Floral and Morphological Characterization of Old Tea Genetic Resources in Hakgala Botanic Garden, Sri Lanka

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Abstract. To overcome the narrow genetic diversity in commercial tea cultivations, it is essential to introduce new genetic resources into the existing germplasm. The Hakgala Botanic Garden (6.54°N, 80.40°E) in Sri Lanka harbors a valuable collection of ancient tea genetic resources, comprising approximately 150 unique introduced tea accessions. A study was conducted to assess the phenotypic diversity of these resources and identify unique genotypes for conservation and utilization in plant breeding. A comprehensive investigation was carried out on the old seedling tea (OST) population at Hakgala Botanic Garden using 15 morphological descriptors and 17 floral descriptors. Morphological characterization revealed significant heterogeneity among the OST genotypes. Diversity indices, including Shannon's index and evenness values, indicated notable phenotypic differentiation, with internode length showing the highest diversity ($H' = 1.22$). A Neighbor-Joining (NJ) tree based on morphological data grouped the genotypes into three major clusters, with several OST accessions displaying morphological similarities to reference tea cultivars. Floral characterization showed a significant variation in certain floral characteristics, such as stigma position and style length. Three accessions, HKG63, HKG1, and HKG53, displayed unique floral morphologies and, hence, came out as distinct clusters. The possibility of using these special accessions in breeding programs is strengthened by these phenotypic differences. These findings highlight the significance of the Hakgala OST population as a reservoir of untapped genetic diversity. The study identifies several genotypes as promising candidates for future tea breeding and conservation efforts, contributing to the sustainable utilization of Sri Lanka's tea genetic resources.

Keywords: Germplasm Conservation, Phenotypic Variation, Old Seedling Tea, Floral characterization, Morphological Characterization

1 Introduction

Tea which is derived from *Camellia sinensis*, is the most famous beverage around the world after water (Hicks, 2001). It originated in China more than 60 million years ago, has been consumed for over 3,000 years (Meng *et al.*, 2018).

The genus *Camellia*, especially section *Thea*, serves as the main source of tea plants, with *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica* recognized as the most extensively cultivated varieties (Mondal, 2012). *C. sinensis* can be classified into three main types: China, Assam, and Cambod according to their leaf size and genetic characteristics (Roy & Chakraborty, 2009; Pan *et al.*, 2022).

China, India, and Sri Lanka are major tea producers in Asia, while Kenya and various African nations also making substantial contributions (Majumder *et al.*, 2011). Tea cultivation in Sri Lanka commenced during British colonization with the introduction of the first tea plant from China in 1824 and

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cultivated at the Royal Botanic Gardens, Peradeniya. Tea cultivation in Sri Lanka expanded rapidly, increasing from 10 acres in 1867 to more than 300,000 acres by 1895 (Richards, 1978). Currently, tea is a vital export, substantially impacting Sri Lanka's economy and providing over 1.5 million job opportunities (Sandika, 2018).

Even though tea industry is such an important industry, it faces challenges, including increasing competitiveness, shrinking cultivation areas, low productivity, and high production costs. In addition to these reducing the genetic diversity of tea, has become a major threat to the sustainability of tea industry in worldwide (Huang *et al.*, 2006). The limited genetic diversity of cultivated tea in Sri Lanka renders it vulnerable to various stresses, such as pests, diseases, and drought (Ranatunga *et al.*, 2017). Continuous selection for desirable traits and planting limited number of cultivars in commercial lands potentially narrowing the genetic diversity highlighting the necessity for conservation and expansion of the genetic base (Goonetilleke *et al.*, 2009).

To address this issue, researchers have explored various approaches to characterize and utilize tea germplasm (Kottawa-Arachchi *et al.*, 2013). Regularly, tea germplasm has been characterized by morphological, biochemical and molecular characterization methods and have been incorporated suitable cultivars as parents to the breeding program.

To overcome this narrow genetic diversity of cultivated tea, the identification and utilization of untouched tea genetic resources into the tea breeding program is vital. The Hakgala region of Sri Lanka is a treasure trove of ancient tea genetic resources, boasting a remarkable collection of approximately 150 unique tea varieties that have been introduced in to the country during colonial period. These tea genetic resources, which have been preserved over the decades, represent a unique and invaluable source of genetic diversity that holds immense potential for tea research, breeding, and conservation efforts. This untapped treasure trove of genetic diversity holds the promise of enhancing the resilience and productivity of the island nation's renowned tea industry (Karunarathna *et al.*, 2018).

The study was conducted with the objectives of to characterize tea genetic resources in Hakgala Botanic Garden using phenotypic traits and to identify potential and unique genetic resources for conservation and utilization in tea breeding program.

2 Materials and Methodology

2.1 Planting Materials

Total of 105 tea accessions were used for the present study. Based on the external visual appearance and growth habit, 71 accessions were selected from the Hakgala Botanic Garden (6.93°N 80°82°E, 1745 m above mean sea level, annual average rainfall about 2300 mm). Additionally, 34 accessions were selected from the ex-situ gene bank of the Tea Research Institute of Sri Lanka (6.54°N, 80.40°E, 1380 m above mean sea level, annual average rainfall about 2300 mm) for comparative analysis and clustering purposes. Both locations are belonged to same agro-ecological zone (Up-Country Wet Zone -UCWZ).

2.2 Sample collection

All selected tea accessions from the Hakgala Botanic Garden were initially tagged using a systematic code system for proper identification. For the morphological characterization, three well-grown branches bearing at least five healthy leaves were collected from each selected accession, serving as replicates. Due to the unavailability of suitable branches in some accessions, only 66 accessions were selected for morphological characterization.

Floral characterization was conducted during the flowering season. Data collection was conducted between 7:00 and 10:00 in the morning to ensure the freshness of floral samples and five fully bloomed flowers were carefully collected from each selected accession. Due to the unavailability of flowers in some accessions, only 105 accessions were selected for floral characterization.

2.3 Data collection and the descriptors used for floral and morphological characterization

Based on tea descriptors, IPGRI (1997) and UPOV (2008), 15 characters for morphological characterization and 17 characters for floral characterization were recorded. Both qualitative and quantitative characters were used. The recorded data were converted into numerical values, according to the standard format of IPGRI (1997) and UPOV (2008) guidelines. Then the obtained data were further converted into binary format as suggested by Crisci and Armengol (1983). A Neighbor Joining tree and a dendrogram were constructed from those binary data (Rohlf 1997).

2.4 Statistical Analysis of the data

PAST (PAleontological STatistics) is used to measure the "Shannon Diversity Index" and "Evenness Diversity Index". Floral and morphological data were analyzed using the "DARwin" software, and the hierarchical tree was constructed based on evolutionary dissimilarities.

3 Results and Discussions

3.1 Morphological Characterization

The morphological characterization in Hakgala Botanic Garden of 66 seedling tea accessions revealed significant phenotypic variability as shown in Table 1. Traits such as young shoot color, leaf shape, leaf margin, internode length, and leaf size showed a substantial degree of morphological variability among the population. In a previous study, a similar diversity pattern of morphological traits was identified for Sri Lankan tea germplasm (Kottawa-Arachchi et al., 2024).

Table 1. Morphological descriptors and diversity indices.

No.	Descriptor	No. of individuals	Frequency	Shannon Diversity Index	Evenness
1	Young shoot color			1.09	0.74
	Whitish	0	0.00		
	yellow green	34	51.52		
	Brown	15	22.73		
	Medium green	16	24.24		
	Purple	1	1.52		
2	Mature shoot color			0.34	0.70
	Whitish	0	0.00		
	yellow green	7	10.61		
	Brown	0	0.00		
	Medium green	59	89.39		
	Purple	0	0.00		
3	Young leaf pubescence			0.93	0.85
	Absent	0	0.00		
	Sparse	31	46.97		
	Medium	29	43.94		
	Dense	6	9.09		
4	Young shoot pigmentation			0.08	0.54
	Present	1	1.52		

	Absent	65	98.48		
5	Petiole pigmentation			0.40	0.74
	Present	9	13.64		
	Absent	57	86.36		
6	Undulation of leaf margin			0.69	1.00
	Absent	34	51.52		
	Weak	0	0.00		
	Medium	32	48.48		
	Strong	0	0.00		
7	Leaf apex habit			0.61	0.92
	Straight	20	30.30		
	Downturn	46	69.70		
8	Leaf shape			0.57	0.88
	Eliptic	49	74.24		
	Lanceolate	17	25.76		
9	Leaf apex shape			0.37	0.72
	Acute	58	87.88		
	Acuminate	8	12.12		
10	Leaf base shape			0.40	0.74
	Acute	57	86.36		
	Obtuse	9	13.64		
11	Serration of leaf margin			0.76	0.71
	Weak	1	1.52		
	Medium	33	50.00		
	Strong	32	48.48		
12	Leaf Waxiness			0.66	0.97
	Present	47	71.21		
	Absent	27	40.91		
13	Texture of leaf upper surface			0.64	0.94
	Smooth	44	66.67		
	Rugose	22	33.33		
14	Internode Length			1.22	0.85
	very short	6	9.09		
	Short	27	40.91		
	Medium	24	36.36		
	Long	9	13.64		
15	Leaf size			0.79	0.55
	Very Small	4	6.06		
	Small	48	72.73		
	Medium	13	19.70		
	Large	1	1.52		

Internode length showed the highest Shannon’s diversity index (H' = 1.22), indicating significant variation. This trait is critical for mechanical harvesting efficiency and high yield potential (Ponmurugan et al., 2019). Young shoot color (H' = 1.09) and mature leaf color (H' = 0.93) showed the second and third highest diversity. These pigments are not only defining visual characteristics but also increase antioxidant capacity, ultraviolet protection, and stress resilience (Tanaka et al., 2008; Xia et al., 2017; Wang et al., 2020). Purple or variegated foliage correlates with increased anthocyanin concentrations and can affect taste and market worth (Li et al., 2023). In contrast, young shoot pigmentation had the lowest diversity (H' = 0.08), though its evenness was 1.00, indicating a dominant phenotype. The undulate leaf margin demonstrated the highest evenness value (E = 0.54), signifying that its various classes were represented in nearly similar quantities. This balanced class distribution reflects a wide and uniform range of variation, offering greater potential for selection in adaptive breeding programs.

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Neighbor-joining tree for the morphological characters. The Neighbor-Joining (NJ) tree (see Fig. 1) which was constructed using morphological data divided, the accessions into three groups. Most old seedling tea (OST) accessions were widely scattered, showing extensive phenotypic divergence. Some OSTs clustered near reference cultivars, suggesting shared ancestry or convergent selection, while others formed distinct clades. Popular commercial cultivars such as TRI 2025 and TRI 3055, both derived from ASM 4/10 (Piyasundara et al., 2009; Ranatunga et al., 2017) were unexpectedly grouped into separate groups in the NJ tree. Notably, TRI 2055 clustered with NAY 3, which is closely related to the China type, suggesting that morphological traits alone may not fully reflect their true genetic backgrounds. This divergence highlights the influence of phenotypic plasticity and convergent traits under similar environmental conditions. In contrast, TRI 3055 and AI 6, both of Assam origin, were placed in the same group, supporting their shared ancestry. These observations emphasize the need for molecular characterization to further clarification of genetic relationships and complement the morphological findings. Cluster C comprised PBGT accessions, Yabukita accessions, and DT1, which exhibited morphological characters associated with China-type accessions, indicating a close relationship with them. Therefore, the OST accessions grouped within Cluster C are likely to have a close genetic affinity with China-type tea.

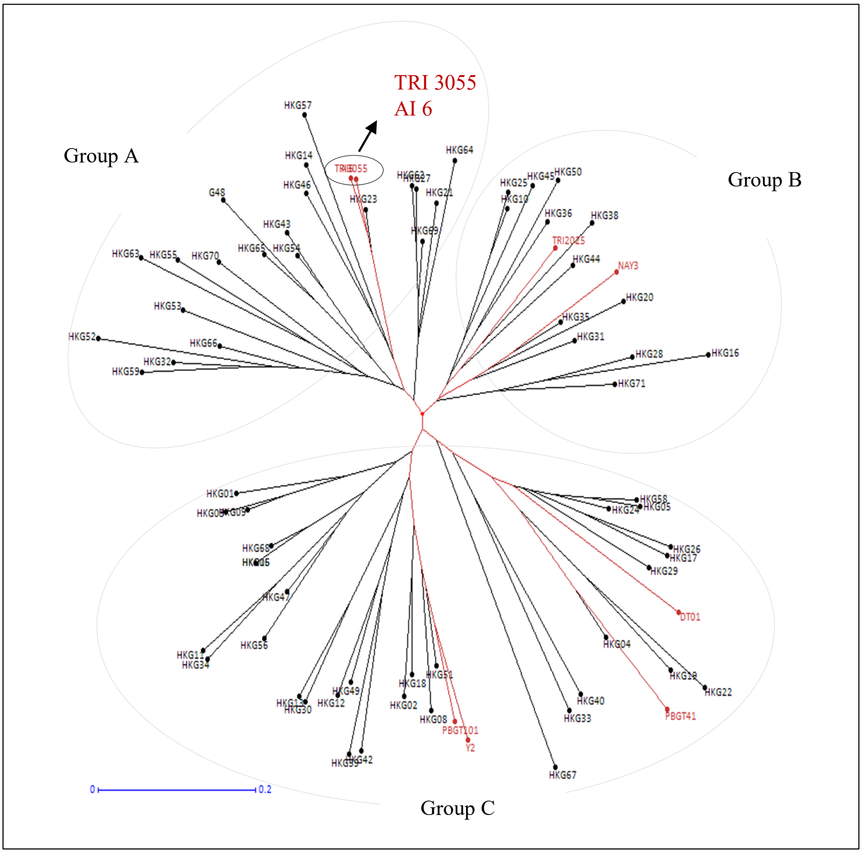

Fig. 1. Neighbor-joining tree for the morphological characters in Hakgala old seedling tea population and the reference accessions

3.2 Floral Characterization

The results, as measured by the Shannon diversity index (H') and evenness (E), showed an abundance of variation among features (Table 2).

Table 2. Morphological descriptors and diversity indices.

No	Descriptors	No of individuals	Frequency	Shannon Diversity Index	Evenness
1	Flower Diameter			0.90	0.82
	Small (<3)	27	25.71		
	Average (3-4)	65	61.9		
	Large (4<)	13	12.38		
2	Petal whorls			0.55	0.87
	Single	80	76.2		
	Semi double	25	23.81		
3	Petal count			0.60	0.60
	Few (<5)	9	8.57		
	Average (5-7)	86	81.9		
	Many (7<)	10	9.52		
4	Sepal whorls			0.05	0.53
	One	1	0.95		
	Two	104	99.04		
5	Sepal Count			0.30	0.45
	Few (>4)	1	0.95		
	Medium (4-5)	97	92.38		
	Many (5<)	7	6.67		
6	Stigma position			0.9701	0.8794
	Below	35	33.33		
	Same level	56	53.33		
	Above	14	13.33		
7	L/W			0.59	0.60
	Small (0.02-0.99)	12	11.43		
	Average (1-2)	86	81.9		

	Large (2.01-2.65)	7	6.67		
8	Petal Curvature			0.76	0.71
	In-curved	28	26.67		
	Flat	5	4.76		
	Re-curved	72	68.57		
9	Shape of apex of the Petal			0.22	0.62
	Obtuse	6	5.71		
	Rounded	99	94.3		
	Retuse	0	0		
10	Main Color of the petal			0.64	0.63
	White (White group 155 D)	81	77.14		
	Cream (Yellow – white group 158 B)	20	19.04		
	White with red purple (pinkish) tinge (yellow-white group 158 B with red-purple group 62A)	4	3.81		
11	Ovary position – in relation to stamens			0	1
	Same height	0	0		
	Androecium higher that gynoecium	0	0		
	Gynoecium higher that androecium	105	100		
12	Ovary hair			0.09	0.55
	Absent	2	1.9		
	Present	103	98.09		
13	Style Splitting			0.94	0.85
	Low	34	32.38		
	Medium	12	11.42		
	High	59	56.19		
14	Column Length of the style			0.73	0.69
	Short (0<5)	25	23.8		
	Average (5-10)	75	71.42		
	High (10<)	5	4.76		
15	Arm Length of the style			0.54	0.57
	Short (0<5)	84	80		
	Average (5-10)	20	19.04		
	High (10<)	1	0.95		

16	Style length			0.92	0.84
	Short (<10)	82	78.1		
	Average (10-20)	20	19.04		
	High (20<)	30	28.57		
17	Stylar arms			0.64	0.63
	Horizontal	16	15.23		
	Semi-cleft/ vertical	83	79.04		
	Spiral	6	5.71		

The stigma position showed the most variety (H' = 0.9701), indicating its evolutionary significance and usefulness in tea breeding for improving cross-pollination efficiency and adaptation. On the other hand, there was no diversity in the accessions' ovary location in relation to the stamens (H' = 0), which is in line with earlier research on the stability of floral traits in tea (Wilson *et al.*, 2004).

High variety was also seen in traits including style length and style splitting (H' = 0.9184 and 0.9369, respectively), which may have an effect on pollination success. On the other hand, ovary hair (H' = 0.0943) and sepal whorls (H' = 0.0538) showed little diversity, indicating that these features may be evolutionarily conserved and have limited variability.

The sepal count had the lowest evenness (E = 0.4491), suggesting a skewed distribution of traits that may be the result of genetic or environmental selection pressures. As a durable morphological marker, the ovary location trait, on the other hand, demonstrated the highest evenness (E = 1).

While characteristics like ovary position can be used as diagnostic tools for cultivar identification, these findings highlight the need for different qualities like stigma position for breeding work. While features with low diversity and high evenness indicate selective stabilizing within the population, traits with both high variety and uneven distribution may represent continuing evolutionary changes (Gupta *et al.*, 2010).

Box Plot Analysis. Nine quantitative descriptors, such as flower diameter, style length, and style column length, were used in a box plot analysis. Key statistical metrics, including the median, range, outliers, and interquartile range (IQR), were displayed in the boxplots (see Fig. 2).

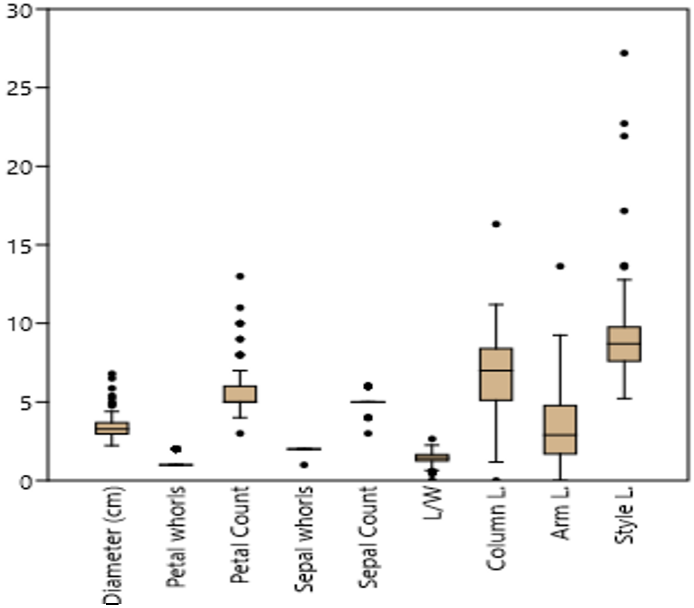

Fig. 2. Box plot of nine quantitative floral descriptors

Higher phenotypic variation is indicated by a broader Inter Quartile Range (IQR) and the higher number of outliers in a trait indicate greater phenotypic variation Breeding programs may target these features because they may provide genetic benefits or environmental adaptability (Kumar et al., 2018). Rare alleles or distinctive phenotypes that are useful for breeding innovation may be represented by outliers. They still need more research, though, as they could potentially be the consequence of measurement errors or environmental factors.

Cluster Analysis. Based on morphological features, a dendrogram generated by the UPGMA method of NTSYS-PC software divided the 105 accessions into three separate clusters (see Fig. 3).

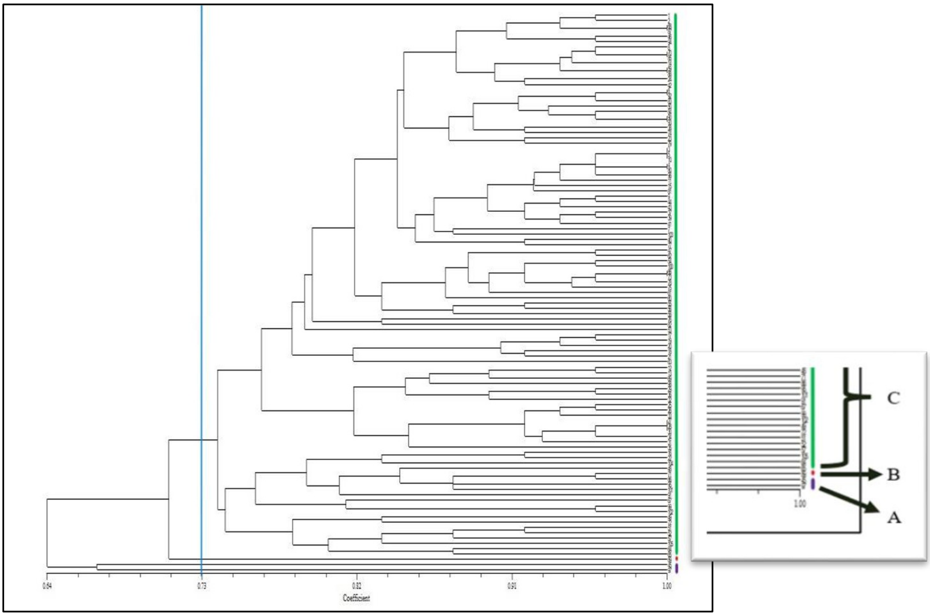

Fig. 3. Relationship among 105 tea accessions based on 17 morphological descriptors in the UPGMA dendrogram

Two accessions (HKG63 and HKG1) from the Hakgala Botanic Garden were included in Cluster A. These accessions were distinguished by their blunt ends, recurved petals, and spiral stylar arm features that are advantageous for enhancing pollination success and seed yield (Wijeratne *et al.*, 2017).

Only HKG53, an ancient accession with the largest petal L/W ratio and flat curvature, was found in Cluster B. Its division into a distinct cluster might indicate genetic distinctiveness and could be useful for introducing new characteristics into breeding populations. The remaining accessions were included in Cluster C, suggesting that they have traits in common and may have shared ancestry or adaptation to similar environmental conditions.

To increase fruit set and seed yield in tea breeding, style width and stigma position are crucial, particularly for cultivars whose pistils are longer than their stamens (Ariyarathna *et al.*, 2011). More insect mediated pollen transport, particularly by bees, is made possible by wider styles, which increases genetic diversity and cross pollination (Chen & Yamaguchi, 2005).

This observed morphological diversity emphasizes the value of OST in Hakgala Botanic Garden as a valuable reservoir of untapped genetic variation. Their long-term persistence under minimal human intervention suggests adaptation to local conditions and potential for stress tolerance or unique metabolite profiles (Aziz *et al.*, 2011). Commencement of both *in situ* and *ex situ* conservation of these accessions is essential. Morphological features are frequently affected by environmental factors, complicating the differentiation between genetic and environmental variation (Bhagat *et al.*, 2010). Consequently, the combination of morphological analysis with molecular marker techniques can enhance the precision of diversity evaluations and assist in validating the uniqueness of important accessions (Rajkumar *et al.*, 2010; Bandara *et al.*, 2024).

4 Conclusion

The present study demonstrates a significant phenotypic diversity within the unutilized seedling tea population at Hakgala Botanic Garden, comparing it to commercial reference cultivars. Morphological characterization revealed substantial heterogeneity across the 66 accessions, especially in traits crucial for agronomic performance. In the Neighbor Joining tree analysis for morphological traits, several accessions were distinguished as genetically distinct, highlighting their potential as valuable genetic resources for future breeding programs. For the floral morphology, the calculated diversity indices confirmed significant phenotypic variation within the traits which are essential in tea breeding programs.

These findings underscore the Hakgala OST population's potential as a crucial reservoir for breeding and the long-term conservation of tea genetic resources in Sri Lanka. The identified distinct accessions, particularly HKG1, HKG53, and HKG63, are promising candidates for future breeding programs. By incorporating these traits and genetic variations, tea breeding efforts can be enhanced, contributing to the development of superior cultivars with improved agronomic properties and greater genetic diversity.

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