Madras Agric. J., 100 (4-6): 287-290, June 2013
https://doi.org/10.29321/MAJ.10.001291

Screening of Papaya (*Carica papaya* L.) Cultivars for Resistance to PRSV Under Polyhouse Conditions

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A pot culture study was conducted during 2009-2010 to screen the papaya cultivars under polyhouse conditions for resistance against ringspot caused by papaya ringspot virus – type P at Tamil Nadu Agricultural University, Coimbatore. Plants were artificially inoculated with PRSV–P strain in the polyhouse. The resistance was assessed by symptom expression, disease score, DAS-ELISA, chlorophyll and biochemical parameters. All the papaya cultivars inoculated with virus tested were ELISA positive, whereas the uninoculated control plants tested were ELISA negative. The chlorophyll pigments were lower in virus inoculated plants than those corresponding uninoculated controls. However, peroxidase, polyphenol oxidase and total phenols were higher in virus inoculated plants than uninoculated controls. The results suggested that the CP 50, a dioecious papaya was identified as tolerant cultivar against PRSV-P under Coimbatore conditions based on the assessment in the polyhouse.

Key words: PRSV, papaya, *Carica papaya,* disease score, ELISA, chlorophyll, enzymes

Papaya (*Carica papaya* L.) is considered one of the most important commercial crops of India and it is a rich source of vitamin A (2020 IU/100g), vitamin C (46g/100g) and flavonoids. 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 has an area of about 80, 000 ha with an annual production of about 2.68 million tonnes during 2007 to 2008 (NHB, 2008).

Papaya ringspot, the most destructive disease is caused by Papaya Ringspot virus – type P (PRSV-P) (Manshardt, 1992). It is transmitted by a number of aphid species in a non-persistent manner to a limited host range of cucurbits and papaya (Purcifull *et al*., 1985). Papaya infected with this virus develop a wide range of symptoms includes 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. When infected at seedling stage, trees do not produce mature fruit.

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 wild type to the cultivar through conventional breeding. None of the *Carica papaya* cultivars in the world tested had natural resistance to PRSV-P. Looking to the importance of papaya cultivation for papain extraction and table purpose in Tamil Nadu, the present study was conducted to identify the resistant or tolerant cultivar of the papaya on the basis of reaction to disease, pigment synthesis and defence related enzymes.

Materials and Methods

Planting materials

The seeds of two gynodioecious papaya cultivars *viz.,* CO 3 and CO 7 and seven dioecious papaya cultivars *viz.,* CO 1, CO 2, CO 4, CO 5, CO 6, Pusa Dwarf and CP 50 were collected from Dept. of Fruit Crops, Horticultural College and Research Institute, TNAU, Coimbatore. The twenty five days old healthy seedlings of uniform growth raised in insect proof polyhouse were selected for this study.

Experimental site

The study was conducted at Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Experiment was laid out in Completely Randomized Block Design with three replications.

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Preparation and artificial inoculation of virus inoculum

The virus inoculum was prepared by homogenizing one gram of PRSV infected leaves in 2 ml of 0.1M chilled sodium phosphate buffer (pH 7.2) containing β-mercaptoethanol and 0.01 M EDTA and applied by gentle rubbing on carborundum dusted leaves. After 5 minutes, the excess sap was washed off by distilled water and plants were observed for symptoms expression kept in insect proof polyhouse. The uninoculated plants were kept as control.

Disease score

The disease intensity score was given based on symptoms in leaves and stem using the scale developed by Dhanam (2006). The scale consists of five levels based on the symptoms exhibited by the plant *viz.,* Resistant (0-1), Tolerant (1 -2), Moderately Susceptible (2-3), Susceptible (3-4) and Highly susceptible (4 and above).

DAS - ELISA

Double antibody sandwich ELISA (DAS-ELISA) was used to detect PRSV. 200 µl of coating buffer (1:1000) was added to each well of a micro titer plate and incubated at 37$_o$C for 2 to 4 hrs. 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$_o$C. The plate was with PBS-T thrice and 200 µl of the anti-virus conjugate (1:500) was added to each well. Plate was incubated at 37$_o$C for 2 hrs and washed thrice. Finally 200 ìl of freshly prepared substrate containing ρ -nitro phenyl phosphate was added to each well and incubated in dark at room temperature for 20-45 minutes to observe the colour development. The reaction was stopped by adding 50 µl of 3M NaOH. Buffer served as negative control. Positive control was also included. Absorbance was read at 405 nm in ELISA reader (EL 800, BIO-TEK Instrument Inc., USA).

Biochemical characters

Chlorophyll 'a', 'b' and total chlorophyll were estimated using 80 per cent acetone as per the method suggested by Arnon (1949) and expressed in mg 100 g^{-1}. Peroxidase activity was assayed as per the method suggested by Hartec (1955). The standard reaction mixture consisted of 1.5ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 30 per cent H_2O_2 and expressed as min.$^{-1}$ g^{-1}. Polyphenol oxidase activity was assayed using the method described by Srivastava (1987). The standard reaction mixture contained 1.5 ml of 0.1 M Phosphate buffer (pH 6.5), 0.5 ml of the enzyme extract and 0.5 ml of 0.01 M catechol and expressed as min.$^{-1}$ g^{-1}. Total phenol was estimated by the method suggested by Malik and Singh (1980). The standard reaction mixture contained 80 per cent ethanol, folin ciocalteau reagent and 20 per cent sodium carbonate and expressed as µg g^{-1}.

Results and Discussion

Disease score

Complete resistance against PRSV was not found in any of the papaya cultivars tested in this study. Typical symptoms of leaf mottling and water soaked lesions on the stem were observed 17 to 24 days after inoculation (Table 1) and the uninoculated controls remained healthy. Among the cultivars screened, CP 50 took the longest days (24.1 days) to express the symptoms, followed by Pusa Dwarf (21.2 days). However CO 7 expressed symptoms within 17.6 days which was on par with CO 3 and CO 1. Disease intensity score of papaya cultivars ranged from 1.55 to 3.75 (Table 1) and this showed that the papaya cultivars tested had a wide range of symptoms. The results revealed that the papaya variety CP 50 had the lowest score of 1.55 based on the symptoms and it was considered as tolerant. The CO 7 registered the highest disease intensity score of 3.75 and it was considered as susceptible to the virus. The tolerance of the genotype CP 50 may be due to inherent genetics, time of infection and climatic conditions. However, this tolerance is strain specific which is prevalent in Tamil Nadu conditions. The findings of present study are in agreement with investigations of Vimla Singh *et al* (2005) and Mohamad Roff (2007) in papaya due to PRSV infection.

Table 1. Evaluation of cultivars under polyhouse conditions for PRSV resistance

Cultivar	Days taken for symptom expression		Disease intensity score		ELISA absorbance value	
	Inoculated plants	Control	Inoculated plants	Control	Inoculated plants	Control
CO 1	19.0	-	3.25	0	0.382	0.052
CO 2	20.6	-	2.75	0	0.367	0.049
CO 3	18.0	-	3.65	0	0.461	0.048
CO 4	19.5	-	3.00	0	0.390	0.050
CO 5	19.1	-	2.75	0	0.298	0.042
CO 6	20.2	-	3.00	0	0.322	0.044
CO 7	17.6	-	3.75	0	0.484	0.051
Pusa Dwarf	21.2	-	1.75	0	0.201	0.044
CP 50	24.1		1.55	0	0.162	0.044
Mean	19.94		3.00		0.340	0.047
SEd	0.66		0.10		0.012	0.001
CD (0.05)	1.39		0.22		0.025	0.003

Confirmation of virus through DAS - ELISA

In DAS-ELISA, all the virus inoculated plants were positive for PRSV-P and the mean absorbance value for all the inoculated plants were many times higher than those of their uninoculated controls. Among the cultivars tested, the genotype CP 50 registered the lowest absorbance value of 0.162 (Table 1), indicating its tolerance against PRSV, followed by Pusa Dwarf (0.201). The maximum absorbance value of 0.484 was registered by CO 7 indicating its susceptibility to the virus.

Chlorophyll

The capacity of photosynthesis depends upon

the amount of chlorophyll present. PRSV-P had the strong influence on the plant pigments *viz.,* total chlorophyll, chlorophyll 'a' and chlorophyll 'b'. The chlorophyll content under PRSV-P infected plants was significantly lower than the uninoculated controls. Among the cultivars selected for evaluation, CP 50 recorded the highest amount of total

Table 2. Evaluation of cultivars for pigments under poly house conditions

Cultivar	Total chlorophyll (mg g_{-1})		Chlorophyll 'a'(mg g_{-1})		Chlorophyll 'b' (mg g_{-1})	
	Inoculated plants	Control	Inoculated plants	Control	Inoculated plants	Control
CO 1	1.77	1.82	1.17	1.23	0.48	0.49
CO 2	1.92	1.90	1.32	1.40	0.51	0.56
CO 3	1.28	1.36	0.73	0.81	0.30	0.37
CO 4	1.64	1.73	1.14	1.20	0.46	0.47
CO 5	1.79	1.92	1.09	1.19	0.53	0.57
CO 6	1.52	1.69	1.01	1.20	0.47	0.43
CO 7	1.12	1.24	0.61	0.79	0.29	0.36
Pusa Dwarf	2.08	2.19	1.46	1.47	0.58	0.61
CP 50	2.10	2.20	1.48	1.49	0.60	0.69
Mean	1.69	1.78	1.12	1.19	0.46	0.50
SEd	0.05	0.06	0.03	0.03	0.01	0.01
CD (0.05)	0.12	0.12	0.07	0.07	0.02	0.02

chlorophyll content (2.10 mg g_{-1}), followed by Pusa Dwarf (2.08 mg g_{-1}) (Table 2). The lowest total chlorophyll content was registered by CO 7 and CO 3. The highest amount of chlorophyll 'a' and 'b' were recorded by CP 50 (1.48 mg g_{-1} and 0.60 mg g_{-1} respectively) and it was at par with Pusa Dwarf (1.46 mg g_{-1} and 0.58 mg g_{-1} respectively). The lowest amount of chlorophyll 'a' and 'b' were recorded by CO 7 (0.61 mg g_{-1} and 0.29 mg g_{-1} respectively) and CO 3 (0.73 mg g_{-1} and 0.30 mg g_{-1} respectively). The degradation of chlorophyll molecules or inhibition of chlorophyll production resulting in the reduction of chlorophyll content in virus infected plants than the healthy plants (Easu, 1956). The results were in accord with the findings of Rahman *et al*. (2008) and Vimla Singh and Shukla (2009) in papaya who reported reduction in chlorophyll content due to PRSV infection. CP 50 recorded the maximum amount of chlorophyll content which might probably be due to its capacity to prevent the destruction of chlorophyll molecules by the virus invasion.

Peroxidase

Peroxidase is the key enzyme which plays an important role in plants defence mechanism. The results of present study revealed that the virus infected plants produced large amount of peroxidase enzyme than the healthy plants. Among the cultivars evaluated, Pusa Dwarf (0.93 min_{-1} g_{-1} in inoculated and 0.79 min_{-1} g_{-1} in healthy plants) had the maximum peroxidase activity and it was on par with respective values in CP 50 (0.91 min_{-1} g_{-1} in inoculated and 0.77 min_{-1} g_{-1} in healthy plants) (Table 3). However, CO 7 (0.39 min_{-1} g_{-1} in inoculated and 0.31 min_{-1} g_{-1} in healthy plants) registered the minimum peroxidase activity followed by CO 3 (0.44 min_{-1} g_{-1} in inoculated and 0.40 min_{-1} g_{-1} in healthy plants). An abrupt rise in the peroxidase activity in PRSV inoculated plants might be due to alteration in redox potential of the plant as a result of virus infection. Peroxidase produced by the plants under virus infected conditions serve as antioxidants and scavenges the toxic substances so as to make the plants survive under stress conditions. The increase in peroxidase activity was observed in disease infected plants by Kavino *et al*. (2008) in banana which is in confirmity with the findings of the present study.

Polyphenol oxidase

Polyphenol oxidase, a copper containing enzyme plays an important role in plant disease resistance. The result of present study revealed that the inoculated plants produced larger amount of polyphenol oxidase than the healthy plants. Difference was significant for polyphenol oxidase enzyme in all the cultivars which were inoculated and healthy. CP 50 had the highest polyphenol oxidase activity (0.070 min_{-1} g_{-1} in inoculated and 0.065 $min_{-1}g_{-1}$ in healthy plants) (Table 3), while CO 7 registered the lowest polyphenol oxidase enzyme of 0.011 min_{-1} g_{-1} in inoculated and 0.010 min_{-1} g_{-1} in healthy plants Higher polyphenol oxidase activity was observed in disease infected plants by Kavino *et al*. (2008) in banana. This is in confirmity with the findings of the present study.

Total phenols

Total phenols play a vital role in disease resistance among the various substances produced. Increased total phenol content was observed in virus inoculated plants. Total phenol content differed significantly among cultivars both in inoculated and healthy plants. Among the cultivars evaluated, CP 50 recorded the highest total phenol

Table 3. Evaluation of cultivars for biochemical characters under poly house conditions

Cultivar	Peroxidase (min_{-1} g^{-1})		Polyphenol oxidase (min_{-1} g^{-1})		Total phenols (µg g_{-1})	
	Inoculated plants	Control	Inoculated plants	Control	Inoculated plants	Control
CO 1	0.59	0.53	0.023	0.021	638.1	593.1
CO 2	0.73	0.67	0.037	0.032	689.2	598.3
CO 3	0.44	0.40	0.013	0.011	570.0	520.1
CO 4	0.57	0.52	0.024	0.020	682.1	612.6
CO 5	0.76	0.68	0.037	0.032	698.5	600.5
CO 6	0.50	0.46	0.027	0.025	690.2	615.0
CO 7	0.39	0.31	0.011	0.010	610.7	598.1
Pusa Dwarf	0.93	0.79	0.048	0.042	811.4	705.0
CP 50	0.91	0.77	0.070	0.065	873.4	720.4
Mean	0.64	0.57	0.032	0.028	699.91	618.81
SEd	0.02	0.01	0.001	0.001	22.98	20.43
CD (0.05)	0.04	0.04	0.002	0.002	48.29	42.92

of 873.4 µg g_{-1} in inoculated and 720.4 µg g_{-1} in healthy plants (Table 3), followed by Pusa Dwarf (811.4 µg g_{-1} in inoculated and 705.0 µg g_{-1} in healthy plants). CO 3 registered the lowest total phenol content of 570.0 µg g_{-1} in inoculated and 520.1 µg g_{-1} in healthy plants which were on par with the respective values of inoculated (610.7 µg g_{-1}) and healthy plants (598.1 µg g_{-1}) in case of CO 7. Among the cultivars evaluated, CP 50 registered the maximum total phenol content. It may be due to

increased synthesis of phenolic compounds despite their destruction due to enhanced polyphenol oxidase activities. Continuous synthesis of phenolics due to invasion by the virus would have made available the substrate for polyphenol oxidase activity which would have resulted in the synthesis of compounds such as quinones which ultimately arrest the entry of virus particles. This would have resulted in a substantial reduction in viral protein which is reflected in the lower absorbance value in ELISA upon stress condition created by virus in the inoculated plants triggering the defence mechanism. An increase in total phenol content was observed by Sundharaiya (2008) in tomato due to leaf curl virus. The results of the present study are in confirmity with the report of the previous worker mentioned earlier.

Conclusion

From the present study, it is concluded that the genotype CP 50 proved to be the best source of field tolerance against papaya ringspot virus under Coimbatore conditions based on the days taken for symptom expression, disease score, ELISA absorbance value, chlorophyll content and defence related enzymes under polyhouse screening. CP 50 is a dioecious type, possessing castor like leaves.

References

Arnon, D.L. 1949. Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris.* Plant Physiol., **24:** 1-15.

Dhanam, S. 2006. Studies on papaya ring spot disease, M.Sc., (Plant Pathology) *Thesis***,** Tamil Nadu Agricultural University, Coimbatore.

Esau, K. 1956. An anatomists view of infected plants. *Am. J. Bot.,* **43**: 739-748.

Hartec, E.F. 1955. Haematin compounds. **In:** Modern methods of plant analysis vol. 4 (Eds.) Peach, K. and M. Tracq, Springer-Verlag. New York, 197-245.

Kavino, M., Kumar, N., Damodaran, T., Harish, S. and Saravanakumar, D. 2008. Biochemical markers as a useful tool for the early identification of *Fusarium oxysporum f. sp. cubense* race 1 resistance banana clones. *Arch. Phytopathol. Plant Prot.,* 1-10.

Malik, C.P. and Singh, M.B. 1980. **In:** Plant Enzymology, Histo-enzymology. Kalyani Publication, Delhi. P53.

Manshardt, R.M. 1992. Papaya. In: F.A. Hammerschlag and R.E. Litz (eds.), Biotechnology in Agriculture No. 8 Biotechnology of Perennial Fruit Crops, CABI, Wallingford Oxon. pp.489-511.

Mohamad Roff, M.N. 2007. Disease rating of papaya cultivars to papaya ringspot virus in Malaysia. *Acta Hort.*, **740**: 277-282.

National Horticulture Board Database. 2008. www.nhb.gov.in/database2008.pdf.

Purcifull, D., Edwardson, J., Hiebert, E. and Gonsalves, D. 1985. Papaya ringspot virus. CMI/AAB *Description of Plant Viruses* (No.84 revised).

Rahman, H., Alam, M.M. and Akanda, A.M. 2008. Alteration of cellular pigments of papaya leaves infected with seven symptomatic isolates of PRSV-P *Int. J. Agric. Res.,* **3:** 69-76.

Srivastava, S.K. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassio) Goid and their implication in disease resistance. *J. Phytopathol.,* **120:** 249-254.

Sundharaiya, K. 2008. Stability analysis for combined resistance to leaf curl virus and root knot nematode *(Meloidogyne incognita* race 3*)* in tomato (*Solanum lycopersicum* L.) Ph.D. (Vegetable Science) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Vimla Singh and Shukla, K. 2009. Effect of PRSV Infection on pigment content and assimilation of carbohydrate in *Carica papaya* L. *Annals Plant Prot. Sci.,* **17:** 152-156.

Vimla Singh, Rao, G.P. and Shukla, K. 2005. Response of commercially important papaya cultivars to papaya ringspot virus in eastern U.P. conditions. *Indian Phytopath.,* **58:** 212-216.

Received: August 22, 2013; Accepted: June 10, 2013