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Research Article

Multivariate Analysis of Tea (*Camellia sinensis* (L.) O. Kuntze) Clones on Morphological Traits in Southwestern Ethiopia

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Information on genetic variability for biochemical characters is a prerequisite for improvement of tea quality. Thirteen introduced tea clones characterized with objective; assessing tea clones based on morphological characters at Melko and Gera research stations. The study was conducted during 2017/18 cropping season on experimental plots in RCBD with three replications. Data recorded on morphological traits like days from pruning to harvest, height to first branch, stem diameter, leaf serration density, leaf length, leaf width, leaf size, petiole length, leaf ratio, internode length, shoot length, number of shoot, canopy diameter, hundred shoot weight, fresh leaf yield per tree. Cluster analysis of morphological trait grouped into four clusters indicated, the existence of divergence among the tested clones. The maximum inter-cluster distance was between clusters I and IV (35.27) while the minimum inter cluster distance was observed between clusters I and II (7.8).Principal components analysis showed that the first five principal components with eigenvalues greater than one accounted 86.45% for 15 morphological traits. Generally, the study indicated presence of variability for several morphological traits. However, high morphological variation between clones is not a guarantee for a high genetic variation; therefore, molecular studies need to be considered as complementary to biochemical studies.

***Keywords**: cluster, divergence, eigenvalue, morphological, principal, yield, tea*

INTRODUCTION

Tea (*Camellia sinensis*(L.) O. Kuntze) is a standout amongst the most prominent and lowest cost type beverages in the world and consumed next to water by a wide range of age groups in all levels of society with more than three billion cups every day around the world (Phong *et al.*, 2016). The original home or 'the primary center of origin' of tea was South-East Asia (Wight, 1959). Tea flourishes well inside the latitudinal ranges between 45° N to 34° S that cross about 52 countries (Mondal *et al.*, 2004). Chinese were the first to utilize tea as medicinal drink, later as refreshment and have been doing so for the past 3000 years (Eden, 1958).

The family *Theaceae* comprises twenty three genera mostly distributed in the tropical and sub-tropical regions of South East Asia and America. The only genus economically important in the family is Camellia that includes about 82 species (Martin, 2007). Tea plants are highly cross pollinated in nature and self-incompatible (Wachira and Kamuya, 2005).The existing cultivated taxa of tea represented by three natural hybrids are encompassing more than 325 species under this genus, presently over 600 popular genotypes of tea are being cultivated worldwide (Mondal, 2011). Many of them are endowed with unique traits, such as improved quality of made-tea, high yield, tolerance to biotic or abiotic stresses (Mondal *et al.,* 2004). In addition, more than 3000 cultivars of *C. japonica* (a wild species of tea) are cultivated due to their excellent floral flamboyance. World tea production (black, green, instant and other) come to 5,954,091 tons while top five producers were China (2,414,802 tons)

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40.56%, India (1,252,174 tons) 21.03%, Kenya (473,000 tons) 7.94%, Sri Lanka (349,308tons) 5.87% and Turkey (243,000 tons) 4.08%. Ethiopia existed at 21th in area 9,727ha and 23th in production from 47 tea delivering nations by producing (10,806tons) 0.18% (FAOSTAT, 2016). In Kenya, the tea clones are capable of yielding 5000-8000 kg of made tea per hectare per year outperforming tea productivity in Ethiopia by 52-129% through the development of improved tea clones and production innovations (EIAR, 2017). Tea introduced to Ethiopia in 1927 is one of the vital industrial crops from which the country has been getting the foreign exchange beyond the fulfilling of residential demands (Yemane*et al.*, 2008). Ethiopian tea is hence quick developing as a competitive product in the European market and is getting to be one of the major sources of income for the national exchequer (EIAR, 2017). A number of suitable multivariate methods like principal component analysis, factor analysis and cluster analysis are presently available for the selection of parent, detection of genetic variability, centre of origin, study of interaction among the environments and tracking the course to crop evolution (Mostafa *et al.*, 2011).

MATERIALS AND METHODS

Description of the Study Site

The experiment was conducted at Jimma Agricultural Research Centers (JARC) Melko and Gera during 2017/2018. Melko is located at 7°46′ N and 36° E Latitude and Longitude, respectively with altitude of 1750 m above sea level, average of last five year temperature was minimum11.7°C and maximum 25.9°C, rain fall of 1511.7 mm, 68.4% relative humidity, wind speed at 1m 2.448 km/hrs, monthly mean soil temperature at 5cm 24.9°C and 73.95 hrs average annual sun shine. Melko characterized by EutricNitosol (reddish brown) with a pH of around 5.2 (Simegn*et al.,* 2016).

Gera is located at 7°7′ N and 36° E Latitude and Longitude, respectively with altitude 1940 m above sea level, average of last five year temperature was minimum 11.1°C and maximum 23.9°C, rain fall of 1558.9 mm, 71.7% relative humidity, wind speed at 1m 1.92 km/hrs, monthly mean soil temperature at 5 cm 22.46°C and 61.76 hrs average annual sun shine. Gera, station also characterized by red soil, which was loam type and quite fertile (Solomon *et al.*, 2014).

Experimental Materials

The experiment was superimposed on thirteen introduced Assam type tea clones that collected from different tea farms (Wushwush, Gumero and Chewaka) and JARC established at Melko and Gera research stations (Table 1).

Table 1: Description of Tea Clones Used for the Study

Serial no.	Country of Introduction	Tea Clones	Sources
1	Kenya	Mlk-2	JARC
2	India	L6	Gummaro
3	Kenya	Mlk-1	JARC
4	India	B9	Gummaro
5	Kenya	11/56	Wushwush
6	India	Chai	Gummaro
7	Kenya	S-15/10	Chewaka
8	Kenya	FNF	Wushwush
9	India	BB-35	Gummaro
10	India	SR-18	Gummaro
11	Kenya	11/4	Wushwush
12	Kenya	6/8	Wushwush
13	Kenya	31/11	Chewaka

Source: Jimma Agricultural Research Center (JARC, 2005)

Experimental Design and Management

The experiment was super-imposed on the effectively settled tea plantation in 2005 at Gera and Melko research station using RCBD with three replications. Twelve plants per plot with 0.6 m by 1.2 m between plants and rows respectively. Twelve years old tea bushes medium pruned with shears at 50 cm height from ground level in December 2017 according to Ahmad *et al.* (2014). After these treatments, tea plants brought back to the normal plucking cycle or shoot replacement cycle; in the spring (pre-monsoon) season during Mid-March to May all morphological data recorded from five sampled plants per plot.

Data Collected

Fifteen different morphological characters (Table 2) were collected from each tea clones using the standard procedures of tea descriptors (IPGRI, 1997).

Table 2: Quantitative Characters Studied and their Description as Per IPGRI, 1997 Tea Descriptor

No.	Characters	Unit	Description
1	Internode length	cm	Measured distance between the 5th and 6th leaves from top of a flush growth, average of 10 shoots exposed to full sunlight
2	Length of mature leaf	cm	Recorded on the 5th leaf below the apical bud, average of five leaves
3	Width of mature leaf	cm	Measured on the 5th leaf from the apical bud of flushing shoot at the maximum breadth, average of five leaves
4	Length of mature leaf petiole	mm	Recorded on the 3rd leaf from the apical bud of flushing shoot, average of five leaves
5	Height up to 1st branching position	cm	Measured length of stem starting from the ground up to the first branch
6	Shoot length	cm	Recorded by measuring the length of harvested shoot of two leaves and a bud at 2/3 height of node between the 2nd and 3rd leaf
7	Canopy diameter	cm	Measured from North-South and East-West using tape meter and taking the average as (NS +EW)/2
8	Fresh leaf yield per tree	g	Recorded from five representative plants and divided by number of sampled plants to obtain yield per tree by undertaking fine plucking
9	Leaf serration density	no./cm	Counted number of serration per one cm at 5th leaves, average of single leave from five sampled plants
10	Days from pruning to harvest	no.	Counted number of days from medium pruning to reach at two leaves and a bud stage on plot based
11	Length leaf to width ratio	cm	Measured by dividing the leaf length value by the leaf width, average of five leaves
12	Leaf size	cm^2	Computed by multiplying leaf length by leaf width values
13	Hundred shoot weight	g	Measured by taking weight of hundred harvestable shoot per plot
14	Number of shoot	no.	Recorded by counting the harvestable shoots from sampled five plants and averaged to get per tree
15	Stem diameter	mm	Measured by using caliper at 10cm height of stem above ground

Statistical Analysis

Analysis of variances for the individual locations computed using SAS Statistical Software package (SAS, 2014). Homogeneity test for error variances of the locations made before combined analysis and error variances of each location found homogenous for all considered traits. The combined analysis was estimated using RCB design. Least significant difference (LSD) at P= 0.05 and 0.01 was employed to identify clones that are significantly different from each other.

Cluster Analysis

In this study morphological characters were used for clustering the clones into homogeneous groups. The data subjected to cluster analysis to determine the variability among the clones. Hierarchical clustering employed using the similarity coefficients among the 13 tea clones. Clustering was performed using the proc cluster procedure of SAS version 9.3 (SAS, 2014) by employing the method of average linkage clustering strategy of the observation. The numbers of clusters were determined by following the approach suggested by Copper and Miligan (1988) by looking into three statics namely Pseudo F, Pseudo t2 and cubic clustering criteria.

Divergence Analysis between Clusters

Genetic divergence between clusters was determined using the generalized MahalanobisD^2 statistics (Mahalanobis, 1936) using the equation:

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

D^2p = the distance between any two groups i and j; Xi and Xj = the p mean vectors of clones i and j, respectively, S^{-1} = the inverse of the pooled covariance matrix. The D^2 values obtained for pairs of clusters tested for significance at 5% and 1% level of significance against the tabulated values of p degrees of freedom, where p is the number of variables considered (Singh and Chaundry, 1987).

Principal Component Analysis

Principal component analysis for morphological traits was performed using correlation matrix by employing SAS version 9.3 (SAS, 2014). The objective of this analysis was to reduce the observed variables into small number of principal components that were accounted for most of the variance in the observed variables. If the alpha value of a specific component is high, it interpreted as, indicating that the component has a strong one-dimensional structure or the dimension can reliably account for the total variance.

Generally, an alpha value of 0.70 or greater considered as reliable (Bland and Altman, 1997).

RESULTS AND DISCUSSION

Cluster Analysis for Morphological Traits

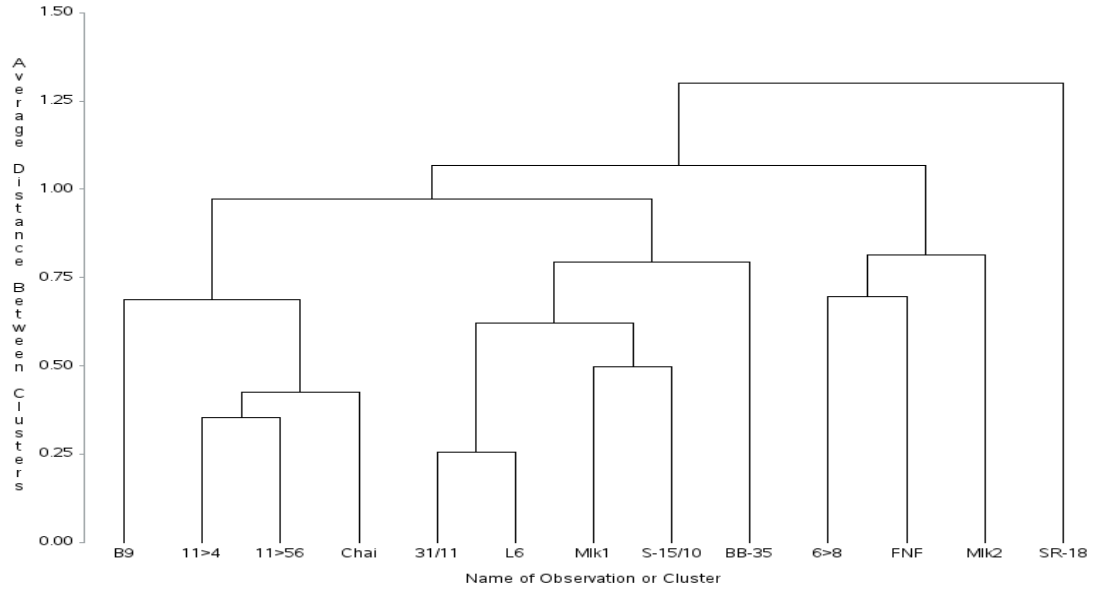
The D^2 value based on the mean of tea clones resulted in classifying the 13 clones into four groups (Table 3 and Appendix Fig. 1). Cluster I was the largest with five clones (38.46%) followed by cluster II with four clones (30.77%), cluster III with three clones (23.07%) and cluster IV with one clone (7.79%) of total populations diversity.

Cluster I consists of tea clones collected from three sources; two tea farms (Chewaka and Gummaro) and one from research station (JARC). This includes, 31/11 and S-15/10 from Chewaka, L6 and BB-35 from Gummaro and Mlk1 from JARC. Cluster II holds 4 tea clones from two farms, thus 11/4 and 11/56 from Wushwush, while Chai and B9 from Gummaro farms). Cluster III includes three tea clones, 6/8 and FNF from Wushwush farm, whereas Mlk2 from JARC. Finally the cluster IV consist only one tea clone (SR-18) from Gummaro tea farm.

Table 3: Distribution of Tea Clones into Four Clusters Based on D^2 Analysis

Cluster number	No of clones	Percent (%)	Clones
1	5	38.46	31/11, L6, Mlk1, S-15/10 and BB-35
2	4	30.77	11/4, 11/56, Chai and B9
3	3	23.07	6/8, FNF and Mlk2
4	1	7.79	SR-18

Figure 1: Dendrogram of the Cluster for Morphological Traits



Cluster Mean Analysis for Morphological Traits

Cluster I exhibited the highest mean value for leaf size, leaf width, leaf length and shoot length. In addition, it showed medium score for the traits such as, mature leaf petiole length, internode length and hundred shoot weight. Cluster II differentiated by highest number of days from medium pruning to first harvest, height to first branch, mature leaf petiole length, and internode length. However, tea clones grouped under this cluster showed medium value for stem diameter, leaf length, leaf size, leaf ratio, shoot length and number of shoots. Cluster III characterized by highest values for stem diameter, number of shoots, canopy diameter and freshleaf yield per tree. Finally, cluster IV identified by highest leaf serration density, leaf ratio and hundred shoot weights. On the other hand, the single clone categorized under, this cluster also identified by medium value for leaf width, canopy diameter and fresh tea leaf yield per plant (Table 4). Generally the lowest values for each clusters were indicated as follow, cluster I for leaf serration density, leaf ratio, number of shoot and canopy diameter, whereas cluster II identified by lowest values of hundred shoot weight and fresh leaf yield per tree. However, the cluster III identified by lowest values for number of days from medium pruning to first harvest, leaf length, leaf width and leaf size, thus cluster III clones characterized by early generation from medium pruning. Finally cluster IV identified by lowest value for height to first branch, stem diameter, mature leaf petiole length, internode length and shoot length.

Table 3: Cluster Mean and Mean Difference for 15 Morphological Traits

	Cluster mean				Cluster mean difference			
Variable	I	II	III	IV	I	II	III	IV
ND	111.53	112.71**	101.82*	104.61	2.41	3.59	-7.30	-4.51
HFB	13.90	15.69**	14.08	11.89*	-0.43	1.35	-0.26	-2.45
SD	79.36	80.35	84.24**	71.45*	-0.82	0.17	4.06	-8.73
LSD	3.61*	3.73	3.78	3.97**	-0.10	0.02	0.06	0.25
LL	14.95**	14.73	13.79*	13.82	0.42	0.21	-0.73	-0.71
LW	6.80**	6.37	6.26*	6.67	0.27	-0.17	-0.28	0.13
LS	104.15**	93.85	85.44*	86.83	8.82	-1.48	-9.88	-8.50
PL	4.72	4.92**	4.47	4.13*	0.04	0.24	-0.21	-0.55
LR	2.11*	2.275	2.19	2.28**	-0.09	0.08	0.00	0.09
IL	6.96	7.44**	6.87	6.69*	-0.11	0.37	-0.19	-0.38
SL	12.44**	11.67	11.56	11.01*	0.55	-0.22	-0.33	-0.88
NS	75.11*	83.44	98.34**	81.00	-8.37	-0.05	14.85	-2.49
CD	90.90*	92.65	99.87**	97.01	-3.08	-1.33	5.89	3.03
HSW	165.47	136.55*	155.92	195.77**	8.77	-20.15	-0.78	39.07
YLD	86.04	84.93*	99.75**	86.82	-2.88	-4.00	10.83	-2.10

***,*= represents maximum and minimum values, respectively, ND= number of days from medium pruning to first harvest, HFB=height to first branch, SD= stem diameter, LSD= leaf serration density, LL=leaf length, LW=leaf width, LS=leaf size, SL=shoot length, NS=number of shoot, CD= canopy diameter, HSW=hundred shoot weight, PL= petiole length of mature leaf, LR=leaf ratio, IL= internode length and YLD= fresh leaf yield per tree*

Genetic Divergence of Tea Clones by Morphological Traits

Genetic divergence as measured by Mahalanobis (1936) generalized distance (D^2) has been one of the important statistical tools to provide a rational basis for selection of parents in breeding programs. Mahalanobis distance (D^2) of the four clusters of 13 tea clones based on 15 quantitative traits presented in Table 5. The maximum inter cluster distance was between clusters I and IV (35.27) followed by III and IV (32.1), II and IV (25.56), II and III (25.29), I and III (25.28) and I and II (7.8). Generally, this study revealed that the tea clones included in this study exhibited moderate divergence. This indicates that, the genetic improvement through crossing among tea clones listed in the above listed cluster distances in the respective order is important in the future tea breeding program. Mehran *et al.* (2007) also indicated the high genetic diversity among eleven Iranian tea clones and grouped them into three clusters that were partly in line with the current study. Ghaderi *et al.* (1984) and Simegn*et al.* (2016) reported that, increasing parental distance implies a great number of contrasting alleles at the desired loci, and then to the extent that these loci recombine in the F_2 and F_3generations following a cross of distantly related parents, the greater will be the opportunities for the effective selection for yield factors.

Table 5: Average Inter-Cluster Divergence Values Obtained for Morphological Traits

Squared distance to cluster				
From class		II	III	IV
I		7.8	25.28*	35.27**
II			25.29*	25.56*
III				32.1**

** = significant at 23.68 and above, ** = highly significant at 29.14 and above*

Principal Component Analysis for Morphological Traits

Phenotypic data that had high contributing component loadings were from such characters as height to first branch (0.95), leaf serration density (0.92), canopy diameter (-0.80), number of shoot (-0.80), hundred shoot weight (-0.79), leaf size (0.78) and number of days from medium pruning to first harvest (0.78). This also partly in lined with Rajanna *et al.* (2011) who reported reliable loading of leaf size and number of shoot of Indian tea clones diversity. Piyasundara *et al.* (2009) also reported that leaf size was contributing significantly to the total phenotypic variation present in the germplasm and hence could be useful in characterizing the tea germplasm. Generally, the first five principal components contributed 86.45% diversity among the tea clones (Table 6)**.** Therefore, the result was relatively in line with Rajkumar *et al.* (2010)that displayed three principal components extraction, which was enough to differentiate more than 90% of 121 accessions maintained in three different geographical and climatic locations. Piyasundara *et al.* (2009) reported the first four principal components accounted for 78% of the total variation of twenty tea

clones in Sri Lanka that was coincide with the conducted study. The first principal component which accounted for 30.27% of the variability among tea clones were attributed to discriminatory traits such as number of days from pruning to harvest, leaf width, leaf size, shoot length, number of shoot, canopy diameter and fresh leaf yield per tree. Likewise, 20% of the total variability among the tested tea clones accounted for the second principal component originated from variation in height to the first branch, internode length and hundred shoot weights. The third principal component, which explained 13.8 % of the total variation among tea clones, was due to the variation in stem diameter, leaf length, mature leaf petiole length and fresh tea leaf yield per tree. The fourth principal component, which explained 11.19% of the total variation, was associated with variation due to leaf serration density and leaf ratio. Finally, quantitative characters leaf length and canopy diameter contributed chiefly to the variation of fifth principal component. Generally, the variation in the first two principal components, which explained the lion share of the observed variation (50.27%), was mainly due to the combined effects of number of days from medium pruning to first harvest, leaf size, number of shoot and canopy diameter, height to the first branch, internode length and hundred shoot weight.

Table 6: Eigenvector and Eigenvalues of the First Five Principal Components for 15 Morphological Traits

Principal Component					
Variables	I	II	III	IV	V
ND	0.77699	0.30991	-0.4336	-0.0502	-0.1301
HFB	0.18563	0.94459	0.04037	-0.0993	0.16381
SD	-0.5192	0.40939	0.61451	-0.2281	-0.0605
LSD	-0.0674	0.28428	-0.1187	0.9234	0.13479
LL	0.46657	0.06083	0.57806	-0.1544	-0.4751
LW	0.60821	-0.5603	0.17527	0.08632	0.37675
LS	0.77862	-0.3168	0.49503	0.05448	-0.0584
PL	0.29606	0.56038	0.62367	0.23354	0.12248
LR	-0.2701	0.5162	0.04223	0.64659	-0.3906
IL	0.42034	0.63853	-0.3273	-0.1033	0.40038
SL	0.69697	0.03794	0.19337	0.15171	0.42547
NS	-0.7989	0.29012	-0.0937	-0.0764	0.01575
CD	-0.8006	-0.1383	0.06155	-0.0945	0.41015
HSW	-0.0068	-0.7864	-0.1606	0.41071	-0.1253
YLD	-0.5764	-0.2932	0.55232	0.19503	0.31257
Eigen value	4.54	3.45	2.07	1.68	1.23
Percent	30.27	20.01	13.80	11.19	8.18
Difference	1.09	1.38	0.39	0.45	0.49

ND=number of days from pruning to harvest, HFB=height to first branch, SD= stem diameter, LSD=leaf serration density, LL=leaf length, LW=leaf width, LS=leaf size, SL=shoot length, NS=number of shoot, HSW=hundred shoot weight, PL=petiole length of mature leaf, LR=leaf ratio, IL=internode length and YLD=fresh leaf yield per tree

CONCLUSION

Principal component analysis for morphological traits also revealed that the first five principal components with eigenvalues greater than one accounted 86.45% for 15 morphological traits. Consequently, the traits played the major role in classifying tea clones into different clusters and must be considered in selecting diverse parents in crossing program. In conclusion, the present study exhibited the presence of considerable genetic diversity for several morphological traits among tea clones. The existence of genetic diversity is potential resource for improvement of the tea crop through selection and hybridization. Therefore, the observed variability for morphological traits must be exploited to improve the yield of this valuable crop.

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