

## Characterization of Tea (*Camellia sinensis* L.) Germplasm in Sri Lanka using Morphological Descriptors

J H N Piyasundara<sup>1</sup>, M T K Gunasekare<sup>1</sup> and I P Wickramasinghe<sup>2</sup>

<sup>1</sup>Plant Breeding Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka

<sup>2</sup>Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka)

### ABSTRACT

Characterization of germplasm is an important initial step towards proper utilization of genetic resources in plant breeding programs. Tea germplasm accessions conserved in field gene banks in Sri Lanka have not been adequately characterized in order to maximize its utilization in tea breeding programs. Hence, present study aims at characterizing 203 tea (*Camellia sinensis* L.) germplasm accessions using 20 standardized morphological descriptors to assess the phenotypic diversity of the existing germplasm collection and to find morphological markers useful in characterization of tea germplasm in Sri Lanka. Principle Component Analysis (PCA) and cluster analysis based on first 9 principle component (PCs), which accounted for 76% of the total phenotypic variation, delineate 203 accessions into 7 major groups. The unique morphological identity of each group was further confirmed using the available information related to their origin, ancestral relationships and locations of selections of the accessions. Moreover, it was able to identify key descriptors contributing to the phenotypic variation of the tea germplasm in Sri Lanka.

This is the first extensive study reported so far on tea germplasm characterization using morphological descriptors, both locally and internationally. The results provide the base for more advanced characterization studies using molecular markers in order to assemble a core germplasm collection in tea. Furthermore, the exceptional morphological descriptors identified in this study would facilitate cultivar identification, which has become one of most demanding needs among tea growers after the introduction of new improved cultivars by the Tea Research Institute of Sri Lanka

**Key words:** *Camellia sinensis*; germplasm; morphological descriptors; Principle Component Analysis; tea;

### INTRODUCTION

Identification of candidate germplasm accessions for inclusion in breeding programs is vital for genetic improvement of future cultivars aiming at higher crop productivity and quality. However, achieving this objective depends largely on the information available on

characterization and evaluation of the accessions. Therefore, better understanding of the genetic diversity present and its distribution is essential for rational utilization of germplasm in crop improvement programs.

Tea (*Camellia sinensis* L.) germplasm accessions which have been conserved *ex-situ* in field gene banks in Sri Lanka have not been characterized adequately for morphological characteristics except in few studies by the early workers (Wickramaratne, 1981; Gunasekare *et al.*, 2001; Piyasundara *et al.*, 2006) using limited number of accessions. Moreover there are no studies reported so far on extensive characterization of tea germplasm in other countries too. This constraints effective utilization of tea genetic resources in breeding programs and related research. Therefore, objectives of the present study were to characterize the tea germplasm accessions using standard morphological descriptors, and to group them into phenotypically diverse groups to define needs for future evaluation.

## MATERIALS AND METHODS

The study is carried out at the *ex situ* tea germplasm of the Tea Research Institute Low Country Regional Centre at St. Joachim estate, Ratnapura, Sri Lanka. A total of 203 tea accessions representing various origins/ ancestry (Table 1) were used in the study.

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

Origin / Ancestry	No. of accessions
Introductions from other countries	07
Accessions selected from existing old seedlings on tea estates located in different agro ecological regions (popularly known as "estate selections")	114
Accessions selected from open pollinated progenies of ASM 4/10	30
Selections from second generation progenies (TRI 2024, TRI 2025 and TRI 2026) of ASM 4/10	11
Cultivars developed through the breeding program of the Tea Research Institute of Sri Lanka	39
Selection from open pollinated seeds of Indo Chinese- Shan Bansang origin	01
Selection made from open pollinated progeny of selection (TRI 3011) made from a St Coombs estate selection	01

Of the 52 descriptors proposed by International Plant Genetic Resource Institute, IPGRI, (Anon, 1997), 20 informative morphological descriptors were selected based on studies carried out by early workers (Wickramaratne, 1981; Gunasekare *et al.*, 2001; Piyasundara *et al.*, 2006) as well as using the field experience. Those 20 descriptors include quantitative as well as qualitative descriptors as indicated below:

### **Six quantitative descriptors**

Leaf length, Leaf width, Leaf length: Leaf width ratio, Leaf size, Leaf angle and Leaf pose were considered as quantitative descriptors.

### **Fourteen qualitative descriptors**

Type of serration of the leaf margin, Waviness of the leaf lamina, Pigmentation in leaf petiole, Pigmentation 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 were chosen as qualitative descriptors.

Five randomly selected plants from a plot of each accession were used to record observations on morphological descriptors. Non parametric data was converted on scales as proposed by IPGRI in descriptors for tea (Anon, 1997). Principle Component Analysis (PCA) was carried out using mean values of morphological descriptors. Average linkage cluster analysis was performed subsequently based on significant number of PCs aiming at grouping 203 accessions. The analysis was performed using Statistical Analysis System (SAS) for Windows Version 8e (Anon 1999 - 2001).

## **RESULTS AND DISCUSSION**

The mean values of 20 morphological descriptors of 203 germplasm accessions were subjected for PCA and the eigenvalues showed that the first 9 principle components accounted for about 76% of the total variation (Table 2).

Table 2. Eigenvalues of the first 9 PCs obtained from the principle component analysis of 20 morphological descriptors

PC	Eigenvalue	Cumulative %
1	3.97266593	19.86
2	2.87184930	34.22
3	1.89619207	43.70
4	1.44067305	50.91
5	1.26610042	57.24
6	1.05364675	62.51
7	0.97718806	67.39
8	0.89496295	71.87
9	0.81146989	75.92

Therefore, first 9 PCs were retained for further analysis. The dendrogram (Figure 1) constructed through average linkage cluster analysis based on those 9 PCs delineated 203 accessions into 7 major clusters (Order of the 203 accessions in the dendrogram has been presented in Table 3)

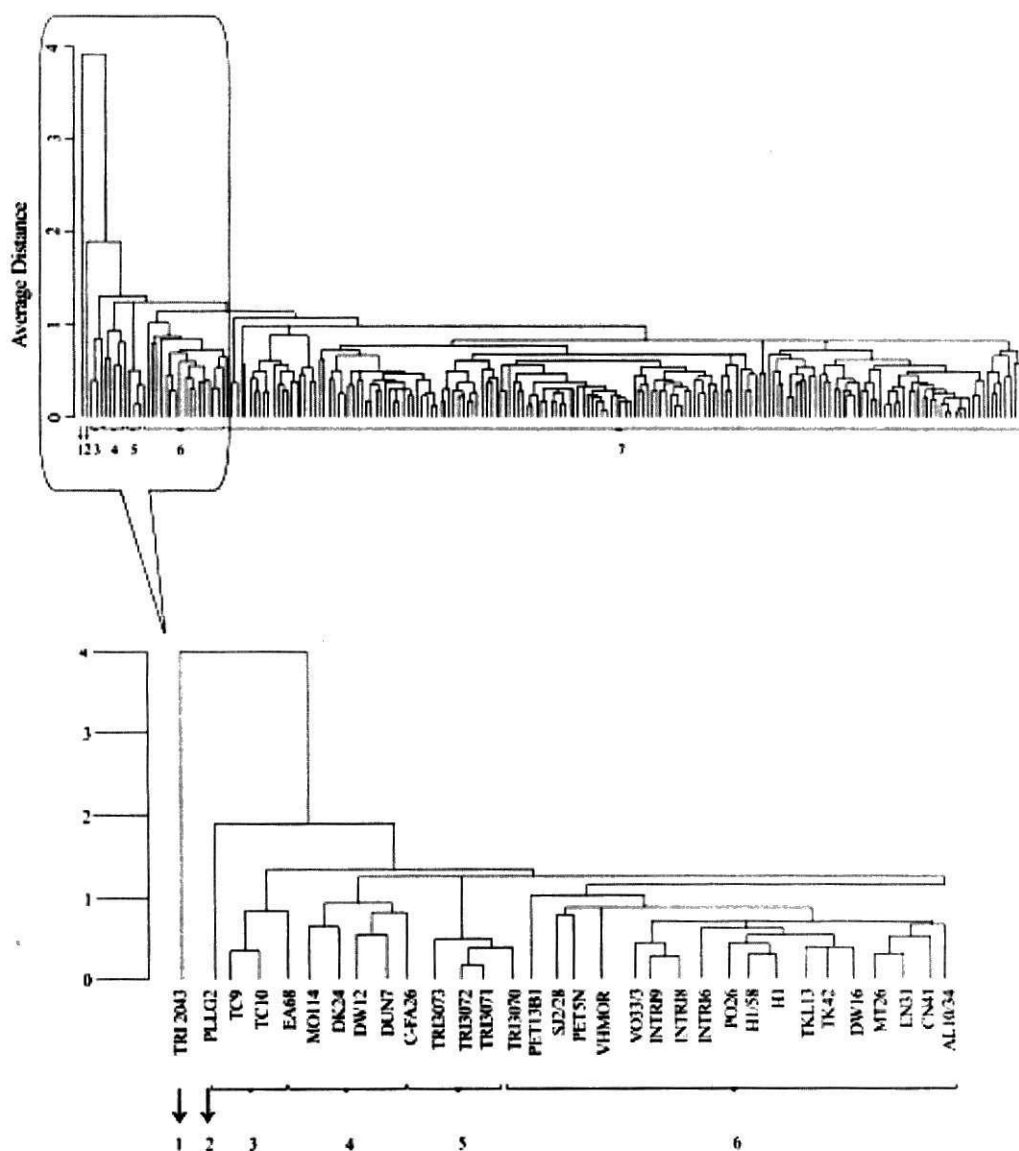


Figure 1. The dendrogram obtained by average linkage cluster analysis of 20 morphological descriptors of 203 accessions

- 1 = Cluster 1 (01 accession- No. 1 in Table 3 )
- 2 = Cluster 2 (01 accession- No. 2 in Table 3 )
- 3 = Cluster 3 (03 accessions- No. 3-5 in Table 3 )
- 4 = Cluster 4 (05 accessions- No. 6-10 in Table 3 )
- 5 = Cluster 5 (04 accessions - No. 11-14 in Table 3 )
- 6 = Cluster 6 (18 accessions- No. 15-32 in Table 3 )
- 7 = Cluster 7 (171 accessions - No. 33-203 in Table 3 )

Table 3. Order of the 203 accessions in the dendrogram beginning from the bottom of the dendrogram

Cluster No.	Accession Identity				
Cluster 1	1 TRI 2043				
Cluster 2	2 PLLG 2 TC9				
Cluster 3	3 MO 114	4 TC 10	5 EA 68		
Cluster 4	6 TRI 3073	7 DK 24	8 DW 12	9 DUN 7	10 C-FA 26
Cluster 5	11 PET 13B1	12 TRI 3072	13 TRI 3071	14 TRI 3070	
Cluster 6	15 INTRI 9	16 SJ 2/28	17 PET 5N	18 VHMOR	19 VO 33/3
	20 H 1	21 INTRI 8	22 INTRI 6	23 PO 26	24 H 1/58
	25 EN 31	26 TKL 13	27 TK 42	28 DW 16	29 MT 26
Cluster 7	30 TRI 2024	31 CN 41	32 AL 10/34	138 B 275	173 GV 35
	33 TRI 2016	68 TRI 3018	103 RG 41	139 TRI 2039	174 TRI 62/10
	34 LLF 14/2	69 TRI 18	104 TRI 3024	140 TRI 4048	175 TRI 62/1
	35 HR 18	70 DEL 40	105 GT 7/4	141 R 80	176 TRI 3019
	36 TRI 4089	71 CAR 7/10	106 TRI 4047	142 P 104	177 TRI 3055
	37 TRI 4014	72 CAR 2/10	107 DUN 3A1	143 TRI 3043	178 TRI 2023
	38 TRI 3060	73 MO 116	108 N4NY	144 TRI 4083	179 TRI 62/4
	39 TRI 62/9	74 TRI 3011	109 GW 19	145 TRI 4053	180 TRI 2021
	40 TRI 3052	75 K 136	110 TRI 4080	146 ASM 4/10	181 TRI 62/19
	41 TRI 3021	76 K 145	111 MT 11	147 TRI 62/6	182 TRI 3042
	42 TRI 3069	77 C 12	112 GT 7/1	148 TRI 2046	183 TRI 3026
	43 TRI 3063	78 RL/E	113 TRI 4085	149 TRI 4079	184 TRI 4042
	44 TRI 3046	79 RL/D	114 GT 1/5	150 TRI 4049	185 TRI 4002
	45 TRI 3025	80 P 63	115 TRI 4020	151 TRI 3020	186 TRI 3051
	46 TRI 2025	81 TRI 4016	116 TRI 3058	152 PBG 1	187 TRI 4024
	47 TRI 2015	82 TRI 3022	117 TRI 396	153 TRI 4059	188 TRI 3029
	48 SM 404	83 NIL 274	118 MT 12	154 TRI 3016	189 TRI 62/14
	49 TRI 216	84 TRI 3050	119 D 95	155 TRI 4055	190 TRI 62/13
	50 HUN 1	85 PW 55	120 TRI 2120	156 TRI 3017	191 TRI 2027
	51 UR 12	86 MT 16	121 P 98	157 TRI 3013	192 KP 204
	52 GF 7/6	87 NEM 9	122 GK 518	158 TRI 4046	193 TRI 4004
	53 TRI 40	88 TRI 2018	123 DEL 231	159 K 150	194 TRI 3047
	54 TK 45	89 T 212	124 NAY 3	160 TRI 4033	195 DW
	55 HR 4/11	90 CR 4	125 DG 39	161 TRI 3014	196 TRI 4071
	56 EBUD 163	91 TRI 43	126 PW 39	162 TRI 2026	197 YF 6/1
	57 TK 43	92 TK 69	127 NIL 12	163 TRI 4056	198 TRI 3015
	58 S 106	93 TRI 862	128 TRI 3065	164 TRI 4052	199 TRI 1114
	59 MO 241	94 TRI 2137	129 TRI 62/3	165 TRI 3057	200 TRI 4061
	60 DW 32	95 TRI 603	130 TRI 1526	166 TRI 2022	201 DG 7
	61 TRI 2020	96 NK4B1	131 TRI 2044	167 MT 20	202 CY 9
	62 KEN 16/3	97 TRI 3062	132 DG 3	168 MT 18	203 A 7/10
	63 MG	98 P 101	133 NL 3/1	169 TRI 62/5	
	64 F 8	99 MT 13	134 NIL 53	170 TRI 4006	
	65 KEN 15/2	100 PA 5	135 C 133	171 TRI 4075	
	66 KEN 15/7	101 PA 22	136 TRI 3041	172 TRI 3037	
	67	102 TRI 4054	137 TRI 4067		

**Table 4. The cluster compositions and the key morphological feature/s responsible for cluster divergence**

Cluster No.	No. of accessions	Key morphological feature/s responsible for cluster divergence
1	01	Pigmentations found in both young leaf and leaf petiole Sharply serrulated leaf margin Wavy leaf lamina
2	01	Pigmentations found only in leaf petiole Denticulate leaf margin Wavy leaf lamina
3	03	Non - pigmented Denticulate leaf margin Large size mature leaf
4	05	Pigmentations found only in young leaves Serrulate leaf margin
5	04	Pigmentations found only in leaf petiole Serrulate leaf margin Large size mature leaf
6	18	Erect leaf angle
7	171	Semi-erect leaf angle

The cluster composition and the key morphological descriptors responsible for cluster divergence in the dendrogram are given in Table 4.

It was able to confirm the grouping of the accessions and their unique morphological identity conserved in each cluster using the available information related to the origin /ancestry and the location of selection of accessions studied and they are as follows:

Cluster 1 comprises of only one accession, TRI 2043 which is the only selection made from the open pollinated progenies of Indo Chinese -Shan Bansang seedlings which had a unique origin of exotic type (Richards, 1965).

Cluster 2 comprises of only one accession, PLLG2. That was the only selection made from existing old seedlings at Poonagala estate considered in this study. This outstanding location of selection further confirms the unique morphological identity of this accession.

Cluster 3 comprises of three accessions (TC9, TC10 and EA68) and two of them (TC 9 and TC 10) were selected from the existing old seedling at the Tillicoultry estate. Accessions selected from the same location falling into the same cluster further confirms their similar

morphological identity. However, EA 68 which was introduced from South Africa also falls into the same cluster owing to the similar morphology and this cannot be supported by the available information related to its origin.

Cluster 4 comprises of five accessions (MO114, DK24, DW12, DUN7 and C-FA26). Four of those accessions (MO114, DK24, DW12 and DUN7) were selections made from the existing old seedling at four different estates (Gunasekare and Kumara, 2005). The other accession C-FA26 is an introduction from an un-known country (details on origin not available). Therefore similar morphological identity of these five cultivars cannot be supported in relation to their origin / ancestry or the location of selection.

Cluster 5 comprises of four accessions (TRI 3070, TRI 3071, TRI 3072 and TRI 3073). All 4 accessions have direct relationship with ASM 4/10 as they were derived from ASM 4/10 in their first and second generation progenies (Gunasekare, 2008).

Cluster 6 comprises of 18 accessions. Of these 18 accessions, four of them are introduction from other countries, but the information about country of origin is not available. The remaining 14 are estate selections. Out of the four introductions, three are from same unknown source (INTR I6, INTR I8 and INTR I9). This same source of origin further confirms the similar morphological identity of these four accessions. Out of the fourteen estate selections two (PET 13 B1 and PET 5 N) were selected from Pettigala estate (agro ecological region WM3). Another three (MT 26, EN 31 and CN 41) accessions have been evolved from the same agro- ecological region (WM3), though they were not selected from the same estate. Another two accessions which were the selection made from Talankanda Estate, namely TKL 13 and TK 42 (Gunasekare and Kumara, 2005) also fall into the same cluster.

Cluster 7 comprised of all the remaining accessions (171) which include ASM 4/10 introduced from India and majority of its descendants. The ancestor and majority of its descendants gathering into same group further confirmed their morphological similarity. In addition Cluster 7 also include the majority of estate selections considered in this study (93 out of 114) selected from various estates located in different agro ecological regions in the country (Gunasekare and Kumara, 2005). Possible explanation for this may be linked to their original source, which could be traced back to single seed source introduced from either China or India (Singh and Gunasekare, 2000).

The presence of a strong resemblance between the morphology of the accessions selected from the same estate or site was evident in tea (Wickramaratne, 1981). Also in several studies conducted in relation to the distribution of genetic variability within a species, the

obvious pattern that emerges was associated with broad ecological regions (Aston and Bradshaw, 1966; Bennett, 1970).

Results obtained from the cluster analysis also revealed that 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 contributing significantly to the total phenotypic variation present in the germplasm and hence could be useful in characterizing the tea germplasm.

## CONCLUSIONS

This study describes and classifies 203 tea accessions of the current germplasm collection maintained at the Tea Research Institute of Sri Lanka into well defined phenotypic groups. PCA using 20 morphological descriptors and cluster analysis based on first 9 principle component (PC) scores delineate 203 accessions into 7 major clusters. The morphological identity within each of the group was confirmed using the available information related to the origin and ancestry of the accessions. Moreover it was also able to identify the key descriptors contributing to the phenotypic variation of the tea germplasm. Being the first systematic and extensive study reported so far on tea germplasm characterization using morphological descriptors, both locally and internationally, this study provides the base for more advance characterization studies using biochemical and molecular markers aiming at assembling a core germplasm collection. Moreover information generated in this study would facilitate cultivar identification programs, which has become one of most demanding needs among tea growers after the introduction of new improved cultivars by the Tea research Institute of Sri Lanka.

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