Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; Sp 10(2): 110-113
Received: 14-01-2021
Accepted: 17-02-2021

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Occurrence of papaya ringspot virus (PRSV) infection in India

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Abstract
Papaya ringspot virus isolates infect papaya as well as cucurbit hosts, and occur as two pathotypes, P & W. The cucurbit hosts are known to be infected by both the pathotypes P & W. Field surveys were conducted to document the relative incidence of the PRSV pathotypes (P and W) infecting cucurbit crops in the 6 states of India. A total of 80 cucurbit samples representing Ash gourd, bottle gourd, bitter gourd, cucumber, musk melon, pumpkin, ridge gourd, squash, sponge gourd and water melon hosts were collected and tested by DAC-ELISA based serological assay, which indicated the association of PRSV with 51 samples. The ELISA positive cucurbit samples were subjected to bioassay to ascertain the pathotypes of PRSV, followed by RT-PCR confirmation for the PRSV association. Among all the symptomatic cucurbits samples collected and tested in this study, a higher incidence (100%) of PRSV was recorded on the bottle gourd samples, whereas no or 0% incidence was recorded on ridge gourd samples. Of 51 ELISA positive samples, upon pathotyping on differential host, a relatively higher incidence of pathotype -P (upto 68.63%) was recorded as compare to that of PRSV W, i.e. 31.37% only. Further, the PRSV-P and -W pathotypes also showed a differential relative incidence on different cucurbit hosts, wherein, PRSV-P was found more predominantly (upto 100%) on three cucurbit hosts namely bitter gourd, cucumber and squash, whereas a least incidence (of 30%)was recorded on muskmelon and pumpkin hosts.

Keywords: Papaya ringspot virus, pathotype P, pathotype W, relative incidence, cucurbit

Introduction
Papaya ringspot virus (PRSV), a member of the genus *Potyvirus* and the family *Potyviridae*, infects papaya as well as cucurbit hosts and poses a major threat for their cultivation. The virions of PRSV are flexuous rod shaped particles possessing a single stranded translatable RNA genome of 10317-10349 nucleotides. The virus is known to be transmitted in nature by different species of aphids.

PRSV was first described in 1945 and the name was coined by Jensen (1949). In India, the natural infection of papaya by PRSV was reported first by Capoor and Varma (1958) [3] from Maharashtra. Subsequently, it was reported from Uttar Pradesh (Khurana and Bhargava, 1970; Khurana, 1984) [8], Rajasthan (Surekha *et al.*, 1977) [14] and Andhra Pradesh, Karnataka, Sikkim and Tamil Nadu (Jagadish Chandra and Samuel, 1999; Roy *et al.*, 1999; Sharma *et al.*, 2005) [4, 11, 7]. The virus seems to be widespread and occur wherever papaya and cucurbits are grown.

The PRSV isolates were classified into two groups, pathotype-W (PRSV-W) infecting members of the family *Chenopodiaceae* and *Cucurbitaceae*, and pathotype-P (PRSV-P), infecting in addition, members of the family *Caricaceae* (Purciful *et al.*, 1984; Yeh *et al.*, 1984) [10, 19]. The PRSV isolates from India have also been classified based on the host range as papaya infecting (pathotype-P) and non-papaya infecting (pathotype-W) types (Roy *et al.*, 1999) [11]. Cucurbit hosts are known to be infected by both the pathotypes of PRSV (P & W) (Basavaraj *et al*., 2020). The symptoms of PRSV infection on cucurbit plants are characterized by mosaic, vein banding, vein clearing, leathery leaf and shoe-stringing which ultimately results in stunted growth as well as loss in flowers and fruit deformation (Gonsalves and Ishii, 1980; Tripathi *et al*., 2008; Mohammed *et al*., 2012; Basavaraj *et al*., 2020) [6, 15, 9]. PRSV has continued to be a major threat for the production of papaya as well as cucurbits in India, by causing the losses of upto 95% as a result of upto 95% disease incidence (Babu *et al* 2018; Capoor and Varma, 1948; 1974; Singh, 1969; Yemewar and Mali, 1980; Verma and Prasad, 1986; Singh, 2006; Turechek *et al*., 2010) [1, 3, 12, 21, 17, 13, 16]. PRSV-W causes significant yield reduction in watermelon, squash, melon, cucumber, and other cultivated cucurbits.

Although both the pathotypes of PRSV (P & W) are known to infect the cucurbit hosts, there are no reports available to indicate the comparative incidence of PRSV pathotypes P and W, which would help in understanding the epidemiology of these pathotypes, and devising the suitable management strategies by knowing the comparative vulnerability of different cucurbit hosts. Thus, the current investigation was taken up with an objective to survey different geographical locations and cucurbit hosts in India and to document the relative incidence of PRSV pathotypes **(**P and W) on different cucurbit crops.

Material and methods

Sample collection and symptomatology: The survey was conducted at 12 locations in 6 states of India over a period of 10 years in three different batches (2009 to 2013, 2014 to 2017, and 2018 to 2020). A total of 80 cucurbit samples exhibiting virus infection-like symptoms were collected from Delhi, Haryana, Karnataka, Madhya Pradesh, Punjab, Uttar Pradesh (Table 1 Figure 1).

DAC-ELISA based diagnosis and PRSV incidence assessment: All the cucurbit samples were subjected to direct antigen-coated enzyme linked immunosorbent assay (DAC-ELISA) (Clark and Bar-Joseph, 1984) to detect the association of PRSV. For DAC-ELISA, the leaf tissues (100 mg each) were tested using the in-house raised polyclonal antibodies (PAbs) to coat protein (CP) of PRSV available at the Advance Center for Plant Virology (ACPV). The previously confirmed plant samples for the PRSV infection and healthy samples were used as the positive and negative controls, respectively.

Pathotyping through bio-assay: To ascertain the association of PRSV pathotype(s) with the DAC-ELISA positive samples (pathotyping) the bio-assay experiment was performed through mechanical sap transmission of the associated virus to the differential hosts (papaya and squash plants; 5 plants each for all the sample). 100 mg leaf tissues were homogenized with the pre-chilled 0.1M sodium phosphate buffer of pH 7.0 (supplemented with 0.2% β-mercaptoethanol and 0.15% Sodium Sulphite) in the pre-chilled mortar & pestles that were placed on ice. The homogenates were smeared by the index finger of the gloved hands on to the cotyledonary leaves of squash (at 1 true leaf stage) and the middle 2nd & 3rd leaves of papaya (at 4-6 true leaf stage) plants dusted with the abrasive agent (carborundum). About 5 minutes' post application of the sap, the inoculated leaves were washed gently with the autoclaved distilled water using the wash bottle. The inoculated plants were then incubated at 28-30°C in the insect proof containment facility. The plants were observed till the symptom expression and recorded the same.

RT-PCR based confirmation of pathotyping: The leaf tissues (100 mg each) from all the bio-assayed plants were used for total RNA isolation by Purelink RNA Mini Kit (Ambion, Invitrogen) following the manufacturers' protocol. For each sample, 500 ng total RNA and 20 nM of PRSV-CP specific reverse primer (RKJ 3 R': GTTGCGCATACCCAGGAGAG) were used for complementary DNA (cDNA) synthesis by Improm-II Reverse Transcriptase (Promega, Madison, WI) following the manufacturer's protocol. For polymerase chain reaction (PCR) assay, 2 ul cDNA as the template and the PRSV CP gene specific primers (HRP 52 F': TCCAAa/gAATGAAGCTGTT GATGCT and RKJ 3 R') were used and performed the reactions by employing a 4 min hot start at 94 °C followed by 35 cycles each of denaturation at 94 °C for 30 sec, primer annealing at 52 °C for 1 min and extension at 72 °C for 40sec and a final extension cycle of 10 min at 72 °C using the Thermo Cycler (Biometra, Germany). The PCR amplified products were visualized using 1% agarose gel under UV trans-illuminator to observe the CP gene amplification.

Further, based on the DAC-ELISA, bio-assay and the RT-PCR results, the relative incidence of PRSV pathotypes –P and –W in different cucurbit samples collected from multiple locations under the current investigation was calculated.

Results and discussion

Sample collection and symptomatology: PRSV is known to induce the mosaic, puckering, blistering, and stunted growth of vines, resulting in small, knobby, malformed, and mottled fruits in different cucurbit hosts (Gonsalves and Ishi 1980, Tripathi *et al* 2008 and Mohammad *et al*. 2012) [6, 15]. The cucurbit samples collected from different states of India under this investigation also revealed a range of symptoms such as mottling (Mo) to mosaic (M), vein banding (VB), vein clearing (VC), leathery leaf (LL), leaf blistering (LB) as well as filiformy (FF). The symptoms that were not observed in the earlier studies could be due to either mixed infections by the other viruses besides PRSV or due to the single infection by the other viruses. (Table.1 and Figure.2).

DAC-ELISA based diagnosis and PRSV incidence assessment: Out of a total 80 samples tested by DAC-ELISA, 51 samples were found associated with PRSV, which constituted about 63.75% incidence. Among them, the highest PRSV incidence of upto 100% each was observed in the samples of three cucurbit hosts, namely, bottle gourd, musk melon and watermelon, followed by upto 75% incidence in the samples of pumpkin host. Whereas, the lowest incidence of upto 33% was recorded in the cucumber samples. It was evident in the previous investigation by Yuki *et al*. (2000) [22] also that the PRSV incidence was much higher in the pumpkin plants (upto 69%) and the cucumber plant were least effected (upto 33%). This kind of differential incidences could be attributed to the differential abilities of different cucurbit host species to fight against or to support the PRSV infections.

Pathotyping through bio-assay: Under the pathotyping studies conducted through bio-assay by inoculating the sap from DAC-ELISA positive cucurbit samples (n=51samples), a total of 35 [Ash gourd (2), Bottle gourd (12), Bitter gourd (2), Cucumber (2), Muskmelon (2), Pumpkin (4), Squash (5) and Watermelon (6)] induced virus infection-like symptoms on the test plants of both papaya as well as squash (cucurbit) hosts. This indicated that the associated pathotype with those samples must be PRSV pathotype P. Whereas, 16 Samples [Ash gourd (1), Bottle gourd (2), Muskmelon (4), Pumpkin (8) and Watermelon (1)] induced the symptoms only on squash test plants upon mechanical sap inoculation (Figure 3).

RT-PCR based confirmation of pathotyping: The bio-assay positive plant samples when subjected to the RT-PCR assay, all the 35 samples were found positive in both the test plants of papaya as well as squash, which confirmed again that the samples that could induce the symptoms on papaya and squash plants were associated with the PRSV pathotype P.

Similarly, those samples that could induce the symptoms on the squash plants only were also found positive in RT-PCR assay and thus confirmed the association of PRSV-W pathotype with those samples. These results confirmed that, all the DAC-ELISA and bioassay positive samples (n=51) were infected with PRSV. Of which, 35 samples were associated with the PRSV pathotype-P, which account for about 68.63% incidence, whereas, only 16 were associated with the PRSV pathotype-W, which accounted only 31.37% incidence.

Among the cucurbit samples that were found positive under DAC-ELISA for PRSV infection, a maximum incidence, (up to 100%) of PRSV-P was observed in the samples each of bitter gourd, cucumber and squash host species, followed by 86% in bottle gourd and 85% in watermelon hosts. Whereas, a minimum of 33% incidence was observed in both muskmelon & pumpkin samples. With regard to the PRSV-W pathotype, a maximum incidence, (upto 67%), each in the muskmelon and pumpkin host samples was recorded, followed by 33% in the samples of ash gourd. Whereas, a minimum of 14% incidence of PRSV-W was recorded in the samples of bottle gourd and watermelon hosts. Among the 10 different samples surveyed and tested under this study, ridge gourd and sponge gourd no or 0% incidence by both the pathotype of PSRV. There is only single report of PRSV infection on sponge gourd from India in 2006 (Verma *et al*, 2006) [18]. However, only a single report of PRSV infection on bitter gourd is available from China (Zhu *et al*, 2016) [23]. Whereas, PRSV infection still not reported on bitter gourd from India. Interestingly, the samples originated from bitter gourd and cucumber hosts showed infection only by PSRV pathotype P but no or 0% incidence by PSRV-W.

Conclusion

The variable incidence of PRSV-P and -W infection was observed among different cucurbit hosts tested in this study. Moreover, there is a significant difference also observed in PRSV. The observed variability could be due to either differential host plant resistance or vector preference to either the virus or hosts, and even the geo-climatic conditions of the location where the crops were grown. Nevertheless, these observations need to be validated through further surveys by collecting and testing the large number of samples originating from almost all the cucurbit hosts grown at all the geo-climatic locations in the India.

Acknowledgements

Authors are highly grateful to Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut (UP) and India and Indian Agricultural Research Institute, New Delhi 110012.

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