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## Host range studies of a *Potyvirus* infecting bottle gourd in Jammu sub-tropics

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### Abstract

Bottle gourd occupies a significant area in the Jammu sub-tropics and PRSV-W, a *potyvirus* belonging to family *Potyviridae*, is one of the major viruses identified responsible for mosaic. A viral isolate was collected during survey from Salaher village of R.S. Pura block in Jammu and was later identified as PRSV-W through RT-PCR. A comparative study was conducted to understand the host range of this virus. Twenty-one plants belonging to different families viz., *Cucurbitaceae*, *Solanaceae*, *Malvaceae*, *Fabaceae*, *Brassicaceae*, *Amaranthaceae* and *Caricaceae*, were inoculated with the isolate and the host reaction was recorded. All the cucurbits were susceptible to PRSV-W but showed varied range of systematic symptoms like mosaic, mosaic mottling, yellowing blisters on leaves, leaf deformation, leaf curling, enations and stunting. However, *Amaranthus* spp. developed localized lesions while all the other plant species were non host.

**Keywords:** *Papaya ringspot virus*, host range, bottle gourd

### 1. Introduction

Bottle gourd (*Lagenaria siceraria* (Mol.) Standl) a white flowered gourd, is a member of *Cucurbitaceae* family and has its roots spread across from Africa to Asia and later America. The pantropical distribution of *Lagenaria* genus can be credited to the oceanic movement of the seeds of the wild genotypes. The history of this crop still needs more exploration. Bottle gourd is one of the first crops to be domesticated is known to be multi-utilitarian crop since ancient times (De Candolle, 1961, Decker-Walters *et al.* 2000; Parle and Kaur, 2011) [3,4,11].

Like all the other cucurbits, *Lagenaria* is exposed to various biotic factors like *Colletotrichum lagenarium*, *Erysiphe cichoracearum*, *Alternaria alternata*, *Fusarium oxysporum* f. sp. *lagenariae*, *Cercospora lagenariae*, *Didymella* spp., *Erwinia tracheiphila*, *Pseudomonas syringae* pv. *lachrymansgae* etc. and many viruses leading to various losses. Viruses have always been the major constraint to bottle gourd. About more than fifty viruses known to cause infection in different cucurbits and also lead to several losses (Lecoq and Desbiez, 2012; Nagendren *et al.*, 2017) [8,10].

One of these viruses is *Papaya ringspot virus*, a member of Family *Potyviridae* is one of the top five important viruses found in cucurbits worldwide (Quiot-Douine *et al.*, 1990) [14]. PRSV is more prevalent in the tropical and sub-tropical areas rather than temperate ones (Purcifull *et al.*, 1984) [13]. This potyvirus is, a positive sense single stranded RNA, linear flexuous rod of 760-800 nm X 12 nm in size. PRSV produces varied set of symptoms on its host plant viz., mosaic mottling, dark green blisters on leaves, ringspots on leaves as well as fruits, interveinal chlorosis or vein clearing, shoe string symptoms, enations.

The virus has two major strains worldwide, strain -W (infects only Cucurbits) and strain -P (can infect both Papaya and Cucurbits). The PRSV-W strain is reportedly responsible for infecting approximately thirty-eight different members of *Cucurbitaceae* (Purcifull *et al.*, 1984) [13]. PRSV is transmitted by at least 20 different aphid species wherein, *Myzus persicae*, *Aphis gosypii* and *Aphis craccivora* are efficient vectors (Kallethwaraswamy and Kumar, 2008) [7]. Pinto *et al.*, (2008) [12] reported 92 per cent transmission efficiency of *M. persicae* and *A. gosypii* as insect vectors for PRSV. In India, this virus in bottle gourd was first time reported by Mantri *et al.*, (2005) [9] from Maharashtra and is responsible for causing 85-90 per cent losses (Hussain and Varma, 1994) [6].

The host range indicates how the virus survives and spreads further. Therefore, the current study was taken up, keeping in view to determine the host range of the PRSV-W isolate collected during the periodic surveys of Jammu district.

## 2. Material and Methods

### 2.1 Raising of test plants for determining the host range

Twenty one plant species belonging to seven different families viz., *Cucurbitaceae* (*Cucurbita pepo*, *C. maxima*, *Cucumis sativus*, *C. melo*, *Citrullus lanatus*, *Luffa acutangula*, *Momordica charantia* & *Praecitrullus fistulosus*), *Solanaceae* (*Solanum lycopersicum*, *S. melongena* & *Capsicum annum*), *Malvaceae* (*Abelmoschus aeculentus*), *Fabaceae* (*Phaseolus vulgaris*, *Vigna unguiculata*), *Brassicaceae* (*Brassica oleracea* var *capitata* & *B. oleracea* var *botrytis*), *Amaranthaceae* (*Amaranthus* spp. and *Chenopodium* spp.) and *Caricaceae* (*Carica papaya*), respectively, were grown under greenhouse conditions in pots containing sterilized potting mixing.

### 2.2 Collection and Maintenance of virus culture

The PRSV-W isolate (J-SA-BG3) was collected from the Salaher village of block, R.S. Pura in Jammu district. The isolate was maintained on a susceptible cultivar 'MAHY-1' sown in 12-inch pots containing the sterilized potting mixture through artificial inoculation.

### 2.3 Screening of the different host plants

Thirty plants of each species were inoculated for our study. The plants belonging to *Cucurbitaceae*, *Malvaceae* and *Leguminaceae* families, were inoculated at the cotyledonary leaf stage i.e., two leaf stage while members of *Solanaceae* and *Chenopodiaceae* families, were inoculated at 4-6 leaf stage. Broccoli and cauliflower plants were inoculated ten days after transplanting. The papaya seedlings were inoculated when they were 1.5 months old. The inoculated plants were then kept up to 30 -40 days to observe the symptoms development. The plants which showed no symptoms were back inoculated on susceptible cultivar 'MAHY-1' to avoid any symptomless carriers. The symptoms produced by the isolates on the test plants were recorded.

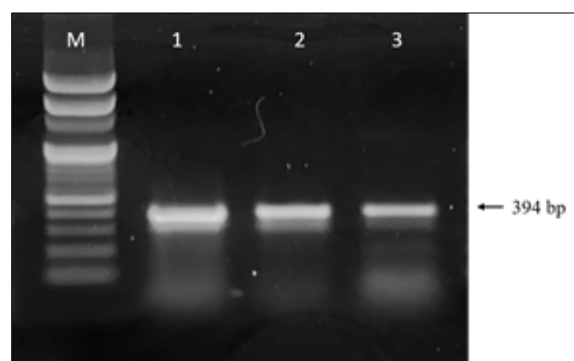
### 2.4 Confirmation of the identity of the virus

The identity of the virus was confirmed through Reverse Transcriptase-PCR using PRSV-W specific primers. Total plant RNA was isolated using "Plant Total RNA kit, Sigma Aldrich" from the infected leaves of susceptible cultivar 'MAHY-1' in which the viral isolate J-SA-BG3 was maintained. The RNA was checked on 1 per cent agarose gel electrophoresed at 90 volts for 1 hour in 0.5 X TAE buffer. Complementary DNA (cDNA) was synthesized using Verso cDNA synthesis kit (Thermo scientific) according to the manufacturer's instructions and amplification was performed as described by Aguiar *et al.*, 2018 [1]. The amplicon was detected through 1.2 per cent agarose gel electrophoresis (in 0.5 X TAE buffer).

## 3. Results

The viral isolate collected from the Salaher village was identified as PRSV-W using coat protein specific primers. The amplicon was of 394 bp in size (Fig. 1). Further, this isolate was used to carry out the host range studies. Out of the twenty-one plant species used, the virus infected all the members of the *Cucurbitaceae* i.e. *C. sativus*, *C. pepo*, *C. melo*, *C. lanatus*, *C. maxima*, *L. acutangula*, *P. fistulosus* and

*M. charantia* and in case of *Amaranthaceae* only *Amaranthus* spp. gave reaction towards PRSV-W isolate. The plants *S. lycopersicum*, *S. melongena*, *C. annum*, *A. esculentus* (L.), *Phaseolus vulgaris*, *Vigna unguiculata*, *Brassica oleracea* var *capitata*, *B. oleracea* var *botrytis*, *Chenopodium* spp. and *C. papaya* were inoculated but did not show any symptoms and failed to infect the susceptible cultivar 'MAHY-1' upon back inoculations. All the plant species that got infected by PRSV-W showed 100 per cent transmission along with varied systemic symptoms except *Amaranthus* spp., which developed local lesions.

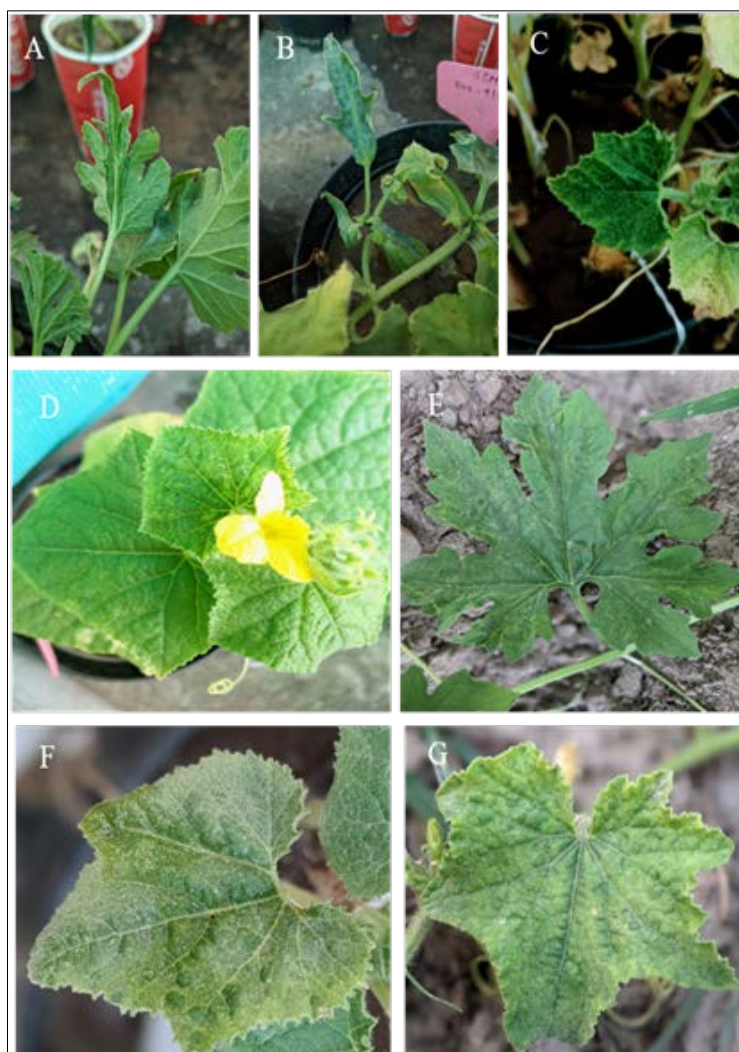


**Fig 1:** Detection of PRSV-W based on RT-PCR using CP specific primers in the bottle gourd mosaic sample: M- DNA ladder 1kb, 1- isolate J-SA-BG3, 2- isolate J-BB-BG1, 3- Positive control

Various symptoms were seen associated with the plant species used in our study (Fig. 2). Mosaic was the most common symptom, as it was recorded from almost all the susceptible host plants especially in the initial stages. In case of cucumber (*C. sativus*), mosaic appeared after 20-25 days of inoculation, while pumpkin (*C. pepo*) plants took 10-15 days for the same. In case of watermelon (*C. lanatus*) mosaic symptom were observed again after 10-15 dpi, this was followed by yellowing. In later stages, all the plants of cucumber, pumpkin and watermelon remained stunted and bore smaller and sterile flowers.

Characteristic dark green blister symptoms associated with PRSV- W was observed in muskmelon (*C. melo*) plants. Initially only mosaic was recorded 10-15 dpi later, 25 days after the inoculation the plants developed blisters. However, round gourd (*P. fistulosus*) plants expressed similar symptoms (mosaic mottling & blisters) after 20-25 dpi.

Out of all the susceptible plant species, the earliest symptoms were observed in summer squash (*C. pepo*) i.e., 7-10 dpi. PRSV-W infection was not only the earliest to appear but was most severe too in squash plants than others. A varied set of symptoms were observed like, mosaic mottle, leaf distortion (filiform) and enations. Just like other cucurbits, mosaic was the very first symptom to be observed. Further, a significant reduction in the leaf lamina and curling was seen and such leaves also had enations, especially in case of severe infections. Meanwhile, in case of winter squash (*C. maxima*) mild mosaic and enation were observed. In *L. acutangula* (ridge gourd) yellowing was observed at 20-25 dpi. In bitter gourd (*M. charantia*), the symptoms were observed after approximately a month of inoculation and that too a very mild mosaic.



A- Enation in Summer squash B- Leaf deformation (filiform) in Summer squash C. Mosaic in Pumpkin D- mosaic in cucumber E- mosaic in Bitter gourd F blisters on muskmelon and G- mosaic in ridge gourd

**Fig 2:** Various symptoms showed by the different host species against PRSV-W

**Table 1:** Observation of bottle gourd mosaic virus in different hosts of various families

Family	Scientific name	Common name	Stage of inoculation	Doi	Transmission (%)	Symptoms observed
Cucurbitaceae	<i>Cucumis sativus</i>	Cucumber	Cotyledonary leaf stage	20-25	100	M
	<i>Cucurbita pepo</i>	Pumpkin	Cotyledonary leaf stage	10-15	100	M
	<i>Cucumis melo</i>	Muskmelon	Cotyledonary leaf stage	10-15	100	B
	<i>Citrullus lanatus</i>	Watermelon	Cotyledonary leaf stage	10-15	100	M, Y
	<i>Cucurbita pepo</i>	Summer Squash	Cotyledonary leaf stage	7-10	100	M, LD, E
	<i>Cucurbita maxima</i>	Winter Squash	Cotyledonary leaf stage	10-15	100	LD, E
	<i>Luffa acutangula</i>	Ridge gourd	Cotyledonary leaf stage	20-25	100	Y
	<i>Praecitrullus fistulosus</i>	Round gourd	Cotyledonary leaf stage	20-25	100	B, M
Solanaceae	<i>Momordica charantia</i>	Bitter gourd	Cotyledonary leaf stage	25-30	100	M
	<i>Solanum lycopersicum</i>	Tomato	4-6 leaf stage	-	0	-
	<i>Solanum melongena</i>	Brinjal	4-6 leaf stage	-	0	-
	<i>Capsicum annuum</i>	Capsicum	4-6 leaf stage	-	0	-
Malvaceae	<i>Capsicum annuum</i>	Chilli	4-6 leaf stage	-	0	-
	<i>Abelmoschus esculentus (L.)</i>	Okra	Cotyledonary leaf stage	-	0	-
Fabaceae	<i>Phaseolus vulgaris</i>	Rajmash	Cotyledonary leaf stage	-	0	-
	<i>Vigna unguiculata</i>	Cow pea	Cotyledonary leaf stage	-	0	-
Brassicaceae	<i>Brassica oleracea var. capitata</i>	Cauliflower	10 days after transplanting	-	0	-
	<i>Brassica oleracea var. botrytis</i>	Broccoli	10 days after transplanting	-	0	-
Amaranthaceae	<i>Amaranthus spp.</i>	Amaranthus	6 leaf stage	20	100	LL
	<i>Chenopodium spp.</i>	Chenopodium	6 leaf stage	-	0	-
Caricaceae	<i>Carica papaya</i>	Papaya	1.5 months old seedling	-	0	-

DOI- days of inoculation, B- Blisters, E- enation, LD- Leaf distortion, LL- Local Lesion, M- Mosaic, MM- Mild Mosaic, Y- Yellowing, -: No symptom

#### 4. Discussion

The viruses being obligate in nature always need a host for its survival. This survival and spread of the virus is determined by the biological assays like host range studies. Once the host range is known appropriate strategies can be adopted like adopting the cropping system, removal of the weed hosts etc. Therefore, during our investigations different hosts viz., *C. sativus*, *C. pepo*, *C. melo*, *C. lanatus*, *C. maxima*, *L. acutangula*, *P. fistulosus*, *M. charantia*, *S. lycopersicum*, *S. melongena*, *C. annuum*, *A. esculentus*, *P. vulgaris*, *V. unguiculata*, *B. oleracea* var *capitata*, *B. oleracea* var *botrytis*, *Amaranthus* spp., *Chenopodium* spp. and *C. papaya* were screened by sap inoculations in order to deduce the host range of the virus causing mosaic in bottle gourd. All the cucurbitaceous hosts produced systemic symptoms (mild mosaic, puckering, mosaic mottling, and enations). All the symptoms were expressed between 7-10 days up to 20-25 days in general, only exception to bitter gourd where the symptoms were seen after 30 days. *Amaranthus* spp. alone produced local lesion whereas, all other plants belonging to *Solanaceae*, *Malvaceae*, *Fabaceae*, *Brassicaceae* and *Caricaceae* were non hosts.

Investigations of the present study are in confirmation to the findings of Raychaudhuri and Varma (1975)<sup>[15]</sup> and Hollings *et al.* (1975)<sup>[5]</sup>. The host range of the bottle gourd mosaic causing virus in different crops like cucurbits, *Nicotiana* spp. were also reported by Mantri *et al.* (2005)<sup>[9]</sup>. Begum *et al.*, 2016 reported the sap transmissible nature of PRSV-W to *Cucurbitaceae*, *Chenopodiaceae*, *Amaranthaceae* and *Leguminosae* except that of *Solanaceae*. They also recorded varied symptoms associated i.e. systemic mosaic, chlorotic & necrotic lesions and leaf distortion. Udvant (2018)<sup>[16]</sup> screened plants of different families (*Cucurbitaceae*, *Leguminaceae*, *Solanaceae*, *Chenopodiaceae*, *Cruciferae*, *Malvaceae* and *Poaceae*) against bottle gourd mosaic disease and found that only from *Cucurbitaceae* family were susceptible to the disease which is in accordance to our findings.

#### 5. Conclusion

The study revealed that the virus i.e., PRSV-W had a narrow host range mostly confined to *Cucurbitaceae*. The identity of the virus was even though confirmed using coat protein specific primers for PRSV- W strain, the fact that our could not infect papaya seedlings cemented its identity as the pathotype -W, which are incapable of infecting papaya, whereas the other pathotype PRSV-P can infect both papaya and cucurbits.

#### 6. Future Prospect

Cucurbits occupy major area under cultivation in Jammu sub-tropics and viruses are the top constraints leading to yield losses every year. The fact that, weeds serve as the reservoir for various viruses, therefore more refined view on the host range can be achieved in future if more plant species especially weeds of varied families could be involved in such studies.

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