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REGULAR ARTICLE

BIOPHYSICAL PROPERTIES OF THE WATERMELON MOSAIC VIRUS-1 CAUSING MOSAIC IN WATERMELON

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SUMMARY

Watermelon mosaic viruse-1 (WMV) is synonymous of papaya ring spot virus strain W. It is considered to be economically important filamentous virus of Indian sub-continent among the top ten viruses of watermelon .It causes severe mosaic mottling, blistering and malformation of leaves of watermelon. Watermelon mosaic virus-1 is a member of potyviridae. The virus contains ribonucleic acid (RNA) with filamentous particles measuring 760-800 x 12 nm size. The Thermal Inactivation Point of WMV-1 is 50-55^{0}C, Dilution End Point between 10^{-3} to 10^{-4} and longevity 24 hrs at room temperature only and remove the at 8 days at 10-12°C.Watermelon mosaic viruse-1 was transmitted through sap and insect vectors in a non persistent manner. Three species of aphids (*Aphis craccivora*, *Myzus persicae* and *Aphis gossypii*) have been found to transmit this virus. *Aphis gossypii* and *Myzus persicae* could transmit all the isolates of WMV-1 and *A. gassypii* was the most efficient vector. It was not transmitted through seeds and soil.

Key words: Watermelon (*Citrullus lanatus* L.), Watermelon mosaic virus-1 TIP, DEP and LIV

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1. Introduction

Watermelon mosaic virus-1 (WMV-1) is a major limiting factor of watermelon yield affecting its production very drastically. Sometime it is difficult to find even a single plant free from infection at the end of the growing season, and thus results in severe yield reduction (Varma and Giri, 1997). Watermelon mosaic viruse-1 is synonymous of papaya ring spot virus strain W (Purcifull *et al.*, 1984 b). It was first reported in India by Bharagava and Joshi (1960). It is considered to be economically important filamentous virus of Indian sub-continent among the top ten viruses of watermelon (Varma, 1988). It causes severe mosaic mottling, blistering and malformation in leaves of watermelon (Greber, 1978; Vani, 1987). Watermelon mosaic virus-1 is a member of potyviridae. The virus contains ribonucleic acid (RNA) with filamentous particles measuring 760-800 x 12 nm size (Purcifull and Hieber, 1979). The Thermal Inactivation Point of WMV-1 is 65-70^{0}C, Dilution End Point between 10^{-3} to 10^{-4} and longevity 24 hrs at room temperature and 8 days at 10-12^{0}C (Vani, 1987).

Watermelon mosaic viruse-1 was transmitted through sap and insect vectors in a non persistent manner. Nearly 24 species of aphids including *Aphis craccivora*, *Aulacorthum saloni, Macrosiphum euphorbiae* and *Myzus persicae* were reported as verctors (Karl and Schmelzer, 1971). *Aphis gossypii* and *Myzus persicae* could transmit all the isolates of WMV-1 and *A. gassypii* was the most efficient vector (Bhargava *et al.*, 1975). It was not transmitted through seeds and soil (Wu and Su, 1977). Verma and Giri (1997) reported that the incidence of watermelon mosaic virus-1 was 80% in Delhi and Uttar Pradesh states of India

2. Materials and Methods

Bio-physical properties

Bio-Physical properties such as thermal inactivation point (TIP), dilution end point (DEP) and longivity *in vitro* (LIV) were

studied to obtain an indication about the nature of the virus.

Thermal inactivation point (TIP)

Young leaves of watermelon plants showing severe disease symptoms were crushed in a sterilized pestle and mortar after this the pulp was strained through a double layered muslin cloth and clear filtrate was centrifuged at 3000 g for 15 minutes. The supernatant was distributed in thin walled test tubes by pouring 2 ml of sap in each tube with the help of a pipette. Care was taken that extract does not touch the sides of the tubes while pouring. The samples were heated at 30^0C, 35^0C 40^0C, 45^0C, 50^0C, 55^0C, 60^0C, 65^0C, 70^0C, 75^0C, 80^0C, 85^0C, and 90^0C in water bath. The water bath was so filled with water that the level was above the level of sap in the test tubes. The tubes were subjected to respective constant temperatures for exactly for 10 minutes. After exposure to desired temperature for 10 minutes ,the tubes were cooled immediately in running water and the test plant were inoculated with sap/inoculum. Plants inoculated with untreated sap served as control. All the plant were washed, labeled and kept for observation.

Dilution end point

The dilution end point exists between two dilutions i.e. between the higher dilution that was still infectious and the next higher the non infectious one. The test was performed by inoculating hypersensitive hosts with sap diluted repeatedly ten times. In case the local lesion host 5 plants, which reacted systemically, were inoculated with each sample.

Symptomatic young leaves were collected from diseased plants and placed In the laboratory, such leaves were washed properly and gently blotted dry with blotting paper ,then took Fifty gram leaves and were ground in mortar and extracts were collected by passing through chees cloth. Dilutions were made in a series like undiluated, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. Eight test tubes were placed in a row in a test tube stand after this Second of these test tubes were field with 9 ml water with. One ml sap which was transferred in the second test tube to make dilution 10^{-1} with the help of a pipette . Sap was mixed thoroughly with water in a test tube and 1 ml of this dilution (10^{-1}) was transferred to the third test tube to be make the dilution (10^{-2}). This procedure was repeated till 10^{-7}. The leaves of *Citrullus lanatus* were inoculated with sap at different dilutions to test infectivity fallowed by five replicates for each dilution level. The plants were washed, labeled and kept for observation for each treatment separately in the insect free glass house.

Longivity *in vitro* (LIV)

Longivity *in vitro* may be defined as "the time expressed in days, weeks, hours for which the virus in crude juice kept at room temperature remained infective. It is usual to store the crude juice in closed tubes and to test a sample on test plant at a series of intervals. LIV was conducted at room temperature (30^0C-35^0C). For this purpose standard extract of the virus prepared from WMV-1 infected leaves of watermelon were distributed in to two sets of tubes. The tubes were plugged with cotton and wrapped with aluminium foil to prevent the evaporation of extract. The sets of tubes were kept at room temperature (30^0C-35^0C) after this The test plants were inoculated with the standard extract kept at room temperature (35^0C) at the interval of 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs, 12 hrs, 14 hrs, 16 hrs, 18 hrs, 20 hrs, 22 hrs, 24 hrs, 36 hrs. The plants inoculated immediately after extraction (i.e. 0.0h.) served as control. After washing and labeling all the plants were kept in the insect proof glass-house for symptom development and observations.

3. Result

Biophysical property of Watermelon mosaic virus -1 causing mosaic in watermelon

Thermal inactivation point

The virus was found active at a temperature upto 50^0C but it was inactivated at 55^0C or more which indicated that the virus was inactivated between 50 - 55^0C as the sap heated at 55^0C for ten minutes could not produce any lesion on *Chenopodium amaranticolor* plant. (Table -1)

Dilution end point

The virus remained infectious in sap extracted from diseased leaves of watermelon at 10^{-3} dilution but not at 10^{-4} dilution, which indicated the dilution end point is between 10^{-3} - 10^{-4} (Table-2).

Longevity *in vitro*

Data presented in Table 3 indicated that virus was infectious upto 24 hrs of storage at room temperature but it was inactivated after 36 hr of storage. The longevity of virus was recorded between 24 - 36 hrs at room temperature.

4. Discussion

The present study showed that dilution end point of the causal virus was 10^{-3} to 10^{-4}, thermal inactivation point between 50 to 55°C longevity *in vitro* upto 24 hrs at room temperature. Similar results were reported by Webb and Scott (1965). Ghosh and Mukhopadhyay (1979), Vani (1987), Raychoudhuri (1973) reported Thermal inactivation point (TIP) of WMV-1 was 60°C. The dilution end point (DEP) was10^{-4} (Webb and Scott, 1965). Vani (1987) reported that TIP of WMV-1 was 65-70^{0}C and DEP lies between 10^{-3} and 10^{-4}. The virus lost its infectively after 24 hours at room temperature and after 8 days at 10-12^{0}C.

Table -1: Thermal inactivation point (WMV-1) of the causal virus

Temperature (^{0}C)	No. of plants inoculated	No. of plants showing symptoms
Room temperature (control)	10	10
30	10	10
35	10	9
40	10	8
45	10	7
50	10	5
55	10	0
60	10	0
65	10	0

Table -2: Dilution end point of (WMV-1)

Dilutions	Types of lesions	Number of lesions
Undiluted	Chlorotic and necrotic local lesions	10
10-1	Chlorotic and necrotic local lesions	8
10-2	Chlorotic local lesions	7
10-3	Chlorotic local lesions	5
10-4	No symptoms	0
10-5	No symptoms	0
10-6	No symptoms	0
10-7	No symptoms	0
10-8	No symptoms	0
10-9	No symptoms	0

Table -3: Longevity *in vitro* (WMV-1)

Period of storage	No. of plants inoculated	No. of plants showing symptoms
Control (0)	10	10
1 hr	10	10
2 hrs	10	10
4 hrs	10	9
6 hrs	10	8
8 hrs	10	9
10 hrs	10	8
12 hrs	10	9
14 hrs	10	9
16 hrs	10	8
18 hrs	10	9
20 hrs	10	8
22 hrs	10	9
24 hrs	10	8
36 hrs	10	0
2 days	10	0
3days	10	0
4 days	10	0
5 days	10	0
6 days	10	0
7 days	10	0

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