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Molecular characterization of hybrids and landraces of sapota by RAPD markers

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ABSTRACT : Molecular characterization of 6 land races and 6 hybrids of sapota were studied 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. 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 were having distinctively round shaped fruits and they were 78 per cent similar. Cricket Ball (ARSA)', 'French Indochina', 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' were closely associated with 'Variegated Sapota' as it is having round shaped fruits. Among hybrids '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.

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

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Sapota (*Achras zapota* L.) 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). It 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.

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). 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).

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, Kittur Rani Channamma 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 Porebeski *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 65^0 C 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 10^0C 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 -20^0C for one hour. The contents of the tube were centrifuged at 6000 rpm at 10^0C 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 37^0C 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 :

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

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 38^0C and extension step at 72^0C for 1.5 minutes after the 38th cycle with final extension step of ten minutes at 72^0C and 4^0C 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 yielding 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 were presented in Table 1.

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

Variation among landraces:

Jaccard's co-efficient of similarity analysis has been

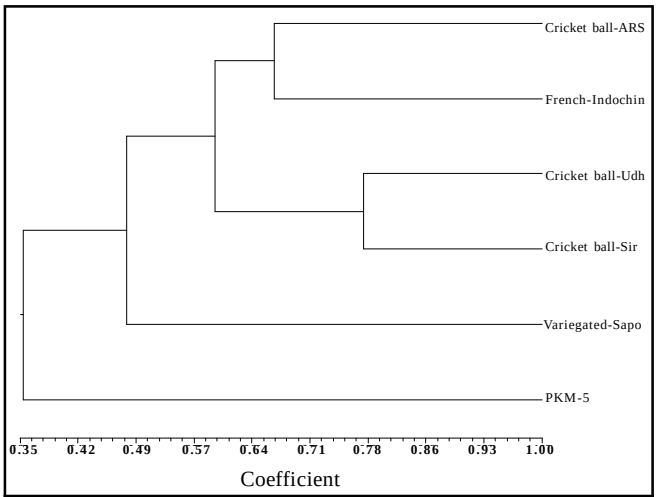

Fig. 1 : Dendrogram of different sapota landraces (6) according to Jaccard's Co-efficient

Table 2 : 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

Table 3 : 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

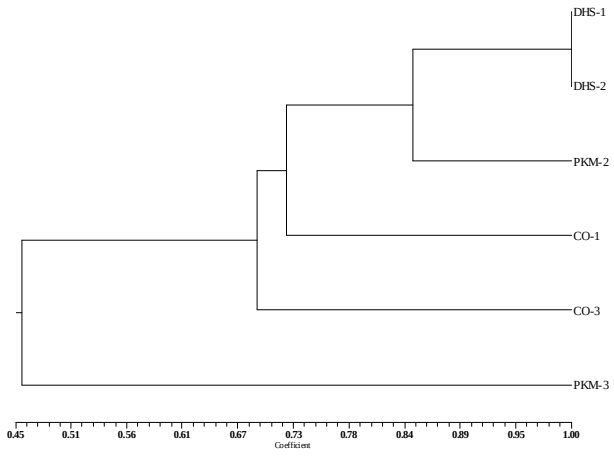

Fig. 2 : Dendrogram of different sapota hybrids (6) according to Jaccard's co-efficient

carried out for landraces also; land races were also distributed based on fruit characteristics (Fig. 1). 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 2). Cricket Ball (ARSA)', 'French Indochina', 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' were closely associated with 'Variegated Sapota' as they were having round shaped fruits. 'PKM-5' placed separately in cluster-II, as it was having oblong shaped fruits. Similar results were found in sweet cherry (Gerlach and Stösser, 2008) and apricot (Ercisli *et al.*, 2009).

Variation among hybrids:

Separate Jaccard's Co-efficient of similarity has been analyzed for development of dendrogram of hybrids (Fig. 2). 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 3) as they were having same parentage ('Kalipatti' X 'Cricket Ball'). 'PKM-2' was closely related with 'DHS-1' and 'DHS-2' as it had 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' was placed in separate cluster (group-II), which had oblong shaped fruits, vertical growth and cluster bearing. Similar discriminative ability of RAPD markers in identifying species, cultivars and hybrids has been demonstrated in many other crops like avocado (Lewis, 1992), apple (Koller *et al.*,1993), mango (Bajpai *et al.*, 2008), pear (Yildirim *et al.*, 2010) and (Pooler and Scorza, 1995).

Conclusion:

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.

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