RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (2) 113-123, 2015

STUDIES ON RESISTANCE TO PAPAYA RINGSPOT VIRUS (PRSV) IN INTERGENERIC POPULATION OF *Carica papaya* L. and *Vasconcellea cauliflora*

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SUMMARY

An intergeneric hybridization program was developed between *Carica papaya* (Var. Pusa Nanha, CP 50 and CO 7) with *Vasconcellea cauliflora* to incorporate the PRSV resistant gene from *V. cauliflora* into the cultivars of papaya. Serological studies suggest that 18 progenies of the cross Pusa Nanha / *Vasconcellea cauliflora,* 5 selected progenies of CP 50 / *Vasconcellea cauliflora* and only 1 hybrid progeny in the cross CO 7/ *Vasconcellea cauliflora* was found to be promising. Among the crosses, Pusa Nanha / *Vasconcellea cauliflora* recorded superior mean performance for number of fruits per tree and fruit yield than other 2 crosses in F_3 generation. Fruit quality parameters were high in CO 7 / *Vasconcellea cauliflora*. Desirable mean performance for days taken for appearance of PRSV symptom after inoculation and disease intensity score were recorded by Pusa Nanha / *Vasconcellea cauliflora.* Based on overall evaluation of F_3 population, 7 progenies from the cross Pusa Nanha / *Vasconcellea cauliflora* and 4 progenies from the cross CP 50 / *Vasconcellea cauliflora* can be forwarded to F_4 generation for further evaluation.

Key words: *Carica papaya*, *Vasconcellea cauliflora*, intergeneric hybridization, PRSV, resistance

Key findings: Our results indicated that *Vasconcellea cauliflora* can be used to develop field tolerance /resistance in *Carica papaya*. These hybrids may be further evaluated to develop PRSV resistant hybrids.

Manuscript received: November 18, 2013; Decision on manuscript: August 26, 2014; Manuscript accepted: February 14, 2015.

Communicating Editor: Bertrand Collard

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important fruits of tropical and subtropical regions of the world. It is believed to be native of Tropical America; probably Southern Mexico (De Candolle, 1884) from where it spread to most of the Caribbean and Asian countries during the 16th century. Papaya fruit is a rich source of nutrients such as provitamin A carotenoids (2020 IU/100 g), vitamin C (46g/100 g), B vitamins, dietary fiber, folate and pantothenic acid; and the minerals, potassium and magnesium.

India stands first in the production of papaya in the world followed by Nigeria, Indonesia, Mexico, Ethiopia and others. The production area for papaya in India is estimated

to be 106,000 ha and production at 4,196,000 metric tons (NHB 2011). The total area under cultivation of papaya has recorded a regular increase in the recent years but its production has not shown corresponding increase. This might be due to the losses caused by various diseases caused by fungi, bacteria, phytoplasma and viruses. There are many economically important diseases of papaya: the most important among these is papaya ring spot virus (Purcifull 1972). Management of PRSV through rouging of infected plants, quarantine regulation of restricting the plant movement, use of insecticides against insect vectors and cross protection generally have not been effective in controlling the disease. Naturally occurring resistance to PRSV-P has not been identified in any papaya cultivar to date. Thus, development of resistant papaya is considered the best strategy for long term virus control. Several species from the related genus *Vasconcellea* exhibit complete resistance to PRSV-P, and present a valuable resource for developing new PRSV-P resistant papaya varieties.

Breeding tolerant varieties with susceptible high yielding commercial varieties has improved yield under infectious conditions (Chan 2004). Numerous efforts have been made to incorporate the resistance genes from other genera in the Caricaceae namely, *Vasconcellea cauliflora, V. quercifolia, V. stipulata* and *V. pubescens*. Intergeneric hybrids between papaya and PRSV resistant species have been produced by number of investigators with aid of embryo rescue techniques (Horovitz and Jimenez 1967; Khuspe *et al*., 1980). However not much progress has been made.

Hence, an intergeneric hybridization programme was developed involving between *Carica papaya* with *Vasconcellea cauliflora* to incorporate the resistant gene from the later into the cultivars of papaya. In this study, attempts were made to evaluate F_3 populations of intergeneric hybrids of *Carica papaya* and *Vasconcellea cauliflora* along with their parents for examination of PRSV resistance.

MATERIALS AND METHODS

Plant material

Inter-generic hybridization was performed using *Carica papaya* (Var. Pusa Nanha, CP 50 and CO 7) as female and *Vasconcellea cauliflora* as male parent to transfer the desirable genes for PRSV resistance. The original cross performed by Jayavalli (2010), produced F_1 plants, seeds were collected from the hybridity confirmed, sib-mated F_1 plants which were used for raising F_2 population. In this investigation, F_3 progenies were raised from the seeds collected from F_2 population and maintained at Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore for evaluation (2011-12).

Mechanical sap inoculation of PRSV

Seedlings of F_3 progenies from 3 cross combinations *viz*., CO 7 / *Vasconcellea cauliflora*, Pusa Nanha / *Vasconcellea cauliflora* and CP 50 / *Vasconcellea cauliflora* along with their parents *viz*., CO 7, Pusa Nanha, CP 50 and *Vasconcellea cauliflora* were artificially inoculated with PRSV under poly house conditions through artificial inoculation method. 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 previously dusted with carborundum powder 600 mesh size. After 5 min, the excess sap was washed off by distilled water.

The disease incidence and intensity scores were given using the scale adopted by Dhanam (2006). The scale consists of 5 levels based on the symptoms exhibited by the infected plants as presented in Table 1.

Transplanting and cultural operations

After observing symptom expression for 27 days after inoculation, apparently healthy F_3 progenies along with their parents were

Table 1. Five levels of scale based on the symptoms exhibited by the infected plants.

Reactions	Intensity scores
Resistant (R)	0-1
Tolerant (T)	1-2
Moderately susceptible (MS)	2-3
Susceptible (S)	3-4
Highly susceptible (HS)	4 and above

transplanted in the main field for evaluation. All the package of practices was followed as per the recommendations of Tamil Nadu Agricultural University (Anon 2004).

Sib-mating

For sib-mating, male and female plants of the same variety/ cross were selected based on the disease intensity score at the time of flowering. In the cross combination Pusa Nanha / *Vasconcellea cauliflora* and CP 50 / *Vasconcellea cauliflora,* female flowers which were about to open the next day were bagged on the previous day evening. Pollen grains were collected from fully mature, unopened flowers of the desired male parent from the same cross combination. Pollination was done between 6.30 and 8.30 AM. At the time of pollination, the bags of the female flowers were removed and the pollen collected from desired male parent was dusted on the stigma and bagged again. In the cross combination CO 7 / *Vasconcellea cauliflora*, the perfect flowers were carefully bagged on the previous day before dehiscence of anther and left as such. Data relating to the fruit characters were taken only from the sibmated fruits. Fully mature, ripe fruits were harvested after 4 months, the seeds were extracted for raising the next generation.

Enzyme Linked Immunosorbant Assay (ELISA)

The Enzyme Linked Immunosorbent Assay (ELISA), a powerful immunological test (Clark and Adams, 1977), is extensively used for detecting, identifying and quantifying viruses in many plant species (Clark, 1994). ELISA test is a more sensitive and convenient than back-inoculation tests when large numbers of plants have to be screened (Miller and Martin, 1988). This test could be an effective component of a reliable method for screening *C. papaya* / *C. cauliflora* hybrid plants for PRSV resistance.

Antibodies for PRSV and their positive samples were obtained from DSMZ, Braunschweing, Germany. Double antibody sandwich ELISA (DAS-ELISA) was performed for the detection of PRSV by following the manufacturer's instructions (DSMZ Gmbh, Braunschweig, Germany). Purified IgG was diluted in coating buffer (1:1000) and 200 µl was added to each well of a micro titre plate (Grainer). The plates were then incubated at 37 ^{o}C for 2 to 4 hrs and thereafter plates were washed with PBS-T using wash bottle, soaked for a few minutes and washing repeated twice. Plates were blotted by tapping upside down on tissue paper. 200 µl of aliquots of the test sample (extracted in sample extraction buffer) were added to duplicate wells. The plates were incubated overnight at 4 ^{o}C. The plates were washed as mentioned earlier and added with 200 µl of the anti-virus conjugate (1:500) to each well and incubated at 37 ^{o}C for 2 hrs. Then the plates were washed 3 times as indicated earlier. Finally 200 µl of freshly prepared substrate in 10 mg ρ-nitro phenyl phosphate (Sigma 104-105) dissolved in 10 ml of freshly prepared substrate buffer) was added to each well and incubated in dark at room temperature for 20-45 minutes or as long as necessary to obtain clear reactions. The reaction was stopped by adding 50 µl of 3M NaOH. Buffer served as negative control. Positive control was also included. Spectrometric measurement of absorbance was then read at 405 nm (EL 800, BIO-TEK Instrument Inc., USA).

Traits under study

First fruiting height was measured from ground level to the height at which first mature fruit appeared and expressed in centimeters. Total soluble solids of the fruit was determined by 'ERMA' hand refractometer and expressed as ^{o}Brix. Total sugars were estimated by the method of Hedge and Horreiter (1962) and expressed in percentage. Total phenol was estimated by the method suggested by Malik and Singh (1980) and expressed as µg g^{-1}. Peroxidase activity was assayed spectrophotometrically (Malik and Singh, 1980) and expressed as min g^{-1}. Polyphenol oxidase activity was assayed using the method described by Esterbaner *et al*. (1977) and expressed as min g^{-1}. Disease intensity score was recorded at 30 days intervals from transplanting till harvest as suggested by Dhanam (2006).

Statistical analysis

The variation of individual F_3s for the characters was analyzed in terms of mean, range and coefficient of variation as per the method described by Sivasubramanian and Madhava Menon (1973) using excel.

RESULTS

Screening of F_3 progenies through artificial inoculation against PRSV under poly house Conditions

In the present investigation, progenies of F_3 population along with parents were raised and artificially inoculated with PRSV under poly house conditions for screening. Observation for PRSV was done 27 days after inoculation. The progeny C1-24 of the cross Pusa Nanha /*Vasconcellea cauliflora* recorded higher per cent of disease free seedlings (44.87 per cent) followed by the progeny C2-15 in the cross combination CP 50 / *Vasconcellea cauliflora* (29.87 per cent). In the cross combination CO 7 / *Vasconcellea cauliflora*, C3-5 had 25.31 per cent of disease free seedlings (Table 2).

Among the 3 crosses, the cross Pusa Nanha / *Vasconcellea cauliflora,* progenies *viz*., C1-10, C1-13, C1-21, C1-24, C1-38, C1-52 and C1-99 and in the cross CP 50 / *Vasconcellea cauliflora,* progenies *viz.*, C2-15, C2-16, C2-26 and C2-28 registered higher number of completely disease free seedlings after 27 days of artificial sap inoculation. Regarding the parents, all female parents were found to exhibit the virus symptoms uniformly after sap inoculation.

ELISA titre value for parents and F_3 Population

In this study, visually symptom free progenies of the cross Pusa Nanha / *Vasconcellea cauliflora viz*., C1-7, C1-10, C1-13, C121, C1-24, C1-28, C1-38, C1-43, C1-46, C1-52, C1-73, C1-82, C1-84, C1-91, C1-92, C1-99, C1-101 and C1-108 from, progenies of the cross CP 50 / *Vasconcellea cauliflora viz.,* C2-15, C2-16, C2-26, C2-27 and C2-28 from and only one progeny, C3-5 from the cross CO 7 / *Vasconcellea cauliflora* were selected to perform ELISA for confirmation of virus free progenies. Parents, negative control papaya plants which were raised under insect proof net house along with selected progenies from the crosses *viz*., Pusa Nanha / *Vasconcellea cauliflora,* CP50 / *Vasconcellea cauliflora* were subjected to DAS- ELISA test.

ELISA titre value varied from 0.150 to 0.974. Among the parents, the resistant male parent *Vasconcellea cauliflora* had recorded the lowest titre value of 0.150. However, the susceptible female parent CO 7 recorded the highest titre value of 0.974, followed by CP 50 (0.970) and Pusa Nanha (0.962). Among the crosses, CO 7 / *Vasconcellea cauliflora* recorded a higher ELISA titre value of 0.285. The cross, Pusa Nanha / *Vasconcellea cauliflora* had ELISA titre value varied from 0.241 to 0.258 and the lowest score was recorded by the progeny C1-24 (0.241). Among the crosses involving CP 50 / *Vasconcellea cauliflora*, ELISA titre value varied from 0.244 to 0.262 and the lowest ELISA titre value was recorded by the progeny C2-15 (0.244) (Table 3).

Eighteen progenies *viz.*, C1-7, C1-10, C1-13, C121, C1-24, C1-28, C1-38, C1-43, C1-46, C1-52, C1-73, C1-82, C1-84, C1-91, C1-92, C1-99, C1-101 and C1-108 from Pusa Nanha /

Table 2. Disease scoring of F_3 population and parents for PRSV under poly house conditions.

Parents/ hybrids	Total number of plants scored	Disease scoring (Number of plants in each category)				Number of plants without symptom 27 days after inoculation
		0	1, 2 & 3	4	5	
Parents						
CO 7	96	0	0	50 (52.08)	46 (47.92)	0
Pusa Nanha	94	0	0	49 (52.13)	45 (47.87)	0
CP 50	96	0	0	51 (53.12)	45 (46.87)	0
Vasconcellea	5	5 (100)	0	0	0	5
Crosses						
C1-7	70	20 (28.57)	0	21 (30.10)	29 (41.33)	20
C1-10	76	26 (34.21)	0	24 (31.59	26 (34.20)	26
C1-13	77	28 (36.36)	0	23 (29.76)	26 (33.88)	28
C1-21	78	30 (38.46)	0	22 (28.00)	26 (33.54)	30
C1-24	78	35 (44.87)	0	18 (23.93)	25 (31.20)	35
C1-28	71	22 (30.98)	0	21 (29.52)	28 (39.48)	22
C1-38	76	26 (34.21)	0	24 (31.49)	26 (34.20)	26
C1-43	75	21 (28.00)	0	22 (30.40)	32 (41.65)	21
C1-46	75	23 (30.67)	0	22 (29.79)	30 (33.75)	23
C1-52	76	25 (32.89)	0	24 (32.15)	27 (34.96)	25
C1-73	72	20 (27.78)	0	22 (31.07)	30 (41.15)	20
C1-82	71	21 (29.58)	0	21 (29.88)	29 (40.53)	21
C1-84	72	22 (30.55)	0	21 (29.68)	29 (39.76)	22
C1-91	70	18 (25.71)	0	23 (32.64)	29 (41.65)	18
C1-92	72	20 (27.78)	0	21 (29.63)	31 (42.59)	20
C1-99	75	24 (32.00)	0	20 (27.25)	31 (40.75)	24
C1-101	71	21 (29.58)	0	20 (28.46)	30 (41.95)	21
C1-108	70	20 (28.57)	0	22 (32.63)	28 (38.80)	20
C2-15	77	23 (29.87)	0	23 (30.06)	31 (40.07)	23
C2-16	76	22 (28.95)	0	23 (30.21)	31 (40.85)	22
C2-26	75	21 (28.00)	0	23 (31.15)	31 (40.90)	21
C2-27	71	18 (25.35)	0	24 (34.49)	29 (40.16)	18
C2-28	75	21 (28.00)	0	23 (30.40)	31 (41.65)	21
C3-5	79	20 (25.31)	0	27 (34.00)	32 (40.69)	20
Mean		19.70		24.26	29.56	19.70
SEd		0.53		0.75	0.66	0.53
CD (P = 0.05)		1.06		1.50	1.33	1.06

Values in the parentheses are in percentage
C1-Pusa Nanha / *Vasconcellea cauliflora*, C2-CP 50 / *Vasconcellea cauliflora,* C3-CO7 / *Vasconcellea cauliflora* *Vasconcellea cauliflora*, 5 progenies *viz.*, C2-15, C2-16, C2-26, C2-27 and C2-28 from CP 50 / *Vasconcellea cauliflora* and only one progeny, C3-5 from the cross CO 7 / *Vasconcellea cauliflora* were selected for further evaluation under field.

Table 3. ELISA titre value for selected progenies of F_2 population and parents against PRSV.

Parents /Crosses	OD value at 405nm
Parents	
Vasconcellea cauliflora	0.150
Pusa Nanha	0.962
CP 50	0.970
CO 7	0.974
Crosses	
C1-7	0.258
C1-10	0.244
C1-13	0.243
C1-21	0.242
C1-24	0.241
C1-28	0.250
C1-38	0.245
C1-43	0.252
C1-46	0.251
C1-52	0.248
C1-73	0.249
C1-82	0.252
C1-84	0.247
C1-91	0.251
C1-92	0.248
C1-99	0.245
C1-101	0.249
C1-108	0.251
C2-15	0.244
C2-16	0.245
C2-26	0.248
C2-27	0.262
C2-28	0.254
C3-5	0.285
Negative control	0.090
Mean	0.249
SEd	0.001
CD (P=0.05)	0.003

Buffer value – 0.015
C1-Pusa Nanha */ Vasconcellea cauliflora, C2-CP 50 / Vasconcellea cauliflora, C3-CO7 / Vasconcellea*

Table 4. Mean and co-efficient of variation of F_3 families for fruit physical, quality and biochemical parameters.

Crosses/ parents	First fruiting height (cm)		Mean fruit weight (kg)		Fruit yield per tree (kg)		TSS (°Brix)		Total sugars (%)		Total phenols (µg g^{-1})		Peroxidase activity (min g^{-1})		Polyphenol oxidase activity (min g^{-1})	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
C1-7	59.2	9.1	1.0 [a]	15.0	20.7	26.2	10.0 [a]	12.5	4.9 [a]	14.2	6559.8*	0.7	0.9*	4.1	0.9*	5.2
C1-10	46.5*	8.57	1.1*	18.8	30.9*	31.8	10.1	8.4	5.0 [a]	12.0	6654.2*	0.3	0.9*	5.7	0.9*	4.8
C1-13	46.5*	18.82	1.1*	26.7	32.3*	19.6	10.4 [a]	11.3	5.1 [a]	15.0	6822.3*	0.6	0.9*	4.9	0.9*	4.1
C1-21	45.1*	9.94	1.1*	7.6	35.2*	18.1	10.6 [a]	11.9	5.2 [a]	9.2	6849.1*	0.3	0.9*	2.8	1.0*	2.9
C1-24	45.0*	7.65	1.1*	11.6	34.7*	14.2	10.4 [a]	10.8	5.2 [a]	16.9	6841.5*	0.7	0.9*	3.2	1.0*	4.3
C1-28	61.0	15.92	1.1 [a]	25.0	21.5 [a]	24.6	9.3	8.7	4.9 [a]	13.3	6333.1*	0.4	0.9*	3.7	0.9*	3.2
C1-38	45.5*	11.41	1.1 *	11.9	31.0*	12.5	10.2	5.6	5.1 [a]	10.2	6758.8*	0.4	0.9*	3.2	0.9*	3.7
C1-43	67.6	12.06	1.0 [a]	12.8	21.6 [a]	27.7	9.7	8.1	4.8	13.1	6533.0*	0.2	0.9*	3.00	0.9*	3.0
C1-46	62.3	15.91	1.0 [a]	11.7	19.8 [a]	25.6	10.1	5.3	4.7	17.6	6452.9*	0.4	0.9*	4.1	0.9*	4.4
C1-52	47.4*	10.03	1.1 *	8.2	31.9*	15.3	10.2 [a]	7.2	5.0 [a]	14.1	6656.7*	0.4	0.9*	4.9	0.9*	3.1
C1-73	67.0	5.89	1.0 [a]	14.8	17.6 [a]	25.7	9.9	10.1	4.9[a]	11.0	6342.7*	0.4	0.9*	5.0	0.9*	4.9
C1-82	60.5	8.66	1.0 [a]	13.8	21.4 [a]	17.7	9.8	7.5	4.8	13.5	6262.3*	0.3	0.9[a]	4.0	0.9*	3.8
C1-84	59.1	9.10	1.1 [a]	8.9	19.8 [a]	9.5	9.6	7.5	4.6	18.8	6341.2*	0.4	0.9*	4.6	0.9*	4.5
C1-91	62.1	10.62	1.0 [a]	10.4	26.5 [a]	39.0	9.3	4.4	4.2	12.3	6440.6*	0.4	0.9*	5.1	0.9*	5.1
C1-92	62.9	18.33	1.0 [a]	22.2	30.0 [a]	23.1	9.5	6.5	4.4	12.6	6517.7*	0.8	0.9*	3.9	0.9*	6.7
C1-99	54.8*	8.27	1.1 *	8.4	31.0*	16.7	10.0	5.0	5.2 [a]	16.9	6547.4*	0.5	0.9*	4.5	0.9*	4.7
C1-101	60.6	9.12	1.1 [a]	11.0	16.2	19.1	9.6	9.5	4.4	17.8	6545.3*	0.5	0.9*	3.9	0.9*	4.3
C1-108	61.3	10.00	1.0 [a]	8.5	20.3 [a]	17.9	9.7	10.2	5.0 [a]	16.4	6545.3*	0.4	0.9*	7.5	0.9*	3.7
Mean	48.7	6.73	1.1	3.4	30.3	10.7	9.9	3.8	4.8	6.3	6555.8	2.7	0.9	1.9	0.9	2.3
C2-15	88.3*	21.00	1.2	13.2	34.6	17.5	9.7 [a]	5.9	4.4 [a]	10.7	4956.9*	0.2	0.8*	4.2	0.7*	9.0
C2-16	93.3*	9.32	1.2	8.4	30.8	22.4	9.5	5.9	4.3	7.6	4949.3*	0.5	0.8*	2.9	0.7 *	4.8
C2-26	94.1*	12.83	1.2	10.6	30.1	24.7	9.2	8.5	4.2	9.9	4912.4*	0.4	0.8*	5.0	0.7*	3.8
C2-27	115.8*	7.39	1.0	14.5	25.7	17.9	8.6	4.9	3.8	12.8	4851.2	0.6	0.8[a]	8.2	0.6[a]	9.8
C2-28	114.4*	11.27	1.2	14.3	30.2	17.0	9.2	7.3	4.1	14.8	4902.0*	7.5	0.8*	7.5	0.7*	4.5
Mean	93.6	4.49	1.2	3.2	29.3	6.5	9.2	4.5	4.2	6.1	4914.4	3.2	0.8	1.3	0.6	1.6
C3-5	96.2	5.25	0.8	8.9	13.7	14.5	10.7	7.2	7.3	4.7	4655.9	0.6	0.4	5.0	0.3	9.3
Pusa Nanha	53.5	8.03	1.0	11.3	19.9	7.1	11.1	5.0	5.3	3.6	5613.90	0.03	0.9	1.1	0.87	1.1
CP 50	121.7	2.10	1.8	1.7	38.3	0.6	10.0	2.7	4.7	4.6	4818.45	0.1	0.8	1.4	0.56	2.7
CO7	113.9	1.55	0.9	12.1	15.7	9.9	12.7	5.9	7.4	5.9	4650.96	0.5	0.3	10.7	0.25	11.4

* Significantly superior than P1(P1- female parent corresponding to the cross; C1- P1 is Pusa Nanha; C2- P1 is CP50; C3-P1 is CO7), [a] Significantly on par with P1; C1-Pusa Nanha / *Vasconcellea cauliflora*, C2-CP 50/ *Vasconcellea cauliflora*, C3-CO7/ *Vasconcellea cauliflora*

Table 5. Disease intensity score and reaction of F_3 population and parents for PRSV under field condition.

Parents/ Crosses	Disease intensity score after transplanting at 30 days intervals								Reaction
	30 & 60	90	120	150	180	210	240	270	
				Parents					
Pusa Nanha	0	0.2	0.6	1.2	1.6	2.2	2.6	3.20	S
CP 50	0	0.4	0.8	1.4	1.8	2.4	2.8	3.60	S
CO7	0	0.6	0.8	1.6	2.0	2.6	3.2	4.60	HS
				Crosses					
C1-7	0	0	0	0.4	0.8	1.0	1.4	1.70	MR
C1-10	0	0	0	0	0.3	0.5	0.	0.95	AH
C1-13	0	0	0	0	0.3	0.4	0.6	0.70	AH
C1-21	0	0	0	0	0.3	0.4	0.6	0.65	AH
C1-24	0	0	0	0	0.3	0.4	0.5	0.80	AH
C1-28	0	0	0	0.5	0.7	0.9	1.5	1.70	MR
C1-38	0	0	0	0	0.2	0.4	0.5	0.90	AH
C1-43	0	0	0	0.5	0.6	1.1	1.5	1.70	MR
C1-46	0	0	0	0.4	0.5	0.8	1.4	1.75	MR
C1-52	0	0	0	0	0.4	0.5	0.6	0.95	AH
C1-73	0	0	0	0.4	0.7	1.3	1.6	1.70	MR
C1-82	0	0	0	0.4	0.6	1.0	1.5	1.60	MR
C1-84	0	0	0	0.4	0.6	1.2	1.4	1.60	MR
C1-91	0	0	0	0.5	0.7	1.5	1.7	1.80	MR
C1-92	0	0	0	0.5	0.8	1.5	1.7	1.80	MR
C1-99	0	0	0	0	0.5	0.7	0.8	0.90	AH
C1-101	0	0	0	0.5	0.8	1.1	1.6	1.70	MR
C1-108	0	0	0	0.4	0.8	1.2	1.5	1.70	MR
C2-15	0	0	0	0	0.4	0.5	0.7	0.85	AH
C2-16	0	0	0	0	0.3	0.5	0.6	0.95	AH
C2-26	0	0	0	0.5	0.7	0.8	0.9	1.00	MR
C2-27	0	0	0	0.5	0.7	0.8	1.15	2.50	MS
C2-28	0	0	0	0.5	0.6	0.7	0.9	1.50	MR
C3-5	0	0	0.6	0.8	0.9	1.5	1.7	2.30	MS
Mean		0.04	0.08	0.43	0.71	1.06	1.32	1.70	
SEd		0.003	0.004	0.012	0.02	0.04	0.01	0.03	
CD (P=0.05)		0.01	0.01	0.02	0.04	0.09	0.02	0.06	

C1-Pusa Nanha / *Vasconcellea cauliflora*, C2-CP 50/ *Vasconcellea cauliflora,* C3-CO7/ *Vasconcellea cauliflora*
1. Apparently healthy (R/AH) 0-1; 2. Moderately resistant (MR) 1-2; 3. Moderately susceptible (MS) 2-3; 4. Susceptible (S) 3-4; 5. Highly susceptible (HS) 4 and above

Disease rating 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).

Evaluation of F_3 population mean performance

The mean value of F_3 progenies were worked out for each cross and presented in Table 4. Progenies of F_3 population were compared with corresponding parents for *per se* performance.

Considering the mean performance, among the 3 crosses, the cross Pusa Nanha / *Vasconcellea cauliflora* had superior mean performance for first fruiting height (48.73 cm), number of fruits per tree (28.56), fruit yield per tree (30.32 kg), total phenols (6555.78 µg/g), peroxidise activity (0.92 min/g) and polyphenol oxidase activity (0.93 min/g). The cross CO 7 / *Vasconcellea cauliflora* had superior mean performance for TSS (10.72°Brix) and total sugars (7.32%) whereas the cross CP 50 / *Vasconcellea cauliflora* had superior mean performance for mean fruit weight (1.18 kg).

Disease intensity score and reaction to PRSV in the field

Among the F_3 progenies, cross combination involving CO 7 and *Vasconcellea cauliflora* exhibited first PRSV symptoms slightly over 4 months after inoculation followed by CP 50 / *Vasconcellea cauliflora.* In the cross CP 50 / *Vasconcellea cauliflora* progenies *viz*., C2-15, C2-16 and C2-26 exhibited symptoms close to 5 months after planting. However, the progenies *viz.,* C1-10, C1-13, C1-21, C1-24, C1-38, C1-52 and C1-99 of the best cross combination involving Pusa Nanha / *Vasconcellea cauliflora* exhibited symptoms 6 months after transplanting (Table 5). Similar observation was also noticed for the appearance of disease on the fruits. Overall disease intensity score at the time of harvest also registered a very similar trend.

DISCUSSION

Screening of F_3 progenies under polyhouse

In a perennial crop like papaya, field screening for diseases is very difficult since it requires a larger area for planting. Hence, screening in polyhouses in the nursery stage proved quick and rapid method. The male parent *V. cauliflora* was reported earlier to be resistant to the strain PRSV prevalent under Coimbatore area of Tamil Nadu, India (Manoranjitham *et al.,* 2008). Studies involved artificial screening suggested that *V. cauliflora* did not show the typical symptoms of PRSV (Magdalita *et al*., 1997; Drew *et al*., 2006). Thirugnanavel (2009) reported that manual inoculation and DAS-ELISA were used in combination to determine whether the plants were resistant to PRSV or not. In the present investigation, the progeny C1-24 of the cross Pusa Nanha / *Vasconcellea cauliflora* recorded higher per cent of disease free seedlings (44.87%). Among the 3 crosses, 7 progenies in the cross Pusa Nanha / *Vasconcellea cauliflora* and 4 progenies in the cross CP 50 / *Vasconcellea cauliflora* registered higher number of completely disease free seedlings after 27 days of artificial sap inoculation. The failures of PRSV symptoms to develop on the manually inoculated hybrid plants indicate the incorporation of genes resistant to PRSV (Jayavalli 2010). Symptom free F_3 hybrids from these crosses (Pusa Nanha / *Vasconcellea cauliflora*, CP 50 / *Vasconcellea cauliflora* and CO 7 / *Vasconcellea cauliflora*) were transplanted in the main field for further evaluation.

Serological test

In the present study, visually symptom free progenies were selected to perform ELISA for confirmation of virus free progenies. The results revealed that the lowest value of 0.150 was recorded by the resistant male parent *V. cauliflora* whereas all the female parents used for this study recorded very high titre values proving their susceptibility. Manoranjitham *et al.* (2008) reported that the wild papaya *V. cauliflora* had the lowest titre value thus indicating its natural resistance to PRSV. They also reported that *V. cauliflora* is resistant to all the strains of PRSV which are prevalent in Coimbatore conditions. Similar results were observed by Jayavalli (2010). Thirugnanavel (2009) also reported that tolerant genotypes recorded lower ELISA absorbance value than the susceptible ones in papaya. Eighteen progenies from Pusa Nanha / *Vasconcellea cauliflora* (C1-7, C1-10, C1-13, C121, C1-24, C1-28, C1-38, C1-43, C1-46, C1-52, C1-73, C1-82, C1-84, C1-91,

C1-92, C1-99, C1-101 and C1-108), 5 progenies from CP 50 / *Vasconcellea cauliflora* (C2-15, C2-16, C2-26, C2-27 and C2-28) and only one progeny from the cross CO 7 / *Vasconcellea cauliflora* (C3-5) were found to record lower titre values ranging from 0.243 to 0.285, proving their tolerance to PRSV and were selected for further evaluation in the field. Similar studies using ELISA test had been conducted previously by Thomas and Dodman (1993) to identify PRSV-P infected *C. papaya*.

Evaluation of F_3 population in field

Mean value is the important factors for selection; mean serves as a basis for eliminating undesirable crosses. Selection for the improvement of quantitative characters can be effective only when the segregating generations possess the potential variability. Many quantitatively inherited characters are fixed rapidly, emphasizing the need to test the character expression in large populations of F_3.

The potentialities of the crosses can also be assessed by the number of desirable sibs or lines that can be selected from the F_3 generation. Since many loci are expected to have become homozygous in the F_3 generation, the mean differences between the sibs may be as minimal as possible. According to Finkner *et al*. (1973), cross or family with higher mean was relatively effective in identifying superior segregants. In the context of the above considerations, mean performance of the F_3 generations of the 3 crosses for the traits are discussed hereunder.

Considering the mean performance, among the 3 crosses, the cross Pusa Nanha / *Vasconcellea cauliflora* had superior mean performance for morphological, biochemical and yield parameters *viz.*, first fruiting height, total phenols, peroxidise activity, polyphenol oxidase activity, number of fruits per tree and fruit yield per tree. This may be due to the early vigor and higher enzyme activity which are considered to be necessary for disease resistance, hence the plants can produce reasonable yield before it is fully infested. The cross CO 7 / *Vasconcellea cauliflora* had superior mean performance for fruit quality parameters *viz*., TSS and total sugars. Involvement of wild parent in the intergeneric hybridization creates ample possibilities for getting poor fruit qualities in the resultant hybrids. In the present study also, reduction in quality characters such as total soluble solids, sugars, acidity and sugar acid ratio were observed in all the 3 crosses due to intergeneric hybridization. Even though, among the cross combinations, the cross CO7 x *Vasconcellea cauliflora* was found to record better in fruit quality attributes. Thirugnanavel (2009) and Jayavalli (2010) also reported similar findings.

In the field condition, among the parents, the gynodioecious female CO 7 was first to exhibit the symptoms closely followed by Pusa Nanha and CP 50. Among the hybrids, cross combination involving CO 7 / *Vasconcellea cauliflora* exhibited first PRSV symptom slightly over 4 months after planting and in the cross CP 50 x *Vasconcellea cauliflora* exhibited symptoms slightly over 5 months after planting. However, the best cross combination involving Pusa Nanha x *Vasconcellea cauliflora* took close to 6 months to exhibit the symptoms. The F_3 progenies *viz.,* C1-10, C1-13, C1-21, C1-24, C1-38, C1-52 and C1-99 of the best cross combination involving Pusa Nanha / *Vasconcellea cauliflora* exhibited delayed symptoms (6 months after transplanting). This may be due to the increased tolerance of the above selected progenies which have been inherited from the wild male parent. This is in close conformity with the results of Jayavalli (2010).

CONCLUSION

The present study suggested that the progenies *viz*., C1-10, C1-13, C1-21, C1-24, C1-38, C1-52 and C1-99 of the cross Pusa Nanha / *Vasconcellea cauliflora* and progenies *viz.,* C2-15, C2-16, C2-26 and C2-28 of the cross CP 50 / *Vasconcellea cauliflora* found to be promising based on the disease intensity score, reaction to the papaya ring spot virus and mean performance for morphological, yield and quality attributes. These hybrids may be forwarded for further evaluation to develop a PRSV resistant hybrids.

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