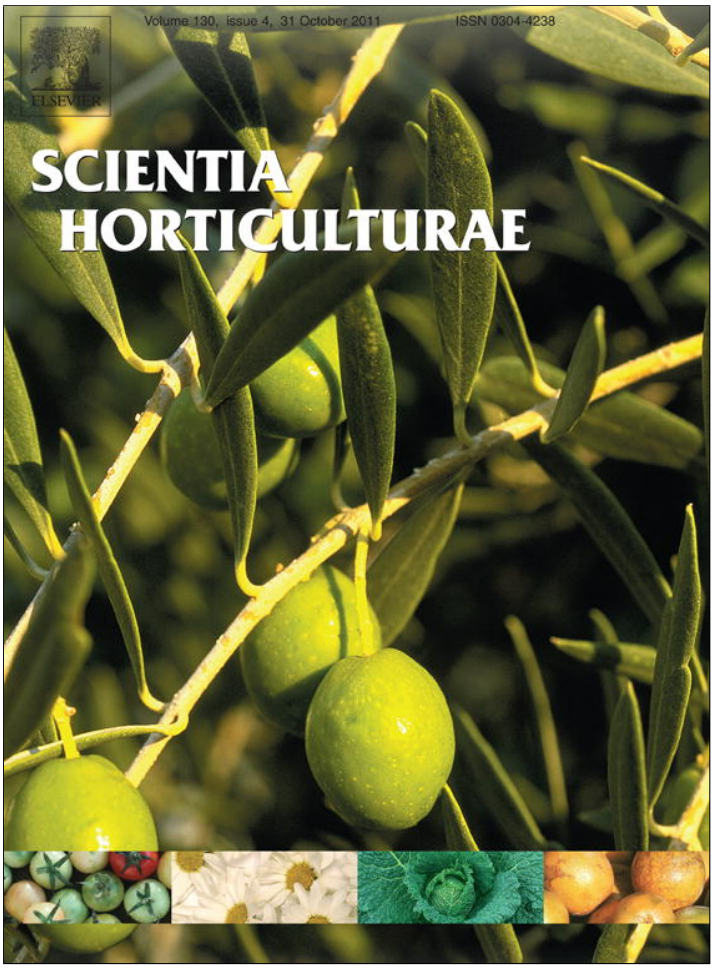

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Breaking the intergeneric hybridization barrier in *Carica papaya* and *Vasconcellea cauliflora*

R. Jayavalli[a,*], T.N. Balamohan[a], N. Manivannan[b], M. Govindaraj[b]

[a] Department of Fruit Crops, Tamil Nadu Agricultural University, Coimbatore 641003, India
[b] Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India

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ABSTRACT

The present investigation was undertaken to develop PRSV (Papaya ringspot virus) resistant hybrids through intergeneric hybridization. Intergeneric hybridization was done involving nine *Carica papaya* cultivars as female and *Vasconcellea cauliflora* as male. To break the intergeneric hybridization barrier, various nutrient combinations were used. Among the combinations used, sucrose 5%, sucrose 5% + boron 0.5% and sucrose 5% + $CaCl_2$ 0.5% improved the fruit set and seed set percentage. A total number of 1197 flowers were pollinated and 308 fruits were obtained. On extraction, 721 seeds were obtained from CO 7, Pusa Nanha and CP 50. Out of 721 F_0 seeds (crossed seeds) sown, 419 seeds germinated and artificial screening for PRSV was carried out 27 days after sap inoculation. Out of 29 F_1 hybrid plants from CO 7 x *V. cauliflora* cross, only six plants namely CO 7V1 to CO 7V6 were found free from PRSV symptoms. Similarly, out of 55 F_1 hybrids from cross involving Pusa Nanha x *V. cauliflora* only 23 plants namely PNV1 to PNV23 were found free from the symptoms and 70 plants namely CPV1 to CPV70 out of 335 plants of CP50 x *V. cauliflora* cross were found free from PRSV symptoms. Among the crosses, Pusa Nanha x *V. cauliflora* had higher yield under PRSV infected conditions, however, total soluble solids and total sugars were found lesser than the CO 7 x *V. cauliflora* cross. The hybridity of the progenies were confirmed by using ISSR (Inter Simple Sequence Repeats) primers by the amplification of DNA from progenies and their parents. ISSR primers UBC 856, UBC807 and ISSR primer combinations UBC 856-817, UBC 810-817, UBC 861-817, UBC 856-810, UBC 861-810 and UBC 856-817 clearly amplified specific bands of the male parent, which were present in F_1 progenies, but it was absent in female parents.

1. Introduction

Papaya (*Carica papaya*) is a tropical fruit having commercial importance because of its high nutritive and medicinal value. Papaya cultivation had its origin in South Mexico and Costa Rica (De Candolle, 1884). It was introduced to India during 16th century by Portuguese travellers. Total annual world production is estimated approximately 6 million tonnes of fruits. India ranks first in world papaya production with an annual production of about 3 million tonnes from 98,000 ha (NHB, 2009). Other leading producers are Brazil, Mexico, Nigeria, Indonesia, China, Peru, Thailand and Philippines. In India, it is commercially cultivated in several states such as Andhra Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Assam, Orissa, Madhya Pradesh, Manipur, Tamil Nadu and Bihar and certain extent in Kerala. However, Andhra Pradesh is the leading state in papaya production about 1500.7 million tones with an area of about 18,800 ha (NHB, 2010).

The papaya cultivation affected by number of diseases caused by various pathogens and viruses. Nowadays the most destructive disease of *C. papaya* worldwide is papaya ring spot caused by papaya ringspot virus-type P Litz (1984), Manshardt (1992), a definitive potyvirus species in the *Potyviridae* (Shukla et al., 1994). PRSV is grouped into two types, Type P (PRSV-P) infects cucurbits and papaya and type W (PRSV-W) infects cucurbits but not papaya (Gonsalves, 1998). At present almost all cultivated varieties are highly susceptible. *Carica cauliflora* J., a wild species having non-edible fruits is well known to be resistant for this viral disease (Jimenez and Horovitz, 1957). Now the species *cauliflora* has been grouped under the genera *Vasconcellea* (Vegas et al., 2003). Various control measures to check the viral incidence against PRSV-P include cultural practices, cross-protection and planting of tolerant cultivars (Gonsalves, 1994). None of these were very successful, since new races of PRSV are being reported. Evolving transgenic plant through coat protein method is one of viable solution but it could address only in short term because of genetic base of the cultivars get narrow down with accumulation of specific resistance genes. Hence, the development of virus resistant cultivars through conventional breeding strategies is the more reliable method in

* Corresponding author. Tel.: +91 9626967403.
E-mail address: jayavallirajappa@yahoo.co.in (R. Jayavalli).

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Table 1
Number of flowers from different cultivars pollinated with different nutrient solutions.

Nutrient solutions	Varieties									
	CO 1	CO 2	CO 4	CO 5	CO 6	CO 7	Pusa Dwarf	Pusa Nanha	CP 50	*Vasconcellea cauliflora*
	No. of flowers pollinated									
Sucrose 5%	10	25	15	17	25	55	40	45	75	Source of pollen
Sucrose 5% + Boron 0.5%	12	22	24	15	29	25	22	15	45	Source of pollen
Sucrose 5% + $CaCl_2$ 0.5%	15	26	15	18	26	28	15	22	42	Source of pollen
Sucrose 5% + Boron 0.5% + $CaCl_2$ 0.5%	21	35	18	22	20	24	16	24	46	Source of pollen
Control	25	35	16	25	26	25	22	26	48	Source of pollen
Total	83	143	88	97	126	157	115	132	256	=1197
Mean	16.6	28.6	17.6	19.4	25.2	31.4	23.00	26.40	51.20	
SE	2.8	2.7	1.7	1.8	1.5	5.9	4.5	5.0	6.0	
SD	6.3	6.0	3.8	4.0	3.3	13.3	10.0	11.2	13.5	
Confidence Level (95%)	7.8	7.5	4.7	5.0	4.1	16.5	12.5	13.9	16.7	

order to control for long term. None of the *C. papaya* cultivars has natural-resistance to PRSV-P, however, several related wild species of *Carica* have been reported as resistant to PRSV-P. Even though interspecific hybridization of *C. papaya* with other species attempted, a very little work has been done so far by using *Vasconcellea cauliflora* which has been identified as a desirable source of resistance for PRSV. Under these circumstances, breaking the barrier in intergeneric hybridization, a crossing programme was initiated between *C. papaya* and *V. cauliflora* to evolve progenies resistant to 'PRSV' by breaking the intergeneric crossability barrier by using various nutrients concentrations and to confirm the hybridity of these progenies using DNA marker (ISSR) at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India.

2. Materials and methods

2.1. Hybridization and field evaluation

Intergeneric hybridization of *C. papaya* with *V. cauliflora* was performed to transfer the desirable gene from *V. cauliflora* to *C. papaya* for PRSV resistance. The varieties CO 1, CO 2, CO 4, CO 5, CO 6, CO 7, Pusa Dwarf, Pusa Nanha and CP 50 were selected and used as female for crossing with *V. cauliflora* as male. Nutrients *viz*., sucrose, boron, calcium chloride and their combinations were smeared on the surface of the stigma before dusting of pollen to overcome the pollination barrier between *C. papaya* and *V. cauliflora* at the time of crossing. Cultivars and different nutrients solutions used are presented in Table 1. Female flowers which were about to open in the next day were bagged on the previous day evening. Pollen grains were collected from fully mature, unopened flowers of *V. cauliflora*. Pollination was done between 6.30 a.m. and 8.30 a.m. in every day. At the time of pollination, the bags on the female flowers were removed and the pollen were dusted on the stigma and bagged again. In gynodioecious varieties, andromonoecious flowers in tree were carefully emasculated on the previous day before dehiscence of anther and pollinated during the next day. Fruit set was observed 5–6 days after pollination, which were carried to maturity in 5–6 months. Matured fruits were harvested at colour break stage for seeds extraction. Collected seeds were treated with GA (Gibberellic acid) at 100 ppm for 2 h a day prior to sowing as it helps for better germination. Then the GA treated seeds were washed gently with running water to remove the traces of GA solution before sowing. Seedlings thus raised were used for screening. All the seedlings were artificially inoculated with PRSV through artificial inoculation method (Manoranjitham et al., 2008). The seedlings that are shown initial resistance (seedling resistance) alone were taken to field for further evaluation. One gram of infected leaves was ground in a pre-chilled mortar and pestle using 1 ml of 0.1 M chilled sodium phosphate buffer (pH 7.2) containing β-mercaptoethanol and 0.01 M EDTA. The sap was rub inoculated using the pestle or glass rod on the young leaves of seedlings at 3 leaves stage which are previously dusted with carborundum powder 600 meshes. After 5 min, the excess sap was washed off by distilled water.

Number of fruits borne on the tree during first crop was taken and expressed as number of fruits per tree. Mean fruit weight of five fruits was worked and expressed in kilograms (kg). The fruit yield per tree was arrived at by multiplying number of fruits harvested with mean fruit weight and expressed as kilograms. Total soluble solids of the fruit was determined by hand refractometer and expressed as ºBrix. Total sugars were estimated as per the method of Hedge and Horreiter (1962) and expressed in percentage. Reducing sugars were estimated by the method suggested by Somogyi (1952) and expressed in percentage. Non-reducing sugars were estimated by subtracting the percentage of reducing sugars from the total sugars and expressed as percentage. Acidity was estimated as per the A.O.A.C method (1960) and expressed as percentage of citric acid equivalents. Ascorbic acid content of the fruit was estimated as per the method of Rosenberg (1945) and expressed in milligrams per 100 g of pulp. Carotene were estimated by the method of Roy (1973) and expressed in milligrams per 100 g. Ratio between the total sugars and titrable acidity was calculated. The least squares differences (LSD), standard error (SE) and test of significance were performed by following Singh and Chaudhary (1999). All this statistic were done by using Genstat (12 edition) programme.

2.2. Molecular markers analysis

To confirm the hybridity of these intergeneric hybrids, ISSR marker analysis was carried out using six plants from CO 7 x *V. cauliflora,* twenty three from Pusa Nanha x *V. cauliflora* and seventy plants from CP50 x *V. cauliflora*. DNA from hybrid progenies and their parents' leaves was extracted following CTAB method (Doyle and Doyle, 1987). PCR reaction was performed using 6 (ISSR) primers. The details of the primers are presented in Appendix A. The reagents that required for performing PCR reaction are as follows (Benbouza et al., 2006). PCR reaction was carried out in total volume of 10 μl in 96 tubes PCR plates. Following were the master mix of solution for one reaction. For ISSR primers reagents of 10× Taq buffer + $MgCl_2$ (15 mM) on 1.0 μl, dNTP (2 mM) on 1.0 μl, Primers 10 μM 1.0 μl (0.5 μl each for combination), Taq polymerase (3 IU/μl) on 0.1 μl, Sterile double distilled water on 4.9 μl and Template DNA 10 ng/μl on 2 μl. Cycling profile – Touch down protocol was followed for all the primers. PCR cycles included initial denaturation at 94 °C for 3 min followed by 19 cycles of 30 s (−0.5 °C) denaturation at 94 °C, annealing at 63 °C for 30 s and 1 min in extension at 72 °C. Again 19 cycles of 15 s denaturation at 94 °C, annealing

Table 2
Analysis of variance for flower pollinated and fruit set percentage in intergeneric hybridization between *Carica papaya* and *Vasconcellea cauliflora*.

Source of variation	df	SS	MS
Different solution	4	755.2	188.8**
Flower pollinated	8	4400	550**
Error (a)	32	2050	64.05
Different solution	4	907.623	226.91**
Fruit set %	8	903.583	112.95**
Error (b)	32	1548.55	48.39

Error (a) for flower pollinated; (b) = fruit set %; ** = significant at $P \leq 0.01$.

at 55 °C for 30 s, 1 min in extension at 72 °C, 10 min in final extension at 72 °C and infinitive final hold at 4 °C. Electrophoresis was performed in 1.5% agarose with 120 V for 2 h.

3. Results and discussion

3.1. Breaking barriers in the intergeneric hybridization

Previous studies (Thirugnanavel, 2010) experienced difficulties of using pollen from *V. cauliflora* to cross with *C. papaya*. Those crosses were showed that either no seed set or very poor seed set. Thus, to improve the seed set in this intergeneric cross, different nutrient solutions were used to enhance the pollen tube growth and there by enhancing good seed set. Prior to pollination, the stigma of *C. papaya* were smeared with nutrients *viz*., sucrose 5%, sucrose 5% + boron 0.5%, sucrose 5% + $CaCl_2$ 0.5%, sucrose 5% + boron 0.5% + $CaCl_2$ 0.5%. Eight varieties of papaya *viz*., CO 1, CO 2, CO 4, CO 5, CO 6, CO 7, Pusa Dwarf, Pusa Nanha and a genotype CP 50 were used as female parents and these were crossed with *V. cauliflora*. A total of 1197 flowers were pollinated using different varieties with different nutrient combinations. Table 2, suggested that means square due to fruit set percentage was highly significant. This result indicates that the fruit setting among the intergeneric hybrids has remarkable differences. Also the effect of different nutrient solution had statistically significant further indicate the differences in each cross. Out of 1197 crosses effected, fruit set percentage was the highest in the cross combination involving Pusa Nanha x *V. cauliflora* (46.15%) followed by CP 50 x *V. cauliflora* (45.83%). However, another cross involving Pusa Dwarf x *V. cauliflora* had the minimum of 12.50 percent. (Table 3). Although the fruit set percentage varied (2.50 to 46.15%), among the combinations, the cross involving CP 50 x *V. cauliflora* had the maximum number of fruits harvested. Hence, on the whole, out of 1197 flowers pollinated using different nutrient combinations, there were 676 fruits initially formed of which 308 fruits were finally harvested (data were not presented).

Table 4
Number of seeds, germination percentage and number of seedlings obtained from intergeneric crosses.

Nutrient solutions	CO 7 x *V. cauliflora*	Pusa Nanha x *V. cauliflora*	CP 50 x *V. cauliflora*
	Number of seeds obtained		
Sucrose 5%	45.00	67.00	400.00
Sucrose 5% + Boron 0.5%	–	–	200.00
Sucrose 5% + $CaCl_2$ 0.5%	–	–	9.00
Total number of seeds = 721			
	Number of seedlings obtained		
Sucrose 5%	29.00	55.00	228.00
Sucrose 5% + Boron 0.5%	–	–	107.00
Total number of seedlings = 419			
	Germination percentage		
Sucrose 5%	64.44	82.08	57.00
Sucrose 5% + Boron 0.5%	–	–	53.35

From 308 fruits, 721 intergeneric F_0 seeds were obtained from three varieties *viz.*, CO 7, Pusa Nanha and CP 50. The cross combinations *viz*., CO 7 x *V. cauliflora*, Pusa Nanha x *V. cauliflora* and CP 50 x *V. cauliflora* produced 45, 67 and 400 numbers of seeds respectively with sucrose 5%. However, CP 50 x *V. cauliflora* crosses smeared with sucrose 5% + boron 0.5% had 200 seeds and sucrose 5% + $CaCl_2$ 0.5% produced 9 seeds (Table 4). Intergeneric cross combinations, CO 7 x *V. cauliflora*, Pusa Nanha x *V. cauliflora* and CP 50 x *V. cauliflora* smeared with sucrose 5% recorded 64.44, 82.08 and 57.00% seed germination respectively. However, CP 50 x *V. cauliflora* smeared with sucrose 5% + boron 0.5% produced 53.35% seed germination. Seeds obtained from a cross involving CP 50 x *V. cauliflora* using sucrose 5% + $CaCl_2$ 0.5% did not germinate. This might be due to either immature seeds or lack of seed food reserve for germination, indicates that formation of seed was not a serious problem in this intergeneric cross however hybrid sterility must be a reason but this is to be validated with further studies. On the other hands, intergeneric cross involving CO 7 x *V. cauliflora* had 29 seedlings, Pusa Nanha x *V. cauliflora* had 55 seedlings and CP 50 x *V. cauliflora* had 228 seedlings. All these combinations were the out come of 5% sucrose smeared. Whereas CP 50 x *V. cauliflora* smeared with sucrose 5% + boron 0.5% produced 107 seedlings.

Intergeneric hybridization is a valuable tool for creating variability in plant breeding by broadening the genetic base of the germplasms (Singh, 2003). Incorporation of resistant genes from wild relatives to the cultivated varieties is often hampered because of poor crossability, early embryo abortion, hybrid seed inviability, hybrid seedling lethality, and hybrid sterility due to incompatibility between the two genera. However, these post fertilization barriers in intergeneric hybridization could overcome by (1) the assemblage of diverse germplasm; (2) application of growth hormones

Table 3
Fruit set percentage of intergeneric hybrids with different combination of nutrient solutions.

Nutrient Solutions	Crosses								
	CO 1 x *V. cauliflora*	CO 2 x *V. cauliflora*	CO 4 x *V. cauliflora*	CO 5 x *V. cauliflora*	CO 6 x *V. cauliflora*	CO 7 x *V. cauliflora*	Pusa Dwarf x *V. cauliflora*	Pusa Nanha x *V. cauliflora*	CP 50 x *V. cauliflora*
Sucrose 5%	30.00	20.00	20.66	41.17	24.00	12.72	12.50	22.22	20.00
Sucrose 5% + Boron 0.5%	25.00	27.27	20.83	26.66	31.03	16.00	27.27	13.33	26.66
Sucrose 5% + $CaCl_2$ 0.5%	26.67	30.76	33.33	33.33	19.23	21.42	40.00	22.72	28.57
Sucrose 5% + Boron 0.5% + $CaCl_2$ 0.5%	38.09	25.71	22.22	36.36	35.00	20.83	43.75	29.16	21.74
Control	40.00	25.71	37.50	36.00	26.92	20.00	36.36	46.15	45.83
Mean	31.95	25.89	26.91	34.70	27.24	18.19	31.97	26.72	28.56
SE	3.02	1.74	3.54	2.37	2.73	1.66	5.58	5.47	4.59
SD	6.75	3.88	7.92	5.31	6.11	3.72	12.48	12.24	10.26
Confidence Level (95%)	8.38	4.82	9.84	6.59	7.58	4.62	15.50	15.19	12.74
Variance	45.62	15.09	62.85	28.21	37.33	13.85	155.92	149.75	105.41

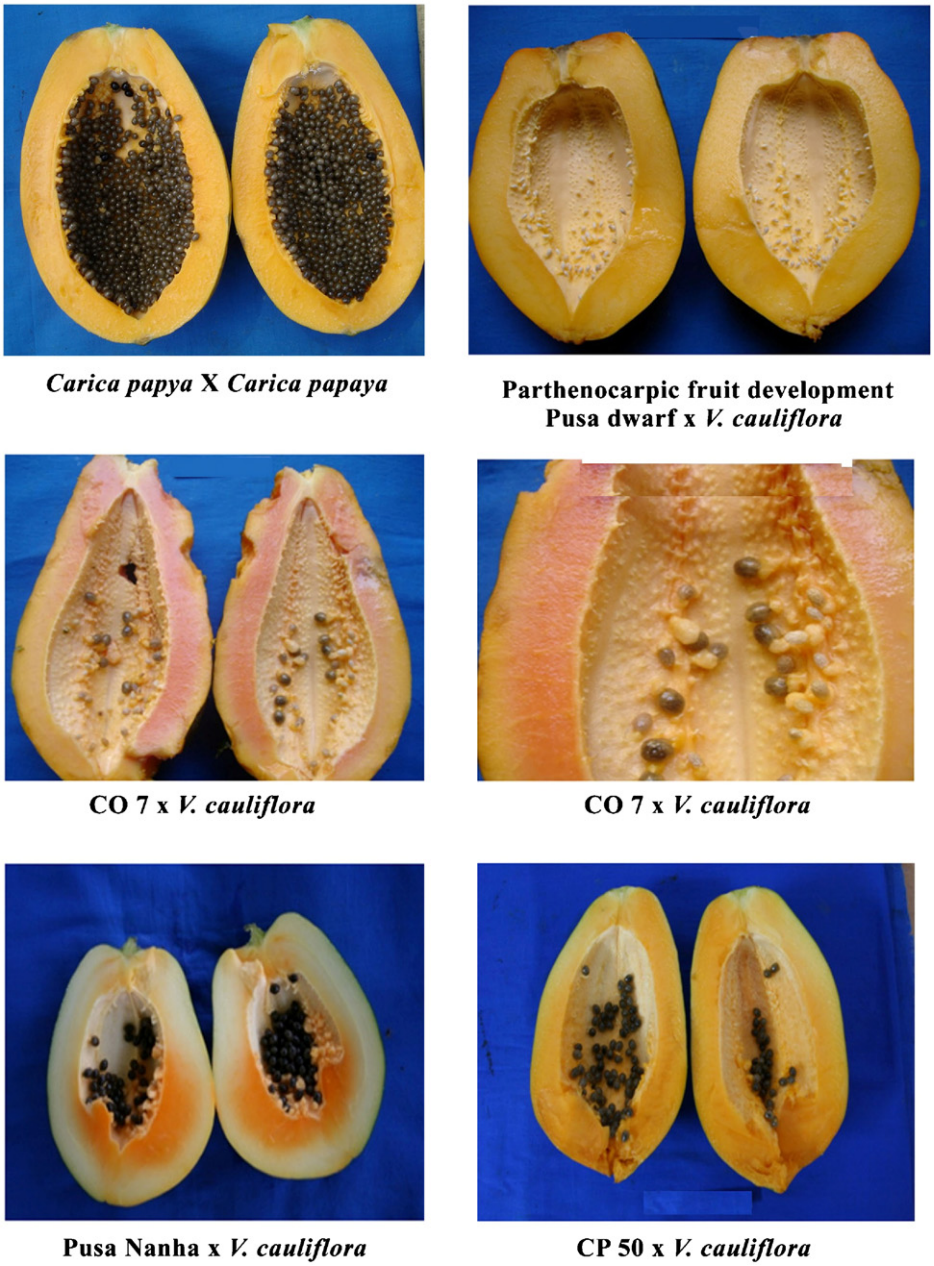

Fig. 1. Seed set in intergenric hybridization using different nutrients solutions.

to reduce embryo abortion; (3) improved culture conditions; (4) restoration of seed fertility by doubling the chromosomes of sterile F_1 hybrids; and (5) utilization of bridge crosses where direct crosses are not possible. In the present study, nutrients *viz.*, sucrose, boron, calcium chloride and their combinations were used to overcome the intergeneric hybridization barrier between *C. papaya* and *V. cauliflora*. Most of the harvested fruits had 5–10 seeds, and many cases the seed did not have embryos. Lack of proper endosperm development was evident in a large number of seeds without embryos. Instead of endosperm, a clear mucous like substances, possibly degraded carbohydrate, was found in the seeds. Small translucent partly developed seeds were observed in the seed cavity in most of the F_1 fruits (Drew et al., 1998; Thirugnanavel, 2010). The findings of present investigation are in close conformity with these findings.

A total of 1197 crosses were affected from which 308 developed fruits were harvested. The result revealed that the seed set was increased considerably with the use of different combinations of nutrients. For instance, among the nutrients combinations, application of sucrose 5% on the stigmatic surface had produced 512 seeds, sucrose 5% + boron 0.5% had produced 200 seeds and sucrose 5% + $CaCl_2$ 0.5% had produced nine seeds (Fig. 1). This may be due to the beneficial effect of sucrose which serves as the best carbohydrate source for pollen germination. This is in close conformity with the earlier findings of Dinesh et al. (2007), Balamohan et al. (2008) and Thirugnanavel (2010). They also reported that application of sucrose on the stigmatic surface of the female flowers may help to break the intergeneric hybridization barrier in papaya by increasing the pollen germination and pollen tube growth.

3.2. Screening of F_1 progenies against PRSV under glass house conditions

Intergeneric hybrid seedlings along with their parents were raised in controlled conditions (glass house experiments). All the entries were artificially (manually) inoculated with PRSV

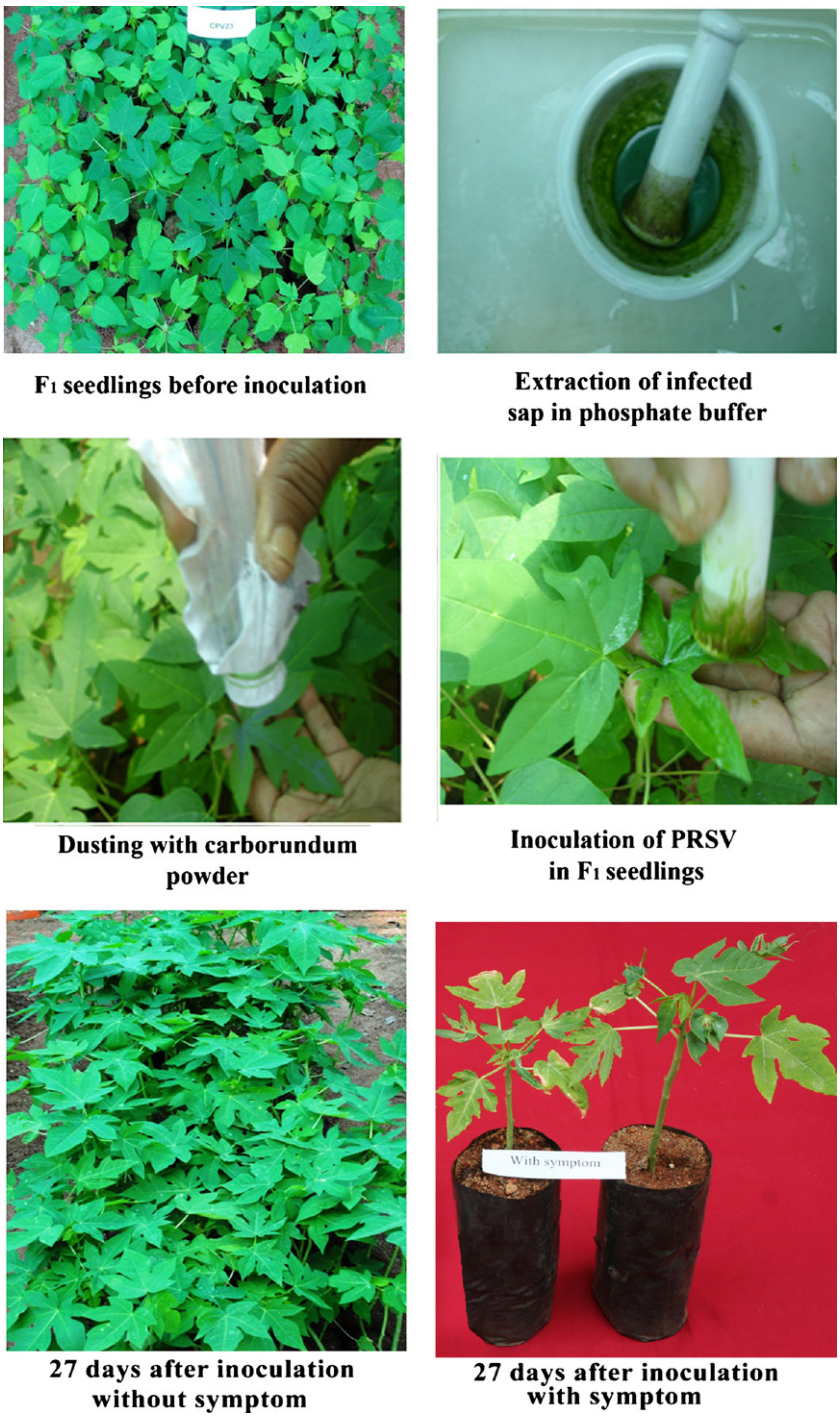

Fig. 2. Confirmation of PRSV resistance in F_1 seedlings.

for screening and observation for PRSV was done 27 days after inoculation. Out of 29 intergeneric hybrid seedlings involving CO 7 x *V. cauliflora*, only six were found to be apparently free from the disease symptoms. Similarly in the cross combination Pusa Nanha x *V. cauliflora*, 23 seedlings (of the 55 seedlings) were found to be apparently free from PRSV. Similarly, another cross combination CP 50 x *V. cauliflora*, showed 70 seedlings out of 335 seedlings were apparently free from PRSV disease. However, all the parents except *V. cauliflora* showed typical PRSV symptoms after artificial inoculation (Table 5).

In general, crop like papaya (perennial nature), field evaluation and screening for diseases is very difficult since, it requires a larger planting area, cost-ineffective and specific field design. On the other hand, when breeders desire to screen a large set of germplasm and this is not an effective due to influence of uncontrolled variation. Hence, screening in glass houses in the nursery stage proved quick and rapid method of evaluation and selection. Regarding the female parents, all were found to exhibit the virus symptoms uniformly after sap inoculation. Symptom free F_1 hybrids were transplanted in the main field for further evaluation. The failures of PRSV symptoms to develop on the manually inoculated hybrid plants indicate the incorporation of genes resistant to PRSV (Fig. 2). Further, the wild genus *V. cauliflora* was found to be completely resistant to the strain

Table 5
Screening of F_1 progenies through artificial inoculation against PRSV under glass house conditions.

Parents/Hybrids	Total number of plants inoculated	Disease scoring (number of plants in each category)						Number of plants without symptom[a]
		0	1	2	3	4	5	
CO 7	5	0	0	0	0	0	5	0
Pusa Nanha	5	0	0	0	0	0	5	0
CP 50	5	0	0	0	0	0	5	0
Vasconcellea cauliflora	5	5	0	0	0	0	0	5
CO 7 x *Vasconcellea cauliflora*	29	6	0	0	0	10	13	6
Pusa Nanha x *Vasconcellea cauliflora*	55	23	0	0	0	15	17	23
CP 50 x *Vasconcellea cauliflora*	335	70	0	0	0	100	165	70

Disease scoring was 0–5 (0 = no disease symptoms; 1 = slight mosaic on leaves; 2 = mosaic patches and/or necrotic spots on leaves; 3 = leaves near apical meristem deformed slightly, yellow, and reduced in size; 4 = apical meristem with mosaic and deformation; 5 = extensive mosaic and serious deformation of leaves, or plant dead).
[a] 27 days after innoculation.

Table 6
Mean performance of parents and F_1 hybrids for fruit yield.

Parents/Hybrids	Number of fruits per tree	Mean fruit weight (kg)	Fruit yield per tree (kg)
Parents			
CO 7	17.00	0.98	16.66
Pusa Nanha	22.00	0.92	18.98
CP 50	21.00	1.82	38.27
Vasconcellea cauliflora	19.00	0.11	2.66
Hybrids			
CO 7 x *Vasconcellea cauliflora*	19.00	0.74	13.99
Pusa Nanha x *Vasconcellea cauliflora*	35.00	1.11	38.85
CP 50 x *Vasconcellea cauliflora*	26.00	1.21	31.38
General mean	22.71	0.98	22.97
SEd	0.90	0.01	0.73
CD ($P = 0.05$)	1.97	0.03	1.61

PRSV which is prevalent in Coimbatore area of Tamil Nadu, India (Manoranjitham et al., 2008).

3.3. Fruit yield and quality performance of intergenric hybrids

In any hybridization programme, yield is the prime character, which decides the supremacy of any selection or hybrid for further advancement. In the present hybridization programme, among the female parents, the genotype CP 50 had maximum fruit weight and yield/tree followed by Pusa Nanha and CO 7. However, among the cross combinations, Pusa Nanha x *V. cauliflora* had higher number of fruits per tree and fruit yield/tree (Table 6 and Fig. 3). This cross combination has many other positive characters

Fig. 3. Intergeneric F_1 hybrid involving Pusa Nanha x *Vasconcellea cauliflora*.

Table 7
Mean performance of parents and F_1 hybrids for fruit quality traits.

Parents/Hybrids	Total Soluble Solids (°Brix)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Titrable acidity (%)	Ascorbic acid (mg/100g)	Carotene (mg/100g)	Sugar acid ratio
Parents								
CO 7	13.5	7.83	7.11	0.72	0.11	30.33	1.93	71.21
Pusa Nanha	11	5.14	4.83	0.31	0.14	39	1.44	36.16
CP 50	9.73	4.35	3.92	0.43	0.21	40.66	1.43	20.74
Vasconcellea cauliflora	5.83	3.95	3.75	0.21	0.24	43.33	1.38	16.49
Hybrids								
CO 7 x *Vasconcellea cauliflora*	10.83	7.63	6.94	0.69	0.12	31.73	1.86	65.53
Pusa Nanha x *Vasconcellea cauliflora*	10.33	5.07	4.55	0.52	0.15	41	1.41	33.3
CP 50 x *Vasconcellea cauliflora*	9.47	4.32	3.77	0.54	0.22	41.67	1.39	19.66
General mean	10.1	5.47	4.98	0.49	0.17	38.23	1.55	37.58
SEd	0.59	0.03	0.01	0.02	0.01	0.72	0.01	1.74
CD ($P = 0.05$)	1.29	0.06	0.02	0.05	0.02	1.58	0.02	3.78

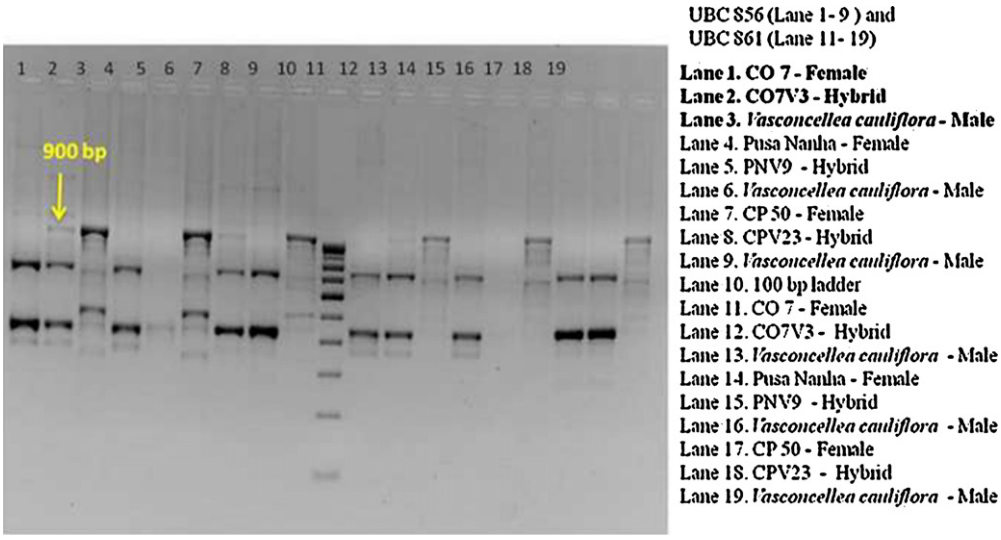

Fig. 4. ISSR markers profile detailing parents and intergeneric F_1's using UBC 856 and UBC 861 primers.

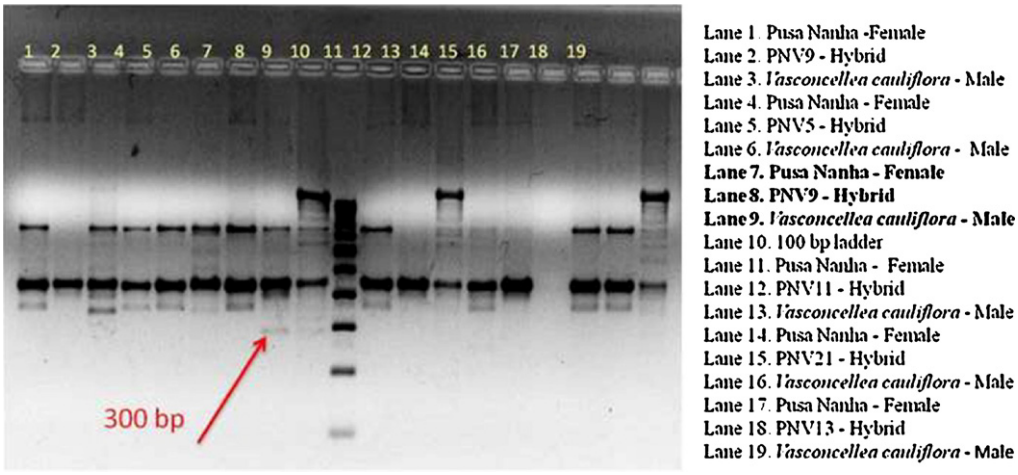

Fig. 5. ISSR markers profile (UBC 856) detailing parents and intergeneric F_1's.

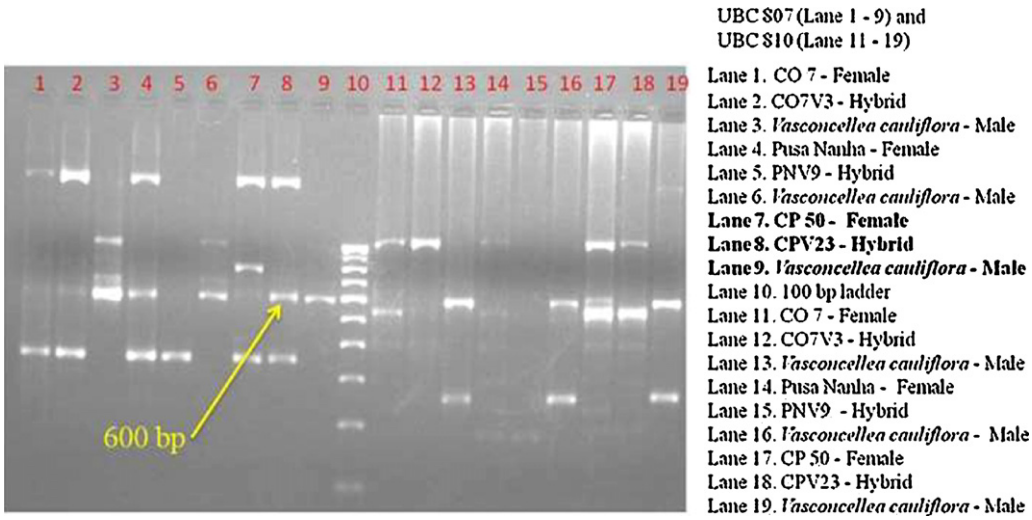

Fig. 6. ISSR markers profile detailing parents and intergeneric F_1's using UBC 807 and UBC 810 primers.

viz., earliness, dwarf stature, maximum number of fruits and yield in spite of the virus disease very much prevalent in the experiment plot. Praveen (2005) also recorded higher fruit weight and yield in one of the interspecific hybrids evaluated for PRSV disease.

While integrating a wild parent in the intergeneric hybridization, there are amble of possibilities for getting poor fruit qualities in the resultant hybrids. In the present study also observed the fact that the quality traits in all the hybrids where marginally affected due to intergeneric hybridization. This may be due to mismatch or inter-chromosomal contradictory between the species. Some time though absence of aforesaid barriers translation of genes in to a specific trait will be affected by one wild receive genes by another dominant wild or domesticated genes. In the present study also found this fact, quality traits in all the hybrids where marginally affected due to intergeneric hybridization. However, among the cross combinations, CO7 x *V. cauliflora* were found better in fruit qualities than the other two cross combinations. As a whole, the quality parameters were not drastically affected in the resultant hybrids due to the presence of wild male parent, *V. cauliflora* (Table 7). Praveen (2005) also reported that the crosses involving *V. cauliflora* as male in the interspecific hybridization programme produced better quality fruits. However, further studies yet to be validated to support this finding.

3.4. Hybridity confirmation using ISSR markers

Three intergeneric hybrids from CO 7 x *V. cauliflora* crosses, eight hybrids from Pusa Nanha x *V. cauliflora* crosses and seven hybrids from CP 50 x *V. cauliflora* crosses were tested for hybridity through ISSR markers. The primer UBC – 856 produced unique banding patterns in *V. cauliflora* (male parent) in which five bands were prominent, out of which third and fifth were absent in female parent (Fig. 4) but these are present in CO 7 x *V. cauliflora* (CO7V3). The same primer produced distinguishable band between Pusa Nanha x *V. cauliflora* (PNV9) which was used for the identification of true hybrid (Fig. 5). In case of UBC – 807 primer, one prominent band was observed in male parent which was absent in female parent but present in CP 50 x *V. cauliflora* (CPV23) hybrid (Fig. 6). These primers were helpful to identify F_1s in cross CO 7 x *V. cauliflora*, Pusa Nanha x *V. cauliflora* and CP 50 x *V. cauliflora.* Those intergeneric F_1 hybrids which is confirmed the hybridity of plants were forwarded to F_2. Ruas et al. (2003) used ISSR markers and successfully evaluated the genetic divergence among the eight *Coffea* species. Praveen (2005) also used ISSR markers to confirm the hybridity of intergeneric hybrids involving *C. papaya* x *V. cauliflora*.

4. Conclusion

The present investigation made a substantial effort to check feasibility of intergeneric hybrids development in papaya. The results showed that it is possible to get fertile progenies while integrating the wild geneome into the cultivated lines by enhancing the pollen tube growth. This might be a good idea that with a whole observation intergeneric crosses perform well in both yield and quality parameters (not severely affected). Such derivative would be taken into further genetical investigation to find out combining ability and the yield heterosis (heterosis estimation beyond the scope of our objectives). Hence, the observed results of diseases resistance of these intergeneric hybrids need to confirm with further studies at the field levels in various growth stage, so that a cultivars can be developed in a fast-track manner by using genetic and breeding information.

Appendix A. List of ISSR primers with sequences used in the analysis

S. No.	Name of the primers	Sequence of the primers
1.	UBC – 807	5′ AGA GAG AGA GAG AGA GT 3′
2.	UBC – 810	5′ CAC ACA CAC ACA CAC AA 3′
3.	UBC – 815	5′ CTC TCT CTC TCT CTC TG 3′
4.	UBC – 817	5′ GAG AGA GAG AGA GAG AT 3′
5.	UBC – 856	5′ ACA CAC ACA CAC ACA CYA 3′
6.	UBC – 861	5′ ACC ACC ACC ACC ACC ACC 3′

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