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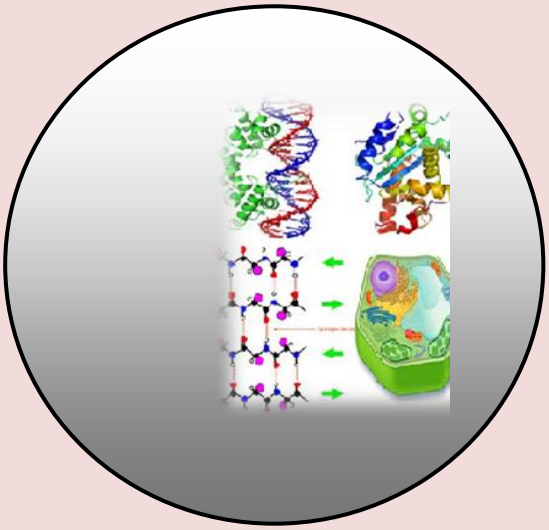

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RESEARCH PAPER

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Genetic Diversity among Coffee Hybrids for Quality Characters Tested under Jima-Tepi Environments in South-Western Ethiopia

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ABSTRACT

An experiment was conducted to determine the extent of genetic diversity among arabica coffee hybrid genotypes for quality characters at two locations in South-western region of Ethiopia. Genetic diversity among 15 Arabica coffee hybrids and one commercial hybrid check variety for 11 quality (three green bean and eight organoleptic cup quality characters were studied across four environments. The randomized complete block design was used in each environment (location-by-year combinations). The quantitative data were analyzed and the significance of effects declared using MIXED procedure of SAS and their pool data across genotypes were used for multivariate analysis. Genetic diversity of Hybrid coffee genotypes was assessed by multivariate analyses (Principal component, Genetic distance and Cluster). Variation for mean performance of coffee hybrids for all quality attributes was significant except aromatic intensity and body tastes. The highest distance was formed between check Hybrid HYCK and HC13 (8.81) while the least was formed between Hybrid HC2 and HC4 (1.39). Cluster anlysis (CA) and Principal component analysis (PCA) biplot classified the hybrids in to four main groups in most cases according to their germplasm compositions. The role of acidity, flavor and overall quality characters for the three vectors was positive across three axes (PC's, 90.74% of variance) which is the indication of the important components of genetic divergence in these materials. Hybrid HC13 and variety check was discriminated for their lower green bean physical characteristics and high cup quality attributes (PC1, 53 % of variance) and hybrid related to Sidamo coffee HC9, HC10 and HC11 for their higher aromatic intensity and aromatic quality tastes and lower mean value for most of quality attributes discriminated (PC2, 26%) from other groups. Green bean physical quality characteristics were inversely related to organoleptic quality attributes in PCA biplot indicating simultaneous gain in both characters becoming difficult. The study indicates the presence of moderate variation for coffee quality characters among the tested hybrid genotypes.

Keywords: Cluster, Cup quality characters, Genetic distance, Green bean characters, Hybrid coffee (Coffea arabica), Principal component.

INTRODUCTION

Coffee plays a key role in Ethiopian Economy in term of production, export earnings and creating job opportunity. Jima which representing midland and Tepi representing lowland coffee growing agro-ecologies stand out as the major coffee environments in country as well as regional wises found in Southwest Ethiopia. These climates are highly conducive for coffee plant & berry development, however, the quality is low especially at lowland (Wrigley, 1988, 2003).

The quality of coffee beans is affected by genetic characteristics, edapho-climatic conditions, post-harvest and roasting process and the final preparation of coffee brews (Leroy *et al*., 2006). Cup quality (Beverage quality), often referred to as liquor quality is an important attribute of coffee and acts as yardstick for price determination (Agwanda *et al*., 2003; Kathurima *et al*., 2009). *Coffea arabica* L. which represents 70% of the commercial coffee of the world and known for its quality attributes superior to Robusta coffee and thus more valued in the market (Pereira *et al.,* 2010).The breeding has the objective to transfer resistance genes from robusta coffee to *arabica* interspecific hybrid (Bertrand *et al*., 2008) and/or within arabica coffee, interaspecific hybrid (Mesfin and Bayetta, 1984). Besides conferring resistance to pests and diseases and an improvement of agronomic characteristics, these crosses may also affect the bean compositions and sensory quality of the coffees (Kitzberger *et al*., 2012) suggesting the need of monitoring the quality status of the newly developed crosses in regular basis in the breeding program.

Germplasm characterization which includes the study of divergence provides estimates for identification of genotypes/genes that may improve the phenotypic features of a cultivar after crossing. However, due to the intrinsic difficulties of a standardized assessment technique for cup quality, there is dearth of information and only few studies presented potential genetic materials to be used in this aspect.

Sobreira *et al*. (2016), studying the genetic diversity for quality among 101 arabica coffee genotypes, assessed from the Germplasm Active Bank of Minas Gerais in Brazil, verified significant divergence among the formed three clusters for all sensory quality attributes, suggesting the possibility of quality gains with the selection of promising parents. Similarly in Kenya Kathurima *et al*. (2009) assessed beverage quality of 42 elite genotypes of arabica coffee, verifying the formation of two main sensory clusters with nearly 47% dissimilarity. Despite the narrow genetic basis of Arabica coffee and the genetic similarity of the cultivars in most of the cases (Lashermes *et al*., 1996; Anthony *et al*., 2002, Setotaw *et al*., 2013), several authors found green bean physical quality and/or sensory diversity among Arabica coffee germplasm collected from different localities in Ethiopia. In these genetic materials and environments they identified two to four genetically divergent clusters and/or uncorrelated principal components (Yigzaw, 2005; Abeyot, *et al*., 2011; Olika, *et al*., 2011). The current coffee breeding program in Ethiopia had identified some elite hybrids derived from three coffee diversity groups viz., Harar, Sidamo and Southwestern Ethiopian coffee (Behailu *et al*., 2008). However, it is not clear how distant these hybrids are from each other and commercial check regarding their potential quality attributes, it understood as the genotype higher score for quality in a given environment. Prior knowledge of this information increases the probability of gains in quality after a selection and recombination process. The genetic consistency within varieties is also essential to quality assurance for any agricultural products (Hue, 2005) apart from exploiting the existing genetic diversity and heterosis in coffee bean quality. The aim of this study was to analyze the genetic divergence among hybrid coffee (*Coffee arabica* L.) genotypes regarding the potential green bean physical and cup quality characteristics under Mid-lowland environments.

MATERIAL AND METHODS

The study was conducted in two different locations in South-western region of Ethiopia, namely at Jimma Agricultural Research Center (JARC) and Tepi National Spice Research Center (TNSRC). The Jima site represents the midland and Tepi represent lowland humid coffee growing agro-ecologies. Fifteen F1 hybrids along one commercial hybrid check variety were evaluated in this study. Three main group was formed based on germplasm composition and figures followed the same letter also indicate their half-sib relationships (Table 1).

The experimental material was laid out in a Randomized Block Design (RBD) with three replications and established in July, 2008 at both locations with comprising of sixteen coffee trees of each genotype in each plot. Recommended cultural practices were followed and observations were made on the green bean physical and organoleptic cup quality parameters for two seasons (2014 and 2016). The coffee sample preparation procedures for quality analysis and data collection techniques for three green bean physical and eight organoleptic cup quality characteristics as described by Abrar *et al*. (2014) and elaborated by Fekadu *et al*. (2019) were adopted. The quality data (green bean and cup quality characters) were subjected to Analysis of Variance (ANOVA) using Proc GLM with the MIXED procedure of SAS (SAS, 2008) and effects declared significant at 5% level. The analysis of variance was performed on data from all sites. Least significant difference (LSD5%) test was used to separate the means. Multivariate analyses were conducted using mean values across environments from the restricted maximum likelihood (REML) analysis of each character using SAS (SAS, 2008). Means of each quality character was standardized before subjecting it to the multivariate analysis as was suggested by Milligan and Cooper (1987). The standardized data of quality characters were then used as an input for the Euclidian distances computing hierarchal cluster analysis (HCA) and principal component analysis (PCA). PCA and HCA were performed using SAS software package SAS (2008). Average linkage strategy and Euclidean distance was used to construct the dendrogram. PCA biplots enabled assessment of the genotypic variation on a multivariate scale, and to visualize the phenotypic correlations among characters through singular value decomposition (SVD) of a genotype by character two-way table (Yan and Tinker, 2005). The characters were centered and standardized before SVD.

Table 1. Description of the coffee hybrids and commercial check used for the study.

#	Code-name	Germplasm Composition*	Group	Cross categories†
1	HC-1	SW X Harrar	1a	CBD res x Harrar +HY
2	HC-2	SW X Harrar	1a	CBD res x Harrar +HY
3	HC-3	SW X Harrar	1a	CBD res x Harrar +HY
4	HC-4	SW X Harrar	1b	CBD res x Harrar +HY
5	HC-5	SWX Harrar	1ab	CBD res x Harrar +HY
6	HC-6	SW X SW	3ch	CBD res x CBD res +Q
7	HC-7	SW X Harrar	1b	CBD res x Harrar +HY
8	HC-8	SW X SW	3c	CBD res +Q xCBD res +Q
9	HC-9	SWX Sidama	2df	CBD res x Sidama +HY
10	HC-10	SWX Sidama	2d	CBD res x Sidama +HY
11	HC-11	SWx Sidama	2d	CBD res x Sidama +HY
12	HC-12	SWx Sidama	2e	CBD res x Sidama +HY
13	HC-13	SW X SW	3f	CBD res x high yielder
14	HC-14	SW X SW	3g	CBD res x high yielder
15	HC-15	SWx Sidamo	2eg	CBD res x Sidama +HY
Commercial hybrid check variety				
16	Aba-Buna (HYCK)	SW X SW	3bh	CBD res x high yielder

*SW=south-western Ethiopian coffee type; Harrar= Harrar coffee type; Sidamo= Sidamo coffee type

†CBD res = CBD resistant; Q = good quality; HY=high yielder; HC =Hybrid coffee, HYCK=hybrid check

RESULTS AND DISCUSSION

Analysis of variance and mean performances

Mean performances result showed significant variation for all quality parameters except aromatic intensity and body tastes among the hybrid and check variety (Table 2) indicating the opportunity to select the genotypes with desirable characters.

The mean values of the test hybrid HC7 and HC8 were desirably higher than the best check for bean size, shape and make appearance and comparable for color attribute with best check, while least score for these three green bean physical characteristics was recorded by Hybrid HC13 and HC9. On the contrary, the hybrid HC13 had highest mean score for all desirable cup quality attributes (aromatic intensity, aromatic quality, acidity, body, flavor and overall quality tastes) ranged from 3.75 to 4.06 which is moderate to strong tastes, while the lower score for four of these attributes other than aromatic intensity and aromatic quality was recorded by Hybrid HC9, HC 10 and HC11 with a range of 3.33-3.60 which is medium value for cup quality attributes. Hybrid HC1 followed by HC2 and HC7 combined above average value for both green bean physical characteristics and desirable cup quality attributes.

Table 2. Mean green bean physical and organoleptic quality traits of fifteen hybrids and hybrid check evaluated across four Jimma-Tepi environments.

Hybrids/ checks	SC(14)	SM	Color	AI	AQ	AC	AS	BI	BO	FL	OAQ
HC1	97.06	4.32	4.36	3.83	3.85	3.85	0.67	0.75	3.75	3.66	3.72
HC2	97.08	4.28	4.44	3.75	3.88	3.65	0.56	0.77	3.67	3.60	3.58
HC3	97.56	4.42	4.29	3.67	3.50	3.77	0.79	0.67	3.65	3.59	3.58
HC4	97.86	4.39	4.44	3.81	3.75	3.67	1.04	1.00	3.58	3.60	3.61
HC5	97.70	4.33	4.18	3.73	3.63	3.60	0.75	0.67	3.60	3.56	3.56
HC6	97.61	4.32	4.33	3.67	3.42	3.54	0.79	0.54	3.63	3.52	3.52
HC7	98.53	4.63	4.36	3.75	3.63	3.54	0.63	0.58	3.60	3.56	3.56
HC8	98.24	4.51	4.63	3.79	3.75	3.48	0.75	0.79	3.50	3.41	3.40
HC9	95.03	4.06	4.00	4.08	4.04	3.60	0.98	0.96	3.52	3.47	3.51
HC10	97.52	4.11	4.04	4.21	3.67	3.40	0.88	0.88	3.50	3.33	3.33
HC11	97.56	4.15	4.13	3.81	3.75	3.42	0.75	0.75	3.50	3.33	3.38
HC12	96.78	4.19	4.19	3.67	3.69	3.69	0.56	0.50	3.63	3.63	3.71
HC13	93.24	3.86	4.03	3.90	4.06	3.92	0.50	0.46	3.85	3.75	3.92
HC14	94.69	4.17	4.14	3.50	3.54	3.68	0.48	0.52	3.71	3.65	3.67
HC15	97.62	4.19	4.35	3.63	3.33	3.42	0.94	0.94	3.58	3.38	3.35
HYCK	97.44	4.31	4.42	3.44	3.44	3.47	1.00	0.96	3.45	3.39	3.34
Mean	96.97	4.27	4.27	3.77	3.68	3.61	0.75	0.73	3.61	3.53	3.55
LSD(0.05)	0.85	0.26	0.24	NS	0.30	0.22	0.28	0.28	NS	0.24	0.22
C.V%	1.08	7.64	7	14.47	10.05	7.48	44.91	47.40	8.25	8.23	7.64

SC14% = percent of above screen 14(5.60mm), SM = Shape and make, AI =Aromatic Intensity, AQ =Aromatic Quality, AC = Acidity, AS= Astringency, BI= Bitterness, BO = Body, FL = Flavor and OAQ = Overall Quality; NS, non-significant

The existing variation among the genotypes was further examined by removing the effects of environments and evaluators. These high CV values for these two characters (astringency and bitterness tastes) and high proportion of error variance for most of low heritability exhibited characters could be emerged from three main sources of variability. One source of variation is the differences among the evaluators in how they perceived, judged and scored each quality parameter. The second of variation is the environments. The other source of variation is obviously the actual differences between genotypes for each of these quality parameters. Nevertheless, the presence of genotypic differences has been clearly reflected after differences due to environments and evaluators were averaged out (Table 2) and displayed in dendrogram (Figure 2). The two Characters (AS and BI) with low experimental precision (high CV values >44%) and that found highly influenced by environment (Table 2) were deselected for multivariate analysis.

Divergence analyses

Principal Component Analysis (PCA), Genetic distance analysis and Clustering Analysis (CA) were used to the simultaneous evaluation of the mean value of green bean physical and organoleptic quality characters for different arabica coffee hybrids and commercial varieties for standardized data (Table 3, Figure 1, Figure 2 and Table 4 and 5).

Principal Component Analysis (PCA)

The interpretation of data from sensory analysis, using the Principal Component Analysis (PCA), is demonstrated as a clear example of the versatile method in coffee (Maeztu *et al.*, 2001).

Table 3. Eigenvectors and eigenvalues of the three principal components (PCs) for quality characters of coffee genotypes evaluated under Jima and Tepi environments.

Characters	Eigenvectors		
	PC1	PC2	PC3
Bean sizes in above screen 14 % (SC14)	-0.378	0.210	0.311
Shape and Make(SM)	-0.253	0.442	0.376
Color	-0.221	0.461	0.298
Aromatic Intensity (AI)	0.080	-0.476	0.571
Aromatic quality(AQ)	0.286	-0.250	0.566
Acidity(AC)	0.408	0.234	0.116
Body(BO)	0.398	0.229	-0.069
Flavor (FL)	0.386	0.331	0.060
Overall quality(OAQ)	0.426	0.208	0.065
Eigenvalue	4.76	2.32	1.08
Difference	2.44	1.24	0.69
Percent of total variance explained	52.94	25.79	12.01
Cumulative percent of total variance explained	52.94	78.73	90.74

In this way, the first three PC having greater than one eigen values explained 90.74% of the variation, 52.94% explained by the first component, and 25.79% by the second component, and 12.01% by the third component (Table 3). The first PC describes the coffee cultivars mainly in relation to the cup quality attributes positively and green bean characteristics (negatively). The second PC describes mainly in reverse way, green bean characteristics (positively) in contrast to aromatic intensity and aromatic quality. The third PC describes with greater contribution of aromatic intensity and aromatic quality (Table 3). These coefficients were further transformed into PC scores to describe genotype and quality attributes relationships in PC biplot using the first two PC's. Moreover, the PC biplot is used to display genotype by trait combination to identify genotypes that are best for certain traits and reveals interrelationships between traits to be used as independent selection criteria (Yan and Rajcan, 2002). Eigenvector coefficients of a relatively large magnitude translate into larger correlations with the original variable and vice versa and reveal its discrimination powers (Table 3 and Figure 1). The first component (PC1) was positively correlated to, aromatic quality, acidity, body, flavor and overall quality values, and negatively correlated to all green bean physical characteristics (SC14, SM and Color). The PC2 was positively correlated to green bean physical characteristics. The orthogonal solutions (uncorrelated) also confirmed that high loading of cup quality characters in PC1 and leveled as cup quality profile component. Three green bean physical characters mainly also loaded on the second component, which was labeled as the raw bean quality describing components. The roles of AC, FL and OAQ for all the three vectors were positive across three axes which is the indication of the important components of genetic divergence in these materials (Table 3).

Genetic distance analysis

The data matrix of the tested quality attributes formed the basis of Euclidean genetic distance calculations. The Euclidean distance between all 120 pairs of hybrid genotypes ranged from 1.39-8.81 (Table 4) with the mean value of 3.94. The highest distance formed between check hybrid HYCK and HC13, while the least formed between hybrid HC2 and HC4. The distribution of the genetic distance values indicates that 69% and 43% of the pair comparisons had values greater than 3.0 and average distance (3.94), respectively. The existence of such moderate to wide range of genetic distance among the hybrids showed the presence of moderate to wide range of genetic variations among them and an opportunity to improve the genetic basis of arabica coffee by implementing crossing.

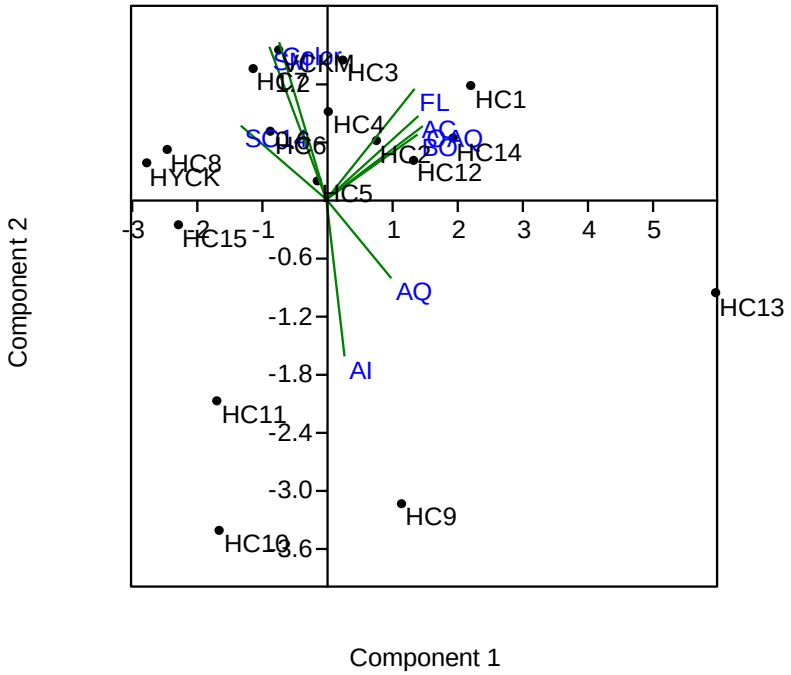

Figure 1. PCA Biplot of the coffee genotypes considering green bean physical and organoleptic quality characters evaluated at Jima-Tepi Environments.

Table 4. Estimates of genetic distance based on quality data for all pair-wise combinations of 16 coffee genotypes.

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HC1	0.00														
HC2	1.91	0.00													
HC3	2.50	2.34	0.00												
HC4	2.36	1.39	1.91	0.00											
HC5	2.97	2.12	1.62	1.79	0.00										
HC6	3.62	2.59	1.78	2.28	1.49	0.00									
HC7	3.58	2.71	2.23	1.87	2.05	2.17	0.00								
HC8	4.84	3.33	3.84	2.76	3.44	3.16	2.56	0.00							
HC9	4.69	4.07	4.97	4.38	3.88	4.84	5.28	5.41	0.00						
HC10	5.99	4.99	5.27	4.80	4.03	4.40	4.95	4.68	3.30	0.00					
HC11	5.23	3.87	4.21	3.80	2.88	3.20	3.92	3.55	3.33	2.22	0.00				
HC12	2.28	2.04	2.01	2.25	1.66	2.48	3.25	4.55	3.88	5.02	3.91	0.00			
HC13	4.60	5.56	6.30	6.39	6.25	7.00	7.64	8.68	5.39	7.96	7.60	4.89	0.00		
HC14	3.41	3.32	2.94	3.84	3.04	3.23	4.45	5.70	4.73	6.04	4.90	2.05	4.68	0.00	
HC15	5.31	3.97	3.56	3.81	2.98	1.96	3.58	3.36	5.28	3.98	2.69	4.02	8.23	4.45	0.00
HYCK	5.68	4.21	3.80	3.86	3.37	2.67	3.61	2.97	5.69	4.89	3.16	4.34	8.81	4.83	1.87
>3.0	10	8	7	7	6	6	8	7	7	5	4	3	3	2	-

Cluster analysis

The HCA indicated four main groups of coffees in most case according to germplasm composition (Figure 2) and the average value for green bean physical and organoleptic quality characters for each group (Table 5). It could be observed that there was genetic similarity among the coffees in each group. The first main group formed by three subgroups, the first subgroup comprised hybrid HC1, HC2 and HC4 whose pedigree contains Harrar coffee with Southwestern Ethiopian coffee germplasm composition, hybrid HC1 and HC2 also related in their genetic back ground, the second group comprised hybrid HC3, HC5, HC6 and HC7, while the third subgroup formed by hybrid HC12 and HC14 (Figure 2).

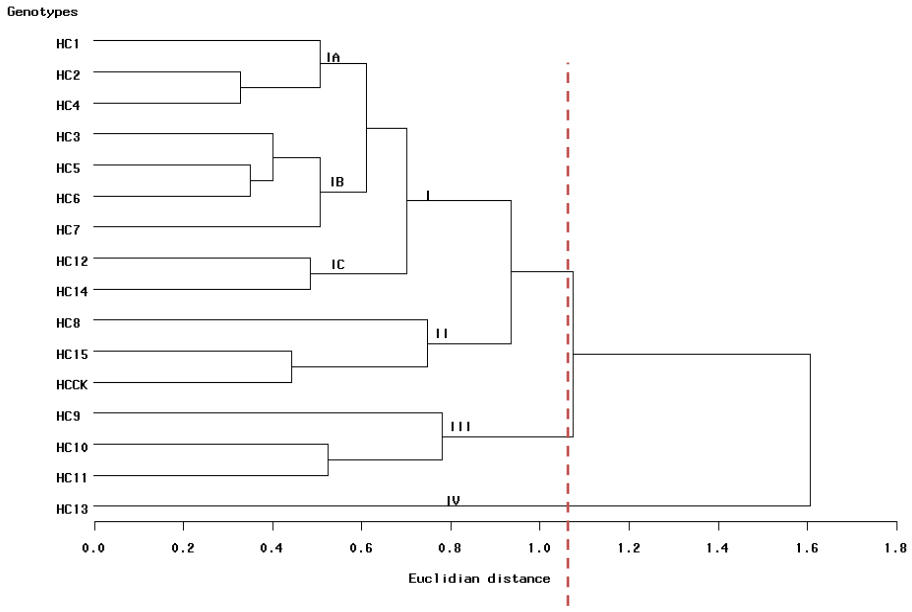

Figure 2. Dendogram of the coffee genotypes considering green bean physical and organoleptic quality characters evaluated at Jima and Tepi environments in Southwestern Ethiopia.

Table 5. Average values, for green bean physical and organoleptic quality characters for each group formed by Cluster analusis.

Group	SC14	SM	Color	AI	AQ	AC	BO	FL	OAQ
1	97.21	4.34	4.30	3.71	3.65	3.67	3.65	3.60	3.61
2	97.70	4.34	4.47	3.62	3.51	3.46	3.51	3.39	3.36
3	96.70	4.11	4.06	4.03	3.82	3.47	3.51	3.38	3.41
4	93.24	3.86	4.03	3.90	4.06	3.92	3.85	3.75	3.92
Mean	96.23	4.16	4.22	3.82	3.76	3.63	3.63	3.53	3.58
CV%	2.12	5.50	4.95	4.85	6.30	5.96	4.43	5.05	7.11

SC14% = percent of above screen 14(5.60mm), SM = Shape and make, AI =Aromatic Intensity, AQ =Aromatic Quality, AC = Acidity, BO = Body, FL = Flavor and OAQ = Overall Quality

Sub-clustering, however, continued with closely related genotypes grouping together down the dendrogram. The first group in generally characterized for higher level of green bean physical characteristics, lower score of aromatic intensity and aromatic quality and for their average scores for other four cup quality attributes. Cluster II contained three hybrids (HC8, HC15 and HCCK) whose pedigree contains purely south-western Ethiopian coffee germplasm except HC15. The third group was formed by the genetic back ground associated to sidamo coffee (HC9, HC10 and HC11) (Figure 2) and was mainly discriminated for its low level of cup quality attributes except AI (Figure 1 and Table 5). The fourth group was formed by singleton hybrid HC13 (Figure 2). These coffee types were allocated at the right positive side of PC1 and were discriminated for their lower green bean physical characteristics (bean size, shape and make, and color) and high cup quality attributes (aromatic intensity, aromatic quality, acidity, body, flavor and overall quality attributes) (Table 5).

It was possible to infer from the factor coefficients and scores those green bean physical characteristics (SC14, SM and color), were inversely related to aromatic intensity, aromatic quality, acidity, flavor and overall quality notes (Table 3 and Figure 1). Similar inverse relationships in cocoa raw quality characteristics with fruity, acid and floral flavors, attributes were reported by Sukha *et al.*, 2007; in other studies lack of significant correlation between the two group of quality characteristics in coffee reported by Dessalegn, *et al*., 2008 and Kathurima, *et al*., 2009.

However, in the present study strong positive relationships were observed between green bean physical characteristics among themselves, and between cup quality characters, flavor, body and overall quality standard, while aromatic intensity and aromatic quality showed lack of association with the former cup quality attributes. Such positive correlation among desirable cup quality characters in hybrid coffees was also reported by Kathurima, *et al*., 2009, Gichimu,*et al*., 2012; Gimase *et al*., 2014.

CONCLUSION

Mean performances result showed significant variation for all quality parameters except aromatic intensity and body tastes among the hybrid and check varieties indicating the opportunity to select the genotypes with desirable characters. This quality variation among the hybrids, further, substantiated with PCA biplot and CA grouped fifteen hybrids and one hybrid check variety into four main clusters. These results also confirm that not only the geographical background, but also intervarietal hybridization significantly contribute to creating genetic variations. The first three components of PCA having greater than one eigen values contributed 94.74% of the variability in the green coffee bean physical and cup quality attributes. This study indicated the presence of moderate levels of genetic diversity among the genotype for evaluated characters.

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