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Genetic diversity analysis of papaya (*Carica papaya* L.) genotypes in Andaman Islands using morphological and molecular markers

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In Andaman and Nicobar Islands, wide diversity of papaya germplasm exists and has not been characterized. Therefore, there is difficulty in differentiating the papaya accessions in different regions of the Islands. Hence, the present investigation was undertaken for characterizing *Carica papaya* L. genotypes using morphological and DNA marker technology. Seventy three genotypes of *C. papaya* collected from different parts of South and Little Andaman Islands were used for analysis. Accessions collected from different parts of Islands showed widest morphological diversity in terms of fruit weight, fruit length, fruit girth, flesh thickness and total suspended solids content. A set of 24 ISSR primers were taken for DNA fingerprinting, among them 6 inter-simple sequence repeats (ISSRs) primers produced 212 amplicons out of which 62 gave 29.2% polymorphism. The maximum number of polymorphic bands was obtained from primer ISSR 15. Cluster analysis revealed that the genotypes are grouped into three clusters with 37% similarity. The present study has shown that the genotypes from various islands differ from each other of which the genotypes collected from Hut Bay (Ac.49) and Neil Island (Ac.14 and Ac.16) were distinct.

Key words: Inter-simple sequence repeats (ISSRs), genetic diversity, *Carica papaya*.

INTRODUCTION

Papaya (*Carica papaya* L.) belongs to the family Caricaceae. It is native to Tropical America from where it is spread to most of the Caribbean and Asian countries during the 16th century. Today, it is a widely distributed fruit crop throughout the tropical as well as the warmer subtropical regions of the world. The importance of papaya to agriculture and the world's economy is well understood by its wide distribution and substantial production in the tropical countries. According to the estimates of National Horticultural Board, India ranks first among papaya producers in the world with an annual production of about 41.96 million MT from an area of 1.06 lakh hectares with the productivity of 39.6 thousand MT/ha in India (NHB, 2011). In Andaman and Nicobar Islands, papaya is considered an important fruit crop, being produced for local consumption. Andaman and Nicobar Island's papaya production in 2011 was estimated at 2200 tons (Anonymous, 2011).

Several papaya varieties developed in different parts of India have been introduced to Andaman and Nicobar Islands by the settlers and Agricultural Department. Despite these introductions, no attempts to maintain varietal distinctness or develop varieties uniquely suited to Andaman and Nicobar Island conditions, have been documented. The open pollinating nature of papaya further tends to decrease varietal purity from one

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generation to the next.

Papaya germplasm shows considerable phenotypic variation for many horticultural traits (Ocampo, 2006). The different criteria which can be used to estimate genetic diversity include pedigree records, morphological traits and molecular markers (Weising et al., 2005). Plant taxonomy is traditionally dependent upon the comparative external morphological characters (Baxy, 2009), however these are environment and developmental stage dependent. Therefore, molecular markers are preferred choice for plant identification as they are detectable in all tissues and independent of environmental change (Ahmad et al., 2004; Tapia et al., 2005). Among the molecular markers Inter-Simple Sequence Repeats (ISSRs) are considered useful and have been extensively used for the identification of species or cultivars in a wide range of plants (Ahmad et al., 2010; Mariniello et al., 2002; Conner et al., 2001). ISSR amplifies inter-microsatellite sequences at multiple loci throughout the genome (Li and Xia, 2005) and permits the detection of polymorphism in microsatellites and intermicrosatellite loci without previous knowledge of DNA sequences.

Therefore, the main objective of the study is on the diversity analysis of diversity of papaya genotypes with an aim to select the pre-breeding materials which could be used for the improvement of papaya specifically suited for Andaman and Nicobar Islands conditions.

MATERIALS AND METHODS

Collection of papaya germplasm

A total of 73 genotypes (Table 1) were collected from different parts of South Andaman Islands, Neil Island, Havelock Islands and Little Andaman (Figure 1). The samples were collected from August to December 2012.

Morphological characterization of papaya

The physico-chemical data collected from the papaya germplasm included fruit length, fruit girth, fruit weight, flesh thickness, flesh colour and TSS. Fruit length was measured from the stylar end to pedicel end. Circumference of the fruit was measured with a cotton twine at the midpoint of the fruit and read accurately on meter scale. After cutting the fruits into two longitudinal halves, the pulp thickness was measured at mid point. Total soluble solids of the fruit were determined by 'ERMA' hand refractometer.

Molecular characterization of papaya

The total genomic DNA was extracted from disease free fresh young leaves by CTAB method (Murray and Thomson, 1980) with slight modification. Purity of DNA was checked by UV spectrophotometer and running in 1% agarose gel. The quantitation of DNA was done using UV spectrophotometer. PCR reaction was performed in final volume of 20 µl containing 10x assay buffer, 2.5 mM dNTPs, 0.5 unit of *Taq* DNA polymerase obtained from Bangalore Genei (Bangalore, India), 10 pmols/reaction primer and 100 ng of template DNA. 24 ISSR primers were procured from (Clonitec). The PCR was performed by initial denaturation at 94°C for 5 min followed by 45 cycle of denaturation at 94°C for one min, annealing at 52°C for one min, and extension at 72°C for two min and final elongation at 72°C for 7 min. The PCR products were resolved on 2% Agarose gel prepared in 1x TAE buffer containing 0.5 µg/ml of ethidium bromide at 100 V for 2.5 h. All the genotypes were scored for presence and absence of the ISSR bands and the data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of character and this data matrix was subjected to further analysis for calculating similarity matrix based on Jaccard's coefficient. The dendrogram was constructed using SAHN based UPGMA to infer genetic relationship (Rohlf, 1998; Sneath and Sokal 1973).

RESULTS AND DISCUSSION

Morphological characterization

Papaya accessions of different parts of Andaman display a wide variation in the fruit characteristics, including fruit length, fruit girth, fruit weight and flesh thickness (Table 2). The highest fruit length of 38 cm and minimum fruit length of 18 cm was recorded in Acc. No. 8 (Burmanallah) and ACC. No. 20 (Ferrargunj), respectively. Maximum fruit girth (45.20 cm) and fruit weight (2.10 kg) was recorded by ACC. No. 62 (Ramkrishna pur, Little Andaman) and Acc. No. 71 (Shyam Nagar, Havelock Island), respectively. Highest flesh thickness of 3.40 cm was observed in ACC. No. 66 from Vijaya Nagar, Havelock Island while highest TSS content of 13.60° Brix was recorded in ACC. No. 64 from Gandhi Nagar, Havelock Island. Substantial morphological variation between the various accessions may be attributed to pollination, sexual recombination and perhaps mutation followed by intensive selection by isolated human communities in diverse environments (Martin, 1976). Problems in pollination, fruit set and production are intimately associated with sex expression resulting from genotype-environment interactions. Cultivar and environmental differences have also produced a wide array of modified forms, so the number and types of modifications have varied in reports by various researchers (Nakasone and Paull, 1998). The analysis using morphological characters revealed considerable amount of diversity among papaya accessions from different parts of Andaman that can be used in selecting diverse parents in breeding programme. However, there is the need for complementing similar work with other techniques such as DNA genetic markers to further accurately classify papaya germplasm existing in different parts of Andaman. Molecular study contributes a great deal in the detection, characterization and evaluation of genetic diversity (Tapia et al., 2005).

Molecular characterization

A total of 24 ISSR primers were used to analyze the genetic diversity among 73 different genotypes of *Carica*

Table 1. Different genotypes of Carica papaya collected from different part of Andaman and Nicobar Islands

Genotype code	Location	Genotype code	Location
Genotypes of South Andaman		Ac.No.38	Wandoor-1
Ac.No.1	Burmanallah-1	Ac.No.39	Wandoor-2
Ac.No.2	Burmanallah-2	**Genotypes of Little Andaman**	
Ac.No.3	Burmanallah-3	Ac.No.40	Kitchadnallah-1
Ac.No.4	Calicut -1	Ac.No.41	Kitchednallah-2
Ac.No.5	Calicut -2	Ac.No.42	Harmindar Bay-1
Ac.No.6	Calicut -3	Ac.No.43	Harmindar Bay-2
Ac.No.7	Lalmitti	Ac.No.44	Harmindar Bay-3
Ac.No.8	Birdline-1	Ac.No.45	Hathidera-1
Ac.No.9	Birdline-2	Ac.No.46	Hathidera-2
Ac.No.10	Birdline-3	Ac.No.47	Hathidera-3
Ac.No.11	Tushnabad	Ac.No.48	V.K. Pur-1
Ac.No.12	Colinpur-1	Ac.No.49	V.K. Pur-2
Ac.No.13	Colinpur-2	Ac.No.50	Ongitikri-1
Ac.No.14	Cattlegunj-1	Ac.No.51	Ongitikri-2
Ac.No.15	Cattlegunj-2	Ac.No.52	Ongitikri-3
Ac.No.16	Bloomsdale-1	Ac.No.53	Ongitikri-4
Ac.No.17	Bloomsdale-2	Ac.No.54	Ongitikri-5
Ac.No.18	Bloomsdale-3	Ac.No.55	Kalinagar
Ac.No.19	Bloomsdale-4	Ac.No.56	Breakwater-1
Ac.No.20	Ferrargunj-1	Ac.No.57	Breakwater-2
Ac.No.21	Ferrargunj-2	Ac.No.58	Sundarpur
Genotypes of Neil Island		Ac.No.59	Butler Bay
Ac.No.22	Laxmipur-1	Ac.No.60	Netaji Nagar
Ac.No.23	Laxmipur-2	Ac.No.61	Farm tikri
Ac.No.24	Laxmipur-3	Ac.No.62	Ramakrishnapur
Ac.No.25	Laxmipur-4	Ac.No.63	Baratpur
Ac.No.26	Baratpur-1	**Genotypes of Havelock Island**	
Ac.No.27	Baratpur-2	Ac.No.64	Gandhi nagar
Ac.No.28	Baratpur-3	Ac.No.65	1 No.
Ac.No.29	Baratpur-4	Ac.No.66	Vijaya Nagar
Ac.No.30	Baratpur-5	Ac.No.67	Krishna Nagar-1
Ac.No.31	Baratpur-6	Ac.No.68	Krishna Nagar-2
Ac.No.32	Baratpur-7	Ac.No.69	Krishna Nagar-3
Ac.No.33	Baratpur-8	Ac.No.70	3 No.
Genotypes of Wandoor and Manjeri		Ac.No.71	Shyam Nagar-1
Ac.No.34	Manjeri-1	Ac.No.72	Shyam Nagar-2
Ac.No.35	Manjeri-2	Ac.No.73	Radha Nagar
Ac.No.36	Manjeri-3		
Ac.No.37	Manjeri-4		

papaya. Among 24 ISSR primers used, 6 primers produced amplification and produce a total of 212 amplicons across 73 genotypes of which 62 amplicons were found to be polymorphic with the level of polymorphism of 29.2% (Table 3). The amplicons size with ISSR primers varies from 0.8 to 0.2 kb. The primers varied in the number of bands produced which ranged from 6 in ISSR 15 to 2 in ISSR 7 with an average of 4.17 bands per primer. Range of polymorphic bands per primer was 6 in ISSR 15 to 1 in ISSR 7. Marker ISSR 15 had the highest PIC value of 0.44 whereas marker ISSR 1 had the lowest PIC of 0.13. The level of polymorphisms in papaya is ranging from of 2 to 6 alleles per primer pair in 73 papaya accessions. This may be due to cross pollination and wide geographical diversity from which the papaya accessions were collected.

★ Surveyed area (South Andaman and Little Andaman)

Figure 1. Map of Andaman Islands showing the sample sites.

Table 2. The standard deviation calculated comparing the measured physico-chemical characteristics.

Characters	Maximum	Minimum	Mean	Standard deviation
Fruit length (cm)	38.00	18.00	28.16	0.44
Fruit girth (cm)	45.20	21.00	33.29	5.64
Fruit weight (kg)	2.10	0.350	1.09	6.03
Flesh thickness (cm)	3.40	1.50	2.42	0.50
TSS (^{O}Brix)	13.60	6.50	10.57	2.39

Table 3. ISSR primers used and polymorphism generated.

ISSR primer No.	Primer name	Sequence	Total no. of bands	Average no. of bands across genotypes	Total No. of bands per genotypes	No. of polymorphic bands	PIC value
1	UBC 840	$(GA)_6$ YT	144	28.8	5	4	0.13
2	UBC 807	$(AG)_8$T	175	58.33	3	2	0.21
7	UBC 842	$(GA)_8$G	124	62.00	2	1	0.21
12	UBC 841	$(GA)_8$YC	179	44.75	4	4	0.40
15	UBC 836	$(AG)_8$YA	212	35.33	6	6	0.44
16	UBC 857	$(AC)_8$YC	127	25.40	5	5	0.42
	Total		961	254.61	25	22	1.81
	Average		274.57	42.44	4.17	3.67	0.30

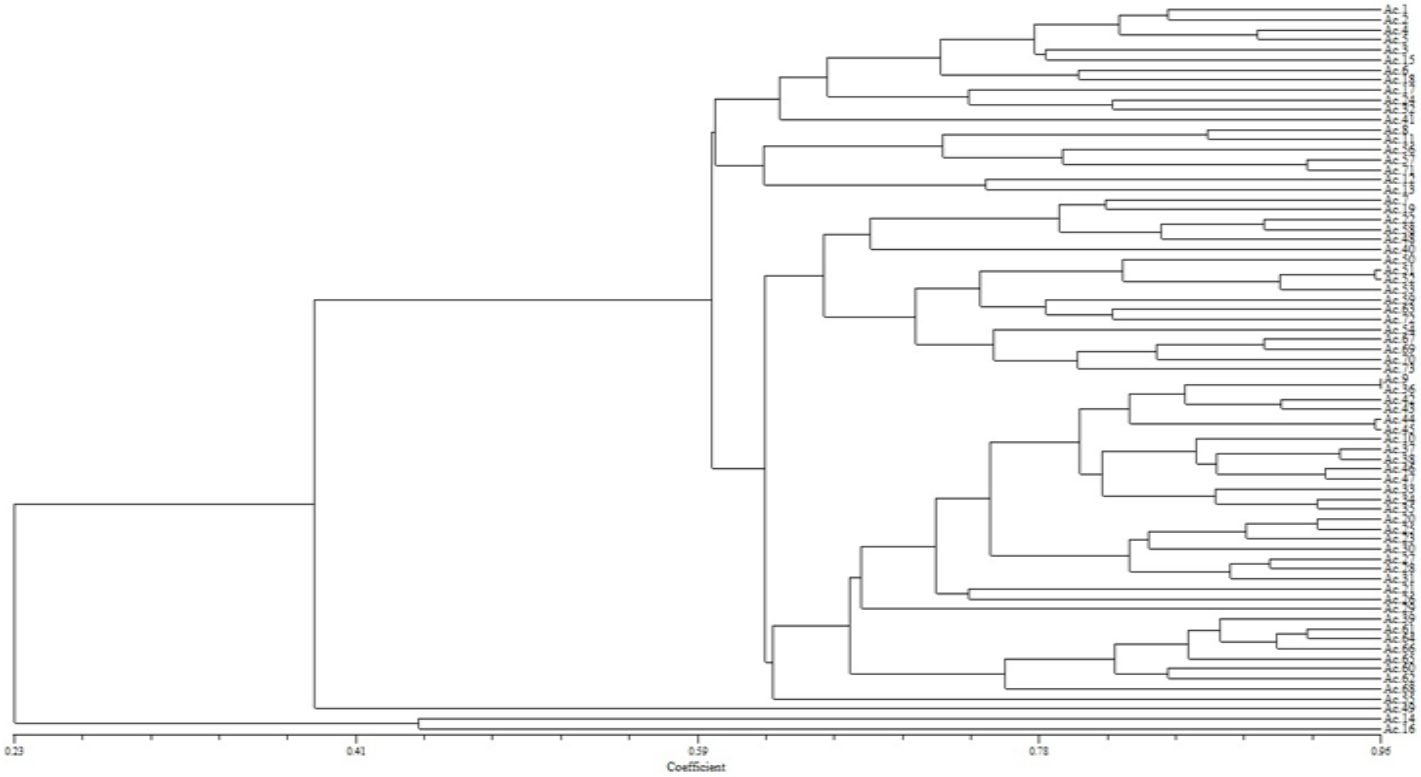

Figure 2. Dendrogram showing genetic diversity amongst 73 genotypes of *Carica papaya* by ISSR primers.

Dendrogram generated by UPGMA (Figure 2) differentiated all the 73 genotypes at 37% similarity. Dendrogram could be divided into three clusters namely cluster A, Cluster B and Cluster C. Cluster A had 70 genotypes at 58% similarity. Cluster B had only one genotype. Cluster C had Ac. 14 and Ac.16 at 43% similarity. Cluster A, subgrouped into four sub-clusters namely sub-cluster I, II, III and IV at 64% similarity. Sub cluster I had 19 genotypes and most were collected from South Andaman while sub cluster II composed 17 genotypes and represented the collections from Neil Island. Sub cluster III had 24 genotypes and most of the genotypes were collected from little Andaman and sub cluster IV comprised 9 genotypes, of which 8 genotypes were collected from Havelock Island. The little Andaman genotypes showed the widest diversity, as they are scattered all over the dendrogram. Generally, the relationships among accessions in the cluster could be attributed to their diversity, geographical locations and also due to exchange of plant genetic resources among farmers within and between the provinces.

In the present study, 73 genotypes collected from the

same geographical regions normally grouped together and depict high similarity (37% with ISSR). This high genetic similarity suggested that there is more gene flow within the agro ecological zone. High gene flow may be due to random mating with very little selection. These findings are in accordance with Shakya et al. (2010) in *Syzygium cuminii*, and Kingdom et al. (2007) in *Annona* spp*.* Selection of germplasm on the basis of the dendrogram can be used for collection of appropriate parental material to improve horticultural traits. In this study genotypes Ac.No. 49 collected from Hut bay and Ac.No.14 and Ac.No. 16 collected from Neil Island showed maximum diversity with other Island genotypes. Hence, this genotype could be utilized to generate sufficient genetic variability by crossing with other Island genotypes. In conclusion, this is the first report of *carica papaya* on “analysis of biodiversity” by using molecular markers in Bay Islands. ISSR markers were valuable for the determination of genetic diversity and relationships amongst 73 genotypes of *carica papaya* collected from different parts of Andaman Islands. The data obtained in this study provided valuable genetic information, especially in the absence of comprehensive studies on the *Carica papaya.*

Conclusions

After conducting both the molecular and morphological analysis of the papaya varieties, analysis of the morphological characters was found to be less informative. More differences could be assessed from the molecular study and they were found to be reliable for the differentiation of the different papaya varieties. These preliminary results will pave the way to more in depth studies on the characterization of the papaya germplasm in Andaman Islands which will eventually facilitate breeding programmes for the development of new cultivars using the elite local cultivars.

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