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Assessment of Genetic Diversity in Hybrid Litchi (*Litchi chinensis* Sonn.) Revealed by RAPD Marker

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The genetic diversity in 10 selected hybrids of litchi, *Litchi chinensis* Sonn. using 15 decamer random primers. Out of 15, 10 RAPD primers revealed polymorphism while the remaining 5 primers reflected no polymorphism. The primers produced a total of 52 bands of which 33 were polymorphic (61.11%). The number of polymorphic fragments for each primer varied from 1-7 with an average of 1.84 polymorphic fragments. The RAPD data were analysed to determine the genetic similarity coefficients which ranged from 0.57 to 0.83. Cluster analysis was performed using the Jaccard's similarity coefficient. This method dendrogram resolved the selected litchi hybrids into two major clusters. The results reveal the genetic relationship of hybrids of litchi developed at BAU, Sabour-Bhagalpur (Bihar).

Keywords: Litchi hybrids; genetic variation; RAPD; genetic similarities; PCA.

1. INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is an essential subtropical evergreen fruit tree belongs to the family Sapindaceae, native to South China. It is considered as the queen of the fruits due to its excellent quality, delicate whitish pulp, characteristic pleasant flavour, luscious red colour and nutritional value.

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Litchi is an introduced crop in India and has limited genetic variability characterised by differences in flushing pattern, leaf, panicle and fruit traits. Genetic diversity in litchi is indicated by a large number of cultivars, which provides the bases of development of new cultivars. There are different characteristics which are used to identify the cultivars. However, there is widespread confusion regarding litchi cultivar nomenclature. The same cultivars may have different names in different localities (synonyms), and different cultivars may have the same name (homonyms) [1]. Traditionally, litchi cultivar characterisation has been based on morphological traits such as floral and fruit characteristics, and the harvest season [2]. The shape of skin segments and protuberances are the reliable and stable genetic characteristics used for discriminating the varieties [3]. The usefulness of this approach is limited due to the interaction of morphological traits with environmental conditions [4], and it is even more difficult to distinguish between closely related germplasm using only morphological characters. Therefore, litchi cultivar names must be standardised.

In recent years molecular biology has allowed solving the problem of cultivar identification through molecular techniques. DNA based molecular markers have proven to be powerful tools in the characterisation of germplasm, varietal identification, clonal fidelity testing, assessment of genetic diversity, elucidation of genetic relationships and marker-assisted selection. Accurate identification of genotypes is a requisite for efficient conservation, maintenance and utilisation of the existing germplasm of litchi. A number of systems have been used in previous studies for litchi germplasm, including random amplified polymorphic DNA (RAPD) [5,6,7,8], amplified fragment length polymorphism (AFLP) [9,10], sequence-related amplified polymorphism (SRAP) [11], inter-simple sequence repeat (ISSR) [12], and simple sequence repeat polymorphism (SSR) [13,14,15]. Choice of a marker system to be used for a particular application depends on its ease of use and the specific objectives of investigation [16]. Compared to all molecular markers, RAPD technique does not require DNA probes or prior sequence information. This procedure is simple, largely automated, requires only a small amount of DNA and can be performed without the use of radioactivity [17]. The present investigation was undertaken for the assessment of genetic diversity among the selected litchi hybrids developed at BAU, Sabour- Bhagalpur, Bihar with the help of RAPD markers.

2. MATERIALS AND METHODS

2.1 Planting Material

Leaf of ten litchi hybrids developed at Bihar Agricultural University, Sabour, Bhagalpur, Bihar (India) through hybridisation programme was collected from the litchi orchards of BAU, Sabour (Table 1).

Table 1. Details of 10 hybrids litchi (*Litchi chinensis* Sonn.) and their parents

Sl No.	Treatments (Hybrids)	Parentage
1.	H-140	China X Bedana
2.	H-141(W)	China X Bedana
3.	H-141(E)	China X Bedana
4.	H-400	Dehrarose X Purbi
5.	H-503	Ojhauli X Purbi
6.	H-515	Purbi X Bedana
7.	H-517	Purbi X Bedana
8.	H-518	Purbi X Bedana
9.	H-520	Purbi X Bedana
10.	H-524	Late Bedana X China

2.2 DNA Isolation

Freshly harvested young and tender leaf samples were used for DNA extraction. In brief, collected leaf samples (0.5 g) were ground by adding 1.5 ml of 2XCTAB extraction buffer (warm 65ºC) in using mortar and pestle. Approximately, 600 µl of the liquid were transferred into 1.5 ml of microcentrifuge and an equal volume (W/V) (600 µl) of ice-cold chloroform: isoamyl alcohol (24:1) was added, mixed well by inversion and centrifuged for 5 min at 12,000 rpm at 4ºC. The top (aqueous) phase was collected into a new microcentrifuge tube and 1/5th volume of 5% CTAB solution was added mixed well by gentle inversion. Then, an equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed well by inversion and centrifuged at 12,000 rpm for 5 min at 4ºC. The top (aqueous) phase was collected using cut tips into a new microcentrifuge tube and an equal volume of CTAB precipitation buffer was added and incubated on ice for 5 min. After incubation, microcentrifuge tube was centrifuged at 12,000 rpm for 5 min at 4ºC and supernatant was discarded. Fifty microlitres of high salt solution were added into the microcentrifuge to

dissolve the pellet. DNA was precipitated by addition of 125 µl ice-cold ethanol and mix gently by inversion. Microcentrifuge tubes were centrifuged at 12,000 rpm for 15 min at 4°C and supernatant was discarded. DNA pellet was washed with 70% ethanol, air dried, dissolved in 25 µl of 0.1X TE buffer.

2.3 RAPD Amplification Procedure

RAPD analysis of litchi hybrids was conducted using 10 decamer arbitrary primers obtained from Operon Technologies, California. RAPD amplification was performed in 20 µl volume containing 10 µl of Premix Taq® Version 2.0 (Xcelris Lab Ltd. Ahmedabad, Gujarat), 0.5 µM primer and 50 ng of template DNA in a thermal cycler (Agilent Technologies). The PCR cycles comprised an initial 94ºC for 5 min for denaturation followed by 35 cycles of denaturation at 94ºC for 1 min, primer annealing at 34ºC for 30 sec, extension at 72ºC for 2 min and a final extension at 72ºC for 3 min. PCR product was subjected to electrophoresis in 1.2% agarose gel and visualised using a gel documentation system (UVITEC, Cambridge). Each experiment was repeated three times with each primer to test the reproducibility of RAPD primer.

2.4 Data Analysis

The polymorphism was documented on the basis of presence (1) or absence (0) of bands, in addition to variation in respect of number and position of bands among the hybrids. The binary data matrix was also subjected to further analysis and genetic similarities among entries were calculated depending on the presence and absence of common bands. Using the software NTSYS-pc [18], the genetic relationship among hybrids was established by calculating the similarity coefficient. The method used for tree building in the cluster analysis involved sequential agglomerative hierarchical non-overlapping clustering based on similarity coefficients. The dendrogram based on similarity indices was obtained by the un-weighted pair group method using arithmetic mean (UPGMA). Computational analysis for determining the major allele frequency, gene diversity, polymorphism information content and heterozygosity was performed using the software Power Marker [19]. Heterozygosity was expressed as the proportion of heterozygous genotypes in the population at a single marker locus in question. Gene diversity was estimated as the probability that the two randomly chosen alleles from the population are different at the marker locus in question. Principal component analysis (PCA) following to construct a correlation similarity matrix afterwards Eigenvalue and Eigenvector matrices. Two-dimensional plots were created using two principal components selected by the NTSYS-pc software.

3. RESULTS

The results of the present study indicated a considerable level of genetic diversity among the cultivars selected. Among 15 primers used in this study, results of 10 primers were taken into consideration since they had given reproducible bands. The polymorphism percentage varied from 25 (OPA-13) to 100 (OPB-07). The percentage of polymorphism was calculated as 60.61% (Table 2). The level of polymorphism with primers differed between the hybrids.

The size of the average amplified fragments ranges between 480 bp to 2300 bp. A total of 384 DNA fragments were generated by 10 primers out of which 184 were polymorphic with an average of 18.4 polymorphic bands per primer (Tables 3 and 4). The amounts of polymorphism detected among litchi hybrids as observed by RAPD primer are shown in Fig. a.

The PIC ranged from 0.50 (OPA-10) to 0.81 (OPB-05). Data for observed maximum major allele frequency, genotype number, number of alleles, genetic diversity and heterozygosity for all the ten hybrids of litchi were analysed using ten RAPD markers, and their respective values were found as 0.45, 5, 7, 0.84 and 1 (Table 5).

A dendrogram based on UPGMA analysis grouped ten litchi hybrids in the two clusters (Fig. 2), with Jaccard's similarity coefficient of 0.57–0.83 (Table 6). These Jaccard's average similarity estimates were mostly clustered in frequency class 50–60, which included 1 pair of hybrids. Similarly, 16 pairs of hybrids were found in frequency class 60–70, 25 pairs of hybrids were in frequency class 70–80, while 2 pairs of hybrids were in frequency class 80–90.

The relationship among the 10 hybrids was also detected by PCA based on RAPD data. Hybrids grouped within the same cluster in the dendrogram were occupying the same positions in the three-dimensional scaling also (Fig. 3).

Table 2. List of 10 selected RAPD primers used for analysing 10 hybrids of litchi

Sr. no.	Primer (Operon code)	Sequences (5-3)	Fragment size (base pair)	Total no. of amplified fragment	Monomorphic bands	Polymorphic bands	Polymorphism (%)
1.	OPA-03	AGTCAGCCAC	500-3000	4	2	2	50.00
2.	OPA-07	GAAACGGGTG	1000-3000	5	0	5	100.00
3.	OPA-08	GTGACGTAGG	200-2000	5	3	2	40.00
4.	OPA-10	GTGATCGCAG	500-1000	4	1	3	75.00
5.	OPA-13	CAGCACCCAC	200-2000	4	3	1	25.00
6.	OPA-14	TCTGTGCTGG	1000-2000	4	2	2	50.00
7.	OPB-01	GTTTCGCTCC	200-3000	7	2	5	71.43
8.	OPB-05	TGCGCCCTTC	500-3000	7	3	4	57.14
9.	OPB-08	TGCTCTGCCC	200-2000	8	1	7	87.50
10.	OPB-10	GTCCACACGG	500-2000	4	2	2	50.00
Total				52	19	33	61.11
Average			480-2300	5.2	1.90	3.30	**60.61**

Table 3. The nucleotide sequence of the decamer random primers, the total number of DNA fragments amplified (A) and the number of polymorphic bands (P) for each hybrid with individual primers

Primer	Nucleotide sequence	Litchi Hybrids																			
		H-140		H-141W		H-141E		H-400		H-503		H-515		H-517		H-518		H-520		H-524	
		A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
A-03	AGTCAGCCAC	2	0	3	1	3	1	3	1	3	1	4	2	3	1	3	1	2	0	3	1
A-07	GAAACGGGTG	4	4	3	3	5	5	3	3	2	2	5	5	3	3	5	5	4	4	4	4
A-08	GTGACGTAGG	4	1	4	1	4	1	4	1	5	2	4	1	4	1	5	2	5	2	5	2
A-10	GTGATCGCAG	3	2	3	2	2	1	3	2	3	2	4	3	3	2	3	2	3	2	3	2
A-13	CAGCACCCAC	4	1	4	1	4	1	3	0	3	0	3	0	4	1	3	0	3	0	3	0
A-14	TCTGTGCTGG	2	0	2	0	2	0	2	0	4	2	3	1	3	1	3	1	3	1	3	1
B-1	GTTTCGCTCC	5	3	5	3	4	2	6	4	6	4	5	3	4	2	4	2	4	2	4	2
B-5	TGCGCCCTTC	4	1	5	2	6	3	6	3	5	2	4	1	5	2	5	2	6	3	3	0
B-8	TGCTCTGCCC	6	4	4	2	6	4	7	5	4	2	5	3	3	1	4	2	5	3	5	3
B-10	GTCCACACGG	4	2	4	2	3	1	4	2	3	1	4	2	4	2	3	1	4	2	4	2
Total:		38	18	37	17	39	19	41	21	38	18	41	21	36	16	38	18	39	19	37	17

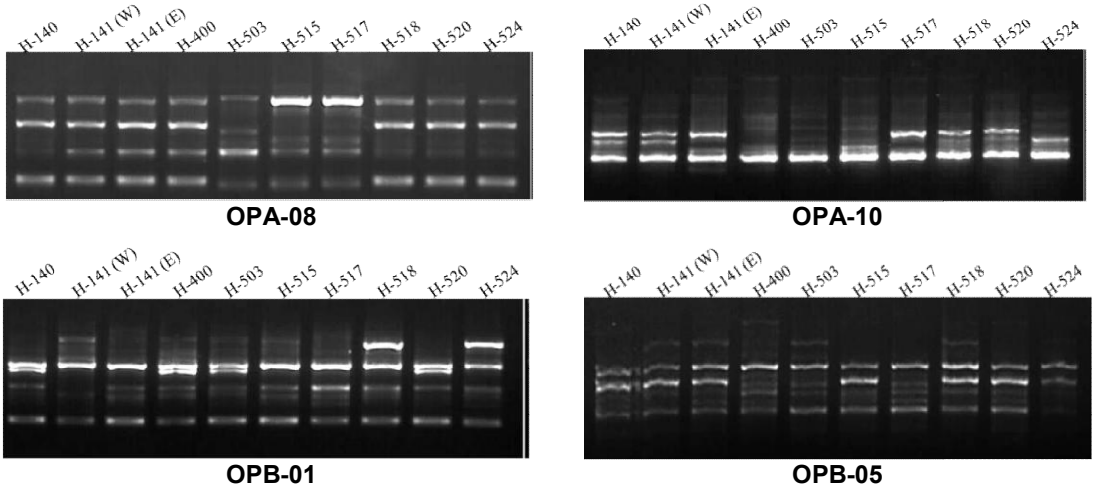

Fig. a. Molecular profiles of 10 hybrid litchis obtained using different RAPD markers

Table 4. Level of polymorphism observed in hybrid litchi using RAPD primers

Sr. no.	Name of Litchi Hybrids	Number of total fragments	Average fragments per primer	Number of polymorphic fragments	Average polymorphic fragments per primers
1.	H-140	38	3.8	18	1.8
2.	H-141(W)	37	3.7	17	1.7
3.	H-141(E)	39	3.9	19	1.9
4.	H-400	41	4.1	21	2.1
5.	H-503	38	3.8	18	1.8
6.	H-515	41	4.1	21	2.1
7.	H-517	36	3.6	16	1.6
8.	H-518	38	3.8	18	1.8
9.	H-520	39	3.9	19	1.9
10.	H-524	37	3.7	17	1.7

Table 5. Analysis of primer pairs used for the amplification in the ten hybrids of litchi

Sr.no.	Primer (Operon code)	Major allele frquency	Genotype no.	No. of allele	Gene diversity	Heterozygosity	PIC
1.	OPA-03	0.35	3	5	0.72	1.00	0.67
2	OPA-07	0.30	4	4	0.72	0.00	0.67
3.	OPA-08	0.30	2	4	0.74	1.00	0.69
4.	OPA-10	0.45	3	4	0.59	1.00	0.50
5.	OPA-13	0.30	2	4	0.74	1.00	0.69
6.	OPA-14	0.25	3	5	0.77	1.00	0.73
7.	OPB-01	0.35	3	5	0.76	1.00	0.72
8.	OPB-05	0.20	4	7	0.84	1.00	0.81
9.	OPB-08	0.25	5	7	0.82	1.00	0.80
10.	OPB-10	0.35	2	4	0.71	1.00	0.66

Table 6. Jaccard's similarity coefficient calculated from RAPD data among 10 litchi hybrids

Hybrids	H-140	H-141 (W)	H-141 (E)	H-400	H-503	H-515	H-517	H-518	H-520	H-524
H-140	1.00									
H-141(W)	0.74	1.00								
H-141(E)	0.75	0.65	1.00							
H-400	0.80	0.70	0.74	1.00						
H-503	0.69	0.67	0.57	0.65	1.00					
H-515	0.72	0.70	0.70	0.71	0.76	1.00				
H-517	0.76	0.70	0.70	0.67	0.64	0.79	1.00			
H-518	0.73	0.70	0.67	0.65	0.77	0.80	0.68	1.00		
H-520	0.83	0.65	0.66	0.78	0.71	0.70	0.74	0.71	1.00	
H-524	0.67	0.68	0.62	0.63	0.74	0.81	0.70	0.79	0.69	1.00

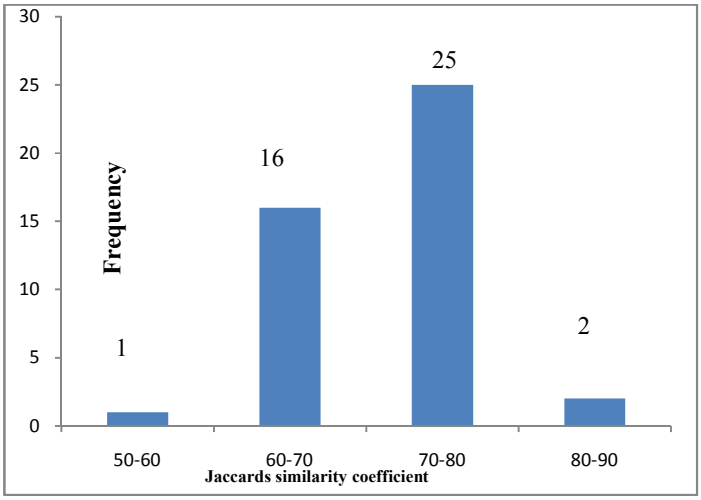

Fig. 1. Jaccard's similarity coefficient

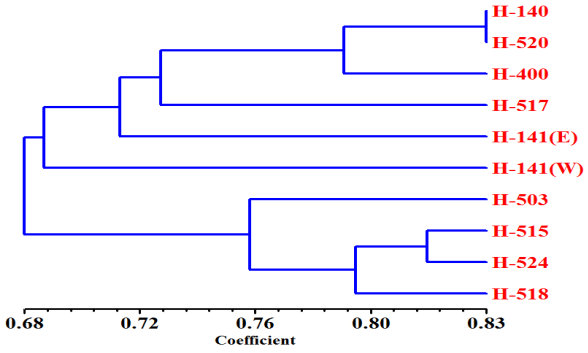

Fig. 2. Dendrogram

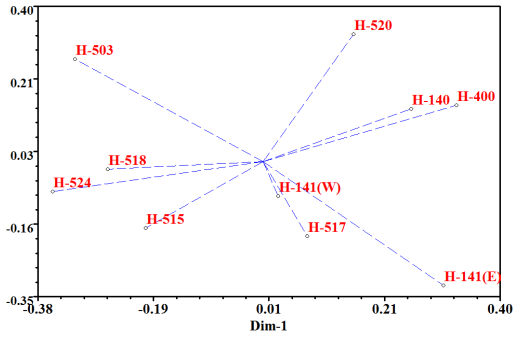

Fig. 3. Principal component analysis

The dendrogram classified the hybrids into two distinct clusters. The first cluster included six hybrids, H-140, H-520, H-400, H-517, H-141(E) and H-141(W). The second cluster included 4 hybrids, H-503, H-515, H-524 and H-518.

4. DISCUSSION

The use of different types of RAPD molecular markers has proven positive to distinguish and establish the genetic relationship among different litchi hybrids [5,20,21,6,7,8,22,23]. The percentage (60.61%) of polymorphic bands revealed in the present study indicates that

RAPD fragments are polymorphic and particularly informative in the evaluation of genetic relationships. Similar levels of a polymorphism associated with RAPD markers have been reported in earlier studies involving litchi [8]. Wang et al. [8] reported that the percentage of polymorphic bands (PPB) was 64.1% by using twelve random primers from eighty ones selected litchi cultivars. The similarity coefficient of most of the litchi germplasm ranged from 55-80% which indicates that the genetic relationships between them were closely related [23]. Present investigation showed genetic similarity coefficients were in the range of 0.57–0.83. This is compatible with earlier studies that reported genetic similarities of 0.55–0.80 using RAPD analysis. The dendrogram generated by this studied classified the whole hybrids into two major clusters. Our findings are also following the previous studies which showed dendrogram grouped the genotypes into three clusters, which correspond well with their pedigree relationship [20,8].

5. CONCLUSION

The present investigation is the footstep for characterisation of hybrid litchi developed by hybridisation program in BAU, Sabour. RAPD generated an informative polymorphism for the genotypic discrimination. This finding will considerably facilitate marker validation for agronomically important characters, genome mapping and recombination breeding programmes aiming at the development of new cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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