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Comparative Host Range and Molecular Studies of Papaya Ringspot Virus

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ABSTRACT: *Papaya ring spot virus* isolate from Bagalkot, Karnataka was evaluated for host range and molecular studies using artificial inoculation and PCR based methods. PRSV-P isolate had a limited host range. The experimental results showed that PRSV isolate was pathogenic to *Carica papaya* (Caricacea) along with *Chenopodium quinoa* (Chenopodiaceae) and *Cucumis sativus* (Cucurbitaceae) producing local lesion and mosaic symptoms respectively. However apathogenic to *Phaseolus vulgaris* (Fabaceae). *Datura stramonium, Datura metel, Capsicum annum cv. California Wonder, Nicotiana tabacum* and *Nicotiana glutinosa* (all Solanaceae).

Keywords: *Carica papaya*, *Chenopodium quinoa, Cucumis sativus* and Papaya ringspot virus

INTRODUCTION

Carica papaya L. belongs to the family Caricaceae, commonly known as "Papaya" it is a popular and economically important fruit tree of tropical and subtropical countries in the World. It is also known as paw paw, papaw tree melon, the fruit of angels and poor man's fruit (Aykroyd, 1951). The cultivation of papaya has significantly increased across the globe in the recent past. During 1985 the world papaya production was only 3.16 Mt with an area of 2.20 lakh ha having productivity of 14.3 t/ha but it production has increased readily up to 13.74 Mt in an area of 4.62 lakh ha having 29.69 t/ha of productivity in 2019. Similarly in India during 1985 the production was 0.24 Mt in an area of 0.31 lakh ha having 7.74 t/ha productivity, whereas in 2019 the production is 6.05 Mt with in an area of 1.49 lakh ha having 40.6 t/ha productivity (Anon., 2019). India is the largest and leading producer of papaya in the world and shares 44.04 per cent of global production. The major papaya growing Indian states are Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Chhattisgarh, West Bengal, Assam, Tamil Nadu and Jharkhand. Karnataka stands 3rd with respect to area and production (Anon., 2018). In Karnataka major papaya growing districts are Bellary, Bidar, Chamarajanagar, Chitradurga, Mandya, Raichur, Kolar, Chikballapur, Koppal, Ramanagara and Tumkur (Anon., 2018).

Papaya is an economically important edible fruits crop and is considered to be as one of the most important sources of vitamins A and C. In addition, papaya contains enzyme papain and chymopapain, both of which are widely used in the food industry, medical purposes and also used for the preparation of value added products.

Papaya is badly affected by many biotic factors such as fungi, bacteria, nematodes and viruses. Besides these papaya viruses cause diseases of global significance with serious damage in fruit production as well as the devastation of the entire crop (Akhter and Akanda, 2008). More than 29 different important virus diseases affecting papaya cultivation have been reported worldwide which belongs to different viral groups (Purcifull and Hiebert, 1978). Among the viruses *Papaya ringspot virus*, *Papaya leaf curl virus* have gained global importance in all the papaya growing countries. In India, *Papaya ringspot virus* (PRSV) is

one of the greatest concern, potentially causing a 100 per cent loss in yield (Sharma and Tripathi, 2014) throughout India *Papaya ringspot virus* is a member of the genus *Potyvirus* in the family Potyviridae and manly infect papaya, cucurbits and other plants. Particles of PRSV are flexuous rod measuring 760-800 nm × 12 nm (Yeh and Gonsalves, 1984a). PRSV consists of single stranded positive sense RNA with having 9,000 to 10,326 nucleotides in length excluding the poly 'A' tail (Wang *et al.,* 1978) and encapsulated with 30-36 kD coat protein. According to the host range specificity, PRSV is classified into two biotypes, (a) PRSV-W, formerly water mosaic virus 1, which naturally infects Cucurbitaceae crops but is unable to infect papaya and (b) PRSV-P, which naturally infects papaya (*Carica papaya*) and can be transmitted experimentally to cucurbits. In order to determine the symptomatology and host rage indexing studies of PRSV were carried out under lab conditions.

MATERIALS AND METHODS

Virus indexing of PRSV to identify reservoir hosts was done by using eight plant species belonging to four families as indicator hosts. The seeds of indicator hosts were collected from ICAR- National Bureau of Plant Genetic Resources, New Delhi such as *Chenopodium quinoa, Cucumis sativus, Phaseolus vulgaris, Datura stramonium, Datura metel, Capsicum annum* cv. California Wonder, *Nicotiana tabacum* and *Nicotiana glutinosa.* The viral inoculation is done at different crop growth stages.

The healthy host plants were artificially inoculated with PRSV infected plant sap under the controlled condition at a different stage of the crop. Mechanical sap inoculation was done by using a 0.005 M phosphate buffer.

Preparation of 0.005 M phosphate buffer

Reagents: 0.05 M phosphate buffer, pH7.0 (to prepare 1 liter)

KH_2PO_4:2.4 g

K_2HPO_4:5.4 g

Thioglycerol:0.75 ml

0.02M 2-mercaptoethanol (1.56 ml/lt)

Dissolved in 1litre of distilled water, by adjusting pH 7.0.

Selection of PRSV infected tissue: Young infected tissue showing primary symptoms was used to those from older plants because of high infective virus concentration and fewer inhibitory compounds. The leaf stage used for inoculation is given in Table 1.

Preparation of infected leaf extracts: The PRSV infected leaf tissue was triturated in a sterilized pre-cold mortar and pestle in chilled 0.05M Phosphate buffer. Grind the tissues in 1:9 dilution *i.e* 1gm tissue in 9 ml buffer till a fine homogenate was obtained. Keep the inoculums chilled till it was used for inoculation. The homogenate was sieved through a muslin cloth. Before inoculation, celite was added to the inoculum (0.025 gm/ml) to serve as an abrasive. Mechanical inoculation was carried out by swabbing with a small piece of sterilized absorbent cotton wool soaked in the inoculum on the upper and lower surface of the young leaves of the host crop. Inoculated plants will be observed daily for the development of phenotypic symptoms. Different kinds of symptoms developed on individual plant species will be recorded separately.

Extraction of total RNA and cDNA synthesis from virus infected papaya plant leaves: In order to conform the presence of viral inoculam in host crops molecular detection was carried out. Isolation of total RNA from the leaf from papaya and host crop was done by using Spectrum™ Plant Total RNA Kit from Sigma-Aldrich (Catalog No.: STRN50). Extracted total RNA samples were quantified and dilute the RNA to 1000ng/ul. The diluted RNA was taken for reverse transcription for synthesizing cDNA using PrimeScript™ 1st strand cDNA Synthesis Kit (Catalog No.: #6110A). cDNA synthesized by reverse transcription was subjected to RT-PCR using a set of primers MB 11A/MB 11B (Bateson *et al*., 1994) to detect the presence of PRSV with PCR conditions, initial denaturation 94°C for 5 min with 35 cycles of 94°C for 2 min (denaturation), 55°C for 1 min (annealing), 72°C for 1 min (extension) and 72°C for 10 min (final extension).

RESULTS

Host range studies helps to detect and identify the type of viruses, based on the expression of typical symptoms like localized lesions and mosaic on indicator hosts. Indexing of PRSV virus was studied by inoculating the crude sap mechanically at a specific leaf stage on the eight species of indicator host plants belonging to different families *viz.*, *Chenopodium quinoa* (Chenopodiaceae), *Cucumis sativus* (Cucurbitaceae), *Phaseolus vulgaris* (Fabaceae). *Datura stramonium, Datura metel, Capsicum annum cv. California Wonder, Nicotiana tabacum* and *Nicotiana glutinosa* (all Solanaceae).

Symptomatic study revealed that *Chenopodium quinoa* expressed local lesions on leaves while *Cucumis sativus* expressed mosaic symptoms due to PRSV infection. Both the indicator hosts took 10 days to produce symptoms after inoculation. Other indicator hosts did not produce any symptoms.

Further PCR analysis was carried out for the inoculated indicator host crops using a set of primers MB 11A/MB 11B (Bateson, *et al*., 1994) to detect the presence of PRSV. Results confirmed the presence of PRSV as there was positive amplification in *Chenopodium quinoa* and *Cucumis sativus* (Table 1, Fig. 1 and Plate 1).

Table 1: Symptoms expression by PRSV on different indicator hosts.

Sr. No.	Plant species	Common name	Family	Leaf stage for Inoculation	Symptoms	Days taken for expression	RT-PCR Detection
1.	*Chenopodium quinoa*	Chenopodium	Chenopodiaceae	4th	Local lesion symptoms	10	+ ve
2.	*Cucumis sativus*	Cucumber	Cucurbitaceae	2nd	Mosaic symptoms	10	+ ve
3.	*Phaseolus vulgaris*	Beans	Fabaceae	2nd	No symptoms	—	– ve
4.	*Datura stramonium*	Datura	Solanaceae	6th	No symptoms	—	– ve
5.	*Datura metel*	Datura	Solanaceae	6th	No symptoms	—	– ve
6.	*Capsicum annum* cv. *California Wonder*	Chilli	Solanaceae	8th	No symptoms	—	– ve
7.	*Nicotiana tabacum*	Tobacco	Solanaceae	6th	No symptoms	—	– ve
8.	*Nicotiana glutinosa*	Tobacco	Solanaceae	6th	No symptoms	—	– ve

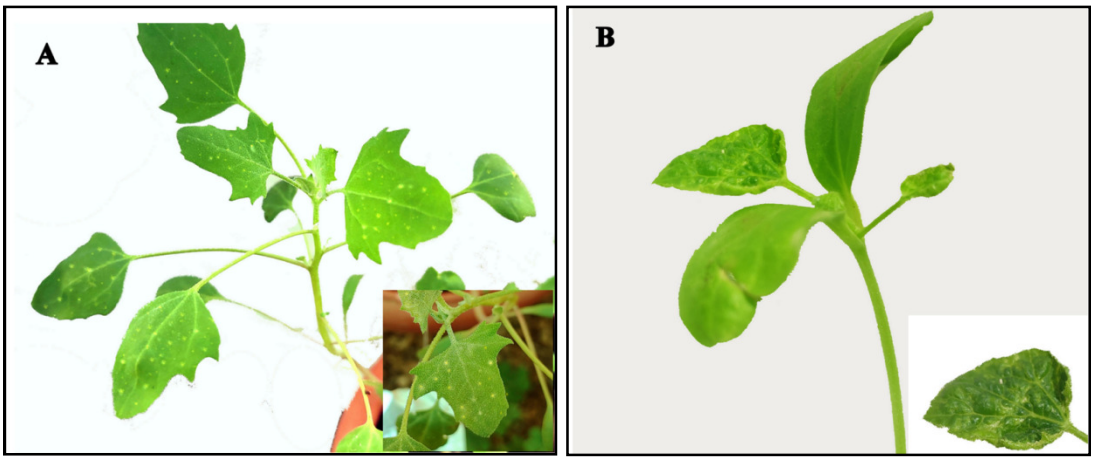

Fig. 1. Symptom expressed by PRSV on *Chenopodium quinoa* - local lesion (A) *Cucumis sativus* - mosaic symptoms (B) on hosts.

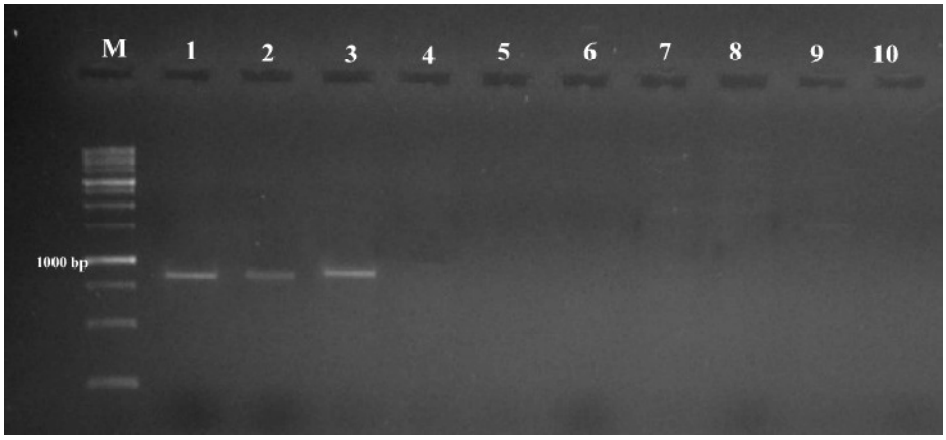

Plate 1: Agarose gel photograph showing amplification of coat protein fragment of PRSV.
(Lane 1: +ve control), *Chenopodium quinoa* (Lane 2) and *Cucumis* sativus (Lane 3) by using a set of primer MB 11A/MB; 11B (~905 bp). Lane L: 1 kb lader (StepUp™ 1 kb DNA Ladder)

DISCUSSION

The efforts on indicator host rang studies were made to ascertain the role of collateral hosts belonging to families other than *Caricaceae*. The results of the host indexing study revealed that PRSV can infect hosts belonging to the families of Chenopodiaceae and Cucurbitaceae. In the case of *Chenopodium quinoa* belonging to Chenopodiaceae, only 'local lesions' were observed on the inoculated leaves at 10 days after inoculation. However, on *Cucumis sativas* belong to the family Cucurbitaceae expressed 'mosaic' symptoms were noticed.

This host range is based on the genetic diversity among the strains of PRSV. These strains were grouped into two, PRSV-P and PRSV-W types. The virus grouped into the PRSV-P (infecting type papaya) affects both papaya and cucurbits and the PRSV-W (cucurbit infecting type) affect only cucurbits but not papaya. It

also has been reported earlier that, PRSV-P is mostly restricted to Chenopodiaceae and Cucurbitaceae families other than Caricaceae (Conover, 1962; Purcifull *et al.,* 1984; Yeh and Gonsalves, 1984b; Thomas and Dodman, 1993; Dahal *et al.*, 1997; Perera *et al.*, 1998; Parmar, 2000; Lakshminarayana Reddy, 2000; Kelaniyangoda and Madhubashini, 2010; Kunkalikar, 2003; Tripathi *et al.,* 2008; Limkar, 2017; Singh *et al.*, 2017; Navanath *et al.*, 2017; Harish, 2018; Kumar *et al.,* 2021). Host range studies of virus and the symptoms it produces often provide an important clue to its identity. It also helps to know the best host for propagation, assaying and maintenance of virus isolates. This study helps to understand virus host interaction in crop plants based on symptomatic and molecular basis.

CONCLUSION

PRSV isolate was pathogenic to *Carica papaya* (Caricacea) along with *Chenopodium quinoa* (Chenopodiaceae) and *Cucumis sativus* (Cucurbitaceae) producing local lesion and mosaic symptoms respectively. However apathogenic to *Phaseolus vulgaris* (Fabaceae). *Datura stramonium, Datura metel, Capsicum annum cv. California Wonder, Nicotiana tabacum* and *Nicotiana glutinosa* (all Solanaceae).

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Conflict of Interest. Nil.

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