International Journal of Plant Breeding ©2009 Global Science Books

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

Mani Ramakrishnan[1*] • Lingaiah Rajanna[2] • Narayanaswamy Papanna[3] • Luke Simon[3,4]

[1] Department of Biotechnology, CMR Institute of Management Studies, 6th 'A' Main, HRBR Lay out, 2nd Block, Kalyan Nagar, Bangalore – 560043, India
[2] Department of Botany, Bangalore University, Jnana Bharathi Campus, Bangalore - 560056, India
[3] Plant Molecular Biology Laboratory, Division of Horticulture, University of Agricultural Sciences, G.K.V.K, Bangalore-560065, India
[4] Current address: School of Medicine, Dentistry and Biomedical Sciences, Institute of Clinical Sciences, Queens University Belfast, Belfast, BT12 6BJ, United Kingdom

Corresponding author: * maniramiyer@yahoo.com

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). [illegible] 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 [illegible] identification (Balasa- [illegible] analysis (Jung et al. 1995; 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

Received: 29 March, 2009. Accepted: 30 June, 2009.

Original Research Paper

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% □-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 □g template DNA, 150 □M 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

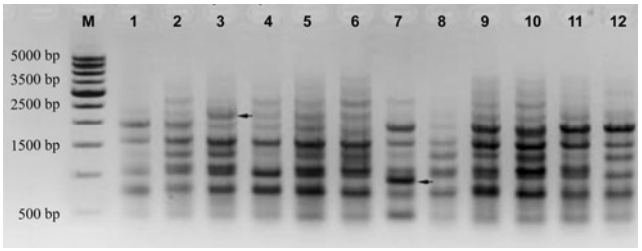

Fig. 1 Gel profile of tea varieties according to RAPD-PCR primer OPC-07. Lanes 1-12 contain the amplification profile of tea varieties 'SA-6', 'UPASI-9', 'UPASI-2', 'UPASI-3', 'CR-6017', 'UPASI-17', 'Assam Seedling', 'TRF-2', 'UPASI-10', 'TRI-2025', 'BSS-1' and 'TRF-1'. Lane M: 500 bp standard DNA marker.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

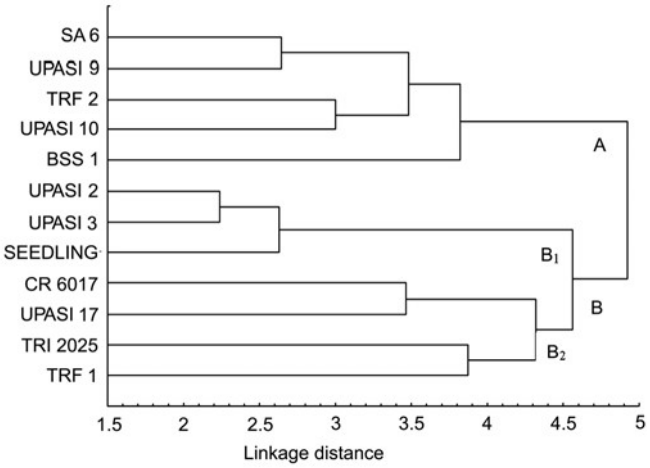

Fig. 2 Cluster analysis. Dendrogram showing RAPD-marker based genetic relationships of twelve tea varieties into three clusters 'A', 'B_1' and 'B_2' segregated as China type, Assam type and Cambod type, respectively.

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.

ACKNOWLEDGEMENTS

The authors are thankful to UPASI-Tea Research Institute, Valparai, Tamilnadu, India for providing the plant material, University of Agricultural Sciences, GKVK, Bangalore, India for providing lab facilities and Dr. A. K. Mandal, UPASI-TRI, Valparai, Tamil Nadu, India for his support throughout the study.

REFERENCES

Ariyarathna C, Gunasekare K (2006) Genetic base of tea (Camellia sinensis L.) cultivars in Sri Lanka as revealed by pedigree analysis. Journal of Applied Genetics 48 (2), 125-128

Babu S (2007) A new high quality clone TRF-2. Planters' Chronicle 103, 6-7

Balasaravanan T, Pius PK, Kumar RR (2001) Parentage of TRF 1 revealed by using RAPD markers. Newsletter of UPASI Tea Research Foundation 11, 1

Balasaravanan T, Pius PK, Kumar RR, Muraleedharan N, Shasany K (2003) Genetic diversity among south Indian tea germplasm (Camellia sinensis, C. assamica and C. assamica spp. sub species lasiocalyx) using AFLP markers. Plant Science 165, 365-372

Balasubramanian S, Satyanarayana N, Sasidhar R, Radhakrishnan B, Sibi M, Marimuthu S, Senthilkumar RS (2001) A new high yielding clone, TRF-1. Bulletin of the UPASI Tea Research Foundation 54, 57-72

Banerjee B (1992) Tea: cultivation to consumption. In: Wilson KC, Clifford

MN (Eds) Botanical Classification of Tea, Chapman and Hall, London, pp 25-51

Cannel MGR, Njuguna CK, Ford ED (1977) Variation in yield among competing individuals within mixed genotype stands of tea: a selection problem. Journal of Applied Ecology 14, 969-985

Chen J, Wang PS, Xia YM, Xu M, Pei SJ (2005) Genetic diversity and differentiation of Camellia sinensis L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemistry and allozyme studies. Genetic Research and Crop Evolution 52, 41-52

Chen L, Yamaguchi S (2002) Genetic diversity and phylogeny of tea plant (Camellia sinensis) and its related species and varieties in the section Thea genus Camellia determined by randomly amplified polymorphic DNA analysis. Journal of Horticultural Science and Biotechnology 77, 729-732

Chen L, Yamaguchi S (2005) RAPD markers for discriminating tea germplasms on the inter-specific level in China. Plant Breeding 124, 404-409

Chen L, Yang YJ, Yu FL, Gao QK, Chen DM (1998) A study on genetic diversity of 15 tea cultivars (Camellia sinensis (L.) O. Kuntze) using RAPD markers. Journal of Tea Science 18, 21-27

Chen L, Yao M-Z, Yang Y-J, Yu F-L (2006a) Collection, conservation, evaluation and utilization of tea genetic resources (Camellia spp.) in China. In: Teixeira da Silva JA (Ed) Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues (1st Edn, Vol I), Global Science Books, Isleworth, UK, pp 578-582

Chen L, Yao M-Z, Zhao L-P, Wang X-C (2006b) Recent research progresses on molecular biology of tea plant (Camellia sinensis). In: Teixeira da Silva JA (Ed) Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues (1st Edn, Vol IV), Global Science Books, Isleworth, UK, pp 426-437

Chen L, Yu FL, Tong QQ (2000) Discussions on phylogenetic classification and evolution of Section Thea. Journal of Tea Science 20, 89-94

Chen L, Zhou ZX, Yang YJ (2007) Genetic improvement and breeding of tea plant (Camellia sinensis) in China: from individual selection to hybridization and molecular breeding. Euphytica 154, 239-248

Dhanraj AL, Rao EVVB, Swamy KRM, Bhat MG, Prasad DT, Suresh NS (2002) Using RAPDs to assess the diversity in Indian cashew (Anacardium occidentale L.) germplasm. Journal of Horticultural Science and Biotechnology 77, 729-732

Duan HX, Shao WF, Wang PS (2004) Study on the genetic diversity of peculiar tea germplasm resource in Yunnan by RAPD. Journal of Yunnan Agricultural University 19, 246-254

Freeman S, West J, James C, Lea V, Mayes S (2004) Isolation and characterization of highly polymorphic microsatellites in tea (Camellia sinensis). Molecular Ecology Notes 4, 324-326

Gul S, Ahmad H, Khan IA, Alam M (2007) Assessment of genetic diversity in tea genotypes through RAPD primers. Pakistan Journal of Biological Sciences 10, 2609-2611

Gupta PK, Balyan HS, Sharma PC, Ramesh B (1996) Microsatellites in plants: A new class of molecular markers. Current Science 70 (1), 45-54

Hamrick JL, Godt MJ, Murawski DA, Loveless MD (1991) Correlations between species traits and allozyme diversity: Implications for conservation biology. In: Falk DA, Holsinger KF (Eds) Genetics and Conservation of Rare Plants, Oxford University Press, New York, pp 75-86

Jung C, Pillen K, Frese S, Fahr S, Melchinger AE (1993) Phylogenetic relationships between cultivated tea and wild species of the genus Beta revealed by DNA fingerprinting. Theoretical and Applied Genetics 86, 449-457

Karthigeyan S, Rajkumar S, Sharma RK, Gulati A, Sud RK, Ahuja PS (2008) High level of genetic diversity among the selected accessions of tea (Camellia sinensis) from abandoned tea gardens in western Himalaya. Biochemical Genetics 46, 810-819

Kaundun SS, Matsumoto S (2002) Heterologous nuclear and chloroplast microsatellite amplification and variation in tea, Camellia sinensis. Genome 45, 1041-1048

Kaundun SS, Matsumoto S (2003) Identification of processed Japanese green tea based on polymorphisms generated by STS-RFLP analysis. Journal of Agricultural and Food Chemistry 51, 1765-1770

Kaundun SS, Park YG (2002) Genetic structure of six Korean tea populations as revealed by RAPD-PCR markers. Crop Science 42, 594-601

Kaundun SS, Zhyvoloup A, Park YG (2000) Evaluation of the genetic diversity among elite tea (Camellia sinensis var. sinensis) accessions using RAPD markers. Euphytica 115, 7-16

Khanuja SPS, Shasany AK, Daroker MP, Kumar S (1998) DNA fingerprinting of plant genetic resources: the need of time. Journal of Medicinal and Aromatic Plant Sciences 20, 348-351

Lewis A, Caroniti M, Morvillo N (2004) Investigating the identity of rose varieties utilizing random amplified polymorphic DNA (RAPD) analysis. Proceedings of the Florida State Horticultural Society 117, 312-316

Li J, Jiang CJ, Wang ZX (2005) RAPD analysis on genetic diversity of the preconcentrated core germplasms of Camellia sinensis in China. Hereditas (Beijing) 27, 765-771

Luo JW, Shi ZP, Shen CW, Liu CL, Gong ZH, Huang YH (2004) The genetic diversity of tea germplasms [Camellia sinensis (L.) O. Kuntze] by RAPD analysis. Acta Agronomica Sinica 30, 266-269

Matsumoto S (2006) Studies on differentiation of Japanese tea cultivars (Camellia sinensis var. sinensis) according to the genetic diversity of phenylalanine ammonia-lyase. Yasai Chagyo Kenkyujo Kenkyu Hokoku 5, 63-111

Matsumoto S, Kiriiwa Y, Takeda Y (2002) Differentiation of Japanese green tea cultivars as revealed by RFLP analysis of phenylalanine ammonia-lyase DNA. Theoretical and Applied Genetics 104, 998-1002

Matsumoto S, Kiriiwa Y, Yamaguchi S (2004) The Korean tea plant (Camellia sinensis): RFLP analysis of genetic diversity and relationship to Japanese tea. Breeding Science 54, 231-237

Mohanan M, Sharma VS (1981) Morphology and systematics of some tea (Camellia species) cultivars. Proceedings of Annual Symposium on Plantation Crops IV, Central Coffee Research Institute, Chikmagalur, India, pp 391-400

Mondal TK (2002) Assessment of genetic diversity of tea (Camellia sinensis (L.) O. Kuntze) by inter-simple sequence repeat polymerase chain reaction. Euphytica 128, 307-315

Nayak S, Rout GR, Das P (2003) Evaluation of the genetic variability in bamboo using RAPD markers. Plant, Soil and Environment 49 (1), 24-28

Nei M, Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonuclease. Proceedings of the National Academy of Sciences USA 76, 5296-5273

Ni S, Yao M, Chen L, Zhao L, Wang X (2008) Germplasm and breeding research of tea plant based on DNA marker approaches. Frontiers of Agriculture in China 2, 200-207

Park YG, Kaundun SS, Zhyvoloup A (2002) Use of the bulked genomic DNA-based RAPD methodology to assess the genetic diversity among abandoned Korean tea plantations. Genetic Resources and Crop Evolution 49, 159-165

Paul S, Wachira FN, Powell W, Waugh R (1997) Diversity and genetic differentiation among populations of Indian and Kenyan tea (Camellia sinensis L.) revealed by AFLP markers. Theoretical and Applied Genetics 94, 255-263

Provost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics 98, 107-112

Rajesh MK, Nagarajan P, Jerard BA, Arunachalam V, Dhanapal R (2008) Microsatellite variability of coconut accessions (Cocos nucifera L.) from Andaman and Nicobar Islands. Current Science 94, 1627-1631

Rao NM, Soneji JR, Chen C, Huang S, Gmitter FG (2008) Characterization of zygotic and nucellar seedlings from sour orange-like citrus rootstock candidates using RAPD and EST-SSR markers. Tree Genetics and Genomes 4, 113-124

Sambrook J, Fristch EF, Maniatis T (1989) Molecular Cloning. A laboratory Manual (2nd Edn), Cold Spring Harbor Laboratory Press, NY, 1659 pp

Saravanan M, John KMM, Kumar RR, Pius PK, Sasikumar R (2005) Genetic diversity of UPASI tea (Camellia sinensis L.) clones on the basis of total catechins and their fractions. Phytochemistry 66, 561-565

Satyanarayana N, Sharma VS (1993) UPASI biclonal seed stocks, Proceedings of the Sixth Joint Area Scientific Symposium (JASS VI), UPASI Tea Research Institute Bulletin 46, 144-154

Shen C-W, Huang Y-H, Huang J-A, Luo J-W, Liu C-L, Liu D-H (2008) RAPD analysis on genetic diversity of typical tea populations in Hunan Province. Chinese Journal of Agricultural Biotechnology 5, 67-72

Simon L, Shyamalamma S, Narayanaswamy P (2007) Morphological and molecular analysis of genetic diversity in jackfruit. Journal of Horticultural Science and Biotechnology 82, 764-768

Singh D, Ahuja PS (2006) 5S rDNA gene diversity in tea (Camellia sinensis (L.) O. Kuntze) and its use for variety identification. Genome 49, 91-96

Sokal RR, Sneath PHA (1973) Principles of Numerical Taxonomy, W. H. Freeman and Co., San Francisco, CA, USA, 359 pp

Venkataramani KS, Sharma VS (1974) The tea clone: 'Sundaram'. Planters' Chronicle 69, 353-355

Vos P, Hogers R, Bleeker M, Reijians M, Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP, a new technique for DNA fingerprinting. Nucleic Acid Research 23, 4407-4414

Wachira FN, Powell W, Waugh R (1997) An assessment of genetic diversity among Camellia sinensis L. (cultivated tea) and its wild relatives based on randomly amplified polymorphic DNA and organelle-specific STS. Heredity 78, 603-611

Wachira FN, Tanaka J, Takeda Y (2001) Genetic variation and differentiation in tea (Camellia sinensis) germplasm revealed by RAPD and AFLP variation. Journal of Horticultural Science and Biotechnology 76, 557-563

Wachira FN, Waugh R, Hackett CA, Powell W (1995) Detection of genetic diversity in tea (Camellia sinensis) using RAPD markers. Genome 38, 201-210

Ward JH (1963) Hierarchic grouping to optimize an objective function. Journal of the American Statistical Association 58, 236-239

Wight W (1962) Tea classification revised. Current Science 31, 298-299

Williams JG, Kubelki AR, Livak KJ, Rafaleski JA, Tingy SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18, 6531-6535

Wood DJ, Barua DN (1958) Species hybrids of tea. Nature 181, 1674-1675

Yao MZ, Chen L, Liang YR (2008) Genetic diversity among tea cultivars from China, Japan and Kenya revealed by ISSR markers and its implication for parental selection in tea breeding programmes. Plant Breeding 127, 166-172