THE ASIAN JOURNAL OF HORTICULTURE
Volume **8** | Issue 2 | December, 2013 | 526-533

Research Paper

Article history **:**
Received : 08.06.2013
Revised : 17.09.2013
Accepted : 30.09.2013

Molecular characterization and genetic diversity analysis of sapota genotypes by RAPD markers

SUHASINI JALAWAADI, R.C. JAGADEESHA[1], D. KIRANSHANKAR[1], KULAPATI HIPPARAGI[1], G. PRABHULING[1] AND **H.R. BASAVARAJAPPA[1]**

Members of the Research Forum

Associated Authors:
[1]K.R.C. College of Horticulture, Arabhavi, BELGAUM (KARNATAKA) INIDIA

Author for correspondence :
SUHASINI JALAWAADI
K.R.C. College of Horticulture, Arabhavi, BELGAUM (KARNATAKA) INIDIA
Email : plantdoctorkrishna@gmail.com

ABSTRACT : To study the molecular characterization of 31 accessions of sapota comprising 19 cultivars, 6 land races and 6 hybrids of sapota using PCR based Random Amplified Polymorphic DNA (RAPD) markers. DNA isolated by CTAB method was used for amplification of 48 markers by using 7 RAPD primers. All 48 polymorphic fragments were used to generate the similarity matrix and construct a dendrogram. In this matrix highest genetic similarity of 100 per cent was observed between the 'DHS-1' and 'DHS-2', while least (23 %) was between 'PKM-3' and 'Culcutta Round'. UPGMA (Unweighted Pair Group Method with Arithmetical averages) cluster analysis using Jaccard's co-efficient of similarity of 31 genotypes showed medium to high diversity, which are distributed between the ranges of 35-100 per cent. For the 19 cultivars, the maximum similarity of 91 units was found between 'Murabba' and 'Oval'. 'Pala' and 'Virudhnagar' were closely placed due to 71 per cent oval shaped fruits. Among landraces the round shaped fruit bearing landraces were grouped in cluster-I, and 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' were closely associated with each other as they are having distinctively round shaped fruits and they are 78 per cent similar. Among hybrids 'DHS-1', 'DHS-2', 'PKM-2', CO-1' and 'CO-3' which are grouped together because of their oval and round shaped fruits with spreading and single bearing habit.

KEY WORDS : RAPD, Molecular characterization, Jaccard's co-efficient

HOW TO CITE THIS ARTICLE : Jalawaadi, Suhasini, Jagadeesha, R.C., Kiranshankar, D., Hipparagi, Kulapati, Prabhuling, G. and Basavarajappa, H.R. (2013). Molecular characterization and genetic diversity analysis of sapota genotypes by RAPD markers. *Asian J. Hort.*, **8**(2) : 526-533.

Sapota (*Achras zapota* L.) is one of the delicious fruit of humid tropical and subtropical regions, belonging to the family Sapotaceae. It is also called by other names, such as chikku, sapota plum, sapodilla or prickly pear.

Sapota is a hardy crop and fairly less susceptible to pest and disease which may be due to the presence of endogenous phenolics substances (Lakshminarayana and Subramanyam, 1966). India is a leading producer of sapota with an area and production of 1, 58,000 ha and 13, 46,000 metric tonnes, respectively (Anonymous, 2010).

Since, sapota is an open pollinated crop, a great deal of variability was thrown up in the population. A few of the cultivars have been selected based on local preferences for different fruit characteristics and later cultivated by farmers through vegetative propagation. Therefore, the high genetic variability in Indian sapota cultivars might have originated through seedling segregation, inter crossing among cultivars or because of a large number of cultivars or genotypes were introduced. Despite its wide cultivation throughout the world, the genetic diversity of the crop has not been studied using molecular markers. In sapota, most of the work on variability has been carried out using morphological characteristics such as tree shape, structure, leaf colour, shape, fruit size and shape (oval and round).

This study appears to be very arbitrary because morphological characteristics are influenced by agro-climatic conditions. So far, no studies were carried out to estimate the extent of genetic variability in sapota cultivars using either isozymes or DNA markers.

RAPD (pronounced "rapid") stands for Random Amplification of Polymorphic DNA. RAPD markers are attractive, because of their simplicity, versatility, modest cost and ability to detect even the relatively small amount of variation (Ragot and Hoisington, 1993).

RESEARCH METHODS

Plant material :

Leaf samples of the 31 sapota genotypes studied in this research were obtained from All India Co-ordinated Research Project on Tropical Fruits, K.R.C. College of Horticulture, Arabhavi (Karnataka).

Sample preparation:

The leaf samples for DNA extraction were prepared according to Tai and Tanksley (1990) with some modifications. The recently matured leaves that were free from damage caused by pests and diseases and available throughout the year were used for isolation of DNA. Approximately 150- 200 mg of leaf samples were grounded in effendorf tube by micro pestle for 5 – 10 minutes using extraction buffer.

DNA extraction:

Isolation of total genomic DNA of sapota was carried out according to Porebski *et al.* (1997), using CTAB with some modifications. 200 mg leaf samples were grounded with 750 ml extraction buffer (2 % CTAB, 100 mm Tris pH 8.0, 25 mm EDTA pH 8.0, 2m Nacl, 2% PVP and 2% β-marcaptoethonal) was incubated at 65 oC for 1 hour, the mixture was cooled to room temperature and 200 µl of chloroform: isoamyl alcohol (24:1 v/v) was added and the mixture was gently vortexed and spun at 6000 rpm at 10^0 C for 15 minutes and the supernatant was decanted and 500 µl chloroform: isoamyl alcohol (24:1 v/v) was added and contents were mixed and spun at 6000 rpm at 10 oC for 15 minutes. The supernatant was carefully decanted. This step was again repeated for one more time. To supernatant solution 0.6 (300 ml) volume of isopropanal was added, that tubes were incubated at -20^0 C for one hour. The contents of the tube were centrifuged at 6000 rpm at 10^0 C for 10 minutes, the supernatant was discarded and the pellet was washed twice with 70 per cent ethanol. The DNA pellet was dried at 37^0 C for 30 minutes the dried DNA pellet was dissolved in 125 µl of Tris EDTA (pH 8.00) buffer containing RNase (20 µg) and incubated at 37^0 C for one hour. Later DNA sample was treated with to remove RNA. The amount of DNA of all the samples was measured by spectrophotometer. The transmitance or absorbance red at A_{260}/A_{280}. The quantity of DNA was calculated by using formula :

$$\text{DNA mg/ml} = \frac{A_{26} + 50 + \text{dilution factor}}{1000\ \text{Dilution}} = 200$$

DNA amplification:

The basic protocol reported by Williams *et al.* (1990) for PCR was followed with slight modifications. The PCR reaction was carried out in 20 µl reaction mixture containing 40 ng template DNA, 0.3 µM primer, 0.3 units of *Taq* polymerase and 100 mM each dNTPs. Amplification was performed in a thermal cycler (Thermo Electro Corporation) for 38 cycle after on initial denaturtion at 94^0C for 5 minutes. In each cycle, denaturation for one minute at 94^0C, annealing for one minute at 38^0 C and extension step at 72^0 C for 1.5 minutes after the 38th cycle with final extension step of ten minutes at 72^0 C and 4^0 C hold.

DNA electrophoresis:

Amplified DNA fragments were separated out on 0.8 per cent agarose gel stained with ethidium bromide (10 mg/ml). Running buffer containing Tris- base, boric acid and EDTA (pH 8.0) was used for electrophoresis and for preparing gels. Wells were loaded with 20 µl of reaction volume and 5 µl of bromophenol blue together. Electrophoresis was conducted at 80-90 volts for 3-4 hours and gel was photographed under UV light by using a gel dock system (Bio-Vis).

DNA analysis:

The presence of a RAPD band in each position was recorded as 1 and its absence as 0. RAPD bands for each primer were scored separately. 'NTSYS' Ver 2.0i package (Rohlf, 1993) a computer application was used to generate Jaccard's co-effecient of similarity matrix, which was later used to construct a dendrograme of UPGMA (Unweighted Pair Group Method with Arithimetical averages). Co-efficient of Jaccard's (SJ) = $n_{AB}/n_A+n_B = n_{AB}$ is the number of bands common for samples A and B, n_A is the total number of bands in sample A and n_B is the total number of bands in sample B.

RESEARCH FINDINGS AND DISCUSSION

Recently matured leaf samples of different sapota genotypes which are available throughout the year were collected. The DNA obtained was amplifiable and of high quality. Spectrophotometer reading of 1.8-1.9 (260 nm per 280 nm) confirmed the good quality DNA. DNA isolated from 200 mg leaf using 750 µl of extraction buffer yielded

Table 1 : Synthetic deoxyribonuclotide 10-mer random primers used for genetic analysis of sapota

Primers	Nucleotide sequence (5' to 3')	Number of fragments amplified
OPA 04	AATCGGGCTC	04
OPB 03	CATCCCCCTG	05
OPB 06	TGCTCTGCCC	05
OPB 08	GTCCACACGG	07
OPC 02	GTGAGGCGTC	06
OPC 08	TGGACCGGTG	12
OPC 05	GATGACCGCC	09
Total		48

good quality.

In this study, 20 Operon random ten-base long, single stranded primers (OPA to OPG) were screened among these seven selected primers amplified unambiguous, readable and reproducible polymorphic bands. A total of 48 amplification products were produced from the selected 7 primers and the number of bands varied from 5-12 with an average of 7.5 bands per primer and the size ranged from 0.1 to 1 kb. The bands which were more than 100 bp are selected for scoring. The details are presented in Table 1. Each and every individual could be identified using the gel profiles (Fig. 5 and 6).

In the analysis, all 48 polymorphic fragments were used to generate the similarity matrix and construct a dendrogram. In this matrix highest genetic similarity of 100 per cent was observed between the 'DHS-1' and 'DHS-2', while least (23 %) was between 'PKM-3' and 'Culcutta Round' (Table 2). UPGMA (Unweighted Pair Group Method with Arithmetical averages) cluster analysis using Jaccard's co-efficient of similarity of 31 genotypes showed medium to high diversity, which are distributed between the ranges of 35-100 per cent (Fig. 1). Genotypes were categorized into two distinctive groups based on fruit characteristics (shape) and canopy shape. Where oval and round shaped fruit bearing genotypes were grouped in first cluster and oblong fruit bearing genotypes are grouped in second cluster. Among oval and round shape fruit bearing genotypes, the round shape fruit bearing genotypes have been grouped in separate sub clusters which includes, 'DHS-1', 'DHS-2', 'Cricket Ball', 'PKM-1', 'PKM-2', 'Gavarayya', 'Guthi', 'Oval', 'Murabba', 'CO-1', 'Kirthbharti', 'French Indochina', 'CO-2', 'Cricket Ball (ARSA)', 'Tagarampudi', 'CO-3', 'Cricket Ball (Udupi)' and 'Cricket-Ball (Sirsi)'. In this round shaped fruit cluster 'DHS-1' and 'DHS-2' were closed together because of their same parentage, again these two were closed with one of their parent 'Cricket-ball'. 'Oval' and 'Murabba' were closed together and they were 91 per cent similar due to their canopy shape. Guthi and Oval were closely associated due to oval shaped fruits. 'CO-1' and 'French-Indochina' were

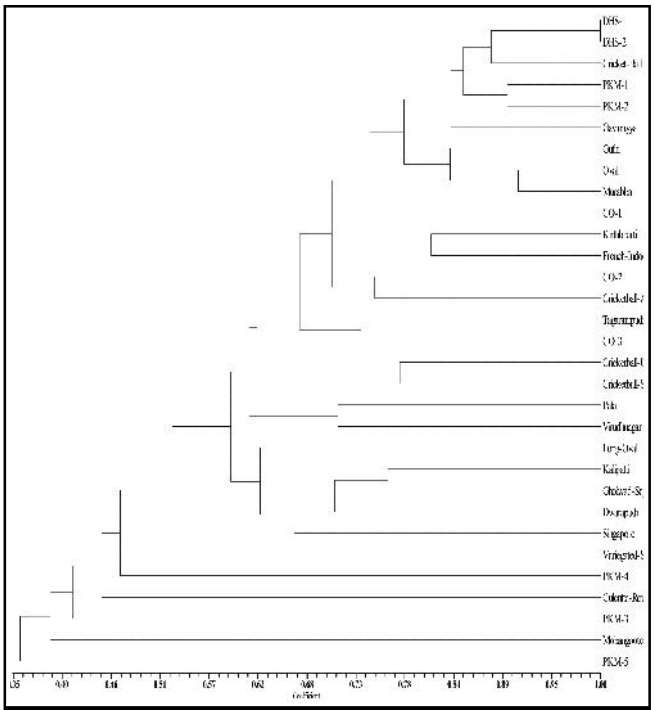


Fig. 1 : Dendrogram of different sapota genotypes (31) according to Jaccard's co-efficient

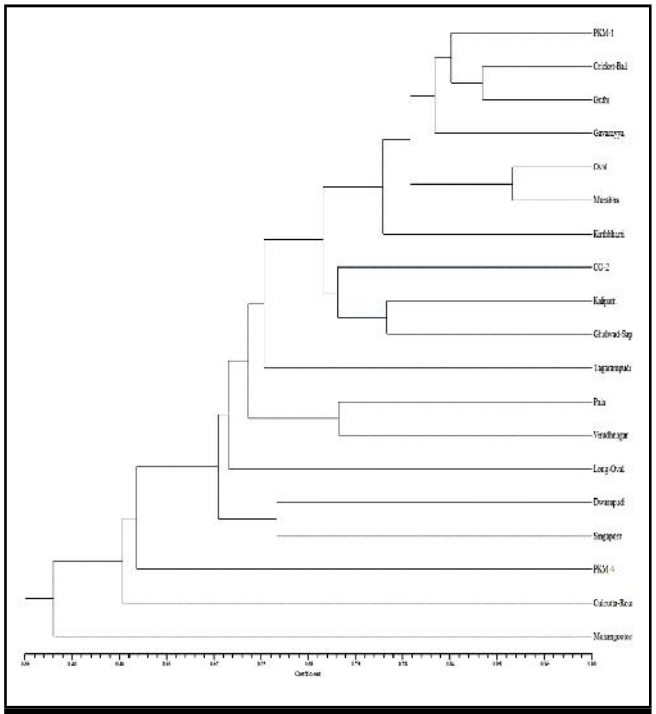


Fig. 2 : Dendrogram of different sapota cultivars (19) according to Jaccard's co-efficient

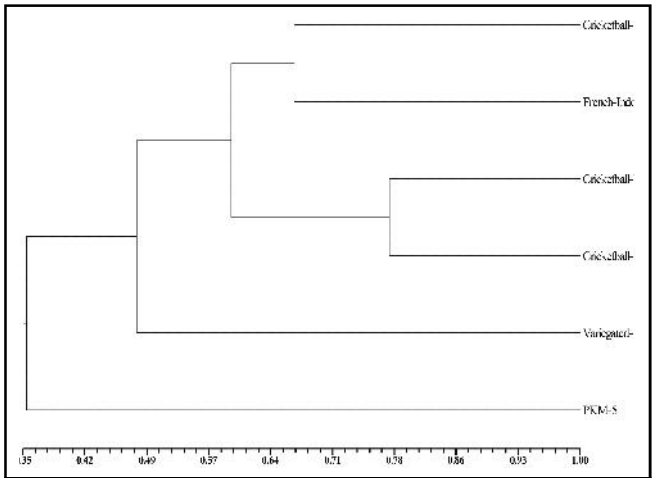


Fig. 3 : Dendrogram of different sapota landraces (6) according to Jaccard's co-efficient

Table 2 : Jaccard's similarity co-efficient of 31 genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	1.00																														
2	1.00	1.00																													
3	0.87	0.87	1.00																												
4	0.84	0.84	0.90	1.00																											
5	0.48	0.48	0.50	0.48	1.00																										
6	0.74	0.74	0.73	0.67	0.41	1.00																									
7	0.67	0.67	0.65	0.63	0.36	0.73	1.00																								
8	0.88	0.88	0.87	0.79	0.48	0.84	0.76	1.00																							
9	0.56	0.56	0.58	0.56	0.37	0.51	0.56	0.61	1.00																						
10	0.58	0.58	0.56	0.58	0.38	0.61	0.75	0.67	0.76	1.00																					
11	0.82	0.82	0.81	0.73	0.47	0.78	0.70	0.88	0.59	0.66	1.00																				
12	0.84	0.84	0.83	0.81	0.43	0.71	0.68	0.84	0.61	0.63	0.78	1.00																			
13	0.79	0.79	0.78	0.76	0.50	0.72	0.73	0.79	0.68	0.74	0.84	0.76	1.00																		
14	0.82	0.82	0.75	0.68	0.47	0.74	0.66	0.82	0.46	0.57	0.76	0.73	0.74	1.00																	
15	0.76	0.76	0.70	0.73	0.42	0.66	0.75	0.76	0.61	0.71	0.71	0.73	0.74	0.63	1.00																
16	0.71	0.71	0.64	0.67	0.45	0.60	0.61	0.71	0.48	0.51	0.70	0.62	0.68	0.60	0.70	1.00															
17	0.78	0.78	0.76	0.69	0.45	0.79	0.76	0.83	0.62	0.72	0.82	0.74	0.91	0.77	0.73	0.62	1.00														
18	0.70	0.70	0.64	0.67	0.42	0.68	0.65	0.75	0.50	0.61	0.69	0.71	0.63	0.61	0.74	0.74	0.71	1.00													
19	0.63	0.63	0.56	0.59	0.32	0.62	0.54	0.63	0.50	0.57	0.62	0.64	0.56	0.57	0.59	0.56	0.59	0.71	1.00												
20	0.41	0.41	0.38	0.36	0.39	0.42	0.38	0.45	0.34	0.31	0.44	0.41	0.38	0.35	0.39	0.42	0.39	0.40	0.30	1.00											
21	0.53	0.53	0.46	0.45	0.32	0.60	0.64	0.53	0.49	0.51	0.51	0.49	0.62	0.55	0.53	0.54	0.66	0.59	0.40	0.34	1.00										
22	0.47	0.47	0.44	0.43	0.42	0.44	0.47	0.51	0.57	0.55	0.50	0.52	0.58	0.38	0.57	0.48	0.53	0.50	0.33	0.44	0.41	1.00									
23	0.71	0.71	0.70	0.68	0.47	0.78	0.66	0.82	0.55	0.66	0.76	0.68	0.74	0.81	0.67	0.65	0.72	0.61	0.62	0.44	0.48	0.46	1.00								
24	0.55	0.55	0.53	0.51	0.34	0.71	0.59	0.64	0.61	0.68	0.68	0.56	0.61	0.54	0.52	0.49	0.65	0.58	0.50	0.41	0.57	0.43	0.63	1.00							
25	0.68	0.68	0.66	0.64	0.44	0.74	0.67	0.77	0.61	0.76	0.76	0.74	0.69	0.67	0.68	0.57	0.78	0.75	0.63	0.41	0.49	0.56	0.71	0.74	1.00						
26	0.39	0.39	0.44	0.38	0.35	0.32	0.28	0.39	0.48	0.37	0.41	0.43	0.35	0.32	0.33	0.26	0.36	0.33	0.36	0.32	0.24	0.31	0.32	0.38	0.39	1.00					
27	0.41	0.41	0.38	0.36	0.23	0.46	0.50	0.50	0.44	0.53	0.48	0.41	0.42	0.44	0.39	0.52	0.43	0.44	0.43	0.36	0.34	0.39	0.53	0.55	0.50	0.26	1.00				
28	0.66	0.66	0.59	0.62	0.40	0.68	0.56	0.66	0.53	0.60	0.65	0.57	0.68	0.65	0.54	0.59	0.71	0.64	0.71	0.38	0.58	0.32	0.70	0.62	0.61	0.39	0.42	1.00			
29	0.48	0.48	0.45	0.48	0.24	0.55	0.63	0.51	0.51	0.71	0.54	0.51	0.61	0.46	0.56	0.38	0.64	0.50	0.46	0.33	0.49	0.47	0.50	0.64	0.63	0.30	0.50	0.53	1.00		
30	0.67	0.67	0.70	0.68	0.44	0.65	0.57	0.67	0.59	0.61	0.61	0.68	0.64	0.56	0.58	0.50	0.63	0.60	0.56	0.41	0.50	0.44	0.61	0.68	0.67	0.44	0.46	0.59	0.53	1.00	
31	0.67	0.67	0.70	0.63	0.44	0.65	0.53	0.67	0.44	0.47	0.66	0.63	0.59	0.61	0.58	0.50	0.63	0.56	0.52	0.46	0.42	0.35	0.61	0.58	0.62	0.44	0.41	0.55	0.45	0.78	1.00

1-DHS-1	8-Cricket-Ball	15-Cricketball-ARSA	22-PKM-4	27-Culcutta Round
2-DHS-2	9-Long-Oval	16-Tagarampudi	23-French-Indochina	28-Virudhanagar
3-PKM-1	10-Kalipatti	17-Murabba	24-Dwarapudi	29-Singapore
4-PKM-2	11-Guthi	18-CO-3	25-Gholwad-Sapota	30-Cricketball-Udupi
5-PKM-3	12-Gavarayya	19-Pala	26-PKM-5	31-Cricketball-Sirsi
6-CO-1	13-Oval	20-Mohangootee		
7-CO-2	14-Kirthbharti	21-Variegated-Sapota		

closely associated with each other as they were having pinkish pulp. 'Tagarampudi' and 'CO-3' were closely grouped each other due to round shaped fruits and 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' were grouped together due to their round shaped fruits.

Sub cluster of oval shaped fruit bearing genotypes were 'Pala', 'Virudhanagar', 'Long Oval', 'Kalipatti', Gholwad Sapota', 'Dwarapudi' and 'Singapore', they were all clustered together with same fruit shape (oval) except 'Dwarapudi', (round) but it was similar in canopy shape.

The oblong shape fruit bearing genotypes were narrowly associated with the round and oval shaped fruit bearing genotypes, which included 'PKM-4', 'Culcutta Round', PKM-3'and 'Mohangootee' and all these genotypes were having oblong shaped fruit except 'Culcutta Round', whereas 'PKM-5' has been made distinctive cluster in the dendrogram because of its distinctive oblong shaped fruit which was closely associated with 'PKM-4', 'PKM-3' and 'Mohangootee'. Similar discriminative ability of RAPD markers in identifying species, cultivars and hybrids has been demonstrated in many other crops like mango (Schnell *et al.,* 1995; Hemanth *et al.*,2000), passion fruit (Cerqueira-Silva *et al.*,2010), pomegranate (Ercisli *et al*., 2011), pear (Yildirim *et al.,* 2010), jack fruit (Anburaj and Sudarmani, 2010), guava (Bajpai *et al*., 2008), citrus (Baig *et al.*, 2008). fig (Salhihannach *et al*., 2006), apricot (Ercisli *et al*., 2009).

Table 3 : Jaccard's similarity co-efficient of 19 sapota cultivars

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1.00																		
2	0.65	1.00																	
3	0.87	0.76	1.00																
4	0.58	0.56	0.61	1.00															
5	0.56	0.75	0.67	0.76	1.00														
6	0.81	0.70	0.88	0.59	0.66	1.00													
7	0.83	0.68	0.84	0.61	0.63	0.78	1.00												
8	0.78	0.73	0.79	0.68	0.74	0.84	0.76	1.00											
9	0.75	0.66	0.82	0.46	0.57	0.76	0.73	0.74	1.00										
10	0.64	0.61	0.71	0.48	0.51	0.70	0.62	0.68	0.60	1.00									
11	0.76	0.76	0.83	0.62	0.72	0.82	0.74	0.91	0.77	0.62	1.00								
12	0.56	0.54	0.63	0.50	0.57	0.62	0.64	0.56	0.57	0.56	0.59	1.00							
13	0.38	0.38	0.45	0.34	0.31	0.44	0.41	0.38	0.35	0.42	0.39	0.30	1.00						
14	0.44	0.47	0.51	0.57	0.55	0.50	0.52	0.58	0.38	0.48	0.53	0.33	0.44	1.00					
15	0.53	0.59	0.64	0.61	0.68	0.68	0.56	0.61	0.54	0.49	0.65	0.50	0.41	0.43	1.00				
16	0.66	0.67	0.77	0.61	0.76	0.76	0.74	0.69	0.67	0.57	0.78	0.63	0.41	0.56	0.74	1.00			
17	0.38	0.50	0.50	0.44	0.53	0.48	0.41	0.42	0.44	0.52	0.43	0.43	0.36	0.39	0.55	0.50	1.00		
18	0.59	0.56	0.66	0.53	0.60	0.65	0.57	0.68	0.65	0.59	0.71	0.71	0.38	0.32	0.62	0.61	0.42	1.00	
19	0.45	0.63	0.51	0.51	0.71	0.54	0.51	0.61	0.46	0.38	0.64	0.46	0.33	0.47	0.64	0.63	0.50	0.53	1.00

1. PKM-1	6. Guthi	11. Murabba	16. Gholwad Sapota
2. CO-2	7. Gavarayya	12. Pala	17. Culcutta Rounr
3. Cricket Ball	8. Oval	13. Mohangootee	18. Virudhanagar
4. Long Oval	9. Kirthbharti	14. PKM-4	19. Singapore
5. Kalipatti	10. Tagarampudi	15. Dwarapudi	

Table 4 : Jaccard's similarity co-efficient of 6 landraces

	CB-ARSA	F-Indochina	CB-Udupi	CB-Sirsi	V-Sapota	PKM-5
CB-ARSA	1.00					
F-Indochina	0.67	1.00				
CB-Udupi	0.58	0.61	1.00			
CB-Sirsi	0.58	0.61	0.78	1.00		
V-Sapota	0.53	0.48	0.50	0.42	1.00	
PKM-5	0.33	0.32	0.44	0.44	0.24	1.00

CB- Cricket Ball, F- French, V- Variegated

Variation among cultivars:

In the analysis, all 48 polymorphic fragments were used to generate the similarity matrix and construct a dendrogram (Fig. 2). Jaccard's co-efficient of similarity analysis has been carried out for cultivars. In the present investigations cultivars were categorized into two groups based on fruit characteristics and canopy shape. Among all cultivars, round and oval shaped fruit bearing cultivars were grouped in first cluster which included, 'PKM-1', Cricket Ball', 'Guthi', 'Gavarayya', 'Oval', 'Murabba', 'Kirthbharthi', 'CO-2', 'Kalipatti', 'Gholwad Sapota' and 'Tagarampudi', 'Pala', 'Virudhanagar', 'Long Oval', 'Dwarapudi' and 'Singapore', within this 'PKM-1', Cricket Ball', 'Guthi', 'Gavarayya', 'Oval', 'Murabba', 'Kirthbharthi' were grouped together, and 'CO-2', 'Kalipatti' and 'Gholwad Sapota', were grouped together and 'Kalipatti' and 'Gholwad Sapota' were closely associated with each other as they were having oval shaped fruits. 'Oval' and 'Murabba' were closely placed due to their canopy shape and they were 91 per cent similar. 'PKM-1' was closely associated with 'Guthi' because 'PKM-1' was selected from 'Guthi'. In sub-cluster, the oval shaped fruit bearing genotypes were 'Pala', 'Virudhanagar', 'Long Oval', 'Dwarapudi' and 'Singapore' these were all grouped together as they were having oval shaped fruit bearing genotypes, except 'Dwarapudi' but it was similar in canopy shape. 'Pala' and 'Virudhnagar' were closely placed due to 71 per cent oval shaped fruits (Table 3).

In second cluster were included, 'PKM-4', 'Culcutta Round' and 'Mohangootee' as they were having oblong shaped fruits, except 'Culcutta Round'. These oblong shaped fruit bearing genotypes were narrowly distributed to round and oval shaped fruit bearing genotypes. (Schnell *et al.*, 1995; Hemanth *et al.*,2000), Passion fruit (Cerqueira-Silva *et al.*,2010), Pomegranate (Ercisli *et al.*, 2011), Pear (Yildirim *et al.*, 2010), Jack fruit (Anburaj and Sudarmani, 2010), Guava (Bajpai *et al.*, 2008), Citrus (Baig et *al.*, 2008), Fig (Salhi-hannach *et al.*,2006), Apple (Yae *et al.*,1995), Pomegranate (Hasnaoui *et al.*, 2010), Mango

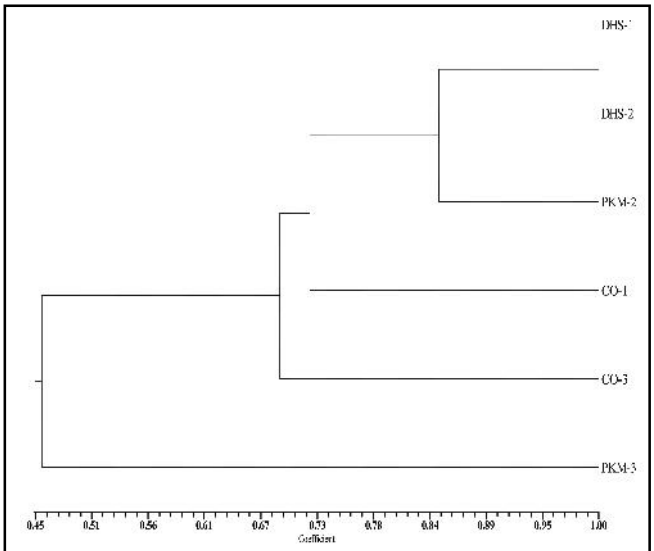
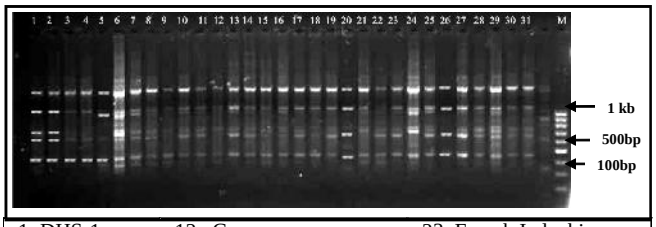


Fig. 4 : Dendrogram of different sapota landraces (6) according to Jaccard's co-efficient



1. DHS-1	12. Gavarayya	23. French Indochina
2. DHS-2	13. Oval	24. Dwarapudi
3. PKM- 1	14. Kirthbharti	25. Gholwad Sapota
4. PKM-2	15. Cricket Ball (ARSA)	26. PKM-5
5. PKM-3	16. Tagarmpudi	27. Culcutta Round
6. CO-1	17. Murabba	28. Virudhanagar
7. CO-2	18. CO-3	29. Singapore
8. Cricket Ball	19. Pala	30. Cricket Ball (Udupi)
9. Long Oval	20. Mohangootee	31. Cricket Ball (Sirsi)
10. Kalipatti	21. Varigated Sapota	
11. Guthi	22. PKM-4	

Fig. 5 : RAPD gel profile of 31 sapota genotypes according to Operon Primer C – 02

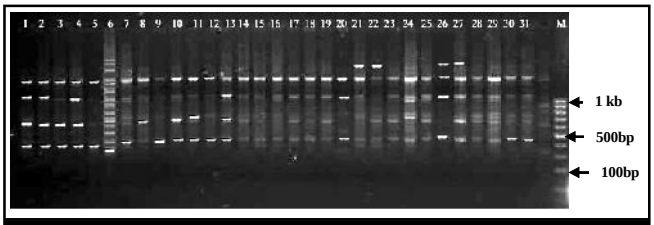


Fig. 6 : RAPD gel profile of 31 sapota genotypes according to Operon Primer C – 08

Table 5 : Jaccard's similarity co-efficient of 6 hybrids

	DHS-1	DHS-2	PKM-2	PKM-3	CO-1	CO-3
DHS-1	1.00					
DHS-2	1.00	1.00				
PKM-2	0.84	0.84	1.00			
PKM-3	0.48	0.48	0.48	1.00		
CO-1	0.74	0.74	0.67	0.41	1.00	
CO-3	0.70	0.70	0.67	0.42	0.68	1.00

(Hemanthkumar *et al*., 2001), Apricot (Ercisli *et al*., 2009), Strawberry (Graham and Mcnicol, 1995), Peach (Xiang *et al.*,1996), Loquat (Hussain *et al*., 2009).

Variation among landraces:

Jaccard's co-efficient of similarity analysis has been carried out for landraces also; land races are also distributed based on fruit characteristics (Fig. 3). Among landraces, the round shaped fruit bearing landraces were grouped in cluster-I, which included 'Cricket Ball (ARSA)', 'French Indochina', 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' and 'Variegated Sapota', within this cluster 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' were closely associated with each other as they were having distinctively round shaped fruits and they were 78 per cent similar (Table 4). Cricket Ball (ARSA)', 'French Indochina', 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' were closely associated with 'Variegated Sapota' as it was having round shaped fruits. 'PKM-5' placed separately in cluster-II, as it was having oblong shaped fruits, Sweet cherry (Gerlach and Stösser, 2008).

Variation among hybrids:

Separate Jaccard's co-efficient of similarity has been analyzed for development of dendrogram of hybrids (Fig. 4). Hybrids were distributed based on fruit shape, growth habit, parentage and bearing habit, which made two distinctive groups. Group-I contained 'DHS-1', 'DHS-2', 'PKM-2', CO-1' and 'CO-3' which were grouped together because of their oval and round shaped fruits with spreading and single bearing habit. Within this group 'DHS-1' and 'DHS-2' were 100 per cent similar (Table 5) as they were having same parentage ('Kalipatti' x 'Cricket Ball'). 'PKM-2' were closely related with 'DHS-1' and 'DHS-2' as it was having oval shape fruits as that of 'DHS-1', 'CO-1' and 'CO-2' were closely linked with 'DHS-2' due to round shaped fruits. 'PKM-3' has been placed in separate cluster (group-II), which have oblong shaped fruits, vertical growth and cluster bearing (Warburton *et al.*, 1996, Pooler and Scorza, 1995, mango (Bajpai *et al*., 2008).

In any introduced crop such as sapota, genetic variation depends on the number of introduced cultivars or genotypes. Generally it is expected to be very narrow, due to small number of introductions. Since, sapota is an introduced crop to India and no information is available on the number of cultivars introduced or the origin of these cultivars, it was expected that the variability in the germplasm would be less. However, results of this study contradict this. In dendrogram there were no closely formed groups, and the highest genetic similarity was indicating the presence of wide genetic diversity.

Conclusion:

The present study is to report on the molecular characterization of sapota. Since, sapota is an open pollinated crop, a great deal of diversity was thrown up in the population. A few of the cultivars have been selected based on local preferences for different fruit characteristics and later cultivated by farmers through vegetative propagation. Therefore, the high genetic variability in sapota might be originated through seedling segregation.

REFERENCES

Anburaj, J. and Sudarmani, D.N.P. (2010). Estimation of genetic diversity in jackfruit employing PCR based D-19 RAPD Fingerprinting. *Adv. Biotech.*, **10**(6) : 34-36.

Baig, M.N.R., Grewa, S. and Dhillon, S. (2008). Molecular characterization and genetic diversity analysis of citrus cultivars by RAPD markers. *Turky J. Agric*., **33** : 375-384.

Bajpai, A., Chandra, R., Rajan S. and Srivastava, N. (2008). RAPD and minisatellite markers for genetic diversity and relationship in guava varieties. *Indian J. Genet*., **68**(4): 441-445.

Cerqueira-Silva, C.B.M., Cardoso-Silva, C.B., Santos, E.S.L., Conceição, L.D.H.C.S., Pereira, A.S., Oliveira, A.C. and Corrêa, R.X. (2010). Genetic diversity in wild species of passion fruit (*Passiflora trintae*) based on molecular markers. *Genet., Mol. Res*., **9** (4): 2123-2130.

Ercisli, S., Agar, G., Yildirim, N., Esitken, A. and Orhan, E. (2009). Identification of apricot cultivars in Turkey (*Prunus armeniaca* L.) using RAPD markers. *Romanian Biotechnol. Letters,* **14**(4) : 4582-4588.

Ercisli, S., Gadze, J., Agar, G., Yildirim, N. and Hizarci, Y., (2011). Genetic relationships among wild pomegranate (*Punica granatum*) genotypes from Coruh Valley in Turkey. *Genet. Mol. Res*., **10** (1): 459-464.

Gerlach, H.K. and Stösser, R. (2008). Sweet cherry cultivar identification using RAPD-derived DNA fingerprints. *Acta Hort.*, **468** : 63-70

Graham, M.J. and Mcnicol, R.J. (1995). An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. *Theor. Appl. Genet*., **90** (7-8) : 1128-1132.

Hasnaoui, N., Mars, M., Chibani, J. and Trifi, M. (2010). Molecular polymorphisms in Tunisian pomegranate (*Punica granatum* L.) as revealed by RAPD fingerprints. *Diversity,* **2** (1) : 107-114.

Hemanth, K.N.V., Narayanaswamy, P., Suresh, N. Sondur, and Theerthaprasad, D. (2000). Paternity analysis of mango (*Mangifera indica* L.) hybrids through RAPD markers. National Seminar on Hi-Tech. Horticulture, Atria Hotel, Bangalore, 26-28th June, 2000

Hemanthkumar, N.V., Narayanswamy, P., Theerth Prasad, D., Mukunda, G.K. and Suresh, N.S. (2001). Estimation of genetic diversity of commercial mango (*Mangifera indica* L.) cultivars using RAPD markers. *J. Hort. Sci. & Biotech*., **76**(5): 529-533.

Hussain, A., Abbasi, N.A., Hafiz, I.A., Ahmad, Z. and Naqvi, S.M.S., (2009). Molecular characterization and genetic relationship among loquat (*Eriobotrya japonica* Lindl.) genotypes of Pakistan assessed by RAPD markers. *Pakistan. J. Bot*., **41**(5): 2437-2444.

Lakshminarayana, S. and Subramanyam, H. (1966). Physical, chemical and physiological changes in sapota fruit (*Achras sapota* L.) family Sapotaceae, during development and ripening. *J. Food Sci., Tech.,* **3** (4) : 151-154.

Pooler, M.R and Scorza, R. (1995). Aberrant transmission of RAPD markers in haploids, doubled haploids, and F1 hybrids of peach: observations and speculation on causes. *Scientia Hort.,* **64** (4): 233-241.

Porebeski, S., Bailey, G. and Baum, B.R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep.,* **15**(1): 8-15.

Ragot, M. and Hoisington, D.A. (1993). Molecular markers for plant breeding comaparisons of RFLP and RAPD genotyping cost. *Theor. Appl. Genet.,* **86** (8) : 975-984

Rohlf, F.J. (1993). NTSYS-pc Numerical taxonomy and multivariate system, version 2.0. *Appl. Biostatistics*. New York, USA.

Salhi-hannach, A., Chatti, K., Saddoud, O., Mars, M., Rhouma, A., Marrakchi, M. and Trifi, M. (2006). Genetic diversity of different Tunisian fig (*Ficus carica* L.) collections revealed by RAPD fingerprints. *Hereditas,* **143**: 15-22.

Schnell, R.J., Ronning, C.M. and Knight, J.Jr. (1995). Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. *Theor. Appl. Genet*., **90** (2): 269-274.

Tai, H.T. and Tanksley, D.S. (1990). A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Bio. Rep*., **8**(4): 297-303.

Warburton, M.L., Bercrra, V.V.L., Goffreda, J.C. and Bliss, F.A. (1996). Utility of RAPD markers in identifying genetic linkages to genes of economic interest in peach. *Theor. Appl. Genet.,* **93** (5-6) : 920-925.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., **18** (22) : 6531-6535.

Xiang, L.Z., Reighard, G.L., Baird, W.V., Abobott, A.G., Rajapakse, S. and Lu, Z.X. (1996). Identification of peach rootstock cultivars by RAPD markers. *Hort Sci.,* **31** (1) : 127-129.

Yae, B., Ko, K., Yae, B.W. and Ko, K.C. (1995a). Classification of *Malus domestica* cultivars by random amplified polymorphic DNA markers. *J. Korean Soc. Hort. Sci.,* **36**(6): 824-828.

Yildirim, N., Ercisli, S., Agar, G., Orhan, E. and Hizarci, Y. (2010). Genetic variation among date plum (*Diospyros lotus*) genotypes in Turkey. *Genet, Mol Res*., **9** (2): 981-986.

■ WEBLIOGRAPHY

Anonymous (2010). *www. nhb.gov.in/statics/area-production-statistics.htm/.*