Unlocking Natures Secret: Revealing the Culprit behind Maharashtra Papaya Ringspot Disease

ABSTRACT

The papaya ringspot disease (PRSD) in Western Maharashtra, India, it is a big threat to cause complete loss in papaya cultivation and the symptomatology of this disease is still insufficient to identify with accuracy to manage the disease. Therefore, the current research was conducted during year 2020-21 with objective to check the occurrence and severity of disease in five major papaya growing districts (Ahmednagar, Pune, Sangli and Satara and Solapur) by using 0-4 disease rating scale. The further studies on Transmission Electron Microscopy (TEM) were employed for identification of virus associated with it. The result of the survey shows that, Ahmednagar having the highest incidence at 87.50%, followed by Solapur (68.75%). In surveyed area Taiwan-786 papaya cultivar found everywhere and revealing an average disease incidence ranging from 10.41 to 87.50%. Common symptoms were found including light leaf discoloration, mosaic patterns, chlorotic patches, leaf curling, stunting, blisters, and fruit deformation. The ringspot virus is characterized by distinctive features such as green ice-land, shoe string formation, pale oily greasy streaks, and ringspots on leaves, fruits, and stems. The TEM studies confirmed the presence of flexible rod-shaped particles of papaya ringspot virus (PRSV) in infected samples.

Keywords: *Carica papaya*, Disease intensity, PRSV detection, TEM

INTRODUCTION

The papaya plant (*Carica papaya* L.), is a fast-growing, short-lived plant that is cultivated for its fruit, papain, pectin and antibacterial qualities in tropical and subtropical climates worldwide [7, 22]. Two of the family *Caricaceae* extant species are still found in Africa, where they first appeared. The third-most-cultivated tropical crop worldwide is the papaya. Although Mexico is the primary exporting nation, Brazil and India are the two biggest producers [9]. When it comes to the proportion of vitamin A, vitamin C, potassium, folate, niacin, thiamine, riboflavin, iron, calcium, and fiber, papaya is the most nutritious fruit among common ones [11]. About 13.74 MT of papaya are produced worldwide, with 4.62 lakh hectares of land used for cultivation [3]. With an area of 1.49 lakh hectares, India is the largest grower, contributing roughly 44.04 percent of the world papaya production (6.05 MT) and it has become a viable alternative to bananas as a cash crop. In India major crop growing states are Andhra Pradesh, Gujarat, Maharashtra, Karnataka, Madhya Pradesh, an Chattisgarh [3,31-33]. Diseases and pests pose serious risks to papaya production everywhere. Papaya ringspot and leaf curl infections are the most common viral maladies [20, 23]. The most destructive disease in the world that affects papaya production in practically every place where it is farmed is papaya ringspot disease (PRSD) [2], which poses a serious threat to the papaya industry. Although begomovirus-related papaya leaf curl disease (PLCD) is more common in Asian nations, recent data suggest that begomovirus infection of papaya has also been noted in American nations, but not on a large scale [2, 1]. Early vegetative stage infection can cause papaya plants to remain stunted, occasionally develop bunchy tops, and never bear fruit, which would result in a 100% yield loss [13, 17]. On the other hand, when plants are infected during the reproductive stage, all of their leaves become yellow and the fruits develop "ring spots" which results in 85.0–90.0% yield losses [12, 18].

At present papaya ring spot disease has assumed serious proportion and became a major constraint in papaya cultivation, thereby threatening the cultivation of papaya in India, including Maharashtra [16, 24, 14]. For the effective control of PRSV disease in papaya we have known about the perfect identification of that disease for effective management to avoid losses

caused by the disease. Considering this alarming issue, the experiment was designed.

MATERIAL AND METHODS

Survey for the record of severity and distribution of PRSD in major papaya growing areas of Western Maharashtra during *Kharif* and *Summer* 2020-21-2021-22

Farmers field in different villages of Ahmednagar, Pune, Sangli and Satara and Solapur districts were covered under survey programme. In each village five Papaya fields were selected randomly on both sides of the road and 60 days old papaya crops were assayed for PRSV disease when symptoms were visible. In each field ten papaya plants were randomly selected and disease incidence were scored using 0-4 rating scale of Mohamad and Mohamad [2007] [21] based on leaf area covered by ring spot symptoms as mentioned in below Table 1. Scores of 0-4 were classified as below.

Table 1. detailed description of PRSV disease rating scale Mohamad and Mohamad [2007]

Rating Scale	Description	Symptoms
0	0 % infection	No any symptoms;
1	0-25 % infection	Very mild mottling/mosaic symptoms & water-soaked streaks on stem, petiole & under leaf surface;
2	26-50 % infection	Severe mottling or mosaic & water-soaking streaks on stem, petiole & under leaf surface;
3	51-75 % infection	Leaf distortion & water-soaking streaks on stem, petiole & under leaf surface;
4	75 % infection	Shoestring & water-soaking streaking on stem, petiole & under leaf surface.

Further these scales were converted to per cent diseases index using formula given by Wheeler (1969) [29].

$$\text{Disease index (\%)} = \frac{\text{Sum of all numerical rating}}{\text{Number of leaves examined x Maximum grade}} \times 100$$

Disease Incidence was calculated by

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

In the meantime, at each studied area, affected leaf samples from infected papaya plants displaying a variety of symptoms were gathered throughout the survey. These samples were gathered individually, and brought in polythene bags. The samples were sent right away to the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri, India. They were then snap-frozen in liquid nitrogen and kept at 80 °C for additional viral identification and analysis.

Kochs assay

Seeds of the papaya cultivar (Taiwan-786) were sown in 30 cm diameter earthen pots under insect-proof glasshouse conditions. The pot mixture was made up of a 2:1:2 (w/w/w) ratio of sterilized soil, sand, and compost. The seedlings were raised in insect-proof glasshouses. After forty days, seedlings were transplanted into polythene bags and maintained in an insect-proof glasshouse. The virus inoculums used in the experiment were kept on the Taiwan-786 papaya cultivar and the different Aphid species was used for inoculation of healthy seedlings as a virus

transmission vector and also mechanical hand inoculations carried out for other experimental purposes.

Detection of PRSV by Transmission Electron Microscope

The detection of PRSV in artificially inoculated plants was undertaken by using the leaf dip method and confirmed its presence. Brandes [1957] [6] described a leaf-dip approach was adopted for detection of PRSV using transmission electron microscopy. A TEM [JEOL JEM-1011 CoolSNAP1] was used to evaluate the method used to prepare leaf-dip extracts. Grids covered with carbon were used to absorb the samples, and 2% [w/v] sodium phosphate state [pH 6.8] was used for negative staining. The estimation of particle sizes was done using internal magnification standards. 1 mm squares from affected tissues of leaves were taken out for ultrastructural investigations. They were then fixed, embedded in Epon resin, and examined under an electron microscope following uranyl acetate and lead citrate staining, as previously described by Marys *et al*. [2000] [30]. Pieces of leaf from plants that were not affected served as controls.

RESULTS AND DISCUSSION

Survey, incidence and symptomatology of PRSD

Total of 75 villages from 5 different tehsils were surveyed across the five districts. To calculate the per cent disease incidence, all plants in the designated plot area [20m×20m] of the fields were counted and the number of plants exhibiting ringspot symptoms were recorded separately [fig 1 and fig 2]. The overall disease incidence was recorded on the basis of symptoms on plants observed. Among the five districts, Ahmednagar found highest [55.55 per cent] disease incidence.

The symptoms of naturally infected and artificially inoculated papaya plants were more or less similar such as light discoloration of leaves turning towards pale yellow, Mild mosaic, mosaic, mottling, chlorotic spots, chlorotic rings, vein clearing, leaf curling, stunting, blisters, leaf distortion, fruit distortion, green ice-land, shoe string formation in leaves, pale oily greasy streaks on stem and ringspots are prominently seen on leaves, fruits, stem and hence the virus named as ringspot virus. Depending on the stage of the crop and infection, the infected plants produced few or no fruits. Many indications of papaya ringspot virus discovered during the study are depicted in the [Fig 1 and fig 2].

Detection of PRSV by transmission electron microscopy [TEM]

The samples of PRSV infected papaya leaves brought from the glasshouse (artificially inoculated plant) and were observed under transmission electron microscope. The results revealed the presence of virus as flexuous rod-shaped particle in the sample and the size was found 760 nm to 800 nm long and 12 nm in diameter [Fig 3].

Worldwide, PRSV has been recognized as the most destructive viral pathogen on papaya [4]. Despite its importance, the national economies of many papaya-cultivated countries are threatened by the incidence of PRSD. The disease affects almost all stages of the crop and spreads very quickly to the whole orchard within three to seven months, which leads to yield losses of up to 100 percent [28, 27, 25]. Although PRSV occurs in different countries, higher levels of diversity were observed among Indian isolates compared to the rest of the world [8, 5, 19]. This might be due to a lack of resistant varieties, the fast evolution of the new strains of PRSV through recombination, and the occurrence of different aphid species [15, 10]. In the current study, the incidence of PRSD was observed to range from 55.55 to 27.49 per cent across different districts surveyed in Maharashtra State, India. The findings showed that the disease is common and has variable incidence rates throughout India. This could be because of the continual larger-scale cultivation of sensitive cultivars including "Red Lady," "Sunrise Solo," and "Arka Surya," as well as fluctuations in relative humidity and temperature, all of which may have contributed to the development of the viral infection in the papaya.

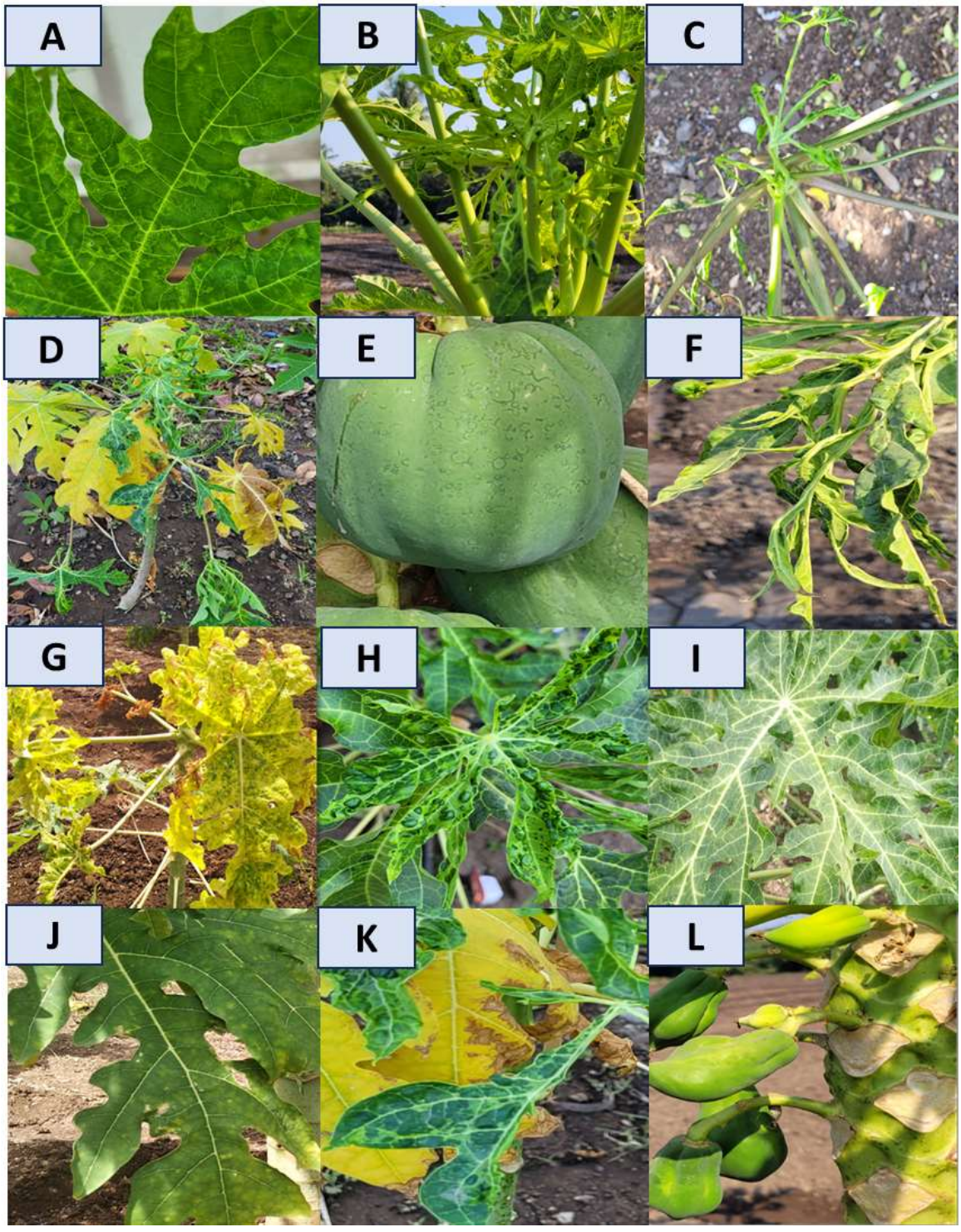

Fig 1: Typical symptoms of PRSD on leaves and fruits of papaya plant A] Mild Mosaic B] Chlorosis C] Shoe string D] Stunting E] Ring spots F] Leaves Distortion G] Severe Mosaic H] Green Ice-land I] Vain clearing J] Local Lesion K] Leaf distortion L] Fruits distortion

Fig 2: A] Healthy fruits B] PRSV infected [misshaped] fruits

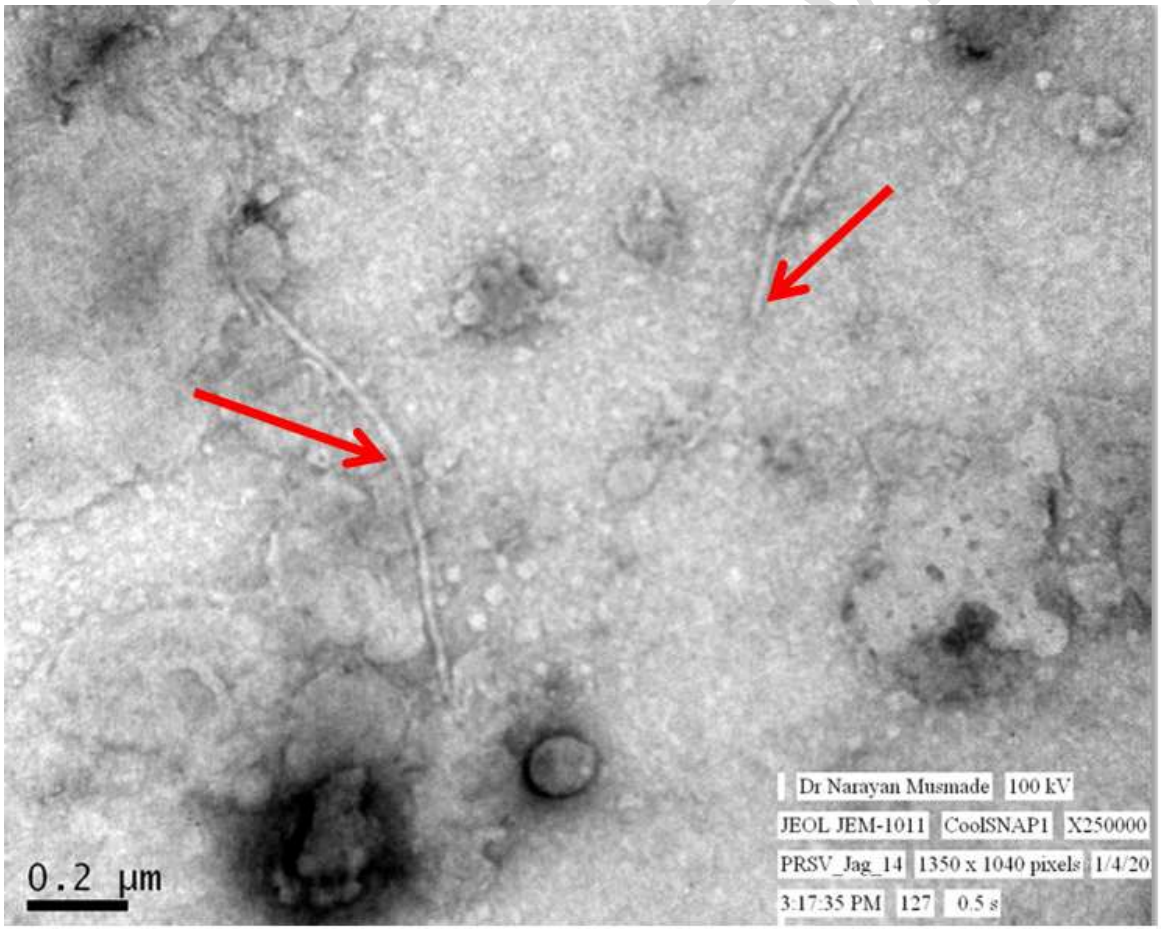

Fig 3: Transmission Electron Microscope [TEM] photos of PRSV, both arrow shows the flexuous rod- shaped particles of PRSV

Table 2. Survey details showing PRSV incidence score in Western Maharashtra State during 2020-21-2021-22 [Pooled data]

District	Taluka	Village	Longitude [N]	Latitude [E]	Crop stage	Incidence [%]	Severity score	Prominent symptoms
Ahmednagar	Shrirampur	Taklibhan	19º36’26"	74º47’56"	Vegetative	68.75	4	Cl, Pc, Ld
		Khandala	19º37’32"	74º36’01"	Flowering	43.75	4	Cl, Mo, Gi
		Haregaon	19º39’30"	74º05'55"	Vegetative	58.33	4	Cl, Mo, Pc
		Belapur	19º33’16"	74º42’28 "	Vegetative	68.75	3	Cl, Ss, Mo
	Sangamner	Nandur	19º30'02"	74º06'53"	Fruiting	87.50	4	Cl, Fd, Ss, Mo,Ld, Rs, Pc
		Vadgaon Landga	19º34’44"	74º06’25"	Vegetative	45.83	4	Cl, St, Mo,
		Nimaj	19º32’16"	74º08’44"	Vegetative	54.16	4	Cl, Mo, Gi
		Khandgaon	19º32’22"	74º11’20"	Fruiting	75.00	4	Cl, Mo, Ss, Fd,Ld, Rs, Pc
	Shrigonda	Dhandarphal Kh.	19º31’10"	74º07’08"	Fruiting	41.66	4	Cl, Ss, Rs
		Chikhali	19º33’06"	74º08’47"	Vegetative	50.00	4	Cl, Mo, Ld
		Kansewadi	18º31’44"	74º40’24"	Vegetative	45.83	4	Cl, Mo, St
		Chikhalthanwadi	18º 32’49"	74º40’54"	Fruiting	75.00	4	Cl, Mo, Fd, Ss
		Adhalgaon	18º38’42"	74º46’16"	Vegetative	29.16	3	Cl, Pc, Gi,
		Ghodegaon	18º35’59"	74º44’46"	Fruiting	43.75	4	Cl, Mo, Rs, Ss
		Belwandi Kothar	18º39’44"	74º42’49"	Fruiting	45.83	4	Cl, Rs, Fd
						Average	55.55	
Solapur	Malshiras	Piliv	17º41’01"	74º59’51"	Fruiting	58.33	4	Cl, Fd, Pc
		Kusmod	17º41’03"	74º59’56"	Fruiting	68.75	4	Cl, Mo, Ld,Ss,
		Dahigaon	17º57’34"	74º44’03"	Vegetative	56.25	4	Cl, Mo, Ss
	Pandharpur	Chandapuri	17º43’48"	74º57’36"	Fruiting	33.33	3	Cl, Mo, Rs
		Tandulwadi	18º07’04"	75º34’30"	Vegetative	41.66	4	Cl, Mo, Ld
		Chale	18º32’56"	73º36’04"	Fruiting	58.33	4	Cl, Mo, Gi
		Wakhari	18º27’28"	74º20’35"	Fruiting	33.33	4	Cl, Fd, Rs
		Hole	17º46’44"	75º18’34"	Vegetative	27.08	4	Cl, Mo, St
		Gursale	17º43’56"	75º18’38"	Vegetative	37.05	4	Cl, Mo, Pc
	Madha	Shirdhon	17º42’39"	75º16’36"	Fruiting	41.66	3	Cl, Ld, Fd
		Tadavale	18º04’45"	75º28’29"	Fruiting	66.66	4	Cl, Mo, Ss, Rs
		Shindewadi	18º01’46"	75º28’40"	Fruiting	50.00	4	Cl, Mo, Ss
		Jadhavwadi	18º00'53"	75º29'37"	Vegetative	41.66	4	Cl, Mo, Pc
		Vetalwadi	18º03'44"	75º28'34"	Fruiting	54.16	3	Cl, Ld, M, Fd
		Chincholi	18º00'31"	75º27'08"	Fruiting	29.16	4	Cl, Mo, Fd
						Average	46.49	
Pune	Indapur	Nhavi	18º09’59"	74º00’45"	Vegetative	37.50	4	Cl, Mo, Gi

	Shirur	Kandalgaon	18º06’02"	75º06’34"	Fruiting	45.83	4	Cl, Mo, Rs, Ss
		Babhulgaon	18º03’35"	75º04’36"	Vegetative	33.33	4	Cl, St, Mo
		Rui	18º01’31"	75º03’24"	Fruiting	41.66	4	Cl, Pc, Rs
		Karewadi	18º09’02"	74º56’11"	Vegetative	37.50	4	Cl, Mo, Pc
		Hivre	18º46’28"	74º07’33"	Vegetative	27.08	4	Cl, Mo, St
		Ranjangaon	18º44’55"	74º14’16"	Fruiting	31.25	4	Cl, Mo, Rs
		KanhoorMehasai	18º50’05"	74º08’28"	Fruiting	41.66	3	Cl, Mo, Fd
		Pimpale Khalsa	18º44’55"	75º04'24"	Vegetative	37.50	4	Cl, Mo, Ss, Gi
		Pabal	18º49’35"	74º02’57"	Fruiting	31.25	4	Cl, Fd, Ss
		Rajuri	19º09’07"	74º08’24"	Fruiting	25.00	4	Cl, Mo, Rs
	Junner	Kumshet	19º12’45"	73º54’52"	Vegetative	20.83	4	Cl, Mo, Pc
		Otur	19º15’31"	73º59’19"	Fruiting	33.33	3	Cl, Fd, Ld
		Golegaon	19º12’46"	73º53’22"	Flowering	37.50	4	Cl, Mo, St
		Nimgaon sava	19º05’00"	74º08’07"	Fruiting	45.83	4	Cl, Mo, Fd,
						Average	35.13	
Sangli	Khanapur	Vita	17º15’54"	74º31’41 "	Fruiting	41.66	4	Cl, Mo, Fd
		Karve	17º11’59"	74º32’32"	Vegetative	37.50	4	Cl, Mo, Ss
		Ghanwad	17º19’28"	74º31’17"	Vegetative	33.33	4	Cl, Mo, Ld
		Bhambarde	17º18’03"	74º34’19"	Fruiting	29.16	4	Cl, St, Pc
		Kurli	17º14’07"	74º33’55"	Vegetative	10.41	3	Cl, Mo
	Miraj	Bhose	16º57’29"	74º44’42"	Flowering	20.83	4	Cl, Mo, Gi
		Soni	16º56’59"	74º41’13"	Vegetative	16.66	4	Cl, Mo, Ld
		Bedag	16º48’04"	74º44’33"	Vegetative	20.83	4	Cl, Mo
		Kalambi	16º53’58"	74º41’15"	Vegetative	37.50	4	Cl, Mo, Ld
		Budhgaon	16º54’50"	74º35’51"	Fruiting	27.08	3	Cl, Pc, Fd
	Walwa	Kasegaon	17º07’21"	74º11’05"	Vegetative	31.25	4	Cl, Mo
		Walwa	17º01’20"	74º22’31"	Fruiting	43.75	4	Cl, Gi, Rs, Fd
		Ahirwadi	17º01’01"	74º20’3 4"	Vegetative	37.50	4	Cl, Mo, Ss
		Shirgaon	17º01’48"	74º23’11"	Vegetative	18.75	4	Cl, Mo, Pc
		Nagthane	17º01’35"	74º24’24"	Fruiting	31.25	4	Cl, Mo, Fd
						Average	31.66	
Satara	Satara	Limb	17º47’31"	74º00’42"	Flowering	16.66	4	Cl, Mo
		Salvan	17º47’55"	73º59’59"	Vegetative	29.16	4	Cl, Mo
		Kidgaon	17º43’39"	73º57’06"	Fruiting	37.50	3	Cl, Mo, Ld
	Koregaon	Arphal	17º46’29"	74º03’27"	Flowering	29.16	4	Cl, Mo
		Kanher	17º43’48"	73º55’10"	Vegetative	33.33	4	Cl, Mo
		Rahimatpur	17º35’22"	74º11’29"	Vegetative	20.83	4	Cl
		Kumthe	17º43’18"	74º11’18"	Fruiting	25.00	4	Cl, Mo
		Pimpode Bk.	17º44'16"	74º10'59"	Vegetative	18.75	4	Mo,
		Lhasurne	17º43'54"	74º10'33"	Flowering	37.50	4	Cl, Mo
		Bhakarwadi	17º43'34"	74º09'35"	Vegetative	10.41	3	Cl

	Wai	Wai	17º57’54"	73º53’40"	Vegetative	33.33	4	Cl, Ld
		Bhopegaon	17º55’44"	73º57’37"	Fruiting	37.50	4	Cl, Fd
		Ozarde	17º55’14"	73º57’10"	Flowering	12.50	4	Cl, Ld
		Bavdhan	17º56’51"	73º53’35"	Vegetative	33.33	4	Cl, Ld
		Kavathe	17º56’46"	73º59’04"	Flowering	37.50	4	Cl, Mo
						Average	27.49	

Cl=Chlorosis
Mo=Mosaic
St=Stunted Growth
Gi=Green Iceland’s
Rs=Ring spots
Ss=Shoestring of leaves
Ld=Leaf distortion
Fd= Frut distortion
T-786=Tiwan-786

Variations in the sources of inoculums, vector population, current climate circumstances, and crop plant phase may further contribute to the disparities in disease incidence observed in surveyed locations [26]. A pathogenicity test conducted as part of the sampling survey for this study showed that PRSV incidence was mostly seen in all assessed areas. Furthermore, all 75 survey samples that were gathered from significant papaya-growing regions in Maharashtra, India, showed no signs of mixed virus infection in natural field circumstances, according to our early TEM studies.

Detection of PRSV by transmission electron microscopy [TEM]

The samples of PRSV infected papaya leaves brought from the glasshouse (artificially inoculated plant) and were observed under transmission electron microscope. The results revealed the presence of virus as flexuous rod-shaped particle in the sample and the size was found 760 nm to 800 nm long and 12 nm in diameter [Fig 3].

Worldwide, PRSV has been recognized as the most destructive viral pathogen on papaya [4]. Despite its importance, the national economies of many papaya-cultivated countries are threatened by the incidence of PRSD. The disease affects almost all stages of the crop and spreads very quickly to the whole orchard within three to seven months, which leads to yield losses of up to 100 percent [28, 27, 25]. Although PRSV occurs in different countries, higher levels of diversity were observed among Indian isolates compared to the rest of the world [8, 5, 19]. This might be due to a lack of resistant varieties, the fast evolution of the new strains of PRSV through recombination, and the occurrence of different aphid species [15, 10]. In the current study, the incidence of PRSD was observed to range from 55.55 to 27.49 per cent across different districts surveyed in Maharashtra State, India. The findings showed that the disease is common and has variable incidence rates throughout India. This could be because of the continual larger-scale cultivation of sensitive cultivars including "Red Lady," "Sunrise Solo," and "Arka Surya," as well as fluctuations in relative humidity and temperature, all of which may have contributed to the development of the viral infection in the papaya. Variations in the sources of inoculums, vector population, current climate circumstances, and crop plant phase may further contribute to the disparities in disease incidence observed in surveyed locations [26]. A pathogenicity test conducted as part of the sampling survey for this study showed that PRSV incidence was mostly seen in all assessed areas. Furthermore, all 75 survey samples that were gathered from significant papaya-growing regions in Maharashtra, India, showed no signs of mixed virus infection in natural field circumstances, according to our early TEM studies.

CONCLUSION

Our findings show that, regardless of the crop age, PRSD is common in nearly every part of the Maharashtra State, India, locations that cultivate papaya. The typical symptoms included light discoloration of leaves turning towards pale yellow, Mild mosaic, mosaic, mottling, chlorotic spots, chlorotic rings, vein clearing, leaf curling, stunting, blisters, leaf distortion, fruit distortion, green ice-land, shoe string formation in leaves, pale oily greasy streaks on stem and ringspots are prominently seen on leaves, fruits, stem and hence the virus named as ringspot virus. The identification and detection of PRSV was done by transmission electron microscopy [TEM]. The results revealed the presence of virus Flexuous rod- shaped particle in sample, including the presence of virus in infected samples.

Disclaimer [Artificial intelligence]

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

1. Alabi OJ, Rwahnih MAl, Brown JK, Idris AM, Gregg L, Kmieciak E, Setamou M, Jifon JL. First report of papaya (*Carica papaya*) naturally infected with the introduced tomato yellow leaf curl virus-Israel. *Plant Dis.* 2016.100, 1959.

2. Alcala-Briseno RI, Casarrubias-Castillo K, Lopez-Ley D, Garrett KA Silva-Rosales L. Network analysis of the papaya orchard virome from two agroecological regions of Chiapas, Mexico. *Msystems* 2020. 5, e00423-19.

3. Anonymous. FAOSTAT Data Base. Available online: http://nhb.gov.in/database (accessed on 1 September 2022).

4. Azad M, Kalam A, Amin L, Sidik NM. Gene Technology for papaya ringspot virus disease management. *Sci. World J.* 2014, 2014, 768038.

5. Babu KS, Banerjee A. Biological and molecular evidence of papaya ringspot virus pathotype P from mid-hills of Meghalaya, India. *Indian Phytopathol.* 2018. 71, 611–620.

6. Brandes J. Ziachrbl. Deut. PJlanzenschutzdienst. Braunschweig1957 pp. 9.

7. Carvalho FA, Renner SS. Molecular phylogenetics and evolution a dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history. *Mol. Phylogenet. Evol.* 2012. 65, 46–53.

8. Castillo XO, Fermin G, Tabima J, Rojas Y, Tennant PF, Fuchs M, Sierra R, Bernal AJ, Restrepo S. Phylogeography and molecular epidemiology of papaya ringspot virus. *Virus Res.,* 2011. 159, 132–140.

9. Evans EA, Ballen FH. An Overview of Global Papaya Production, Trade, and Consumption: FE913/FE913, 9/2012; EDIS 2012; University of Florida: Gainesville, FL, USA, 2012.

10. Hamim I, Rwahnih MAl, Borth WB, Suzuki JY, Melzer MJ, Wall MM, Green JC, Hu JS Papaya ringspot virus isolates from papaya in Bangladesh: Detection, characterization, and distribution. *Plant Dis*. 2019. 103, 2920–2924.

11. Huerta-Ocampo JA, Osuna-Castro JA, Lino-Lopez GJ, Barrera-Pacheco A, Mendoza-Hernandez G, De Leon-Rodriguez A, de la Rosa APB. Proteomic analysis of differentially accumulated proteins during ripening and in response to 1-MCP in papaya fruit. *J. Proteom*. 2012. 75, 2160–2169.

12. Hussain S, Varma A. Occurrence of papaya ringspot virus from Amritsar (Punjab) India. *J. Phytopathol* 1994*. Res*. 7, 77–78.

13. Jain RK. Nasiruddin KM, Sharma J, Pant RP, Varma A. First report of occurrence of papaya ringspot virus infecting papaya in Bangladesh. *Plant Dis*. 2004, 88, 221.

14. Kale SV. Studies on *papaya ring spot virus* (PRSV) isolate in Marathawada. M. Sc. Thesis, submitted to M.A.U. Parbhani, M.S. India1999.

15. Kalleshwaraswamy CM, Kumar NKK. Transmission Efficiency of papaya ringspot virus by three aphid species. *Phytopathology*. 2008. 98, 541–546.

16. Khurana SMP, Bhargava KS. Induced apocarpy and "double papaya fruit" formation in papaya with distortion ring spot infection. *P1. Dis. Reptr*. 1970. 54: 181-183.

17. Kunkalikar SR, Poojari S, Arun BM, Rajagopalan PA, Chen TC, Yeh SD, Naidu RA, Zehr UB, Ravi KS. Importance and genetic diversity of vegetable-infecting tospo-viruses in India. *Phytopathology*, 2011. 101, 367–376.

18. Lokhande NM, Moghe PG, Matte AD, Hiware BJ. Occurrence of papaya ringspot virus (PRSV) in Vidharbha regions of Maharashtra. *J. Soils Crop*. 1992, 2, 36–39.

19. Maina S, Barbetti MJ, Edwards OR, Minemba D, Areke MW, Jones RAC. Genetic connectivity between papaya ringspot virus genomes from Papua New Guinea and Northern Australia, and new recombination insights. *Plant Dis*. 2019.103, 737–747.

20. Mishra R, Patil S, Patil A, Patil BL. Sequence diversity studies of papaya ringspot virus isolate in South India reveal higher variability and recombination in the 50-terminal gene sequences. *Virus Dis*. 2019. 30, 261–268.

21. Mohamad Noor, Mohamad Roff. Disease Rating of Papaya Cultivars to *Papaya Ringspot Virus* in Malaysia. *Acta Horticulturae*. 2007: 277-281. 10.17660/*ActaHortic*.740.34.

22. Niklas KJ, Marler TE. *Carica papaya* (Caricaceae): A case study into the effects of domestication on plant vegetative growth and reproduction. *Am. J. Bot*. 2007, 94, 999–1002.

23. Premchand U, RK Mesta, Basavarajappa MP, Venkataravanappa V, Devappa V, Narasimha Reddy LRC, Shankarappa KS. Identification of novel begomoviruses associated with leaf curl disease of papaya (*Carica papaya* L.) in India. *Agronomy*. 2023: 13, 3.

24. Rao RP. Studies on *papaya ring spot virus* infecting *Carica sp.* Ph.D. Thesis, Submitted to Marathwada Agric, Univ., Parbhani, M.S., India 1988.

25. Sharma SK, Tripathi S. Papaya ringspot virus-P: Overcoming limitations of resistance breeding in Carica papaya L. In Plant Virus–Host Interaction; Academic Press: Cambridge, MA, USA, 2014; pp. 177–194.

26. Taylor LR. Distribution of virus disease and the migrant vector aphid. In Plant Virus Epidemics: Monitoring, Modelling and Predicting Outbreaks; McLean, G.D., Garrett, R.G., Ruesink, W.G., Eds.; Academic Press: Sydney, Australia, 1986; pp. 35–57.

27. Tripathi S, Suzuki JY, Ferreira SA, Gonsalves D. Papaya ringspot virus-P: Characteristics, pathogenicity, sequence variability and control. *Mol. Plant Pathol*. 2008: 9, 269–280.

28. Ventura JA, Costa H, Tatagiba da JS. Papaya diseases and integrated control. In Diseases of Fruits and Vegetables: Volume II; Naqvi, S.A.M.H., Ed.; Springer: Dordrecht, The Netherlands, pp. 2004: 201–268.

29. Wheeler BEJ. *An Introduction to Plant Diseases*. John Wiley and Sons. Ltd. London. pp. 1969: 301.

30. Marys E, Carballo O, Izaguirre-Mayoral ML. Occurrence and relative incidence of viruses infecting papaya in Venezuela. Ann Appl Biol. 2000, 136: 121–124.

31. Besong, Paul Ndip, and Tonjock Rosemary Kinge. 2021. “Fungi Diversity on Some Fruits and Biological Control Using Two Plants Extracts”. Journal of Advances in Biology & Biotechnology 24 (4):24-38. https://doi.org/10.9734/jabb/2021/v24i430209.

32. Rodrigues, Gerlan do Nascimento, Edna Peixoto da Rocha Amorim, José Gomes Filho, Valdeir Nunes Carvalho, Samuel Silva de Lima, Erisson Marques da Silva, José Alexandre da Silva Filho, Jorge Luiz Xavier de Lins Cunha, Lucas Alceu Rodrigues de Lima, and Alessandra da Silva Araújo. 2019. “Control of Peduncular Rot in Post-Harvest Mango Fruits With Hydrotherapy and Refrigeration”. Journal of Experimental Agriculture International 37 (4):1-7. https://doi.org/10.9734/jeai/2019/v37i430276.

33. Lius S, Manshardt RM, Fitch MM, Slightom JL, Sanford JC, Gonsalves D. Pathogen-derived resistance provides papaya with effective protection against papaya ringspot virus. Molecular Breeding. 1997 Jun;3:161-8.