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Genetic diversity among coffee hybrids for quality characters tested under highland environments in southwestern 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 different sites in South-western region of Ethiopia. Genetic diversity among eight Arabica coffee hybrids and two standard check 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 site (site-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 taste. The highest distance was formed between Hybrids HC4 and HC5 (8.56) while the least was formed between Hybrids HC2 and HC3 (0.93). CA and PCAbiplot classified the hybrids in to two main group. The role of aromatic quality, acidity, body, flavor and overall quality characters for the two vectors was positive across three axes (PC's, 81.18% of variance) which is the indication of the important components of genetic divergence in these materials. Hybrid HC5 and variety check was discriminated for their lower green bean physical characteristics and high cup quality attributes (PC1, 65.56 % of variance) and hybrid HC4 for its opposite behavior (PC2, 15.62%) from other groups. Green bean physical quality characteristics were inversely related to organoleptic quality attributes in PCAbiplot indicating simultaneous gain in both characters becoming difficult. The study indicates the presence of low to moderate variation for coffee quality characters among the tested coffee genotypes.

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

Introduction
Coffee, as export commodity, plays key role in national economies of nearly 80 developing world countries including Ethiopia. Its role, in Ethiopian Economy, especially in term of production, export earnings and creating job opportunity is also great. High land coffee growing agro-climate constitute, one of the major coffee growing agro-ecology in south-western Ethiopia. These climates are highly conducive for coffee plant & berry development to produce quality coffee beans.

However, the quality of coffee beans is affected by so many factors vis-a-vis genetic characteristics, edapho-climatic conditions, post-harvest and roasting process and the final preparation of coffee brews (Leroy *et al*., 2006) [14]. Coffee breeding programs in Ethiopia has identified a number of heterotic interaspecific hybrids for yield and resistance to major diseases mainly to coffee beery disease (CBD) which is the major production constraint of highland coffee environment. An improvement of the agronomic characteristics of these crosses may also affect the bean compositions and sensory quality of the coffees (Kitzberger *et al*., 2012) [12] suggesting the need of monitoring the quality status of the newly developed crosses in regular basis in the breeding program.

Determination of genetic diversity 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. Kathurima *et al.* (2009) [11], studying the genetic diversity for quality among of 42 elite genotypes of arabica coffee, verifying the formation of two main sensory clusters with nearly 47% dissimilarity. Sobreira *et al*. (2016) [19]

assessed beverage quality of 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.

Several authors found green bean physical quality and/or sensory diversity among Arabica coffee germplasm collected from different localities in Ethiopia, 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) [13, 3, 18]. Yigzaw, 2005; Abeyot, *et al*., 2011 and Olika, *et al*., 2011, in their genetic materials and environments, have identified two to four genetically divergent clusters and/or uncorrelated principal components.

The current coffee breeding program in Ethiopia had identified some elite hybrids derived from Southwestern Ethiopian coffee diversity (Behailu *et al*., 2008) [14]. 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) [10] 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 highland Gera environments.

Material and Methods

The study was conducted at two different sites for two production seasons in Gera district of the south-western region of Ethiopia. The sites were Gera Research Station and on-farm location around the station. The study sites represent the highland humid coffee growing agro-ecology and are well known as hot spot for coffee berry disease (CBD). Eight F_1 hybrids along two standard check varieties were evaluated in this study (Table 1). The experimental material was laid out in a Randomized Block Design (RBD) with three replications and established in July, 2008 at both sites 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. 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) [1] and elaborated by Fekadu *et al*. (2019) [6] were adopted. The quality data (green bean and cup quality characters) were subjected to Analysis of Variance (ANOVA) using ProcGLM with the MIXED procedure of SAS (SAS, 2008) and effects declared significant at 5% level. 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). 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) [22]. The characters were centered and standardized before SVD.

Table 1: Description of the coffee hybrids and commercial checks used for the study

Code-name	Parent	Cross*	Cross categories†
HC¶1	P_1, 7455	P_1 X P_2	CBD res +Q x CBD res +Q
HC2	P_2, 7530	P_1 X P_3	CBD res +Q x CBD res +Q
HC3	P_3, 74153	P_1 X P_4	CBD res +Q x CBD res +Q
HC4	P_4, 74167	P_5 X P_2	CBD res +Q x CBD res +Q
HC5	P_5, 74158	P_3 X P_5	CBD res +Q x CBD res +Q
HC6		P_3 X P_2	CBD res +Q x CBD res +Q
HC7		P_1 X P_5	CBD res +Q x CBD res +Q
HC8		P_4 X P_2	CBD res +Q x CBD res +Q
Ababuna (HCCK)		hybrid check	CBD res x high yielder
74110 (VCK)		variety check	CBD res

* P=Parent, †CBD res = CBD resistant; Q = good quality; HY = high yielder; ¶HC =Hybrid coffee

Results and Discussion

Analysis of variance and mean performances

Mean performances result showed significant variation for all quality parameters except aromatic intensity taste among the hybrid and check varieties (Table 2) indicating the opportunity to select the genotypes with desirable traits. In assessment of quality attributes, their mean range values largely varied between 3.8 to 4.3 for aromatic quality and least varied 3.9 to 4.0 for aromatic intensity (Table 2).

Table 2: Mean and range values of hybrids and checks of arabica coffee evaluated at two locations for two years.

Entry	SC(14)	SM	Color	AI	AQ	AC	AS	BI	BO	FL	OAQ
Range	94.1-98.7	4.1-4.7	4.4-4.8	3.9-4.0	3.8-4.3	3.4-4.0	0.50-0.79	0.33-0.75	3.7-3.9	3.4-3.9	3.4-3.9
Mean	97.30	4.53	4.66	3.95	4.01	3.74	0.63	0.48	3.76	3.69	3.68
LSD(0.5)	0.66	0.23	0.26	NS	0.29	0.22	0.25	0.19	0.17	0.21	0.21
Experimental C.V%	0.83	6.31	6.98	7.99	9.03	7.21	48.89	50.21	5.49	7.07	7.12

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

On other hand, higher experimental coefficient of variation (CV) were observed for two characters (astringency and bitterness tastes) and high proportion of error variance for most of low heritability exhibited characters also recorded (data not shown). Similar manner of high CV and low heritability values for these two quality characters attributes

was reported by Fekadu *et al*. (2019; 2020) [6] among hybrid coffee genotypes evaluated under Jimma-Tepi mid-lowland environments in Southwestern Ethiopia. The existing variation among the genotypes was further examined by removing the effects of environments and evaluators in multivariate analysis. The two Characters (AS and BI) with low experimental precision (high CV values >48%) and that found highly influenced by environment (Table 2) were deselected for multivariate analysis.

Divergence analyses

Principal Component Analysis (PCA) and 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, Fig. 1, Table 4 and 5).

The principal component analysis (Table 3) revealed that two principal components PC1and PC2 with eigenvalues 5.90 and 1.41, respectively, have accounted for 81.18% of the total variation. Eigenvector coefficient of a relatively large magnitude translates into larger correlations with the original variable and vice versa and reveals its discrimination powers (Fig. 1and Table 3).

PC biplot is used to display genotype by trait combination to identify genotypes that are best for certain traits (Yan and Rajcan, 2002) [22]. The first two components of PCA explained 81.18% of the variability in the green coffee beans, thus it is adequately possible to explain the variation using scatter plots. The HCA, on other hand, classified the coffee genotypes into three main groups. The orthogonal solutions (uncorrelated) also confirmed that high loading of cup quality traits in PC1 and leveled as cup quality profile component. Three green bean physical traits also loaded on the second component, which was labeled as the raw bean quality describing components. The role of acidity, flavor and overall quality attributes for both the vectors were positive across two axes which is the indication of the important components of genetic divergence in these materials (Table 3). The first component (PC1) accounted for 65.56% of variance and was positively correlated to aromatic intensity, aromatic quality, acidity, body, flavor and overall quality values, and negatively correlated to all green bean physical characteristics (bean size, shape and make, and color). Coffee hybrid HC5 and check variety VCK were discriminated for their higher cup quality attributes mainly (aromatic quality, acidity, flavor and overall quality attributes) and lower for green bean physical attributes in group three (Fig. 1 and Table 4). The PC2 (15.62%) was positively correlated to green bean physical characteristics and negatively for aromatic intensity and body tastes. Coffee hybrid HC4, located at the bottom of the plan, were discriminated for its lower desirable cup quality attributes mainly (aromatic quality, acidity, flavor and overall quality attributes) in biplot (Fig. 1) and group two (Table 4). The first group in general characterized for their average performance for most of the quality attributes, beside that the two hybrids, HC1 was characterized for its large bean size (SC14) and placed on the top side, while the second hybrid H8 for its relatively low aromatic intensity on bottom side of PC plane from their group (Fig. 1 and Table 4).

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

Characters	Eigenvectors	
	PC1	PC2
Bean sizes in above screen 14 % (SC14)	-0.344	0.394
Shape and Make(SM)	-0.282	0.346
Color	-0.296	0.485
Aromatic Intensity (AI)	0.314	-0.207
Aromatic quality(AQ)	0.364	0.23
Acidity(AC)	0.337	0.434
Body(BO)	0.296	-0.081
Flavor (FL)	0.367	0.304
Overall quality(OAQ)	0.385	0.275
Eigenvalue	5.9	1.41
Difference	4.49	0.56
Percent of total variance explained	65.56	15.62
Cumulative percent of total variance explained	65.56	81.18

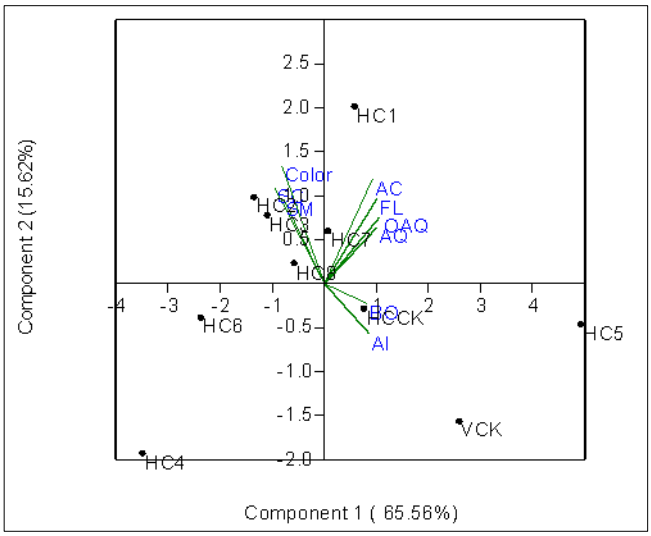

Fig 1: PCA Biplot of the coffee genotypes considering green bean physical and organoleptic quality characters evaluated at highland Gera Environments in Southwestern Ethiopia

Table 4: Average values, for green bean physical and organoleptic quality characters for each group formed by HCA.

Group	SC14	SM	Color	AI	AQ	AC	BO	FL	OAQ
1	97.64 (0.72)	4.563 (4.03)	4.70 (1.17)	3.95 (1.27)	3.99 (2.12)	3.76 (2.14)	3.74 (1.31)	3.70 (1.77)	3.69 (1.88)
2	98.19 (0)	4.64 (0)	4.61 (0)	3.88 (0)	3.81 (0)	3.44 (0)	3.75 (0)	3.42 (0)	3.44 (0)
3	94.10 (0.08)	4.17 (1.87)	4.49 (2.20)	4.07 (1.04)	4.18 (4.57)	3.87 (3.29)	3.82 (1.48)	3.80 (1.85)	3.84 (2.95)
Mean	96.65	4.46	4.60	3.96	3.99	3.69	3.77	3.65	3.66
CV%	2.30	5.64	2.29	2.45	4.63	6.05	1.16	5.74	5.53

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

Genetic distance analysis

The data matrix of the tested quality attributes formed the basis of Euclidean genetic distance calculations. The

Euclidean distance between all 45 pairs of hybrid genotypes ranged from 0.93-8.56 (Table 5) with the mean value of 3.92. The highest distance formed between hybrid HC4 and HC5, while the least distance formed between hybrid HC2 and HC3. It was low to moderate (<10.0). The distribution of the genetic distance values indicates that 64% and 47% of the pair comparisons had values greater than 3.0 and average distance (3.92), respectively. This type of genetic distance distribution was also reported by Fekadu *et al*. (2020) [7]. The existence of such moderate range of genetic distance among the hybrids showed the presence of moderate range of genetic variations among them and an opportunity to improve the genetic basis of arabica coffee by implementing crossing.

Table 5: Estimates of genetic distance based on quality data for all pair-wise combinations of ten coffee genotypes

Genotypes	1	2	3	4	5	6	7	8	9
HC1	0.00								
HC2	3.09	0.00							
HC3	2.57	0.93	0.00						
HC4	5.71	4.24	3.98	0.00					
HC5	5.11	6.75	6.36	8.56	0.00				
HC6	3.91	2.19	1.88	2.16	7.37	0.00			
HC7	2.01	2.65	2.09	4.48	5.08	2.92	0.00		
HC8	3.61	2.87	2.74	4.69	6.22	3.22	3.02	0.00	
HYCK	2.69	2.77	2.25	4.87	4.53	3.27	2.35	3.27	0.00
VCK	4.61	4.82	4.47	6.54	3.49	5.30	4.02	4.34	2.56
>3.0	6	3	3	5	5	3	2	2	-

The PC biplot is also used to display genotype interrelationships between traits to be used as independent selection criteria (Yan and Rajcan, 2002) [22]. Thus, it was possible to infer from the factor coefficients and scores those green bean physical characteristics (bean size, shape and make, and color), were inversely related to aromatic intensity, aromatic quality, acidity, flavor and overall quality notes (Table 3 and Fig. 1). Similar inverse relationships in cocoa raw quality characteristics and flavor attributes with fruity, acid and floral flavors, attributes were reported by Sukha *et al.* (2007) [20], while lack of significant correlation between the two quality characteristics in coffee reported by Dessalegn *et al*. (2008) [5] and Kathurima *et al*. (2009) [11]. However, in the present study the above two quality groups were independently positively related among themselves. Such positive correlation among desirable cup quality traits in hybrid coffees have also been reported (Dessalegn *et al*., 2008; Kathurima *et al*., 2009; Gichimu *et al*., 2012; Gimase *et al.*, 2014) [5, 11, 9, 8]. However, the present finding partly deviate from Walyaro (1983) [21] who reported that body notes lacks strong correlation with overall quality and concluded as unimportant for the assessment of liquor quality in Arabica coffee. Such discrepancy could be attributed to the variation in coffee materials and test environments used in different studies. In view of the above points simultaneous selection for both beverage quality and desirable green bean physical characteristics is difficult in agreement to Kathurima *et al*. (2009) [11], however, it would be possible to find the coffee genotypes that exhibit average performance for two attributes, in present study the hybrids placed on the right top side of PC plane (Fig. 1) would possibly met this requirements.

Conclusion

Mean performances result showed significant variation for all quality parameters except aromatic intensity taste 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 PCAbiplot and CA grouped eight hybrids and two check varieties into three main clusters. The first two components of PCA having greater than one eigenvalues contributed 81.18% of the variability in the green coffee bean physical and cup quality attributes. This study indicated the presence of low to moderate levels of genetic diversity among the genotype for evaluated characters.

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