and each genotype within a cluster depending on the specific objective of the hybridization program. Crosses involving genotypes belonging to the most divergent cluster distances could be used for hybridization programs to obtain good manifestations of heterosis and wide variability (Singh and Chaudhary, 1987).

To get a greater heterotic response in the hybrids and a wide range of variance among the segregating population, various genotypes with distant clusters could be used in the hybridization program. Since clusters II and VI had the greatest Inter-cluster distance (142.82), followed by clusters I and VI (100.94), crossing between these clusters' desired lines can result in a superior hybrid or recombinant. Moreover, depending on the specific objectives of the hybridization program, the special benefits of each cluster and each genotype within a cluster should be taken into account while choosing parents. To get good manifestations of heterosis and a wide range of variability, hybridization procedures could use crosses involving genotypes

from the most dissimilar cluster distances (Singh and Chaudhary, 1987).

Table2. *Inter cluster distance for 10 quantitative traits of Bale and Wet Arsi zone coffee collection in Gera southwest Ethiopia.*

	I	II	III	IV	V	VI
I	0	5.528 ^{ns}	8.718^{ns}	2.377 ^{ns}	43.831**	100.935**
II		0	26.242**	13.993 ^{ns}	73.890**	142.817**
III			0	2.553 ^{ns}	15.906 ^{ns}	53.762**
IV				0	27.713**	76.058**
V					0	19.723*
VI						0

***,** ***=Highly significant, ($p<0.01$) $x^2=21.67$, ($p<0.05$) $x^2=16$*

Principal Component Analysis

The first four principal components with Eigen values greater than unity explained 70.55 percent of the total variation among the 137 genotypes for the 10 quantitative characters measured (Table 3). Principal component analysis showed 4 PC (PC1, PC2, PC3, and PC4) exhibited greater than one Eigen value (2.59, 2.09, 1.31 and 1.07) (Table 2). Accordingly, the first PCA accounted 25.88% of total variation, followed by the second (20.86%), the third (13.12%) and the fourth (10.7%). However, the first two principal components (PC1 and PC2) were contributed more to the total variation. The first PC contributes higher to the total variation (25.88%) due to greater contribution of positive discriminatory traits of fruit length (0.75%), fruit thickness (0.87%), fruit width (0.86%), coffee berry disease (0.42%) and number of secondary branch (0.32%). Variation in the second PC (20.86 %) was mainly influenced by fruit length (0.75%), fruit width (0.86%), and coffee berry disease (0.60), percent (%) of bearing primary branches (0.39%) and canopy diameter (0.42%). The third PC (13.12%) variation was exhibited also due to greater contributory traits of clean coffee yield (0.3), number of secondary branch (0.75%), canopy diameter (0.46%) and percent (%) of bearing primary branch (-0.53%). Likewise, the fourth PC variation revealed by coffee leaf rust (-0.30%) and height up to first primary branch (0.90%).

However, Chahal and Goal (2002) revealed that characters with the largest absolute values closer to unit within the first principal component influence the clustering more than those with lower absolute values closer to zero. Accordingly, fruit length (0.75%), fruit thickness (0.87%), fruit width (0.86%), coffee berry disease (0.42%) and number of secondary branch (0.32%), percent (%) of bearing primary branches (0.39%) and canopy diameter (0.42%) had more contribution to the total variation and were the one that most differentiated the clusters and should be considered in selection diverse of parent for future crossing and breeding program. However, in PC2 and PC4 coffee berry disease (-0.72) and coffee leaf rust (-0.3) were negatively contributed for total variation, respectively.

This finding partially agrees with Masreshaw, (2018) who had reported that first principal component that accounted the highest total variation (21.99%) was due to the chief contribution of positive discriminatory traits like average length of primary branches, fruit width, fruit thickness and hundred bean weights. The result partially coincided with Tounekti et al., (2017) who report that PC1 accounted for 51.01% of the total variation, which were due to greater contribution of fruit length (0.29), fruit width (0.30), fruit thickness (0.30), bean length (0.30), bean width (0.26) and bean thickness (0.22). Similarly, Yigzaw (2005) also reported characters contributing for variation for coffee genotypes includes inter-node lengths, tree height, canopy diameter, number of branches, bean and fruit character.

Table3. *Eigen values and Eigenvectors of the first four principal components (PCA) for some important traits.*

Variables	PCA1	PCA2	PCA3	PCA4
Bean yield (kg/ha)	-0.20	0.79	**0.30**	0.17
Fruit length (mm)	**0.75**	**0.33**	-0.23	0.00
Fruit thickness (mm)	**0.87**	0.17	-0.09	-0.07
Fruit width (mm)	**0.86**	**0.31**	-0.15	-0.14
Coffee berry disease (%)	**0.42**	**-0.72**	0.17	-0.11
Coffee leaf rust (%)	-0.29	**0.60**	0.22	**-0.30**
Number of secondary branch(no)	**0.32**	-0.13	**0.75**	-0.21
Height up to first primary branch (cm)	0.18	-0.05	0.00	**0.90**
Canopy diameter	0.22	**0.42**	**0.46**	0.22
% of bearing Primary branch	-0.17	**0.39**	**-0.53**	-0.12
Eigenvalue	**2.59**	**2.09**	**1.31**	**1.07**
Difference	0.50	0.77	0.24	0.23

Percent of variation (%)	**25.88**	**20.86**	**13.12**	**10.7**
Cumulative variance (%)	**25.88**	**46.74**	**59.85**	**70.55**

CONCLUSION

Most of measured quantitative morphological characters and two years yield data showed significant ($p < 0.05$) difference between each other. The accession collected from the same districts cluster into different group indicates existence of variability with accession collected the same kebele. Grouping of accessions based on multivariate methods is important to select representative accessions for breeders to get heresies segregates. The significant inter-cluster distance indicated the existence of variability and which helps to gate the erotic segregates by crossing accessions belonging to these clusters. However, the maximum inter cluster distance was observed between clusters II and VI (142.82), Followed by cluster I and VI (100.94), which help to get a superior hybrid or recombinant desirable lines. 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 selection and hybridization programs.

Principal components analysis showed that about 70.55% of the total variation among genotypes through PC1to PC4 and the total variation loaded largely by traits like canopy diameter, height up to first primary branch, percentage of bearing primary branch and coffee leaf rust. Furthermore, in order to confirm the present encouraging result, the current findings must be further studied with physiological, quality and biochemical analysis with the support of advanced molecular techniques which provides immense potential to ensure effective utilization, conservation and development of improved varieties.

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