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Pistil Related Morphological Traits Reflect Genetic Diversity of Tea in Sri Lanka

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ABSTRACT

Intense cross hybridization among the different tea taxa has created high heterogeneity in the tea germplasm. Pistil morphology, defined by the style and ovary characters is known to be distinctive in the different taxa, and provides reliable criteria for tea taxonomy. The objective of the present study was to assess the variability of pistil related characters to be used as a measure of genetic diversity in tea germplasm collection in Sri Lanka. A representative sample consisting of 27 tea accessions were characterized using six pistil related morphological traits. Seventy five percent of the total variability in pistil morphology was explained by the first two principle components that were loaded to the greatest extent by the traits, number of arms in the style, length of style arms and length of the style column. It was also revealed the possibility of using those traits as indices to estimate the genetic and genealogical diversity in tea. Five well defined groups identified based on the Principle Component Analysis, confirmed the presence of high variation in pistil morphology. The wide variation captured herein indicates that the Sri Lankan tea germplasm is a result of extensive inter-hybridization among the major tea taxa. This is an important finding to confirm the richness of the local tea germplasm having genes introgressed from all three tea taxa.

Key words: *Camellia sinensis*, genetic diversity, pistil, tea

INTRODUCTION

The currently available tea gene pool has its ancestry in three different taxa, *Camellia sinensis* (L) (China type), *C. assamica* (Assam type), and *C. assamica* spp. *lasiocalyx* (Cambod type) (Sealy, 1958; Wight, 1962). However, merge of other non-tea *Camellia* species into tea gene pool has also been reported (Wight and Barua, 1957; Wight, 1962; Sharma and Venkataramani, 1974). Predominantly out-cross breeding system and the absence of absolute crossing barriers within the genus *Camellia* has resulted in innumerable intergrades of phenotypes (clines) through intense hybridization and intercrossing (Banerjee, 1992).

Many of the vegetative descriptors used for characterization of tea (Piyasundara *et al.*, 2006) express continuous variation that overlaps among different groups or taxa which therefore has limited applications in taxonomy (Wickramarathne, 1981; Banerjee, 1992).

Reproductive morphology, being more distinctive and less plasticize to environment effects, are attractive and reliable criteria in taxonomy (Harper, 1977) specifically in tea (Banerjee, 1992). The classification system proposed by Wight (1962), which is regularly practised in the Indian subcontinent, primarily relies on pistil characters and generally accepted to be more realistic and practical in working with highly variable tea populations of heterogeneous origins (Banerjee, 1992). Accordingly, in *C. sinensis* (L.), commonly known as China type, the styles remain free for a greater part of the length, style arms 3 - 4 numbered and spread horizontally. In C. *assamica* commonly known as Assam type, style is united to a greater part having three horizontally spreading arms. In *C. assamica var. lasiocalyx* commonly known as Cambod type, styles are free up to one third of the length and have three style arms spreading ascending.

Hence, the objective of the present study was to assess the variability of pistil related characters with a view to explore the possibility of using them as a measure of genetic diversity present in tea germplasm collection in Sri Lanka.

MATERIALS AND METHODS

The study was carried out during the peak flowering period starting from January - April 2007 in the field gene bank established at Tea Research Institute of Sri Lanka (TRISL) in Talawakelle (Lat. 6° 54' N, Long. 80° 42' E, 1382 m amsl; average minimum temperature 14.6 °C, average maximum temperature 24.3 °C, average number of sunshine hours 5 hours per day, total annual rainfall 2140 mm). A representative sample of 27 accessions in the working collection of the tea germplasm was used to study pistil related morphological traits.

Three vegetatively propagated twenty year old bushes were sampled for each of the accession. Ten freshly bloomed flowers were collected from each accession. Sampling was repeated three times at monthly intervals. Following pistil related morphological traits were recorded adopting the "Descriptors of Tea" developed by IPGRI (Anon, 1997): 1. position where the style splits, 2. number of style arms, 3. position of stigma, 4. length of style column 5. length of style arms and 6. number of locules. All the length measurements were taken using a vernier caliper and hand lenses. Number of locules was counted after taking cross sections of the ovary, and by observing the sections under light microscope (x 4).

The data was analyzed by variable reduction multivariate techniques, correlation analysis followed by Principal Component Analysis (PCA) using SAS software (SAS, 1985), in order to determine the important traits and those that were highly correlated.

RESULTS

Variability present in pistil related morphology is summarized in Table 1. The number of style arms varied from 3 - 5. Style column length was in the range of 1.67 - 0.89 cm whereas the style arm length was in the range of 0.96 - 0.10 cm. The proportion of style column length to style arms length varied independently of the total length of the style.

Table 1. Summary of pistil related characters of the accessions studied

Accession	Style arm length (mm)	Style column length (mm)	*Stigma position	Style splitting	No.of style arms	No. of locules
TRI 3020	0.10±0.00	1.00±0.01	1	3	3	3
TRI 777	0.11±0.01	0.98±0.02	1	1	3	3
TRI 3025	0.18±0.00	1.05±0.02	2	3	3-4	3-4
TRI 2023	0.18±0.01	1.15±0.01	1	3	3-4	3-4
TRI 2025	0.19±0.00	1.09±0.01	1	3	3	3
KEN 16/3	0.19±0.01	1.11±0.01	1	3	3-4	3-4
TRI 3013	0.19±0.01	1.23±0.01	1 or 2	3	3-4	3-4
DT 1	0.26±0.02	1.08±0.01	2	3	3	2-3
TRI 3035	0.27±0.01	0.86±0.02	1 or 2	2	3	3
MT 18	0.30±0.01	0.91±0.03	2	2 or 3	3	3
TRI 2026	0.30±0.01	1.04±0.03	1	3	3	3
TRI 3018	0.31±0.01	0.74±0.04	1 or 2	2	3	3
TRI 62/9	0.33±0.01	1.05±0.01	1	3	3-4	3-4
TRI 3055	0.34±0.01	0.89±0.01	1 or 2	2	3	3
TRI 4061	0.35±0.01	1.11±0.01	3	2	4-5	4-5
DT95	0.37±0.01	1.35±0.01	1	3	3	3
PK2	0.40±0.01	1.26±0.01	1	2	3-4	3-4
CY9	0.41±0.01	1.08±0.04	1 or 2	2	3	3
TRI 4052	0.47±0.01	1.13±0.03	2	2	4-5	4-5
DN	0.51±0.01	1.05±0.01	1	2	3-4	3-4
N2	0.55±0.01	1.33±0.02	1	3	3	3
TRI 3041	0.57±0.03	1.07±0.02	2	3	3-4	3-4
TRI 2024	0.62±0.02	1.02±0.01	2	2	3-4	3-4
TRI 3016	0.62±0.02	1.67±0.02	1 or 3	2	3-5	3-5
Assam/ Cambod	0.65±0.01	1.20±0.02	1	2	3-4	3-4
TRI 3047	0.89±0.13	0.99±0.02	2	2	3-5	3-5
TRI 3019	0.96±0.03	1.33±0.02	1 or 2	1	5	5

* : Relative height between androecium and gynoecium
1: same height, 2: androecium high, 3: gynoecium high
Style splitting – 1: geniculate, 2: ascending, 3: united

Correlations among the traits are given in the Table 2. Length of style arms was correlated with all the other traits and the number of style arms is highly correlated to the number of locules.

Table 2. Pearson correlation coefficients of pistil related traits

	Length of style	Length of style column	Length of style arms	*Stigma position	No. of style arms	Style splitting
Length of style	1.000					
Length of style column	0.492	1.000				
	0.009					
Length of style arms	0.338	-0.522	1.000			
	0.085	0.005				
*Stigma position	0.216	-0.218	0.488	1.000		
	0.278	0.274	0.001			
Number of style arms	0.432	-0.114	0.713	0.408	1.000	
	0.025	0.572	0.001	0.035		
Style splitting	-0.558	-0.188	-0.418	-0.292	-0.489	1.000
	0.003	0.349	0.030	0.140	0.010	

*: Relative height between androecium and gynoecium

Seventy five percent of the total variability in the pistil related traits was described by the first two principle components (Table 3). Principle component (PC) 1 was loaded to the greatest extent by two traits, *viz.* number of style arms and the length of style arms whereas length of the style column had the major contribution to PC2.

Table 3. PC loadings and axes

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Length of style	0.381	0.497	-0.014	0.339	-0.557	-0.412
Length of style column	-0.077	0.727	0.176	0.175	0.268	0.575
Length of style arms	0.506	-0.314	-0.254	0.116	-0.345	0.668
*Stigma position	0.382	-0.198	0.899	0.004	0.068	-0.006
Number of style arms	0.508	-0.046	-0.281	0.369	0.689	-0.221
Style splitting	-0.433	-0.287	0.123	0.839	-0.082	0.051
Eigen value	2.776	1.703	0.647	0.460	0.341	0.070
Total variability explained	0.463	0.234	0.108	0.077	0.057	0.012
Cumulative variability explained	0.463	0.747	0.855	0.931	0.988	1.000

*: Relative height between androecium and gynoecium

Five different putative categories of style types were distinguished in the Principle Component Analysis (Figure 1). The styles of accessions categorized in the group I was characterized by having less number (3) of style arms, and longer style columns relative to the length of arms. This type of style morphology corresponds to the 'Assam' archetype

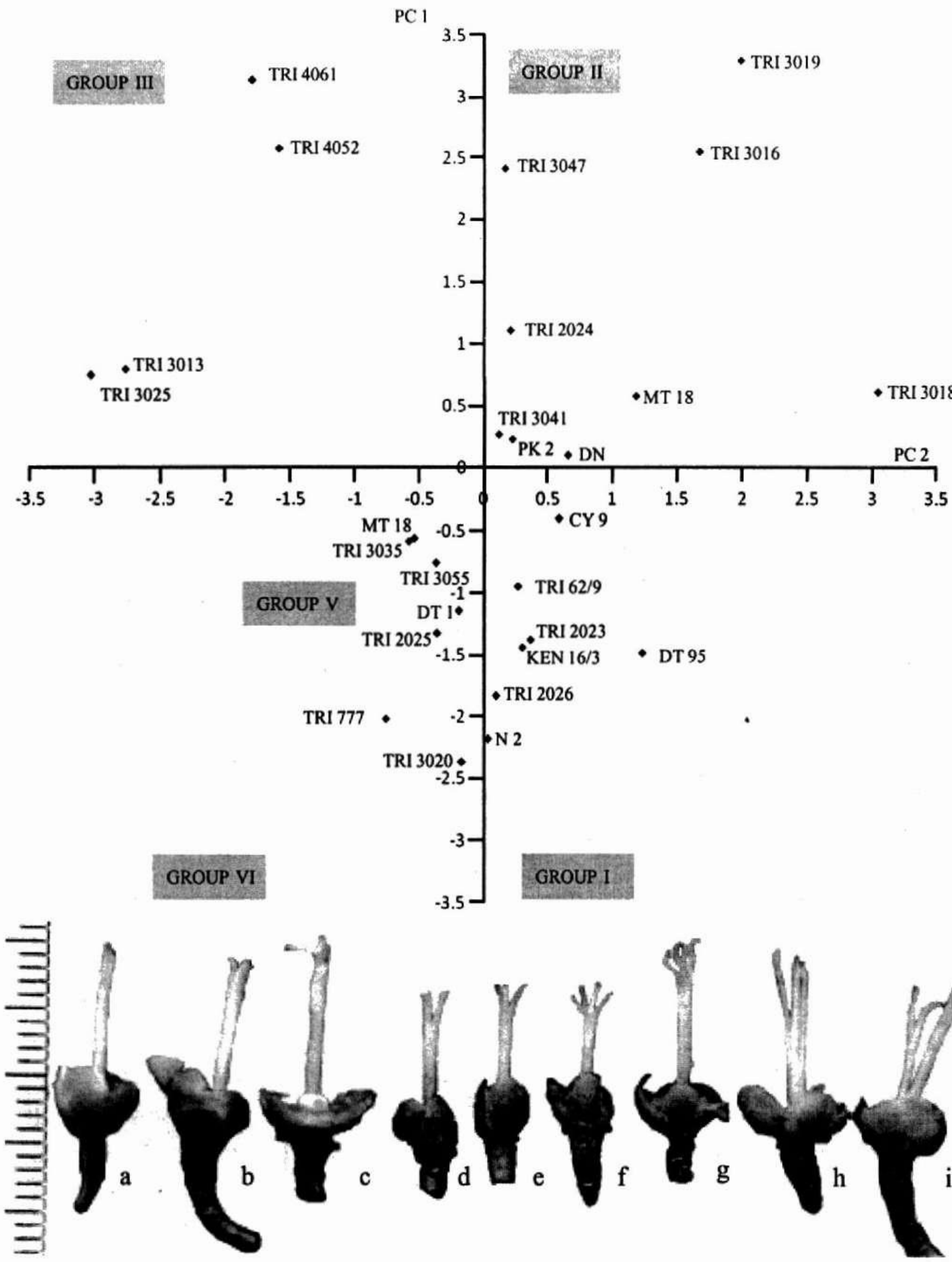

Figure 1. (i) PC grouping of the accessions studied (ii) variability recorded in the stylemorphs: (a) TRI 3020 (b) DT 95 (c) TRI 2025 (d) TRI 3025 (e) TRI 3013 (f) TRI 4052 (g) TRI 4061 (h) TRI 3016 (i) TRI 3019

described by Wight (1962). Similarly, the accessions that were categorized into group II have styles with more number of style arms (4 - 5), and longer style columns. This style morphology resembles Cambod archetype. Assam / Cambod introduction which is the ancestor of majority of the commercial tea cultivars was included in this group to be reminiscent of more affinity to Cambod ancestry. Group III includes accessions where the styles are distinguished by having number of style arms in between the above two groups (3 - 4). The style arms were longer relative to the length of style columns. This group resembles China archetype described in the Wight's classification. Style type represented in group IV has shorter style arm length as well as style column length and less number of arms. This is the mirror group of group II and does not correspond to any of the archetype styles. Group IV was not represented by any of the accessions used in the study. Most of the accessions studied were categorized in group V. Accessions in group V resemble those in groups I, II and III to varying degrees and are likely to be hybrids or clines of the archetypes.

DISCUSSION

Style morphological types that were observed in the accessions studied deviate in different degrees, and do not directly correspond with any of the style types of the archetypes described by Wight (1962). However, the range of variation observed in style column length and number of style arms distributed within the range described in the respective taxa. Although, pistil morphs of the taxa were absent in their true form, it was possible to recognize the individual archetype traits in combination (hybrids). Existence of the major three tea taxa in their true forms is debatable either in wild or cultivated germplasm (Kingdon-Ward, 1950). However, remarkably high variation in pistil morphology captured in the present study reveals that the Sri Lankan germplasm was a result of an extensive inter-hybridization among major three tea taxa. This is an important finding to confirm the richness of the local tea germplasm having genes introgressed from all three tea taxa.

Richness of the germplasm in terms of genetic diversity is exceedingly important in genetic enhancement of crops. In cross breeding species both heterozygosity of parental lines and the level of segregation in the progeny contribute to the genetic diversity of the breeding stock (Sneller *et al.*, 2005). The commercial tea germplasm of TRISL has Assam/ Cambod origin, with minute contribution from a few estate selections of unknown ancestry (Ariyarathna and Gunasekare, 2007). As evident from the present study, the wide variability in pistil morphology emanates that those parental lines were extremely heterozygous and the parental gene pool has been segregated extensively in the subsequent generations. This phenomenon is strikingly manifested in the pedigree group that forms the progeny TRI 3019, TRI 3016, and TRI 3017 and their parental lines *viz.* Assam/Cambod and DT 95 (Ariyarathna and Gunasekare, 2007). Measurements made on pistil morphological traits of the above progeny transgress to extreme values compared to their parents.

In the absence of archetypes that makes the ideal out-group (reference group used for comparison),.it was not possible to derive an absolute scale to estimate the extent of genetic diversity or hybridity. However, with proper validation, the diversity scale based on the PCs presented in this study, can be used as a relative index to gauge genetic variability among accessions. Use of this index has high expediency since the morphological traits such as number of style arms, length of style arms and length of style column, that were identified as important are distinct, less plastic and easily scorable with minimum human error. Developing such a scale for large number of accessions capturing comprehensive range of the germplasm, would enable broader applications and more accuracy. Further, the same can be used along with genealogical, phenotypic, biochemical and molecular information, as a composite index to measure genetic diversity with enhanced precision.

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