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Genetic diversity study in papaya (*Carica papaya* L.) cultivars using RAPD and ISSR markers

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Thirteen papaya cultivars and lines were used to assess genetic diversity through dominant PCR based markers (16 RAPD and 12 ISSR primers). RAPD (random amplified polymorphic DNA) primers gave 58 polymorphic bands out of 126 with 47.19% polymorphism and ISSR (Inter simple sequence repeats) primers produced 49.80% polymorphism with 37 polymorphic bands out of 65 bands. Dendrogram showed clear grouping ancestrally related papaya cultivars. Principle coordinate analysis (PCoA) showed congruence with dendrogram pattern while bootstrap values at major nodes in both marker systems as well as in pooled data indicated robustness of cluster pattern in dendrogram construction. Analysis of molecular variance (AMOVA) indicated that highly significant genetic variability was obtained through both marker systems while cultivars showed noticeable variation in case of RAPD markers. Mantel's test between similarity and cophenetic coefficient matrices in different combination showed poor to very good correlation between matrices. This study indicates relation between papaya cultivars based on their ancestral relationship which can be utilized for conservation of important cultivars and development of future strategies for crop improvement programme.

Keywords: Genetic diversity, *Carica papaya*, RAPD, ISSR, AMOVA

Introduction

Papaya (*Carica papaya* L.) belongs to family *Caricaceae* and considered to originate from Central to South America[1,2]. It is cultivated in tropical and subtropical region[2,3] of the planet with annual world production of more than 12.4 million tons[4], making it economically valuable fruit crop[5,6]. Papaya is a perennial fruit crop which produces monoecious, dioecious and hermaphrodite sex flower[7,8]. The fruit has low calorie value (39 Kcal) and is a good source of carotenoid (precursor of vitamin A) and lycopene (an antioxidant)[9-10]. Papaya fruit latex contains proteases which help to cure digestive track problems as well as have industrial importance[9-10]. Phenolic compounds from various part of plant showed anti-bacterial, anti-fungal and anti-helminthic activity[9-10]. It has other important medicinal properties such as, anti-cancer, act as contraceptive and abortion of pregnancy[9-10].

Papaya is a well characterized plant and considered an important model fruit crop for basic and molecular research as its genome is completely decoded[11]. However, the information on its sexual and genetic variation is still limited[11]. Papaya showed different sex forms as well as variation in fruit flesh colour, height, susceptibility to biotic and abiotic stresses which are related to genetic level variation. Papaya improvement programs are going on at many places through intergeneric breeding programme with wild relative species. Studies on phylogenetic and genetic variation showed relation of papaya to herbaceous plant which can help breeder to improve their breeding programs[12,13]. Molecular markers (RAPD and ISSR) were used to study the genetic and phylogenetic relationship of papaya in different part of the world by various workers[1,2,5,7].

Rodríguez *et al*[2] tested eighteen *Carica cubensis* with nine RAPD primes and found them as a subspecies of *Carica papaya*. Shobhana *et al*[1] analysed eleven commercial papaya cultivars with two wild relatives, using eleven ISSR and sixteen RAPD primers and found ISSR markers superior than RAPD. Shudha *et al*[14] screened seventy-three papaya genotypes of Andaman and Nicobar Island with twenty-four ISSR primers and found sufficient distinctness between them. Dinesh *et al*[3], Jayavalli *et al*[12] and Shudha *et al*[13,14] used ISSR primers to screened *Papaya ring spot virus* resistant plant from breeding population.

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In present study, we have characterized thirteen Indian papaya cultivars to find out genetic diversity through molecular markers which can help in improving Indian papaya breeding program in future.

Table 1 — Information of the papaya cultivars used in the study

Cultivar	Information of Origin	Source
Pusa Delicious	Selection and sibmating of Ranchi	IARI, New Delhi
Pusa Giant	Selection of Ranchi	IARI, New Delhi
Pusa Dwarf	Selection and sibmating of Ranchi	IARI, New Delhi
Pusa Nanha	Mutation of Pusa Delicious	IARI, New Delhi
Pusa Majesty	Selection of Ranchi	IARI, New Delhi
Nanha Munna	Selection from Pusa Nanha	IARI, Pune
Pune Selection-1	Selection from Madhubala	IARI, Pune
Pune Selection-3	Selection from Madhubala	IARI, Pune
Pune Selection-5	Selection from Madhubala	IARI, Pune
CO-2	Selection from Peradeniya, a Sri Lanka genotype.	TNAU, Coimbatore
CO-7	Hybid between Coorg Honey Dew and CP58	TNAU, Coimbatore
Honey dew	Local adaptive genotype, Karnataka	CHES, Chettalli
Madhubindu	Selection from Honey Dew	JAU, Junagadh

Materials and Methods

Plant Material and DNA Extraction

Four different young leave from seedlings of one month old thirteen papaya cultivars (Table 1) were collected under ice condition during 2014 at Indian Agricultural Research Institute, Regional Station, Pune, Maharashtra. One hundred milligram pooled leaves were used for DNA isolation by Qiagene plant DNA isolation minikit. Quantity was checked in molecular device Spectramax 450 using DNA quantify slide and quality was evaluated on 0.8% agarose gel.

Random Amplified Polymorphic DNA (RAPD)

Twenty-three decamer oligonucleotide primers for RAPD were screened out of which sixteen RAPD primers gave good result (Table 2). Reaction mixture for RAPD was prepared according to Urasaki *et al*[15] and PCR condition was maintained as described by Rodriguez *et al*[2] with higher annealing temperature. Each 20 µl reaction mixture for RAPD contain 20 mM Tris-HCL (pH 8.4), 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM of each dNTPs, 20 pmoles primers, 50 ng genomic DNA and 1 unit *Taq* DNA polymerase (Bangalore Genei, Bangalore). PCR was performed in Eppendorf vepoprotect thermocycler with cycle condition for RAPD; initial denaturation step of 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 38°C for 1 min and 72°C for 2 min and final

Table 2 — Band size, number of bands and fragments, percentage of polymorphism, PIC, RPI and Rp obtained by RAPD primers

Sr. No.	RAPD primers	Allele/band size (bp)	No. of polymorphic bands / No. of total bands	Percent polymorphism	Total no. of fragments amplified	PIC	RPI	Resolving power
1	BB-18	168-4505	5/8	62.50	80	0.860	6.88	2.46
2	BC-201	170-4200	5/6	83.33	43	0.790	4.74	3.08
3	I-08	481-2617	2/5	40.00	51	0.758	3.79	0.46
4	IBRC-RP07	168-2823	3/9	33.33	97	0.875	7.88	1.38
5	OPA-05	370-4552	5/9	55.55	85	0.873	7.86	3.54
6	OPA-11	294-2465	4/6	66.67	58	0.799	4.79	1.38
7	OPB-07	242-1287	3/7	42.86	68	0.834	5.84	2.31
8	OPB-08	100-3466	6/10	60.00	79	0.860	8.60	2.77
9	OPB-10	140-3108	3/10	30.00	104	0.885	8.85	2.00
10	OPF-02	146-2096	2/8	25.00	94	0.870	6.69	1.38
11	OPY-07	229-2604	6/8	75.00	63	0.842	6.74	3.38
12	OPW-12	164-1955	2/10	20.00	118	0.895	8.95	1.08
13	T-01	232-2561	4/8	50.00	75	0.843	6.74	1.38
14	T-12	610-3589	3/7	42.86	76	0.846	5.92	2.00
15	W-11	342-2427	3/7	42.86	77	0.849	5.94	2.15
16	W-15	139-5195	2/8	25.00	94	0.867	6.93	0.77
Total			58/126	-	1262	-	-	-
Average			3.63/7.88	47.19	78.88	0.847	6.70	1.97

elongation step at 72°C for 10 min. PCR products were loaded on 1.5% agarose gel, using 2.5 µl 6X gel loading buffer and electrophoresis were performed at constant 100V for 2 h in 1X TBE buffer. The gel was stained with ethidium bromide and visualized under UV light.

Inter-Simple Sequence Repeat (ISSR)

Eighteen UBC series primers for ISSR were screened out of which twelve ISSR primers gave good result (Table 3). Reaction mixture for ISSR primers was prepared according to Jayavalli *et al*[12] and PCR condition was maintained as described by Dinesh *et al*[3] with slight modification. Each 20 µl reaction mixture for ISSR contain 20 mM Tris-HCL (pH 8.4), 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM of each dNTPs, 20 pmoles primers, 50 ng genomic DNA and 1 unit *Taq* DNA polymerase (Bangalore Genei, Bangalore). PCR was performed in Eppendorf vepoprotect thermocycler with cycle condition for ISSR; initial denaturation step of 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, Ta for 1 min and 72°C for 2 min and final elongation step at 72°C for 5 min. PCR products were loaded on 1.5% agarose gel, using 2.5 µl 6X gel loading buffer and electrophoresis were performed at constant 100V for 2h in 1X TBE buffer. The gel was stained with ethidium bromide and visualized under UV light.

Gel Documentation and Scoring

Gels stained with ethidium bromide were documented using GENESYS gel documentation system (Syngene, UK). Molecular size of amplicons was analysed by using a 100 bp DNA ladder (Bangalore Genei, Bangalore). The banding patterns obtained from both RAPD and ISSR for all 15 cultivars were scored using binary codes presence (1) or absent (0), each of which treated as independent characters.

Analysis of Genetic Diversity Study

Pairwise Jaccard's similarity coefficient between all thirteen cultivars was calculated[16]. Jaccard's similarity matrix was subjected for cluster analysis using an unweighted pair group method with arithmetic means (UPGMA)[17]. NTSYSpc version 2.02 software[18] was used to generate a dendrogram and to test its robustness, bootstrap analysis was carried out using 1000 resamplings in WINBOOT software[19]. Number of amplified products, polymorphic band and percentage of polymorphism was calculated for each primer. Average polymorphic information content (PIC) values for marker was calculated by taking the mean of PIC value for all the markers produce by primer. PIC = $1- (p^2-q^2)$, where p^2 is the proportion of accessions having an amplicons and q^2 is the proportion of accessions not having the amplicons. Resolving power of primer was calculated using formula $Rp = \sum Ib$, where Ib (band informativeness) = $1 - [2 \times (0.5-p)]$, p being the proportion of cultivars containing the band[20]. PIC and Rp gives an idea about the primer which could better distinguish the cultivar. The utility of each marker system was determined by calculating a diversity index (DI), effective multiplex ratio (EMR) and marker index (MI) as describe by Powell *et al*[21] DI for genetic marker was calculated as: $DI = 1-\sum pi^2$ (where

Table 3 — Band size, number of bands and fragments, percentage of polymorphism, PIC, RPI and Rp obtained by ISSR primers

Sr. No.	ISSR primers	Allele/band size (bp)	No. of polymorphic bands / No. of total bands	Percent polymorphism	Total no. of fragments amplified	PIC	IPI	Resolving power
1	UBC-808	629-2862	2/6	33.33	63	0.808	4.85	0.92
2	UBC-810	375-699	1/3	33.33	28	0.564	1.69	0.30
3	UBC-817	267-1176	1/5	20.00	58	0.788	3.94	0.92
4	UBC-818	247-2812	4/8	50.00	82	0.859	6.87	2.15
5	UBC-824	180-1991	3/7	42.86	65	0.811	5.68	0.62
6	UBC-826	280-1269	4/4	100.00	28	0.686	2.74	2.00
7	UBC-829	395-1889	3/7	42.86	87	0.857	6.00	0.62
8	UBC-834	148-630	3/5	60.00	55	0.789	3.95	1.38
9	UBC-857	280-2116	5/6	83.33	61	0.806	4.84	0.92
10	UBC-864	213-1847	10/10	100.00	48	0.820	8.20	3.23
11	UBC-873	228-544	1/4	25.00	40	0.683	2.73	0.15
Total			37/65	-	615	-	-	-
Average			3.64/5.91	49.80	55.91	0.770	4.68	1.20

'pi' is the allele frequency of the ith allele). DI for polymorphic marker is: (Di_{av}) p = $\sum Di_n/n_p$ (where Di_{av} is arithmetic mean of DI for that marker assay, n_p is the number of polymorphic loci and n is the total number of loci). EMR can be described as a product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay. EMR = n_p (n_p/n).

Analysis of molecular variance (AMOVA), principal coordinated analysis (PCoA) and Mantel's test was performed using GenAlex software[22]. AMOVA provide information regarding variation between cultivars and within cultivars[23]. The relation between distance matrix elements based on their first two principle coordinate was inferred from PCoA analysis and presented graphically. The similarity matrix from RAPD and ISSR marker was subjected to Mantel's test which provides cooperative analysis between both markers[24].

Results

Random Amplified Polymorphic DNA (RAPD)

Highest polymorphic bands (6) were given by OPB - 08 and OPY - 07 while lowest number of polymorphic bands (2) was observed in I - 08, OPF - 02, OPW - 12 and W - 15 (Table 2). Primer OPB - 08, OPB - 10 and OPW - 12 gave highest total number of bands (10); however, I - 08 gave lowest five total numbers of bands. Primer BC - 201 gave 83.33% polymorphism which was the highest and the lowest value in OPW - 12 (20%). Primer OPW - 12 gave highest 118 total number of fragments amplified. At the same time, BC - 201 produced 43 total number of fragments amplified. Primer OPW - 12 gave highest polymorphic information content (0.895) and RAPD primer index (8.95) while I-08 gave lowest polymorphic information content (0.758) and RAPD primer index (3.79). Highest and lowest resolving power was found with OPA-05 (3.54) and I - 08 (0.46), respectively. In this study, OPW - 12 to be proved very good primer for highest number of total band production, total number of fragments amplified, polymorphic information content and RAPD primer index. Primer BC - 201 proved better for highest number of polymorphic bands production and percentage of polymorphism. Primer I - 08 gave poor result for number of polymorphic bands, total number of bands, polymorphic information content (PIC), RAPD primer index and resolving power. RAPD primers amplified bands with size range of 100 bp (OPB - 08) to 5159 bp (W - 15). They amplified 58 polymorphic bands and 126 total numbers of bands with an average of 3.63 and 7.88, respectively. They produce 1262 total number of fragments amplified with an average of 78.88 fragments. Average values for percentage polymorphism, PIC, RAPD primer index and resolving power (Rp) was 47.19%, 0.847, 6.70 and 1.97, respectively. Diversity index (DI), effective multiplex ratio (EMR) and marker index (MI) values were calculated as 0.845, 1.92 and 1.63, respectively.

Many researchers carried out diversity study in papaya. Shobhana *et al*[1] found 118 bands with 91 polymorphic bands. Saxena *et al*[25] reported 138 total numbers of bands and 68 polymorphic bands. Similarly, Madarbokus and Ranghoo-Sanmukhiya[5] interpreted 149 total number of bands and 38 polymorphic bands. Shobhana *et al*[1] and Saxena *et al*[25] obtained 7.37 and 7.67 values for average total number of bands, respectively. In case of average polymorphic bands, Shobhana *et al*[1], Madarbokus and Ranghoo-Sanmukhiya[5] and Saxena *et al*[25] calculated 5.69, 4.75 and 3.77 average polymorphic bands, respectively. Shobhana *et al*[1] and Saxena *et al*[25] who obtained RAPD band range of 200-3500 bp while Rodriguez *et al*[2] interpreted range of 95-5490 bp. For percentage of polymorphism, Shobhana *et al*[1], Madarbokus and Ranghoo-Sanmukhiya[5] and Somsri and Bussabakornkul[8] who obtained 37.2%, 25.5% and 21.71% polymorphism while Saxena *et al*[25] reported 50.7% polymorphism. Rodriguez *et al*[2] calculated 0.703 values for PIC. Saxena *et al*[25] discovered DI, EMR and MI values as 0.41, 2.89 and 1.18, respectively, during their study. Mir *et al*[26] found average value of 1.65 and 0.75 for Rp and MI during their RAPD marker study on apricot.

Dendrogram constructed from RAPD data showed Jaccard's similarity range from 0.703 to 0.919 Figure 1(I). Pusa Delicious and Pusa Giant fall in group A with an average of 77.00% similarity with other cultivars among which Pusa Delicious showed least similarity with PS-01 and of 70.30% while Pusa Delicious and Pusa Giant had 81.60% similarity. Group B was subdivided in to two group of B_1 and B_2 among which B_2 contained Honey Dew and Madhubindu with 85% similarity with each other and an average 78% similarity with group B_1 which was further divided in to two subgroups of B_{1a} and B_{1b}. Subgroup B_{1a} contained Pusa Dwarf and CO-7 which had highest similarity with each other of 83.70% and around 83.50% with B_{1b} which was subdivided at 80% similarity with two groups of $B_{1b(i)}$ and $B_{1b(ii)}$. $B_{1b(i)}$

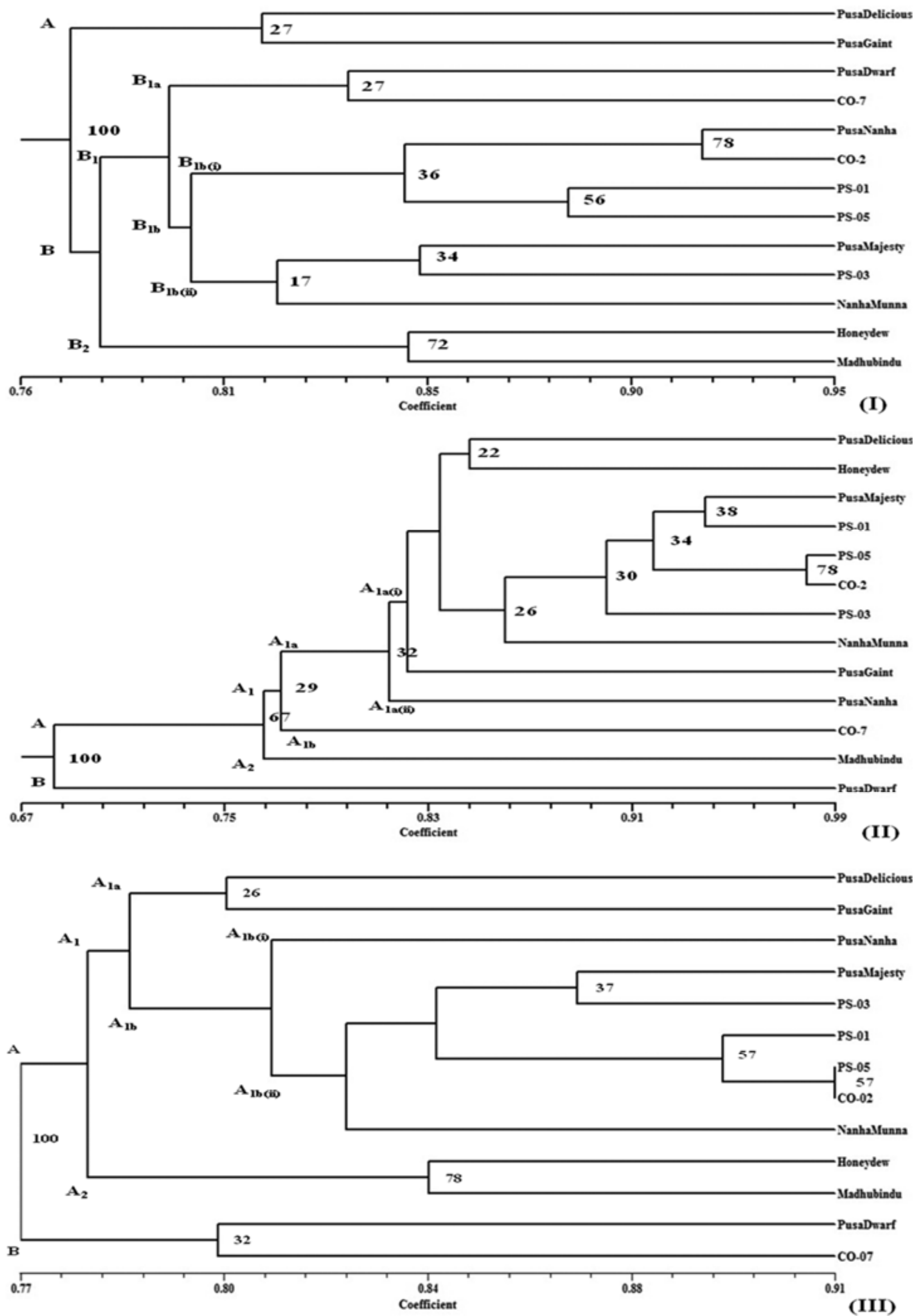

Fig. 1 — Dendogrograms generated using UPGMA for (I) RAPD (II) ISSR and (III) RAPD + ISSR for thirteen papaya cultivars.

contained Pusa Nanha, CO-2, PS-01 and PS-05 with similarity of approximately 85%. Pusa Nanha and CO-2 formed one group with similarity of 91.90% and PS-01 and PS-05 formed another with 88.80%. $B_{1b(ii)}$ contained three cultivars Pusa Majesty, PS-03 which formed one group of similarity of 85.30% while Nanha Munna showed separate lineage with an average of 82% with other two cultivars. Somsri and Bussabakornkul[8] found more than 90% similarity during their study on papaya. Shobhana *et al*[1] reported Pusa Delicious and Pusa Giant in same group during their study of RAPD markers which support our grouping result for these two cultivars while Saxena *et al*[25] also support our result for CO-2 and CO-7 which indicated these two cultivars in same group but different sub clusters.

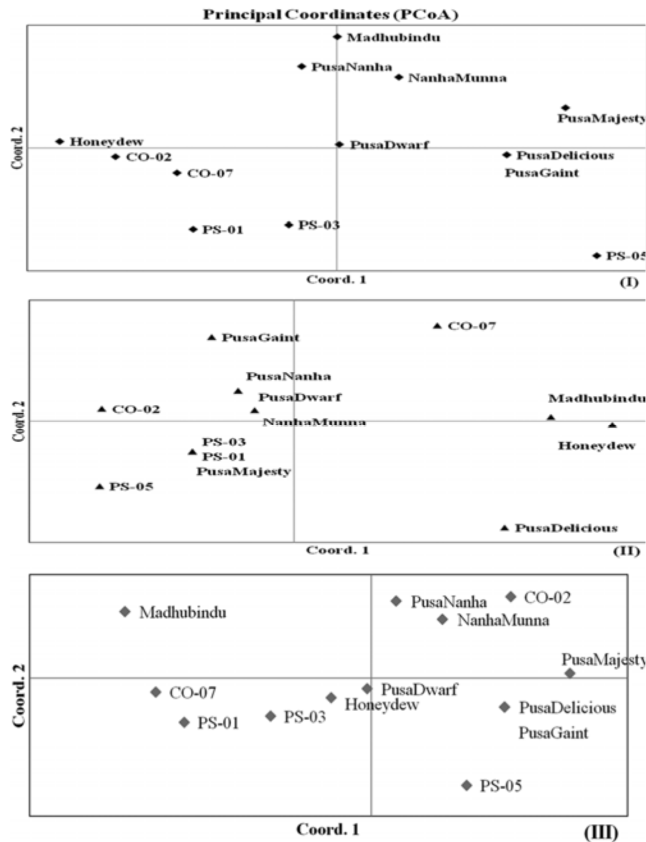

Fig. 2 — Two-dimension plot of principal coordinate analysis (PCoA) of (I) RAPD (II) ISSR and (III) RAPD + ISSR for thirteen papaya cultivars.

Bootstrap analysis was performed to showed robustness of tree and values were indicated at node for >15. Main node showed 100 values for showing good result while group A and $B_{1b(ii)}$ poor strength. At the same time, node showing >30 values were of good strength. Shobhana *et al*[1], Rodriguez *et al*[2] and Saxena *et al*[25] also calculated bootstrap values and found similar result with poor strength at initial node and significant strength at sub-sub-group level. Principal coordinate analysis (PCoA) showed similarity with dendogram as Pusa Delicious and Pusa Giant at same place and Pusa Majesty and Pusa Nanha in same plane [Fig. 2 (I)]. PCoA gave 82.06% of total genetic diversity with first three component PC1, PC2 and PC3 values of 0.335 (34.27%), 0.301 (30.75%) and 0.166 (17.04%), respectively.

Inter-Simple Sequence Repeats (ISSR)

Twenty-two UBC series ISSR primers were screened among which eleven gave polymorphic results (Table 3). The highest numbers of polymorphic and total number of bands were observed in UBC-864 (10) while the lowest number of polymorphic bands (1) was produced in UBC-810, UBC-817 and UBC-873. Primer UBC-810 also produced the lowest number of total bands (3). Primers UBC-826 and UBC-864 showed 100% polymorphism while UBC-817 indicated lowest 20% polymorphism. UBC-829 produced highest 87 total number of fragments while lowest of 28 were found in UBC-810. Maximum and minimum PIC were calculated for UBC-818 and UBC-810 with values of 0.859 and 0.564, respectively. UBC-810 gave lowest values for ISSR polymorphic index (1.69) and Rp (0.30) while UBC-864 gave highest values of 8.20 and 3.23 for same. Band size ranged from 148 bp to 2862 bp for ISSR primers in this study. ISSR primers produced 37 polymorphic and 65 total numbers of bands with average of 3.64 and 5.91, respectively. They produced 615 total numbers of fragments with an average of 55.91 fragments per primer. The average values for percentage of polymorphism, PIC, ISSR primer index and Rp were 49.80%, 0.770, 4.68 and 1.20, respectively. DI, EMR and MI were calculated as 0.770, 2.63 and 1.82 respectively, for ISSR primers.

For ISSR study, Shobhana *et al*[1] obtained 75 total bands with eleven ISSR primers while Saxena *et al*[25] produced 74 total numbers of bands with seven ISSR primers. Carrasco *et al*[27] produced 114 bands with seven ISSR primers and Rebelo da Costa *et al*[28] observed 94 bands with nine ISSR primers. Carrasco *et al*[27], Rebelo da Costa *et al*[28] and Saxena *et al*[25] found 87, 63 and 45 number of polymorphic bands, respectively. Saxena *et al*[25] recorded 61% polymorphism while Shobhana *et al*[1] and Sudha *et al*[13] reported 35% and 29.20% polymorphism, respectively. Sudha *et al*[13] calculated average value of 0.30 for PIC. Sudha *et al*[13] found bans size range from 200 bp to 800 bp. Shobhana *et al*[1] and Saxena *et al*[25] reported band size of 250 - 2500 bp while Rebelo da Costa *et al*[28] found it 180 bp to 1900 bp. Saxena *et al*[25] calculated MI of 2.19 for ISSR primers.

Dendrogram showed Jaccard's similarity range from 0.621 to 0.979 with two main groups A and B [Fig. 1 (II)]. Group B contained only Pusa Dwarf which showed approximately 68% similarity with group A which was divided in to subgroup A_1 and A_2. Madhubindu belonged to subgroup A_2 with 76.50% similarity with group A_1. Group A_1 is further subdivided in to A_{1a} and A_{1b} in which A_{1b} contained CO-7 with approximately 77% similarity with group

A_{1a}. Subgroup A_{1a} was divided in to $A_{1a(i)}$ and $A_{1a(ii)}$. Pusa Nanha represented group $A_{1a(ii)}$ with more than 82% similarity with group $A_{1b(ii)}$. Group $A_{1a(i)}$ was divided in to two group in which Pusa Giant form separate lineage of approximately 83% similarity with remaining eight cultivars. Honey Dew and Pusa Delicious formed one group of 84.60% similarity with each other and around 84% similarity with remaining six cultivars. Nanha Munna and PS-03 individually formed their separate lineage with approximately 86% and 90% similarity with remaining cultivars which they had 86.80% similarity with each other. Pusa Majesty and PS-01 formed one group while PS-05 and CO-2 formed second group with more than 91% similarity with each other. Pusa Majesty and PS-01 had 93.90% similarity while PS-05 and CO-2 had highest 97.90% similarity. Shobhana *et al*[1] also found more than 80% similarity between Pusa Delicious and Pusa Giant and Saxena *et al*[25] found CO-2 and CO-7 in different groups which support our study. Sudha *et al*[13] found more than 37% similarity which was better than our result.

Bootstrap analysis was done and values more than 15 were considered for presentation on node. Values more than 30 were shown at many nodes which considered significant strength for that node while values around 20 were comparatively poor strength. Shobhana *et al*[1] and Saxena *et al*[25] were also found poor strength at sub-group level while significant strength was found at initial and sub-sub-group level.

Principal coordinate analysis showed congruence with dendrogram construction [Fig. 2 (II)]. PS-03, PS-01 and Pusa Majesty found on similar position with PS-05 in same plane and CO-2 at proximity as they all fall in same group. Honey Dew and Pusa Delicious were found in same plane, differently as they formed one group. Pusa Nanha, Pusa Dwarf and Nanha Munna were located on same and nearby position indicating their close relations. Madhubindu and Honey Dew were found at proximity as they have 82.50% similarity. First three components of PCoA covered more than 87.83% of total diversity with PC1, PC2 and PC3 values of 0.452 (46.79%), 0.226 (23.39%) and 0.170 (17.64%), respectively.

Pooled molecular marker analysis showed dendrogram grouping similar to RAPD data analysis. Pusa Dwarf and CO-07 formed one group (B) with similarity of 80% while Pusa Delicious and Pusa Giant also showed approximately 80% similarity, Showing ancestral relationship (A_{1a}). Madhubindu depicted 84% similarity with ancestor Honey Dew (A_2). Nanha Munna reported more than 80% similarity with Pusa Nanha. Again PS-03 and Pusa Majesty fall in same group and showed more than 85% similarity. PS-05 and CO-02 showed 91% similarity with each other and more than 89% similarity with PS-01. Bootstrap values were shown at node as strength was good. Again PCoA showed same what similar distribution of papaya cultivars as reported in RAPD analysis and showed congruence with dendrogram.

Analysis of molecular variance (AMOVA) was performed and result showed highly significant performance for both molecular markers but only during RAPD, cultivars showed significant behaviour (Table 4). Mantel test analysis showed poor value of 0.271 for cophenetic metric comparison of RAPD and ISSR while very good value of 0.929 was obtained during comparison of similarity and cophenetic metric of ISSR (Table 5).

Table 5 — Correlations between matrices (Mantel test)[a]

	RAPD	ISSR	RAPD + ISSR
RAPD	0.691	0.271	0.667
ISSR	0.308	0.929	0.761
RAPD + ISSR	0.832	0.308	0.771

a diagonal: similarity/cophenetic; above diagonal: cophenetic; below diagonal: similarity

Table 4 — Analysis of molecular variance (AMOVA) for RAPD and ISSR markers

Molecular marker	Source of variation	df	Sum of square	Mean sum of square	t_{cal}	t_{tab} at 5%	t_{tab} at 1%
RAPD	Cultivar	12	16.13	1.36	2.11*	1.81	2.28
	Primer	15	452.60	30.17	46.76**	1.72	2.14
	Error	180	116.15	0.65	-	-	-
ISSR	Cultivar	12	11.16	0.93	1.52	1.83	2.34
	Primer	10	283.45	28.35	46.31**	1.91	2.47
	Error	120	73.45	0.61	-	-	-

Discussion

In case of PAPD markers, Pusa Delicious and Pusa Giant formed one group as they were selected from Ranchi cultivar. Similarly, PS-01 and PS-05 showed relatedness due to selection from Madhubala. Pusa Majesty and PS-03 showed similarity because both contained some defence mechanism against PRSV as Pusa Majesty is considered to be resistant against PRSV from Bihar region while PS-03 is considered tolerant for PRSV from Maharashtra region, PS-03 also showed tolerant against our Junagadh virus strain as mentioned in this study. Madhubindu showed relation with its ancestor Honey Dew due to its selection from it. At subgroup level, Nanha Munna showed 78.90% similarity with Pusa Nanha from which it was selected while PS-1, PS-03 and PS-05 were related due to their ancestor Madhubala. Pusa Dwarf, Pusa Nanha and Nanha Munna fall in same subgroup which may be correlated with their similarity with height character. Again, primers and species both showed considerable result in case of RAPD marker which can be seen from AMOVA. So, it can be concluding that RAPD markers are good molecular marker for diversity study.

Dendrogram of ISSR data showed conserved repeat region for PS-1, PS-03 and PS-05 as they came from same ancestor and fall in same subgroup. Pusa Majesty, PS-1, PS-03, PS-05 and CO-2 were showed more than 91% similarity which can be correlated as their use as promising cultivar for future purpose. Similar relation was also found during RAPD analysis. Except CO-2, remaining four cultivars are used for cultivar development at regional level against PRSV infection while CO-2 considered among good papain producer which is a protease and currently protease related work is going on for transgenic development against PRSV so, this may be considered a chance for their relation. Their relation showed congruence with PCoA result as PS-1, PS-03 and Pusa Majesty showed location at same position and PS-05 in a same sector of graph while CO-2 found at a close position from PS-05 and other three promising cultivar as compared to other. Though Pusa Nanha, Pusa Dwarf and Nanha Munna fall in different subgroup but PCoA showed their relatedness due to grouping at proximal distance on graph. Madhubindu and Honey Dew also showed close location on graph indicating their relation.

Pooled marker analysis showed relatedness between Honey Dew and Madhubindu during clustering. Similarly, Pusa Giant and Pusa Majesty indicated their ancestral relationship. PS-01 and PS-05 fall in one subgroup, showing their common genetic background of Madhubala which was also supported by PS-03 as it also found in the same group. Here, Nanha Munna also showed good relation with its predecessor Pusa Nanha by following same group. Pusa Delicious and Pusa Dwarf also found in same group indicating their precedent relationship. CO-7 showed intimate relation with either Honey Dew or Madhubindu, during grouping in dendogram or location on PCoA graph as it one of the parents was Coorg Honey Dew which was also developed from Honey Dew.

From this study, diversity study through dominant molecular markers like RAPD and ISSR are still useful to identify genetic relationship between related species which are originated from same ancestry and based upon this relationship it would be helpful to conserve cultivars as well as development of breeding programmes between genetically divers cultivars to improve and develop new germplasms for future breeding programmes.

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