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# Evaluation of morphological diversity in south Indian tea clones using statistical methods

**Lingaiah Rajanna [1], Mani Ramakrishnan [2,*] and Luke Simon [3,4]**

[1] Department of Botany, Bangalore University, Jnana Bharathi Campus, Bangalore - 560056, India
[2] Post Graduate Department of Biotechnology, Chikka Muniyappa Reddy Institute of Management Studies, 6th 'A' Main, HRBR Lay out, 2nd Block, Kalyan Nagar, Bangalore – 560043, India
[3] Plant Molecular Biology Laboratory, Division of Horticulture, University of Agricultural Sciences, G.K.V.K, Bangalore-560065, India
[4] Current address: School of Medicine, Dentistry and Biomedical Sciences, Institute of Pathology, Grosvenor Road, Royal Victoria Hospital, Queens University Belfast, Belfast, BT12 6BJ, United Kingdom
***** Corresponding author, e-mail: maniramiyer@yahoo.com

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**Abstract:** Morphological diversity of three *Camellia* (Theaceae) taxa conserved in an ex situ gene bank was studied and the importance of different descriptors in categorising accessions into distinct groups was also determined. Twelve accessions were characterised using 15 morphological descriptors of IPGRI guidelines. The results of principal component analysis (PCA) on morphological characters showed that the first two principal components accounted for 44.77 % of the total variance. In the evaluated quantitative characters, all three taxa had a coefficient of variation (CV) greater than 24.85%, and within the taxon the CV was greater than 9.59%. The qualitative characters showed a wide range of variations and yielded significant differences ($p<0.05$). Phenotypic data had high contributing component loadings from characters such as leaf area, weight of harvested shoots, stem colour, leaf pubescence and young shoot colour. Cluster analysis delineated the accessions into three groups. The implications of our results hold promise for assessing genetic diversity in germplasm collections, which is a prerequisite for their utilisation, effective management and crop improvement.

**Keywords:** *Camellia* spp., morphological diversity, numerical descriptors, tea germplasm

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## INTRODUCTION

Tea [*Camellia sinensis* (L.) O. Kuntze] has been known for more than 2000 years in China and is naturally distributed throughout tropical Asia [1]. Tea production in India started in Assam in 1823 by Robert Bruce. South Indian tea has diverse genetic resources, since all existing plantation stocks are the progeny of the plants or seed stocks brought from Assam, China and other sources. Initially, seed stocks were imported from China through Kolkata botanical garden and planted in Nilgiris in 1832 for experimental purposes [2]. Subsequently, a few more plants from Assam and China were also planted in different areas in southern India. Later, the tea scientific department of united planters association of southern India (UPASI) produced accessions from natural populations and also through breeding methods [3]. The genetic resource of tea in UPASI is undoubtedly one of the most important sources of tea germplasm resources in India. A large number of controlled hybridisation were attempted and some of the progeny were also recommended for planting [4]. The existing diversity will have to be preserved and characterised for future crop improvement programmes that constitute the fundamental support structure for the tea industry.

Leaf morphology has an important role in identifying taxa in which variation in floral structures is uninformative or in which flower specimens are infrequent owing to a limited flowering season, for example [5]. The use of morphological characters is cost-effective when compared to that of biochemical and molecular markers for preliminary characterisation of many individuals to identify morphologically similar groups and for simple varietal identification of phenotypically distinguishable cultivars [6]. In tea, morphological characters have been used to study genetic diversity [7-8], variation [9-11], phylogeny and classification [12-15]. Leaf features have been largely unexploited in taxonomic studies, resulting from a belief that they respond in a plastic manner to environmental factors. However, in ex situ gene banks, the plant materials are grown under similar environmental conditions and farming practices, making it possible to compare taxa.

Statistical methods have been reported [16-18] and there are two main types of techniques to represent taxonomic structure: cluster analysis and principal component analysis (PCA). It should be useful for both the breeding programme and the germplasm conservation of tea plants to understand the diversity and differentiation of morphology among those taxa. In the present study, cultivated tea clones of *Camellia sinensis*, *C. assamica* and *C. assamica* subsp. *lasiocayx* in south Indian germplasm are morphologically described and assessed for their diversity by applying statistical methods. The aim is to detect intra-specific boundaries and to identify reliable distinguishing characters. This study will provide a basis for further investigations of systematic classification using the data from morphological characters.

## MATERIALS AND METHODS

### Plant Materials

Twelve tea cultivars belonging to *Camellia sinensis* (China type), *C. assamica* (Assam type) and *C. assamica* subsp. *lasiocalyx* (Cambod type) were collected from the UPASI-TRI (United Planters Association of Southern India - Tea Research Institute) germplasm collection centre at Valparai, Tamil Nadu (Table 1). Five randomly selected plants from one plot of each accession were

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used to record observations on morphological characters. Young shoots from each cultivar with two leaves and a bud fully exposed to sunlight were collected and morphologically described. Fifteen important characteristics of stem, 4$^{th}$ leaf and young shoots were analysed qualitatively and quantitatively following the guidelines of IPGRI (International Plant Genetic Resources Institute) [19]. For characterisation, IPGRI descriptors were adopted. A total of 17 characters were scored. For each specimen, five mature, healthy looking leaves were scored and averaged. Upon further examination, it was found that two characters were constant (not informative) and were eliminated from the analyses. Finally, 15 characters were selected (Table 2). In our study, due to the homogeneity of local climatic conditions and the similarity of farming techniques applied, the effect of those factors on the morphological characteristics was not considered or interpreted. For quantitative characters, coefficients of variation (CV = standard deviation / mean) among the clones at inter- and intra-specific levels were calculated. A Kruskal-Wallis test [20] was used to determine the differences of qualitative characters among the three taxa.

**Table 1.** List of evaluated *Camellia* stock and their clones

| Camellia species and their clones (type) | Accession number | Source of material |
|---|---|---|
| *Camellia assamica* (Assam) | | |
| 'UPASI-2' | B/ 4/142 (Jayaram) | Brooklands Estate, the Nilgiris |
| 'UPASI-3' | B /5/63 (Sundaram) | Brooklands Estate, the Nilgiris |
| 'Assam Seedling' | -- | Assam |
| *Camellia sinensis* (China) | | |
| 'UPASI-9' | B/6/61 (Athrey) | Brooklands Estate, the Nilgiris |
| 'UPASI-10' | B /6/62 (Pandian) | Brooklands Estate, the Nilgiris |
| 'TRF-2' | NLT/17/10 | The Nullatanni Estate, Munnar |
| 'SA-6' | -- | High Wayves, Tea Estates India |
| *Camellia assamica* subsp. *lasiocalyx* (Cambod) | | |
| 'UPASI-17' | B /6/203 (Swarna) | Brooklands Estate, the Nilgiris |
| 'TRF-1' | Selection A | Arrapetta, Wynaad |
| 'CR-6017' | -- | Craigmore, the Nilgiris |
| 'BSS-1' | Biclonal seed stock | UPASI-10 x TRI-2025 |
| 'TRI-2025' | -- | TRI, Sri Lanka |

**Principal Component Analysis**

To explore the pattern of variations in measured characters and to find those decisive characters for distinguishing taxa, PCA was carried out using mean values of morphological observations. PCA can be used for transforming attributes of a dataset into a new set of uncorrelated attributes (principal components) while still retaining as much of the variability of the dataset as possible. It can also handle variables of different types (nominal, ordinal and numerical)

simultaneously and deal with relationships between variables. In addition, Cronbach's alpha [21] was calculated for each of the components extracted.

### Cluster Analysis

Clones of the three *Camellia* taxa were grouped by cluster analysis using the unweighted pair group method analysis (UPGMA) based on the similarity matrix of Euclidean distances of the morphological data. To trace the relationship among the tea clones, the data were standardised before clustering and a dendrogram was constructed. The statistical analyses were performed using the STATISTICA software version 4.5.

## RESULTS AND DISCUSSION

### Morphological Diversity and PCA

The variation in morphological characters of *C. sinensis*, *C. assamica* and *C. assamica* subsp. *lasiocalyx* is summarised in Table 2 by applying statistical methods. Multivariate statistical techniques such as PCA and cluster analysis are commonly used methods for characterisation and genetic diversity analysis of germplasm and can increase the accuracy of interpretation of information generated in characterisation studies [14]. Characters were chosen with respect to variations among taxa mentioned in the literature and also based on careful observation of specimens. The PCA results on morphological characters showed that the first two components accounted for 44.77% of the total variance in the dataset (Table 2). The principal component of single trait accounted for 29.84% of the total variance and was highly interpretable (Cronbach's alpha = 0.80). If the alpha value of a specific component is high, it is 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 is considered to be reliable [22]. Phenotypic data that had high contributing component loadings were from such characters as leaf area (0.88), weight of harvested shoot (0.83), stem colour and leaf pubescence (over 0.7), and young shoot colour with negative loading (-0.71).

A morphometrical analysis of leaf morphology is a useful and rapid method for identification of species [15]. Morphometric studies on *Taxus* (Taxaceae), a taxonomically complex genus with many sterile specimens like *Camellia*, showed that leaf characters are a powerful tool in separating and identifying species in this morphologically labile plant group [23-24]. Pi et al.[15] investigated 54 species of *Camellia*. In their study, PCA results accounted for 63.2 and 20.6% of the total variance for component 1 and component 2 respectively. The sum of the two components accounted for most of the total variance, whereas in our study, 44.77% of the total variance was observed in the data set with a sample size of three *Camellia* taxa comprising 12 clones. In their report, the average values of lamina vertical length, horizontal width, width-length ratio, leaf area and leaf veins were transformed before they were used for PCA. The results of this study showed that the qualitative and quantitative characters with high component loadings are in conformity. Su et al. [11] compared morphological characters of *Camellia sinensis* (*formosensis*) and two closely related

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**Table 2.** Variation in morphological characteristics of investigated tea samples. Loadings of the morphological characters on first two components are from PCA. Eigenvalues, percentages of variance explained and cumulated, and Cronbach's alpha are given for each component.

| Character | Range of variation (Standard deviation) | Data type | Component | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Weight of harvested shoot (g) | 1.23 (0.343) - 3.70 (0.500) | Quantitative | 0.83 | -0.01 |
| Leaf area ($cm^2$) | 18.5 (1.000) - 46.5 (6.782) | Quantitative | 0.88 | 0.04 |
| Internodal length (cm) | 2.20 (0.158) - 5.50 (0.454) | Quantitative | 0.65 | 0.02 |
| Petiole length (cm) | 0.25 (0.500) - 0.50 (0.084) | Quantitative | 0.56 | 0.26 |
| Leaf serration density (number/cm) | 8.00 (0.836) - 20.0 (1.140) | Quantitative | 0.59 | -0.70 |
| Stem colour | Green – dark green | Multistate | 0.71 | -0.29 |
| Immature leaf colour | Yellow green – green | Multistate | -0.42 | -0.54 |
| Mature leaf colour | Yellow green – green | Multistate | 0.13 | -0.14 |
| Leaf blade shape | Ovate-elliptic | Multistate | 0.32 | 0.61 |
| Leaf apex shape | Acute-obtuse | Multistate | -0.05 | -0.38 |
| Leaf blade pubescence | Sparse – intermediate | Binary | 0.70 | 0.16 |
| Leaf blade base shape | Obtuse – acute | Binary | 0.20 | -0.61 |
| Leaf waxiness | Absent – present | Binary | -0.25 | -0.59 |
| Petiole colour | Green – yellow green | Multistate | 0.20 | -0.12 |
| Young shoot colour | Green - yellow green | Multistate | -0.71 | 0.14 |
| | | Eigenvalue | 4.48 | 2.24 |
| | | Variance explained (%) | 29.84 | 14.93 |
| | | Variance cumulative (%) | 29.84 | 44.77 |
| | | Cronbach's alpha | 0.80 | 0.55 |

taxa of Taiwan native wild tea plants using numerical methods. In their studies, characters with high loadings were bud pubescence, young branchlet pubescence, abaxial midrib pubescence and petiole pubescence (over 0.7). Thus, significant values of the Cronbach's apha and character component loadings in our investigation are on a par with their findings. Similarly, Hu [25] used 15 leaf characters measured on a tea germplasm collection of Taiwan to evaluate inter-taxa variations among *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica* and *C. sinensis* var. *formosensisi*.

The evaluated morphological qualitative characters showed a wide range of variations and yielded significant differences ($p<0.05$) among the three taxa (Table 2). Among the qualitative characters, stem colour ($n = 12$, $p = 0.05$) and young shoot colour ($n = 12$, $p = 0.01$) exhibited significant differences. Similarly, significant qualitative characters showed high factor loadings in the first component of PCA (Table 2). Quantitative and qualitative characters showed that clear morphological differences exist within and among taxa in our study (Tables 2-3). Chen et al. [12] reported significant differences ($P<0.05$) in the seven qualitative characters of *C. sinensis* (cultivated tea) and its wild relatives in Yunnan province, China.

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**Table 3.** Variations of quantitative morphological characters of the three *Camellia* taxa

| ***Camellia*** **Species and their clones** | **Weight of harvested shoot (g)** | **Internodal length (cm)** | **Leaf area ($cm^2$)** | **Petiole length (cm)** | **Margin serration per cm** |
|---|---|---|---|---|---|
| *Camellia assamica* | | | | | |
| UPASI 2 | 2.72 ± 0.370 | 3.1 ± 0.587 | 46.5 ± 6.782 | 0.5 ± 0.083 | 9 ± 1.581 |
| UPASI 3 | 3.56 ± 0.531 | 5.5 ± 0.545 | 36 ± 3.847 | 0.5 ± 0.122 | 20 ± 1.141 |
| ASS SEED | 3.7 ± 0.370 | 3.75 ± 0.336 | 45 ± 1.581 | 0.4 ± 0.070 | 18 ± 1.581 |
| | 3.3 ± 0.503 | 4.10 ± 1.217 | 42.4 ± 5.907 | 0.46± 0.052 | 15.5 ± 5.714 |
| C V (%) | 15.25 | 29.65 | 13.94 | 11.5 | 36.78 |
| *Camellia sinensis* | | | | | |
| UPASI 9 | 2.15 ± 0.223 | 4.5 ± 0.791 | 22.5 ± 1.322 | 0.4 ± 0.083 | 13 ± 1.581 |
| UPASI 10 | 1.53 ± 0.482 | 3.35 ± 0.414 | 32.3 ± 1.923 | 0.3 ± 0.1 | 16 ± 0.707 |
| TRF 2 | 1.81 ± 0.114 | 2.2 ± 0.158 | 32.2 ± 1.351 | 0.5 ± 0.071 | 8 ± 0.836 |
| SA 6 | 2.29 ± 0.614 | 3.5 ± 0.371 | 26.3 ± 1.188 | 0.3 ± 0.072 | 15 ± 0.707 |
| | 1.95 ± 0.334 | 3.4 ± 0.942 | 28.3 ± 4.761 | 0.4 ± 0.094 | 13 ± 3.652 |
| C V (%) | 17.21 | 27.72 | 16.82 | 25.54 | 28.2 |
| *Camellia assamica* subsp. *lasiocalyx* | | | | | |
| CR 6017 | 1.25 ± 0.343 | 3 ± 0.547 | 24.5 ± 0.901 | 0.2 ± 0.05 | 12 ± 0.707 |
| UPASI 17 | 1.97 ± 0.632 | 3.25 ± 0.207 | 30.6 ± 1.673 | 0.5 ± 0.071 | 9 ± 0.836 |
| TRF 1 | 1.23 ± 0.343 | 3.55 ± 0.671 | 18.5 ± 1 | 0.4 ± 0.071 | 9 ± 0.707 |
| TRI 2025 | 1.57 ± 0.279 | 2.95 ± 0.23 | 22.1 ± 0.831 | 0.3 ± 0.044 | 16 ± 0.707 |
| BSS 1 | 1.65 ± 0.594 | 2.75 ± 0.261 | 28.3 ±1.404 | 0.3 ± 0.07 | 15 ± 0.836 |
| | 1.54 ± 0.303 | 3.13 ± 0.299 | 24.8 ± 4.821 | 0.35 ± 0.097 | 12 ± 3.364 |
| C V (%) | 19.72 | 9.59 | 19.44 | 27.64 | 27.57 |
| Total | 2.12 ± 0.825 | 3.45 ± 0.857 | 30.4 ± 8.736 | 0.38 ± 0.096 | 13.3 ± 3.961 |
| C V (%) | 38.96 | 24.85 | 28.74 | 24.68 | 29.71 |

Results in Table 3 show that differences, i.e. CV, in morphological characters according to IPGRI guidelines exist among *C. sinensis, C. assamica* and *C. assamica* subsp. *lasiocalyx*. In the evaluated quantitative characters, all three taxa have a CV greater than 24.85%, and within a taxon the CV is greater than 9.59%. Weight of harvested shoot shows the highest percentage of CV (38.96) and the lowest is in internodal length (24.85%). The internodal length character exhibits the lowest CV (9.59%) in *C. assamica* subsp. *lasiocalyx* and the highest is in margin serration of *C. sinensis* (36.78%). Among the three *Camellia* taxa, 28.74% diversity is detected in leaf area. Chen et al. [12] presented the variation in the morphological characters of *C. sinensis* var. *sinensis*, *C. taliensis* (W. W. Sm.) Melc., and *C. sinensis* var. *assamica* in Yunnan province, China. In their study, quantitative characters exhibited a CV of more than 20% in leaf area, weight of harvested shoot, period of flowering, pericarp thickness and seed weight across all three taxa. Incidentally, variations in such characters as leaf area and weight of harvested shoot seem to support our findings. Similarly, Yu and Xu [26] evaluated diversity in tea germplam resources of China using morphological characters. In our study to estimate the diversity in the current UPASI-TRI

germplasm collections (Tables 2-3), the diversity (CV) among the three *Camellia* taxa based on morphological characters is between 24.85-38.96%. Within taxa it is 11.5-36.78% for *C. assamica*, 17.21-27.72% for *C. sinensis*, and 9.59-27.64% for *C. assamica* subsp. *lasiocalyx* (Table 3). This is much lower than results from other studies based on amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) markers.

Vegetatively propagated clones began to replace seed propagation in the 1960s and probably reduced the genetic diversity within tea cultivation [27]. Wachira et al. [28] reported that 72% of the variation resided among individuals within populations of *C. sinensis* and its wild *Camellia* relatives based on RAPD and AFLP markers. Kaundun and Park [29] stated that 16% of the total diversity of RAPD-PCR markers was observed among populations of Korean tea. Balasaravanan et al. [2] assessed the genetic diversity among tea cultivars from southern India using AFLP markers and found a narrow genetic diversity (less than 37.76%), which supports our findings. Significant variations occurred for the quantitative characters among the investigated taxa. Each clone was relatively distinct from one another in their phenotypic characters by multivariate analysis. Hu [25] used 15 leaf characters measured on 132 tea germplasms to evaluate inter-taxa variation among *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica* and *C. sinensis* f. *formosensis*. Further, there were attempts to study *C. sinensis* and its closely associated species based on numerical methods [30-31]. However, these studies aimed at exploring the variation among populations of *C. sinensis* and its closely related species rather than solving the fundamental taxonomic problems.

In our study we have summarised the morphological variation among the three *Camellia* taxa by applying statistical methods. We have found the intra-specific boundaries and reliable distinguishing morphological characters. A sound knowledge of taxonomy is a prerequisite for the success of any germplasm conservation programme of tea plants. The results summarised here using morphological descriptors of UPASI clones can form a basis for further identification and selection of elite clones from the existing tea germplasm for tea improvement programmes.

**Cluster Analysis**

A dissimilarity matrix based on Euclidian distances for the three *Camellia* taxa is presented in Table 4. The clones of the *Camellia* taxa exhibited a large variation between and within the taxa. The estimated dissimilarity of 4.58% was highest between the clones UPASI 2 (Assam tea) and CR 6017 (Cambod type) while the least dissimilarity (2.00 %) was between two China clones, namely SA 6 and UPASI 10. A phenogram based on the Euclidian distances from the morphological data divides the section *Camellia* into three clusters, viz. cluster $C_1$ and sub-clusters $SC_1$ and $SC_2$, which can be recognised as *Camellia assamica* (Assam type), *Camellia sinensis* (China tea) and *Camellia assamica* subsp. *lasiocalyx* (Cambod type) respectively (Figure 1). This type of clustering pattern is generally consistent with existing knowledge on the morphology and systematics of *Camellia* species [2, 32-34]. The clustering of tea clones is also in congruence with a recent report of Roy and Chakraborty [35] based on ISSR markers.

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**Table 4.** Dissimilarity matrix of 12 tea clones based on morphological characters

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **UPASI 2** | 0 | | | | | | | | | | | |
| **UPASI 3** | 2.83 | 0 | | | | | | | | | | |
| **Assam Seedling** | 3.16 | 3.16 | 0 | | | | | | | | | |
| **UPASI 9** | 2.83 | 4.00 | 3.16 | 0 | | | | | | | | |
| **UPASI 10** | 3.74 | 4.24 | 4.00 | 3.16 | 0 | | | | | | | |
| **TRF 2** | 4.24 | 3.74 | 4.00 | 3.74 | 2.83 | 0 | | | | | | |
| **SA 6** | 3.74 | 4.24 | 4.00 | 3.16 | 2.00 | 2.83 | 0 | | | | | |
| **CR 6017** | 4.58 | 4.12 | 4.36 | 3.61 | 3.32 | 2.65 | 3.87 | 0 | | | | |
| **UPASI 17** | 4.00 | 4.00 | 3.74 | 3.46 | 4.00 | 2.83 | 3.46 | 3.00 | 0 | | | |
| **TRF 1** | 3.46 | 3.46 | 3.74 | 3.16 | 3.46 | 2.83 | 2.83 | 3.87 | 3.16 | 0 | | |
| **TRI 2025** | 3.61 | 4.12 | 3.87 | 3.32 | 2.24 | 3.32 | 3.00 | 3.74 | 4.12 | 3.00 | 0 | |
| **BSS 1** | 3.74 | 4.24 | 4.00 | 2.83 | 3.16 | 3.74 | 3.16 | 3.00 | 3.46 | 3.16 | 3.32 | 0 |

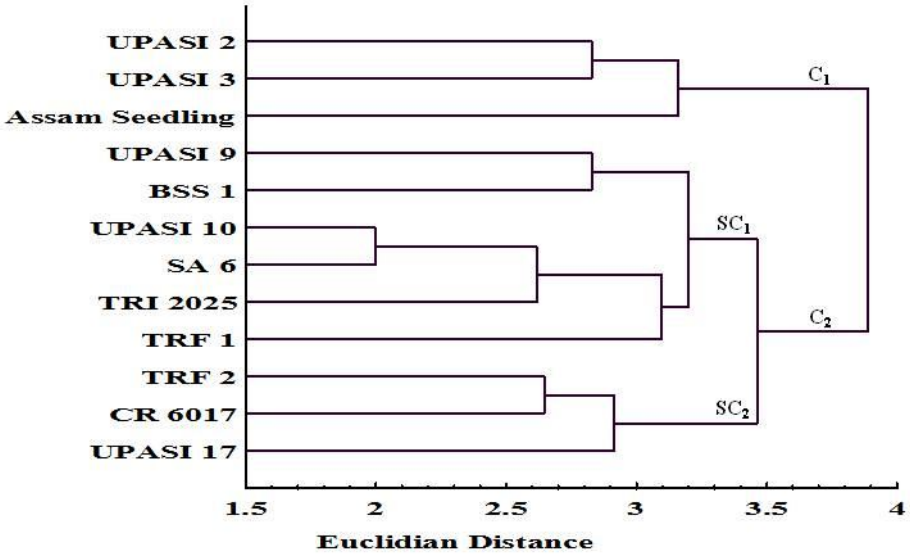


**Figure 1.** Dendrogram of the 12 tea clones based on Euclidean distances of morphological characters

Cluster 1 ($C_1$) consists of clones of only one species, *C. assamica* (UPASI-2, UPASI-3 and Assam seedlings), and cluster 2 ($C_2$) contains clones of two remaining taxa. Thus, $C_2$ has two subclusters, $SC_1$ and $SC_2$, with clones of *C. sinensis* and *C. assamica* subsp. *lasiocalyx* respectively. Subcluster $SC_1$ comprises China type (*C. sinensis*) clones, namely UPASI-9, UPASI-10 and SA 6. The results obtained in our study confirm the report of Saravanan et al. [36], in which the total leaf catechins and their fractions were used for genetic diversity studies. The clone TRF 2 of Cambod type (*C. assamica* subsp. *lasiocalyx*) in subcluster 1 is an exception. Morphological and molecular studies revealed that TRF 2 should be grouped under China varieties [37]. In the cluster $SC_1$, along with China teas, hybrid tea BSS1 and TRI 2025 clones of Cambod tea are also grouped.

Interestingly, UPASI 10 and TRI 2025 are female and male parents of the hybrid BSS 1 respectively, developed by UPASI-TRI. BSS 1 is morphologically more similar to Cambod tea clustered in the group of China teas. These results confirm the morphological explanations and

genealogical data of the hybrid and its parents as reported by Satyanarayana and Sharma [4] and Mohanan and Sharma [33]. In our earlier report, the BSS 1 hybrid tea clustered in the China tea group [34]. Vo et al. [13] reported a similar kind of clustering; a hybrid between Indian and Chinese clones (LDP1) clustered with its morphologically similar mother (PH1), which is *C. sinensis* var. *assamica* (Indian tea).

Subcluster $SC_2$ has Cambod tea clones CR 6017, UPASI 17 and TRF 2 of *C. sinensis* (China type). Clustering of certain clones in our study coincides with results of Paul et al. [38], in which the clone UPASI 17 is grouped under the Cambod type based on genetic diversity studies. All tea clones form three groups in our study based on a statistical methods. Clone TRF 1, grouped in the China cluster along with TRI 2025, is an exception. TRF 1 is similar to TRI 2025 based on morphology [39]. In our earlier study using RAPD marker, the clustering of TRF 1 was found to be in the Cambod group along with TRI 2025 [34]. Vo et al. [13] studied the morphological diversity of Vietnamese tea at Lamdong and produced similar results in the grouping of some clones.

## CONCLUSIONS

The present study has shown that phenotypic characters such as leaf area, weight of harvested shoot, stem colour, leaf pubescence and young shoot colour can be used to distinguish between the three *Camellia* species and their accessions into well-defined phenotypic groups similar to the genetic diversity determined by RAPD markers. Therefore, information on morphological diversity should also be useful for future breeding programmes as well as for proper conservation of genetic diversity in the adapted germplasm.

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