

## Phenotypic Diversity of Sri Lankan Tea (*Camellia sinensis* L.) Germplasm Based on Morphological Descriptors

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**ABSTRACT.** *The characterization of germplasm collections is important to plant breeders for its utilization and the efficient and effective management of gene banks. Objectives of this study were to estimate morphological diversity present in some tea (*Camellia sinensis* L.) accessions conserved in an ex situ gene bank and to determine the importance of different descriptor traits in categorizing accessions into distinct groups. Twenty accessions were characterized using 13 standardized morphological descriptors. Principle Component Analysis (PCA) using 13 traits and clustering on first four Principle Component (PC) scores delineated the twenty accessions into 3 clusters. First four PC scores were accounted for 78% of the total variation. Except mature leaf petiole length and the appearance of leaf upper surface, all other 11 morphological descriptors significantly contributed to the total phenotypic variation. Of the 11 discriminate traits, leaf width, leaf shape, leaf pigmentation and petiole pigmentation had the highest contribution to morphological variability. This study permits the categorization of tea accessions into major groups based on the identification of traits contributed for variation.*

### INTRODUCTION

Identification of superior germplasm accessions for inclusion in breeding programs is vital for genetic enhancement of improved cultivars aiming at higher crop productivity. This objective depends largely on the information available on characterization and evaluation of the accessions. The information can also be used to help understand patterns of genetic variation existing in crop species (Fernando, 2000; Hagedimitriou *et al.*, 2005; Kumaran *et al.*, 2000; Perera and) and to identify accessions with high genetic variability and also to select genetically close and distant accessions as the success of any hybridization program depends on selecting genetically distant/different parents (Anandappa, 1993). Plant breeders should have a manageable number of accessions to use in search for new characters or character combinations and a structural way to evaluate whole germplasm collections. Therefore, the concept of the core collection appears to offer a number of potential benefits to users of genetic resources. A core collection is intended to contain a minimum repetitiveness of the genetic diversity of a crop species and its wild relatives. Hodgkin *et al.* (1999) used morphological variation to develop a core collection in sesame.

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Sri Lankan tea (*Camellia sinensis* L.) accessions which are conserved under the *ex-situ* conservation strategy have not been adequately characterized for morphological traits except in few studies by Gunasekare *et al.* (2001) and Wickramaratne (1981). Furthermore, when the global tea germplasm is considered, there are no proper studies reported for characterization of tea germplasm using morphological descriptors and interpretation of data based on biometrical approach to identify patterns of variation. Hence, the present study was undertaken to; (i) estimate the extent of phenotypic variation of germplasm accessions using morphological descriptors, (ii) identify the contribution of different traits to the total variation and (iii) categorize *ex situ* germplasm collection for rational utilization in tea crop improvement programs.

In this study, data was recorded based on the tea descriptors developed by the International Plant Genetic Resource Institute, IPGRI (Anon, 1997). This internationally agreed format would help better utilization of the information.

## MATERIALS AND METHODS

Twenty germplasm accessions used in this study consisted of representative samples originated from introductions, selections made from existing old seedling tea populations on different Agro Ecological Regions, altitude classes and materials derived from breeding programs. Those accessions were among the 245 accessions conserved in the *ex situ* gene bank in the low country region. In order to standardize the methodology in characterization, the IPGRI Descriptors for tea (Anon, 1997) was adopted. Five randomly selected plants from one plot of each accession were used to record observations on morphological characters. Non-parametric data were converted on a scale as indicated in Table 1, enabling its use in the statistical analysis. Principle Component Analysis (PCA) was carried out using mean values of morphological characters. Cluster analysis was performed based on significant Principle Components (PC) using SAS - Version 8.1 (2001).

**Table 1. Scores allotted for non-parametric characters.**

Descriptor	Score/Scale
Leaf shape	Broadly elliptic: <b>2.5</b> ; Elliptic: <b>3</b> ; Elliptic - Lanceolate: <b>3.5</b> ; Lanceolate: <b>4.0</b>
Appearance of the leaf upper surface	Shiny: <b>1</b> ; Dull: <b>2</b>
Pigmentation on petiole and leaf	Pigmented: <b>1</b> ; Non-pigmented: <b>0</b>
Young shoot pubescence	Sparse: <b>3</b> ; Intermediate: <b>5</b> ; Dense: <b>7</b>
Leaf apex habit	Down turn: <b>1</b> ; Straight: <b>2</b>
Immature and Mature leaf color*	2.5GY6/6: <b>1.1</b> ; 2.5GY5/6: <b>1.2</b> ; 7.5GY6/6: <b>2.1</b> 7.5GY5/6: <b>2.2</b> ; 7.5GY4/4: <b>2.3</b> ; 7.5GY4/6: <b>2.4</b> 7.5GY3/2: <b>2.5</b> ; 7.5GY3/4: <b>2.6</b>

**Note:** \*According to Munsell color charts for plant tissue, (1952), Maryland, U.S.A.

## RESULTS AND DISCUSSION

The means of morphological data used for characterization of accessions in this study is presented in Table 2.

**Table 2. Means of morphological traits used for characterization of accessions.**

Accession	Character code												
	1	2	3	4	5	6	7	8	9	10	11	12	13
TRI 62/13	16.40	6.58	2.49	3.0	118	1.2	2.4	9.4	0	1	1	3	1
TRI 62/19	15.48	6.04	2.56	3.0	94	1.1	2.4	4.4	0	1	1	3	1
TRI 2044	13.24	4.08	3.25	3.0	72	2.2	2.4	8.6	0	0	2	3	1
TRI 3029	15.00	5.96	2.52	2.5	74	1.1	2.2	7.4	0	1	2	3	2
TRI 3070	16.16	6.22	2.60	3.0	97	1.2	2.4	6.8	0	1	1	3	1
TRI 4071	15.20	4.76	3.19	3.5	57	2.1	2.2	6.0	0	0	2	3	2
AL 10/34	11.90	4.64	2.56	3.0	75	2.4	2.6	6.4	0	0	1	5	1
DK 24	11.26	3.52	3.20	4.0	69	1.2	2.3	4.6	1	0	2	5	2
DW 12	14.74	4.92	3.00	3.0	73	1.2	2.4	7.8	0	0	1	3	2
DW 16	12.28	4.18	2.94	3.0	54	2.2	2.3	4.4	0	0	1	3	2
GT 1/5	15.28	5.10	3.00	3.0	77	1.1	2.4	6.6	0	0	2	3	2
GV 35	14.78	5.78	2.56	3.0	83	1.2	2.4	6.2	0	0	2	3	2
HUN 1	11.36	5.02	2.26	3.0	81	1.2	2.4	4.6	0	0	1	3	2
KEN 15/2	10.20	4.16	2.45	3.0	74	2.2	2.5	7.2	0	0	2	3	1
MO 114	9.78	3.42	2.86	3.5	41	2.2	2.3	5.0	0	0	2	7	2
MO 116	12.42	3.42	3.63	4.0	70	2.2	2.3	4.4	0	0	2	7	1
MT 26	11.02	4.64	2.38	3.0	42	1.1	2.3	4.6	0	0	2	3	2
NIL 3/1	14.62	4.86	3.01	3.0	81	1.1	2.4	5.6	0	0	1	3	1
N4NY	12.06	4.68	2.58	3.0	59	2.2	2.6	8.0	0	0	1	5	2
SJ 2/28	9.94	4.52	2.20	3.0	68	1.2	2.4	3.8	0	0	2	3	2

**Note:** 1 - Leaf Length (cm); 2 - Leaf Width (cm); 3 - The Ratio of Leaf Length to Leaf Width; 4 - Leaf Shape; 5 - Leaf pose<sup>\*</sup>(degrees); 6 - Immature leaf color, 7 - Mature leaf color; 8 - Length of the mature Leaf petiole (mm); 9 - Leaf pigmentation; 10 - Petiole pigmentation; 11 - Appearance of the Leaf Upper Surface; 12 - Young Shoot pubescent; 13 - Leaf apex habit; <sup>\*</sup>The angle between internodes axis and the tip of the leaf.

Eigen values of the correlation matrix obtained from the PCA of the 13 morphological descriptors are given in Table 3. Eigen values of the first 4 PCs were greater than one, indicating that those 4 PCs significantly contributed to the variation existing in the genotypes studied. Furthermore, first 4 PCs accounted for approximately 78% of the total variation.

According to Eigen vectors of the first 4 PCs (Table 4), leaf length, leaf width, leaf shape, leaf pose, petiole pigmentation and young shoot pubescence were important in PC 1 while in PC 2 they were immature leaf color as well as mature leaf color and leaf apex habit. In PC 3 the ratio of leaf length to leaf width and leaf shape was the important characters. In PC 4 only a single character was found important and it was leaf pigmentation. Of the 13

characters studied, length of the mature leaf petiole and appearance of the leaf upper surface did not show any significant contribution to the variation.

**Table 3. Eigen values of the correlation matrix based on the PCA of the 13 morphological descriptors.**

PC	Eigen value	Proportion	Cumulative
1	4.952	0.381	0.381
2	2.052	0.158	0.539
3	1.923	0.148	0.687
4	1.185	0.091	0.778

**Table 4. Eigen vectors for first four PCs of the 13 morphological descriptors.**

Character	PC 1	PC 2	PC 3	PC 4
Leaf length	0.327	-0.106	0.361	0.275
Leaf width	0.428	-0.128	0.015	0.003
Ratio of leaf length to leaf width	-0.222	0.050	0.509	0.319
Leaf shape	-0.329	-0.053	0.414	-0.204
Leaf pose	0.354	0.087	0.299	-0.247
Immature leaf color	-0.235	0.478	-0.015	0.296
Mature leaf color	0.103	0.530	-0.223	-0.318
Length of the mature leaf petiole	0.228	-0.267	0.107	0.385
Leaf pigmentation	-0.174	-0.228	0.284	-0.492
Petiole pigmentation	0.333	-0.108	0.240	-0.072
Appearance of the leaf upper surface	-0.245	-0.280	0.020	0.323
Young shoot pubescent	-0.303	0.235	0.201	-0.141
Leaf apex habit	-0.148	-0.428	-0.330	0.095

A dendrogram (Fig. 1) constructed using cluster analysis conducted on the first 4 PC (which accounted for about 78% of the total variation), clearly indicated that the 20 accessions used in this study were grouped into three well differentiated clusters at the average distance of 1.0. The cluster composition of different accessions based on 13 morphological descriptors are given in Table 5.

Possible explanations for the grouping of accessions found in the study could be related to their origin or ancestry and also to the location of selection. Cluster I comprised of 3 accessions, MO 114, MO 116 and DK 24. Of them, MO 114 and MO 116 originated from Mooloya (MO) Estate. Since they have originated from one source, there is less variation between the accessions and this is further confirmed by grouping them into one cluster in the dendrogram (Fig. 1). However, DK 24 which originated from a different

source (selected from Diyanillakelle Estate) also falls into the same cluster, but forming a different branch. It is suggested that the inclusion of more characters than what was used in the present study could be useful in differentiating this accession to explain its deviation.

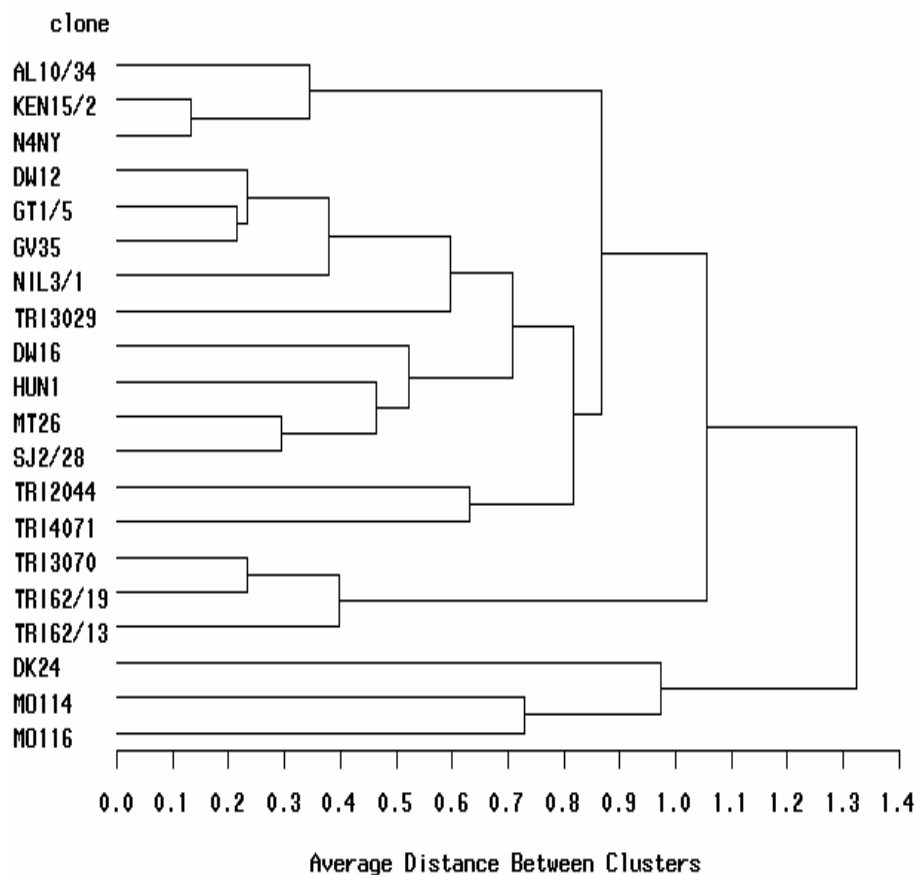


Fig. 1. Dendrogram of 20 accessions based on average linkage cluster analysis using 13 morphological descriptors.

Table 5. The cluster composition of different accessions based on 13 morphological descriptors.

Cluster No.	Number of Accessions	Accession identity
I	03	DK 24, MO 114 and MO 116
II	03	TRI 3070 , TRI 62/19 and TRI 62/13
III	11	AL 10/34, KEN 15/2, N4NY, DW12, GT1/5, GV35, NIL3/1, TRI 3029, DW16, HUN1, MT26, SJ2/28, TRI 2044, TRI 4071

All 3 cultivars presented in the Cluster II consist of accessions, TRI 3070, TRI 62/19 and TRI 62/13, which have a similar origin and could be traced back to open pollinated progeny of an introduced source, ASM 4/10.

The remaining 14 accessions were mostly estate selections (denoted with acronym other than TRI) and formed a major cluster (Cluster III) in the dendrogram. The accessions falling into this cluster show strong resemblance between the morphology of the accessions and the agro-ecology from where it has been originally selected. In Sri Lanka, tea-growing regions fall into four broad categories owing to the differences in climatic and edaphic factors. The accessions which have been selected for their ecological variation is in agreement with their origin based on the spatial distribution (Gunasekare and Kumara, 2005). There is evidence to confirm that initial tea populations on various estates are not from the same geographical regions in Sri Lanka, but were introductions from India from a common source/seed garden. This suggests that the extent of morphological diversity among the accessions selected from similar regions is less than that could be seen among populations derived from different sources such as introductions.

Although the morphological analysis does not necessarily reflect the genetic diversity of the tea germplasm as revealed by biochemical or molecular markers (Liyanage *et al.*, 2003), the present analysis of morphological traits provides a basis for broad categorization of tea germplasm. PCA identifies the variables contributing to most of the phenotypic diversity, while clustering helps in grouping of accessions based on their degree of relationship to each other. Hence, the grouping based on PCA analysis further confirms the results obtained as there is a relationship among groups based on their pedigree or origin.

The characterization of the germplasm based on morphological descriptors can also be used to establish a representative core collection. Number of accessions selected per cluster may be based on the presence of unique traits which would allow 100% of the known morphological diversity to be retained in the core collection (Zewdie *et al.*, 2004). Data also suggest that some accessions in each group possess unique variations and they could be used as an exceptional character identification necessary to differentiate the accessions.

## CONCLUSIONS

PCA using 13 morphological descriptors and clustering based on the first four PC scores delineate twenty accessions of TRI into 3 defined clusters. Out of the 13 morphological descriptors used, except mature leaf petiole length and the appearance of leaf upper surface, all other 11 descriptors significantly contributed to the total phenotypic variation of the germplasm. Of the 11 descriptors, leaf width, leaf shape, leaf pigmentation and petiole pigmentation were found to be highly discriminating descriptors.

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