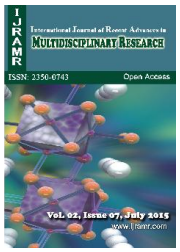

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Research Article

DETECTION OF GENETIC POLYMORPHISMS AMONG COMMERCIAL TEA POPULATION AND ENDEMIC WILD TEA PLANT (*GORDONIA OBTUSA. EXUT AND ARN*) AS REVEALED BY RAPD MARKERS

[1,*]Shalimol, A., [1]Arumugasamy, K., [1]Nantha kumar, R., [1]Abdul kaffoor, H., [1]Muthuraj, K., [2]Midhusha Johny and [2]Minu Venugopal

[1]Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641029, Tamil Nadu, India
[2]Department of Biotechnology, Kongunadu Arts and Science College (Autonomous), Coimbatore-641029, Tamil Nadu, India

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ABSTRACT

Genomic fingerprinting of 4 tea plant genotypes was carried out using 7 RAPD multiloci primers. All the primers showed significant amplification in PCR Analysis. A total of 303 bands were produced in all the 4 clones with an average of 43.29% RAPD bands per primer. Among all the bands 16.83% were polymorphic in nature. The molecular size of the amplified DNA fragments ranged from 100 to 6000 bp. Dendrogram was constructed based on the genetic similarity matrix using the UPGMA algorithm, which showed two main clusters. Cluster 1 contains only one clone i.e., U3 and the reminder 3 clones in cluster 2. Cluster 2 was further sub-divided into 2 sub-clusters. In which wild type tea plant categorized in 1 subcluster and the remaining two tea clones are categorized to form sub-sub clusters. Cluster analysis revealed that all the four genotypes were widely diverse genetically. All the four genotypes were showed identical polymorphism while generating phylogenetic tree.

INTRODUCTION

Tea is the oldest, non-alcoholic, caffeine containing beverage produced from the young shoot tips (two and a bud) of different tea varieties i.e., *Camellia sinensis* (L.) O.Kuntze (China type), *C.sinensis var. assamica* (Assam type) and *C.sinensis* subspecies lasiocalyx (Planch.) wight (Cambod type). All the three varieties of tea are highly cross pollinated and intercrossible without any reproduction barrier. Thus the existing population is a mixture of three categories of tea (Banerjee, 1992; Biswas *et al*., 2009). Apart from these varieties, tea family (Theaceae) also comprise of wild species. *Gordonia obtusa* wall. exut and Arn is a wild tea plant in the tea family, Theaceae. *Gordonia* is a genus of flowering plants in the family Theaceae, of the roughly 40 Sps. all two are native to South east Asia in Southern China, Taiwan. The remaining species are native to South East North America. Plant breeders need genetic markers of the varieties, which can be used for the development of new improved varieties.

Corresponding author: Shalimol, A.,
Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641029, Tamil Nadu, India.

DNA based molecular markers are effecting at evaluating genetic diversity among the species and cultivars, and the data are easier to obtain than classical morpho anatomical descriptors. Until that time, several types of molecular markers i.e., AFLP (Amplified Fragment Length Ploymorphism) based marker analysis (Chen *et al*., 2000), RFLP (Restriction Fragment length Polymorphiam) based fingerprinting (Chen *et al*., 2000), RAPD (Random Amplified Polymorphic DNA) profiles (Devarumath *et al*., 2002; Dos Santos *et al*., 1994; Gul *et al*., 2007) and ISSR (Inter Single Sequence Repeating) based analysis (Chen *et al*., 2000; Kaundan *et al*., 2000) have been engaged to quantify the genetic diversity within tea germplasm collections of various countries. In the present investigation, use of RAPD markers for assessing the genetic diversity and relationship among the wild type and 3 cultivated clonal genotypes from three different tea varieties.

MATERIALS AND METHODS

Plant Material

Clonal genotypes of three tea varieties and one wild endemic tea plant (*Gordonia obtusa* wall.

Exut and Arn) were used for the present study (Table 1). These genotypes were collected from the United Planter's Association of Southern India (UPASI), Valparai, Tamilnadu, India.

Table 1. Tea genotypes from four categories of tea population

.Name of the species	Varieties	Tea Genotypes
Camellia sinensis (L.) O. Kuntze	China type	UPASI 9
C.sinensis var. Assamica	Assam type	UPASI 3
C. sinensis subspecies lasiocalyx	Cambod type	UPASI 17
Gordonia obtusa wall. Exut and Arn	Wild type	

Genomic DNA Isolation and Purification

Tender unfolded leaf samples from four different genotypes were collected from the clonally propagated plants and stored immediately at -800C for DNA extraction. The total genomic DNA was extracted from the stored leaf samples using DNeasy Plant Mini Kit (Qiagen) as per the manufacturer's instructions. The concentration of the purified genomic DNA in each case was adjusted to 10ng/µL in different aliquots and stored at -200C for use in PCR amplification.

PCR Amplification for RAPD Genotyping

Seven decamer random RAPD primers (OPERON – Qiagen U.S.A) OPA 03, OPB 07, OPB 10, OPB 20, OPD 02, OPD 07 and OPD 13 (Table 2) were used for PCR amplification of the genomic DNA of 4 tea clones. Polymerase chain reaction was carried out in eppendorf thermal cycler using ten decamer random primers (Table 2).

Table 2. Primer sequences used as RAPD markers

Primer Name	Sequence (5'-3')
OPA 03	AGTCAGCCAC
OPB 07	GGTGACGCAG
OPB10	CTGCTGGGAC
OPB 20	GGACCCTTAC
OPD 02	GGACCCAACC
OPD 07	TTGGCACGGG
OPD 13	GGGGTGACGA

Each 25 µl reaction mixture contained 1 unit of *Taq* DNA polymerase (Takara), 0.2 mM each d*NTPs,* 1X PCR buffer, 3 mM MgCl2, 10 pmole of primer (OPERON-Qiagen,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 and reaction was ended with 4°C .

The amplified products were separated on 2% agarose/EtBr gel using 1X TBE buffer. Bands were visualized under UV light, photographed in gel documentation system and bands were scored from photographs.

Scoring and analysis of data

Each polymorphic band was considered as a binary character and 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) as a standard. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix.

RESULTS AND DISCUSSION

The present study was conducted to reveal the genetic variation among the 4 tea clones, China, Assam, Cambod, and wild type by reproducible amplification of DNA through RAPD analysis using 7 random decamer primers. The primer sequences used for amplification are enumerated in Table.2.The total DNA isolated from 4 clones were visualized using agarose EtBr gel electrophoresis (Figure 1). The results of the DNA fingerprinting of 4 clones are shown in Figure 2. The polymorphisms obtained from the DNA fingerprinting data were summarized in Table 3. A total of 303 distinct bands were scored from 7 different fingerprinting profiles of which 51 were polymorphic. An average of 43.29% bands per primer was amplified showing 16.83% polymorphic amplification which indicates the high level of polymorphism present among the 4 tea clones (Table 3).

A diverse level of polymorphism in different crops has been reported in *Brassica* (Chen *et al*. 2000), tomato (Moonmoon 2006, Tabassum *et al*. 2013), egg plant (Biswas *et al*. 2009) and chili (Paran *et al*. 1998). Extensive polymorphism in tea was reported earlier in several genetic diversity studies using RAPD markers (Wachira *et al*. 1995, Kaundun *et al*. 2000, Jorge *et al*. 2003). Mondal *et al*. (2000) characterized 25 Indian and 2 commercial tea cultivars using RAPD markers and found 95.2% genetic variability. Among 7 primers used, OPB 10 produced maximum number of polymorphic bands (81 bands) that indicated a high level of polymorphism. On the other hand primer OPD 13 generated the least number of polymorphic bands (20 bands). The RAPD banding profiles of the amplified products of these seven primers on four different clones are shown in Fig. 2. The band size ranged from 100 to 6000 bp. In earlier reports, Lai *et al.* (2001) and Gul *et al*. (2007) observed approximately 200 to 1000 bp and 250 to 1500 bp fragment sizes in different tea clones respectively.

Table 3. Analysis of polymorphism obtained with RAPD primers in tea varieties

Primer	Total No. of RAPD bands (a)	Number of polymorphic bands (b)	Polymorphism (%) [(b/a)*100]	Band size (bp)	
				Minimum	Maximum
OPA 03	39	4	10.25	100	6000
OPA O7	27	6	22.22	600	3000
OPA 10	81	7	8.64	300	2500
OPA 20	41	10	24.40	300	3500
OPD 02	44	7	15.90	250	2000
OPD 07	51	8	15.70	300	2500
OPD 13	20	9	45.00	200	2000
Total : 7	Total : 303	Total : 51	Average : 16.83 %		

Figure 1: Lane 1- Sample 1; Lane 2 – genomic DNA from Assam type (UPASI 3); Lane 3 – genomic DNA from China type (UPASI 9).; Lane 4 – genomic DNA from Campod type (UPASI 17).

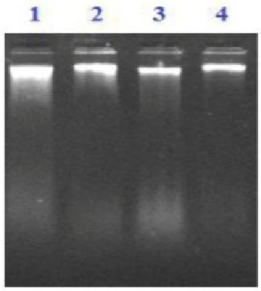


Figure 2: Results of RAPD Profiling. Lane M – DNA ladder (1 Kb); Lane 1- Sample 1; Lane 2 – genomic DNA from Assam type (UPASI 3); Lane 3 – genomic DNA from China type (UPASI 9).; Lane 4 – genomic DNA from Campod type (UPASI 17).

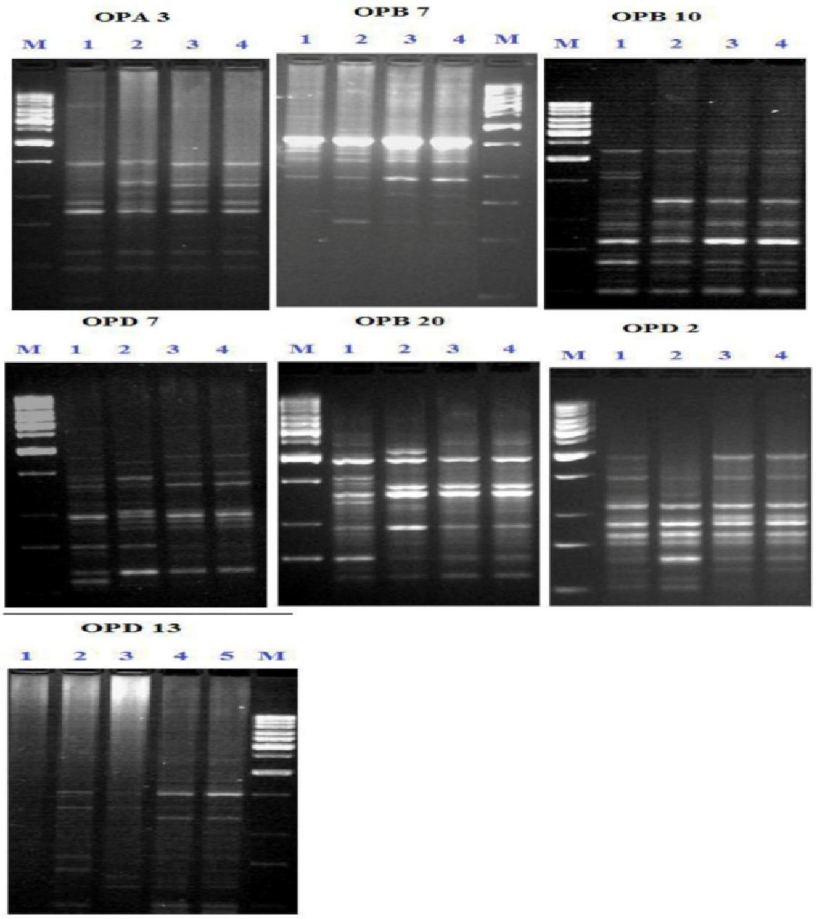
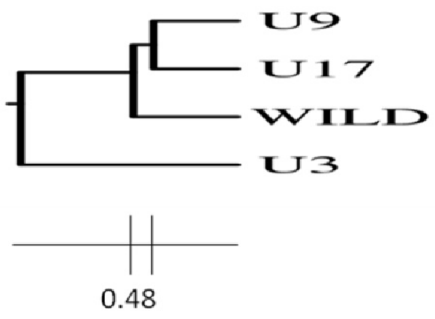


Figure 3: Phenetic Dendrogram constructed among the 4 varieties of tea plant



The similarity matrix obtained in Table 3 was used in an UPGMA algorithm for cluster analysis. The phenetic dendrogram constructed on the basis of genetic identity obtained from RAPD matrix was represented in Figure 3. The dendrogram constructed based on Nei's (1972) genetic distance separated out the 4 clones in to two major clusters (Figure 3). Cluster 1 contains only one clone i.e., U3 and the reminder 3 clones in cluster 2. Cluster 2 was further sub-divided into 2 sub-clusters. In which wild type tea plant categorized in 1 subcluster and the remaining two tea clones are categorized to form sub-sub clusters. From this investigation it is clearly indicating that each of the 18 tea clones possessed specific fingerprinting profile which could be used for their authentic identification. The detection of high level of genetic variability also supports the findings of Lai *et al*. (2001), Chen *et al*. (2000), and further strengthens the reports of Welsh and MacClelland (1990) and Dos Santos *et al*. (1994) who concluded that RAPD markers are effective for visualizing high level of genetic polymorphism in plant species.

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