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Evaluation of F_2 intergeneric population of papaya (*Carica papaya* L.) for resistance to papaya ringspot virus (PRSV)

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ABSTRACT

The present investigations were undertaken to evaluate the F_2 intergeneric population of *Carica papaya* (var. Pusa Nanha, CP 50 and CO 7) and *Vasconcellea cauliflora* for PRSV resistance. Investigations were also made to estimate the mean performance, genetic parameters governing different traits and reaction to the PRSV in the F_2 population. Among the crosses, Pusa Nanha × *V. cauliflora* recorded superior mean performance for fruit yield, number of fruits per tree and fruit biochemical parameters than other two crosses in F_2 generation. Mean performance for papain recovery per fruit and the enzyme activity were high in CP 50 × *V. cauliflora*. Fruit quality parameters were high in CO 7 × *V. cauliflora*. Desirable mean performance for days taken for disease after inoculation and disease intensity score were recorded by Pusa Nanha × *V. cauliflora.* Among the various characters studied, higher GCV, PCV, heritability and genetic advance as percent of mean were registered by the cross Pusa Nanha × *V. cauliflora* for all the morphological characters and fruit yield parameters. From the F_2 population 24 plus trees were selected for further evaluation based on their performance *viz*., morphological, yield, quality and biochemical characters and reaction to PRSV.

1. Introduction

Papaya (*Carica papaya* L.), one of the major fruit crops, belongs to the family Caricaceae. 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. It is cultivated throughout tropical and subtropical regions worldwide (Storey, 1953). Papaya fruit is a rich source of nutrients such as provitamin A carotenoids (2020 IU/100 g), vitamin C (46 g/100 g), B vitamins, dietary fibre, folate and pantothenic acid; and the minerals, potassium and magnesium (Popenoe, 1974; Samson, 1986). The ripe fruit of the papaya is usually eaten raw and the unripe green fruit can be eaten cooked, usually in curries, salads and stews. Papaya yields a valuable proteolytic enzyme 'papain' which is used for tenderizing meat, preparation of chewing gum, pre shrinking of wool, degumming natural silk, in cosmetics *etc.* (Chan and Tang, 1978).

India stands first in the production of papaya in the world and currently the area under papaya in India is estimated at 96,000 ha and production at 3,913,000 metric tonnes (National Horticulture Board Database, 2010). The total area under cultivation of papaya has recorded a regular increase in the recent past but its production has not shown corresponding increase. This might be due to the losses caused by various diseases incited by fungi, bacteria, phytoplasma and viruses. Among these, viral diseases are the limiting factors of papaya cultivation particularly papaya leaf curl (*Begomovirus*) and papaya ring spot virus (*potyvirus*) are more prevalent in India. Papaya ring spot cause heavy loss of 40–90 percent depending upon the time of infection and age of the plant (Manshardt, 1992).

PRSV is a member of the genus *potyvirus*, with flexuous, filamentous particles about 780 nm × 12 nm. It is transmitted mechanically and by many species of aphids in a nonpersistent manner. PRSV is grouped into papaya-infecting type-P (PRSV-P) and non-papaya infecting type-W (PRSV-W). PRSV infection is reported to occur in every region where papaya is grown irrespective of the agroclimatic conditions. In the 60s and early 70s, PRSV was a constraint in parts of Western and Northern India. Tamil Nadu was relatively free from PRSV infection till 2003; however from the year 2004 papaya orchards were severely affected by this virus (Sharma et al., 2005). Control of the disease including rouging of diseased plant, cultural practices, cross protection, quarantine regulations

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restricting plant movement and use of insecticides against insect vector has been attempted through various strategies. An ideal approach for controlling PRSV is through incorporation of PRSV-resistant gene *via* breeding. Numerous efforts have failed to identify PRSV resistant genes in papaya. Interpretation of PRSV-P resistance segregation in F_1 intergeneric hybrids of *V. quercifolia*, *V. cundinamarcensis* (Drew et al., 1998) and *V. cauliflora* (Magdalita et al., 1997) points to the existence of several genetic sources for PRSV-P resistance in the *Vasconcellea* gene pool. Resistance conferred by *V. quercifolia* appears to be controlled by multiple genes with Co-dominance (Drew et al., 1998), whereas a lack of F_1 segregation suggests that *V. cauliflora* and *V. cundinamarcensis* carry a single dominant gene for this trait (Manshardt and Wenslaff, 1987; Magdalita et al., 1997). Multi-genic inheritance of resistant to PRSV, which uniquely exhibit inter-genic interactions (Jayavalli, 2010). Conflicting findings are probably due to genetic differences in local virus strains and plant material (Horovitz and Jimenez, 1967), environmental conditions, and deficient diagnostic method. Jimenez and Horovitz (1957) reported that *Carica cauliflora* J., a wild species having non-edible fruits is known to be resistant for this viral disease. Now the species *cauliflora* has been grouped under the genera *Vasconcellea* (Vegas et al., 2003). Manoranjitham et al. (2008) observed that *Vasconcellea cauliflora* plants are resistant to the strain PRSV prevalent in Coimbatore area of Tamil Nadu, India. Work on intergeneric hybridization against PRSV in papaya is being attempted in many countries by conventional means. However, not much progress has been made in this direction. Hence, an intergeneric hybridization programme was initiated involving *C. papaya* (varieties Pusa Nanha, CO 7 and CP 50) with *V. cauliflora* to incorporate PRSV resistance and to evaluate the F_2 population of these progenies along with their parents.

2. Materials and methods

2.1. Plant material

Inter-generic hybridization was made using *C. papaya* as female and *V. cauliflora* as male parent to transfer the desirable genes for PRSV resistance. The original cross performed by Jayavalli (2010) produced F_1 plants which were maintained at the college orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore prior to this study. F_1 seeds were collected to raise the F_2 population.

2.2. Evaluation of F_2 population

Seeds obtained from hybridity confirmed F_1 population (through ISSR and SSR markers) were raised along with the parents in nursery bags. A single seed was sown in each poly bag of 20 cm × 10 cm size, filled with a mixture of red earth, sand, farm yard manure in 1:1:1 ratio. The bags were kept under partial shade and irrigated through rosecan regularly.

2.2.1. Sap inoculation

Seedlings thus raised were used for screening. All the seedlings were artificially inoculated with papaya ring spot virus through artificial sap 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 three leaves stage previously dusted with carborundum powder 600 meshes. 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 five levels based on the symptoms exhibited by the infected plants as shown below.

Sl. No.	Reactions	Intensity scores
1	Resistant (R)	0–1
2	Tolerant (T)	1–2
3	Moderately susceptible (MS)	2–3
4	Susceptible (S)	3–4
5	Highly susceptible (HS)	4 and above

2.2.2. Transplanting and cultural operations

After observing symptom expression for 27 days after inoculation, apparently healthy F_2 progenies along with their parents were transplanted in the main field at a spacing of 1.8 m × 1.8 m. Two months after transplanting, thinning was done to ensure presence of at least one pistillate plant in the case of dioecious population and a hermaphrodite tree in each pit in the case of gynodioecious population. A standard package of practices was followed as recommended by Tamil Nadu Agricultural University such as 50 g in each of N, P and K per plant were applied once in two months interval and irrigation given at weekly interval. Observations were recorded for biometrical and biochemical characters (Tables 3–4) for all the hybrids and parents.

2.2.3. Sib mating

In the cross combination Pusa Nanha × *V. cauliflora* and CP 50 × *V. 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 × *V. 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 four months, the seeds were extracted.

2.3. Traits under study

First flowering height was measured from ground level to the node of first flower and expressed in centimetres. Height of the plant was measured from a fixed point of 15 cm above the ground level to terminal crown and expressed in centimetres. Stem circumference was measured 15 cm above the ground level and expressed in centimetres. First fruiting height was measured from ground level to the height at which first mature fruit appeared and expressed in centimetres. Mean fruit weight of five fruits was worked and expressed in kilograms. Procedure suggested by Moore (1984) was used for measuring the proteolytic activity with tyrosine as standard and expressed as tyrosine units per mg of papain.

Total soluble solids of the fruit was determined by 'ERMA' hand refractometer and expressed as °Brix. Total sugars were estimated by the method of Hedge and Horreiter (1962) and expressed in percentage. Acidity was estimated as per the AOAC, 1960 and expressed as percentage of citric acid equivalents. Total phenol was estimated by the method suggested by Malik and Singh (1980) and expressed as $\mu g\,g^{-1}$. 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).