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Physiological and Biochemical Characterization of Intergeneric Hybrids of Papaya

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Successful intergeneric crosses have been achieved between *Carica papaya* and *Vasconcellea cauliflora* and break the intergeneric hybridization barrier 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 + $CaCl_2$ 0.5 per cent improved the fruit set and seed set percentage. A total number of 1197 flowers were pollinated resulted in 308 fruits. On extraction, 721 seeds were obtained from CO 7, Pusa Nanha and CP 50. Out of twenty nine F_1 hybrid plants of CO 7 x *Vasconcellea cauliflora,* only six plants namely CO 7V1 to CO 7V6 were found free from PRSV symptoms. Similarly, out of fifty five F_1 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. Physiological and biochemical characters were found to be higher in the male parent *Vasconcellea cauliflora* and also the cross combination involving Pusa Nanha x *Vasconcellea cauliflora* recorded the highest chlorophyll content and the enzymes like peroxidase and polyphenol oxidase also played a major role in plant productivity and resistance to PRSV at the time of harvest.

Keywords: *Carica papaya*, *Vasconcellea cauliflora*, intergeneric hybrids, papaya ringspot virus (PRSV), peroxidase(PO), polyphenol oxidase (PPO).

Papaya (*Carica papaya* L.), a delicious fruit tree, is affected by number of diseases caused by various pathogens and viruses. Virus diseases are widespread and are very serious among the diseases of papaya. Most destructive disease of *Carica papaya* worldwide is papaya ring spot caused by papaya ring spot virus (Manshardt, 1992). Almost all cultivated varieties are found to be highly susceptible. Control measures to check Papaya Ring Spot Virus-P (PRSV-P) include cultural practices or cross-protection or planting of tolerant cultivars (Gonsalves, 1994). None of the has been very successful so far against PRSV. PRSV-P tolerant cultivar *'Cariflora'* was a first developed cultivar in Florida by Conover *et al*. (1986) in an effort to combat the problem of PRSV-P. The cultivars '*Cariflora'*, Tainung No.5 and Eksotika were used in breeding programs around the world to serve as source of PRSV-P tolerance. Thirugnanavel (2010) screened the germplasm collection of papaya for PRSV resistance, and found the genotype CP 50 as a best field tolerance one for PRSV at Coimbatore conditions. For the present investigation, the genotype CP 50 is used as one of the female parents for intergeneric hybridization for PRSV resistance.

At present, development of virus resistant cultivars through conventional breeding is a reliable tool for long term control. Several related wild species of *Vasconcellea* have been reported as resistant to PRSV-P. Even though intergeneric hybridization of *Carica papaya* with other species was attempted, very little progress has been made by using *Vasconcellea cauliflora* which has the desirable gene for PRSV resistance. Hence, present investigation was carried out to transfer the resistance gene to the popular cultivars of papaya. The biochemical and physiological characters were studied to find out the characters responsible with disease resistance.

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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 for field evaluation for resistance against PRSV. Six F_1 hybrid seedlings of CO 7 x *Vasconcellea cauliflora*, 23 F_1 hybrid seedling of Pusa Nanha x *Vasconcellea cauliflora* and 70 F_1 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 package of practices were followed. 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, Braunschweing, Germany. DAS-ELISA was performed for the detection of PRSV by following the manufacturer's instructions (DSMZ Gmbh, Braunschweig, Germany).

Physiological and biochemical characters at harvest

Chlorophyll a, b and total chlorophyll contents were estimated using 80 per cent acetone as per the method suggested by Arnon (1949) and expressed as mg g^{-1}. Total phenol and peroxidase activity were estimated by the method suggested by Malik and Singh (1980) and expressed as µg g^{-1} and min g^{-1} respectively. Polyphenol oxidase activity was assayed using the method described by Esterbaner *et al*. (1977) and expressed as min g^{-1}.

Results and discussion

Intergeneric hybridization for PRSV resistance

Transfer of PRSV resistance from *Vasconcellea cauliflora* to *Carica papaya* by intergeneric hybridization involving *V. cauliflora* was earlier attempted and reported (Sawant, 1958; Horovitz and Jimenez, 1967; Litz and Conover, 1983; Chen *et al*., 1991 and Thirugnanavel, 2010). The genus *Vasconcellea* contains 21 species that were formerly in *Carica* distributed from Argentina and Chile to South Mexico (Manshradt, 1992; Badillo, 1993). In contrast to papaya, the 21 related species have demonstrated resistance to most of the diseases that infect papaya. Disease resistances that have been identified in *Carica* species for PRSV-P resistance are *C*. *cauliflora, C*. *pubescens, C*. *quercifolia* and *C*. *stipulate* (Conovar, 1964; Horovitz and Jimenez, 1967). Phytophthora tolerance in *C*. *goudotiana,* papaw dieback resistance in *C*. *parviflora* (Drew *et al.*, 1998) and resistance to *Asperisporium caricae* (black spot) in *C*. *pubescens* have been reported. Consequently, there have been numerous attempts to transfer useful disease resistance genes from wild relatives to papaya through intergeneric hybridization.

The aim of the present study is to transfer the desirable genes for PRSV resistance from *Vasconcellea cauliflora* by crossing *Carica papaya* to incorporate the resistant gene into the cultivars of papaya. In the present investigation large scale crossing programme 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, (gynodioecious) were employed as female 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 (Fig.1). Though the pollination and fertilization were

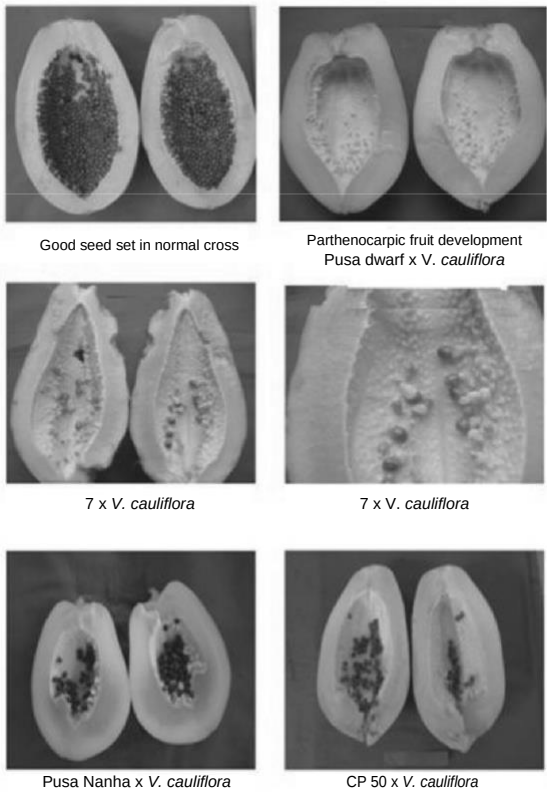

Fig. 1. See set in intergeneric hybridization using different nutrients solutions

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) and Magdalita *et al*. (1988), Sawant (1958), Zamir and Tadmore (1986) also confirmed the similar results.

In the present study, out of 721 F_0 seeds sown in the nursery, 419 seed germinated with a germination percentage of 57. In general, the intergeneric crosses recorded a very low germination percentage when compared to parents. 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 from 222 seeds (two from CO 2 and four from CP 50).

Table 1. 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)						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
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

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).

Screening of F_1 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 was made after 27 days of 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*, out of 55 seedlings, 23 seedlings (PNV1 to PNV23) were found to be apparently free from PRSV. In the cross combination CP 50 x *Vasconcellea cauliflora*, out of 335 seedlings, 70 seedlings (CPV1 to CPV70) were apparently free from PRSV disease. However, all the parents except *Vasconcellea cauliflora* showed typical PRSV symptoms after artificial inoculation (Table 1).

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 F_1 hybrids were transplanted in the main field for further evaluation. The failure of PRSV symptoms to develop on the manually inoculated hybrid plants indicate the

Table 2. Mean performance of parents and F_1 hybrids for physiological and biochemical characters at first harvest

Parents / Hybrids	Total chlorophyll (mg g^{-1})	Chlorophyll 'a' (mg g^{-1})	Chlorophyll' b' (mg g^{-1})	Total phenols (µg g^{-1})	Peroxidase activity (min g^{-1})	Polyphenol oxidase activity (min g^{-1})
Parents						
CO 7	2.07	1.53	0.54	4759.67	0.36	0.29
Pusa Nanha	2.66	1.72	0.93	5428.67	0.87	0.88
CP 50	2.82	1.89	0.91	4811.33	0.84	0.55
Vasconcellea cauliflora	2.98	2.00	0.98	6690.33	0.96	0.95
Hybrids						
CO 7 x *Vasconcellea cauliflora*	2.27	1.66	0.58	4963.00	0.46	0.40
Pusa Nanha x *Vasconcellea cauliflora*	2.96	1.98	0.97	6548.33	0.91	0.94
CP 50 x *Vasconcellea cauliflora*	2.79	1.85	0.94	4993.00	0.88	0.65
General mean	2.65	1.80	0.84	5456.33	0.75	0.67
SEd	0.02	0.01	0.01	102.96	0.01	0.01
CD (P = 0.05)	0.04	0.03	0.02	224.34	0.02	0.02

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).

Physiological characters

Chlorophyll plays a major role in plant productivity. The capacity of photosynthesis depends upon the amount of chlorophyll present. Development of mosaic, yellowing and chlorosis *etc*. in virus infected plants have attributed two causes namely stimulation of chlorophylase which degrades chlorophyll into chlorophyllide and phytol; and inhibition of chloroplast development (Esau, 1956).

In the present study, chlorophyll content varied among the parents and their hybrids. Among the parents, the male parent *Vasconcellea cauliflora* recorded the maximum total chlorophyll content. Among the hybrids, the cross combination involving Pusa Nanha x *Vasconcellea cauliflora* recorded the maximum total chlorophyll content at harvest proved its supremacy in accumulation (Table 2). Thirugnanavel (2010) also reported higher chlorophyll content in the tolerant papaya genotypes screened for PRSV.

Biochemical characters

Enzyme activity is one of the important tools to confirm the resistance to pathogens. When a pathogen infects the host tissue, a small number of specific genes are induced to produce mRNA's that permit synthesis of similar number of specific proteins (Vera and Conejero, 1989). Many of these proteins are enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, peroxidase and β-1-3 glucanase (Vidhyasekaran, 1993). These enzymes are involved in the synthesis of low molecular weight substances such as phytoalexins, phenols and lignin, which are inhibitory to the invading pathogens (Vidhyasekaran, 1993). Hence, estimation of these biochemical markers, which provide mechanism for resistance to pathogens is highly essential. Among the various enzymes, peroxidase is considered as one of the important defence related enzymes due to its role in catalyzing the condensation of phenolic compounds into lignin.

Estimation of peroxidase in the present study revealed that, the resistant parent *Vasconcellea cauliflora* had the maximum peroxidase activity followed by Pusa Nanha among the parents. In the hybrid combination, Pusa Nanha x *Vasconcellea cauliflora* crosses recorded the maximum peroxidase activity at harvest. The decrease in peroxidase content was observed in disease infected plants by Dhanumjaya Rao *et al*. (2007) in grapes and Kavino *et al*. (2008) in banana. This is in confirmity with the findings of the present study.

Polyphenol oxidase (PPO) oxidises the phenols to highly toxic quinines and hence considered to play an important role in disease resistance. In the present study, among the parents, the resistant male *Vasconcellea cauliflora* had the maximum polyphenol oxidase followed by Pusa Nanha. The crosses involving Pusa Nanha and *Vasconcellea cauliflora* had the maximum polyphenol oxidase activity (Table 2). Dhanumjaya Rao *et al*. (2007) in grapes and Kavino *et al*. (2008) in banana have reported similar findings in resistant plants.

Vidhyasekaran (1988) described the occurrence of many kinds of phenolics in plants. In the present study, maximum total phenol was observed in *Vasconcellea cauliflora* followed by Pusa Nanha. The cross combination involving these two parents recorded the maximum total phenol content. Thirugnanavel (2010) also observed higher total phenol content in the papaya genotype CP 50 which was identified as one of the tolerant genotypes for PRSV in the germplasm collections.

Conclusion

Physiological and biochemical characters were found to be higher in *Vasconcellea cauliflora* and the cross combination involving Pusa Nanha x *Vasconcellea cauliflora*. 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 better performers and the same have been advanced to F_2 generations.

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