



Analysis of Papaya Intergeneric Hybrids for Morphological Traits

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Successful crosses have been achieved in intergeneric hybridization between *Carica papaya* and wild *Vasconcellea cauliflora*, to break post zygotic barriers by using various nutrients. Among the nutrients used, sucrose 5 per cent, sucrose 5 per cent + boron 0.5 per cent and sucrose 5 per cent + CaCl₂ 0.5 per cent improved the fruit set and seed set percentage. A total of 1197 flowers were pollinated resulted 308 fruits. On extraction, 721 seeds were obtained from CO 7, Pusa Nanha and CP 50. Out of twenty nine F₁ hybrid plants of CO 7 x *Vasconcellea cauliflora*, only six plants namely CO7V1 to CO7V6 were found free from PRSV symptoms. Similarly, out of fifty five F₁ hybrid plants of Pusa Nanha x *Vasconcellea cauliflora* only twenty three plants namely PNV1 to PNV23 were found free from the symptoms and seventy plants namely CPV1 to CPV70 out of 335 plants of CP50 x *Vasconcellea cauliflora* were found free from PRSV symptoms. F₁ hybrids exhibited very clear morphological marker in the form of pink vein colour. The number of days for first flowering, first flowering height (cm), plant height (cm), stem girth (cm), number of leaves, first fruiting height (cm), fruit set (%), days to first harvest, fruit yield per tree (kg), were intermediate between the two parents.

Key words: *Carica papaya*, *Vasconcellea cauliflora*, intergeneric hybrids, papaya ring spot virus (PRSV)

Papaya, also called papaw or pawpaw, is an edible fruit of tropical softwood tree (*Carica papaya*) of the family Caricaceae. It is native to Central America and has spread to many other tropical and sub-tropical lands including India, the Philippines, and parts of Africa by way of early trade routes. Papaya is considered one of the most economically important and nutritious fruits for rich source of antioxidants, carotenes, vitamin C and flavonoids; the B vitamins folate and pantothenic acid; the minerals potassium and magnesium; and fiber. In addition, papaya is the source of the digestive enzyme papain, which is an industrial ingredient used in brewing, meat tenderizing, pharmaceuticals, beauty products and cosmetics. India and Indonesia have consistently been the top producers, contributing more than 71% of the total world production (FAOSTAT, 2004). At present, it is cultivated throughout the world. India is one of the largest producer of papaya in the world has an area of about 98, 000 ha with an annual production of about 3629 million tonnes (NHB, 2009). In India, it is commercially cultivated in Andhra Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Assam, Orissa, Madhya Pradesh, Manipur, Tamil Nadu and Bihar and certain extent in Kerala.

Papaya crops suffer from several diseases and pests, the most ubiquitous and widespread of which is papaya ringspot virus (PRSV). Almost all cultivated

varieties are found to be highly susceptible. This virus affects production and productivity in every region of the world by decreasing photosynthetic capacity of the plants, which subsequently display stunted growth, deformed and made it fruit inedible and eventually, plant mortality.

Incidence of PRSV has been reported to be more than 90 per cent in India (Varma, 1996) and rendering papaya orchards economically unviable (Hema and Theertha Prasad, 2003). In Tamil Nadu, the disease was first noticed in Coimbatore during 2003 (Jyoti Sharma *et al.*, 2004).

Existing management technologies are no way successful to check the disease. The conventional breeding is the only reliable tool for long term control of the disease. None of the *Carica papaya* cultivars has natural-resistance to PRSV-P. Several related wild species of *Carica* have been reported as resistant to PRSV-P. However, several wild *Carica* species such as *C. cauliflora*, *C. pubescens* and *C. quercifolia* are resistant to PRSV. Conventional interspecific hybridization of *Carica papaya* with other species has been difficult because of interspecific reproductive barriers (Manshardt and Wenslaff, 1989). In Australia, hybrids between *C. papaya* and *Vasconcellea cauliflora* were produced via embryo rescue technique. Dinesh *et al.* (2007) produced intergeneric hybrids in *Carica papaya* and *Vasconcellea cauliflora* using sucrose 5 % as an

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agent to break reproductive barrier. Keeping the above points in consideration the present study was contemplated to produce the F₁ hybrids between different varieties of *C. papaya* and *Vasconcellea cauliflora* for the resistance against PRSV.

Materials and Methods

Source of parental material and Production of F₁ hybrids

Different varieties of *C. papaya* and a PRSV resistant male parent, *Vasconcellea cauliflora* were obtained from the Department of Pomology, Horticultural College, Coimbatore. F₁ hybrids were developed through intergeneric hybridization by adopting the procedure given by Dinesh *et al.* (2007).

Glass house screening of F₁ hybrids and Parents

A total of three hybrids, one male parent and three female parents were screened for PRSV resistance under glass house conditions by sap inoculation method. One gram of infected leaves was ground in a pre-chilled mortar and pestle using 1 ml of 0.1M 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 previously dusted with carborundum powder 600 meshes. After 5 minutes, the excess sap was washed off by distilled water. The disease incidence and intensity score was given using the scale developed by Dhanam (2006).

Field evaluation

The cross combinations and parents were subjected to field evaluation for resistance against PRSV. Six F₁ hybrid seedlings of CO 7 x *Vasconcellea cauliflora*, 23 F₁ hybrid seedling of Pusa Nanha x *Vasconcellea cauliflora* and 70 F₁ hybrid seedlings of CP50 x *Vasconcellea cauliflora* along with parents (6 seedlings each) were transplanted in the main field for evaluation. Seedlings were planted in main field in Randomized Block Design and standard practices were adopted. The disease intensity in each combination was scored according to the scale developed by Dhanam (2006).

DAS ELISA of hybrids and parents

Apparently virus free 99 plants from different hybrid combinations and parents were subjected for ELISA confirmation using PRSV specific antibody. Antibody for PRSV and their positive samples were provided from DSMZ, Braunschweig, Germany. AS-ELISA was performed for the detection of PRSV by following the manufacturer's instructions (DSMZ GmbH, Braunschweig, Germany).

Morphological analysis

Intergeneric F₁ hybrids exhibited very clear morphological marker in the form of vein colour and colour of petiole was observed by visual appearance. Morphological characters (Days to first flowering,

First flowering height (cm), Plant height (cm), Stem girth (cm), Number of leaves, First fruiting height (cm), Fruit set (%), Days to first harvest, Fruit yield per tree (kg), were intermediate between the two parents) were examined. Days taken for the first flower to appear from the date of planting was counted and expressed in numbers. Height of the plant was measured from ground level to the node of first flower and expressed in centimeters. Stem girth was measured 15 cm above the ground level and expressed in centimeters. Fully developed, opened leaves were counted from the tip to the base of the tree and expressed in numbers. Fruit set per cent was calculated by the formula.

$$\text{Fruit set per cent} = \frac{\text{No. of fruits harvested}}{\text{No. of fruit set}} \times 100$$

The days taken from the date of planting to the date on which the fruit was first harvested from the tree at colour break stage was counted and expressed in number. The fruit yield per tree was arrived at by multiplying number of fruits harvested with mean fruit weight and expressed as kilograms.

Results and Discussion

Inter-generic hybridization of *Carica papaya* with other genus *Vasconcellea cauliflora* was started with the aim to transfer the desirable genes for PRSV resistance into the cultivars of papaya. In the present investigation, a large scale crossing programme was initiated using nine papaya varieties viz., CO 1, CO 2, CO 4, CO 5, CO 6, Pusa Dwarf, Pusa Nanha, CP 50 all dioecious and CO 7 a gynodioecious type, as females with *Vasconcellea cauliflora* as male. Among the nine varieties used for crossing, only three cross combinations viz., CO 7 x *Vasconcellea cauliflora*, Pusa Nanha x *Vasconcellea cauliflora* and CP 50 x *Vasconcellea cauliflora* had produced viable seeds and the rest produced only parthenocarpic fruits. Though the pollination was enhanced by using different nutrient solutions, the success of hybridization varied considerably with the varieties/genotypes used. Litz and Conover (1983) also reported that the success in intergeneric hybridization between *C. papaya* and *V. cauliflora* varied depending on the genotype used for crossing. Manshardt and Wenslaff (1989), Sawant (1958), and Zamir and Tadmire (1986) also confirmed the same.

In the present study, out of 721 F₀ seeds sown in the nursery, 419 seed germinated with a percentage of 57 due to intergeneric crosses. Intergeneric cross involving CO 7 x *Vasconcellea cauliflora* had 29 hybrid seedlings out of 45 seeds, Pusa Nanha x *Vasconcellea cauliflora* had 55 hybrid seedlings out of 67 seeds and CP50 x *Vasconcellea cauliflora* had 335 hybrid seedlings out of 609 seeds. Thirugnanavel (2010) also obtained six seedlings

Table 1. Screening of F₁ 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)					No. of plants without symptom after 27 days of inoculation	
		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
<i>Vasconcellea cauliflora</i>	5	5	0	0	0	0	0	5
CO 7 x <i>Vasconcellea cauliflora</i>	29	6	0	0	0	10	13	6
Pusa Nanha x <i>Vasconcellea cauliflora</i>	55	23	0	0	0	15	17	23
CP 50 x <i>Vasconcellea cauliflora</i>	335	70	0	0	0	100	165	70

Data not statistically analyzed, Disease scoring was 0 to 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).

from 222 seeds (two from CO2 and four from CP 50).

Screening of F₁ progenies through artificial inoculation against PRSV under glass house conditions

Intergeneric hybrid seedlings along with parents were raised and artificially inoculated with PRSV under glass house conditions for screening. Observation for PRSV was done 27 days after inoculation. Out of 29 intergeneric hybrid seedlings involving CO 7 x *Vasconcellea cauliflora*, six (CO

7V1 to CO 7V6) were found to be apparently free from the disease. Similarly in the cross combination Pusa Nanha x *Vasconcellea cauliflora*, 23 out of 55 seedlings (PNV1 to PNV23) were found to be apparently free from PRSV. In the cross combination CP 50 x *Vasconcellea cauliflora*, 70 out of 335 seedlings (CPV1 to CPV70) were apparently free from PRSV disease. However, the female parent showed typical PRSV symptoms and the male parent *V. cauliflora* was free from PRSV after artificial inoculation (Table 1).

Table 2. Morphological markers exhibited by the hybrids

Characters	Male parent	Female parents			Hybrids		
	<i>Vasconcellea cauliflora</i>	CO 7	Pusa Nanha	CP 50	CO 7 x <i>V. cauliflora</i>	Pusa Nanha x <i>V. cauliflora</i>	CP 50 x <i>V. cauliflora</i>
Texture of petiole	Pubescent	Smooth	Smooth	Smooth	Variation in the extent of hairiness was observed, that was not measured	Variation in the extent of hairiness was observed, that was not measured	Variation in the extent of hairiness was observed, that was not measured
Colour of petiole	Light pink with green	Green	Green	Green	Variation in the extent of colour of petiole was observed, that was not measured	Variation in the extent of colour of petiole was observed, that was not measured	Variation in the extent of colour of petiole was observed, that was not measured
Vein colour	Pink	Light yellow to green	Light yellow to green	Light yellow to green	Variation in the extent of colour of petiole was observed, that was not measured	Variation in the extent of colour of petiole was observed, that was not measured	Variation in the extent of colour of petiole was observed, that was not measured
Number of vein	5	8	8	7	8	8	7

In a perennial crop like papaya, field screening for diseases is very difficult since, it requires a larger area for planting. Hence, screening in glass houses in the nursery stage proved quick and rapid method. Regarding the female parents, all were found to

exhibit the virus symptoms uniformly after sap inoculation. Symptom free F₁ 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

Table 3. Mean performance of parents and F₁ hybrids at first flowering

Parents / Hybrids	Days to 1 st flowering	1 st flowering height (cm)	Plant height at 1 st flowering (cm)	Stem girth at 1 st flowering (cm)	No. of leaves at 1 st flowering
Parents					
CO 7	122.33	94.36	116.33	11.66	18.00
Pusa Nanha	99.66	40.42	67.00	15.00	20.00
CP 50	114.33	103.33	126.33	12.33	14.66
<i>Vasconcellea cauliflora</i>	91.00	31.66	51.00	18.00	18.33
Hybrids					
CO 7 x <i>Vasconcellea cauliflora</i>	96.66	71.95	100.32	13.00	20.66
Pusa Nanha x <i>Vasconcellea cauliflora</i>	93.00	37.13	61.66	16.00	22.66
CP 50 x <i>Vasconcellea cauliflora</i>	97.00	93.82	103.33	14.00	21.00
General mean	102.00	67.53	89.43	14.28	19.33
SEd	0.81	3.08	1.10	0.72	0.98
CD (P = 0.05)	1.76	6.71	2.40	1.56	2.14

incorporation of genes resistant to PRSV. Further, the wild genus *V. cauliflora* was found to be completely resistant to the strain PRSV prevalent in Coimbatore area of Tamil Nadu, India Manoranjitham *et al.*, 2008).

Hybrid confirmation through morphological analysis

Hybrid confirmation by morphological markers

The morphological markers identified by the breeders to confirm the hybridity in the male parent *Vasconcellea cauliflora* has very distinctive character of pubescent petiole with light pink colour and five pink colour veins found in *Vasconcellea cauliflora* against seven to eight veins with light yellow to green found in *C. papaya* (Table 2).

Among the hybrid combinations, the crosses involving Pusa Nanha x *Vasconcellea cauliflora* exhibited very clear morphological markers in the form of vein colour. The crosses had light pink veins against light yellow to green found in female parent. Similarly, the colour of the petiole and texture of the petiole were intermediary in nature in the hybrids (Fig.1). Morphological characters could be used as a reliable visual marker for preliminary identification of the hybrid prior to genetic analysis. Intermediate morphological characters have been used previously for the identification of *C. papaya* x *C. cauliflora* interspecific hybrids by Chen *et al.* (1991).

In the present study, among the cross

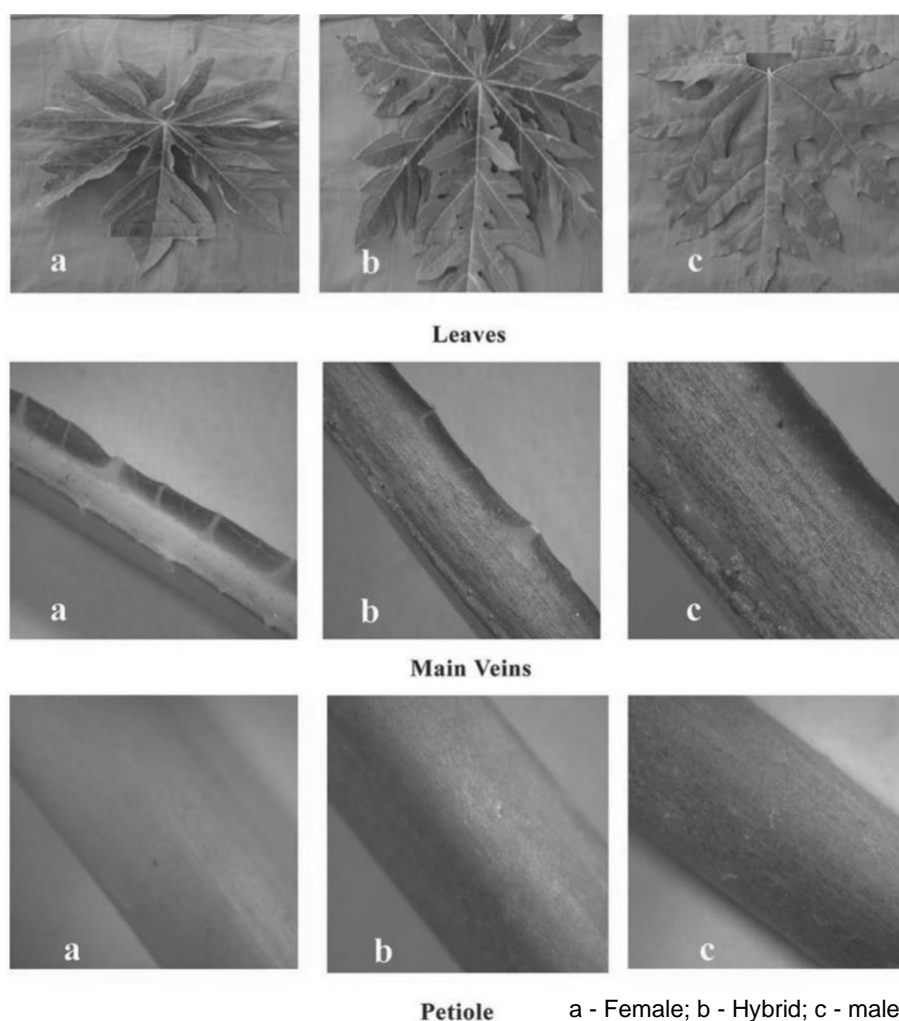


Fig. 1. Morphological marker in the F₁ intergeneric population

combinations, Pusa Nanha x *Vasconcellea cauliflora* crosses were found to be early in flower and fruit production, flowers at lowest height with maximum leaves at first flowering and at harvest. Early vigour is an important morphological character which is considered to be a necessary trait for disease resistance, because, before the plant is fully infested, it can able to give a reasonable yield (Thirugnanavel 2010). Among the cross combinations,

Pusa Nanha x *Vasconcellea cauliflora* had recorded the maximum fruit set percentage, however for days to harvest, not much difference was observed among the crosses. (Table 3, 4 and 5) Manshardt *et al.* (1995) also evaluated several F₁ interspecific hybrids and found better growth and vigoures among them. Hybridization of *V. cundinamarcensis* and *V. parviflora* produced vigorous F₁ plants (Drew *et al.*, 1998). These

Table 4. Mean performance of parents and F₁ hybrids at first harvest

Parents / Hybrids	First fruiting height (cm)	Plant height at first harvest (cm)	Stem girth at first harvest (cm)	No. of leaves at first harvest	Fruit set (%)	Days to first harvest
Parents						
CO 7	106.00	217.00	25.00	16.00	65.82	242.33
Pusa Nanha	52.00	125.33	28.00	18.00	68.55	240.33
CP 50	119.17	231.47	27.00	15.33	64.49	241.33
<i>Vasconcellea cauliflora</i>	37.00	106.00	31.66	16.33	68.33	254.33
Hybrids						
CO 7 x <i>Vasconcellea cauliflora</i>	91.66	163.00	25.00	18.66	55.87	263.33
Pusa Nanha x <i>Vasconcellea cauliflora</i>	46.33	117.00	39.00	22.66	81.96	261.67
CP 50 x <i>Vasconcellea cauliflora</i>	99.16	186.00	31.00	17.67	71.15	264.00
General mean	75.57	163.68	28.10	17.81	68.03	252.48
SEd	0.67	1.11	0.87	0.63	0.83	1.75
CD (P = 0.05)	1.47	2.42	1.90	1.38	1.81	3.82

findings are in close conformity with the findings of the present study

Table 5. Mean performance of parents and F₁ hybrids for fruit yield

Parents / Hybrids	No. 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
<i>Vasconcellea cauliflora</i>	19.00	0.11	2.66
Hybrids			
CO 7 x <i>Vasconcellea cauliflora</i>	19.00	0.74	13.99
Pusa Nanha x <i>Vasconcellea cauliflora</i>	35.00	1.11	38.85
CP 50 x <i>Vasconcellea cauliflora</i>	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

Conclusion

Intergeneric hybrids in *Carica papaya* and *Vasconcellea cauliflora* using sucrose 5 per cent, sucrose 5 per cent +boron 0.5 per cent and sucrose 5 per cent + CaCl₂ 0.5 per cent improved the fruit set and seed set percentage. Morphological characters could be used as a reliable visual marker for preliminary identification of the hybrid prior to genetic analysis. Pusa Nanha x *Vasconcellea cauliflora* exhibited very clear morphological markers. Based on the disease intensity score, reaction to the PRSV and performance, the cross combinations viz., CO 7 x *Vasconcellea cauliflora* (CO7V3), Pusa Nanha x *Vasconcellea cauliflora* (PNV9) and CP 50 x *Vasconcellea cauliflora* (CPV23) were advanced to F₂ generations.

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