International Journal of the Physical Sciences Vol. 6(5), pp. 727-743, 9 February, 2011
Available online at http://www.academicjournals.org/IJPS
DOI: 10.5897/JMPR11.1243
ISSN 1996-0875©2011 Academic Journals

Full Length Research Paper

Identification of superior varieties of tea (*Camellia sinensis* (l.) o. kuntze) in the selected UPASI germplasm using biomarkers

S. Ramkumar[1], P. Sureshkumar[2]*, A. K. A. Mandal[3], K. Rajaram[2] and P. Mohankumar[1]

[1]Plant Physiology and Biotechnology Division, UPASI TRF, UPASI Tea, Research Institute, Nirar Dam BPO, Valparai 642 127, Coimbatore District, Tamilnadu, India.
[2]Department of Biotechnology, Anna University of Technology, Tamil nadu, Triuchirappalli- 620 024, India.
[3]School of Bioscience and Technology, Vellore Institute of Technology, Vellore, Tamilnadu, India.

Accepted 8 November, 2011

Biomarkers are used as a vital tool in cultivar improvement programme for woody perennial tree crops such as (*Camellia sinensis* (L.) O. Kuntze). Commercially important fifteen accessions were selected and investigated for total polyphenol oxidase (PPO) activity (U/mg of protein), based on the PPO activity range, the accessions were further separated into three groups viz; high (P/11/10, UPASI-16, UPASI-18, UPASI-14, I/30/17), moderate (UPASI-3, UPASI-17, UPASI-13, UPASI-1, UPASI-21) and low (I/30/9, p/11/15, I/30/30,MGL-16, MGL- 8) PPO activity. To study the pattern of genetic diversity, random amplified polymorphic DNA (RAPD) analysis was performed using twenty decamer primers. The RAPD amplification results revealed that genetic similarity (GS) among the accessions tested ranged 0.64 to 0.918 with an average of 0.28%. RAPD dendogram showed three distinct clusters of high, moderate and low using un-weighed pair-group method for arithmetic averages analysis (UPGMA) method. This genetic diversity studies on tea showed effectively for the initial assessment of partitioning the intra specific level of genetic variation correlated to the total PPO enzyme activity.

Key words: *Camellia sinensis*, random amplified polymorphic DNA (RAPD), polyphenol oxidase, genetic diversity.

INTRODUCTION

Tea (*Camellia sinensis* (L) O. Kuntze), beverage is made from tender leaves of the tea plant. India is the largest producer of tea (an important economic crop). Polyphenol oxidase (PPO) is an important enzyme in tea plants, especially for tea quality. High PPO activity is necessary for the enzymatic oxidation during process of the black tea manufacture and it should be deactivated promptly in the green tea making process. Polyphenol oxidase (EC1. 10.3.2) is also known as phenol oxidase, tyrosinase, o-diphenol oxidase, catechol oxidase, phenolase, and chlorogenic acid oxidase. These enzymes in higher plants oxidize a great variety of monophenolic and o-diphenol compounds and catalyze two types of reactions (Yelena et al., 1996). First reaction involves the hydroxylation of a monophenol to give a diphenol and second involves the removal of hydrogen's from diphenol to give quinone (Robertson and bendall, 1983). During the enzymatic oxidation PPO enzyme leads to catechin for production of black tea pigments, viz; theflavins (TF) and thearubigis (TR). This polyphenol oxidase enzyme provides an important role in plant metabolism; provide some defense against predators by their astringency. This enzyme mostly found in higher plants like apple, peach, mushroom tobacco, coffee and tea (whitaker,

*Corresponding author. E-mail: sureshbiotech2003@yahoo.co.in.

Abbreviations: GS, Genetic similarity; **PPO,** polyphenol oxidase; **RAPD,** random amplified polymorphic DNA; **UPASI,** United Planters Association of South India; **UPGMA,** un-weighed pair-group method for arithmetic averages analysis.

Table 1. Basic information regarding the tea origin and descriptive characters of prominent south Indian tea cultivars used in the study.

S/N	Clone	Varital type	Source of the material
1	P/11/10	Assam	Paraley estate selection, valparai
2	UPASI-16	Assam (B/6/182)	Brooklands estate, the nilgiris.
3	UPASI- 18	Cambod (B/6/57)	Brooklands estate, the nilgiris.
4	UPASI -14	Cambod S/6/99 (Singara)	Singara estate, the nilgiris.
5	I/30/17	Nil	Iyerpadai estate selection, valparai
6	UPASI-3	Assam B/5/63 (Sundaram)	Brooklands estate, the nilgiris.
7	UPASI-17	Cambod B/6/203 (Swarna)	Brooklands estate, the nilgiris.
8	UPASI-13	Assam (B/6/137)	Brooklands estate, the nilgiris.
9	UPASI-21	Assam (B/4/198)	Brooklands estate, the nilgiris
10	UPASI-1	Assam (B/4/141 (Ever green))	Brooklands estate, the nilgiris.
11	I/30/9	Assam	Iyerpadai estate selection, valparai
12	P/11/15	Assam	Paraley estate selection, valparai
13	I/30/30	Assam	Iyerpadai estate selection, valparai
14	MGL – 8	China	Murugaley estate selection, valparai
15	MGL - 16	China	Murugaley estate selection, valparai

1994). Genetic diversity assessments at inter and intra-species levels are important basis for collection, conservation, evaluation, and utilization of tea germplasm. Some traditional methods, such as morpho-logical characteristics, chemical components, esterase, isozymes, and karyotype, have been employed to describe the phylogenetic relationships among tea plants. Molecular markers are valuable tools in the characterization and evaluation of genetic diversity within and between species and populations.

In tea different molecular markers have been used by different workers to study the genetic diversity. In a pre-liminary study, Tanuka et al. (1995) attempted to detect variations among Korean, Japanese, Chinese, Indian and Vietnamese tea using 10-mer and 12-mer primers. They concluded that China, Korean tea has undergone some genetic diversification and Japanese tea showed a close similarity with the Chinese tea. Recently, random amplified polymorphic DNA (RAPD) have also been used for the investigation of genetic relationship (Wacheria et al., 1997), identification of parentage (Tanaka et al., 2001), genetic diversity (Kaundun et al., 2000; Mondal, 2000; Chen et al., 2005) and genetic mapping (Hackett et al., 2000) of tea plants (*C. Sinensis*). Genetic variability of *in vitro* raised tea plants were investigated by Mondal and Chand (2002), who reported that while both Assam and China have specific band, Japanese tea are more closer to Chinese tea than others and same of the tea varieties from Vietnam are the hybrids of Assam and China. Lai et al. (2001) have studied the genetic relationship of 37 tea samples that comprised 21 clones of china, 3 clones of Assam, and 6 individual samples of native Taiwanese wild tea by using RAPD and ISSR markers. The evaluation of genetic diversity in tea is a prerequisite for screening superior variety of tea. Hence, the present work investigates to identify the superior varieties of tea among the 15 commercially available tea accessions (UPASI germplasm) by using biomarkers like biochemical (total PPO activity) and molecular markers (RAPD).

MATERIALS AND METHODS

Plant material

The 15 tea accessions (UPASI germplasm) were collected from Valparai (UPASI) and the Nilgiris estates for the present study (Table 1).

Preparation of crude polyphenol oxidase (PPO) enzyme

The soluble and bound component of the PPO crude enzyme was extracted using acetone from 25 g of crop shoots by homogenizing the tissue of the shoots using chilled acetone (–20°C) with acid washed sand powder using a pre chilled pestle and mortar. The homogenate was filtered through Whatmann No.1 filter paper. The retentate was washed free of phenolics by passing through chilled acetone then with cold aqueous acetone (80:20 acetone/water, v/v) and finally washed with acetone. The previous homogenate slurry was filtered through Whatmann No.1 paper filter. The powder was then stored in evacuated desiccators to allow complete drying. The dried white powder called as an acetone powder, is used for preparation of the enzyme extracts. All the enzyme preparation and its activity were done at 25°C.

Enzyme preparation

The soluble component of the enzymes was extracted with 5 g

acetone powder by gentle grinding in a pestle and mortar with distilled water (1:10 w/v) and incubated 10 min followed by centrifugation at 4000 rpm for 10 min. The supernatant obtained was soluble enzyme. Subsequently, the residue was extracted by regrinding with 5 mL of 0.2 M sodium sulphate (Na_2SO_4) solution and incubated at 30 min followed by centrifugation for 10 min at 10000 g. The supernatant obtained was ionically bound enzyme. PPO activity was calculated using formula according to previously published protocol by Singh and Ravindranath et al. (1990).

Polyphenol oxidase (PPO) enzyme assay

Polyphenol oxidase activity was determined using UV-Spectrophotometer (Ultra spec 2100 pro, GE Healthcare Biosciences Ltd). The principle is based on the initial increase in the rate of the absorbance at 380 nm. Firstly, Mix 1.5 ml of 0.1M sodium phosphate buffer (pH 5.6) with 100 µl of 10 mM of catechin as substrate in a 3 ml cuvette, then extracted 100 µl of crude enzyme was added in 1.0 cm path length cuvette and the absorbance was recorded immediately 30 s at 3 min interval at 25°C. The instrument was tarred using the same mixture without an enzyme. Triplicate measurement was recorded for each assay. One unit of PPO enzyme activity was defined as that amount which caused a rate of change of 0.001 absorption unit's min^{-1} at 380 nm. Overall enzyme activity was expressed as U mg/protein.

Protein estimation

Protein content was determined according to the coomassie blue binding method of Bradford (1976).

Statistical analysis

All extractions and determinations were conducted 3 times at least. Data were expressed as means ± standard error of the mean of the mean of three independent experiments carried out in duplicate. A one way ANOVA with Duncan's test was employed to evaluate the significance of results. A probability (p) value <0.05 was considered significant (Gomez and Gomez, 1976).

Random amplified polymorphic DNA (RAPD) Analysis

DNA isolation from tea leaves

Young and fresh leaves were collected from the 15 accessions with similar age and uniformly pruned at 26" (about 60 cm) above ground level were selected and subjected for RAPD analysis. Leaves were washed thoroughly in sterile distilled water. Equal quantity (100 mg) of leaf tissue was weighed and used for DNA isolation. DNA was extracted from the young tea leaves using the CTAB method 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 ^{0}C for one hour. 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 mg/ml) for two hour at 37°C and purified using 500 ml of equilibrated phenol and 750 ml 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. Observed gel under in a UV light transilluminator.

List of primers used Polymerase chain reaction (PCR) amplification

Fifteen decamer random primers (from OPERON - Qiagen Company, U.S.A.) were used for all PCR reactions. List of primers used and their sequences are presented in Table 2.

Polymerase chain reaction (PCR) and gel electrophoresis

Polymerase chain reaction was carried out in peltier thermal cycler (PTC-200, MJ Research, Inc., and U.S.A) using ten decamer random primers (Table 2). Each 25 µl reaction mixture contained 1 unit of *Taq* DNA polymerase, 0.2 mM each d*NTPs,* 1X PCR buffer, 3 mM $MgCl_2$ (Bangalore Genei Pvt. Ltd., India), 10 pmole of primer (OPERON-Qiagen Company, U.S.A.) and approximately 50 ng of template genomic DNA. PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 45 cycles of denaturation at 94°C for 45 s, annealing at 36°C for 60 s and extension at 72°C for 120 s followed by final extension at 72°C for 10 min. The amplified products were separated on 2% agarose gel using 1X TBE buffer followed by staining in ethidium bromide solution (1 µg/ml) and documentation was carried out by placing of the stained gel on UV-Transilluminator. The reproducibility of the amplification products was checked thrice for each polymorphic primer. Bands were scored from photographs.

Scoring and analysis of data

Bands were scored as present (1) or absent (0) in all the samples. All the DNA samples were repeated at least twice and only reproducible bands were scored. Molecular weight of each band was estimated using 1 kb DNA ladder (Fermentas Life science, Germany) as a standard. Similarity coefficient matrix was constructed by calculating Jaccard's similarity coefficient values for each pair wise comparison between samples (Jaccard, 1908). A dendogram was generated (using average linkage procedure) from this matrix following un-weighed pair-group method for arithmetic averages analysis (UPGMA) method using NTSYS 2.1 (Rohlf, 2002).

RESULTS

Polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO) enzyme activity of fifteen accessions (UPASI germplasm), eight from UPASI released clones and seven accessions from Paraley, Iyerpadai and Murugaley estates selections were assayed. Activity of PPO as bound and soluble form and total activity has been shown in Table 3.

UPASI released clones showed higher PPO activity compared to selections from other estates (Paraley and Murugaley estate, Valparai). The fifteen accessions were divided into three groups (high, moderate and low) based on the total PPO (soluble and bound) enzyme activity, each groups having five clones each. The high group

Table 2. List of primers along with their sequence used in the present study.

S/N	Primer	Sequences5'
1	OPA 02	TGCCGAGCTG
2	OPA 03	AGTCAGCCAC
3	OPA 05	AGGGGTCTTG
4	OPA 17	GACCGCTTGT
5	OPB 03	CATCCCCCTG
6	OPB 07	GGTGACGCAG
7	OPB 10	CTGCTGGGAC
8	OPB 18	CCACAGCAGT
9	OPB 20	GGACCCTTAC
10	OPD 02	GGACCCAACC
11	OPD 04	TCTGGTGAGG
12	OPD 07	TTGGCACGGG
13	OPD 13	GGGGTGACGA
14	OPD 19	CTGGGGACTT
15	OPK 02	GTCTCCGCAA
16	OPK03	CCAGCTTAGG
17	OPK 07	AGCGAGCAAG
18	OPK 11	AATGCCCCAG
19	OPK 13	GGTTGTACCC
20	OPK 17	CCCAGCTGTG

(700 to 1300 U/mg of protein) viz; P/11/10, UPASI-16, UPASI-18, UPASI-14, I/30/17, moderate (300 to 500 U/mg of protein) viz; UPASI-1, 3, 13, 17 and 21, low (50 to 90 U/mg of protein) viz; MGL-8, 16, I/30/9, I/30/30 and P/11/15, respectively (Table 3).

Our results showed out of 15 accessions screened for total PPO activity, UPASI-18 (Cambod Type) showed that highest activity (1233.57 U/mg of protein) followed by UPASI - 16 (Assam type; 1151.29 U/mg of protein) and P/11/10 (Paraley estate; 1085.14 U/mg of protein).

Random amplified polymorphic DNA (RAPD) analysis

The amplification profiles of total genomic DNA of the fifteen accessions, which were grouped into high, moderate and low based on their PPO activity, were tested for genetic diversity studies. Out of the twenty primers tested only 14 provided good amplifications (Figures 2 to 7, 9, 10, 13, 14, 16 to 18 and 20) primers which detected good polymorphisms remaining six primers (Figures 8, 11, 12, 15, 19 and 21) had showed low reproducibility in all accessions. A total of six hundred and eighty one amplicons were obtained with 100 to 1500 bp in size and nearly 70% were monomorphic. The number of bands ranged 30 to 35 per primer.

Similarity coefficiency between the accessions was derived by Nei's correlation. The pair wise (Nei and Li, 1979) genetic distance co-efficient values for fifteen accessions ranged 0.64 to 0.918 indicating the diversity (Table 4). The UPGMA analysis of the scored data and the Jaccard's similarity co-efficient values were used for clustering to develop the dendogram. The cluster analysis indicated that fifteen different accessions belong to (*C. sinensis* (L.) O. Kuntze) formed two major clusters based on similarity index (Figure 1). First major clusters were further divided into two minor sub clusters. The first minor sub clusters contained five accessions that is, UPASI-18, UPASI-16 and UPASI-14 (similarity indics 0.86) grouped with P/11/10 and I/30/17. The second minor sub cluster contained five accessions, four accessions like UPASI-13 UPASI-17 UPASI-13 and UPASI-21 are grouped together with similarity index of 0.91 and this was grouped with UPASI -1.

The second minor cluster was divided into two minor sub cluster, the first contained I/30/9 and MGL-16 with similarity index of 0.81 grouped with second minor sub cluster. The second minor group contained three accessions, two of them I/30/30 and MGL-8 (with similarity index 0.85) grouped with P/11/15.

DISCUSSION

The genetic diversity among the germplasm resource has been demonstrated using morphological character was described by Sealy (1958), Chang (1984) and Ming (1992). Chen et al. (2000) reported that *Thea*

Table 3. Summary of results of tea PPO polyphenol oxidase.

Sample	Soluble PPO ([a]) (U/mg of protein)	Bound PPO ([a]) (U/mg of protein)	Total PPO ([a]) (U/mg of protein)	Groups (PPO activity range)
P/11/10	12.68 ± 2.68	1072.46 ± 3.68	1085.14 ± 1.37[c]	
UPASI -16	103.35 ± 4.59	1047.94 ± 5.97	1151.29 ± 3.41[b]	
UPASI - 18	190.82 ± 4.32	1042.75 ± 3.04	1233.57 ± 4.56[a]	High
UPASI - 14	84.97 ± 1.80	744.08 ± 5.43	829.05 ± 2.23[d]	
I/30/17	7.97 ± 0.93	781.18 ± 1.28	789.15 ± 2.02[e]	
UPASI - 3	12.78 ± 1.43	432.89 ± 3.81	445.68 ± 2.84[f]	
UPASI - 17	8.97 ± 0.04	369.51 ± 5.79	378.48 ± 4.0[ih]	
UPASI - 13	13.35 ± 0.55	387.24 ± 1.76	400.59 ± 1.76[h]	Medium
UPASI - 1	3.53 ± 0.83	391.30 ± 2.13	394.82 ± 1.03[i]	
UPASI - 21	113.38 ± 0.23	315.84 ± 0.54	434.99 ± 1.28[g]	
I/30/9	4.36 ± 1.21	79.76 ± 0.58	84.12 ± 0.57[jk]	
P/11/15	5.21 ± 0.80	38.75 ± 2.60	43.96 ± 3.14[l]	
1/30/30	8.86 ± 0.43	54.53 ± 1.07	63.39 ±1.03[k]	Low
MGL-16	1.67 ± 0.17	88.62 ± 2.18	90.29 ± 3.08[jk]	
MGL- 8	3.02 ± 0.22	88.04 ± 1.47	91.06 ± 1.35[j]	

Enzyme Specific activity ([a]) - One unit of enzyme defined as the amount which caused a rate of change of 0.001/OD units min^{-1}at 380nm using catechin as substrate. *Means followed by the same letter are not significantly different at P<0.005 by Duncan's test.

Table 4. Genetic similarity matrix of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) based on RAPD analysis.

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
A1	1.000														
A2	0.793	1													
A3	0.800	0.857	1												
A4	0.782	0.831	0.864	1											
A5	0.787	0.827	0.834	0.801	1										
A6	0.682	0.718	0.691	0.701	0.733	1									
A7	0.665	0.708	0.700	0.718	0.697	0.889	1								
A8	0.656	0.705	0.698	0.702	0.714	0.873	0.918	1							
A9	0.640	0.708	0.674	0.705	0.691	0.854	0.883	0.889	1						
A10	0.670	0.760	0.712	0.743	0.728	0.828	0.848	0.846	0.872	1					
A11	0.667	0.684	0.683	0.667	0.686	0.709	0.706	0.697	0.687	0.723	1				
A12	0.648	0.686	0.651	0.648	0.648	0.705	0.682	0.654	0.644	0.693	0.815	1			
A13	0.642	0.687	0.665	0.663	0.643	0.700	0.690	0.694	0.671	0.663	0.771	0.833	1		

Table 4. Contd.

A14	0.667	0.691	0.697	0.673	0.673	0.684	0.681	0.698	0.662	0.686	0.797	0.811	0.846	1	
A15	0.647	0.711	0.676	0.673	0.667	0.709	0.725	0.723	0.726	0.757	0.801	0.791	0.0.779	0.804	1

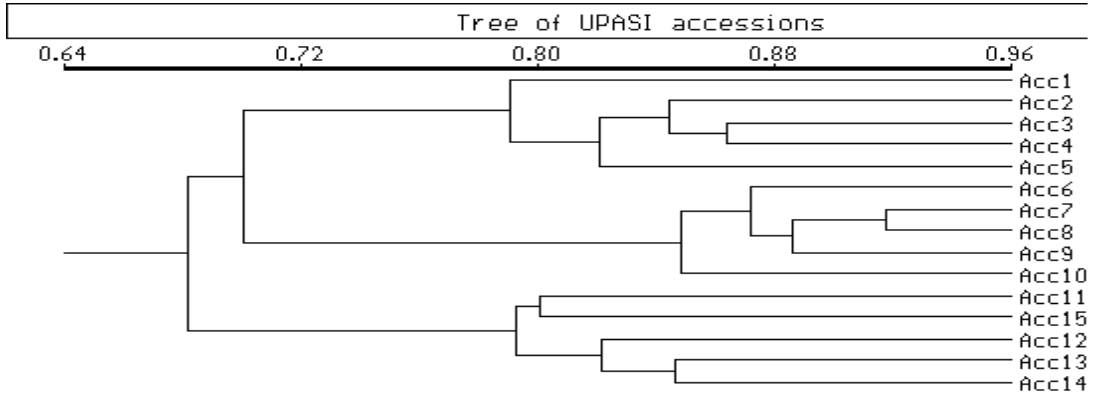

Figure 1. DNA based genetic relationship elaborated through the dendogram of 15 tea clones (*Camellia sinensis* (L.) O. Kuntze) constructed through bivariate data matrix generated using 20 RAPD primers.

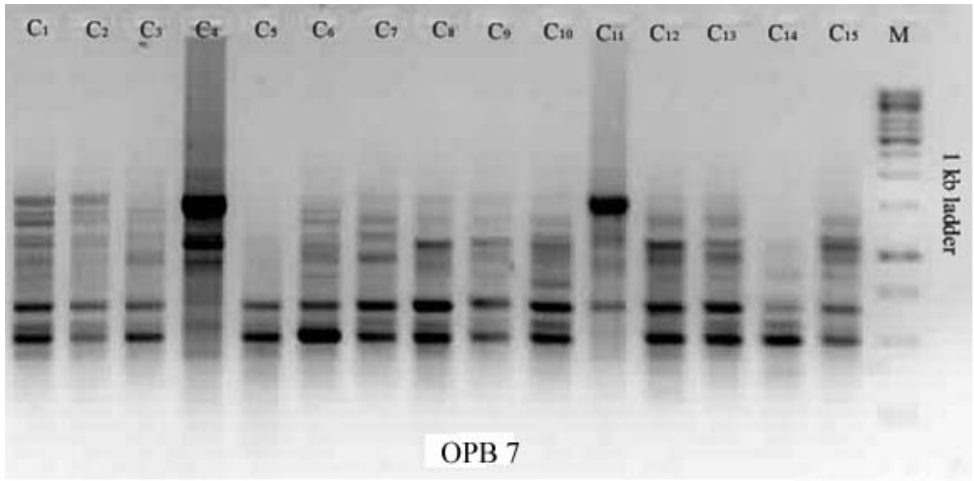

Figure 2. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPB7 primer.

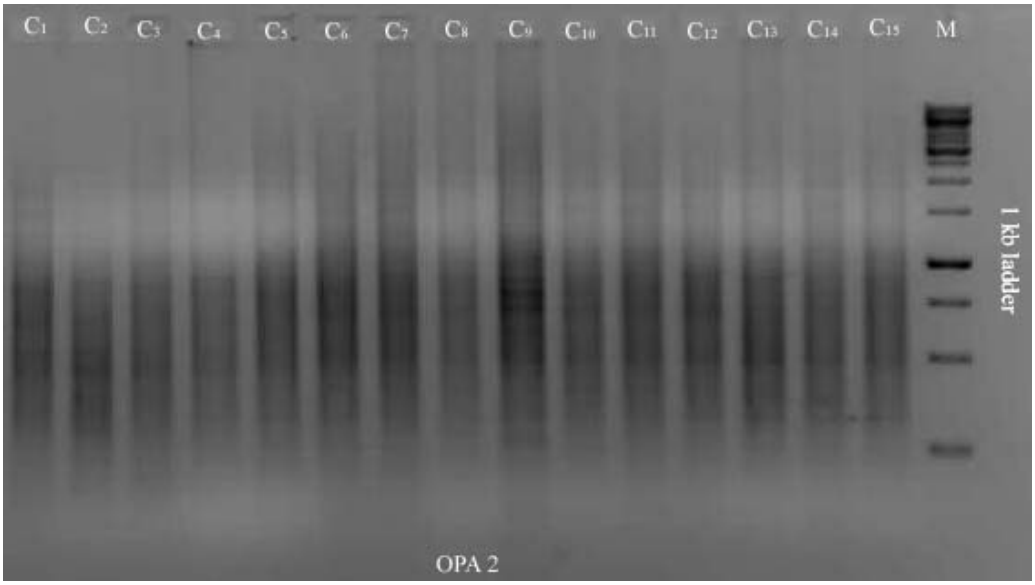

Figure 3. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPA 2 primer.

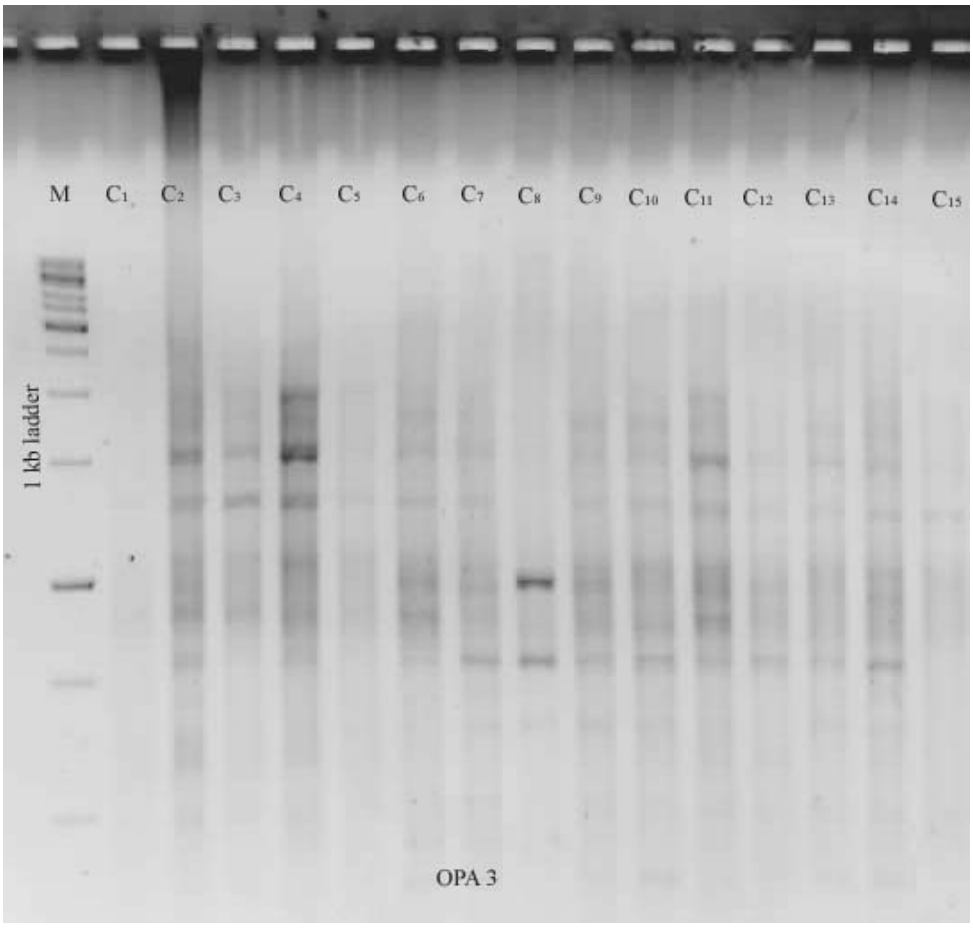

Figure 4. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPA 3 primer.

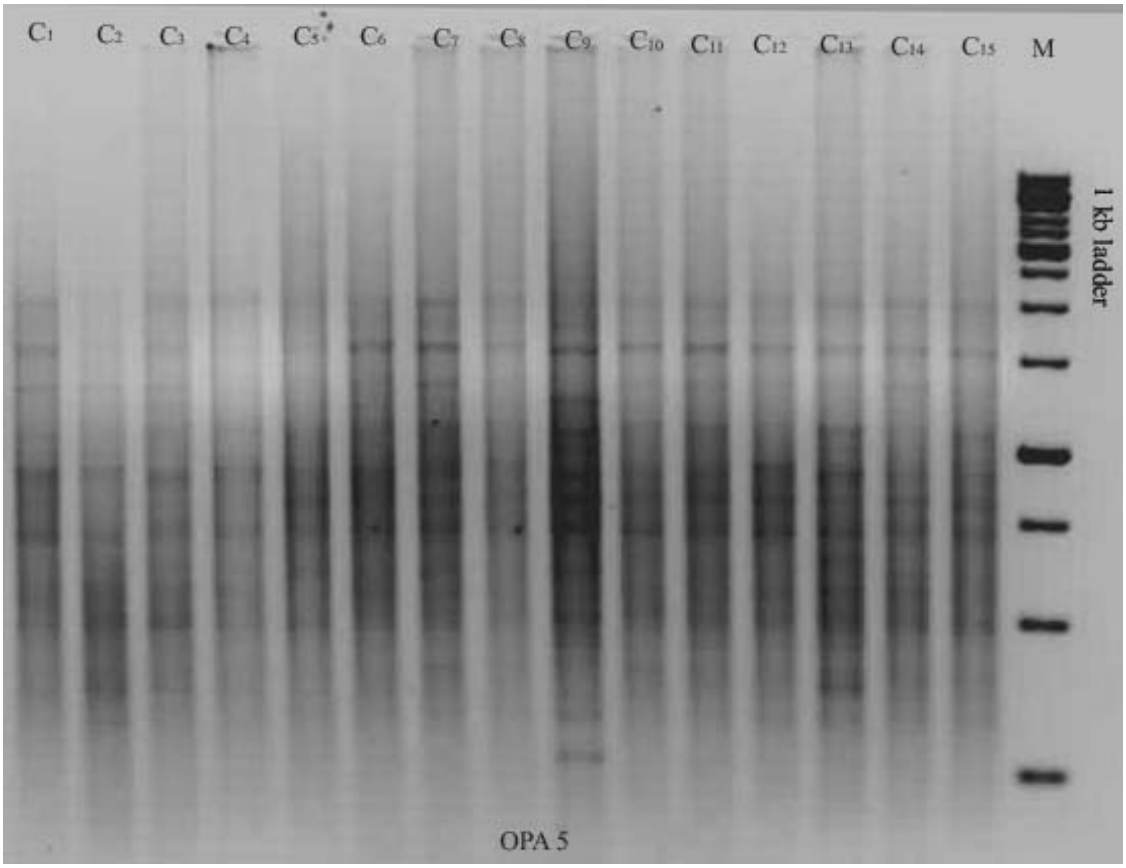

Figure 5. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPA 5 primer.

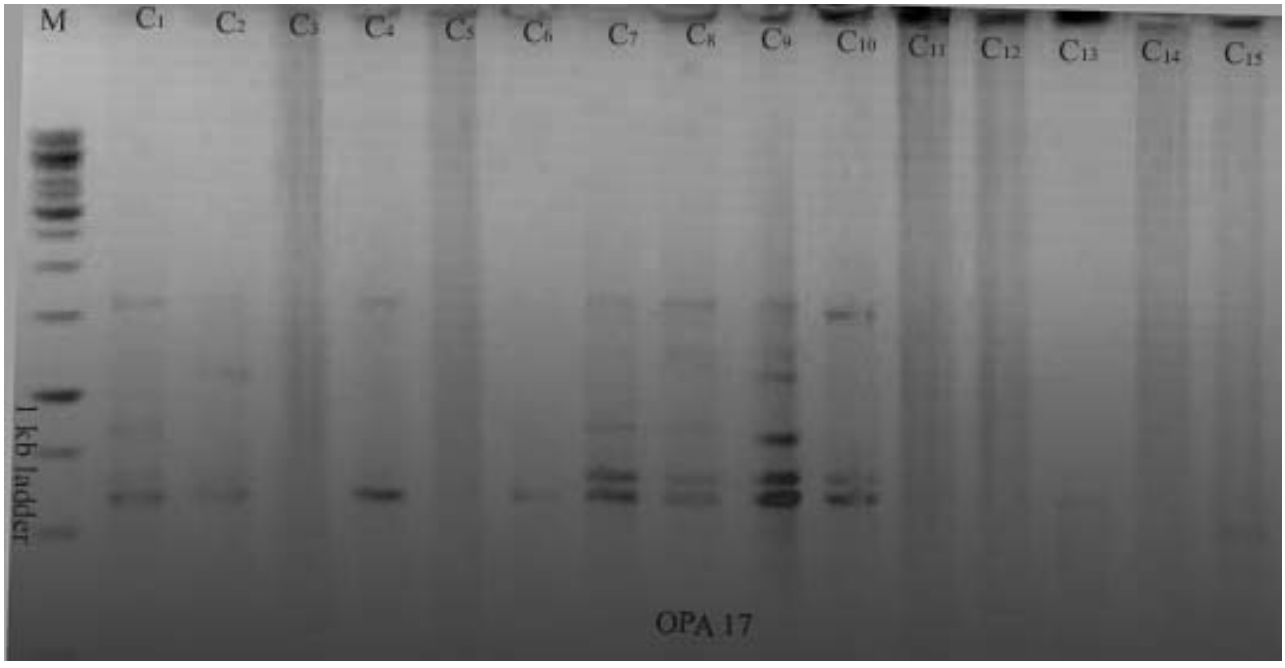

Figure 6. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPA 17 primer.

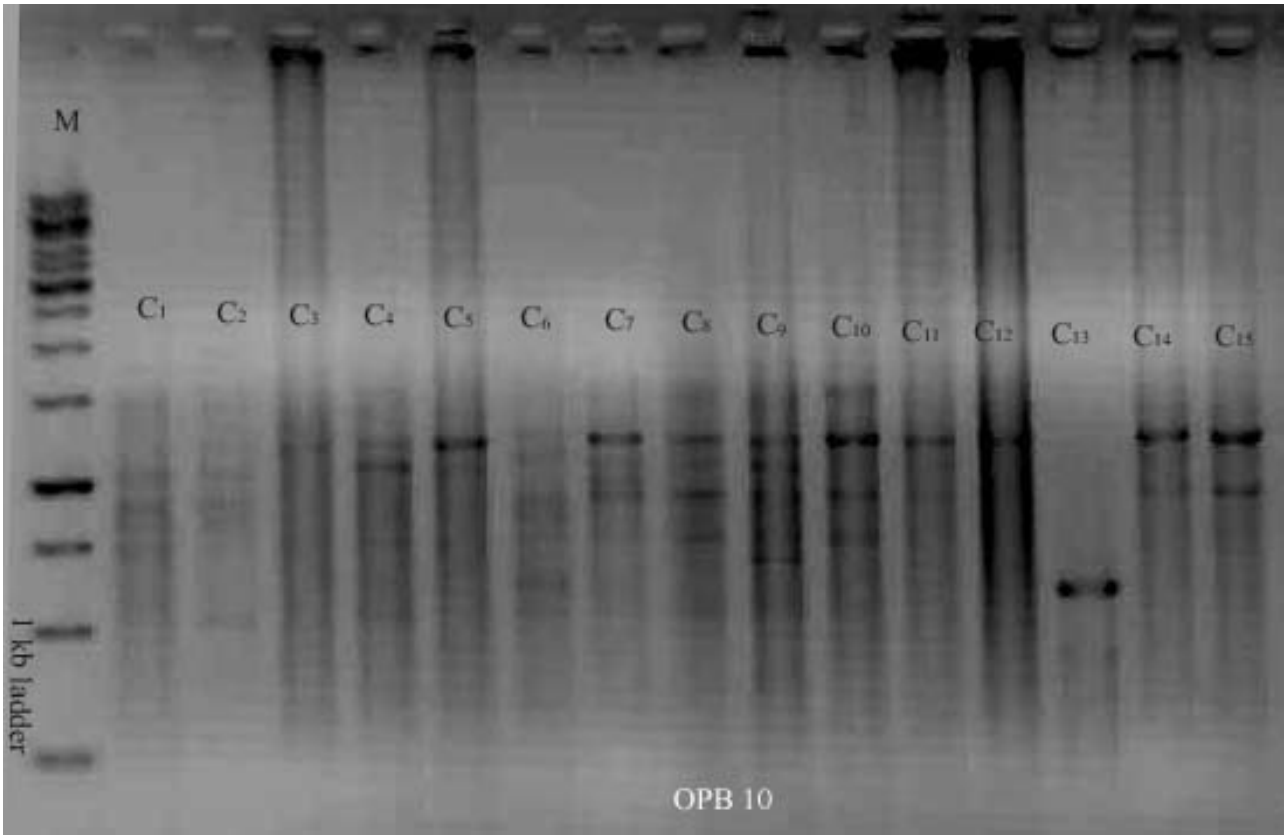

Figure 7. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPB10 primer.

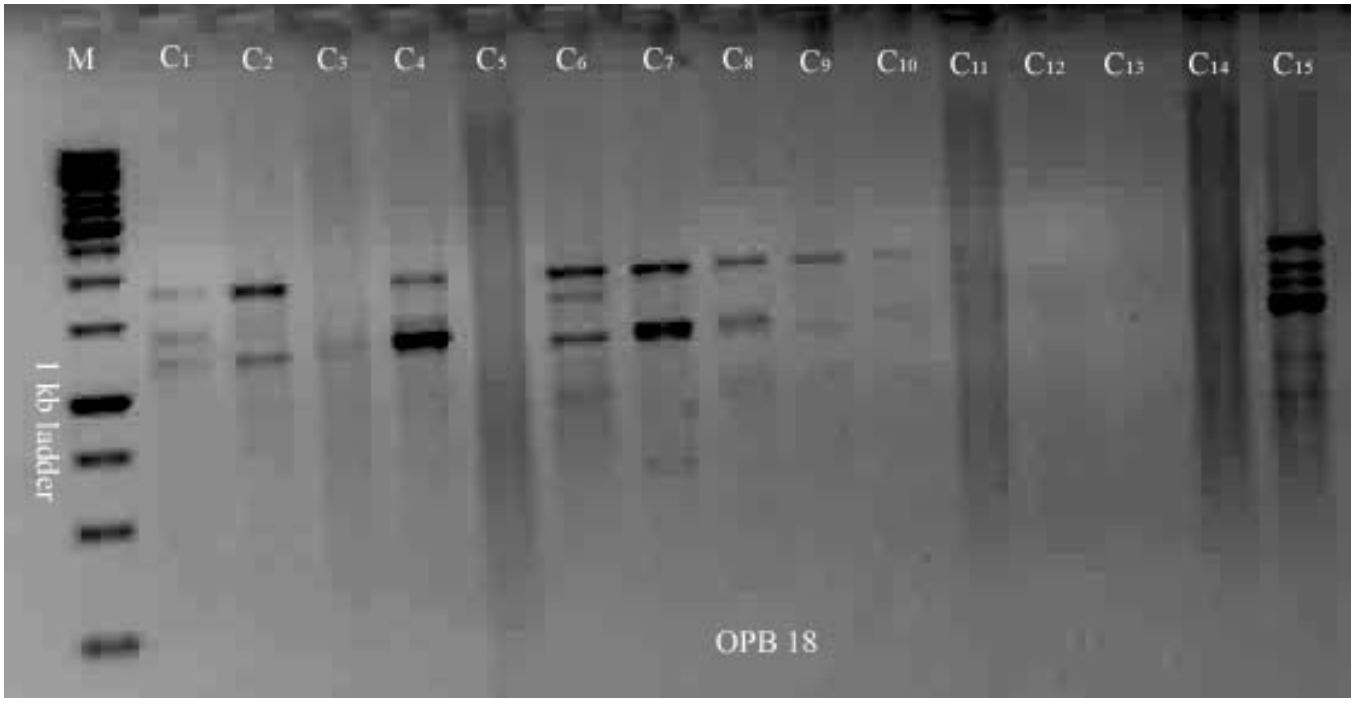

Figure 8. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPB18 primer.

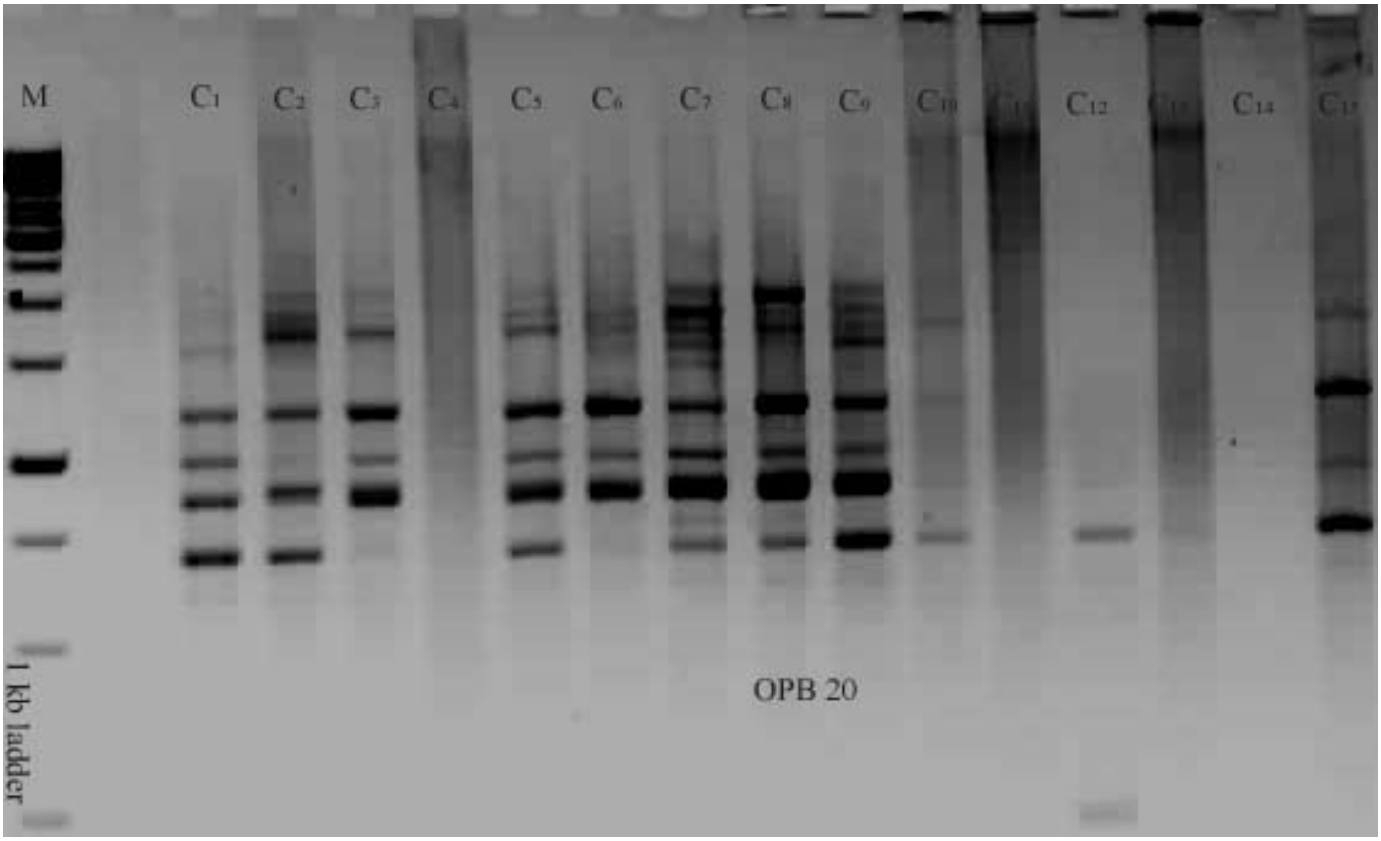

Figure 9. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPB 20 primer.

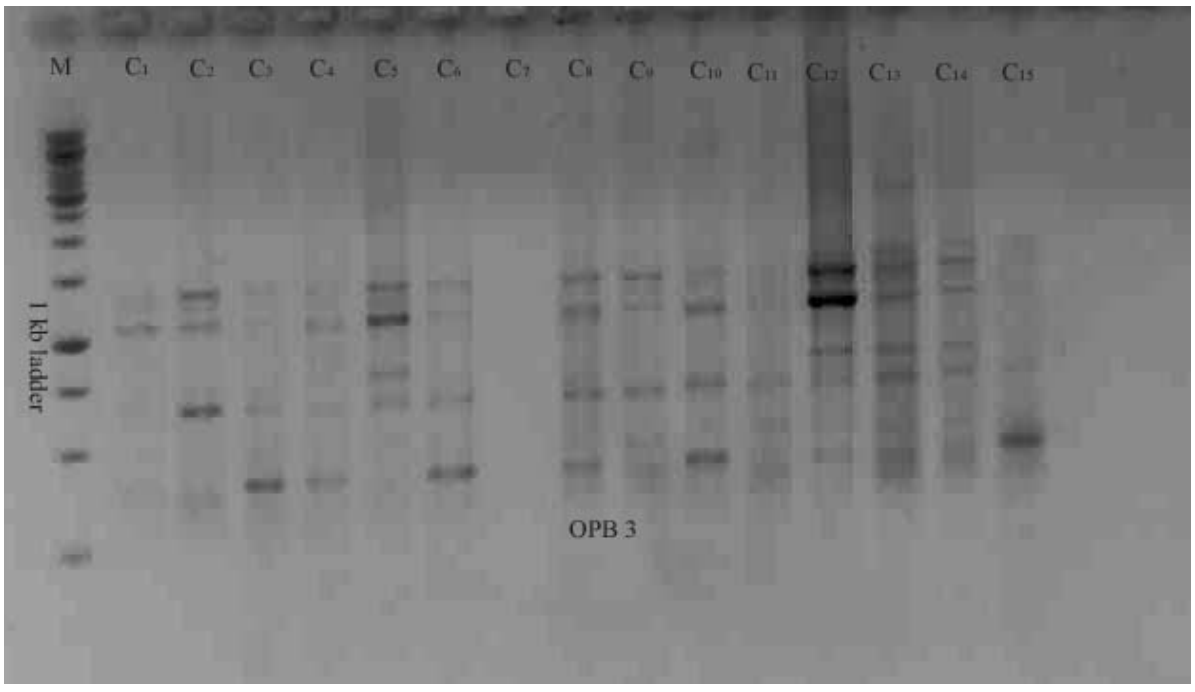

Figure 10. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPB3 primer.

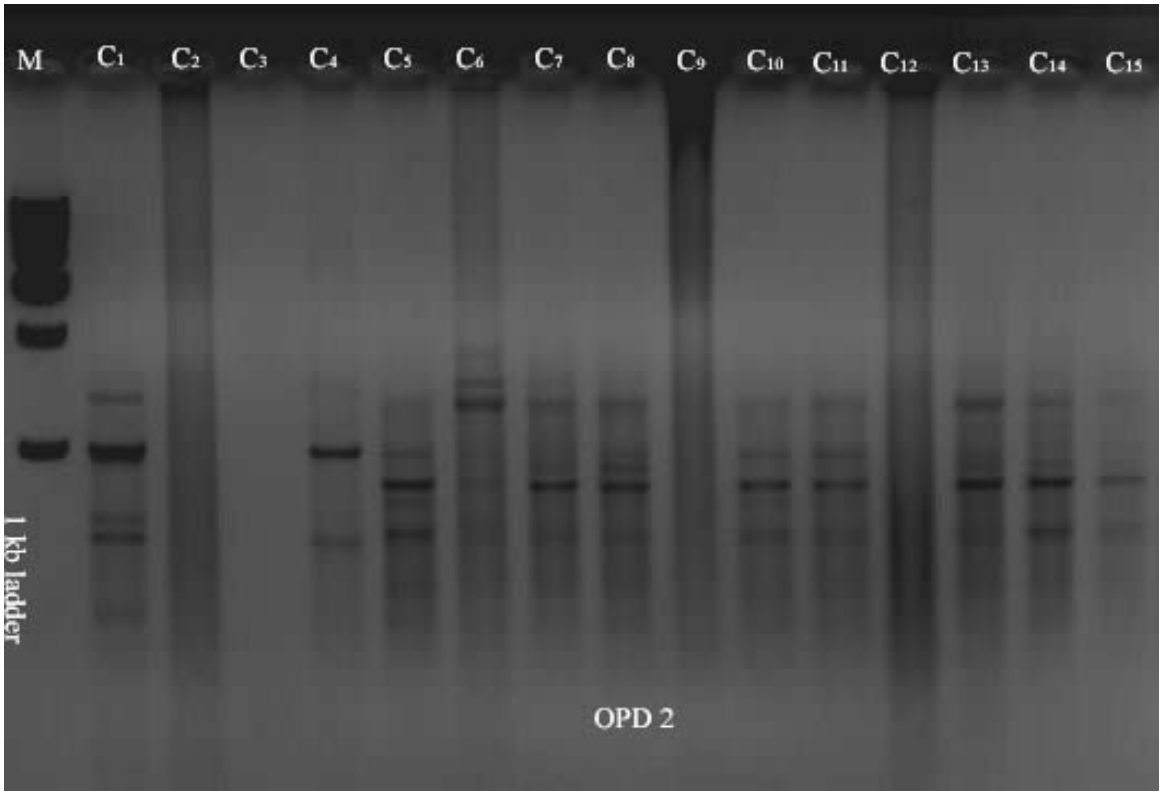

Figure 11. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPD2 primer.

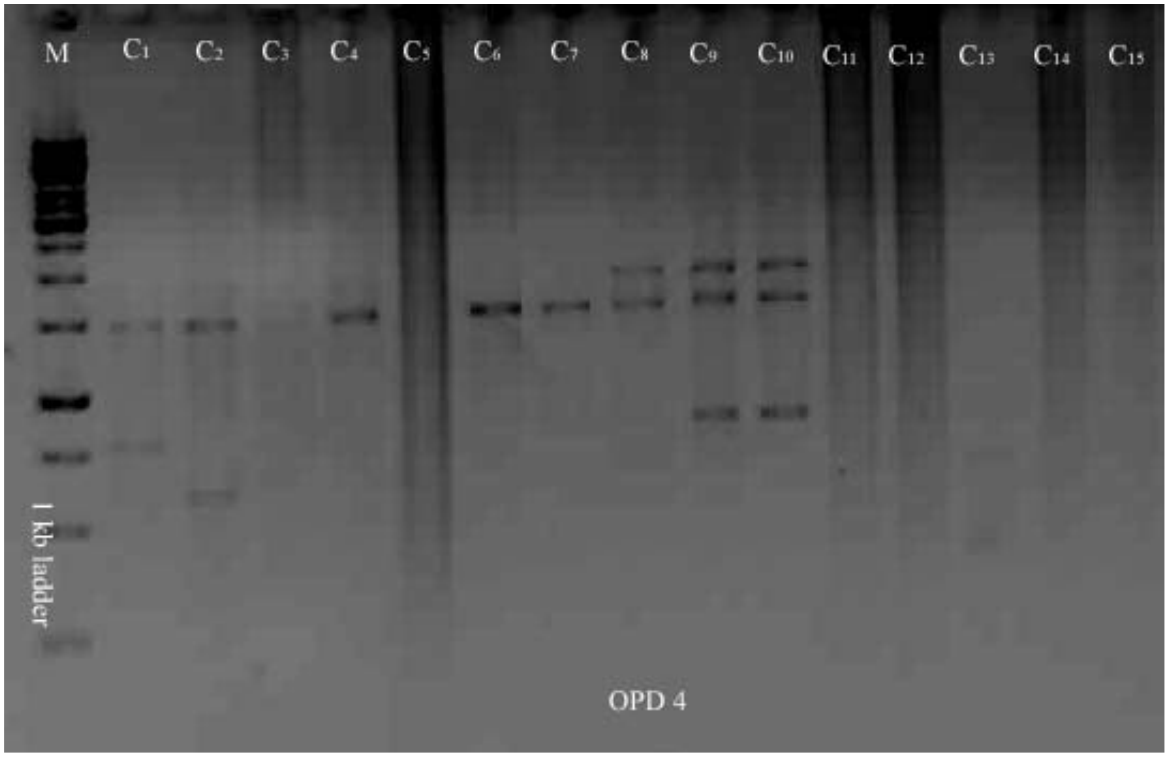

Figure 12. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPD4 primer.

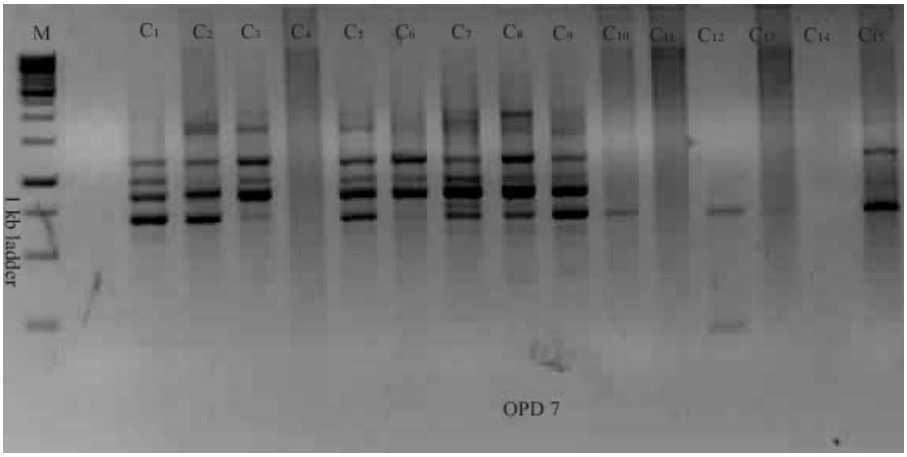

Figure 13. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPD7 primer.

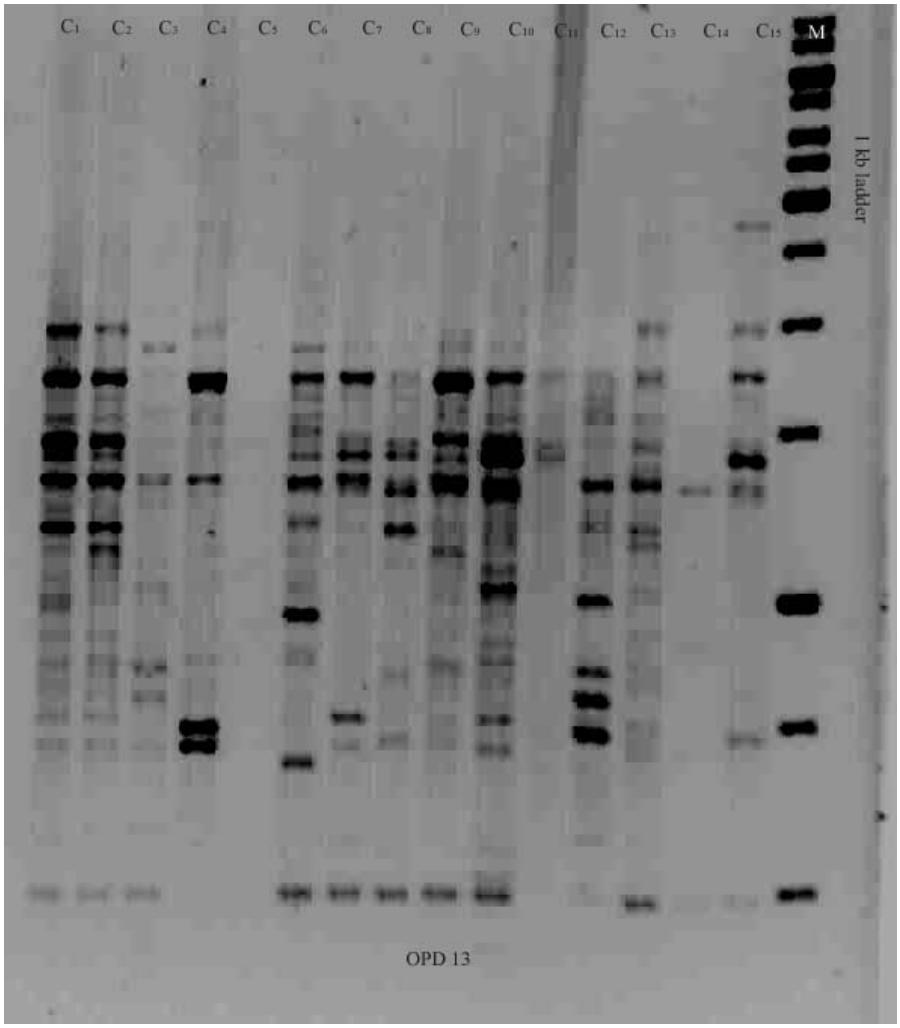

Figure 14. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPD13 primer.

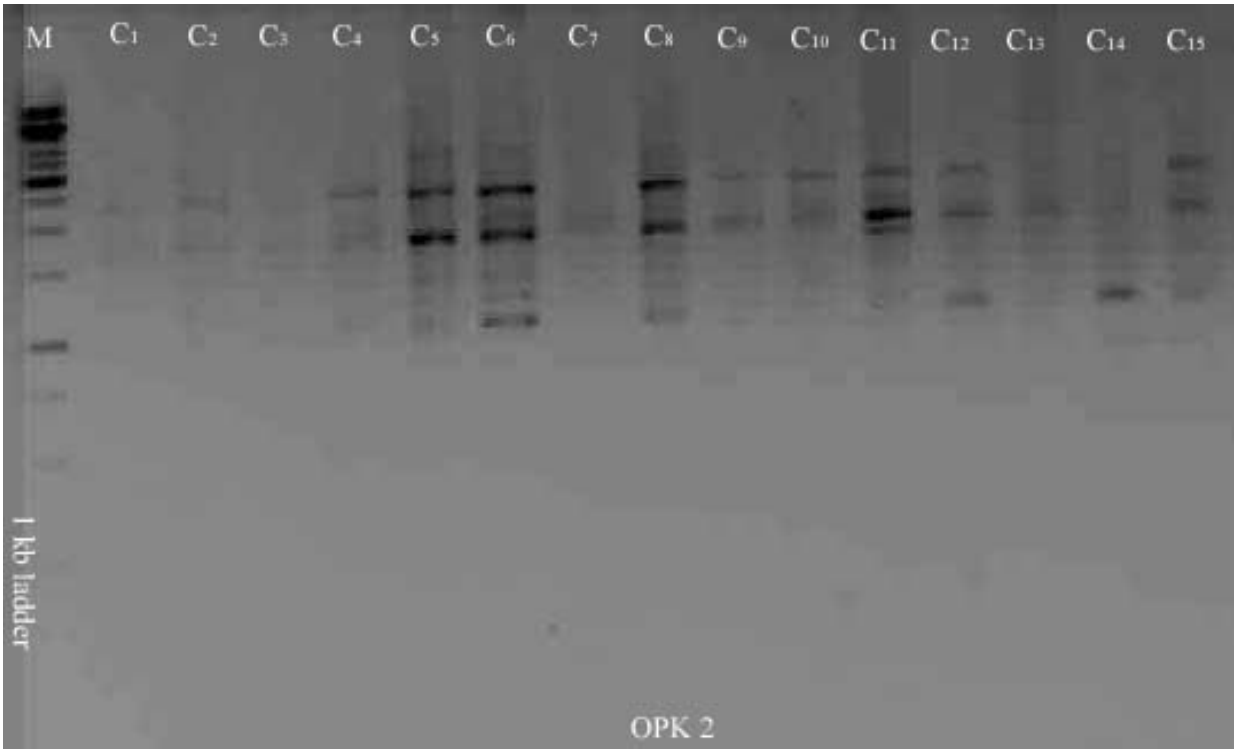

Figure 15. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPK 2 primer.

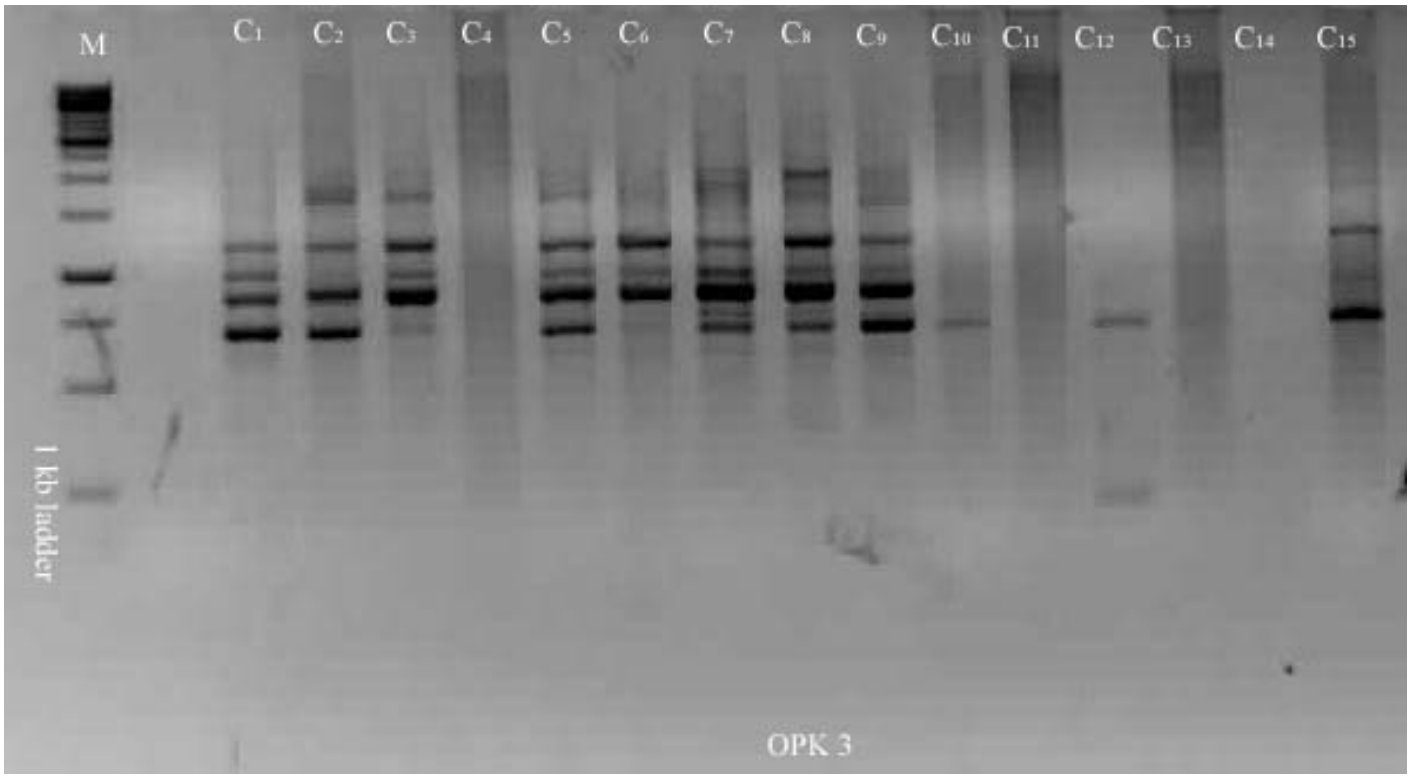

Figure 16. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPK 3 primer.

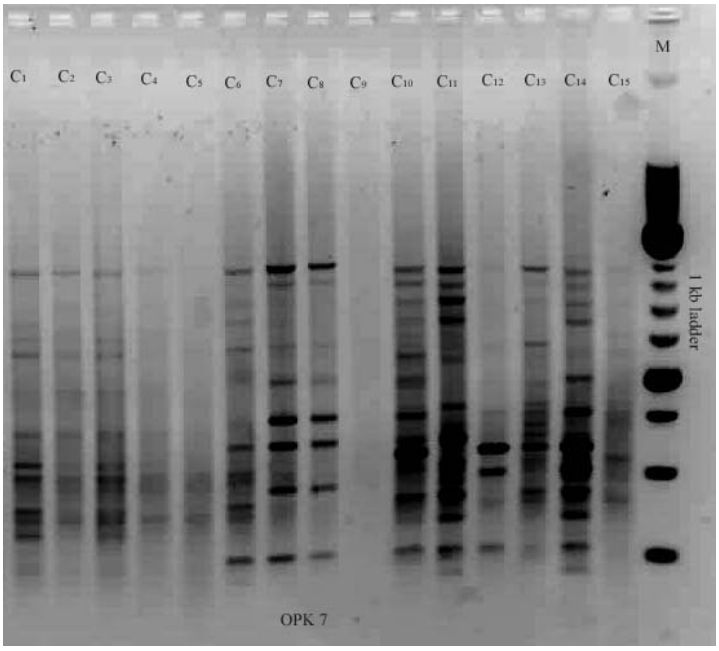

Figure 17. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPK7 primer.

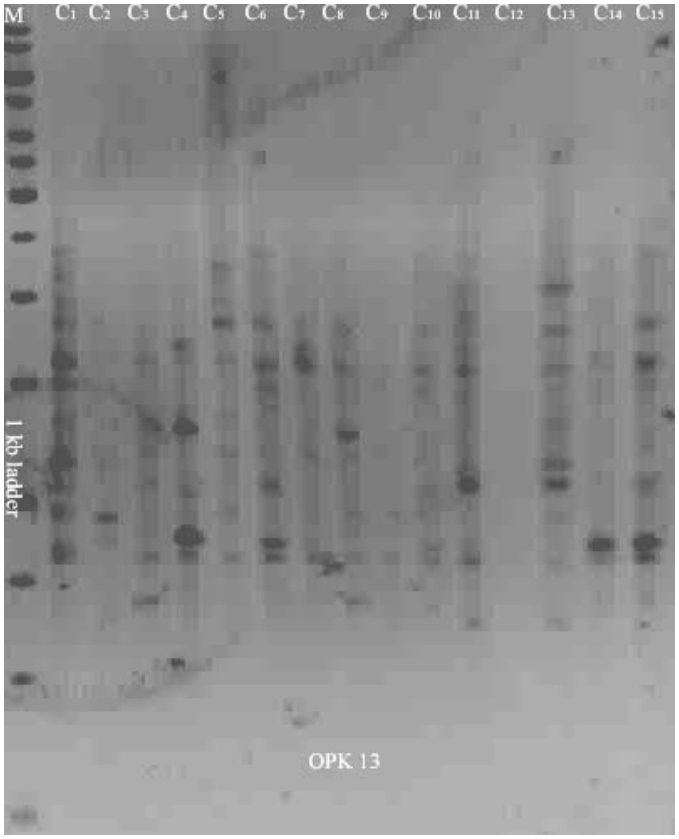

Figure 18. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPK13 primer.

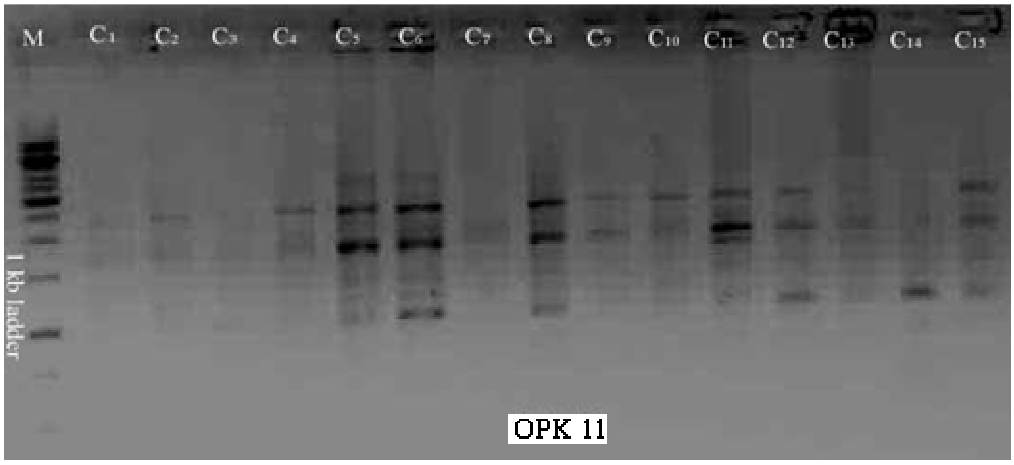

Figure 19. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPK 11 primer.

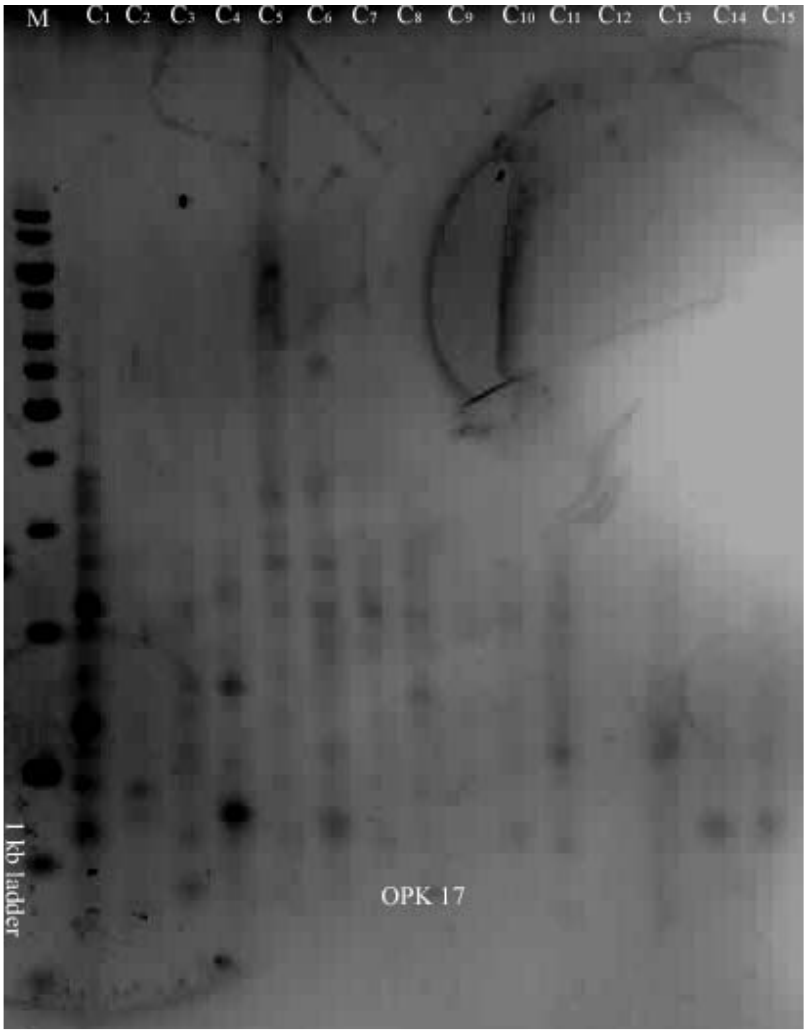

Figure 20. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPK 17 primer.

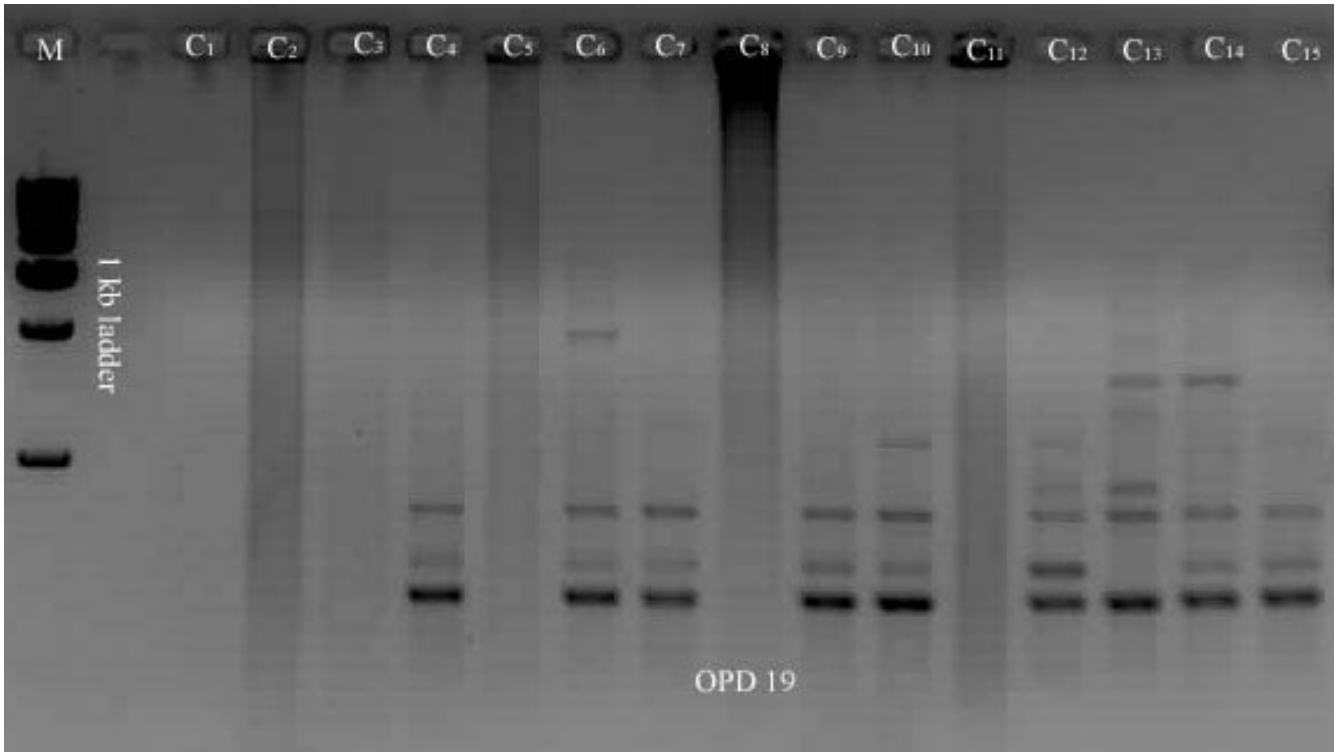

Figure 21. RAPD profile of 15 accessions of tea (*Camellia sinensis* (L.) O. Kuntze) using OPD 19 primer.

Showed wide morphological variations in tree height, tree habit, leaf, size and shape of flower and fruit characters. The biochemical characterization also plays an important role in the diversity like, biochemical compositions (Du et al., 1990), esterase isozymes (Lu et al., 1992) and polyphenol oxidase (Singh and Ravindranath, 1990)

The karyotype (Liang et al., 1994) was used to discriminate tea plants and their wild allied species. However, it was found that all were not reproducible because of different growing environments, developing stages, seasons and even experimental conditions. Hence, the biochemical like enzyme activity related to RAPD analysis can be used to identify the high quality tea varieties. Molecular markers like RAPD discriminate germplasm at intra and inter specific levels. Conner and Wood (2001) described DNA fingerprinting analysis also provide a good method for the intra specific level of germplasm.

The biochemical marker (PPO activity) results showed three different range of activity (high, moderate and low), each containing 5 accessions. In order to correlate the biochemical marker study with molecular marker, all the 15 accessions were analysed (RAPD) using 20 decamer primers. The primers were selected based on their ability to produce maximum number of bands, reproducibility and the ability to produce polymorphism. Among the 20 RAPD primers were used, fourteen primers showed high level of polymorphism. The OPD13, OPB20 and OPB10 primers were capable of producing high level of polymorphism. This could be explained by the capability of individual primers to amplify the less conserved and highly repeated regions of the genomic DNA. There is high possibility for the amplified fragments to contain repeated sequences.

In cluster analysis based on the dendogram, the shared fragments divided into three groups, high, moderate and low. Among the two major clusters based on similarity index, first major clusters were further divided into two minor sub clusters. The first minor sub clusters contained five accessions (high), the second minor sub cluster contained five accessions (moderate) and the second minor (low).

The UPASI 18 (“Cambod”) was found to be 0.28% different from the other accessions. This shows that there is considerable variability among the accessions selected and can be further utilized for crop improvement.

In this analysis of genetic diversity within the population showed most variable in “Cambod” types of tea plants. However, other accessions were collected from the same geographic region (Brooklands Estate) exhibited high level of similarities, presumably because of selection being made on the same natural populations as reported (Wachira et al., 1995). The genomic diversity between the Assam and China clones showed wide variations in

their geographical origin. Mondal et al., (2002) described that an average, 57% within and 43% between population's variability and the Chinese clones as more genetically diverse than Assam clones.

Conclusion

The present investigation concludes that, results of biochemical markers studies correlated well in RAPD analysis. The pattern of clusters segregated very well in the groups (high, moderate and low) which related to the total PPO activity of the selected UPASI germplasm. This is the first report showing the identifications of superior clones performed by using total PPO and RAPD analysis.

ACKNOWLEDGEMENT

The authors thank UPASI TRF, UPASI Tea Research Institute, Nirar Dam BPO, Valparai 642 127, Coimbatore District, Tamilnadu, India for providing financial assistances.

REFERENCES

Bradford M (1976). "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding", Anal. Biochem., 72: 248-254.

Chang HT (1984). A revision of the tea resource plants. Acta. Sientiarum. Naturalium Universitatis Sunyatseni. pp. 1-12.

Chen L, Yu FL, Tong QQ (2000). Discussions on phylogenetic classification and evolution of sect *Thea*. J. Tea. Sci., 20: 89-94.

Conner PJ, Wood BW (2001). Identification of pecan cultivars and their genetic relatedness as determined by randomly amplified polymorphic DNA analysis. J. Am. Soc. Hort. Sci., 126: 474-480.

Du QZ, Li MJ, Liu WH, Wang HS (1990). Chemical and numerical taxonomies of section *Thea* plants. J. Tea. Sci., 10: 1-12.

Gomez KA, Gomez AA (1976). Statistical procedures for agricultural research with emphasis of rice. International Rice Research Institute, Los Banos, Philippines.

Hackett CA, Wachira FN, Paul S, Powell W, Waugh R (2000). Construction of a genetic linkage map for *Camellia sinensis* (tea). Heredity, 85: 346-355.

Jaccard P (1908). New research on the floral distribution. Vaudoise Bulletin of the Society of Natural Sci., 44: 223-270.

Kaundun S, Zhyvoloup AS, Park YG (2000). Evaluation of the genetic polymorphism among elite tea (*Camellia sinensis* var. sinensis) accessions using RAPD markers. Euphytica, 115: 7-16.

Lai JA, Yang WC, Hsiao JY (2001). Anassessment of genetic relationships in cultivated tea clones and native wild tea in Taiwan using RAPD and ISSR markers. Bot. Bull. Acad. Sin., 42: 93-100.

Liang GL, Zhou CQ, Lin MJ, Chen JY, Liu JS (1994). Karyotype variation and evolution of sect *Thea* in Guizhou. Acta. Phytotaxonomica. Sinica, 32: 308-315.

Lu CY, Liu WH, Li MJ (1992). Relationship between the evolutionary relatives and the variation of esterase isozymes in tea plant. J. Tea. Sci., 12: 15-20.

Ming TL (1992). A revision of Camellia sect. *Thea*. Acta. Botanica. Yunnanica., 14: 115-132.

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.

Mondal TK, Chand PK (2002). Detection of genetic instability among the miocropropagated tea (*Camellia sinensis*) Plants. In. vitro. Cell. Dev. Biol., 37: 1-5.

Nei M, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonuclease, Proc. Natl. Acad. Sci. USA., 76: 5296-5273.

Rohlf FJ (2002). NTSYS-pc:numerical taxonomy system ver.2.1.Exeter publishing Ltd., Setauket, Newyork.

Sealy JR (1958). A Revision of the Genus *Camellia*. Royal Horticultural Society, London.

Singh HP, Ravindranath SD (1990). Occurrence and Distribution of PPO activity in floral organs of some standard and local cutivars of tea. J. Sci. Food. Agric., 64: 117-120.

Tanaka J, Yamaguchi N, Nakamura Y (2001). Pollen parent of tea cultivar Sayamakaori with insect and cold resistance may not exist. Breed. Res., 3: 43-48.

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

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.

Whitaker JR (1994). Principles of Enzymology for the Food Sciences, New York: Marcel Dekker.

Yelena G, Sheptovitsky, Brudvig GW (1996). Isolation and characterization of spinach photosystem II membrane-associated catalase and polyphenol oxidase. Biochemistry, 35: 16255-16263.