Indian Journal of Agricultural Sciences **85** (8): 1069–73, August 2015/Article

Effect of papaya ringspot virus on growth, yield and quality of papaya (*Carica papaya*) cultivars

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Received: 25 October 2013; Accepted: 8 April 2015

ABSTRACT

The field experiment was conducted during 2008 – 2010 to study the effect of papaya ringspot virus – type P on growth, yield, and quality of papaya (*Carica papaya* L.) varieties under PRSV infected conditions at Tamil Nadu Agricultural University, Coimbatore. Significant variation was observed for disease score, DAS-ELISA, tree growth, fruit parameters, yield, and quality characters among the varieties evaluated. The results revealed that all the papaya varieties tested were ELISA positive and PRSV adversely affected the growth, yield and quality of papaya varieties. Among the cultivars evaluated, CP 50 performed better even under PRSV infected conditions and recorded the lowest disease score (1.9), ELISA value (0.198), highest plant height (255.60 cm), stem girth (41.76 cm), number of leaves (41.78), maximum yield (29.26 fruits/plant), dry papain production (8.3 g dry papain/fruit) and tyrosine unit (139.2).

Key words: CP 50, Papain, Papaya ringspot virus type –P (PRSV-P), Papaya varieties, Yield

Papaya (*Carica papaya* L.), native of Tropical America, is one of the most important commercial fruit crops of India. Papaya is a rich source of vitamin A (2020 IU/100g), vitamin C (46g/100g), dietary fibre, and minerals (Sampson 1986). The unripe fruit is a good source of papain, an enzyme used in the brewing of beer; as an ingredient in the manufacture of drugs and cosmetics; as an agent for degumming natural silk; and as a shrink resistance treatment for wool. India is the largest producer of papaya in the world and currently it is cultivated in an area of about 105600 ha with an annual production of about 4.19 million tonnes during 2010 to 2011 (NHB 2011). The total area under cultivation increases annually, but the productivity is not increasing as expected. This might be due to the occurrence of pest and diseases.

Commercial papaya cultivation is affected by many fungal and viral diseases and few pests, of which papaya ringspot viral disease type P (PRSV-P) is the most destructive disease (Manshardt 1992). The virus, which is transmitted by a number of aphid species in a non-persistent manner to a limited host range of cucurbits and papaya, belongs to *potyvirus* family of single stranded RNA virus (Purcifull *et al*. 1985). Papaya infected with this virus develops a wide range of symptoms: mosaic and chlorotic leaf; water soaked oily streaked stem and petiole; mottled and distorted young leaves; and ring spotted fruits. Infected plants loss vigour and become stunted. Harvests are virtually nil when the virus is transmitted at seedling stage. If the transmission occurs before flowering, the flower production will be affected which could cause severe yield loss up to 85-90% (Lokhande *et al*. 1992, Hussain and Varma 1994). PRSV was first reported in Hawaii (Jensen 1949) and was found to occur in tropical and subtropical areas where papaya is grown (Purcifull 1972). In India, PRSV-P was first reported from North India during 1960 (Khurana and Bhargava 1970), but only during 1995 in South India (Byadgi *et al*. 1995). Within a span of 5 to 6 years, PRSV had spread throughout South India (Verghese *et al*. 2002).

Several attempts have been made world over to curb the PRSV incidence in papaya including transgenic approach. Few tolerant lines like 356-3 (Zee 1985), Cariflora (Conover *et al*. 1986), L 248 (Chan 2004), and Solo × Cavite, Kapoho × Tainung and Cavite × 4172 hybrids (Magdalita *et al*. 2007) had been identified by selection and hybridization. Intergeneric hybridization between *C. papaya* as female and resistant *Vasconcellea cauliflora* as male parent resulted in some advanced lines using embryo rescue techniques (Magdalita *et al.* 1997, Drew *et al*. 2006).

Control measures, including rouging of diseased plant, cultural practices, cross protection, quarantine regulations restricting plant movement and use of insecticides against insect vector, generally have been ineffective in eliminating the disease. Development of genetically resistant cultivar to this virus is the most reliable solution for long term control which includes screening and identification of resistant cultivars in the germplasm or transfer of resistant gene from

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wild type to the cultivar through conventional breeding. The purpose of present study was to determine the impact of PRSV-P on the growth, yield, quality and biochemical changes in papaya varieties.

MATERIALS AND METHODS

The field experiment was carried out at orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2008–2010. Eight varieties, one of which was gynodioecious (CO 7) and seven were dioecious (CO 1, CO 2, CO 4, CO 5, CO 6, Pusa Dwarf and CP 50) were tested. The trail was laid out in randomized block design with three replications. The observations recorded were disease severity, growth, yield, quality, and biochemical parameters. In order to assess the disease severity, an observation scale with five levels was adopted (Dhanam 2006): resistant (0-1), tolerant (1-2), moderately susceptible (2-3), susceptible (3-4) and highly susceptible (4 and above).

Double antibody sandwich ELISA (DAS-ELISA) test was carried out to detect PRSV. 200 il of coating buffer (1:1000) was added to each well and incubated at 37 °C for 4 hr. Plate was washed with PBS-T (Phosphate Buffer Saline containing Tween 20) thrice at three minutes interval. 200 µl of aliquots of the test sample (extracted in sample extraction buffer) were added and incubated overnight at 4 °C. The plate was washed with PBS-T thrice and 200 µl of the anti-virus conjugate (1:500) was added to each well. Plate was incubated at 37 °C for 2 h and washed thrice. Finally 200 µl of freshly prepared substrate containing P-nitro phenyl phosphate was added to each well and incubated in dark at room temperature for 20-45 min to observe the colour development. The reaction was stopped by adding 50 µl of 3M NaOH. Absorbance was read at 405 nm in ELISA reader (EL 800, BIO-TEK Instrument Inc., USA).

The growth parameters such as plant height (cm) stem girth (cm), and number of leaves was recorded at first harvest. The yield and other yield components like fruit length (cm), circumference (cm), cavity index, pulp thickness (cm), and fruit weight (kg) were measured in ten fruits in each replication and the mean was worked. The number of fruits per plant and yield per plant (kg) were recorded in five trees in each replication and the mean was taken. The cavity index was calculated using the following formula.

$$\text{Cavity index} = \frac{\text{Cavity volume}}{\text{Fruint Volume}} \times 100$$

Total soluble solids was determined by 'ERMA' hand refractometer and expressed as °Brix. Procedure suggested by Hedge and Horreiter (1962) was used for estimating total sugars and expressed as percentage. Reducing sugars was estimated as per the procedure by Somogyi (1952) and expressed as percentage. Non reducing sugars were calculated by subtracting reducing sugars from total sugars. Titrable acidity was estimated by AOAC, (1960) and expressed as citric acid equivalent. The papain was extracted in 85–90 days old fruit in dual purpose papaya varieties, viz. CO 2, CO 5, Pusa Dwarf and CP 50 at 8.00 scaps to 9.00 scaps and the papain activity was calculated using the method described by Moore (1984) and expressed as tyrosine units per mg of papain. Data were subjected to statistical scrutiny as per the methods suggested by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The present study in the field under high disease pressure revealed that all the eight varieties tested for PRSV-P exhibited typical symptoms of PRSV-P. Substantial variability was observed for all the varieties tested (Fig 1) and the intensity varied from tolerant to highly susceptible among the varieties. Furthermore, serological test confirmed that all the cultivars tested were ELISA positive and susceptible to the disease. Out of eight varieties tested, CP 50 exhibited the lowest disease score (1.90) and ELISA value (0.198) which indicated that it had some degree of field tolerance to PRSV-P. CO 7 obtained highest disease score (4.75) and ELISA value (0.402) that confirmed CO 7 was highly susceptible. This may be due to inherent genetic makeup of the genotype, time of infection and climatic conditions. Dhanam (2006), Mohamad Roff (2007), Magdalita *et al*. (1997) and Vimla Singh *et al.* (2005) also reported PRSV tolerant papaya varieties recorded lowest disease score and lowest ELISA value.

Varieties appeared to be well differentiated for vigour characters such as plant height, stem girth and number of leaves per plant and the magnitude of the vigour varied significantly among the varieties evaluated (Table 1). The highest plant height (255.60 cm), stem girth (41.76 cm) and number of leaves per plant (41.78) were recorded for CP 50 that indicated it was vigorous even under PRSV-P affected conditions. Cooper and Jones (1983) stated that vigorous growth under stress condition is an indication of disease tolerance. Pusa Dwarf, a dwarf cultivar registered the minimum plant height of 146.61 cm. Drastic reduction in vigour was noticed in susceptible cultivars and the least stem girth (30.46 cm) and minimum number of leaves/plant (31.46) was recorded for CO 7. In these cultivars, the growth was reduced and in severe cases, the growth was completely arrested. Similar kind of findings was observed in papaya due to PRSV infection by Mowlick *et al*. (2008). Multiplication of virus at faster rate in the susceptible cultivars could have caused a substantial reduction in cell

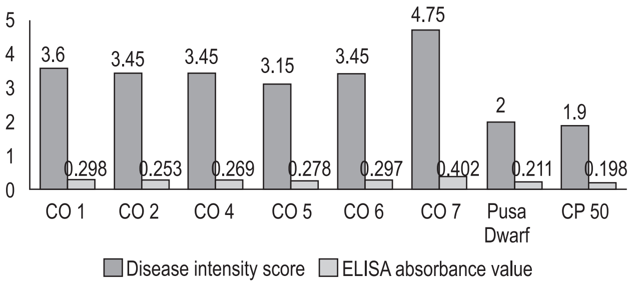

Fig 1 Disease intensity score and ELISA values of papaya cultivars for PRSV

Table 1 Growth, fruit and yield characters of papaya cultivars under PRSV infected conditions

Cultivar	Plant height (cm)	Stem girth (cm)	No of leaves	Fruit length (cm)	Fruit circumference (cm)	Cavity index	Pulp thickness (cm)	No. of fruits/ plant	Mean fruit weight (kg)	Fruit yield/ plant (kg)
CO 1	198.66	38.13	35.62	21.9	33.6	28.7	2.61	19.79	1.38	27.31
CO 2	226.50	40.32	34.12	27.6	45.2	26.8	2.90	27.95	1.53	42.76
CO 4	248.00	34.15	38.86	21.9	34.3	29.0	2.76	22.47	1.45	32.58
CO 5	241.62	41.57	34.87	26.1	42.6	26.3	2.93	24.21	1.68	40.67
CO 6	243.95	40.72	40.72	24.2	41.7	27.4	2.97	20.62	1.79	36.90
CO 7	223.50	30.46	31.46	23.5	31.3	21.0	2.45	16.16	1.02	16.48
Pusa Dwarf	146.61	39.12	36.72	29.7	51.7	24.1	3.81	21.93	2.35	51.53
CP 50	255.60	41.76	41.78	29.2	47.2	25.1	3.31	29.26	2.05	59.98
Mean	222.80	38.23	36.72	25.48	40.91	26.02	2.96	22.77	1.65	38.50
CD (P = 0.05)	13.38	2.17	2.10	1.45	2.34	1.46	0.16	1.32	0.09	2.42

division and elongation thereby decreased plant vigour.

Fruit characters such as length, circumference, cavity index, and pulp thickness were significantly varied among the varieties (Table 1). Pusa Dwarf recorded the maximum fruit length (29.7 cm) and circumference (51.7 cm). CO 1 and CO 4 recorded the minimum fruit length (21.9 cm) whereas, CO 7 recorded the minimum circumference (31.3 cm). Khurana (1970), Vimla Singh *et al.* (2005) and Rahman and Akanda (2008) reported that fruit length and circumference were reduced by PRSV infection. These findings are in confirmity with the findings of the present study. Lower cavity index is beneficial in papaya as it directly relates with more pulp thickness. The lowest cavity index was registered by CO 7 (21.0) and the highest cavity index was registered by CO 4 (29.0). However, CO 7 registered the minimum pulp thickness of 2.45 cm. The cavity index is mainly depends on cavity volume and fruit volume that was decided by the size of the fruits. CO 7 was highly susceptible to PRSV and the virus affected the photosynthate accumulation in fruits that drastically reduced the fruit size and pulp thickness of CO 7. Reduction in fruit size reduced the cavity volume thereby CO 7 recorded the lowest cavity index with less edible portion.

The yield components like number of fruits, fruit weight and yield/plant were adversely affected by the virus and varied significantly among the varieties evaluated (Table 1). Reduction in yield and its attributing characters in all the varieties evaluated were observed in the present study. Out of eight varieties evaluated, the highest number of 29.26 fruits/plant was recorded by CP 50 and the lowest number of 16.16 fruits/plant was registered by CO 7. Pusa Dwarf recorded the maximum fruit weight of 2.35 kg/fruit and CO 7 registered the minimum fruit weight of 1.02 kg/fruit. CP 50 recorded the highest fruit yield of 59.98 kg/plant followed by Pusa Dwarf (51.53 kg/plant), while susceptible CO 7 registered the lowest fruit yield of 16.48 kg/plant. Reduction in number of fruits, fruit weight and yield in papaya due to severe PRSV infection was well documented by Crane *et al.* (1995), Vimla Singh *et al.* (2005), Rahman and Akanda (2008) and Mowlick *et al.* (2008). Although PRSV-P negatively affected the yield and its components, there are many additional factors including inherent genetics, adaptability to climatic and edaphic soil conditions, time of initiation of fruiting, and cultural practices that influence this parameters. Moreover, PRSV-P reduced the leaf area; thereby it reduced the chlorophyll biosynthesis, which ultimately affects the chlorophyll production and photosynthesis and finally produced less photosynthates in plant.

The perusal of the data on fruit quality characters indicated that PRSV-P drastically reduced the quality of the fruits and the quality characters varied significantly among the varieties (Table 2). Among the varieties tested, CO 7 registered the maximum total sugars (6.93%) and reducing sugars (6.62%). CO 1 registered the minimum total sugars (5.12%) whereas CO 5 (4.49%) registered the lowest reducing sugars. The maximum non-reducing sugars of 0.57 % was recorded by CO 5 and CP 50 recorded the lowest non reducing sugar of 0.21%. The highest total soluble solids content of 12.31° Brix was recorded by CO 5 while the lowest total soluble solids of 10.10° Brix was recorded by CP 50. The highest fruit acidity of 0.19 per cent was recorded

Table 2 Fruit quality of papaya cultivars under PRSV infected conditions

Cultivar	Total sugars (%)	Reducing sugars (%)	Non reducing sugars (%)	T.S.S (°Brix)	Acidity (%)	Sugar acid ratio
CO 1	5.12	4.86	0.26	10.57	0.16	32.00
CO 2	6.30	5.81	0.49	11.11	0.12	52.50
CO 4	6.14	5.72	0.42	12.27	0.17	36.11
CO 5	5.06	4.49	0.57	12.31	0.16	31.62
CO 6	5.98	5.61	0.37	11.48	0.19	31.47
CO 7	6.93	6.62	0.31	11.82	0.17	40.79
Pusa Dwarf	5.32	5.09	0.23	11.92	0.17	31.29
CP 50	5.17	4.99	0.21	10.10	0.19	27.21
Mean	5.74	5.39	0.35	11.43	0.16	35.34
CD (0.05)	0.32	0.30	0.02	0.62	0.01	2.05

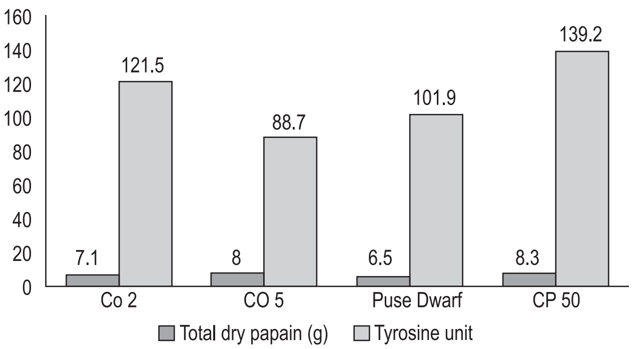

Fig 2 Performance of papaya cultivars for yield and quality of papain

by CP 50 and CO 6. However, CO 2 registered the lowest acidity of 0.12 per cent. The highest sugar acid ratio of 52.50 was observed in CO 2, whereas CP 50 recorded the lowest sugar acid ratio of 27.21. Khurana (1970), Dhanam (2006), Rahman and Akanda (2008) and Vimla Singh and Shukla (2009) reported that total soluble solids and sugar content of the papaya fruits were reduced when plants infected with ringspot virus. This may be due to poor translocation of metabolites to the fruits (sink) from the source due to interference by virus. The results of the present study also confirmed the findings of the earlier work done by above mentioned authors.

Papain, a proteolytic enzyme obtained from immature papaya fruit is having high industrial value was measured in latex yielding varieties and the characters varied significantly among the varieties (Fig 2). Of the four varieties evaluated for papain, CP 50 recorded the highest papain recovery of 8.3 g/fruit followed by CO 5 (8.0 g/fruit), while Pusa Dwarf registered the lowest papain recovery of 6.5 g/fruit. Among the genotypes evaluated for papain activity, CP 50 recorded the highest tyrosine unit of 139.2 followed by CO 2 (121.5), while CO 5 registered the lowest unit of 88.7. PRSV had a marked effect on papain production which considerably reduced the yield of papain. Khurana (1970) and Vimla Singh *et al*. (2005) reported that the papain yield was drastically reduced due to severe PRSV infection. In the present study also, reduction in papain yield was observed. This may be due to reduction in fruit size, since papain recovery is directly related to high fruit surface area. At virus free condition, CO 2, a dioecious variety is commercially cultivated for papain production produced a yield of 7.29 g dry papain/fruit (Kamalkumar 2003). But the papain yield was reduced to 6.8 g dry papain/fruit due to severe virus infection. The genotype CP 50 recorded the highest yield of papain and tyrosine unit because of its largest fruits which is an essential trait for total papain recovery per fruit. Although CP 50 yielded more fruits, the inherent genetics of the plant, ability to tolerate the virus infection and climatic factors that influences the papain yield and activity which is highly essential for the papain industry.

From the present study, it was concluded that papaya ringspot virus type P adversely affected the growth, yield and quality of all the varieties evaluated under field conditions. Serological test also proved that all the varieties evaluated were susceptible to the virus. In general, CP 50 was found to be superior to the rest as it had some degree of tolerance to ringspot virus and produced high yield. The quality of the fruits is low. However, this variety has the potential of yielding more papain even under high disease pressure. CP 50 may be used for future breeding program to develop virus tolerant types. Further it may be forwarded to multi location trials for papain production.

ACKNOWLEDGEMENTS

The authors are grateful to M/s Senthil Papain and Foods Pvt Ltd, Coimbatore, Tamil Nadu for providing the financial support. The authors wish to thank Dean, HC and RI, TNAU, Coimbatore for his valuable suggestions during the investigation. The authors are thankful to lab 40, CPPS, TNAU, Coimbatore to assist in virus testing.

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