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Genetic relationship among papaya (*Carica papaya*) and wild papaya (*Vasconcellea* species) using RAPD and ISSR markers*

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Papaya (*Carica papaya* L.) is the most economically important fruit crop of the family Caricaceae, growing in the tropical and sub-tropical regions of the world. Originating from Central and South America, it bears highly nutritious fruits which are rich source of vitamins (A, folate, pantothenic acid and C) and minerals, low in sodium, fat and calories and contains no starch. The annual worldwide production of papaya amounts to 6.9 million tonnes, of which 36.1% is produced in India (NHB 2009). Despite this high production, most of the Indian production is based on few varieties resulting in restricted genetic variability. The cultivated papayas belong to genus *Carica* in family Caricaceae. Earlier this genus included both wild and cultivated papaya species until Badillo separated them into two based on assessment of morphological (Badillo 2000) and genetic markers (Aradhya *et al.* 1999). Futher genetic studies of Caricaceae supported the diversity of the two genera (Kim *et al*. 2002, Van Droogenbroeck *et al*. 2004). The wild papayas have been placed in genera *Vasconcellea* and are referred to as Highland or mountain papayas. These have 21 species of which many produce edible fruits and serve as potential source of genetic resistance against papayas several diseases. The two species of *Vasconcellea* included in this study, viz *V. cauliflora* and *V. gouditiana* are known to be source of resistance to papaya ring spot virus (Manshardt 1992) and a novel apple flavour respectively.

In recent years hybrids of papaya are gaining commercial importance in domestic and international markets. According to Chan (1992), the productivity of hybrids surpasses that of parental lines by 199.6%. Also valuable traits like resistance to diseases, ornamental features and good flavour etc. can be brought into cultivated papaya by intergeneric hybridization with *Vasconcellea* leading to development of new varieties.

In the present study, the genetic diversity and molecular profiles of important cultivars and hybrids grown over wide acreage in India along with two important species of *Vasconcellea* were generated. Two different markers namely RAPD, ISSR were used in order to achieve different information. RAPD profiles are known to represent the whole genome, while ISSR targets microsatellite rich regions of the genome. Therefore, both the methods were included to provide a comprehensive assessment of inter- and intra-species affinities in papaya.

The study was undertaken during 2009–10 with two wild papaya species, namely, *Vasconcellea caulifora* (VC), *Vasconcellea gouditiana* (VG), and eleven commercial cultivars, viz. Surya (SU), Sunrise Solo (SS), Pusa Delicious (PD), Pusa Giant (PG), Arka Prabath (AP), Shantha (SH), CO1, CO2, Dwarf Lily (DL), Pink Flesh (PF) and Tainung 1 (T1). These cultivars were maintained in the field gene bank of Indian Institute of Horticultural Research, Bangalore. DNA was extracted from fresh leaf samples using modified CTAB method (Doyle and Doyle 1990). At least three independent DNA preparations were made from leaf tissue collected from each plant. DNA concentration and purity was estimated by recording their absorbance at 260nm, 280nm in GeneQuart UV/Visible spectrophotometer. Quality was checked by running on 0.8% agarose gel. The eleven ISSR primers used for the study were UBC 807, UBC 810, UBC 814, UBC 815, UBC 817, UBC 834, UBC 836, UBC 841, UBC 844, UBC 856, UBC 889 while the RAPD primers were OPB17, OPD02, OPD20, OPE06, OPF12, OPG10, OPN09, OPO10, OPO15, OPR03, OPR15, OPS03, OPS12, OPW02, OPAG03, OPAG11. The ISSR primers were synthesized by Bangalore

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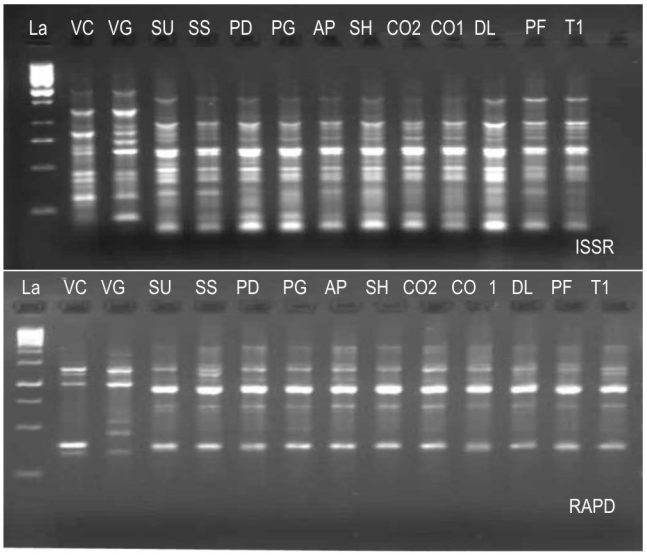
Genei while RAPD primers were obtained from Operon Tech. Inc. Alameda, CA, USA.

Initially, RAPD primers from OP-B, D, E, F, G, N, O, R, S, W and AG series were screened in reactions with selected papaya DNA templates in triplicate. Primers that resulted in discrete, well separated bands on agarose gels were then selected to amplify all the papaya and two *Vasconcellea* species. RAPD reactions were carried out in 25ul volumes and contained 20ng of template DNA, 0.3uM of RAPD primer, 1mM dNTP, 2.4mM (MgCl2), 10 × Incomplete reaction buffer with 1 U *Taq* DNA Polymerase (Bangalore Genei, Bangalore, India). Amplification reactions were carried out using a programme to include pre- denaturation at 95°C for 1 min., followed by 45 cycles of denaturation at 94 °C for 1 min., annealing at 36°C for 1 min. and extension at 72 °C for 7 min. The final cycle had an additional 7 min. extension at 72 °C. The PCR products were analyzed by electrophoresis in 1.5% (w/v) agarose gels in 1 × TAE buffer at constant voltage (5V cm-1) for 3 hr. After electrophoresis the gels were visualized and photographed on Uvi-Pro software of UV Pro Platinum Gel Documnetation. The PCR conditions for ISSR were similar to that of RAPD with modification that the concentration of DNA was increased to 50ng and primer concentration was 10 pmoles. The reaction conditions were similar to RAPD with few modifications like the predenaturation was increased to 4 min. and annealing temperature was 54 °C for 45 sec. The number of cycles was 35. PCR products were resolved on 1.5% agarose and visualized and photographed like RAPD gels.

The sizes of all amplification products were estimated by comparison with standard molecular weight DNA ladder (1kb). Bands were scored as discrete variables using "1" to indicate the presence and "0" to indicate the absence of a particular band. The data was then statistically analyzed by Winboot software to obtain distance matrix and the corresponding dendrogram was drawn to obtain clusters by NTSYS PC 2.11 software.

The total number of primers screened in this study was eleven ISSR and sixteen RAPD; all the primers produced good results in terms of discrete and consistent band patterns. The typical gel profile obtained by each method is shown in Figure 1 A, B.

RAPD profiles for all 16 primers resulted in total of 118 bands, ranging between 200–3500 bp, of which 91 (37.2%) were polymorphic. It is evident from the dendrogram (Fig 2) that papaya accessions were grouped into two major clusters (A and B) indicating that they are genetically different. The two *Vasconcellea* species separated into one group (cluster A) while all the eleven cultivars of cultivated papaya grouped into another cluster. The values in the distance matrix (Table 1) ranged from 0.17 (between Shanta and *V. cauliflora*, CO1 and *V. cauliflora)* to 0.94 (between Pusa Giant and Pusa Delicious). The bootstrap analysis of tree robustness was



La - 1 Kb ladder, VC: *Vasconcellea cauliflora,*
VG: *Vasconcellea gouditiana,* SU: Surya, SS: Sunrise Solo,
PD: Pusa Dwarf, PG: Pusa Giant, AP: Arka Prabhat, CO2: CO2,
CO1: CO1, DL: Dwarf Lify, PF: Pink Flesh, T1: Tainung 1

Fig 1 A and B showing the gel profile for the PCR amplified products using ISSR 817 primer and RAPD OPF12 primer

performed and the values are indicated on the nodes of the dendrogram. The two species of *Vasconcellea*, viz. *cauliflora* and *gouditiana* are placed very closely with bootstrap significance of 100 indicating a high similarity between these two species. Morphologically also they share some common traits like broad leaves of caster type, small fruits with high papain content and dioecious sex form. In the cluster B, consisting of the commercial cultivars of papaya, again two major groups were formed. One cluster consisted of Dwarf Lily, Pink Flesh and Tainung 1 while the other consisted of Surya, Sunrise Solo, Pusa Delicious, Pusa Giant, Shanta, CO2, CO1 and Arka Prabhat.

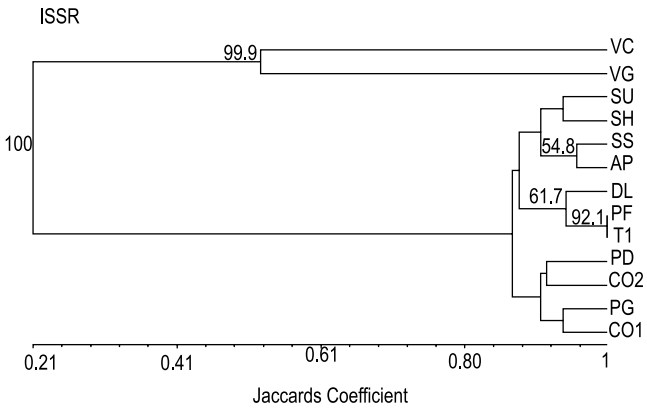


Fig 3 Denderogram generated by UPGMA cluster analysis showing the relationships among papaya genotypes and cultivars using ISSR markers

Table 1 Pair-wise genetic distance by ISSR and RAPD method. [a]Papaya cultivars are abbreviated as in Text, [b]Prefixes I and R indicate that the genetic distance values were determined by ISSR or RAPD respectively

	[a]VC	VG	SU	SS	PD	PG	AP	SH	CO2	C01	DL	PF
[a]VG	[b]I0.52											
	R0.51											
SU	I0.20	I0.20										
	R0.18	R0.18										
SS	I0.22	I0.22	I0.94									
	R0.21	R0.20	R0.94									
PD	I0.17	I0.17	I0.89	I0.88								
	R0.20	R0.20	R0.85	R0.88								
PG	I0.18	I0.18	I0.91	I0.86	I0.93							
	R0.19	R0.19	R0.84	R0.85	R0.94							
AP	I0.23	I0.21	I0.93	I0.96	I0.87	I0.85						
	R0.18	R0.18	R0.84	R0.87	R0.89	R0.89						
SH	I0.21	I0.23	I0.94	I0.88	I0.84	I0.86	I0.88					
	R0.17	R0.17	R0.84	R0.85	R0.92	R0.94	R0.89					
CO2	I0.20	I0.20	I0.94	I0.88	I0.91	I0.94	I0.88	I0.88				
	R0.20	R0.19	R0.81	R0.81	R0.90	R0.88	R0.85	R0.90				
CO1	I0.19	I0.23	I0.86	I0.84	I0.88	I0.94	I0.80	I0.85	I0.88			
	R0.17	R0.18	R0.82	R0.82	R0.92	R0.86	R0.86	R0.92	R0.93			
DL	I0.23	I0.23	I0.84	I0.86	I0.86	I0.88	I0.82	I0.83	I0.83	I0.90		
	R0.21	R0.20	R0.80	R0.81	R0.82	R0.80	R0.85	R0.82	R0.84	R0.85		
PF	I0.24	I0.24	I0.90	I0.92	I0.84	I0.86	I0.88	I0.88	I0.88	I0.88	I0.94	
	R0.19	R0.19	R0.78	R0.79	R0.78	R0.78	R0.80	R0.81	R0.85	R0.81	R0.84	
T1	I0.24	I0.24	I0.90	I0.92	I0.84	I0.86	I0.88	I0.88	I0.88	I0.88	I0.94	I1.00
	R0.18	R0.18	R0.82	R0.83	R0.80	R0.82	R0.87	R0.82	R0.83	R0.82	R0.86	R0.89

The ISSR primers used in the study were 15–18 nucleotides in length and represented the dinucleotide motifs. The eleven primers used produced discrete band patterns for DNA from the papaya cultivars and *Vasconcellea* species. A typical profile, obtained after amplification with primer 814, is shown in figure 1B. A total of 75 bands, between 250 and 2500 bp, were scored for the eleven primers, of which 35% were polymorphic. The distance matrix values ranged from 0.17 (between Pusa Delicious and *V. cauliflora* , Pusa Delicious and *V. goudatiana)* to 1.00 (between Pink Flesh and Tainung 1). The consensus tree (Fig 3) had two major groups among the cultivars of papaya- one group consisted of seven cultivars, viz., Surya, Shanta, Sunrise Solo, Arka Prabhat, Dwarf Lily, Pink Flesh and Tainung 1 while the other had Pusa Delicious, Pusa Giant, CO1 and CO2.

zIn the present study the comprehensive representation of the affinities between cultivars is consistent with few morphological characters that could possibly distinguish cultivars in the groups. Dwarf Lily, Pink Flesh and Tainung 1 are all pink pulp cultivars of exotic origin while Sunrise Solo is a parent of Surya, Pusa Delicious and Pusa Giant share a common origin and CO1 and CO2 are medium tall plants with yellow- orange flesh and dioecious nature. Both RAPD and ISSR give banding profiles that represent several disparate regions of the genome, but the ISSR gave more meaningful result. It is interesting to note that Arka Prabhat which is a selection from cross between Surya and (Tainung 1 × Dwarf Lily) and Surya which is a cross between Sunrise Solo and Pink Flesh came together in a group. This relationship is more clearly defined by ISSR primers which has clustered all the parents of Arka Prabhat into one group. Cultivars Pusa Delicious, Pusa Giant, CO2 and CO1 also formed a group which is consistent with the observation that all the four cultivars are dioecious in nature; they are medium

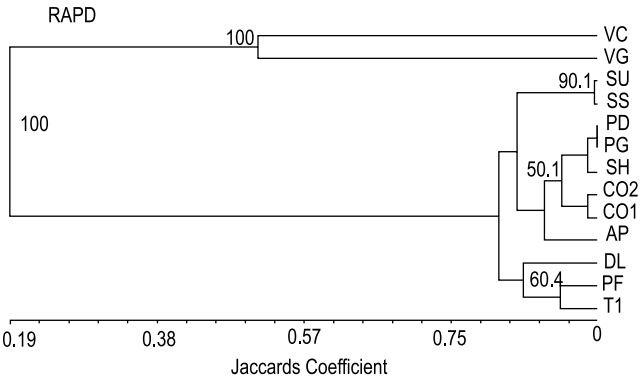


Fig 2 Denderogram generated by UPGMA cluster analysis showing the relationships among papaya genotypes and cultivars using RAPD markers

tall, producing fruits with yellow flesh colour. The *Vasconcellea* genera formed a separate cluster for both the markers. This proves that this genus is distantly related to *Carica papaya* as reported previously by several workers (Van Droogenbroeck *et al.* 2002, Kyndt and Gheysen, 2005). Also, among the two species of *Vasconcellea, V. gouditiana* grouped closer to Surya than *V. cauliflora*. This is consistent with the finding that among more than twenty species of *Vasconcelea*, *V. cauliflora* is most distantly related to *C. papaya* (Drew *et al*. 2007). This aspect should be kept in mind while breeding for papaya ring spot virus resistance by making intergeneric crosses between papaya cultivars and *V. cauliflora*. The draft papaya genome has been released and large number of microsatellite markers have been identified therefore, for future studies these primers should be used be used to distinguish between cultivars also, since they are codominent in nature they would be useful to identify hybrids that have resulted from crosses between landraces, or to analyze molecular markers linked to traits that are useful in breeding programmes.

SUMMARY

Two different molecular methods namely, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) were used to measure genetic diversity among thirteen commercially important cultivars of papaya and two species of useful wild papaya. Eleven ISSR and 16 RAPD primers were screened to amplify a total of 75 and 118 bands respectively. Of these, approximately 35% and 37.2% of the bands were polymorphic in each. Genetic similarity was computed and results displayed graphically as dendrogram generated by UPGMA cluster analysis showing the relationships among wild and cultivated papaya cultivars. Both methods revealed different groupings of the eleven cultivars of papaya. On the basis of correlation of data with morphological grouping based on fruit pulp colour and plant height, ISSR was judged to be best method for analyzing all papaya germplasm.

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