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Original Research Article

Physical Properties of Papaya Ring Spot Virus

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A B S T R A C T

Papaya, the poor man's fruit of the tropics encompasses most of the desirable qualities of a fruit. The carpaine, an alkaloid produced by papaya leaves has been utilized as a diuretic and a heart stimulant. Papaya belongs to the family *Caricaceae*. The papaya ring spot virus disease (PRSV) is a well-known aphid and sap transmissible plant pathogenic virus in the genus Potyvirus and family Potyviridae. Results on bio-physical properties such as thermal inactivation point revealed that, the virus was found active at a temperature up to 50°C, but it was inactivated at 55°C or more, which indicated that, the virus was inactivated between 50 and 55°C. The virus remained infective in sap extracted from diseased leaves of papaya at 1: 1000 dilutions but not at 1: 10000 dilutions, which indicated the dilution end point between 1: 1000 and 1: 10000. Virus was infectious upto 8 hrs of storage at room temperature and it was inactivated after 10 hrs of storage. The longevity of virus was recorded between 8 and 10 hrs at room temperature. Result on the electron microscopy of the purified preparations of PRSV revealed the presence of flexuous rod shaped particles at different magnifications. The size of the virus particles measured average length was 750 nm in and 12-13 nm in width.

Introduction

Papaya, the poor man's fruit of the tropics encompasses most of the desirable qualities of a fruit. The carpaine, an alkaloid produced by papaya leaves has been utilized as a diuretic and a heart stimulant (Singh *et al.*, 1983). Papaya belongs to the family *Caricaceae*. The papaya ring spot virus disease (PRSV) is a well-known aphid and sap transmissible plant pathogenic virus in the genus Potyvirus and family Potyviridae. Among viral diseases, papaya ring spot virus is a wide spread pathogen that can cause up to 90% yield losses in papaya. Papaya ring spot virus is transmissible by means of mechanical and several aphid species. The aphid species *Myzus persicae* was considered as the most efficient vector of papaya ring spot virus. The virus had narrow host range, infecting plant species belonging to Cucurbitaceae and Caricaceae family.

Materials and Methods

Seeds of papaya cultivar and different host like cucurbits and *Chenopodium* for experimental purpose were purchased from local market, Latur. The papaya ring spot disease samples were collected from the farmers' fields of various villages in Latur district, where papaya fields were found infected with PRSV. Experiments were conducted in the glasshouse of Department

of Plant Pathology, College of Agriculture, Latur during 2016 and electron microscopy carried out at Advanced Centre for Plant Pathology, Division of Plant Pathology, IARI, New Delhi.

Physical properties

The studies on physical properties *viz*., thermal inactivation point (TIP), dilution end point (DEP) and longevity *in-vitro* (LIV) were carried out as per Bose *et al.*,(1960), using papaya (Cv. Redlady) leaves infected with different strains / isolates of papaya ring spot virus source and *Chenopodium amaranticolor* as an assay host.

Thermal inactivation point

The thermal inactivation point of a virus in crude juice is "the temperature required for the complete inactivation of a virus in untreated crude juice during a 10 minutes exposure" to heat. The term is used to state one temperature as the inactivation temperature or to mention two temperatures in between at which the virus is inactivated completely. Symptomatic young leaves were collected from diseased plants. In the laboratory such leaves were washed properly and gently blotted dry with blotting paper. Fifty gram infected leaves were ground in a mortar and pressed through cheese cloth. Two ml of sap was transferred in 16 test tubes separately. The water bath was filled with water until the level was at least 3 cm above the level of the sap in the test tube. Water was heated to 30^0 , 35^0 , 40^0 , 45^0 , 50^0 , 55^0 , 60^0 , 65^0 , 70^0 , 75^0 , 80^0 , 85^0 , 90^0 , 95^0 , and 100^0 C temperature, respectively. One test tube was placed in the rack of water bath when water reaches at 30^0 C temperature (lowest). A thermometer was placed in water bath close to test tube at same level. The temperature in each case was maintained for 10 minutes. Test tube was removed from bath after 10 minutes and cooled in running water. After heating, the bath to the next temperature treated a second tube in the same manner. When all test tubes were treated at specified temperatures, the leaves of *Chenopodium amaranticolor* were inoculated with each sample separately, including one untreated control, kept at ambient temperature (20±°C). Regular observations were recorded for the appearance of symptoms in different treatments.

Dilution end point (DEP)

This experiment was conducted with a view to determine up to what extent, the sap could remain infective when subjected to serial solutions. The 34 dilution end point exist between two dilution i.e. between the higher dilution that was still infectious and the next higher was the non-infectious one. The test was performed by inoculating hypersensitive hosts with sap diluted repeatedly x 10. In case the local lesion host was not known, at least 5 plants, which reacted systemically, were inoculated with each sample. Symptomatic young leaves were collected from diseased plants. In the laboratory, such leaves were washed properly and gently blotted dry with blotting paper. Fifty gram leaves were ground in a mortar and extracts were collected by passing through cheese cloth. Dilutions were made in a series like undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, $10^{-6,}$ 10^{-7} and 10^{-8}. Eight test tubes were placed in a row in a test tube stand. Second of these test tubes were filled with 9 ml water with help of a pipette. One ml sap was transferred in the second test tube to make dilution 10^{-1}. Sap was mixed thoroughly with water in test tube and 1 ml of this dilution (10^{-1}) was transferred to the third test tube to be made the dilution (10^{-2}). This procedure was repeated till 10^{-7}. The leaves of

Chenopodium amaranticolor were inoculated with sap at different dilutions to test infectivity. There were five replicates for each dilution level. Symptoms were observed after 10-15 days and data were recorded for each treatment separately.

Longevity *in- vitro* (LIV)

In order to know the longevity of the papaya ring spot virus in crude sap, an experiment of ageing *in-vitro* was carried out. The standard leaf extract of the papaya were prepared in 1:1 ratio of tissues to buffer (neutral 0.05 phosphate buffer of pH-7.4 containing 0.02 M 2-mercaptoethanol), were kept at room temperature. It is usual to store the crude juice in closed tubes and to test a sample on test plants at a series of intervals. The inoculums was prepared as earlier and two ml sap was pipetted to each test tube and stored at room temperature for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hrs. After the specified duration of storage the samples were inoculated on the leaves of *Chenopodium amaranticolor*. 35 Regular observations were made for the appearance of symptoms and data were recorded from each plant separately.

Leaf-dip preparations

Leaf dip preparations of the virus were made by following the procedure of Brandes (1956). For leaf-dip preparations freshly harvested young leaves of papaya were observed under transmission electron microscope (JEM) to study the particle morphology and to determine most common particle size using the following procedure (Hichborn and Hills., 1965).

Grid preparation

Copper grids (400 mesh, 3mm diameter) (BIORAD, Microscience Division, US) were cleaned with Acetic Acid and loaded on a filter paper placed at the bottom of a clean Petri dish with sterile distilled water. A clean slide was then carbon coated in vaccum coating unit (BIORAD, E6440, Evaporation PSU) and gently brought in contact with the water in the Petri dish containing grids. The carbon filter was gently allowed to float off on the water surface and was watered on the grids, when the filter paper having them was lifted. Grids were either air dried in the dark at room temperature for 12-24 hrs or in an oven at 37^0 C for 30 minutes.

Mounting and staining

Diseased leaf bits were first chopped in a few drops of negative stain Uranyl acetate 2% (pH 4.2) and then carbon coated grids were allowed to float for 2 minutes. Excess stain was removed with filter paper and grids were air dried. Negatively stained grids were finally examined under electron microscope (EM).

Grid examination

Negatively stained grids were loaded on the grid holder, introduced into the EM and scanned. Electron micrographs were either taken on cut film or 35mm film. Morphology and size of virus particles were studied from negatives with good contrast. About 100 particles were carefully measured from their image drawn on a white paper.

Results and Discussion

Physical properties

The physical properties such as thermal inactivation point (TIP), Dilution end point (DEP) and longevity in vitro (LIV) of the virus isolate were determined according to the standard procedure as described in

Material and methods. The results thus obtained are produced herein as under.

Thermal inactivation point (TIP) of papaya ring spot virus isolate

The virus was found active up to temperature 50°C but was inactivated at 55°C or more, which indicated that the virus was inactivated between 50 and 55°C, as the sap treated at 55°C for ten minutes could not produce any lesions on *Chenopodium amaranticolor* plants (Fig. 1 and Table 1).

The number of local lesion produced on *Chenopodium amaranticolor* at temperature 30^0 , 35^0 , 40^0 ,45^0 and 50^0 C was 25.8, 22.5, 20.4, 16.2 and 4.4 respectively. From above result it is also observed that, the no. of local lesions decreased with increase in temperature. Similar result on thermal inactivation point was reported by Gude *et al.*, (2008).

Dilution end point of papaya ring spot virus isolate

Results on dilution end point of virus were presented in table 2 and figure 2. The result indicated that the virus remained infective in sap extracted from diseased leaves of papaya at 1: 1000 dilutions and lost its infectivity at 1: 10000 dilutions and more, which indicated the dilution end point between 1: 1000 and 1: 10000. The number of local lesions produced at crude sap was more (24.4 lesion). However, at 1:10, 1:100, 1:1000 dilution no. of local lesions were 13.2, 8.8 and 3.4 respectively. Results also indicated that the number of local lesions on assay host decreased progressively as the dilution was increased.

Table.1 Thermal inactivation point of papaya ring spot virus isolate

Sr. No.	Temperature(0° C)	No. of local lesions
1.	30	25.8
2.	35	22.5
3.	40	20.4
4.	45	16.2
5.	50	4.4
6.	55	No Lesions
7.	60	No Lesions
8.	65	No Lesions
9.	70	No Lesions
10.	75	No Lesions
11.	80	No Lesions
12.	85	No Lesions
13.	90	No Lesions
14.	95	No Lesions
15.	100	No Lesions

Table.2 Dilution end point of papaya ring spot virus isolate

Sr. No.	Dilution	No. of local lesion
1.	Crude sap	24.4
2.	1: 10	13.2
3.	1: 100	8.8
4.	1: 1000	3.4
5.	1: 10000	No lesions
6.	1: 100000	No lesions
7.	1: 1000000	No lesions
8.	1: 10000000	No lesions
9.	1: 100000000	No lesions
10.	1: 1000000000	No lesions

Table.3 Longevity *in vitro* of papaya ring spot virus isolate at room temperature (22-26°C)

Sr. No.	Period of storage (hours) Room temperature	No. of local lesions produced on assay host
1.	0	29
2.	2	24
3.	4	16.5
4.	6	13.5
5.	8	6.8
6.	10	No lesions
7.	12	No lesions
8.	14	No lesions
9.	16	No lesions
10.	18	No lesions
11.	20	No lesions
12.	22	No lesions
13.	24	No lesions

Fig.1 Thermal inactivation point of papaya ring spot virus isolate

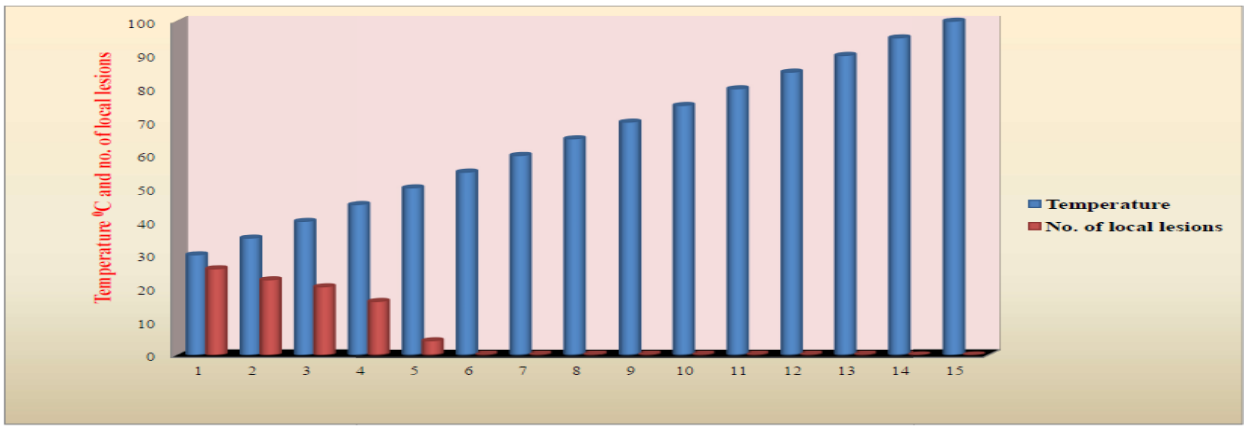


Fig.2 Dilution end point of papaya ring spot virus isolate at room temperature (22-26^0 C)

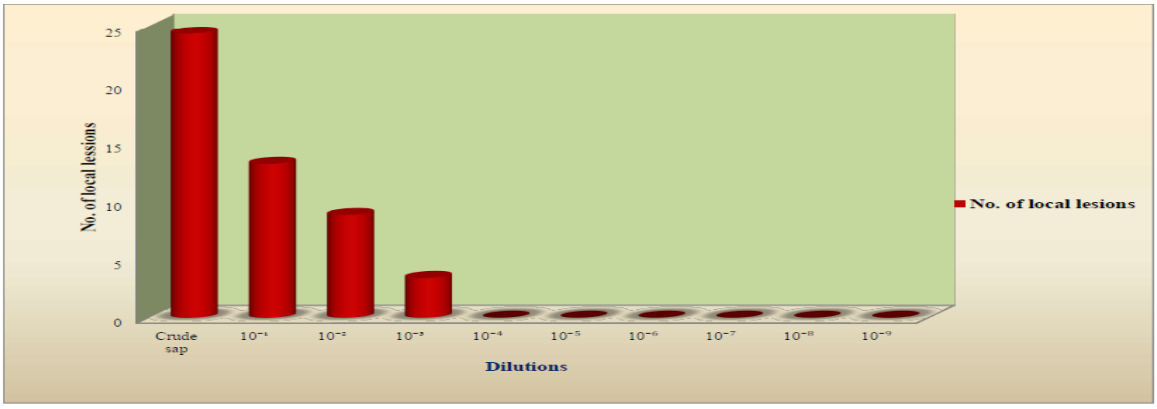


Fig.3 Longevity *in- vitro* of papaya ring spot virus isolate at room temperature (22- 26^{0}C)

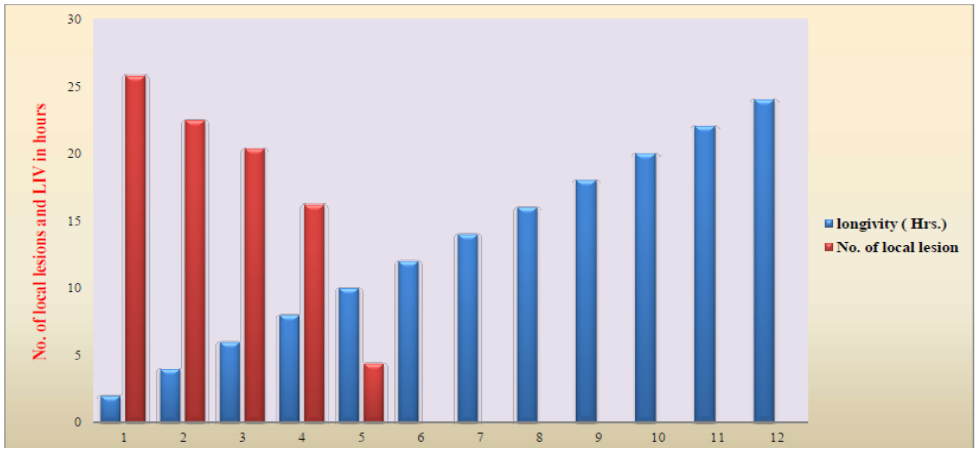
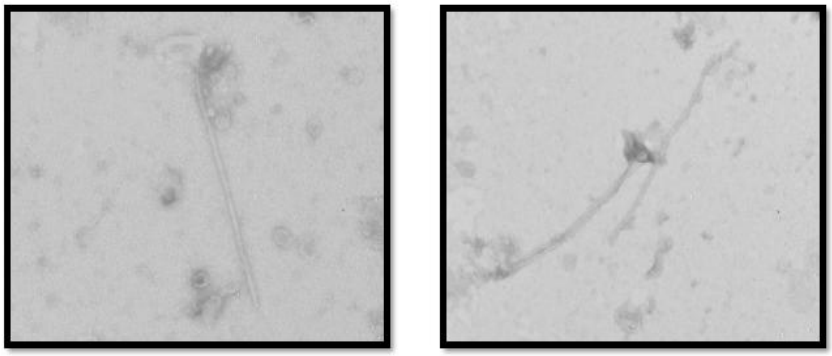


Plate.1 Electron micrograph of flexuous rods of PRSV measuring 750 nm long and 12 – 13 nm in diameter (X 200)



Longevity *in vitro* (LIV) of papaya ring spot virus isolate at room temperature (22-26^0 C)

Results on viability / infectivity of virus in crude sap at different storage hours (period) was observed and presented in table 3 and figure 3. Results on viability of virus in crude sap at different period (hrs.) at room temperature indicated that virus remain infectious up to 8 hrs at room temperature and it was inactivated at 10 hours and more of storage temperature. Number of local lesions was more (29 lesions on assay host) in fresh crude sap. The number of local lesions at 2, 4, 6 and 8 hours storage was 24, 16.5, 13.5 and 6.8, respectively.

Electron microscopy

The electron microscopy of the purified preparation of PRSV by using two per cent uranyl acetate revealed aggregated numerous flexuous rod shaped particles intertwined together which made it difficult to measure. However average length of 750 nm and 12-13 nm diameter was obtained among the measured particles. This partial size closely resembled the observations of 760-800 nm long x 12nm wide (Brunt *et al.,* 1996) and 760-800 nm x 12-13 nm as reported by Purcifull *et al.,* 1996 and Tripathi *et al.,* (2008) reported that virions are filamentous, non-enveloped and flexuous measuring 760–800 nm and 12 nm.

Results on biophysical properties such as thermal inactivation point revealed that, the virus was found active at a temperature up to 50°C, but it was inactivated at 55°C or more, which indicated that, the virus was inactivated between 50 and 55°C as the sap treated at 55°C for ten minutes could not produce any lesions on *Chenopodium amaranticolor* plants. The virus remained infective in sap extracted from diseased leaves of papaya at 1: 1000 dilutions but not at 1: 10000 dilutions, which indicated the dilution end point between 1: 1000 and 1: 10000. Virus was infectious up to 8 hrs of storage at room temperature and it was inactivated after 10 hrs of storage. The longevity of virus was recorded between 8 and 10 hrs at room temperature. In study, we have observed dilution end point in between 1 x 10-3 50 to 1 x 10-4, thermal inactivation point between 50-55°C and longevity *in vitro* up to 8 hrs, respectively. Result on the electron microscopy of the purified preparations of PRSV revealed the presence of flexuous rod shaped particles at different magnifications. The size of the virus particles measured average length was 750 nm in and 12-13 nm in width.

References

Bos, L., Hagedorn, D. J. and Quantz, L. 1960. Suggested procedures for international identification of legume viruses. Tijdschr. Plziekten.66:328-343.

Brandes, J., Uber das Aussehenund die Verteilung das Tabakmosaikvirus im Blattgewebe. 1956. Elecktro mikroskopische untersuchungen an ultradunnen Mikrotomscnitten, Phytopath. Z., 26: 93.

Gude, S. R., Nagaraju, P. N. S., Ravikumar, B. and Anjula, N. 2008. Occurrence of a strain of papaya ring spot virus on cucurbitaceous crops around Bangalore. Environ. Ecol. 26 (3): 1066-106.

Hitchborn, J. H. and Hills, G. J. 1965. The use of negative staining in the electron microscope examination of plant viruses in crude extracts. Virol. 27: 528-40

Purcifull, D. E., Simone, G. W., Baker, C. A. and Hiebert, E. 1996. Papaya ring spot virus. Descr. Pl. Viruses. 293: 7

Singh, U., Wadwani, A. M. and Johri, B. M. 1983. Dictionary of Economic Plants. ICAR

Tripathi, S., Suzuki, J. Y., Ferreira, S. A. and Gonsalves, D. 2008. Papaya ring spot virus characteristics, pathogencity, sequence variability and control. Molecular Pl. Path. 9 (3): 269-280.