IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
ISSN-0973-1547 Volume **15** | Issue 1 | January, 2020 | 55-60
DOI: 10.15740/HAS/IJPS/15.1/55-60
Visit us - *www.researchjournal.co.in*

RESEARCH ARTICLE

Molecular diagnosis, status and temporal incidence of papaya ringspot disease of papaya

■ **Upasna Priya, Monika Karn and Mohammad Ansar**

SUMMARY

Papaya plant is severely affected by different viruses among them papaya ring spot virus is causing considerable yield loss leading to the complete failure of fruiting. Therefore, present investigation commenced with molecular diagnosis, current status and temporal incidence of disease. Various symptomatic plants gave positive amplification in one step RT-PCR with both Potyvirus and PRSV CP gene specific primers indicating an amplicon of ~330bp and 850bp bands, respectively. In leaf samples, 46 to 89 per cent RNA was found positive for PRSV infection. Whereas least infection of 13 per cent was found in seeds collected from infected fruits. The disease was observed at three different experimental plots which ranges from 49 to 75.3 per cent incidence. A rapid increase in the aphid population was noticed from middle of December to February. Further, maximum aphid population was noticed at experimental plot-1 (plant pathology) followed by plot-3 (IFS) with 5.48 and 5.14/plant. Periodic observation of the disease was assessed. The appearance of ringspot was noticed in first week of October with diverse symptom. It slowly increased upto middle of January and exponentially increased upto middle of May month with 65.3 per cent incidence. The peak aphid population was noticed in middle of February (20.5/plant) which was gradually declined upto 3.9/plant in May. The present information will helpful in understanding the epidemiology of ring spot disease and suitable management possibilities.

Key Words : Aphid, Papaya, RT-PCR, PRSV, Potyvirus

How to cite this article : Priya, Upasna, Karn, Monika and Ansar, Mohammad (2020). Molecular diagnosis, status and temporal incidence of papaya ringspot disease of papaya. *Internat. J. Plant Sci.*, **15** (1): 55-60, **DOI: 10.15740/HAS/IJPS/15.1/55-60,** Copyright@ 2020: Hind Agri-Horticultural Society.

Article chronicle : Received : 16.11.2019; **Revised :** 11.12.2019; **Accepted :** 26.12.2019

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
Upasna Priya, Department of Plant Pathology, Bihar Agricultural University, Sabour, **Bhagalpur (Bihar) India**
Email : upriya2014@gmail.com

Address of the Co-authors:
Monika Karn, Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, **Solan (H.P.) India**
Email : karn.monika1990@gmail.com

Mohammad Ansar, Department of Plant Pathology, Bihar Agricultural University, Sabour, **Bhagalpur (Bihar) India**

Papaya orchard critically hindered by various biotic factors; among them viruses are playing significant role which produces complex symptom. Several viruses like papaya ring spot virus (PRSV), papaya leaf distortion virus (PLDV), papaya mosaic virus (PMV), Papaya meleira virus (PMeV), papaya apical necrosis virus (PANV), papaya leaf curl virus (PLCV) and papaya lethal yellowing virus (PLYV) are the important viruses affecting papaya cultivation across the world (Abreu *et al.*, 2015). Ring spot is one of the major disease in India

and poses a serious threat to papaya production. The PRSV belongs to the family Potyviridae having positive ss-RNA genome approximately 10.3 Kb in size. It causes substantial losses in both papaya and cucurbits hosts. The disease has been noticed with 70-95 per cent crop losses in several parts of India (Singh and Shukla, 2009). The virus is transmitted by different aphid species mainly by *Myzus persicae* in non-persistent manner. Papaya cultivation recently expanded in various zones of Bihar state but it is discouraged by severe infection of PRSV. The plants showed typical characteristics symptoms of ringspot on leaves, fruits and even on petioles. Severe mosaic, distorted leaves along with shoestring commonly noticed in the plants. Disease appears with mild appearance in the October month and it severely increases upto May. The plants depicted to complete failure of second year fruiting. The present study elaborated the findings of virus diagnosis and its periodic appearance in papaya orchard. The generated information will be helpful in understanding the disease pattern which may be further employed in disease management module.

MATERIAL AND METHODS

Total RNA isolation and one step RT-PCR:

A varied range of viral symptoms like mild mosaic, distortion, curling, twisting, shoestring, ringspot on leaves and petioles were collected (Fig. A). The floral parts, fruits showing mild and severe ring and seed were also taken into consideration. Three leaves of each typically infected and a healthy leaf selected as control for RNA isolation. Total RNA was extracted using SV total RNA isolation kit (Promega, USA) and followed the manufacturer's protocol. The RT-PCR was performed in 50µl reaction using one step RT-PCR kit (TITANIUM). RNA samples of 2µl as template was used in 45.5µl PCR reaction mixture which consisted of 5 µl of 10x one step RT-PCR buffer, 1µl each primer, 50x dNTP mix 1µl and Taq RT enzyme 1µl. The amplification was performed in Master cycler (Eppendorf, Germany) with standard protocol programmed for 1 cycle of 45°C for 45 min for cDNA synthesis and 35 cycles of following parameters, denaturation at 94°C for 30 sec, annealing temperature as per the Table A for 1 min. The extension at 68°C for 1 min, followed by 1 cycle of final extension for 68°C for 10 min. After completion of cycling, the reaction was hold at 4°C. The amplicons were analysed in 1 per cent agarose gel with 1X TAE buffer containing 0.1 per cent ethidiumbromide and imagine in gel documentation system (UVITECH, UK).

Estimation of disease incidence and aphid population at different locations:

To find the incidence of *PRSV* in papaya three different places like integrated farming system (IFS) plots, Krishi Vigyan Kendra (KVK) and experimental plots (EP) of Department of Plant Pathology, Bihar Agricultural University, Sabour, were considered. To calculate the disease incidence at each location, plant population counted and diseased plants were tagged. In each targeted location (IFS, KVK and EP), randomly selected the papaya plants and monitored the aphid population. Aphid abundance in the field was assessed by using yellow sticky traps (7×12 cm) dimension. The traps were manually prepared with four face plastic containers. Each face was painted with bright yellow colour and coated with a sticky substance (grease). The trapped aphids on each face of the container was counted. Similarly, aphid population was also counted on leaves of papaya plants. Each plant four leaves were considered (1 top, 2 middle and 1 lower leaf) to note vector population. Collection of trapped aphids was done in 1.5 ml collection tube containing 70 per cent alcohol with the help of fine hair brush.

Temporal incidence of PRSV and aphid population in papaya:

In order to assess the temporal incidence of *PRSV* and aphid population papaya plants were maintained. Disease incidence was calculated as earlier described formula. The data for disease incidence and aphid population were recorded at 15 days periodic interval.

Table A : Information about RT-PCR primers used

Primers	Primer sequence (5 '-3 ')	Tm (°C)	Amplic on size (bp)	Source /Remark
oligo 1n	TGGTHTGGTGYATHGGARAAYGG	49	330	Marie-Jeanne *et al.*, 2000
oligo 2n	TGCTGCKGCYTTCATYTG			
PRSV-P CP F	CCAAGACTGAAGCGGTGGAT	49	850	Designed by CP conserved region
PRSV-P CP R	GCATACCCAGGAGAGAGTGC			

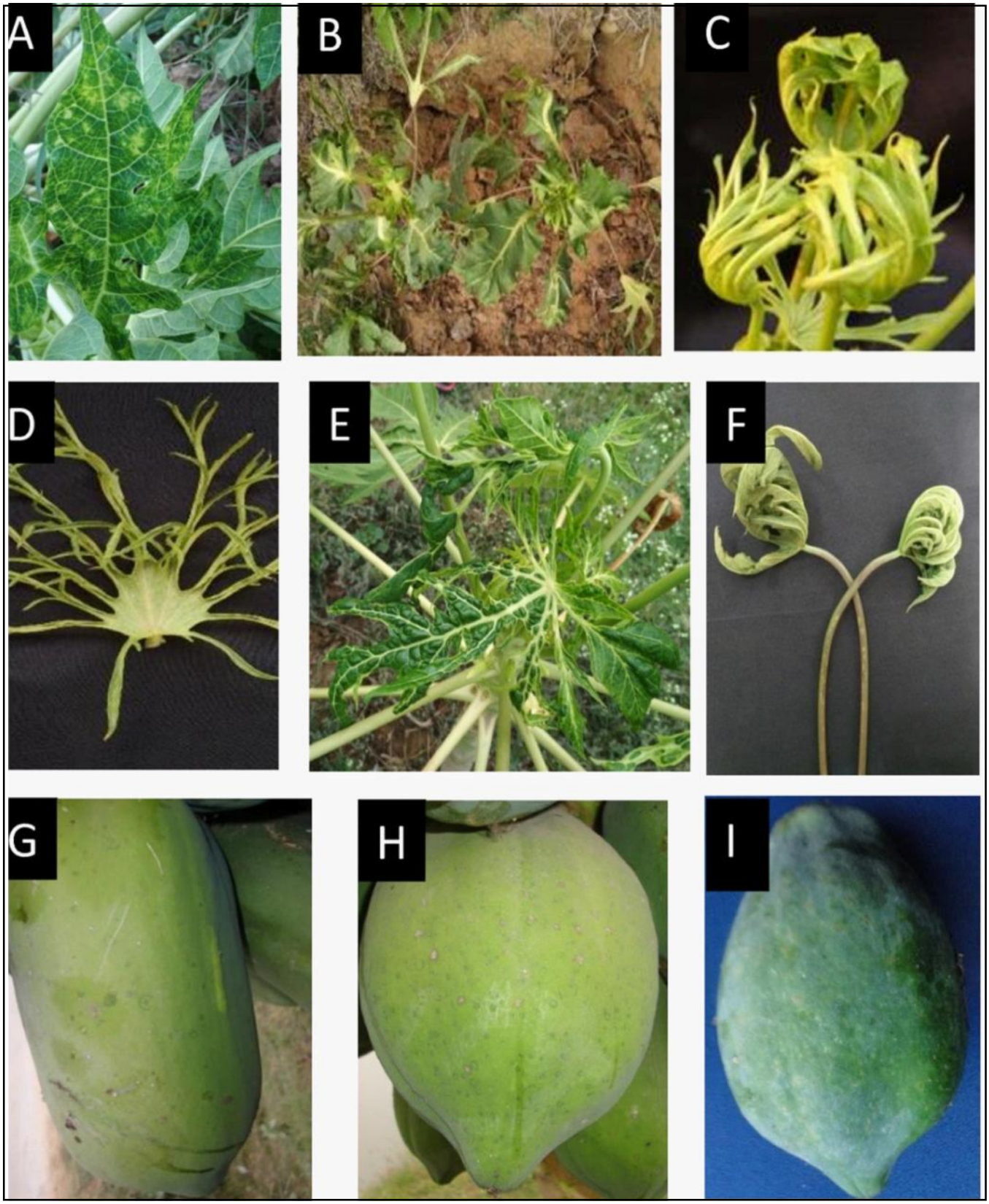

Fig. A: Symptomatic diversity of PRSV, A: mild ring spot, B: distorted leaf, C: curling, D: shoe string, E: puckering, F: rings on petiole, G and H: mild rings on fruit, I: severe symptom on fruit

Aphid population was also counted on leaves as previous described methodology.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

One-step RT-PCR and PCR analysis:

In one step RT-PCR various symptomatic plants were tested. Primarily these samples gave positive amplification with potyvirus specific primers as indicated by the presence of amplicon of ~330bp. Further these samples were also found positive with PRSV CP gene specific primers by producing 850bp band (Fig. 1). In leaf 46 to 89 per cent RNA was found positive for PRSV infection. A least infection 13 per cent was found in seeds collected from infected fruits. Fruits showed severe symptom of ring indicating 87 per cent infection. No any positive amplification was found in healthy leaves and fruits (Table 1).

Disease incidence and aphid population at different location:

Assessment of papaya ring spot disease and associated aphid population were considered at three different location experimental plots. The incidence ranges from 49 to 75.3 per cent. Minimum incidence was observed at experimental plots (Plant Pathology), whereas it was found maximum (75.3) in plants grown at integrated farming plots. The aphid population was monitored in respective papaya plots. A rapid increase in the population was noticed from middle of December to February. An average population of aphid was recorded at all three locations. Maximum was noticed at experimental plot-1 (plant pathology) and plot-3 (IFS) with 5.48 and 5.14/plant (Fig. 2).

Temporal dynamics of aphid population and PRSV incidence:

For periodic observation of the disease, tagged plants were observed periodically (15 days interval). The appearance of ringspot was noticed in first week of October with diverse symptom. The incidence was slowly increased upto middle of January. The disease was found exponentially increased up to middle of May month. Abrupt increase in incidence was recorded from 15th February which was reached upto 15th May with 65.3 per cent (Fig. 3). The aphid population 4 to 5/plant was observed upto December. The peak population was noticed in middle of February (20.5) which was gradually declined upto 3.9/plant in May.

Papaya is widely cultivated in tropical and subtropical regions of the world which is broadly affected by a number of viruses (Tripathi *et al*., 2008). In Indian subcontinent PRSV severely affecting the papaya cultivation which failure the second year fruiting. The virus transmission efficiency by the aphids species has been studied worldwide, where by *A. gossypii*, *A. rumicis* Linnaeus and *Myzus persicae* were found to be the most efficient vectors (Jensen, 1949; Capoor and Varma, 1958; Vegas *et al*., 1985 and Kalleshwaraswamy *et al*., 2007). *PRSV* is affecting the papaya cultivation in south and northern Bihar of India where it commonly

Table 1 : RT-PCR of diverse symptomatic plants/plant parts

Symptomatic plants /plant part	RT-PCR assay		Per cent positive samples
	Potyvirus primer (oligo 1n/ oligo 2n)	CP gene specific primer (PRSV)	
Mild mosaic leaves	+	+	89
Distorted leaves	-	-	67
Curling twisting	+	+	46
Shoestring	+	+	58
Puckered leaf	+	+	87
Petioles	+	+	56
Floral parts	+	+	34
Fruits with mild ring	+	+	67
Seeds	+	+	13
Fruits with severe ring	+	+	87
Healthy leaves	-	-	0
Healthy fruit	-	-	0

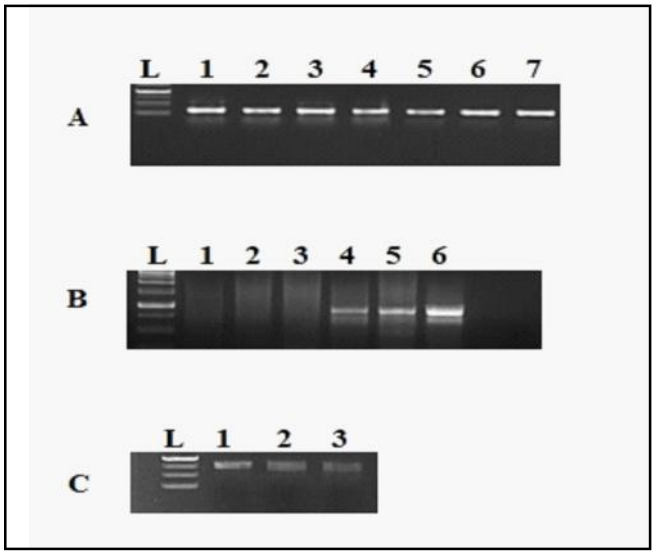

Fig. 1: Gel electrophoresis of PCR amplified products using potyvirus specific primers (A) 1- mild mosaic leaves, 2-curling twisting, 3-shoestring, 4-puckered leaf, 5-petioles, 6-fruits with mild ring, 7- fruits with severe string; PRSV CP gene specific primer papaya leaves (B) 1-distorted leaves, 2-healthy leaves, 3-healthy fruit, 4-mild mosaic leaves, 5-curling twisting, 6-shoestring; (C) 1-puckered leaf, 2-petioles, 3-fruits with mild ring

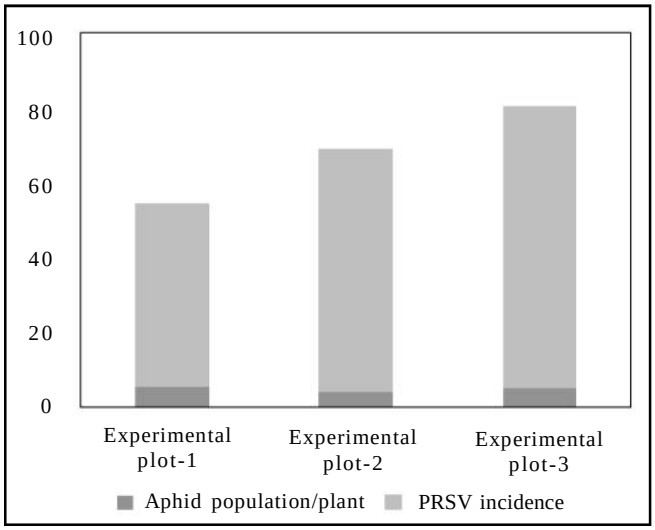

Fig. 2: Papaya ring spot disease incidence and associated aphid population at three different location experi mental plots

grown. The present investigation was undertaken to detect the PRSV infection in papaya. The results of one step RT-PCR tests indicated that various symptomatic plants found positive with CP gene specific primer. The PRSV detection from infected papaya samples using DAS-ELISA followed by one-step RT PCR using CP

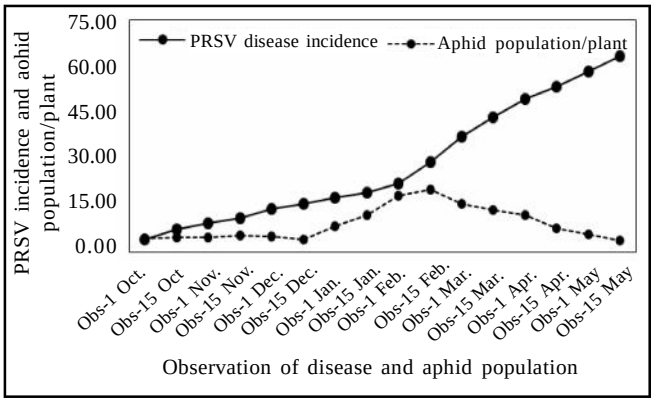

Fig. 3: Temporal dynamics of aphid population and PRSV incidence

gene was carried out by (Ansar and Srinivasaraghavan, 2016). The incidence of PRSV ranges from 49 to 75.3 per cent. at three experimental plots. At all experimental plots infestation of aphids were noticed which clearly indicated its role in virus transmission. There are five common aphid vectors e.g. *Aphis craccivora*, *A. gossypii*, *A. citricola*, *Myzus persicae* and *Rhopalosiphum maidis* are found in transmission of virus, but most efficient was *Myzus persicae* which can transmit 70 per cent disease (Bijaya *et al*., 2006). The temporal dynamic study indicated that abrupt increase in disease was found from middle of February which was reached upto May. The increasing trend of disease synchronised with aphid population might be the possible cause of high disease pressure. The present investigation covers the molecular diagnosis, current status and temporal incidence of PRSV affecting papaya. The generated information will be supportive in understanding disease epidemiology and formulation of management options.

REFERENCES

Abreu, P.M., Antunes, T.F., Magana, A.A., Perez, B.D., Tapia, T.R., Ventura, J.A., Fernandes, A.A. and Fernandes, P.M. (2015). A current overview of the papaya meleira virus, an unusual plant virus. *Viruses*., **8 & 7** (4):1853-1870.

Ansar, M. and Srinivasaraghavan, A. (2016). Papaya ring spot virus: An inducer of chlorotic spots and severe mosaic of papaya in Bihar. ISMPP 37th Annual Conference and National Symposium, pp. 51-52.

Bijaya, P., Devjani, P., Subharani, S. and Singh, T.K. (2006). Spatial distribution of *Myzus persicae* (Sulzer) on cauliflower. *Annals Plant Protect. Sci*., **14**(1): 38-40.

Capoor, S.P. and Verma, P.M. (1958). A mosaic disease of papaya in Bombay. *Indian J. Agric. Sci.*, **28**: 225-233.

Jensen, D.D. (1949). Papaya virus disease with special reference to Papaya ring spot. *Phytopathology.*, **39** (3): 191 – 211.

Kalleshwaraswamy, C.M., KrishnaKumar, N.K., Verghese, A., Dinesh, M.R., Ranganath, H.R. and Venugopalan, R. (2007). Role of transient aphid vectors on the temporal spread of papaya ringspot virus in South India. *Acta Horticulture*, **740** : 251-258.

Singh, V. and Shukla, K. (2009). Effect of PRSV infection on pigment content and assimilation of carbohydrates on *Carica papaya*. *Ann. Pl. Protect. Sci.*, **17**: 152-156.

Tripathi, S., Suzuki, J.Y, Ferreira, S.A. and Gonsalves, D. (2008). Papaya ring spot virus-P: characteristics, pathogencity, sequence variability and control. *Molecular Plant Pathology*, **9** (3): 269-280. DOI:10. 1111/j.1364-3703.2008.00467.x.

Vegas, A., Cermeli, M. and Trujillo, G. (1985). Afidos conel virus de la mancha anillada de la lechosa en Venezuela: presencia, transmission y eficiencia. *Agron. Trop.*, **35** : 25-31.