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Research Article

Floral Diversity and Genetic Structure of Tea Germplasm of Sri Lanka

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The role of tea germplasm in crop improvement, though well recognized, yet lacks sufficient information depriving its optimum use. About 600 accessions are conserved as tea germplasm in Sri Lanka and only 4% have been frequently utilized in breeding. Floral morphological characters are useful descriptors for preliminary characterization of genetic resources and particularly pistil traits are considered as reliable criteria in taxonomical studies of higher plants. The objectives of the present study were to conduct a comprehensive analysis on floral diversity of tea germplasm to determine the nature and extent of genetic structure of tea germplasm and to categorize accessions into major taxa. Eighty-nine accessions from the tea germplasm were characterized using 16 floral traits. Results indicated presence of considerable variation among germplasm accessions. Accessions were categorized into five different groups based on the diversity of floral traits and highly discriminating accessions were identified based on the grouping pattern. Among the traits, pistil traits were highly variable compared to other traits. Tea germplasm is predominantly represented by Cambod type accessions (68%) followed by Assam types (20%). Availability of China type accessions is low. Gaps in the germplasm collection were identified and information generated can be used for decision making in future germplasm exploration missions and breeding programme.

1. Introduction

Tea germplasm is the most valuable fundamental materials for tea breeding and biotechnology with a huge potential for the whole tea industry in the future [1]. The role of germplasm in crop improvement, though well recognized, still lacks sufficient information on the performance of the germplasm collection and has led to its limited use. Currently, about 600 tea germplasm accessions are being maintained at the Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka [2]. However, these accessions are not adequately evaluated for biochemical, agronomic, and molecular traits [3, 4]. Hence, tea crop improvement programmes are being practiced on a narrow genetic base. According to the records, only 23 accessions (4% of the total germplasm) have been frequently utilized in tea breeding programmes as parents in the past couple of decades to develop new cultivars [5]. The limited genetic diversity of tea renders it more vulnerable to stress conditions such as the effect of pests, diseases, and drought. Therefore, there is an urgent need to evaluate the existing germplasm to utilize them in tea breeding programmes.

Tea, *Camellia sinensis* (L) O. Kuntze., has been classified into below species level and they are *C. sinensis* var. *assamica* (Assam type) and *C. sinensis* var. *sinensis* (China type) [6]. The third type referred as Cambod or Southern form as a subspecies of *C. assamica* was recognized by Wight [7]. Tea is an allogamous plant and all tea taxa freely interbreed and thus show many overlapping morphological, biochemical, and physiological attributes that are highly diverse and heterozygous [8]. Because of the extreme hybridization, existence of the pure archetypes of tea is doubtful [8]. Therefore, it is important to characterize accessions in the tea germplasm for genetic structure that would assist in better management and utilization of the available genetic resources.

Plant morphology is considered as the scientific study of the physical form and external structure of plants and can be applied to carve up diversity into its systematic subunits [9]. Morphological characters are useful descriptors for preliminary characterization of genetic resources and varietal identification, as they are cost effective compared to biochemical and molecular markers [10]. Among the morphological descriptors, floral traits are important and can be used as taxonomic indicators which are considered informative in identifying the relationship among species of the same genus [11].

Despite the several disadvantages such as continuous variations and high plasticity, influenced by environmental factors, morphological characters are the most adopted markers used by tea breeders globally. Therefore, in tea, morphological descriptors have been used for characterization [12], genetic diversity and comparison [13, 14], cultivar identification [15], and phylogeny and classification studies [16–18]. A comprehensive phylogenetic analysis of all genera belonging to the plant family Theaceae was conducted [18]. It has been reported that the numerical taxonomy and cladistic analysis based on morphological characters of floral organ are useful in species classification of genus *Camellia* [11]. Also, the taxonomic position of the wild tea plant (*C. sinensis* (L.) O. Kuntze forma *formosensis* Kitamura) was assessed using morphological traits [16] and the potential of the intraspecific classification of tea plant based on the variations of floral morphology and numerical taxonomy was reported [12].

Several attempts have been made to characterize tea genetic resources in Sri Lanka using morphological descriptors [2, 19, 20]. Characterization of 203 tea germplasm accessions using 35 standardized vegetative descriptors to assess the phenotypic diversity has been reported and identified minimum list of descriptors suitable as morphological markers that are useful in characterization of tea germplasm in Sri Lanka [20]. Another study has been conducted to assess the variability of pistil related characters to be used as a measure of genetic diversity in tea germplasm using 27 accessions [21]. However, this study has employed only six floral traits to characterize a limited number of germplasm accessions. Therefore, it is important to use a large number of floral traits and germplasm accessions in order to comprehensively analyze the diversity of tea germplasm in Sri Lanka.

Hence, the objective of the present study was to conduct a comprehensive analysis on floral diversity of tea germplasm in Sri Lanka: (1) to determine the nature and extent of genetic diversity of the tea germplasm and (2) to categorize accessions into major taxa.

2. Material and Methods

2.1. Experimental Location and Plant Materials. A total of 89 accessions (i.e., 15% of the total collection) including exotic origins, estate selections, improved cultivars, unadapted accessions, and other important *Camellia* spp. representing the total diversity of the Sri Lankan germplasm were selected for the study. While selecting accessions, diversity tree of tea germplasm in Sri Lanka as described by [4] was followed. Accessions were selected to represent each branch of the diversity tree and number of accessions from a branch was selected based on the total available. List of accessions selected for the study is given in Table 1.

All planting materials are maintained as a living collection at the field gene bank at the Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka (field number 09, St. Coombs Estate; located Lat 6.94N, Long 80.66E, altitude 1256 amsl). The plants are of the same age and have been maintained under similar growth conditions.

2.2. Data Collection. The study was carried out during peak flowering period starting from January to April in 2012, 2013, and 2014. Ten freshly bloomed flowers and ten flower buds were collected from each accession between 9.00 and 10.00 a.m. from three free growing tea bushes. Measurements were repeated three times during the three years' period. Flower buds were collected just before blooming and flowers bloomed on the same day they were collected for the study. Experiment was conducted as a CRD.

The flowers were studied in detail. Thirteen quantitative floral traits, bud length and width, corolla diameter, total number of petals and sepals, number of whorls of sepals and petals, petal length and width, style length, style column length, style arm length, and number of style arms, were recorded. The three qualitative floral traits studied together with the character states are given in the Table 2. The character ovary pubescence was observed under the low power of a stereo microscope and rated as density per microscopic field.

All traits were measured as per the IPGRI [22] and UPOV [23] guidelines as described below.

Bud length and width: length and width of 10 flower buds were measured using a venire caliper and average measurement was taken; diameter of corolla: average diameter of 10 freshly bloomed flowers was taken; number of petals and sepals: petals and sepals of 10 freshly bloomed flowers were counted manually; petal length and width: length and width of petals were measured using a venire caliper; three pistil traits, namely, style length, style column length, and style arm length, were measured using a Vernier caliper. Number of style arms, total number of sepals, total number of petals, and number of sepal and petal whorls of 10 freshly bloomed flowers were counted manually.

2.3. Data Analysis. The mean values for 13 quantitative traits were used for statistical analysis and descriptive statistics parameters were generated for each trait. Data collected among different years were analyzed separately and there was no significant difference among years. Hence, average data were used for further analysis. Data were subjected to analysis of variance and mean separation was done for each trait to investigate variations among three tea taxa. Thirteen quantitative traits were correlated among each other and subjected to Principle Component Analysis (PCA) for developing new variables. In order to reveal the relationships among germplasm accessions, a dendrogram was constructed

Table 1: List of accessions selected for the study and their pedigree.

Number	Accession	Pedigree/origin
1	MT 18	Estate selection from Balangoda Est. (WM1)
2	DG 39	Estate selection from Balangoda Est. (WM3)
3	DG 7	Estate selection from Balangoda Est. (WM3)
4	CV 4/B1	Estate selection from Cannaverella Est. (IU3)
5	CH 13	Estate selection from Craighead Est. (WM2)
6	DK 24	Estate selection from Diyanillakelle Est. (WU2)
7	DN	Estate selection from Dayagama Est. (WU2)
8	DEL 40	Estate selection from Delta Est. (WU2)
9	DT 1	Estate selection from Drayton Est. (WU1)
10	DT 95	Estate selection from Drayton Est. (WU1)
11	DUN 7	Estate selection from Dunsinane Est. (WU2)
12	H 1/58	Estate selection from Hulandawa Est. (WL1)
13	HS 10/A	Estate selection from Hethersett Est. (WU3)
14	K 145	Estate selection from Kirkoswald Est. (WU1)
15	MO 241	Estate selection from Mooloya Est. (WM2)
16	NAY 3	Estate selection from Nayabedda Est. (IU3)
17	NL 8/3	Estate selection from Neluwa Est. (IU3)
18	N 2	Estate selection from Norwood Est. (WU1)
19	PK 2	Estate selection from Park Est. (WU3)
20	PLLG2	Estate selection from Poonagala Est. (IU3)
21	PO 26	Estate selection from Poronuwa Est. (WM1)
22	QT 4/4	Estate selection from Queenstown Est. (IU3)
23	B 275	Estate selection from Ragala Est. (IU2)
24	S 106	Estate selection from Sirikandura Est. (WL1)
25	TRI 2142	Estate selection from St. Coombs Est. (WU1)
26	TRI 2016	Estate selection from St. Coombs Est. (WU1)
27	TRI 1294	Estate selection from St. Coombs Est. (WU1)
28	TRI 3011	Estate selection from St. Coombs Est. (WU1)
29	TRI 26	Estate selection from St. Coombs Est. (WU1)
30	TRI 1114	Estate selection from St. Coombs Est. (WU1)
31	CY 9	Estate selection from Tangakelle Est. (WU1)
32	TC 10	Estate selection from Tillicoultry Est. (WU2)
33	WT26	Estate selection from Waltrim Est. (WU3)
34	W 3	Estate selection from Wootton Est. (WU2)
35	TRI 777	Introduction from Shan Cho Long 777 (1937)
36	TRI 2043	Introduction from Shan Bang Sang 777 (1937)
37	ASM 4/10	Introduction from India (ASM 4/10 seed), 1938
38	TRI 2023	Introduction from India (ASM 4/10 seed), 1938
39	TRI 2024	Introduction from India (ASM 4/10 seed), 1938
40	TRI 2025	Introduction from India (ASM 4/10 seed), 1938
41	TRI 2026	Introduction from India (ASM 4/10 seed), 1938
42	TRI 62/5	Introduction from India (ASM 4/10 seed), 1958
43	TRI 62/9	Introduction from India (ASM 4/10 seed), 1958
44	TRI 3047	Introduction from India (ASM 4/10 seed)
45	TRI 3052	Introduction from India (ASM 4/10 seed)
46	TRI 3055	Introduction from India (ASM 4/10 seed)
47	Yabukita1	Introduction from Japan (seed), 1998
48	Yabukita2	Introduction from Japan (seed), 1998
49	Yabukita3	Introduction from Japan (seed), 1998
50	Yabukita4	Introduction from Japan (seed), 1998
51	Yabukita5	Introduction from Japan (seed), 1998
52	Yabukita6	Introduction from Japan (seed), 1998
53	PBGT55	Introduction from Korea (seed), 2000
54	PBGT53	Introduction from Korea (seed), 2000
55	PBGT48	Introduction from Korea (seed), 2000
56	PBGT73	Introduction from Korea (seed), 2000
57	PBGT12	Introduction from Korea (seed), 2000
58	PBGT49	Introduction from Korea (seed), 2000
59	PBGT60	Introduction from Korea (seed), 2000
60	PBGT67	Introduction from Korea (seed), 2000
61	PBGT61	Introduction from Korea (seed), 2000
62	PBGT41	Introduction from Korea (seed), 2000
63	TRI 3016	ASM 4/10 × DT 95
64	TRI 3019	ASM 4/10 × DT 95
65	TRI 3017	ASM 4/10 × DT 95
66	TRI 4052	CY 9 × ASM 4/10
67	TRI 4053	CY 9 × ASM 4/10
68	TRI 4049	CY 9 × ASM 4/10
69	TRI 4067	CY 9 × NAY 3
70	TRI 4068	CY 9 × NAY 3
71	TRI 4076	N 2 × N2
72	TRI 4078	N 2 × N2
73	TRI 4079	N2 × N2
74	TRI 4061	TRI 2020 × TRI 2023
75	TRI 3022	TRI 2023 × TRI 2026
76	TRI 4085	TRI 2024 × N2
77	TRI 4071	TRI 2024 × N2
78	TRI 3018	TRI 2024 × TRI 1114
79	TRI 3026	TRI 2024 × TRI 1114
80	TRI 3036	TRI 2024 × TRI 777
81	TRI 3041	ASM 4/10 OP
82	TRI 3013	TRI 2024 OP
83	TRI 3072	TRI 2025 OP
84	TRI 3014	TRI 2025 OP
85	TRI 3073	TRI 2025 OP
86	TRI 3015	TRI 2026 OP
87	TRI 3050	TRI 3011 OP
88	VHMOR	Unknown origin
89	INTRI6	Unknown origin

using major Principle Components. The accessions were grouped by average linkage cluster analysis.

All the above statistical analyses were performed by using SAS 9.1 for Windows software [24] and PAST 3.

3. Results and Discussion

3.1. Morphological Diversity of the Tea Germplasm Based on Floral Traits. Distribution of variation of 13 quantitative

Table 2: List of qualitative traits used for the study.

Character	Character state
Ovary pubescence	Densely hairy, slightly (intermediate), sparsely
Stigma position	Below stamen whorl, same level as the stamen whorl, above the stamen whorl
Split pattern of style	Geniculate, free for greater part of their length Ascending, free for about half their length United, united for greater part of their length

Table 3: Descriptive statistics of morphological traits of tea germplasm.

Trait	Min	Max	Mean	CV%
Bud length (mm)	9.91	16.03	12.97	10.17
Bud width (mm)	8.05	15.18	11.48	10.37
Corolla diameter (mm)	12.56	48.21	32.17	12.82
Petal length (mm)	14.19	24.00	17.56	11.23
Petal width (mm)	9.72	54.06	14.38	33.81
Total number of sepals	4	7	5.20	8.78
Total number of petals	5	12	7.38	18.44
Number of whorls of sepals	2	3	2.06	11.26
Number of whorls of petals	2	3	2.73	16.35
Number of style arms	3	4	3.13	10.96
Style length (mm)	3.58	14.61	10.47	15.87
Style column length (mm)	0	10.59	6.72	38.83
Style arm lengths (mm)	1.11	10.23	3.99	57.70

Min = minimum value; max = maximum value; CV = coefficient of variance.

morphological traits measured among germplasm accessions is shown in Table 3.

As illustrated by descriptive statistics, among the traits, petal width, style column length, and style arm length showed higher variation than the other traits having high coefficient of variance (CV) values. Conversely, traits, namely, total number of sepals, bud length, and width, petal length and number of whorls of sepals recorded low CV values indicating less diversity among the germplasm accessions.

The smallest bud length was observed in Yabukita3 (9.91 mm) seedling and the smallest bud width was recorded in TRI 4067 (8.05 mm) cultivar. The largest bud length of 16.03 mm value was recorded in TRI 3014 cultivar and the largest bud width of 15.18 mm value was found in TRI 2043 cultivar.

The size of the tea flower varies extensively among different accessions studied (Figure 1) and could be categorized as small, medium, and large in size. The largest flowers were observed in cultivar TRI 3016 with a corolla diameter of 48.21 mm and the smallest flowers were observed in estate cultivar PO 26 with a corolla diameter of 23.33 mm.

Figure 1: Variation of flower size (corolla diameter) among different accessions (bar = 1 cm).

Figure 2: Variation of style length among different accessions (bar = 1 cm).

Minimum and maximum petal lengths were observed in the accession QT 4/4 and cultivar TRI 2043, respectively, and minimum and maximum petal widths were recorded in estate cultivar DUN 7 and cultivar TRI 3014. Among the 89 accessions studied, flowers with variable number of petals and sepals were observed. Number of petals varied from 5 to 12 and number of sepals varied from 4 to 7. Number of whorls of sepals and petals were 2-3 in all accessions.

It was observed that pistil related morphological traits were highly varied in the tea germplasm. Out of the 89 accessions studied, 77 accessions had three style arms which is common in tea and 12 had four style arms. Also, style column length (Figure 2) and style arm lengths varied among accessions having high CV values of 39% and 58%, respectively. A previous study conducted using 27 accessions from Sri Lankan tea germplasm also revealed that variations of the germplasm could be explained by three pistil traits: number of arms in the style, style column length, and style arm lengths to the greatest extent [21]. Hence, results from the current study are in agreement with those findings.

Stigma position relative to stamens is another parameter that varied among accessions. As explained in Figure 3, 64% of the accessions possess stigma and stamen at the same level and 29% of the accessions are having stigma position above stamen level. However, no correlation was observed among stigma position relative to stamens and major tea taxa.

3.2. Correlation between Floral Traits. Correlation analysis among 13 traits revealed positive and negative correlations among different floral morphological traits as shown in Table 4. Bud length was positively correlated with bud width, corolla diameter, and petal length while bud width showed positive correlation with petal length and corolla diameter. There was a high correlation between corolla diameter and petal width. It was interesting to note that pistil traits are correlated with each other as well as with some flower traits. Style length showed a positive correlation with style column length. However, style column length showed a

Table 4: Pearson correlation coefficients of 13 floral morphological traits.

	Bud length	Bud width	Corolla diameter	Petal length	Petal width	Style length	Style column length	Style arm length	Total number of sepals	Total number of petals	Number of style arms	Number of sepal whorls	Number of petal whorls
Bud length	1.000												
Bud width	0.711^{***}	1.000											
Corolla diameter	0.440^{***}	0.454^{***}	1.000										
Petal length	0.462^{***}	0.430^{***}	0.819^{***}	1.000									
Petal width	0.364^{**}	0.401^{***}	0.344^{**}	0.267	1.000								
Style length	0.382^{**}	0.244	0.608^{***}	0.586^{***}	0.228	1.000							
Style column length	0.060	−0.021	0.066	0.070	0.063	0.567^{***}	1.000						
Style arm length	0.167	0.191	0.366^{**}	0.317	0.074	0.013	-0.752^{***}	1.000					
Total number of sepals	0.120	0.156	0.194	0.038	0.002	−0.023	−0.050	0.091	1.000				
Total number of petals	0.045	0.092	0.175	0.093	0.152	−0.214	−0.267	0.120	0.149	1.000			
Number of style arms	0.24	0.098	0.298	0.181	0.022	0.171	−0.015	0.235	0.114	0.374^{**}	1.000		
Number of sepal whorls	0.048	0.061	0.056	0.113	0.014	−0.035	−0.075	0.043	0.321	0.147	−0.096	1.000	
Number of petal whorls	−0.011	0.107	0.237	0.111	0.142	−0.160	−0.289	0.211	0.215	0.658^{***}	0.240	0.148	1.000

$^{**}p < 0.001$; $^{***}p < 0.0001$.

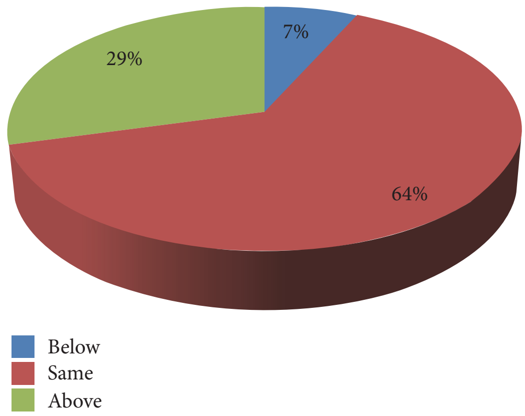


Figure 3: Variation of stigma position relative to stamens in the germplasm.

negative correlation with style arm length. A previous study also reported a negative correlation between style column length and style arm length [21]. Style length was positively correlated with corolla diameter and petal length. Also, a positive correlation was observed between number of style arms and total number of petals. As pistil traits are important in taxonomic classification in tea, it is worthwhile studying these relationships in depth. Therefore, it may be useful to identify few parameters from the study to combine with pistil traits to develop a more reliable methodology to distinguish individual accessions into different archetypes especially when extensive hybridization takes place among tea taxa.

3.3. Principle Component Analysis (PCA) of Floral Traits and Grouping of Accessions. When 13 traits were subjected to PCA, around 76% of the variability in the floral traits was described by first five Principle Components (PCs). As indicated in Table 5, first PC was loaded with two flower traits, corolla diameter, and petal length whereas the loading of the second and third PCs was contributed by pistil traits, namely, style length, style column length, and style arm length. It has been reported that number of style arms contributed to variations [21]; however, in the present study loadings of the fourth PC were affected by number of style arms.

Resultant dendrogram generated by average linkage cluster analysis based on first five PCs is shown in Figure 4. The accessions exhibited large variations and estimated average distance between clusters varied from 0.03 between most similar accessions W3 and TRI 4053 to 1.9 between TRI 3014 and rest of the 88 accessions.

The dendrogram divided accessions into five groups.

Cultivar TRI 3014 separated from other 88 accessions at an average distance of 1.896 being highly discriminated from all others. TRI 3014 is a recommended tea cultivar for mid country wet zone and low elevations of Sri Lanka [25]. TRI 3014 recorded the largest bud width and maximum petal length during the floral trait examination.

The second cluster separated from other accessions with an average distance of 1.46 and two recommended cultivars TRI 2043 and TRI 3016 were included in this group. TRI 2043 is a unique cultivar recommended for silver tip production [25], characteristic with anthocyanin pigmentation and densely arranged pubescence in lower leaf epidermis and buds [2]. A previous study conducted to characterize tea germplasm using vegetative morphological traits reported that TRI 2043 was separated from all other accessions [20]. In the present study, TRI 2043 recorded the largest bud width and maximum petal length among the germplasm accessions. TRI 3016 recorded the largest corolla diameter.

The third cluster with five accessions separated at an average distance of 1.25 from other 81 accessions. The five accessions included two estate selections CY 9 and DG 7 and one recommended cultivar TRI 3055, a recent introduction from Korea PBGT41 and INTRI6 which is an accession with unknown origin. All these accessions belong to Cambod type as shown in Table 6.

There are nine accessions grouped in the fourth cluster which separated at an average distance of 1.13 from the rest. Two estate selections B 275 and NAY 3, one recommended cultivar TRI 4067, and six exotic accessions introduced from Korea (PBGT 48, PBGT 49, PBGT53, PBGT55, PBGT67, and PBGT73) were found in this group. The most interesting feature of the group is that it contains all nine accessions

Table 5: Eigenvalues, individual, and cumulative percentage of variation explained by the first five principal components loadings of the 13 quantitative characteristics.

Character	PC1	PC2	PC3	PC4	PC5
Bud length	0.38	−0.12	−0.08	0.17	−0.29
Bud width	0.37	−0.04	−0.08	0.27	−0.39
Corolla diameter	0.46	−0.05	−0.13	−.09	0.24
Petal length	0.43	−0.11	−0.10	−0.04	0.25
Petal width	0.27	−0.05	0.04	0.08	−0.54
Style length	0.31	−0.40	0.05	−0.15	0.28
Style column length	0.00	−0.52	0.46	−0.12	0.01
Style arm length	0.22	0.34	−0.53	0.01	0.24
Number of style arms	0.21	0.15	0.14	−0.51	0.14
Total number of sepals	0.12	0.17	0.34	0.41	0.28
Total number of petals	0.15	0.42	0.38	−0.25	−0.18
Number of sepal whorls	0.07	0.14	0.30	0.58	0.28
Number of petal whorls	0.16	0.42	0.33	−0.17	−0.09
Eigen value	3.61	2.47	1.40	1.26	1.12
Difference	1.14	1.06	0.14	0.14	
Variance explained (%)	27.75	18.94	10.78	9.68	8.62
Variance cumulative (%)	27.75	46.69	57.47	67.15	75.77

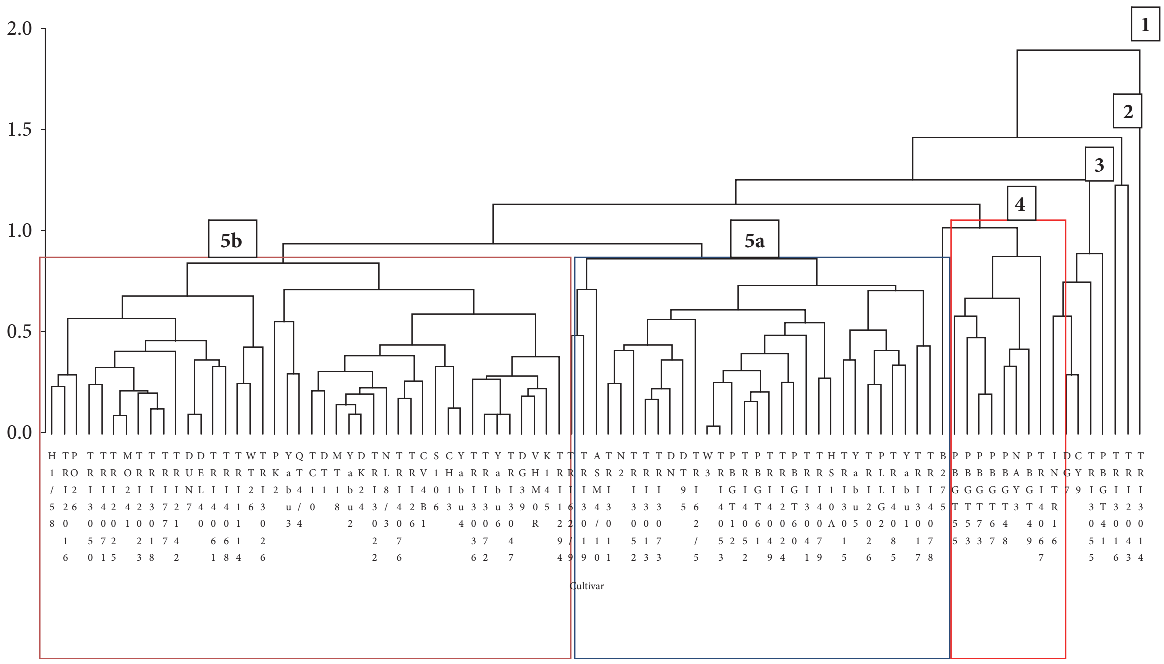


Figure 4: Dendrogram of average linkage cluster analysis based on 13 morphological characteristics.

belonging to China type. As indicated in Table 6, out of the 11 China type accessions used in the study, nine were grouped together based on the overall variation in floral traits.

The fifth cluster has two subclusters 5a and 5b with 30 and 42 accessions, respectively. Both subclusters have mixture of accessions sharing variable floral features. Thirteen Assam type accessions (Table 6) are grouped in subcluster 5b.

The score plot of the first two PCs is shown in Figure 5. It has been found that cultivars TRI 4067, TRI 2043, NAY 3, and DUN 7 are extreme observations along the PC1. Also, two cultivars INTRI 6 and TRI 2026 were found to be different along the PC2. The information present in the loadings is displayed in Figure 6. All 13 variables are represented in the biplot by a vector and the direction and length of the vector indicate how each variable contributes to PC1 and PC2. The PC1 has positive coefficient for the variables bud length and width, diameter of corolla, style length, style column length, and petal length and width. The largest coefficients in the PC1 are two pistil traits style column length and style arm length. The PC2 has positive coefficients for 10 traits except style column length, petal length, and total number of style arms. The largest coefficients in the PC2 are bud length and diameter of corolla.

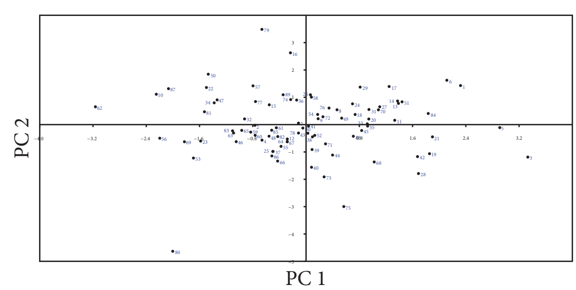


Figure 5: PCA score plot of 89 accessions. (1 = H1/58, 2 = TRI62/9, 3 = INTRI6, 4 = DG7, 5 = TRI3055, 6 = ASM4/10, 7 = PK2, 8 = TRI3011, 9 = W3, 10 = TRI3050, 11 = TRI4053, 12 = TRI4052, 13 = TRI3015, 14 = TRI3013, 15 = TRI2025, 16 = DUN7, 17 = TC10, 18 = TRI62/5, 19 = CY9, 20 = B275, 21 = DT95, 22 = TRI2024, 23 = S106, 24 = DEL40, 25 = TRI4049, 26 = TRI4071, 27 = TRI4079, 28 = TRI3019, 29 = TRI2023, 30 = TRI2026, 31 = DN, 32 = TRI3036, 33 = TRI3052, 34 = TRI3072, 35 = TRI3014, 36 = TRI3018, 37 = TRI4085, 38 = TRI4076, 39 = TRI4061, 40 = MT18, 41 = TRI3017, 42 = TRI3016, 43 = PO26, 44 = CV4B1, 45 = DT1, 46 = CH13, 47 = TRI2142, 48 = DG39, 49 = HS1OA, 50 = TRI2016, 51 = TRI1114, 52 = Yabukita1, 53 = Yabukita3, 54 = Yabukita5, 55 = Yabukita4, 56 = TRI1294, 57 = MO241, 58 = TRI777, 59 = NL8/3, 60 = N2, 61 = TRI3022, 62 = TRI3026, 63 = TRI3047, 64 = TRI4068, 65 = PBGT55, 66 = PBGT53, 67 = PBGT48, 68 = PBGT73, 69 = PBGT12, 70 = TRI3073, 71 = PBGT49, 72 = PBGT60, 73 = PBGT67, 74 = PLLG2, 75 = NAY3, 76 = WT26, 77 = VHMOR, 78 = K145, 79 = TRI2043, 80 = TRI4067, 81 = TRI4078, 82 = TRI26, 83 = PBGT61, 84 = PBGT41, 85 = TRI3041, 86 = QT4/4, 87 = Yabukita6, 88 = Yabukita2, and 89 = DK24.)

3.4. Genetic Structure of Sri Lankan Tea Germplasm Based on Floral Traits. Split pattern of style was used to differentiate between variety and subspecies levels within *C. sinensis* [7]. According to his classification, as shown in Figure 7, China types (*C. sinensis* var. *sinensis*) are having geniculate styles (free for greater part of their length) and Cambod types (*C. sinensis* ssp. *lasiocalyx*) are ascending type in nature (free for about half their length). Wight [7] further stated that styles of Assam types (*C. sinensis* var. *assamica*) are characteristic with united style (united for greater part of their length). Classification by Wight [7] on major tea taxa is generally

Table 6: Grouping of accessions/cultivars based on character split pattern of style.

Cambod type		Assam type	China type
Estate selections			
CY 9	TRI 2016	CH 13	B 275
DEL 40	TRI 26	CV4B1	NAY 3
DG 39	TRI 3011	N 2	
DG 7	W 3	NL 8/3	
DK 24	WT 26	PO 26	
DN	MO 241	QT 4/4	
DT 1	MT 18	S 106	
DT 95	PK 2	TC 10	
DUN 7	PLLG 2		
H 1/58	TRI 1114		
HS1OA	TRI 1294		
K 145	TRI 2142		
Introductions			
TRI 2023	ASM 4/10	Yabukita03	PBGT67
TRI 2024	PBGT60	Yabukita04	PBGT48
TRI 2025	PBGT61	Yabukita06	PBGT49
TRI 2026	PBGT12	TRI 777	PBGT53
TRI 62/5	PBGT41	TRI 2043	PBGT55
Yabukita01	TRI 3052		PBGT73
Yabukita02	TRI 3055		
Yabukita05	TRI 3047		
TRI 62/9			
Improved cultivars			
TRI 4061	TRI 3015	TRI 3013	TRI 3016
TRI 4068	TRI 3017	TRI 3014	TRI 3019
TRI 4071	TRI 3022	TRI 3018	TRI 4067
TRI 4076	TRI 3026	TRI 3036	
TRI 4078	TRI 3041		
TRI 4079	TRI 3073		
TRI 4085	TRI 4049		
TRI 4053	TRI 4052		
Unknown origin			
INTRI6		VHMOR	

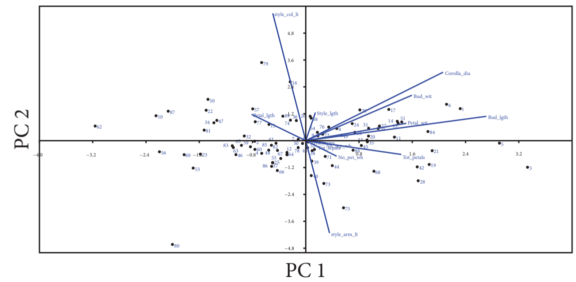


Figure 6: PCA loading plot of 13 variables along PC1 and PC2.

Figure 7: Variation in split pattern of stigma in accessions, TRI62/5 (United), B275 (Geniculate), and TRI2025 (Ascending) representing Assam, China, and Cambod, respectively (bar = 1 cm).

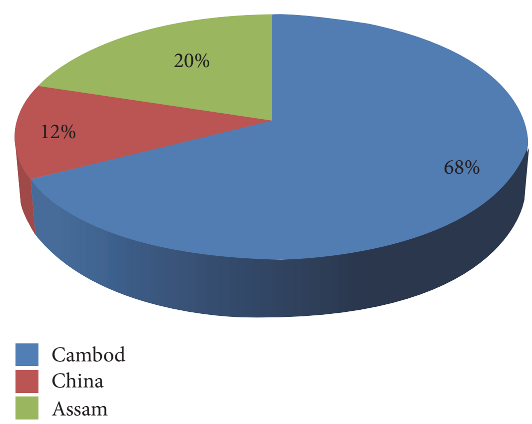


Figure 8: Percentages of accessions that fall into major tea taxa available in the tea germplasm.

accepted and widely adopted globally for identification of genetic structure.

Among the 89 beverage type accessions studied, 60 recorded ascending type styles, 18 reported united styles, and 11 accessions were having geniculate styles. Accordingly, accessions can be categorized into three major groups as indicated in Table 6.

Based on variation in split pattern of stigma, the beverage type accessions of Sri Lankan tea germplasm are predominantly represented by Cambod type (68%) followed by Assam type (20%) and China types are poorly represented (Figure 8). As per the records, during the early stages of the tea industry in Sri Lanka, seeds mainly of China type were (during 1824–1833) introduced for mass cultivation and the exercise was a complete failure as seeds were unable to flourish in the island [26]. A large consignment of Chinese tea seeds was introduced into various regions of Northeast India, and widespread spontaneous hybridization took place with the endemic gene pool of *C. sinensis* var. *assamica* [26, 27]. These hybrids exhibited a significant polymorphism and were better adapted to colonize the new habitats. This may be the reason for availability of a large number of Cambod type accessions in Sri Lankan tea germplasm compared to Assam and China type.

These results are in agreement with previous findings, based on a coefficient of parentage (COP) analysis in commercial tea cultivars in Sri Lanka, where it was reported that ASM 4/10 and CY 9 were the main ancestral lines that contributed to the cultivated tea gene pool [28]. Both these cultivars show more affinity towards Cambod origin (Table 6).

Table 7: Summary of floral characters of major tea taxa.

Floral characters	Statistical parameters					
	China type		Cambod type		Assam type	
	Range	Mean	Range	Mean	Range	Mean
Bud length (mm)	11.03–15.62	13.08	10.68–15.74	13.10	9.91–16.03	12.46
Bud width (mm)	8.05–12.95	11.64	8.61–13.87	11.57	9.24–15.18	11.07
Corolla diameter (mm)	26.68–48.21	32.74	12.56–38.72	32.43	23.33–44.49	29.76
Petal length (mm)	14.26–20.46	17.28	14.58–22.80	17.90	10.10–54.06	15.09
Petal width (mm)	10.62–17.33	13.88	9.72–24.01	14.26	8.16–12.75	9.99
Total number of sepals	5-6	5.27	4–7	5.22	5-6	5.11
Total number of petals	6–10	7.64	5–12	7.47	5–9	6.94
Total number of style arms	3-4	3.18	3-4	3.17	3	3
Number of whorls of sepals	2	2	2-3	2.10	2	2
Number of whorls of petals	2-3	2.90	2-3	2.72	2-3	2.67
Style length (mm)	3.58–12.90	9.17^{b}	7.76–14.61	10.85^{a}	4.81–10.59	8.07^{ab}
Style column length (mm)	0.00–3.33	0.93^{b}	3.75–10.55	7.42^{a}	1.11–4.02	2.23^{a}
Style arm lengths (mm)	3.58–10.01	8.25^{a}	1.80–10.23	4.25^{b}	1.11–4.02	2.23^{b}
Ovary pubescence	Sparsely to densely hairy		Sparsely to densely hairy		Slightly to densely hairy	
Stigma position	Below or above or same level as the stamen whorl		Below or above or same level as the stamen whorl		Below or above or same level as the stamen whorl	
Style	Geniculate		Ascending		United	

Means with the same letter are not significantly different.

An analysis of the distribution of the main tea taxa estate selections, improved cultivars, and introductions in Sri Lankan tea germplasm are given below.

3.4.1. Estate Selections. Estate selections are very vital components in tea germplasm as they adapted from various agroecological regions of the country and have enormous potential in tea crop improvement programmes. Out of the 34 estate selections used in the study, 24 resemble Cambod type and eight showed Assam type pistil characters. Only two estate selections B 275 and NAY 3 belong to China taxa. All six estate selections from St. Coombs Estate, Talawakelle, used for the study showed Cambod type affinity. A previous study conducted using 50 estate selections also revealed that majority of the estate selections in the germplasm are Assam and Cambod types [29]. Therefore, tea germplasm is inadequately represented by China type in estate selections too. Hence, it is necessary to expand future estate selection programmes aiming at selection of accessions related to China taxa [30].

3.4.2. Introductions. The first tea germplasm in Sri Lanka was introduced as tea seeds from India and China during the early stages of the industry and subsequent explorations of exotic germplasm for cultivar development should not be overlooked, as many recommended tea cultivars in use were based on germplasm from introductions [4]. As indicated from the results, a higher percentage of introductions acquired prior to 1960s (83%) from India and Indo-China were reported as Cambod types.

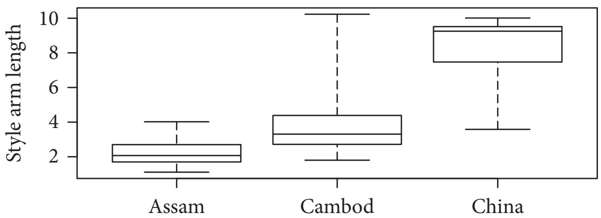
On the contrary, results showed that recent introductions from Korea (PBGT accessions) belong to China (60%) archetype. Inadequate representation of China types in tea germplasm warrants continuation of exploration of germplasm with China characters preferably from exotic origins [30].

3.4.3. Improved Cultivars. Around 72% of the improved cultivars were categorized as Cambod types. Majority of the improved cultivars were reminiscent of more affinity to Cambod taxa and this may be due to the selection for large leaved characters during cultivar development programme to maintain higher productivity levels over small leaved China types giving low yields. However, to maintain appreciable level of diversity in cultivated genepool, it is of utmost importance to concentrate on acquiring or developing cultivars having China characters aiming to use other desirable traits that are not present in high yielding types.

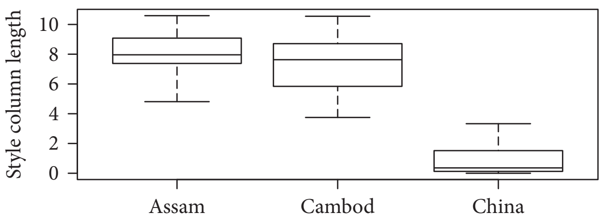
3.5. Variation of Other Floral Traits among Tea Taxa. Summary of floral traits of major three taxa, Cambod type, Assam type, and China type, is given in Table 7.

Among the floral traits of the three taxa, the pistil traits, namely, style length, style column length, and style arm lengths, showed a significant variation. Highest style length and style column length was recorded in Cambod type accessions and China type accessions had highest style arm length. All other traits did not show significant variations among three taxa.

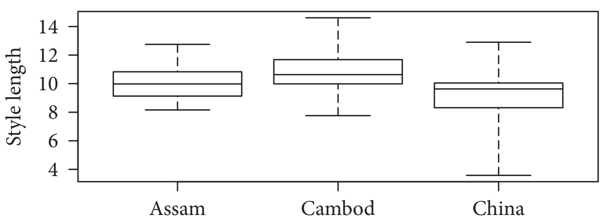
Among the three qualitative traits used, ovary pubescence and stigma position relative to the stamen whorl did not



(a) Variation of style arm length among three taxa



(b) Variation of style column length among three taxa



(c) Variation of style length among three taxa

Figure 9

show any relationship with three taxa. Hence, these two traits cannot be used to distinguish between three taxa.

Variation of pistil traits among major taxa is shown in Figures 9(a), 9(b), and 9(c). It is clear from these figures that pistil traits are significantly varied among three taxa.

4. Conclusions

This is the first comprehensive study to determine the genetic structure of tea germplasm in Sri Lanka using pistil traits and results indicate presence of considerable variation among germplasm accessions. Accessions could be categorized into five different groups based on the diversity of floral traits and it was possible to identify highly discriminating accession such as TRI 3014 based on the grouping pattern. Among the 13 traits, pistil traits are highly variable compared to other traits and they are useful as taxonomic markers in classifying accessions into major tea taxa. Further, grouping pattern of accessions enables separating accessions belonging to China taxa indicating the usefulness of pistil traits in classifying accessions. Overall, 13 floral traits used in the study could be effectively utilized to categorize germplasm accessions in future studies too.

Present study generated valuable information on genetic structure of tea germplasm of Sri Lanka. It was revealed that the Sri Lanka tea germplasm was predominantly represented by Cambod type accessions followed by Assam types. Availability of China type accessions is low. Similar trend can be observed in two major categories of germplasm, estate selections, and improved cultivars indicating selection programmes were more biased towards Cambod and Assam archetypes. On the contrary, recent introductions to the germplasm from Japan and Korea have more affinity towards China types. Hence, the present study identifies gaps in the germplasm collection and information generated can be used for decision making in future germplasm exploration missions. Thus, future exploration missions have to be geared to acquire more China type accessions and it is suggested to concentrate this fact on continuing future estate selection programme. Furthermore, it may be worthwhile developing China hybrids in future crop improvement programmes to enrich the Sri Lankan tea germplasm.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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