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Identification of Discriminating Morphological Descriptors for Characterization of Tea (*Camellia sinensis* L.) Germplasm in Sri Lanka

J.H.N. Piyasundara, M.T.K. Gunasekare[1] and I.P. Wickramasinghe[2]

Division of Plant Breeding
Tea Research Institute
Talawakelle, Sri Lanka

ABSTRACT. *Accessibility of germplasm in breeding programs depends largely on the availability of characterization and evaluation data. Tea germplasm accessions conserved in the ex situ gene bank at Tea Research Institute, have not been characterized systematically to identify morphological markers contributing to phenotypic variation of the local germplasm. Therefore, rationalizing the list of proposed descriptors to identify the most discriminating descriptors relevant for local tea germplasm is a prerequisite for efficient characterization. The present study was undertaken to find out highly discriminating morphological descriptors contributing to the variation in the local tea germplasm through multivariate statistical techniques. Two hundred and three germplasm accessions conserved in the ex situ gene bank collection was characterized using 20 standardized morphological descriptors. Principle Component Analysis (PCA) using 20 descriptors showed that 16 out of 20 descriptors were informative and contributes significantly to the variation present in the germplasm. Cluster analysis based on significant principle components further revealed that descriptors viz. type of serration of leaf margin, waviness of the leaf margin, pigmentations in young leaf, pigmentations in leaf petiole, size of the leaf and leaf angle were the most discriminating descriptors in distinguishing accessions into phenotypically diverse groups. The present study identifies the morphological descriptors that are most relevant for characterization of tea germplasm in Sri Lanka.*

INTRODUCTION

The importance of using genetic resources in breeding programs to enhance crop genetic potential has been well recognized (Thomas and Mathur, 1991). However, the accessibility of the germplasm depends largely on the information available on characterization and evaluation. Use of morphological characters is cost effective when compared to the use of biochemical and molecular markers for preliminary characterization of large number of accession to identify morphologically similar groups and for simple varietal identification of phenotypically distinguishable cultivars (Martinez *et al*., 2003). Among the several activities, the first step for proper characterization of germplasm is to identify the phenotypic variation present in the given germplasm. In order to achieve this objective, the germplasm accessions need to be characterized using a standard set of descriptors. A descriptor list for

1 Division of Plant Breeding, Tea Research Institute of Sri Lanka.

2 Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

tea has been proposed by the International Plant Genetic Resource Institute - IPGRI (Anon, 1997). However, the above descriptor list is prepared considering world-wide tea germplasm collection (Anon, 1997), and hence, all the descriptors proposed by IPGRI may not be equally important for characterizing the local (Sri Lankan) tea germplasm. Therefore, rationalizing the list of proposed descriptors to identify most discriminating descriptors relevant for local tea germplasm is a prerequisite for efficient characterization. Further, the use of minimum list of descriptors would help save resources since characterizing the entire collection using all proposed descriptors require more resources. Therefore, it is necessary to identify highly discriminating descriptors relevant to the local germplasm in order to formulate a minimum list of descriptors for characterizing the tea germplasm in Sri Lanka.

Variable reducing multivariate statistical techniques such as principle component analysis and cluster analysis are the commonly used methods for characterization and genetic diversity analysis of germplasm in various crops such as wild oat (Beer *et al.*, 1993), coconut (Kumaran *et al.*, 2000; Perera and Fernando, 2000), cocoa (Bhat *et al.*, 2000), olive (Hagidimitriou *et al.*, 2005), strawberry (Lavin *et al.*, 2005) and would increase the accuracy of interpretation of information generated in characterization studies. However, in tea, only few studies have been reported on the patterns of morphological variation (Wickramaratne, 1981; Gunasekare *et al.*, 2001; Piyasundara *et al.*, 2006). In these studies, the number of accessions used to study the variation present in the germplasm was limited. Therefore, an extensive study using a larger sample, representative of the whole collection, is necessary to capture the variation present in the collection and to precisely identify a minimum list of descriptors relevant and important to local collection.

Therefore, the objective of the present study was to identify key morphological descriptors contributing to the total phenotypic variation of the tea germplasm in relation to local collection.

MATERIALS AND METHODS

Two hundred and three germplasm accessions representing tea accessions from various ancestry and origins were included in the study. Considering their ancestry and origin, the 203 accessions can be categorized as shown in (Table 1).

Table 1. Origin / ancestry of the accessions considered in the study

Origin /ancestry of the accessions	No. of accessions
Accessions introduced from other countries (Introductions)	07
Accessions selected from existing old seedlings on tea Estates located in different Agro ecological regions (Known as "estate selections")	114
Accessions selected from open pollinated progenies of female parent ASM 4/10	30
Selections from second generation progenies of ASM 4/10 (TRI 2024, TRI 2025 and TRI 2026).	11
Accessions developed through the breeding programme of the Tea Research Institute of Sri Lanka	39
Selection from open pollinated seeds of Indo Chinese- Shan Bansang	01
Selection made from open pollinated progeny of a St Coombs estate selection	01

Twenty morphological descriptors proposed by IPGRI (Anon, 1997) were used to score the 203 accessions. The selection of 20 descriptors which include parametric as well as non-parametric data, were based on the outcome of previous studies conducted in tea (Wickramaratne, 1981; Gunasekare *et al.*, 2001; Piyasundara *et. al.*, 2006). Six parametric descriptors included. Leaf length, Leaf width, Leaf length: width ratio, Leaf size, Leaf angle and Leaf pose. Fourteen non- parametric descriptors included; Type of serrations of the leaf margin, Waviness of the leaf lamina, Pigmentation in leaf petiole, Pigmentations in young leaf, Leaf pubescence, Leaf venation, Leaf apex habit, Leaf apex shape, Leaf base shape, Leaf shape, Leaf petiole colour, Young shoot colour, Immature leaf colour and Mature leaf colour (colour descriptors were measured using Munsell colour chart for plant tissue, 1952).

Five randomly selected plants from plots of each accession were used to score for each of the 20 morphological descriptors. Non parametric data was converted on scales as proposed in the IPGRI descriptor list. Principle Component Analysis (PCA) was carried out using mean values of morphological descriptors scored for 203 accessions. Average Linkage Cluster analysis was performed subsequently, based on significant Principle Components (PCs) to identify the discriminating descriptors responsible for grouping of accessions. The statistical analysis was performed using Statistical Analysis System (SAS) for Windows Version 8e (Anon, 2001). A dendrogram was generated using cluster analysis on first 9 PCs.

RESULTS AND DISCUSSION

The eigen values generated from correlation matrix obtained by PCA using the means of 20 morphological descriptors scored for 203 accessions are given in Table 2.

Eigen values of the principle components (PCs) indicated that the first 9 PCs accounted for 76 % of the total variation present in the accessions. Therefore, only the first 9 PCs were retained for further analysis. Eigen vectors of each of the principle components (Table 3) revealed that only few variables contributed significantly towards deciding the position of each PC.

The first PC which accounted for about 20 % of the total variation was predominantly associated with leaf width and leaf size. The second PC which accounted for 14 % of the total variation was associated with all descriptors related to color such as young shoot color, petiole color and immature leaf color. Leaf shape and leaf length:width ratio was associated significantly with the third PC which accounted for only 9 % . The rest of the 6 PCs, which accounted for 32 % of the total variation, was mainly associated with descriptors such as mature leaf color, leaf angle and leaf pose in the fourth PC; leaf base shape and type of serration in leaf margin in the fifth PC; waviness of the leaf lamina in the sixth PC; leaf venation in the seventh PC; young leaf pigmentation in the eight PC and petiole pigmentation in the ninth PC. Accordingly, of the 20 descriptors scored, only 16 contributed significantly to the total variation present in the germplasm collection.

Based on the the first 9 principle components of the PCA, Average Linkage Cluster Analysis was performed as shown resulted in the dendrogram (Figure 1), in which 7 well defined clusters could be identified.

Table 2. Eigen values of the correlation matrix obtained from the principle component analysis of 20 morphological descriptors.

PC	Eigen value	Difference	Proportion	Cumulative %
1	3.97266593	1.10081663	0.1986	19.86
2	2.87184930	0.97565723	0.1436	34.22
3	1.89619207	0.45551903	0.0948	43.70
4	1.44067305	0.17457263	0.0720	50.91
5	1.26610042	0.21245367	0.0633	57.24
6	1.05364675	0.07645869	0.0527	62.51
7	0.97718806	0.08222511	0.0489	67.39
8	0.89496295	0.08349307	0.0447	71.87
9	0.81146989	0.09124847	0.0406	75.92
10	0.72022142	0.02053873	0.0360	79.52
11	0.69968269	0.03599073	0.0350	83.02
12	0.66369196	0.07000230	0.0332	86.34
13	0.59368965	0.06815974	0.0297	89.31
14	0.52552991	0.00829726	0.0263	91.94
15	0.51723265	0.10997237	0.0259	94.52
16	0.40726028	0.07637305	0.0204	96.56
17	0.33088723	0.08539605	0.0165	98.21
18	0.24549118	0.13964482	0.0123	99.44
19	0.10584637	0.10012815	0.0053	99.97
20	0.00571822		0.0003	100.00

Table 3. Eigen vectors for first nine PCs of the 20 morphological descriptors

Descriptor	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
1	-0.0245	0.2235	0.1016	-0.1909	**0.4580**	0.1328	-0.0726	-0.3871	0.3735
2	0.0234	0.1336	0.0793	0.0491	-0.0673	**0.8318**	0.0979	0.2427	0.1620
3	-0.0107	0.3135	0.0318	-0.0488	0.3376	0.0217	-0.2519	-0.1874	**-0.5296**
4	-0.0951	0.2872	0.0228	0.0211	-0.0503	-0.1861	0.1650	**0.6187**	0.0475
5	-0.2659	0.1527	0.0373	-0.0042	0.3928	-0.1409	0.0233	0.0209	0.2759
6	0.2001	0.0546	-0.0838	0.0947	0.0259	0.0453	**-0.6880**	0.2198	0.4200
7	-0.3272	-0.0060	-0.0062	0.1358	0.0256	-0.1402	0.0484	-0.0902	0.1506
8	0.1289	-0.0699	-0.3902	0.0545	0.1801	-0.3324	0.1374	0.1238	0.3041
9	0.0217	-0.0576	-0.3182	-0.0923	**0.4743**	0.2220	0.4246	0.1366	-0.0970
10	-0.2169	0.0171	**0.4381**	0.0174	0.0803	-0.0585	0.2241	0.1636	0.1767
11	0.2402	0.0827	0.1616	**0.5073**	0.1397	0.0468	0.1480	0.1326	0.1778
12	0.2974	0.0026	0.0838	**-0.4924**	-0.1711	-0.0931	0.1481	-0.0402	0.1464
13	0.3732	0.1776	0.3026	0.2429	0.1220	-0.1081	0.0872	0.0711	-0.0679
14	**0.4386**	0.1471	-0.0402	0.2016	0.0912	-0.0457	0.1125	0.0147	-0.0429
15	-0.1790	0.0166	**0.5559**	0.0380	0.0374	-0.1101	-0.0206	0.0871	0.0229
16	**0.3989**	0.1876	0.1746	0.2197	0.0306	-0.0832	0.0726	0.0335	-0.0320
17	0.0957	**-0.4742**	0.1667	0.0180	0.1567	0.0328	-0.0286	0.0810	0.0108
18	0.1298	**-0.4041**	0.1321	0.0325	0.2415	0.0454	-0.1305	0.0861	0.0385
19	0.1023	**-0.4813**	0.1284	-0.0143	0.1875	0.0039	0.0330	0.0411	-0.0725
20	0.0438	-0.0323	0.0071	**0.5177**	-0.2022	0.0572	0.2826	-0.4545	0.2737

1. type of serrations in the leaf margin	6. leaf venation,	11. leaf angle(degrees)	16. leaf size
2. waviness of the leaf lamina,	7. leaf apex habit	12. leaf pose (degrees)	17. immature leaf colour
3. pigmentations in leaf petiole,	8. leaf apex shape	13. leaf length (cm)	18. young shoot colour
4. pigmentations in young leaf,	9. leaf base shape	14. leaf width(cm)	19. petiole colour
5. leaf pubescence	10. leaf shape	15. leaf length :width ratio	20. mature leaf colour

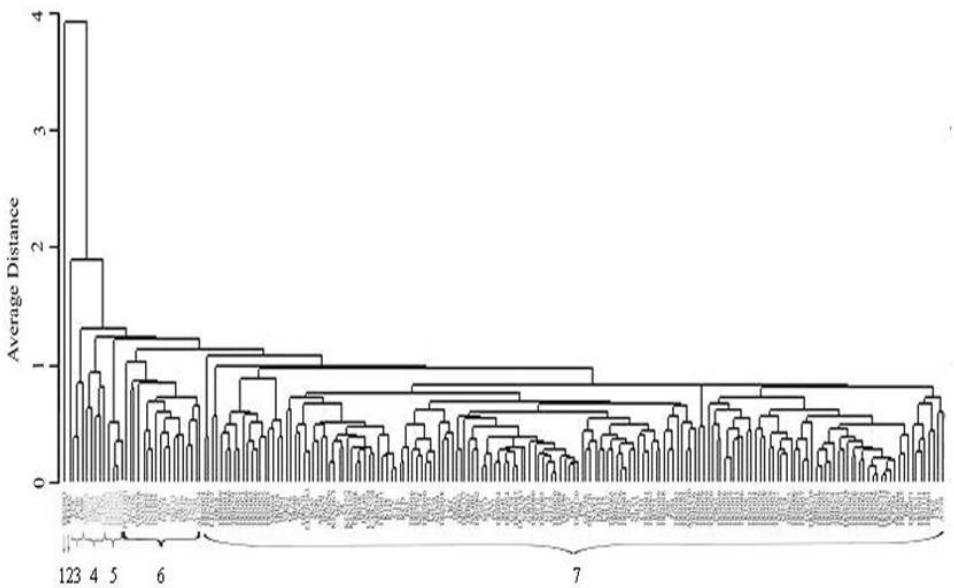

Figure 1. The Dendrogram obtained by cluster analysis of 20 morphological descriptors of 203 accessions

1. Cluster 1 (01 accession)
2. Cluster 2 (01 accession)
3. Cluster 3 (03 accessions)
4. Cluster 4 (05 accessions)
5. Cluster 5 (04 accession)
6. Cluster 6 (18 accessions)
7. Cluster 7 (171 accessions)

The interpretation of the descriptors mainly responsible for cluster divergence is presented in Table 4.

Table 4. The interpretation of the descriptors primarily responsible for cluster divergence

Cluster No.	Morphological descriptors mainly responsible for cluster divergence
Cluster 1	• Pigmentations found in both young leaf & leaf petiole • Sharply serrulate leaf margin with wavy leaf lamina
Cluster 2	• Pigmentations found only in leaf petiole • Denticulate leaf margin with wavy leaf lamina
Cluster 3	• Non- pigmented • Denticulate leaf margin without wavy leaf lamina • Large size leaves
Cluster 4	• Pigmentations found only in young leaves
Cluster 5	• Pigmentations found only in leaf petiole • Large size leaves
Cluster 6	• Erect leaf angle
Cluster 7	• Semi erect leaf angle

According to the above interpretation, only 6 descriptors namely type of serration of leaf margin, waviness of the leaf lamina, pigmentations in young leaf, pigmentations in leaf petiole, size of the leaf and leaf angle were found to be primarily responsible for cluster divergence. Therefore, these 6 descriptors were identified as the most morphological discriminating descriptors for effective characterization of the tea germplasm in Sri Lanka.

CONCLUSIONS

This study describes and estimates the extent of phenotypic variation present among the tea germplasm accessions in Sri Lanka. Of the 20 standard morphological descriptors used to score a representative sample of 203 germplasm accessions, only 6 descriptors, namely, type of serration of leaf margin, waviness of the leaf lamina, pigmentations in young leaf, pigmentations in leaf petiole, size of the leaf and leaf angle were significantly associated with the total phenotypic variation present in the local tea germplasm. Hence, out of the 52 standard list of descriptors proposed by IPGRI (Anon, 1997), the present study enabled the identification of a minimum number of key descriptors most relevant for characterizing the tea germplasm in Sri Lanka in a systematic manner. The information gathered in this study will also be useful in cultivar identification programmes which have become a demanding need of the tea industry after introducing new improved cultivars by the Tea Research Institute of Sri Lanka. This is the first systematic and extensive study reported so far aiming at identification of key descriptors relevant for characterization of tea germplasm, both locally and internationally.

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