

Multivariate Analysis of Phenotypic Diversity in the South Ethiopian Coffee (*Coffea arabica* L.) for Quantitative Traits

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Abstract

Coffee is the most important export crop of the south Ethiopian region with more than 46 percent share of the national market. It covers more than 185 000 ha of land in 50 Woredas (districts) with 11 are high, 7 medium and 32 are low coffee producers. Garden coffee comprises 130000 ha, semi forest 45 000 ha and forest coffee 10000 ha where the semi forest and forest coffee production systems are pertinent to the western part of the region. A field experiment on evaluation of 41 south Ethiopian coffee accessions with 2 standard checks of the southwest Ethiopian origin was conducted using Randomized Complete Block Design at Wonago Research Sub-Station during 1999-2000 cropping seasons. Data on 7 morphological agronomic characters, average of three years data on severity of CBD and CLR infestations and yield was obtained for the 43 genotypes. The germplasm accessions differed significantly for all the 7 morphological agronomic characters and coffee bean yield in the univariate analyses of variances indicating the prevalence of variability among south Ethiopian coffee germplasm accessions. Further, the first four principal components explained 82.63 percent of the total variation prevalent within the germplasm accessions out of which 32.52 percent was explained by the first principal component. Average linkage cluster analysis using Mahalanobis (D^2) distance for the 10 characters grouped the 43 accessions in to 9 clusters. The number of accessions per cluster ranged from 1 in cluster IX to 13 in cluster II. The clustering pattern of the accessions revealed the prevalence of genetic diversity in the south Ethiopian coffee for the characters considered. The maximum inter-cluster distance was observed between clusters V and VII while the minimum was observed between clusters VI and VII. The study highlighted the possibility of using accessions of the distant clusters as potential candidates for the genetic improvement of south Ethiopian coffee through crossing and selection.

Keywords: Cluster analysis; *Coffea arabica*; Genetic diversity; South Ethiopia; Germplasm

Introduction

Coffee is the most important export crop of the south Ethiopian region with more than 46 percent share of the national market. It covers more than 185000 ha of land in 50 Woredas (districts) with 11 are high, 7 medium and 32 are low coffee producers. Garden coffee comprises 130 000ha, semi forest 45 000ha and forest coffee 10 000ha where the semi forest and forest coffee production systems are pertinent to the western part of the region. In 2005 cropping season, the annual coffee production of the region was 131 000 tons out of which 100 302 tons was exported as 60 percent washed and 40 percent dry processed [1]. The average yield of coffee in the region is 500 kg/ha (for local or landrace cultivars) while 800 kg/ha for the released coffee berry disease resistant cultivars. Though the region is highly endowed with suitable environments and immense genetic diversity for coffee production, the productivity of coffee per unit area remains very low as compared to world average. This is attributed mainly due to the lack of improved cultivars for central and eastern coffee growing areas of the region, shortage of improved agronomic technologies and prevalence of diseases mainly Coffee berry disease and coffee wilt disease. Moreover, physiological problems such as die back due to absence of shade trees coupled with minimum use or absence of agricultural inputs in the smallholder coffee orchards of Central and Eastern Zones of the Region is very common [1].

The exploitation of genetic diversity for crop improvement should be the ultimate objective of genetic resources exploration and conservation. The vital stages of evaluation and incorporation of valuable characters such as disease resistance and/or tolerance

to environmental stress factors into new varieties appeared to be justifications of genetic resources conservation, characterization, and evaluation [2-4]. In cognizant of this fact, renewed efforts of coffee germplasm collection were undertaken consecutively for 3 years (1995-1997) from different coffee growing areas of Central and Southeastern part of the South Ethiopian Region by Jima Agricultural Research Center (JARC) and as a result more than 350 accessions were collected and maintained at the center.

Several workers have estimated the extent of genetic diversity present from the different sources of *arabica* coffee germplasm collections. For instance, a study by Catter on second progeny *arabica* coffee collections of Ethiopian origin indicated the prevalence of high level of variability in morphological, agronomic and biochemical characteristics [5]. The genetic diversity analysis conducted by Lashermes, et al. using RAPD markers on cultivated and sub-spontaneous accessions of *arabica* coffee confirmed the narrow genetic base of commercial cultivars (3 typica and 3 bourbon types) [6]. On the other hand, they reported the existence of large genetic diversity within the sub-spontaneous material, which consisted of 11 samples

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Received February 26, 2015; Accepted April 30, 2015; Published May 04, 2015

Citation: Gessese MK, Bellachew B, Jarso M (2015) Multivariate Analysis of Phenotypic Diversity in the South Ethiopian Coffee (*Coffea arabica* L.) for Quantitative Traits. Adv Crop Sci Tech S1: 003. doi:[10.4172/2329-8863.S1-003](http://dx.doi.org/10.4172/2329-8863.S1-003)

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representing the different coffee growing areas in Ethiopia. Further, they have suggested the prevalence of an east-west differentiation in the Ethiopian coffee germplasm. Similarly, Montagnon and Bouharmont characterized 148 *arabica* coffee accessions for phenotype diversity under field condition [7]. They have evaluated the accessions using eighteen different morphological and agronomic traits by employing multivariate analysis and identified two main groups in the coffee accessions. According to them, accessions of group I have a more erect branching habit, narrower leaves, and were more resistant to coffee leaf rust and coffee berry disease than accessions of group II. They further opined that group I mostly contained Ethiopian *arabica* coffee accessions collected from west of Great Rift Valley, whereas group II contained commonly cultivated varieties throughout the world and Ethiopian accessions collected from east of Great Rift Valley in Ethiopia. Kebede and Bellachew studied the genetic diversity of Hararghe coffee (*Coffea arabica* L.) landraces (using quantitative morphological characters), which is characterized as garden coffee located in the eastern Ethiopia and reported the prevalence of enormous genetic diversity among the landrace collections [8]. On the same basis, the present study was conducted in order to estimate the genetic diversity among South Ethiopian coffee germplasm collections and to facilitate for use in the ongoing breeding program.

Materials and Methods

The experiment was carried out at Wonago Agricultural Research Sub-Station (WARSS) located near Wonago town, 99 km south of Awassa at an altitude of 1850 meters above sea level. The sources of test materials were 41 South Ethiopian coffee accessions that were collected from 6 Woredas of Gedeo, Sidama and Wolayta zones and maintained

in the field at WARSS (Table 1). The 41 accessions and 2 released coffee berry disease (CBD) resistant cultivars were planted in July 1999 using Randomized Complete Block Design in 4 replications. Each plot had 10 plants with a spacing of 2m by 2m between plants and rows. All field management practices were applied to all plots uniformly as recommended (JARC, 1996). Four plants were taken at random from each accession and labeled for data collection on different growth characters listed in Table 2. Jima Agricultural Research Center's coffee breeding and genetics conventional methods were employed for data collection [9]. Data on 7 morphological agronomic characters vis-à-vis stem girth, plant height, number of primary branches, number of stem nodes, length of longest primary branches, canopy diameter and internode length of the main stem; percent disease infestation levels on CBD and coffee leaf rust (CLF) and average of 3 years clean coffee yield was obtained on the 43 genotypes (Tables 3 and 4).

Data analysis

A two-way analysis of variance (using MSTATC statistical software package) was computed for each quantitative character in order to identify the variability among accessions. Further, the data were standardized to a mean of zero and a variance of unity, to avoid differences in scales used for analyses before undertaking principal component and divergence analyses. Clustering was performed by average linkage method and the number of clusters was determined by examining the pseudo F statistic and the pseudo t2 statistic using SAS software package [10]. Genetic diversity between clusters, as standardized Mahalanobis D² values between clusters and principal components based on correlation matrix, were calculated using the same software employed in cluster analysis. The D² values obtained

Place of collection (Woreda)	Collection number (genotype identity)	Total number of genotypes per Woreda	Remark
Wonago	85190, 85181, 85188, 85196, 85195, 85193, 85200, 85180, 3170, 3270, 1377, 2077, 2777, 3677, 3977, 2181	16	Gedeo Zone
Yirgachefe	85245, 85238, 85257, 85237, 85241, 85252, 85260, 85246, 85259, 2970, 3070	11	Gedeo Zone
Aleta Wondo	85264, 85296, 85265, 85263, 85288, 85269, 85294	7	Sidama Zone
Dale	3470, 3670	2	Sidama Zone
Bolososore	1681, 2081	2	Wolayta Zone
Sodozuria	1870	1	Wolayta Zone
Southwest Ethiopia	75227, 744	2	Released CBD Resistant cultivars
Unknown	85213, 85232	2	

Table 1: Details of germplasm accessions used in the study.

Characters	PC 1	PC 2	PC 3	PC 4
Stem girth	0.320244	0.218051	0.027805	-0.496615
Plant height	0.365684	0.254400	0.368768	0.270685
Number of primary branches	0.393347	-0.409839	-0.094420	0.040422
Number of nodes on the stem	0.400710	-0.426543	-0.002449	0.062014
Length of longest primary	0.347277	0.267598	-0.0210344	-0.310869
Canopy diameter	0.494893	0.100075	0.041793	-0.085156
Internode length of the stem	0.085595	0.546520	0.348756	0.285654
% CBD infestation	0.003899	-0.290628	0.525117	-0.391957
% CLR infestation	0.187800	-0.237740	0.198156	0.529851
Average yield	0.200743	0.128586	-0.609656	0.234012
Eigenvalues	3.251856	2.091618	1.738643	1.180991
%Total variance	32.52	20.92	17.39	11.81
%Cumulative variance	32.52	53.43	70.82	82.63

Note: PC1, PC2, PC3, and PC4 are the first four principal components with eigenvalues greater than unity.

Table 2: Eigenvalues, total variance, cumulative variance, and eigenvectors for the 10 characters.

Woreda	Clusters									Total genotypes per Woreda
	I	II	III	IV	V	VI	VII	VIII	IX	
Yirgachefe	-	4	4	1	-	1	-	-	1	11
Wonago	3	7	2	1	1	1	1	-	-	16
Dale	-	-	-	-	2	-	-	-	-	2
Aleta Wondo	1	2	-	-	2	-	1	1	-	7
Sodozuria	-	-	-	1	-	-	-	-	-	1
Bolososore	-	-	-	-	-	-	1	1	-	2
Southwest	-	-	-	-	-	1	-	1	-	2
Unknown	1	-	1	-	-	-	-	-	-	2
Total	5	13	7	3	5	3	3	3	1	43

Table 3: Distribution of the 43 genotypes over nine clusters based on the 10 characters considered in the study.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	4.3								
II	26.4	2.4							
III	34.1	30.6	3.6						
IV	42.6	18.7	26.5	5.3					
V	60.6	55.2	37.3	33.2	4.3				
VI	56.4	28.3	30.0	22.9	18.8	5.3			
VII	68.4	41.5	27.9	49.4	33.2	18.6	5.3		
VIII	85.3	31.1	81.2	43.9	134.7	75.8	110.6	5.3	
IX	50.0	42.6	40.0	33.0	58.0	56.8	62.5	74.6	0.0

Table 4: Inter (Bottom) and intra (bold and diagonal) cluster distances among the 43 genotypes.

for pairs of clusters were considered as the calculated values of Chi-squared (X^2) and were tested for significance both at 1% and 5% probability levels against the tabulated values of X^2 for 'P' degree of freedom, where P is the number of characters considered (P=10 in the present case) [11]. The important traits in each principal component that significantly contributed to the variation observed were identified as suggested by Jonson and Wichern [12].

Results and Discussions

Analyses of variances

Univariate analyses of variance were computed using MSTATC version 2.10 statistical software program for the seven quantitative morphological characters and the three years combined yield data. The ANOVA showed a highly significant difference among the genotypes for all the characters considered. Southeast Ethiopian coffee population was stated to be of narrow genetic base [6-13], however, the findings of this study indicates the presence of wide variations among Southeast Ethiopian (Sidama, Gedeo and Wolayta) landrace coffee populations located east of the Great Rift Valley. This might be attributed to the differences in the type of collections used i.e. forest coffee versus landraces. Since landraces are traditional varieties that have evolved over generations of selections by farmers they are characterized by high genetic heterogeneity, good adaptation to local environmental conditions and low productivity. In view of this it may be reasonable to state that there is a good chance to improve Sidama, Gedeo (Yirgachefe) and Wolayta coffees through selection and breeding. Such a view was endorsed by the work of earlier researchers [5,8,14].

Principal component analysis (PCA)

The first four principal components with eigen values greater than unity explained 82.63 percent of the total variation among the 43 genotypes for the 10 quantitative characters measured. Principal component one accounted nearly one third (32.52%) of the total variation. Accordingly, canopy diameter, number of nodes on the

main stem, number of primary branches and plant height in that order are the most important characters that contributed for the variation in the first PC. On the same basis, internode length, number of nodes on the main stem, number of primary branches and plant height had significant contributions for the variation in the second PC. In the 3rd PC yield, severity of coffee berry disease, plant height and internode length are the most important characters that contributed for the variation obtained.

In light of the results obtained from the PCA, it may be possible to deduce that more than half (53 %) of the variation obtained was primarily due to number of nodes, primary branches, and plant height. This perhaps emphasized the significance of these characters to the appraisal of genetic diversity in the south Ethiopian landrace coffee populations. Moreover, these characters could be used as a selection criterion for improving the productivity of the crop since they represent the lion's share in the variability of the coffee population in the specified area. Similar results have been reported for Limu coffee and Hararge coffee types (*Coffea arabica* L.) in Ethiopia [8,15].

Cluster analysis

The 41 southeast Ethiopian coffee selections including 2 southwest Ethiopian CBD resistant cultivars were grouped in to 9 clusters suggesting the prevalence of wide phenotypic variations in the coffee populations. The number of genotypes per cluster varied from 1 in cluster IX to 13 in cluster II. Cluster III contained selections only from Gedeo Zone (Yirgachefe and Wonago Woredas). On the same manner, in cluster V except 1 from Wonago, was composed of selections from Sidama Zone (Dale and Aleta Wondo Woredas). The 2 CBD resistant cultivars (75227 and 744) used as checks were grouped in clusters VI and VII where each cluster had 3 selections.

The selections from Wonago Woreda distributed in to 6 clusters where 7 out of 16 were grouped in cluster II. Similarly the selections from Yirgachefe distributed in to 5 clusters where 4 out of 11 were grouped in cluster III. Relatively low mean yield and higher scores of

both CBD and CLR infestations characterized cluster IX that contains only 1 selection from Yirgachefe.

The cluster analysis failed to clearly show relatedness of the selections due to geographical origin. Rather it is evident that there is overlapping of clustering patterns in respect of all Woredas, which could be explained as lack of differentiation among Woredas arising partly due to gene flow [8,16].

Inter and Intra-cluster distance (D^2) analysis

Almost all clusters showed a highly significant ($P < 0.01$) difference among each other. The smallest inter-cluster distance (18.6) was observed between clusters VI and VII while the highest (134.7) was between clusters V and VIII. In most of the cases, the genotypes among the clusters are significantly ($P < 0.001$) divergent from each other. Considering the intra-cluster (within cluster) distance, no significant genetic dissimilarity was detected.

Since the magnitude of heterosis largely depends upon the degree of genetic divergence in the parental lines, the germplasm selections belonging to the pairs of distant clusters such as V and VIII, VII and VIII and I and VIII could be very useful in hybridization program to obtain a wide variation among the segregates and to maximize heterosis in the F1. Similar view was held by earlier researchers.

Conclusions

Overlapping of the clustering patterns of the accessions from different districts indicated lack of differentiation among districts to a certain extent. Moreover, germplasm accessions from Gedeo Zone were more divergent than selections of Sidama Zone though relatively greater number of selections was considered from Gedeo Zone. Further, it is also possible to state that quantitative characters studied significantly contributed to the elucidation of genetic diversity prevalent in the region.

The significant inter-cluster distances between clusters indicated that there is a high opportunity for obtaining transgressive segregates and maximize heterosis by crossing germplasm accessions belonging to these 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. Further, the quantitative characters vis-à-vis number of stem nodes, primary branches, plant height, length of the longest primary branch and stem diameter could be used as a selection criterion for improving the productivity of the crop since they represent the lion's share in the variability of the coffee population in the specified area.

The number of germplasm accessions, the locations (number of districts) and the number of characters considered for the South Ethiopian coffee were small. Therefore, it is necessary to conduct further study by including more number of germplasm from diverse locations to find best estimate of the genetic diversity within the region.

Furthermore, additional traits of interest and molecular techniques may be very useful in order to further confirm the present encouraging result that indicated the presence of considerable variations within South Ethiopia coffee populations that provides immense potential for the development of improved varieties from the local landraces for the area.

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