International Journal of

Agricultural Research

ISSN 1816-4897

Academic
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www.academicjournals.com

International Journal of Agricultural Research 5 (9): 805-815, 2010
ISSN 1816-4897 / DOI: 10.3923/ijar.2006.390.400
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Genetic Relatedness among Indian Litchi Accessions (*Litchi chinensis* Sonn.) By RAPD Markers*

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Abstract: Genetic relatedness among Indian litchi (*Litchi chinensis* Sonn.) cultivars was investigated using RAPD (random amplified polymorphic DNA) markers. Fourteen RAPD primers which produced consistent profiles were chosen, resulting in amplification of 77 reproducible polymorphic bands. The RAPD analysis produced an average of 15.8% polymorphic and 0.10% monomorphic markers. With the help of RAPD marker system we were able to classify all the accessions into different groups despite of their same or different geographical origins and climatic adaptations. The polymorphism information content scores were calculated for each of the 77 RAPD polymorphic fragments. Unweighted pair-group method with arithmetic average (UPGMA) dendrograms using Jaccard's coefficients reflected no clear cut variation or grouping based on either morphology or climatic adaptation. However, dendrogram showed that 27 accessions of Indian litchi could be classified into groups when the similarity coefficients were shown in a range of 0.11 to 0.47. Two accessions [Chinarose (B) and Late Bedana (B)] were found to be genetically significantly distant from the other accessions using RAPD markers. The information obtained from the present study could be of practical use in mapping the litchi genome as well as for classical breeding.

Key words: Litchi, RAPD, genetic diversity, accessions

Introduction

Litchi chinesis Sonn. is a genus in the Sapindaceae or soapberry family which contains about 140 genera and 2000 species (Chapman, 1984). *Litchi chinensis* (Litchi) is a dense polygamous evergreen tree which grows 10-15 m in height and has been shown to possess variable diploid chromosome numbers where 2n = 28, 30, 32 (Chapman, 1984). The variation in chromosome number is perhaps because the modern species had more than one wild progenitor. Litchi is a popular fruit crop in many tropical and subtropical countries, although it originated in Southern China and Northern Vietnam. Many varieties are densely distributed and grown in India. Bihar is the most important litchi growing state, which contributes 77% of total litchi production in the country. The variability among litchi cultivars is still unknown since breeding for new cultivars is done by growers and is based on low number of parents. Nomenclature of litchi cultivar in its present state suffers from many inconsistencies-the same cultivar may be known 'under several names and different cultivars may appear under the same name (Aradhya *et al.*, 1995). Field collection and preservation in gene banks

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**Originally Published in International Journal of Agricultural Research, 2006*

of Plant Genetic Resources (PGR) has been extensively conducted at the international level. To identify genetic materials that may contain useful traits for germplasm enhancement, a systematic evaluation of genetic diversity is required to understand relationship among accessions and their corresponding collecting –site environment (Steiner and Greene, 1996). Understanding the genetic diversity within a germplasm collection facilitates their use, provided that information is available from characterizing these germplasm collections (Strauss *et al*., 1998). Comparison of parents using difference in DNA markers may be one of the methods by which breeders can increase the probability of selecting those parents with different gene sets. This method will produce progeny with new and more favorable combinations of genes for quality and yield. Recent reports have been focused on using DNA based markers, particularly random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers, to measure genetic diversity in exotic litchi cultivars i.e., Chinese litchi accessions (*Litchi chinensis*) (Ding *et al*., 2000), Thai litchi accessions (*Litchi chinensis*) (Tongpamnak *et al*., 2002), closely related species longan (*Dimocarpus longan*) (Xiang *et al*., 1998) and numerous other fruit species such as lemon (*Citrus lemon* L.) (Machado *et al*., 1996), peach (*Prunus persica* L.) (Lu *et al*., 1996), mango (*Mangifera indicia* L.) (Lopez *et al*., 1997) grape (*Vitis vinifera*) (Sensi *et al*., 1996), currant (*Ribes grossularia*) (Lanham and Brennan, 1999) and pear (*Pyrus* sp.) (Monte-Corvo *et al*., 2000). According to the previous reports RAPD could detect substantial genetic variation within perennial fruit cultivars and generally demonstrate that cultivars can be discriminated on the basis of genetic characteristics (Lopez-Volenzuela *et al*., 1997). Choice of a marker system to use for a particular application depends on its ease of use and the particular objects of the investigation (Rafalski *et al*., 1996. RAPD technique does not require DNA probes or prior sequence information. This procedure is simple, largely automable, requires only small amounts of DNA and can be performed without the use of radioactivity (Karp *et al*., 1996). RAPD markers also have limitations such as their dominant character and reproducibility (Williams *et al*., 1990). Reliability may be increased by replicate analyses and PCR performed at different times. Although, AFLP is also performed in the same fashion but it is more labor intensive and expensive than RAPD analysis.

The objective of this study was to use RAPD analyses to estimate the level of genetic relatedness among 27 litchi accessions collected from different geographical regions of India.

Materials and Methods

Plant Materials

Twenty seven accessions of litchi were collected from five different geographical regions of India and established in Jawaharlal Nehru University nursery. They were the source of DNA samples (Table 1).

DNA Extraction

Young leaves were harvested from the plants grown in the nursery, wiped with hydrogen peroxide (H_2O_2) mopped dry and quickly frozen and powdered in liquid nitrogen. The powders were either used for isolation of DNA immediately, or were stored in a deep freezer (-80°C) for long-term storage. Total DNA was extracted following the method of Doyle and Doyle (1987) with minor modification and adapted to litchi as follows: 1.5 g young leaves were ground in liquid nitrogen to a fine powder and extracted with cetyltrimethylammonium bromide (CTAB) hot extraction buffer [2% CTAB, 50 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.2% (w/v) β-mercaptoethanol, 2% (w/v) polyvinylpyrrolidon (PVP)]. The mixture was incubated at 65C for 45 min, followed by extraction with chloroform/isoamyl alcohol (24:1). Isopropanol (0.6 volume) was used to precipitate nucleic acids and the pellet obtained was dissolved in 500 μL of sterile distilled water and incubated at -80°C for 30 min. Remaining impurities were extracted with equal volume of phenol:chloroform:isoamyl

Table 1: Description of litchi accessions collected from different geographical regions of India

Cultivars/Plants	Parental relation	Source	Origin
Kalkattia, Dehra Rose, Bedana, Late-bedana, Late-large-red, Ajholi, China, Chinarose, Deshi, Purbi, Kasba and Illaichi	Grafted/air layering plants	Sabour Agricultural college	Bhagalpur, Bihar
Kalkattia, Dehradun, Bedana, Late-bedana, Early-bedana, Late-large-red, Rose scented	Grafted/air layering plants	Bihar Agricultural College	Muzaffarpur, Bihar
Seedless, Kalkattia and Dehradun	Grafted/air layering plants	Punjab Agricultural University	Gurdaspur, Punjab
Seedless and Dehradun	Grafted/air layering plants	Regional Horticultural Division	B.Deoghar, Jharkhand
Seedless	Grafted/air layering plants	Regional Horticultural division	Muzaffarnagar, U.P
Dumdum	Grafted/air layering plants	Indian Forest Research Institute	Dehradun, Uttranchal

alcohol (25:24:1). Total DNA was precipitated using 0.1 volume of sodium acetate (pH 5.2) and chilled ethanol (2.0 volumes). The precipitate was washed with 70% ethanol and the pellet was dissolved in TE buffer/MQ. The quantity and quality of purified DNA was estimated by comparing band intensities on agarose gel.

Polymerase Chain Reaction (PCR)

Random Amplified Polymorphic DNA (RAPD) assays were carried out in 25 µL reaction mixtures containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at room temperature), 1.5 mM $MgCl_2$, 0.2 mM each of the deoxyribonucleotide triphosphates (dNTPs), 10 pmoles primer, 0.5 U Taq DNA polymerase (*Bangalore genei*) and 10-50 ng of genomic DNA (depending on the primer, after several initial experiments aimed at optimizing conditions). A control PCR tube containing all compounds, but no template, was run with each primer to check for contamination. DNA amplification was carried out in a PTC 100 thermocycler (Programmable Thermal Controller, MJ Research Inc, Labmate), with the following conditions: 94C (3 min) initial denaturation, 44 cycles of 94C (1 min), 35°C (90 sec), 72°C (90 sec). The final extension cycle allowed an additional incubation for 5 min at 72°C. At least two PCR amplifications were performed for each sample with RAPD to evaluate the reproducibility of the bands obtained. The amplified DNA fragments were separated in a 1% agarose gel using 1X TAE buffer according to Sambrook *et al.* (1989) and stained with ethidium bromide. Gels were visualized and imaged using gel documentation system (Gel Doc Mega, Biosystematica, UK).

Data Analysis

Data from molecular marker techniques requires detailed analysis to establish genetic relations. For each primer, polymorphic bands are scored for their presence or absence in all the accessions by visually assessing photographs of the gels. It is important that duplicate amplifications are performed to confirm the reliability of the bands. The size of amplification product was determined by comparison with Gene ruler (1 kb ladder, MBI, Fermentas). Only distinct, reproducible, well-resolved fragments, in the size range from 200 bp to 2.5 kb, were scored as discrete variables using '1' to indicate presence and '0' to indicate absence of a band for the 27 accessions. From the band data, monomorphic and polymorphic bands were identified for each type of cultivar. Genetic similarity between pairs was estimated by the similarity coefficient (S_M), an algorithm that considers individuals to be genetically similar only when they possess a band in common. This approach seems to be more appropriate for a genetic character, since the lack of a common band should not imply a similarity in genetic terms. Dendrogram was constructed for RAPD markers, using the scorable fragments and assessed by the Jaccard's (1908) coefficient prior to the construction of a final dendrogram using RAPD markers. The similarity matrix was used to construct the dendrogram by using unweighted pair group method with arithmetical averages (UPGMA and SAHN) of the NTSYSpc software package, version 2.02e (Rohlf, 1998).

Results

The analysis of the pre-screening data using 27 accessions of Indian litchi collected from different locations and 14 primers out of 22 RAPD operon primers (OPA-1, OPA-2, OPA-3, OPA-4, OPA-5, OPA-6, OPA-7, OPA-8 OPA-9, OPA-10, OPB-4, OPB-7, OPG-1 and OPG-3) detected the diversity among the cultivars used. Out of 14, 7 primers (OPA-1, OPA-4, OPA-6, OPA-7, OPB-4, OPB-7 and OPG-1) were chosen that had more than 5 scorable bands and 7 primers (OPA-2, OPA-3, OPA-5, OPA-8, OPA-9, OPA-10 and OPG-3) generated less than five bands (Table 2). Using the DNA purification strategies, good and clear amplification pattern could be obtained for the various accessions of litchi cultivars (Fig 1a, b and 2).

From the integration of data obtained from the RAPD technique, using 14 selected primers for all the 27 accessions, 12-77 amplicons per selected RAPD operon primer were scored, originating a total of 538 fragments. Of these, an average of only 15.8% were polymorphic and a least percentage (0.10%) of monomorphic bands (data was not shown) were found (Table 2). The lower level (15.8%) of polymorphic bands generated by the various primers enabled narrow genetic discrimination of all cultivars except V1 and V8.

Accession Specific Bands

RAPD analysis also revealed putative accession-specific amplified products. A DNA banding profile using primers OPA-1, OPA-4 and OPA-7 as shown in Fig. 1a, b and 2 indicated the specific markers of 200, 1031, 1200, 400, 2000 and 1200 bp at 40 ng DNA templates of accessions V15 and V21, respectively.

Table 2: RAPD markers produced by 14 selected primers (out of 22 tested primers) in 27 types of litchi cultivars studied

		RAPD fragments scored			
Primer (Operon code)	Sequences (5'-3)	Total No. of fragments	Polymorphic bands	Polymo-rphism (%)	Fragment size (base pair)
OPA 01	CAG GCC CTT C	38	6	10.5	>800, 800, >700, 600, 500, 400
OPA 02	TGC CGA GCT G	35	5	14.3	>1200, 1200, 1031, <900, 900
OPA 03	AGT CAG CCA C	25	2	08.0	2500, 2000
OPA 04	AAT CGC GCT G	68	7	10.2	2500, <2500, 2000, 1500, 1200, 800, 600
OPA 05	AGG GGT CTT G	29	3	10.4	800, 750, 600
OPA 06	GGT CCC TGA C	35	7	20.0	2000, 2000, 1500, 1200, 1031, 900, <900
OPA 07	GAA ACG GGT G	77	7	0 9.1	1200, 1031, >900, 900, 800, 700, 600
OPA 08	GTG ACG TAG G	42	3	0 7.1	>700, 600, 500
OPA 09	GGG TAA CGC C	35	4	14.2	2500, 2250, 1500, 1200
OPA 10	GTG ATC GCA G	26	3	11.5	600, 500, 200
OPB 01	GTT TCG CTC C	0 0	0	00. 0	-
OPB 02	TGA TCC CTG G	00	0	00. 0	-
OPB 04	GGA CTG GAG T	12	6	5 0.0	>500, <500, 450,400,200, 150
OPB 07	GGT GAC GCA G	51	7	07.4	1200, 1031, 900, 700, 650, 400
OPB 08	GTC CAC ACG G	0 0	0	00. 0	-
OPB 09	TGG GGG ACT C	0 0	0	00.0	-
OPB 16	TTT GCC CGG A	0 0	0	00.0	-
OPB 17	AGG GAA CGA G	0 0	0	00. 0	-
OPG 01	CTA CGG AGG A	32	7	40.5	1350, 1200, 1031, 800, 600, 500
OPG 02	GGC ACT GAG G	00	0	00.0	-
OPG 03	GAG CCC TCC A	33	4	07.7	900, 700, 500, 400
OPG 04	AGC GTG TCT G	0 0	0	00.0	-

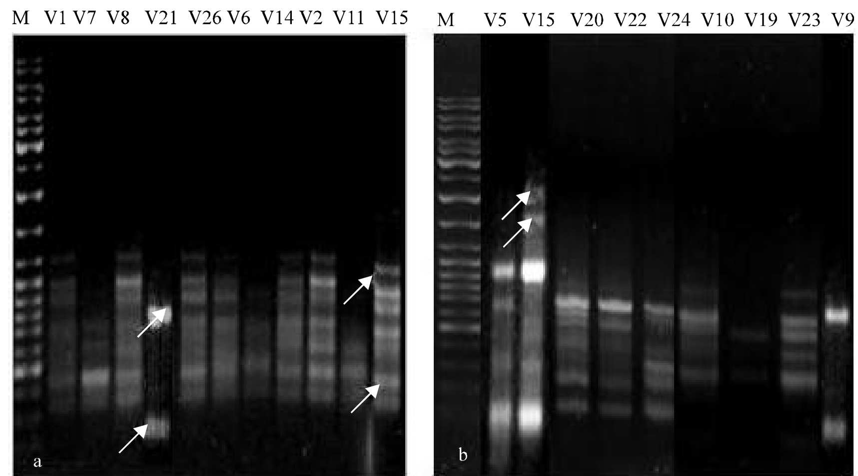

Fig. 1: RAPD agarose gel electropherosis profiles of the lithi accessions using primers OPA-1 (a) OPA-4 (b) Lanes indicated by M represents molecular mass marker (gene ruler 1Kb ladder, MBI, Fermentas). The accessions numbers (V1-V15 and 5-V9) indicated the template DNA from appropriate cultivars as shoen in Table 3. Arrows indicate putative accession- specific marker

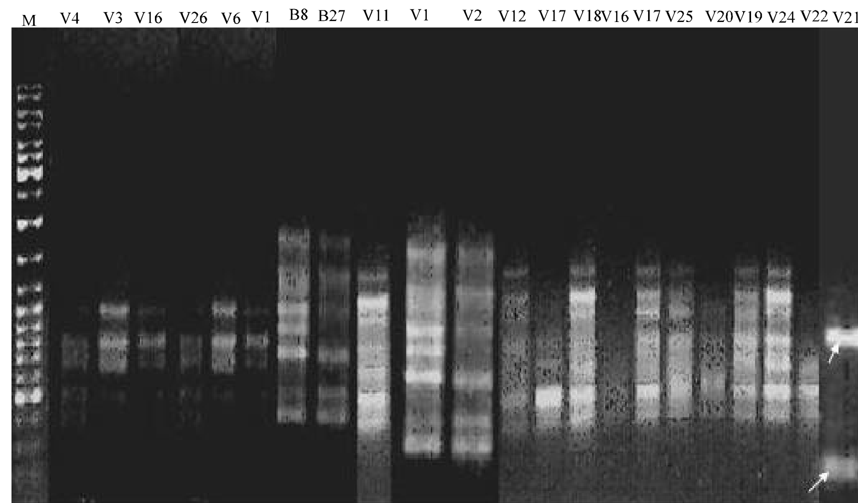

Fig. 2: RAPD agarose gel electropherosis profiles of the lithi accessions using primers OPA-7 Lane indicated by M contains molecular mass marker (Gene ruler 1Kb ladder, MBI, Fermentas). The accessions numbers (V1-V15 and 5-V9) indicated the template DNA from appropriate cultivars as shoen in Table 3. Arrows indicate putative accession- specific marker

Genetic Similarity and Diversity of Litchi Accessions Revealed by UPGMA and SAHN Algorithm

The similarity matrix obtained using Jaccard's coefficient is shown in Table 3. Similarity coefficients ranged from 0.11 to 0.47 in 27 accessions of litchi tested in the present investigation. These similarity coefficients were used to generate a tree for cluster analysis using UPGMA and SAHN

Table 3: Distance matrix values based on RAPD data among 27 accessions of litchi

	V1	V2	V3	V4	V5	V6	V7	V8	V9
V1	1.000								
V2	0.276	1.000							
V3	0.346	0.263	1.000						
V4	0.119	0.140	0.125	1.000					
V5	0.000	0.000	0.000	0.000	1.000				
V6	0.224	0.192	0.261	0.167	0.077	1.000			
V7	0.308	0.186	0.200	0.103	0.000	0.191	1.000		
V8	0.477	0.196	0.260	0.143	0.000	0.293	0.452	1.000	
V9	0.000	0.025	0.000	0.000	0.000	0.000	0.061	0.000	1.000
V10	0.077	0.128	0.081	0.000	0.000	0.000	0.027	0.029	0.125
V11	0.172	0.148	0.179	0.103	0.000	0.244	0.255	0.271	0.000
V12	0.160	0.053	0.244	0.000	0.000	0.122	0.149	0.133	0.000
V13	0.295	0.157	0.222	0.000	0.000	0.000	0.128	0.163	0.000
V14	0.246	0.217	0.278	0.122	0.114	0.341	0.196	0.208	0.000
V15	0.025	0.024	0.054	0.067	0.000	0.115	0.057	0.000	0.000
V16	0.140	0.063	0.068	0.143	0.000	0.156	0.125	0.135	0.000
V17	0.125	0.022	0.049	0.053	0.000	0.065	0.171	0.056	0.200
V18	0.048	0.045	0.050	0.000	0.000	0.067	0.053	0.057	0.000
V19	0.075	0.093	0.100	0.032	0.190	0.216	0.128	0.111	0.091
V20	0.048	0.070	0.050	0.000	0.000	0.185	0.081	0.057	0.222
V21	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.069	0.000
V22	0.051	0.049	0.054	0.000	0.000	0.074	0.088	0.063	0.000
V23	0.098	0.146	0.049	0.111	0.000	0.100	0.079	0.086	0.200
V24	0.063	0.104	0.043	0.040	0.188	0.147	0.093	0.073	0.059
V25	0.152	0.058	0.109	0.077	0.000	0.139	0.195	0.150	0.111
V26	0.264	0.255	0.327	0.105	0.000	0.222	0.260	0.333	0.000
V27	0.231	0.179	0.148	0.147	0.032	0.268	0.091	0.188	0.033

	V10	V11	V12	V13	V14	V15	V16	V17	V18
V1									
V2									
V3									
V4									
V5									
V6									
V7									
V8									
V9									
V10	1.000								
V11	0.027	1.000							
V12	0.000	0.149	1.000						
V13	0.174	0.104	0.132	1.000					
V14	0.000	0.136	0.326	0.077	1.000				
V15	0.000	0.121	0.000	0.000	0.026	1.000			
V16	0.000	0.125	0.061	0.097	0.143	0.000	1.000		
V17	0.000	0.025	0.033	0.000	0.048	0.077	0.000	1.000	
V18	0.000	0.081	0.200	0.074	0.103	0.000	0.000	0.133	1.000
V19	0.080	0.152	0.229	0.000	0.302	0.000	0.030	0.071	0.074
V20	0.167	0.111	0.071	0.000	0.049	0.083	0.000	0.214	0.143
V21	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000
V22	0.222	0.057	0.080	0.083	0.053	0.000	0.059	0.077	0.083
V23	0.154	0.051	0.033	0.000	0.073	0.000	0.000	0.125	0.133
V24	0.000	0.093	0.156	0.000	0.190	0.000	0.077	0.091	0.045
V25	0.000	0.195	0.219	0.027	0.182	0.000	0.154	0.238	0.042
V26	0.057	0.286	0.262	0.106	0.245	0.000	0.048	0.000	0.054
V27	0.133	0.132	0.064	0.114	0.189	0.065	0.051	0.121	0.200

method (Fig. 3). Two types of comparisons were carried out to evaluate the degree of relationship in the litchi cultivars 1) among different accessions collected from the same locations and 2) among common accessions collected from different locations. The cluster analysis indicates that 27 accessions

Table 3: Contined

	V19	V20	V21	V22	V23	V24	V25	V26	V27
V1									
V2									
V3									
V4									
V5									
V6									
V7									
V8									
V9									
V10									
V11									
V12									
V13									
V14									
V15									
V16									
V17									
V18									
V19	1.000								
V20	0.208	1.000							
V21	0.000	0.000	1.000						
V22	0.300	0.000	1.000						
V23	0.071	0.214	0.000	0.077	1.000				
V24	0.286	0.150	0.000	0.053	0.043	1.000			
V25	0.188	0.087	0.000	0.048	0.083	0.143	1.000		
V26	0.182	0.000	0.065	0.000	0.111	0.045	0.231	1.000	
V27	0.167	0.125	0.000	0.065	0.156	0.075	0.098	0.157	1.000

Accessions details: Vi Seedless (P), V2 Seedless (UP), V3 Seedless (J), V4 Kalkattia (P), V5 Kalkattia (B), V6 Kalkattia (M), V7 Dehradun (M), V8 Dehradun (P), V9 Dehraroj (j), V10 Dehraroj (B), V12 Bedana (M), V13 Bedana (B), V14 Bedana (B), V15 Late Bedana (M), V16 Early Bedana (M), V17 Late Large Red (B), V18 Late Large Red (M), V19 Ajholi (B), V20 China (B), V21 Chinarose (B), V22 Deshi (B), V23 Purbi (B), V25 Illaichi (B), V26 Rose scented (M), V27 Muzaffarpur (M), B = Bhagalpur, Bihar, M = Muzaffarpur, Bihar, P = Punjab, UP Uttar Pradesh, D = Dehradun, Uttranchal, J = Jharkhand

of litchi formed five groups based on similarity coefficients (Table 3) except theaccessions V15 [Late Bedana (M)] and V21 [Chinarose (B)] which exhibited only 0 and 3% genetic similarity to others. Therefore both of them are kept independent.

Group I

Large clusters were formed which contained a mixture of 11 litchi accessions obtained from different localities. Cluster analysis shows a close relation within them. Accession V1 (Seedless) and V8 (Dehradun) obtained from Punjab were found to have the highest similarity (SI = 0.47, Table 3) while the other accessions belonging to this group also exhibited a co- similarity based on similarity coefficient (range of SI = 0.11 to 0.45, Table 3). As per the cluster analysis, Dehradun (M) was closely related (about 75%) with Seedless (P) and Dehradun (P). Similarly, accessions Seedless (J), Rose scented (M), Kalkattia (M), Late Bedana (B), Seedless (U.P.), Dehra Rose (B), Muzaffarpur (M) and Bedana (B) were found to be very close (27 to 75%) with Seedless (P) and Dehradun (P) respectively. The genetic similarity was high as per the coefficient similarity and it was possible to correlate them with each other despite their different geographical location. This is a good indication of the fitness of the result that was obtained, which is particularly important for analysis of Indian litchi cultivars, since their genetic diversity and parental relations are unknown.

Group II

In this group, all four accessions were found partially close to each other. Between Late Large Red (B) and Illaichi (B) the similarity was high (SI = 0.15, Table 3) as compared to the

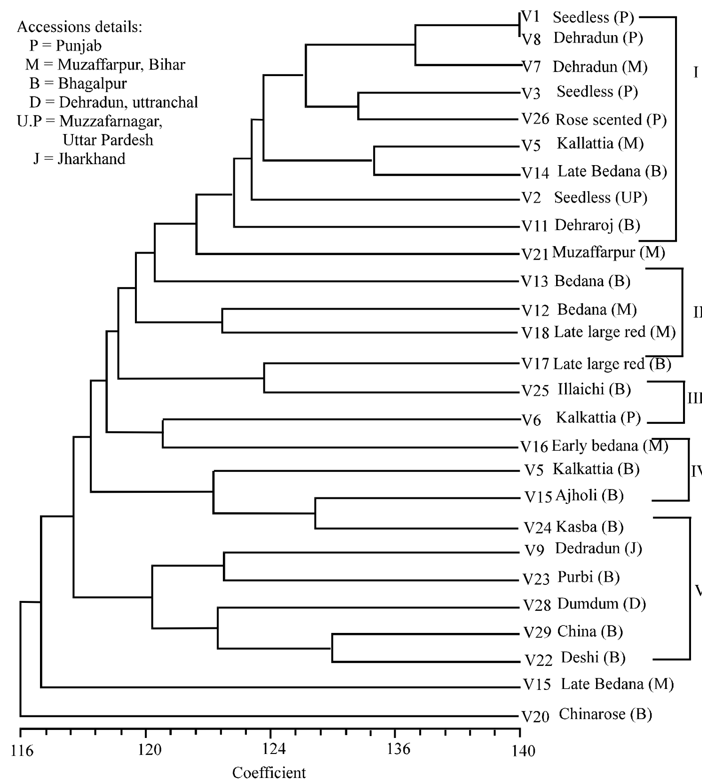

Fig. 3: Dendrogram derived from UPGMA cluster analysis usoing Jaccard's coefficient of RAPD markers

similarity (SI = 0.04, Table 3) between the accessions Bedana (M) and Late Large Red (M). Late Large Red (B) and Illaichi (B) exhibited about 48% similarity with Bedana (M) and Late Large Red (M).

Group III and IV

Group III contains only two accessions Kalkattia (P) and Early Bedana (M). Dendrogram reveals the similarity (SI=0.14, Table 3) between two accessions which reflects a lower level of genetic diversity despite their different geographical locations. Cluster analysis indicated that these two accessions were close (27%) with Late Large Red (B) and Illaichi (B). While group IV contains three accessions Kalkattia (B), Ajholi (B) and Kasba (B) which belong to the same place, these three accessions were found to be associated (35 to 60%) with each other according to their origin and habitat relatedness.

Group V

It was the second largest group generated by cluster analysis which includes five accessions Dehradun (J), Purbi (B), Dumdum (D), China (B) and Deshi (B). They were kept in a separate group as per the genetic relatedness with each other revealed by cluster analysis/similarity coefficient. China (B) and Deshi (B) were most similar (SI= 0.30, Table 3) while Dehradun (J) and Deshi (B) were the least similar (SI = 0.00, Table 3) in this group. Dumdum (D) was found closely related (about 62%) with accessions China (B) and Purbi and 25 % with Dehradun (J) and Deshi (B).

Interestingly, the two accessions China Rose (B) and Late Bedana (M) showed a high genetic distance (SI= 0.00 and 0.02, Table 3) in cluster analysis among all the accessions. Therefore, the relatively more genetic variation obtained from these two accessions seems to be due to a large phylogenetic distance from other groups of accessions.

Discussion

Genetic diversity among 27 selected accessions of Indian litchi was assessed with 77 RAPD polymorphic bands generated by 14 selected operon primers. In the recent past with the same objective, RAPD analyses were carried out by Paran *et al.* (1998) in *Capsicum annuum* and by Tongpamnak *et al*., 2002 in *Litchi chinensis* (Thai litchi accession). The percentage of polymorphic bands (PPB) in each accession ranged from 7.1 to 50.0 % These results more or less agreed with RAPD and AFLP analysis in exotic litchi accessions (Tongpamnak *et al*., 2002) and other species e.g., Japanese plum cultivars (Bellini *et al*., 1998), chilli (Paran *et al*.,1998) and soybean (Choudhary *et al*., 2001).

For similarity coefficients the ranges were 0.11 to 0.47 for RAPD data, indicating the genetic diversity following a descending pattern of polymorphism among the 27 litchi accessions. Only two accessions, Chinarose (B) and Late Bedana (M) were not genetically similar (SI = 0.0 and 0.02) to the rest of the accessions which were classified into five different clusters as per the similarity coefficient of cluster analysis. There was no higher level of similarity observed among the cultivars originating from the same or nearby geographical locations supporting both the hypothesis of authoctonal origin (Tongpamnak *et al.* 2002) as well as the limited diffusion of litchi cultivars from their zones of cultivation (Baril *et al*., 1997).

Litchi is grown in northern parts of India (Punjab, U.P. and Uttranchal) where the climatic conditions (low humidity, low temperature and basic soil) are different from the main litchi growing region (Bihar). Inspite of these differences in the environmental factors under which litchi cultivars are grown, they do not exhibit any difference in flowering and harvesting time. Horticulturists have reported that fruit size, color at maturity, fruit quality and leaf shape of the cultivars varies based on the geographical regions. However, our study with DNA markers did not reveal any clear pattern of grouping based on morphology or putative climatic or geographical origin, as detected in some other crops (Paul *et al*., 1997; Spooner *et al*., 1996). Present results corroborate those of Belaj *et al.* (2001) who also reported similar results in Olive germplasm using RAPD markers.

Estimation of genetic diversity is highly influenced by the genome selected for evaluation and by the number of markers assayed. Since fruit tree cultivars are maintained by vegetative propagation, accurate identification of vegetative materials is crucial for growers and is required for plant breeder's rights. This DNA marker technique can be used to identify genetic variation and detect the relationship between DNA markers and horticultural traits of interest. For this reason, RAPD technique has been employed to screen the germplasm in case of several higher plants. Most of these studies have been carried out in case of cross-pollinated plants and consequently, relatively higher estimates of genetic variability were obtained. In the case of tissue culture, RAPD technique has enabled the testing of fidelity of micropropagated plants (Rani *et al*., 1995). The RAPD profiles, however, could reveal relative variability as well as similarity within the 27 accessions of 19 litchi cultivars. Clearly there is scope for large-scale application of RAPD for analysis of such cross-pollinated/heterozygous plants. The present study reveals that PCR based fingerprinting technique; RAPD is informative for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationships among different accessions of Indian litchi cultivars. The information obtained from the present study could be of practical use in mapping the litchi genome as well as for classical breeding. The markers identified in our studies will be useful in genetic analysis of litchi accessions in germplasm holdings.

The putative cultivar-specific bands can be used as probes to ascertain whether they are in low or high copy numbers in the litchi genome and such specific bands may be used for genotype characterization and grouping germplasm accessions. Further, putative cultivar-specific RAPD markers could be converted to sequence characterized amplification regions (SCARs) after sequencing and designing primer pairs to develop robust cultivar specific markers. The study also provides a basis for litchi breeders to make informed choices on selection of parental material based on genetic diversity to help in overcoming the problems usually associated with a tree crop improvement program.

Acknowledgement

The author's sincere thanks to Department of Biotechnology, Govterment of India (Grant no. BT/PR/1775/Agr/8/122/99) for financial assistance.

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