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Genetic diversity among south Indian tea germplasm (*Camellia sinensis*, *C. assamica* and *C. assamica* spp. *lasiocalyx*) using AFLP markers

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Abstract

Amplified Fragment Length DNA Polymorphism (AFLP) analysis of 49 tea cultivars from south India produced a total number of 1555 unambiguous polymorphic amplified DNA fragments. The dendrogram derived by unweighted pair group method with arithmetic mean algorithm (UPGMA) analysis and the PCO plot drawn using principal component analysis revealed that all these tea cultivars could be clearly distinguished into three distinct groups viz., Assam, China and Cambod as well as an intermediate. Among the populations characterized, the Chinary type showed a maximum diversity index of 0.612 and the minimum of 0.285 was observed within the Assam type. Genetic distance was maximum (0.946), between Assam and Cambod and minimum (0.852) between Assam and China. More than 90% similarity as observed between the cultivars UPASI-22 and UPASI-23. Affinity of each cultivar towards the populations was determined using the similarity index. Analysis and comparison of AFLP fragments revealed distinct segregation of all the cultivars into their respective groups, except UPASI-18 and UPASI-24. Studies on diversity assessment of south Indian tea cultivars using AFLP fingerprinting revealed that the present day commonly grown south Indian tea germplasm has narrow genetic diversity (< 37.76) among the cultivars necessitating a sustained effort to preserve tea germplasm resources and the development of superior varietal material through wide genetic crosses.

Keywords: AFLP; Genetic diversity; South India; Tea cultivars

1. Introduction

India is the world's largest producer and consumer nation for tea. Tea is produced by mechanical and biochemical processing of young shoots of *Camellia* spp. Tea production in India started with the identification of tea germplasm in the state of Assam in 1823 by Robert Bruce. The country has abundant germplasm resources, which not only assist the development of superior material for planting but also provide a genetic resource for other tea producing countries as well [1]. Consequently, the most of the global cultivated area for tea has received its basic planting material directly or indirectly from the enhanced germplasm in India [2]. The cultivated tea varieties of south India belong to different species such as *Camellia sinensis*, *Camellia assamica* and *Camellia assamica* spp. *lasiocalyx* [3]. Although the exact origin of tea cultivars is still a subject of debate, the varietal differentiation relies on their geographical origins in terms of provenance from China, Assam or Cambod. However, all caffeine-producing teas were also classified under the name *C. sinensis* (L.) O. Kuntze, irrespective of their taxonomic variation [4–7].

South India has diverse genetic resource, since all the existing collections are the progenies of the plants or seed stocks brought from Assam, China and other geographically unattributed sources. Initially, seed stocks were imported from China through Botanical Garden, Kolkata and planted in Nilgiris in 1832 for experimental purpose. Subsequently, a few more sets of

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plants from Assam and China were also planted at different areas of south India. Later the Tea Scientific Department of United Planters Association of South India (UPASI) produced accessions from naturally adapted seedling populations and by breeding [1]. South Indian tea accessions are highly genetically variable due to uncontrolled pollination and many of the present day accessions were selected from large populations based on their phenotypic superiority. Although the existing diversity is extensive, the genotypes selected and exploited for extensive planting are very much limited. Most of the cultivars extensively grown in south India are trait specific in terms of yield, quality and resistance to drought and blister blight disease. A large number of controlled hybridizations were attempted and some of the progenies were also recommended for planting [8]. Besides, a few varieties are grown for ornamental purpose. The existing diversity will have to be preserved and characterized for future crop improvement programmes that constitute the fundamental support structure for the tea industry.

Knowledge of genetic diversity among the available tea germplasm is an important prerequisite for future breeding and crop improvement programs. Marker assisted breeding for increasing the gene pool offers great advantages for plant breeders and gene bankers for varietal improvement. Classification of tea germplasm was attempted earlier by using morphological markers, but analysis with this system failed to reveal the taxonomic affinities between accessions, probably due to the environmental plasticity of morphological traits. In the present study, we employed the Amplified Fragment Length DNA Polymorphism (AFLP) technique for fingerprinting 49 distinct tea accessions from south India widely selected for planting in this region. This study was carried out to genetically characterize the south Indian tea cultivars and to determine the genetic relationship among the accessions.

2. Materials and methods

2.1. Plant material

Forty-nine accessions from south India were used in the present study. These included more familiar and widely planted genotypes released by UPASI Tea Research Foundation (UPASI TRF), commonly cultivated estate selections, certain Sri Lankan cultivars and a species of ornamental *Camellia* (Table 1). Most of the plant materials for analysis were collected from the germplasm collection of UPASI TRF, located at Valparai, Tamil Nadu, India.

2.2. DNA isolation

DNA was extracted from the young tea leaves using the CTAB method [9] with some modifications. Young leaf tissues (0.5 g) were ground in liquid nitrogen and mixed with 10 ml of CTAB buffer and incubated at 65 °C for 60 min. Samples were extracted with equal volume of chloroform/isoamyl alcohol (24:1 v/v) and the aqueous phase was mixed with 2/3 volume of chilled isopropanol. Precipitated DNA was collected by centrifugation and washed with 70% ethanol. DNA was air dried and re-suspended in 1 ml of sterile distilled water. Later it was treated with RNase A (1 μg/μl) for 30 min at 37 °C and purified using 500 μl of equilibrated phenol and 750 μl of chloroform/isoamyl alcohol (24:1 v/v). The purified DNA was re-precipitated from the aqueous phase using chilled ethanol, air-dried and re-suspended in sterile water. The high molecular weight DNA was checked for quality and quantity using agarose gel (0.8%) electrophoresis and with DyNA Quant (Pharmacia Biotech).

2.3. AFLP analysis

AFLP analysis was carried out following the AFLP™ plant mapping procedure [10]. Restriction digestion of DNA samples was performed using two restriction enzymes (*Eco RI* and *Mse I*). Simultaneously double stranded adaptors were ligated to the restricted fragments overnight at room temperature. Preselective amplification was performed by combining the *Eco RI* and *Mse I* primers. Initially 64 primer pairs were tested for selective amplification. The *Eco RI* primers contained fluorescent dyes, FAM (6-carboxyfluorescein) for blue or JOE (2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein) for green and NED (N-(1-naphthyl)-ethylene diamine) for yellow, labeled at the 5′ end. From those primer pairs, three were selected [E-ACA/M-CTG (Blue), E-ACC/M-CTG (Yellow) and E-AGG/M-CTG (Green)] for further analysis since they amplified more and distinct polymorphic loci. The PCR products were run in polyacrylamide gel on an ABI prism®377 DNA sequencer. Three different colored dyes (yellow, green and blue) were used to detect the PCR product profiles, which allowed three different reactions to be co-loaded in single lane. In addition, an internal lane size marker (Gene scan Rox-500), labeled with a fourth color (red) was added along with the loading dye for molecular weight reference. Electrophoresis was carried out under 3 V, 60 mA and 200 W electric field at 51 °C.

2.4. Data analysis

Details on molecular weight of PCR products were collected using a gel scanner of ABI prism 377. Different DNA fragments amplified with each primer were treated

Table 1
Origin and descriptive characters of prominent south Indian tea cultivars

Serial number	Clone	Accession	Source of the material	Variety	Reference
1	UPASI-1	B/4/141 (Ever green)	Brooklands Estate, The Nilgiris	Assam	[3]
2	UPASI-2	B/4/142 (Jayaram)	Brooklands Estate, The Nilgiris	Assam	[3]
3	UPASI-3	B/5/63 (Sundaram)	Brooklands Estate, The Nilgiris	Assam	[18]
4	UPASI-4	B/6/10	Brooklands Estate, The Nilgiris	?	[19]
5	UPASI-5	B/6/21	Brooklands Estate, The Nilgiris	?	[19]
6	UPASI-6	B/6/24 (Brooklands)	Brooklands Estate, The Nilgiris	?	[19]
7	UPASI-7	B/6/34	Brooklands Estate, The Nilgiris	?	[19]
8	UPASI-8	B/6/36 (Golconda)	Brooklands Estate, The Nilgiris	?	[19]
9	UPASI-9	B/6/61 (Athery)	Brooklands Estate, The Nilgiris	China	[3]
10	UPASI-10	B/6/62 (Pandian)	Brooklands Estate, The Nilgiris	China	[3]
11	UPASI-11	B/6/127	Brooklands Estate, The Nilgiris	?	[19]
12	UPASI-12	B/6/129	Brooklands Estate, The Nilgiris	?	[19]
13	UPASI-13	B/6/137	Brooklands Estate, The Nilgiris	?	[19]
14	UPASI-14	S/6/99 (Singara)	Singara Estate, The Nilgiris	?	[19]
15	UPASI-15	SP/4/5 (Spring field)	Springfield Estate, The Nilgris	China	[3]
16	UPASI-16	B/6/182	Brooklands Estate, The Nilgiris	?	[19]
17	UPASI-17	B/6/203 (Swarna)	Brooklands Estate, The Nilgiris	Cambod	[3]
18	UPASI-18	B/6/57	Brooklands Estate, The Nilgiris	?	[19]
19	UPASI-19	SP/4/6	Springfield Estate, The Nilgris	?	[19]
20	UPASI-20	B/7/372	Brooklands Estate, The Nilgiris	?	[19]
21	UPASI-21	B/4/198	Brooklands Estate, The Nilgiris	Assam	[3]
22	UPASI-22	B/6/29	Brooklands Estate, The Nilgiris	?	[19]
23	UPASI-23	B/5/148	Brooklands Estate, The Nilgiris	?	[19]
24	UPASI-24	B/5/149	Brooklands Estate, The Nilgiris	?	[19]
25	UPASI-25	K/19/16	Kulikkadu, UPASI TRF, Anamallais	?	[20]
26	UPASI-26	DVS/3A/39	Devarshola Estate, Nilgiri-Wynaad	?	[20]
27	UPASI-27	A/58	Anaimudi Estate, Anamallais	?	[20]
28	AK-1		Akkamalai, Anamallais	?	
29	ATK-1		Attikunna, Nilgiri-Wynaad	China	[3]
30	B/1/101		Brooklands Estate, The Nilgiris	?	
31	BSB-1	Clone selected from UPASI-10 × TRI 2025		?	[21]
32	BSS-1	Biclonal seed stock	UPASI-10 × TRI 2025	?	[8]
33	BSS-2	Biclonal seed stock	UPASI-2 × TRI 2025	?	[8]
34	BSS-3	Biclonal seed stock	UPASI-9 × TRI 2025	?	[8]
35	C-17		BBTC, Singampatty	?	
36	CH-1		UPASI TRF, Anamallais	?	
37	CR 6017		Craigmore, The Nilgiris	Cambod	[3]
38	SA-6		High Wayves, Tea Estates India	?	
39	SMP-1		High Wayves, Tea Estates India	?	
40	TES-34		?	?	
41	TRF-1		Arrapetta, Wynaad	Assam	[22]
42	TRI-2024		TRI, Sri Lanka	?	
43	TRI-2025		TRI, Sri Lanka	Cambod	[3]
44	TRI-2026		TRI, Sri Lanka	?	
45	TRI-2043		TRI, Sri Lanka	?	
46	TTL-1		Tata Tea Limited, Munnar	?	
47	TTL-2		Tata Tea Limited, Munnar	?	
48	TTL-3		Tata Tea Limited, Munnar	?	
49	W-2	*C. japonica*	UPASI TRF, Anamallais	?	

as discrete characters and numbered sequentially. Genotypes were scored for the presence (1) or absence (0) of each fragment. Only those fragments, which had a molecular weight greater than 100 bp and were visualized with medium or high intensity were considered for data analysis. From the binary data, the similarity coefficient values between the genotypes were derived based on the probability that an amplified fragment from one accession will also be present in another with the Nei's estimate [11]. Correlation matrices developed for the three primers used were consolidated in one single matrix and the mean values were presented. Similarity based relationship between the genotypes were presented in the form of a dendrogram, developed by following unweighted pair group method with arithmetic mean algorithm (UPGMA) using SAHN clustering analysis of NTSYSPC, version 2.0 [12]. Principal component analysis was performed using SPSS,

Table 2
Similarity matrix shows the similarity index between the south Indian cultivars (numbers indicate the serial number of genotypes as in Table 1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
1	1.00																																																
2	0.61	1.00																																															
3	0.63	0.77	1.00																																														
4	0.72	0.70	0.78	1.00																																													
5	0.04	0.07	0.11	0.05	1.00																																											
6	0.65	0.66	0.69	0.69	0.05	1.00																																										
7	0.62	0.79	0.81	0.76	0.09	0.71	1.00																																									
8	0.00	0.03	0.05	0.02	0.68	0.02	0.02	1.00																																								
9	0.65	0.78	0.85	0.81	0.04	0.69	0.82	0.00	1.00																																							
10	0.14	0.15	0.09	0.11	0.05	0.11	0.12	0.05	0.09	1.00																																						
11	0.73	0.76	0.78	0.80	0.03	0.70	0.83	0.00	0.85	0.13	1.00																																					
12	0.00	0.10	0.06	0.02	0.12	0.02	0.08	0.13	0.08	0.16	0.12	1.00																																				
13	0.71	0.59	0.59	0.72	0.04	0.59	0.64	0.00	0.68	0.12	0.73	0.03	1.00																																			
14	0.03	0.08	0.02	0.05	0.15	0.02	0.05	0.15	0.04	0.16	0.09	0.68	0.05	1.00																																		
15	0.08	0.10	0.12	0.15	0.05	0.08	0.04	0.04	0.11	0.22	0.09	0.09	0.07	0.14	1.00																																	
16	0.74	0.57	0.61	0.75	0.02	0.61	0.66	0.00	0.66	0.16	0.73	0.00	0.81	0.00	0.08	1.00																																
17	0.05	0.12	0.09	0.10	0.17	0.05	0.12	0.14	0.09	0.16	0.12	0.72	0.09	0.67	0.09	0.03	1.00																																
18	0.03	0.07	0.06	0.03	0.08	0.08	0.04	0.09	0.07	0.00	0.05	0.29	0.03	0.30	0.04	0.00	0.29	1.00																																
19	0.08	0.09	0.09	0.07	0.13	0.07	0.08	0.11	0.09	0.13	0.10	0.68	0.07	0.66	0.10	0.05	0.72	0.29	1.00																															
20	0.18	0.17	0.16	0.20	0.06	0.17	0.16	0.06	0.18	0.27	0.20	0.11	0.19	0.15	0.46	0.20	0.09	0.07	0.11	1.00																														
21	0.64	0.66	0.73	0.71	0.08	0.63	0.77	0.04	0.72	0.12	0.77	0.11	0.73	0.10	0.11	0.78	0.11	0.05	0.10	0.22	1.00																												
22	0.64	0.66	0.73	0.80	0.03	0.64	0.68	0.02	0.80	0.11	0.78	0.05	0.74	0.04	0.09	0.75	0.06	0.03	0.07	0.20	0.80	1.00																											
23	0.68	0.68	0.76	0.83	0.05	0.68	0.75	0.02	0.83	0.11	0.79	0.05	0.77	0.04	0.09	0.76	0.08	0.03	0.07	0.20	0.77	0.92	1.00																										
24	0.05	0.10	0.08	0.08	0.17	0.08	0.10	0.12	0.07	0.05	0.08	0.11	0.09	0.12	0.07	0.07	0.15	0.07	0.11	0.11	0.09	0.06	0.08	1.00																									
25	0.04	0.10	0.05	0.06	0.19	0.03	0.09	0.18	0.08	0.12	0.09	0.68	0.07	0.64	0.06	0.02	0.80	0.26	0.71	0.09	0.09	0.06	0.08	0.17	1.00																								
26	0.56	0.73	0.74	0.71	0.12	0.62	0.74	0.05	0.70	0.11	0.76	0.13	0.59	0.13	0.08	0.61	0.15	0.05	0.10	0.19	0.79	0.72	0.71	0.09	0.11	1.00																							
27	0.74	0.66	0.70	0.81	0.02	0.72	0.72	0.00	0.76	0.13	0.82	0.03	0.75	0.03	0.08	0.76	0.05	0.03	0.07	0.17	0.69	0.80	0.81	0.07	0.03	0.64	1.00																						
28	0.20	0.20	0.21	0.19	0.06	0.18	0.17	0.04	0.21	0.29	0.25	0.12	0.13	0.14	0.64	0.13	0.10	0.04	0.08	0.51	0.21	0.19	0.19	0.12	0.09	0.20	0.22	1.00																			
29	0.70	0.75	0.74	0.81	0.04	0.62	0.70	0.00	0.76	0.11	0.78	0.05	0.71	0.05	0.07	0.73	0.09	0.06	0.09	0.15	0.69	0.76	0.77	0.07	0.07	0.75	0.74	0.12	1.00																	
30	0.11	0.09	0.12	0.15	0.09	0.11	0.07	0.07	0.14	0.23	0.14	0.16	0.09	0.19	0.40	0.09	0.15	0.03	0.11	0.40	0.13	0.12	0.12	0.10	0.12	0.12	0.10	0.42	0.08	1.00																
31	0.68	0.65	0.67	0.72	0.05	0.60	0.72	0.00	0.70	0.16	0.73	0.07	0.75	0.07	0.06	0.73	0.10	0.03	0.08	0.14	0.75	0.68	0.70	0.12	0.08	0.66	0.73	0.16	0.71	0.08	1.00														
32	0.41	0.34	0.40	0.45	0.04	0.50	0.47	0.03	0.43	0.06	0.48	0.07	0.52	0.03	0.00	0.58	0.06	0.09	0.06	0.15	0.49	0.51	0.52	0.03	0.02	0.41	0.52	0.08	0.42	0.05	0.43	1.00													
33	0.17	0.20	0.17	0.20	0.15	0.17	0.16	0.13	0.22	0.23	0.24	0.13	0.21	0.17	0.28	0.22	0.11	0.09	0.13	0.41	0.24	0.20	0.20	0.12	0.10	0.22	0.23	0.42	0.17	0.37	0.19	0.20	1.00											
34	0.39	0.39	0.41	0.39	0.19	0.36	0.39	0.10	0.43	0.09	0.38	0.03	0.40	0.06	0.11	0.44	0.10	0.08	0.14	0.15	0.50	0.44	0.40	0.09	0.10	0.41	0.32	0.10	0.37	0.10	0.39	0.26	0.24	1.00										
35	0.57	0.59	0.63	0.62	0.07	0.63	0.67	0.02	0.67	0.11	0.66	0.06	0.61	0.06	0.03	0.63	0.12	0.10	0.10	0.05	0.61	0.64	0.70	0.13	0.09	0.59	0.64	0.07	0.63	0.00	0.62	0.53	0.10	0.38	1.00									
36	0.15	0.12	0.14	0.15	0.07	0.11	0.09	0.08	0.15	0.24	0.17	0.12	0.16	0.12	0.38	0.17	0.12	0.00	0.08	0.57	0.18	0.15	0.15	0.14	0.10	0.17	0.14	0.45	0.11	0.51	0.14	0.14	0.44	0.10	0.11	1.00								
37	0.00	0.00	0.06	0.03	0.55	0.00	0.02	0.63	0.00	0.05	0.00	0.11	0.00	0.12	0.04	0.00	0.14	0.10	0.10	0.04	0.04	0.02	0.02	0.13	0.12	0.04	0.00	0.04	0.00	0.08	0.00	0.00	0.08	0.09	0.03	0.05	1.00								
38	0.67	0.81	0.82	0.72	0.07	0.66	0.81	0.02	0.82	0.11	0.82	0.08	0.65	0.06	0.08	0.65	0.11	0.04	0.08	0.15	0.76	0.73	0.72	0.12	0.10	0.80	0.73	0.20	0.81	0.10	0.75	0.43	0.19	0.40	0.67	0.15	0.02	1.00							
39	0.00	0.02	0.09	0.04	0.65	0.02	0.05	0.67	0.02	0.08	0.00	0.11	0.00	0.13	0.11	0.00	0.16	0.06	0.11	0.09	0.05	0.02	0.02	0.14	0.16	0.08	0.00	0.11	0.00	0.12	0.02	0.02	0.12	0.17	0.04	0.13	0.70	0.05	1.00						
40	0.67	0.72	0.78	0.73	0.07	0.67	0.76	0.03	0.79	0.09	0.81	0.06	0.67	0.06	0.10	0.67	0.11	0.02	0.08	0.19	0.79	0.74	0.77	0.10	0.08	0.76	0.71	0.22	0.75	0.13	0.76	0.41	0.20	0.41	0.67	0.15	0.04	0.83	0.05	1.00				
41	0.02	0.04	0.07	0.05	0.75	0.03	0.03	0.67	0.01	0.05	0.02	0.12	0.02	0.16	0.07	0.02	0.17	0.08	0.13	0.10	0.05	0.03	0.03	0.15	0.21	0.08	0.02	0.08	0.02	0.09	0.02	0.04	0.12	0.19	0.03	0.07	0.54	0.04	0.63	0.04	1.00			
42	0.64	0.76	0.76	0.69	0.07	0.66	0.76	0.03	0.74	0.11	0.75	0.08	0.64	0.08	0.11	0.67	0.11	0.07	0.08	0.20	0.79	0.73	0.73	0.12	0.10	0.76	0.67	0.21	0.74	0.16	0.72	0.43	0.24	0.42	0.64	0.19	0.04	0.86	0.05	0.80	0.06	1.00		
43	0.63	0.75	0.79	0.73	0.08	0.71	0.78	0.04	0.75	0.14	0.77	0.07	0.64	0.06	0.08	0.63	0.09	0.02	0.06	0.20	0.77	0.70	0.75	0.11	0.07	0.78	0.69	0.21	0.73	0.12	0.70	0.43	0.19	0.37	0.65	0.15	0.04	0.87	0.05	0.83	0.05	0.83	1.00	
44	0.62	0.74	0.75	0.68	0.09	0.71	0.71	0.05	0.74	0.16	0.73	0.11	0.61	0.08	0.08	0.63	0.11	0.07	0.08	0.22	0.76	0.70	0.75	0.09	0.07	0.78	0.64	0.19	0.73	0.13	0.62	0.46	0.22	0.42	0.65	0.18	0.07	0.78	0.07	0.80	0.06	0.81	0.84	1.00
45	0.56	0.62	0.67	0.67	0.13	0.59	0.67	0.04	0.62	0.15	0.65	0.05	0.54	0.08	0.13	0.56	0.11	0.06	0.09	0.21	0.67	0.54	0.57	0.09	0.07	0.64	0.58	0.17	0.62	0.18	0.58	0.40	0.22	0.35	0.55	0.24	0.06	0.70	0.10	0.63	0.07	0.69	0.70	0.65	1.00
46	0.05	0.04	0.10	0.06	0.73	0.04	0.07	0.80	0.05	0.07	0.04	0.15	0.05	0.18	0.04	0.05	0.18	0.08	0.15	0.08	0.07	0.06	0.06	0.12	0.21	0.10	0.05	0.06	0.04	0.09	0.04	0.06	0.11	0.13	0.06	0.07	0.65	0.07	0.72	0.07	0.70	0.07	0.08	0.09	0.09	1.00
47	0.66	0.72	0.74	0.76	0.08	0.70	0.78	0.00	0.79	0.12	0.78	0.07	0.62	0.06	0.09	0.67	0.08	0.02	0.04	0.19	0.77	0.72	0.75	0.08	0.06	0.72	0.70	0.18	0.70	0.11	0.73	0.47	0.21	0.41	0.60	0.16	0.00	0.78	0.02	0.69	0.04	0.75	0.75	0.73	0.66	0.04	1.00
48	0.65	0.76	0.80	0.80	0.06	0.69	0.74	0.02	0.83	0.09	0.79	0.06	0.63	0.04	0.08	0.65	0.08	0.05	0.08	0.16	0.73	0.80	0.81	0.07	0.07	0.78	0.73	0.21	0.87	0.10	0.74	0.42	0.18	0.41	0.65	0.13	0.02	0.83	0.03	0.82	0.03	0.78	0.81	0.79	0.65	0.06	0.74	1.00
49	0.52	0.30	0.31	0.39	0.02	0.44	0.39	0.00	0.34	0.19	0.38	0.00	0.43	0.00	0.00	0.44	0.04	0.00	0.06	0.14	0.38	0.39	0.40	0.03	0.02	0.27	0.48	0.09	0.37	0.07	0.35	0.33	0.09	0.21	0.32	0.08	0.00	0.32	0.00	0.36	0.02	0.34	0.35	0.38	0.39	0.03	0.32	0.33	1.00

version 7.5. The first two components extracted were used to derive the PCO plot. The distance within and between any population was calculated by the mean coefficient values between all the individuals. Affinity index of each accession towards the other members of the population was calculated with the mean similarity co-efficiency of each individual with the other individuals of the respective group.

3. Results and discussion

The AFLP profile of PCR products of 49 genotypes for primer combinations E-ACA/M-CTG, E-ACC/M-CTG and E-AGG/M-CTG were detected by the fluorochromes, FAM, NED and JOE, respectively. Fingerprinting revealed a total number of 1555 unambiguous polymorphic amplified DNA fragments with an average of 518.3 polymorphic loci per combination. The size of loci ranged from 87.43 to 359.03 bp. This confirms that AFLP is capable of detecting substantial numbers of polymorphic loci with a relatively small number of primer pairs. In Lettuce (*Lactuca* spp.), the use of only three AFLP primer combinations allowed the identification of 320 polymorphic loci [13].

Nei's similarity coefficient matrix (Table 2) showed very close correlation (0.92) between the accessions UPASI-22 and UPASI-23, while several cultivars were distantly related. Among the populations characterized, the Chinary type showed a maximum diversity index of 0.612 within the population (Table 3), while minimum diversity was observed within the Assam type (0.285). Studies on Kenyan tea cultivars also revealed that the maximum diversity index among Chinary populations [14]. In the present study, maximum genetic diversity index was observed between Assam and Cambod populations (0.946), while the minimum (0.852) was recorded between Assam and China. Affinity of each of the cultivars with the source populations clearly defined the relationship of the cultivars with the population types (Table 4.). The dendrogram (Fig. 1) and the PCO plot (Fig. 2) revealed that all the south Indian tea cultivars could be clearly distinguished into three distinct groups as well as one intermediate group. Diversity assessment of Kenyan tea cultivars had also resulted in three types of grouping among the accessions studied [14]; but there were no intermediates. The prominent groups were further named as Assam, China and Cambod since the phenotypic characteristics of the individual members possessed well defined taxonomic traits.

As suggested by Mohanan and Sharma [3], the accessions UPASI-3 and UPASI-21 belonging to Assam, UPASI-15 belonging to Chinary and UPASI-17 and CR 6017 belonging to Cambod types segregated

Table 3
Diversity index within and between the populations of tea, cultivated in south India

Population	Assam	China	Cambod
Assam	0.285		
China	0.852	0.612	
Cambod	0.946	0.903	0.611

Table 4
Affinity index of the tea accessions towards the population types

Accession	Assam	China	Cambod	Affinity max.
UPASI-1	0.669	0.146	0.027	Assam
UPASI-2	0.711	0.147	0.062	Assam
UPASI-3	0.743	0.145	0.072	Assam
UPASI-4	0.751	0.164	0.050	Assam
UPASI-5	0.064	0.078	0.465	Cambod
UPASI-6	0.674	0.132	0.032	Assam
UPASI-7	0.747	0.114	0.062	Assam
UPASI-8	0.020	0.069	0.468	Cambod
UPASI-9	0.762	0.156	0.047	Assam
UPASI-10	0.121	0.354	0.099	China
UPASI-11	0.772	0.175	0.055	Assam
UPASI-12	0.062	0.126	0.409	Cambod
UPASI-13	0.680	0.140	0.038	Assam
UPASI-14	0.055	0.153	0.413	Cambod
UPASI-15	0.088	0.483	0.076	China
UPASI-16	0.693	0.149	0.017	Assam
UPASI-17	0.092	0.116	0.442	Cambod
UPASI-18	0.045	0.039	0.175	Cambod
UPASI-19	0.078	0.105	0.409	Cambod
UPASI-20	0.179	0.518	0.089	China
UPASI-21	0.740	0.173	0.075	Assam
UPASI-22	0.738	0.150	0.043	Assam
UPASI-23	0.759	0.152	0.047	Assam
UPASI-24	0.088	0.101	0.135	Cambod
UPASI-25	0.071	0.098	0.446	Cambod
UPASI-26	0.717	0.155	0.099	Assam
UPASI-27	0.729	0.152	0.027	Assam
AK 1	0.185	0.534	0.084	China
ATK 1	0.742	0.115	0.040	Assam
B-1-101	0.110	0.476	0.114	China
BSB 1	0.708	0.130	0.047	Assam
BSS I	0.457	0.097	0.039	Assam
BSS II	0.199	0.450	0.125	China
BSS III	0.399	0.128	0.119	Assam
C-17	0.645	0.068	0.061	Assam
CH-1	0.152	0.513	0.091	China
CR6017	0.021	0.055	0.423	Cambod
SA 6	0.771	0.140	0.063	Assam
SMP 1	0.033	0.108	0.458	Cambod
TES 34	0.755	0.155	0.063	Assam
TRF 1	0.039	0.083	0.461	Cambod
TRI2024	0.746	0.175	0.069	Assam
TRI2025	0.753	0.157	0.062	Assam
TRI2026	0.728	0.171	0.080	Assam
TRI2043	0.638	0.186	0.080	Assam
TTL 1	0.062	0.074	0.497	Cambod
TTl 2	0.731	0.149	0.044	Assam
TTL 3	0.764	0.136	0.050	Assam
W-2	0.372	0.094	0.018	Assam

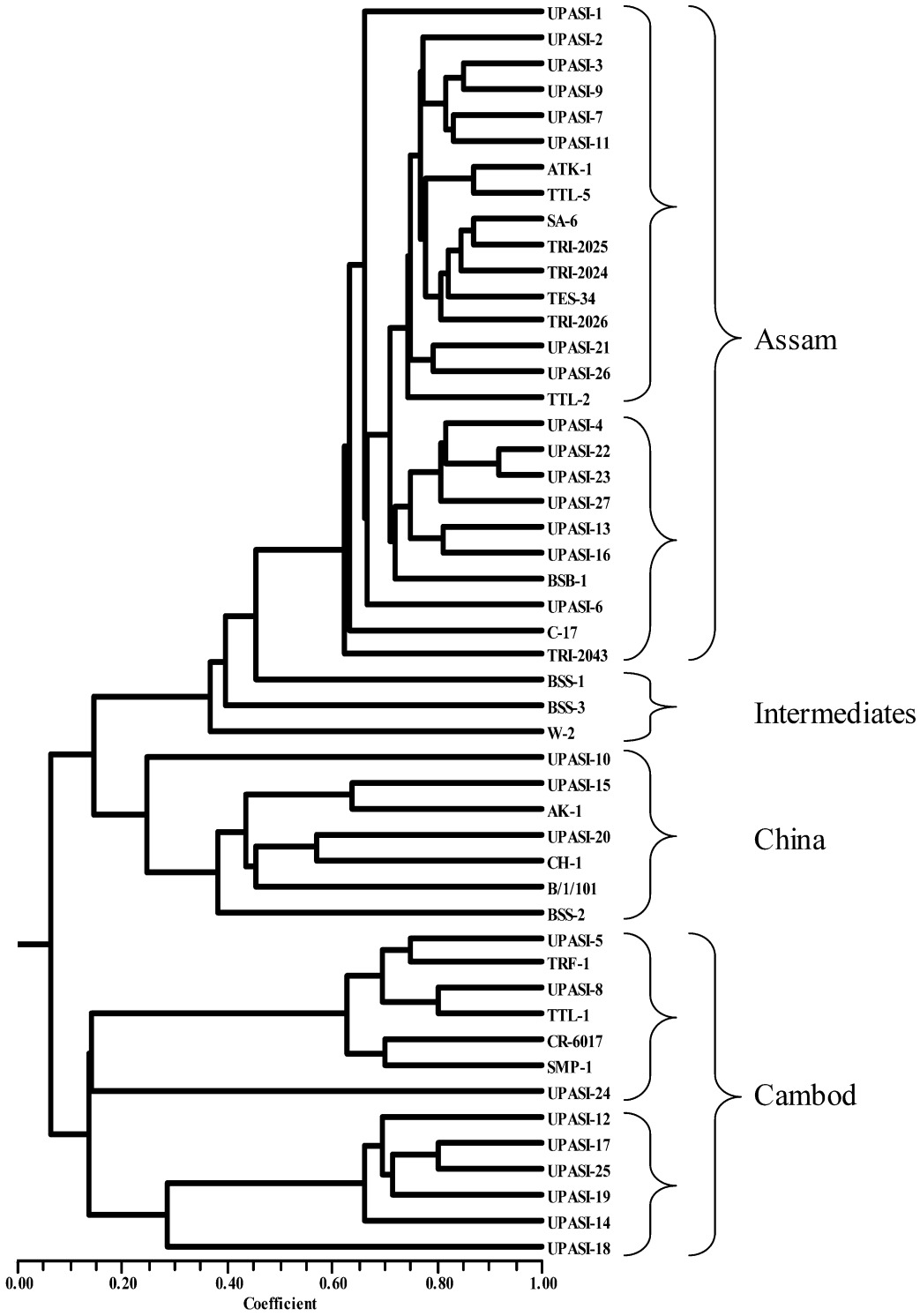

Fig. 1. Dendrogram of south Indian tea cultivars (UPASI released and estate selections) using AFLP analysis.

accordingly into the three groups during the analysis. The well-known pure line Chinaries, CH-1 and B/1/101 clustered under China type. A cultivar released by Tea Research Institute, Sri Lanka and extensively used by south Indian tea researchers for breeding purposes, TRI 2025 (Cambod phenotype) clustered along with Assam in AFLP analysis. Similarly, two popular south Indian clones UPASI-9 and ATK-1 (due to their high yield and drought resistance) characterized as Chinary also grouped under Assam in the present study. Some of the well-distinguished tea cultivars were found to cluster according to their geographical origin. Clones released

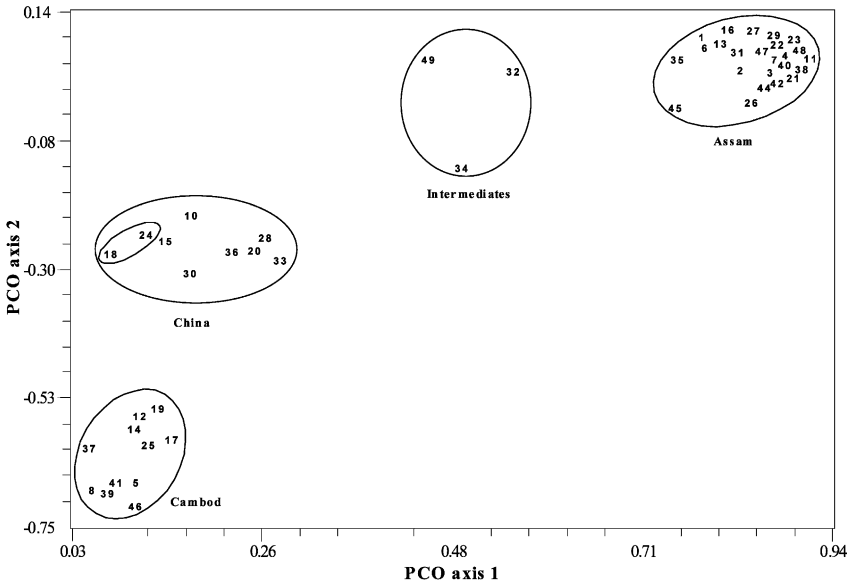

Fig. 2. PCO plot showing the origin and diversity of tea varieties cultivated in south India (numbers indicate the serial number of genotypes as in Table 1).

by Tea Research Institute, Sri Lanka such as TRI 2024, TRI 2025, TRI 2026 and TRI 2043 grouped under Assam, even though TRI 2043 was phenotypically distinct due to high anthocyanin content when compared with other Sri Lankan clones. This confirms the hypothesis that their common ancestry [genetic stock 4/10 (St.124)] was brought from Assam to Sri Lanka [15].

The high yielding biclonal seed stocks, BSS-1, BSS-2 and BSS-3, released by UPASI TRF showed a peculiar pattern of classification. Following cluster analysis, BSS-2 grouped under Chinary, even though its parents, UPASI-2 and TRI-2025, belonged to Assam type. Interestingly, the other two seed stocks, BSS-1 and BSS-3, did not group under any of the major three types and are, therefore, considered intermediates. The ornamental *Camellia* (Wild 2) also segregated as an intermediate along with the two-biclonal seed stocks between Assam and China types. Although these three clustered together, they possessed more than 65% genetic diversity among them and had a distant relationship with the Cambod type ($>85\%$). The clustering of the ornamental genotype with the intermediate group suggests that such genotypes may have either originated independently [16] or as the progeny of Assam and Chinary/Cambod parents [17]. This further suggests that crossing Assam type with Chinary or Cambod parents might help to develop new cultivars with superior traits, which will also be useful for the enhancement of genetic diversity.

Among the cultivars characterized, the maximum genetic similarity was observed between the accessions UPASI-22 and UPASI-23 characterized under Assam type. Although the Assam cultivars constituted a larger population, they possessed a narrow genetic base when compared with Cambod and Chinary cultivars. More than 50% population of the Assam type were related among themselves with less than 20% diversity, while in the case of China and Cambod, all the accessions had a similarity coefficient value <0.8. In general, both Assam and Cambod cultivars clustered into two sub groups, but no subgroups were seen among the Chinaries. Among the two sub groups of Cambod type, one had only UPASI released clones while the other comprised estate selections along with a few UPASI clones. This may be due to the close geographical origin of UPASI clones (selected from a couple of estates in Nilgiris) though minimal segregation was observed. It may be pointed out that these plantations, when initially planted during the latter part of the 19th century, may have obtained seeds from the same original source garden.

Results of the analysis of AFLP fragments using UPGMA and PCO analysis are congruent. But the accessions, UPASI-18 and UPASI-24 clustered as Cambod by UPGMA grouped along with China in PCO analysis. This may be due to their weaker affinity (<0.2) towards both Chinary and Cambod cultivars. However, they may be considered as Cambod type since they have more affinity towards Cambod than Chinaries.

The availability of large numbers of fragments defining independent genetic loci with highly reproducible polymorphism detection enables the efficient evaluation of genetic diversity. Studies on diversity assessment of south Indian tea cultivars using AFLP fingerprinting revealed that it is a powerful tool among the available genetic fingerprinting techniques and it can be adopted for any kind of breeding program in tea. We have for

the first time employed AFLP for the assessment of genetic diversity of south Indian tea cultivars. Diversity assessment revealed a narrow genetic diversity among the cultivars (37.76) due to the popularity of certain cultivars among the commercial planting companies. Among the existing cultivars, the biclonal seedlings BSS-1 and BSS-3, released by UPASI TRF, Valparai were more diverse in their genetic make up. Field evaluation of these cultivars had also proved their enhanced yield behavior [8]. It is also possible that a large proportion of valuable tea germplasm may have been already lost through the continuous removal of older plants, especially seedlings for commercial planting of vegetatively propagated plants. Further extensive planting of a few clones will cause as similar erosion of genetic diversity. The Assam cultivars, which are extensively exploited for higher yield, in particular, showed a narrow genetic diversity. To avoid further degradation of germplasm resources, existing populations must be preserved and further crosses should be made with genetically distant varieties or genotypes of diverse origin.

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