



Unlocking Nature's Secret: Revealing the Culprit Behind Maharashtra Papaya Ringspot Disease

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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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.

1. 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 [1,2]. 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 [3]. 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 [4]. About 13.74 MT of papaya are produced worldwide, with 4.62 lakh hectares of land used for cultivation [5]. 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 [5,6-8]. Diseases and pests pose serious risks to papaya production everywhere. Papaya ringspot and leaf curl infections are the most common viral maladies [9,10]. The most destructive disease in the world that affects papaya production in practically every place where it is farmed is papaya ringspot disease (PRSD) [11], 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 [11,12]. 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 [13,14]. 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 [15,16].

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 [17,18,19]. 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.

2. MATERIALS AND METHODS

2.1 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 [20] based on leaf area covered by ring spot symptoms as mentioned in below Table 1. Scores of 0-4 were classified.

Further these scales were converted to per cent diseases index using formula given by Wheeler [21].

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

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

Table 1. detailed description of PRSV disease rating scale Mohamad and Mohamad [20]

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.

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.

2.2 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.

2.3 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 [22] 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. [23]. Pieces of leaf from plants that were not affected served as controls.

3. RESULTS AND DISCUSSION

3.1 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 [Figs. 1 and 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 [Figs. 1 and 2].

3.2 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 [24]. 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 [25,26,27]. Although PRSV occurs in different countries, higher levels of diversity were

observed among Indian isolates compared to the rest of the world [28,29,30]. 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 [31,32]. 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.

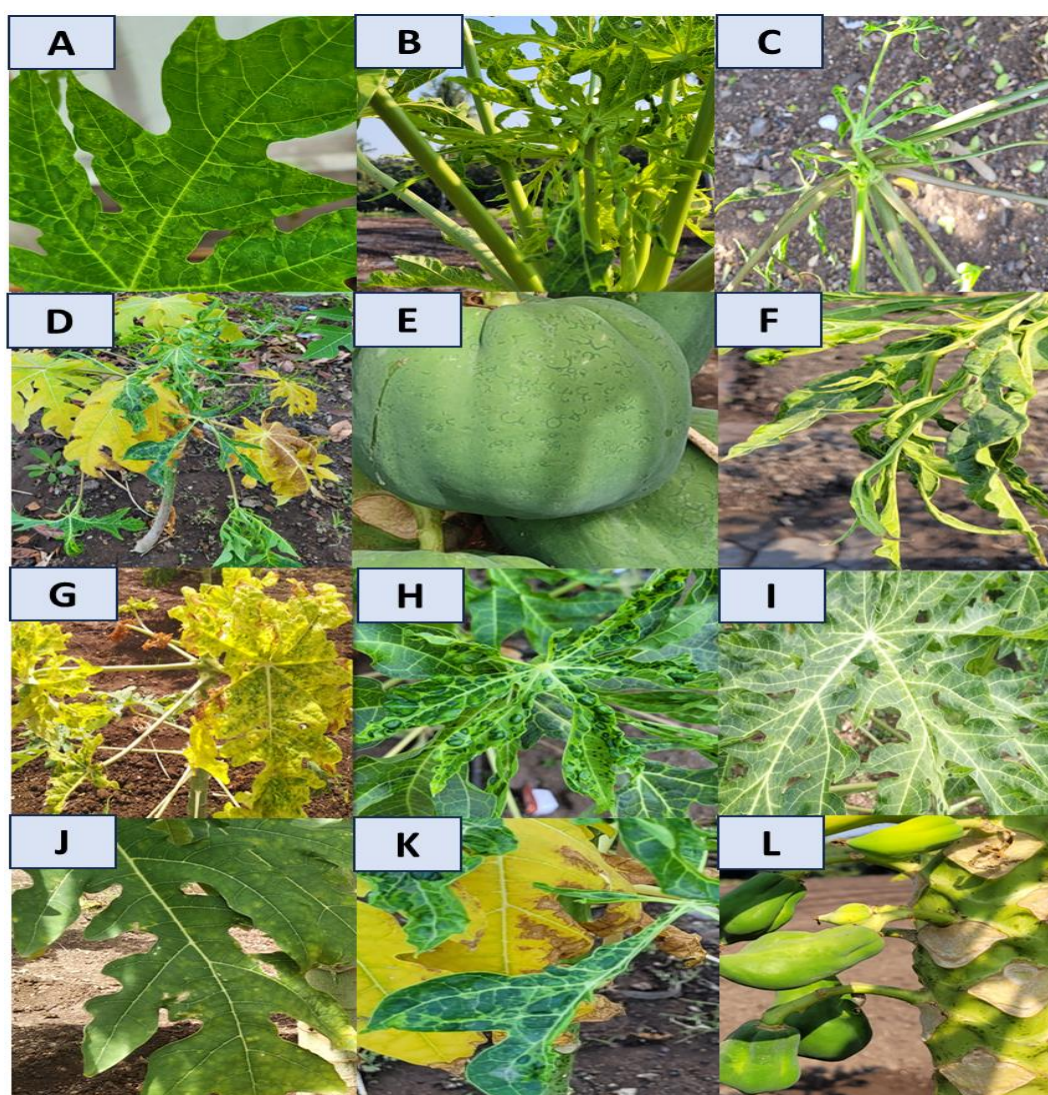


Fig. 1. Typical symptoms of PRSD on leaves and fruits of papaya plant A] Mild Mosaic B] Chlorosis C] Shoe string D] Stunting E] Ring spots F] Leaves Distortion G] Severe Mosaic H] Green Ice-land I] Vain clearing J] Local Lesion K] Leaf distortion L] Fruits distortion



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

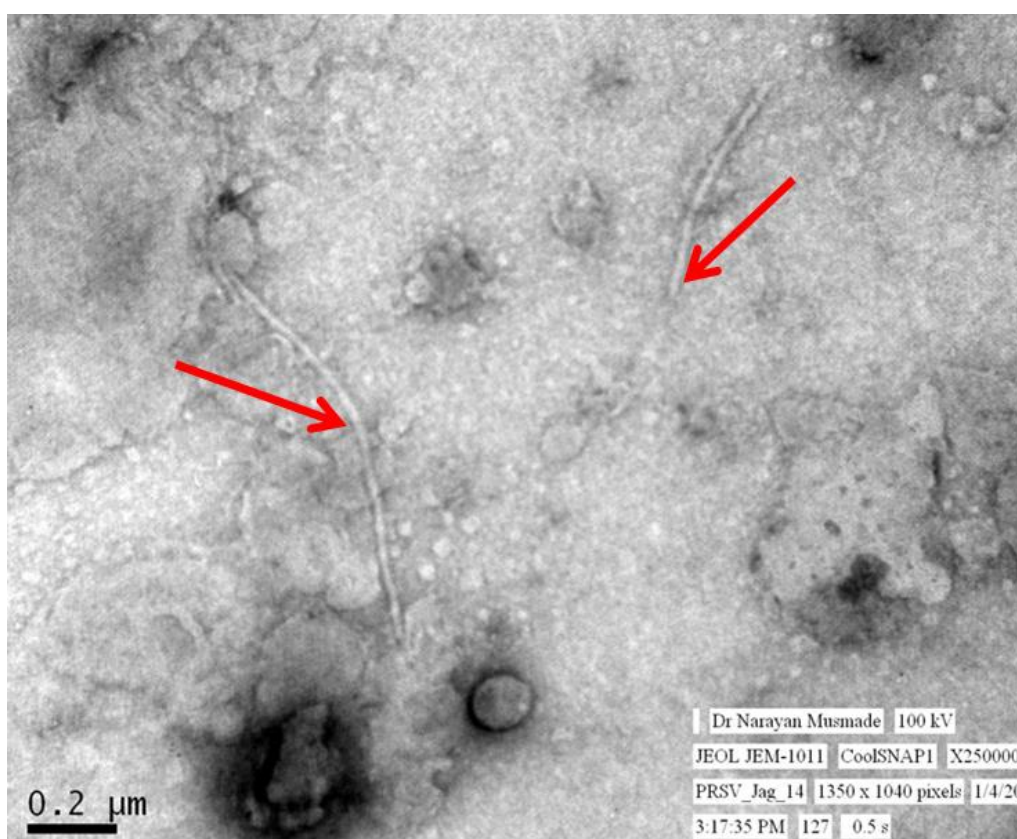


Fig. 3. Transmission Electron Microscope [TEM] photos of PRSV, both arrow shows the flexuous rod- shaped particles of PRSV

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 [33]. 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.

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	19°36'26"	74°47'56"	Vegetative	68.75	4	Cl, Pc, Ld
		Khandala	19°37'32"	74°36'01"	Flowering	43.75	4	Cl, Mo, Gi
		Haregaon	19°39'30"	74°05'55"	Vegetative	58.33	4	Cl, Mo, Pc
		Belapur	19°33'16"	74°42'28 "	Vegetative	68.75	3	Cl, Ss, Mo
		Nandur	19°30'02"	74°06'53"	Fruiting	87.50	4	Cl, Fd, Ss, Mo,Ld, Rs, Pc
	Sangamner	Vadgaon Landga	19°34'44"	74°06'25"	Vegetative	45.83	4	Cl, St, Mo,
		Nimaj	19°32'16"	74°08'44"	Vegetative	54.16	4	Cl, Mo, Gi
		Khandgaon	19°32'22"	74°11'20"	Fruiting	75.00	4	Cl, Mo, Ss, Fd,Ld, Rs, Pc
		Dhandarphal Kh. Chikhali	19°31'10" 19°33'06"	74°07'08" 74°08'47"	Fruiting Vegetative	41.66 50.00	4 4	Cl, Ss, Rs Cl, Mo, Ld
	Shrigonda	Kansewadi	18°31'44"	74°40'24"	Vegetative	45.83	4	Cl, Mo, St
		Chikhalthanwadi	18° 32'49"	74°40'54"	Fruiting	75.00	4	Cl, Mo, Fd, Ss
		Adhalgaon	18°38'42"	74°46'16"	Vegetative	29.16	3	Cl, Pc, Gi,
		Ghodegaon	18°35'59"	74°44'46"	Fruiting	43.75	4	Cl, Mo, Rs, Ss
		Belwandi Kothar	18°39'44"	74°42'49"	Fruiting	45.83	4	Cl, Rs, Fd
	Average							55.55
Solapur	Malshiras	Piliv	17°41'01"	74°59'51"	Fruiting	58.33	4	Cl, Fd, Pc
		Kusmod	17°41'03"	74°59'56"	Fruiting	68.75	4	Cl, Mo, Ld,Ss,
		Dahigaon	17°57'34"	74°44'03"	Vegetative	56.25	4	Cl, Mo, Ss
		Chandapuri	17°43'48"	74°57'36"	Fruiting	33.33	3	Cl, Mo, Rs
		Tandulwadi	18°07'04"	75°34'30"	Vegetative	41.66	4	Cl, Mo, Ld
	Pandharpur	Chale	18°32'56"	73°36'04"	Fruiting	58.33	4	Cl, Mo, Gi
		Wakhari	18°27'28"	74°20'35"	Fruiting	33.33	4	Cl, Fd, Rs
		Hole	17°46'44"	75°18'34"	Vegetative	27.08	4	Cl, Mo, St
		Gursale	17°43'56"	75°18'38"	Vegetative	37.05	4	Cl, Mo, Pc
		Shirdhon	17°42'39"	75°16'36"	Fruiting	41.66	3	Cl, Ld, Fd
	Madha	Tadavale	18°04'45"	75°28'29"	Fruiting	66.66	4	Cl, Mo, Ss, Rs
		Shindewadi	18°01'46"	75°28'40"	Fruiting	50.00	4	Cl, Mo, Ss

District	Taluka	Village	Longitude [N]	Latitude [E]	Crop stage	Incidence [%]	Severity score	Prominent symptoms
		Jadhavwadi	18°00'53"	75°29'37"	Vegetative	41.66	4	Cl, Mo, Pc
		Vetalwadi	18°03'44"	75°28'34"	Fruiting	54.16	3	Cl, Ld, M, Fd
		Chincholi	18°00'31"	75°27'08"	Fruiting	29.16	4	Cl, Mo, Fd
Average							46.49	
Pune	Indapur	Nhavi	18°09'59"	74°00'45"	Vegetative	37.50	4	Cl, Mo, Gi
		Kandalgaon	18°06'02"	75°06'34"	Fruiting	45.83	4	Cl, Mo, Rs, Ss
		Babhulgaon	18°03'35"	75°04'36"	Vegetative	33.33	4	Cl, St, Mo
		Rui	18°01'31"	75°03'24"	Fruiting	41.66	4	Cl, Pc, Rs
		Karewadi	18°09'02"	74°56'11"	Vegetative	37.50	4	Cl, Mo, Pc
	Shirur	Hivre	18°46'28"	74°07'33"	Vegetative	27.08	4	Cl, Mo, St
		Ranjangaon	18°44'55"	74°14'16"	Fruiting	31.25	4	Cl, Mo, Rs
		KanhoorMehasai	18°50'05"	74°08'28"	Fruiting	41.66	3	Cl, Mo, Fd
		Pimpale Khalsa	18°44'55"	75°04'24"	Vegetative	37.50	4	Cl, Mo, Ss, Gi
		Pabal	18°49'35"	74°02'57"	Fruiting	31.25	4	Cl, Fd, Ss
	Junner	Rajuri	19°09'07"	74°08'24"	Fruiting	25.00	4	Cl, Mo, Rs
		Kumshet	19°12'45"	73°54'52"	Vegetative	20.83	4	Cl, Mo, Pc
		Otur	19°15'31"	73°59'19"	Fruiting	33.33	3	Cl, Fd, Ld
		Golegaon	19°12'46"	73°53'22"	Flowering	37.50	4	Cl, Mo, St
		Nimgaon sava	19°05'00"	74°08'07"	Fruiting	45.83	4	Cl, Mo, Fd,
Average							35.13	
Sangli	Khanapur	Vita	17°15'54"	74°31'41 "	Fruiting	41.66	4	Cl, Mo, Fd
		Karve	17°11'59"	74°32'32"	Vegetative	37.50	4	Cl, Mo, Ss
		Ghanwad	17°19'28"	74°31'17"	Vegetative	33.33	4	Cl, Mo, Ld
		Bhambarde	17°18'03"	74°34'19"	Fruiting	29.16	4	Cl, St, Pc
		Kurli	17°14'07"	74°33'55"	Vegetative	10.41	3	Cl, Mo
	Miraj	Bhose	16°57'29"	74°44'42"	Flowering	20.83	4	Cl, Mo, Gi
		Soni	16°56'59"	74°41'13"	Vegetative	16.66	4	Cl, Mo, Ld
		Bedag	16°48'04"	74°44'33"	Vegetative	20.83	4	Cl, Mo
		Kalambi	16°53'58"	74°41'15"	Vegetative	37.50	4	Cl, Mo, Ld
		Budhgaon	16°54'50"	74°35'51"	Fruiting	27.08	3	Cl, Pc, Fd
	Walwa	Kasegaon	17°07'21"	74°11'05"	Vegetative	31.25	4	Cl, Mo
		Walwa	17°01'20"	74°22'31"	Fruiting	43.75	4	Cl, Gi, Rs, Fd
		Ahirwadi	17°01'01"	74°20'3 4"	Vegetative	37.50	4	Cl, Mo, Ss

District	Taluka	Village	Longitude [N]	Latitude [E]	Crop stage	Incidence [%]	Severity score	Prominent symptoms
		Shirgaon	17°01'48"	74°23'11"	Vegetative	18.75	4	Cl, Mo, Pc
		Nagthane	17°01'35"	74°24'24"	Fruiting	31.25	4	Cl, Mo, Fd
Average							31.66	
Satara	Satara	Limb	17°47'31"	74°00'42"	Flowering	16.66	4	Cl, Mo
		Salvan	17°47'55"	73°59'59"	Vegetative	29.16	4	Cl, Mo
		Kidgaon	17°43'39"	73°57'06"	Fruiting	37.50	3	Cl, Mo, Ld
		Arphal	17°46'29"	74°03'27"	Flowering	29.16	4	Cl, Mo
		Kanher	17°43'48"	73°55'10"	Vegetative	33.33	4	Cl, Mo
	Koregaon	Rahimatpur	17°35'22"	74°11'29"	Vegetative	20.83	4	Cl
		Kumthe	17°43'18"	74°11'18"	Fruiting	25.00	4	Cl, Mo
		Pimpode Bk.	17°44'16"	74°10'59"	Vegetative	18.75	4	Mo,
		Lhasurne	17°43'54"	74°10'33"	Flowering	37.50	4	Cl, Mo
		Bhakarwadi	17°43'34"	74°09'35"	Vegetative	10.41	3	Cl
	Wai	Wai	17°57'54"	73°53'40"	Vegetative	33.33	4	Cl, Ld
		Bhopegaon	17°55'44"	73°57'37"	Fruiting	37.50	4	Cl, Fd
		Ozarde	17°55'14"	73°57'10"	Flowering	12.50	4	Cl, Ld
		Bavdhan	17°56'51"	73°53'35"	Vegetative	33.33	4	Cl, Ld
		Kavathe	17°56'46"	73°59'04"	Flowering	37.50	4	Cl, Mo
Average							27.49	
Cl=Chlorosis		St=Stunted Growth		Rs=Ring spots		Ld=Leaf distortion		T-786=Tiwan-786
Mo=Mosaic		Gi=Green Iceland's		Ss=Shoestring of leaves		Fd= Frut distortion		

4. 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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