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Assessment of Genetic Relationship and Hybrid Evaluation Studies in Tea (*Camellia* sp.) by RAPD

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ABSTRACT

The genetic relationships among 12 tea accessions representing three species in the genus *Camellia* were studied using random amplified polymorphic DNA (RAPD) markers. The genetic distance matrix based on Euclidian Distances showed a minimum genetic distance of 2.24 between 'UPASI-2' and 'UPASI-3' clones and the maximum was 4.47 between 'TRF-1' and 'TRI-2025'. The dendrogram based on Ward's method of cluster analysis clearly characterized all 12 tea varieties into three clusters based on their types namely China, Assam and Cambod. Pair-wise genetic similarity index between parent and hybrid clones generated showed a highest mean of 0.59 between 'TRI-2025' and 'BSS-1' and a lowest of 0.34 between 'UPASI-10' and 'BSS-1'. This study revealed that all the varieties analysed fall the present taxonomic framework of *Camellia* species and that the hybrid is of Cambod type. RAPD markers can thus be successfully applied in this taxon for the study of relationships and to confirm hybrid origin. The study offers a sound platform for future tea breeding programmes in tea as well as evolution of hybrids in the commercially important tea varieties.

Keywords: genetic distance, genetic diversity, polymerase chain reaction, STATISTICA, tea clones

INTRODUCTION

Tea is the most popular non-alcoholic beverage across the world, belonging to the family Theaceae. The genus *Camellia* comprises of three species with specific plant types (Wight 1962) *viz*. *Camellia sinensis* (China type), *C. assamica* (Assam type) and *C. assamica* sub species. *Lasiocalyx* (Cambod type). Natural cross-pollination between these species and subspecies, followed by vegetative propagation has resulted in considerable variation in this taxon (Wood and Barua 1958). There is also doubt as to whether or not existing tea populations have resulted from free hybridization between the three main taxa or if other *Camellia* species are also involved (Cannel *et al*. 1977). The numerous hybrids that apparently have resulted are still generally referred to as Assam, China or Cambod type depending on their morphological proximity to the main taxa (Banerjee 1992). Morphological, cytological and chemical classification of *Camellia* has been reported by Chen *et al*. (2000). However, plant variety discrimination and assessment of genetic relationship using morphological traits have several limitations, especially in perennial crops. Molecular differences, using DNA markers are more authentic and unaffected by environmental factors (Dhanraj *et al*. 2002; Rajesh *et al*. 2008; Ni *et al*. 2008). Genetic analysis reduces ambiguities that can arise when examining morphological properties (Lewis *et al*. 2004). Research on plant discrimination has therefore shifted to the use of the more sensitive DNA markers. Development of PCR has allowed the introduction of number of DNA-based markers. The most frequently used DNA markers include restriction fragment length polymorphisms [RFLP] (Sambrook *et al*. 1989), random amplified polymorphic DNA [RAPD] (Williams *et al*. 1990), simple sequence repeats [SSR] (Gupta *et al*. 1996), amplified fragment length polymorphism [AFLP] (Vos *et al*. 1995) and inter simple sequence repeats [ISSR] (Provost and Wilkinson 1999). These molecular markers are based on different principles and are obtained by using procedures of varying complexity and generate different amounts of polymorphic data. RAPD is generally less expensive and more broadly available to breeders than the costlier non-PCR based marker techniques (Rao *et al*. 2008). Also, they gained importance due to their simplicity, efficiency, the relative ease to perform the assay and non-requirement of DNA sequence information (Khanuja *et al*. 1998).

Molecular markers have successfully used to identify *Camellia sinensis* cultivars using protein-based markers (Saravanan *et al*. 2005; Matsumoto 2006), chloroplast DNA (Kaundun and Matsumoto 2002), 5S ribosomal DNA [rDNA] (Singh and Ahuja 2006), ISSR markers (Mondal 2002; Chen *et al*. 2005; Yao *et al*. 2008), AFLP marker (Balasaravanan *et al*. 2003; Karthigeyan *et al*. 2008), RFLP marker (Matsumoto *et al*. 2002; Kaundun and Matsumoto 2003; Matsumoto *et al*. 2004), RAPD marker (Shen *et al*. 2008) and SSR (Freeman *et al*. 2004). RAPDs have also been employed to determine parental identification (Balasaravanan *et al*. 2001), phylogenetic analysis (Jung *et al*. 1993; Chen and Yamaguchi 2002), core collection (Li *et al*. 2005) and to determine genetic diversity (Wachira *et al*. 1995, 1997; Chen *et al*. 1998; Kaundun *et al*. 2000; Wachira *et al*. 2001; Luo *et al*. 2004; Duan *et al*. 2004; Chen *et al*. 2006a, 2006b; Shen *et al*. 2008).

Understanding the genetic relationships of the existing tea germplasm would help to select parental cultivars for current and long-term success of tea breeding (Chen and Yamaguchi 2005; Chen *et al*. 2007). The traditional method of species identifying by morphological characters had now been replaced by more consistent DNA profiling that are simple and reliable (Nayak *et al*. 2003). Genomic analysis of cultivated tea germplasm is essential to determine the genetic relationship and hybrids identification of the existing tea clones. In this study, we have used RAPD markers

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Table 1 Description of tea varieties included in the study

Clone	Accession number	Source of material	Variety	Reference
'UPASI-2'	B/ 4/142 (Jayaram)	Brooklands Estate, The Nilgiris	Assam	Mohanan *et al*. 1981
'UPASI-3'	B /5/63 (Sundaram)	Brooklands Estate, The Nilgiris	Assam	Venkataramani and Sharma 1974
'UPASI-9'	B/6/61 (Athrey)	Brooklands Estate, The Nilgiris	China	Mohanan *et al*. 1981
'UPASI-10'	B /6/62 (Pandian)	Brooklands Estate, The Nilgiris	China	Mohanan *et al*. 1981
'UPASI-17'	B /6/203 (Swarna)	Brooklands Estate, The Nilgiris	Cambod	Mohanan *et al*. 1981
'TRF-1'	Selection A	Arrapetta, Wynaad	Cambod	Balasubramanian *et al*. 2001
'TRF-2'	NLT/17/10	The Nullatanni Estate, Munnar	China	Babu 2007
'SA-6'	--	High Wayves, Tea Estates India	China	Balasaravanan *et al*. 2003
'CR-6017'	--	Craigmore, The Nilgiris	Cambod	Mohanan *et al*. 1981
'Assam Seedling'	--	Assam	Assam	--
'BSS-1'	Biclonal seed stock	UPASI-10 x TRI-2025	Cambod	Balasaravanan *et al*. 2003
'TRI-2025'	--	TRI, Sri Lanka	Cambod	Mohanan *et al*. 1981

to analyse the genetic relationships among the cultivated tea genotypes of South India.

MATERIALS AND METHODS

Plant materials

Twelve cultivars of tea from *Camellia sinensis* (China type), *C. assamica* (Assam type) and *C. assamica* sub species *lasiocalyx* (Cambod type) were collected from the UPASI-TRI (United Planters Association of Southern India – Tea Research Institute) germplasm collections, Valparai, India (**Table 1**). Approximately 20 g of young shoots with two leaves and a bud were randomly sampled from each cultivar, washed using distilled water, wiped with 70% (v/v) ethanol, then dried prior to storage at -80°C in sealed plastic bags.

DNA extraction and purification

All the reagents and chemicals were obtained from Bangalore Genei, Bangalore, India of molecular biology grade. DNA was extracted from the stored leaves by the cetyl trimethyl ammonium bromide (CTAB) method according to a modified protocol of Simon *et al*. (2007). 500 mg of leaf tissue was powdered using liquid nitrogen and was mixed with 10 ml extraction buffer, preheated to 65°C, containing 100 mM Tris-base, pH 8.0, 20 mM EDTA, pH 8.0, 1.4 M NaCl, 3% (w/v) CTAB, 2% polyvinyl pyrrolidone and 1% β-mercaptoethanol, then incubated at 65°C for 30 min with gentle shaking. The mixture was cooled to room temperature, to which 10 ml cold 24: 1 (v/v) chloroform: isoamylalcohol was added and the contents were mixed well. After centrifugation at 6,000 × *g* for 20 min at 4°C, the supernatant was transferred to a fresh tube and the chloroform: isoamylalcohol step was repeated until a clear supernatant was obtained. To the supernatant 5 M NaCl was added (0.5 v/v) and mixed gently followed by addition of 0.8 vol of cold isopropanol to precipitate the DNA. The mixture was incubated overnight at 4°C, and then centrifuged at 8,000 × *g* for 20 min. The resulting pellet was washed with 70% (v/v) ethanol, air-dried, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Two µg RNase (Bovine pancreatic ribonuclease) was added to each sample which was incubated for 3 h at 37°C, mixed with an equal volume of phenol and centrifuged at 6,000 × *g* for 20 min. at room temperature. This step was followed by a washing with an equal volume of 1: 1 (v/v) phenol: chloroform and then with chloroform alone. DNA was precipitated overnight at 4°C with 0.5 vol of 5 M NaCl and 1 vol of cold isopropanol and the resulting pellet obtained after centrifugation was dissolved in TE buffer, analysed on an agarose gel and quantified using a spectrophotometer (ND-8000, NanoDrop Technologies, Wilmington, USA).

PCR amplification

The PCR amplification protocol followed was according to Williams *et al*. (1990) with minor modifications. Of the 50 primers screened using the bulk DNA, 12 showing prominent bands were selected for RAPD-PCR analysis (**Table 2**). Reproducibility of the primers was tested by repeating the PCR amplification three times under similar conditions. PCR reactions were carried out in a volume of 25 µl containing 25 ŋg template DNA, 150 µM each dNTP, 1.5 mM $MgCl_2$, 1.5 unit *Taq* DNA polymerase (Sigma Aldrich Chemicals, Bangalore, India), 5 pmol primer (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPH, OPI, OPJ and OPK series, Operon Technologies, Alameda, CA, US) in PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.05% (v/v) NP40 and 0.05% (v/v) Triton X-100). Amplifications were performed in a Corbett Research Thermocycler (Corbett Research, Mortlake, NSW, Australia), programmed for an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, primer extension at 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were resolved in a 1.2% (w/v) agarose gel, visualised and documented using an Alpha Digidoc system (Alpha Innotech, San Leandro, CA, US).

RAPD profile analysis

Amplified fragments from each primer was manually scored for their presence (1) or absence (0) and a matrix of the different RAPD phenotypes of all 12 was assembled for statistical analysis by STATISTICA computer package (STATISTICA for Windows, StatSoft Inc, Tulsa. OK, USA, 1996). The sizes of the fragments were estimated using 500 bp standard DNA markers (Bangalore Genei, Bangalore, India), co-electrophoresed with the PCR products. A genetic dissimilarity matrix was developed using Squared Euclidean Distances, which estimates all pair-wise differences in the amplification products (Sokal and Sneath 1973) and a cluster analysis was based on Wards method using a minimum variance algorithm (Ward 1963). The genetic similarity was determined between hybrid and their parents by Nei and Li's method (Nei and Li 1979); $S= 2N_{AB}/(N_A + N_B)$, where N_{AB} is the number of bands that individuals A and B shared in common, N_A is the number of bands in individual A and N_B is the number of bands in individual B. The analyses were performed using the software, STATISTICA version 4.5.

RESULTS AND DISCUSSION

The objective of this study was to estimate the extent of the genetic diversity among tea genotypes using RAPD markers. RAPD markers have been successfully used as a tool for assessing genetic variation in various plant species, including tea (Gul *et al*. 2007). The traditional method of identifying species by morphological characters has been replaced by the use of DNA profiling (Nayak *et al*. 2003) which provides more reliable information. PCR amplification was followed by a modified protocol published by our group (Simon *et al*. 2007), which produced good amplifications with 25 ng of template DNA and 1.5 U *Taq* DNA polymerase. Shen *et al*. (2008) had shown good amplification pattern with 40 ng of template DNA and 1.5 U *Taq* DNA polymerase. Of the 50 primers screened, 12 produced clear and readable bands (**Table 2**) ranging from 400 to 3500 bp that were consistent in 3 repeated PCR analyses. The number of bands for each primer obtained varied from 6 to 16 with an average of 10.67 bands per primer. A total of 128 bands were scored, of which 86 (67.2%) were polymorphic and shared within the tea cultivars, 32 (25%) were monomorphic and were common to all the cultivars and only 10

Table 2 RAPD-PCR primers. The sequence, level of polymorphism and pair-wise genetic similarity of selected polymorphic primers in tea varieties.

Primers	Sequence (5′-3′)	№ of amplified fragments	№ of monomorphic bands	№ of polymorphic shared bands	№ of polymorphic unique bands	Genetic similarity index	
						UPASI 10: BSS 1	TRI 2025: BSS 1
OPA-02	TGCCGAGCTG	15	5	9	1	0.34	0.56
OPA-16	AGCCAGCGAA	13	6	6	1	0.24	0.55
OPC-07	GTCCCGACGA	12	3	7	2	0.57	0.88
OPD-03	GTCGCCGTCA	10	4	6	0	0.28	0.54
OPF-01	ACGGATCCTG	9	2	6	1	0.34	0.55
OPF-03	CCTGATCACC	11	3	8	0	0.33	0.77
OPF-05	CCGAATTCCC	8	0	7	1	0.32	0.40
OPF-08	GGGATATCGG	11	2	8	1	0.30	0.65
OPF-13	GGCTGCAGAA	10	2	7	1	0.32	0.60
OPF-18	TTCCCGGGTT	16	4	11	1	0.35	0.47
OPJ-16	GGAGTACTGG	7	1	6	0	0.35	0.54
OPK-07	AGCGAGCAAG	6	0	5	1	0.33	0.57
	Total	128	34	86	10	0.34*	0.59*

* Mean similarity index

Table 3 Genetic dissimilarity matrix of 12 tea varieties based on polymorphism of RAPD markers.

SA-6	0.00											
UPASI-9	2.65	0.00										
UPASI-2	2.83	3.00	0.00									
UPASI-3	3.00	3.16	2.24	0.00								
CR-6017	3.16	3.61	3.16	3.32	0.00							
UPASI-17	3.46	3.87	3.74	3.61	3.46	0.00						
Assam Seedling	3.32	3.16	2.24	2.83	3.00	4.12	0.00					
TRF-2	3.46	3.32	3.46	3.32	4.24	3.46	3.61	0.00				
UPASI-10	3.00	2.83	3.32	3.46	4.12	3.87	3.46	3.00	0.00			
TRI-2025	3.74	3.32	2.74	3.32	3.74	4.24	3.32	4.24	4.12	0.00		
BSS-1	3.46	3.87	3.46	3.61	4.00	4.00	3.87	3.46	4.00	3.87	0.00	
TRF-1	3.61	4.00	3.87	4.00	4.12	3.87	4.00	4.36	3.32	4.47	3.87	0.00

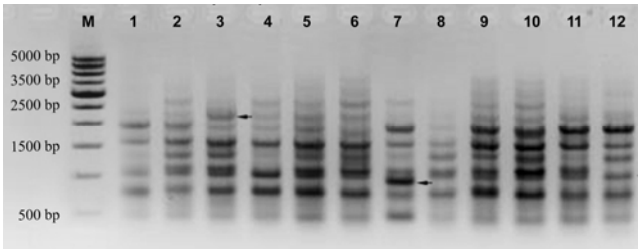

Fig. 1 Gel profile of tea varieties according to RAPD-PCR primer OPC-07. Lanes 1-12 contain the amplification profile of tea varieties 'SA-6', 'UPASI-9', 'UPASI-2', 'UPASI-3', 'CR-6017', 'UPASI-17', 'Assam Seedling', 'TRF-2', 'UPASI-10', 'TRI-2025', 'BSS-1' and 'TRF-1'. Lane M: 500 bp standard DNA marker. Arrows: unique polymorphic bands among vars. 'UPASI-2' and 'Assam Seedling', 2,150 and 940 bp, respectively.

(7.8%) were unique. Similarly, a high percentage of polymorphism has been obtained using RAPD markers by Shen *et al*. (2008) where the genetic diversity and genetic variation of four tea populations of *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica* cv. 'Duntsa', *C. ptilophylla* and *C. sinensis* var. *assamica* cv. 'Jianghua' in Hunan province of China were studied by RAPD markers; their result indicated 88.9% polymorphism. A representative polymorphic gel profile of primer OPC-07 is shown in **Fig. 1**.

The highest genetic dissimilarity of 4.47% was between genotypes 'TRI-2025' and 'TRF-1', which where collected from Sri Lanka and India, respectively (Balasubramanian *et al*. 2001). While the least genetic dissimilarity (2.24%) was between genotypes 'UPASI-2' and 'UPASI-3', and between 'UPASI-2' and 'Assam Seedling', all belonging to the same regions of origin (Assam). Similarly, a low proportion of genetic diversity was revealed by RAPD markers within a Korean tea population (Kaundun and Park 2002; Park *et al*. 2002). A pair-wise genetic dissimilarity matrix was calculated using Euclidian Distance (Sokal and Sneath 1973) and is represented in **Table 3**. In the dendrogram (**Fig. 2**), all 12 varieties were clearly divided into two major clusters 'A' and 'B' at a linkage distance (LD) of 4.93. Cluster 'A' consisted of five varieties clustered at LD = 3.8. Varieties 'SA-6' and 'UPASI-9' were clustered together at LD = 2.64; similarly varieties 'TRF-2' and 'UPASI-10' belonging to China type were clustered together at LD = 3 (Mondal 2002). All the varieties of Chinese origin were clustered together at LD = 3.5 in cluster 'A', except for 'TRF-1' in cluster 'B'. The 5s rDNA gene diversity analysis by Singh and Ahuja (2006) separated 'SA-6' to a hybrid China variety and 'UPASI-9' into China-type. In the same study the clone 'UPASI-2' was grouped under Assam variety. Our present study is consistent with the existing knowledge on the morphology and systematics of *Camellia* species (Wight 1962; Mohanan and Sharma 1981), and 5s rDNA gene diversity analysis (Singh and Ahuja 2006). Chen and Yamaguchi (2005) used RAPD markers for discriminating tea germplasms at the inter-specific level in China. This de-

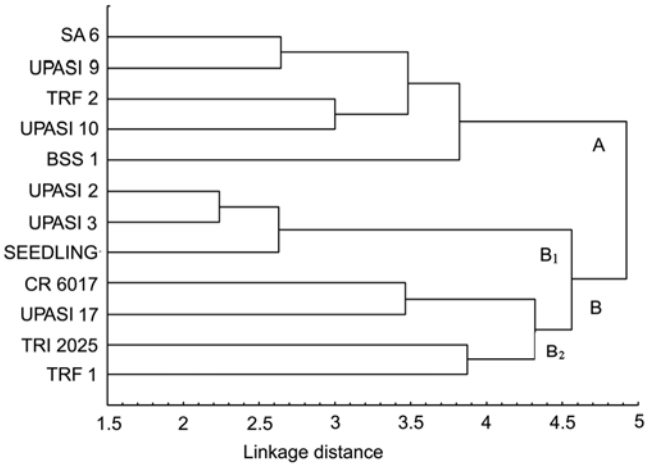

Fig. 2 Cluster analysis. Dendrogram showing RAPD-marker based genetic relationships of twelve tea varieties into three clusters 'A', 'B_1' and 'B_2' segregated as China type, Assam type and Cambod type, respectively.

monstrates that RAPDs offer a suitable method for detection of variability from molecular analysis of tea genotypes and other horticultural crops.

The maximum genetic difference among the varieties of Chinese origin was 4.36% between 'TRF-1' and 'UPASI-10' whereas the minimum genetic difference was observed between the varieties 'SA-6' and 'UPASI-9' (2.65%). Variety 'BSS-1', phenotypically classified under Cambod type, was clustered with varieties from China as an exception in the cluster. The China tea varieties and drought-tolerant clones 'UPASI-9' and 'UPASI-10' were also reported to be clustered together based on morphological markers by Mohanan and Sharma (1981). Similarly the genetic diversity study of UPASI tea clones on the basis of total leaf catechin grouped the 'UPASI-9' and 'UPASI-10' under the China variety (Saravanan *et al*. 2005). The rDNA analysis has shown to cluster 'SA-6' and 'BSS-1' together (Singh and Ahuja 2006). Interestingly, var. 'SA-6' is morphologically classified into China tea but has been considered as Assam tea based on its clustering by Balasaravanan *et al*. (2003) and as hybrid China variety by Singh and Ahuja (2006). The clone 'TRF-2' is a high quality clone with an acceptable level of leaf yield, tolerance to drought and has a low incidence of pests and diseases (Babu 2007). The presence of the variety 'BSS-1' (Biclonal seedstock 1) belonging to the Cambod type with China tea is due to the hybrid nature of 'BSS-1' as it has been developed using 'UPASI-10' (China type, female parent) and 'TRI-2025' (Cambod type, male parent), and hence grouped with its female parent (Wachira *et al*. 1995). This shows that 'BSS-1' is phenotypically similar to the Cambod type but genotypically closer to the China type.

Cluster 'B' consisted of two sub-clusters 'B_1' and 'B_2' with Assam and Cambod type, respectively linked at linkage distance (LD) = 4.6. Sub-cluster 'B_1' grouped all the three Assam types ('UPASI-2', 'UPASI-3' and 'Assam Seedling') together of which varieties 'UPASI-2' and 'UPASI-3' were closely linked together at LD = 2.25 and clustered with 'Assam Seedling' at LD = 2.63. The results of Balasaravanan *et al*. (2003) show that varieties 'UPASI-2' and 'UPASI-3' were clustered together when analysed by AFLP markers. The grouping of the Assam types separately suggest that the clones might have developed from free hybridization between the other two taxa of *Camellia* spp. (Wood and Barua 1958; Cannel *et al*. 1977). Clustering of the clone , 'UPASI-3' in our study is similar to the results obtained by Paul *et al.* (1997), where 'UPASI-3' was grouped with Assam type when analysed with AFLP markers. Primer OPC-07 produced unique bands specific to 'Assam Seedling' and 'UPASI-2', similar to the results of Chen and Yamaguchi (2005) at the inter-specific level of China tea.

Sub-cluster 'B_2' had four varieties that predominantly clustered with Cambod type at LD = 4.3. The Cambod types 'CR-6017' and 'UPASI-17' were clustered together at LD = 3.45 and varieties 'TRI-2025' and 'TRF-1' were clustered at LD = 3.9. Among all the varieties, Cambod types 'TRF-1' and 'TRI-2025' showed maximum genetic dissimilarity (4.47%) because of their origin; 'TRI-2025' is a cultivar identified at the Tea Research Institute, Sri Lanka developed from Assam/Cambod open pollinated progeny (Ariyarathna and Gunasekare 2006) and 'TRF-1' originated at UPASI-TRF, Valparai, India. A similar grouping of Cambod varieties ('CR-6017', 'UPASI-17' and 'TRI-2025') was identified by Mohanan and Sharma (1981) with the phenotypic classification of *C. assamica* sub species *lasiocalyx*, A clustering pattern obtained by an AFLP profile showed that var. 'CR-6017' was more closely associated to 'TRF-1' than to 'UPASI-17', whereas 'TRI-2025' was linked to 'SA-6' (Balasaravanan *et al*. 2003).

RAPD markers have been successfully used to identify hybrids in tea (Balasaravanan *et al*. 2001). However, the use of SSR markers may be preferred as RAPD patterns are inadequate for accurate genotypic scoring (Mondal 2002; Rao *et al.* 2008). In our study, the primers provided clear and reproducible banding patterns unique to groups based on their origin. A pair-wise genetic similarity index between parents ('UPASI-10' and 'TRI-2025') and their progeny ('BSS-1') was calculated individually for each RAPD marker where a range of similarity indices (0.32 to 0.88) was exhibited by these varieties (**Table 2**). The genetic similarity of the hybrid tea variety revealed a higher mean similarity index (0.59) with Cambod type parent 'TRI-2025' and (0.34) with 'UPASI-10' parent. The highest similarity index of 0.88 was observed between 'TRI-2025' and 'BSS-1' with primer OPC-07 and lowest similarity of 0.32 between 'UPASI-10' and 'BSS-1' using primer OPF-05. These results were consistent with previous reports by morphological characterization of the hybrid and its parents (Mohanan and Sharma 1981; Satyanarayana and Sharma 1993). This shows that among the tea varieties RAPD markers are adequate to confirm the genetic similarity between hybrids and parents as well as to determine the genetic relationships among them.

The results of this present study suggest that RAPD markers are potentially useful in genetic relationship studies in tea since the RAPD analysis segregated 12 tea varieties into three clusters *viz.* China, Assam and Cambod type, which confirm the phenotypic classification of Wight (1962). This method of grouping also supports the findings of Paul *et al*. (1997) where AFLP markers were used to investigate Indian and Kenyan tea varieties. This clustering pattern is consistent with the existing knowledge on the tea classification studies (Wight 1962) and studies related to morphology and systematics of tea cultivars (Mohanan and Sharma 1981). Paul *et al*. (1997) separated Indian and Kenyan teas into three major groups (China, Assam and Cambod type) in their genetic diversity and genetic differentiation studies analysed using AFLP markers. In their studies clone 'UPASI-3' was classified as Assam type, which is also supported by our findings. A similar clustering pattern was noticed using SSR markers (Mondal 2002). Our results also show that cvs. 'UPASI-9' and 'UPASI-10' were recognised as China tea. Chloroplast microsatellites primers segregated tea varieties at the intra-specific level and nuclear microsatellites primers classified vars. *assamica* and *sinensis* into two groups, thus demonstrating the value of molecular markers in establishing the genetic relationship between tea varieties (Kaundun and Matsumoto 2002). Such clustering and segregation are essential for germplasm analysis (Dhanraj *et al*. 2002) and understanding the genetic organization of populations is important for the development of strategies designed to preserve genetic variation (Hamrick *et al*. 1991). This helps to maintain the genetic uniformity among hybrid cultivars and is essential to select desirable plants for breeding.

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