IDENTIFICATION OF A STRAIN OF CUCUMBER MOSAIC VIRUS ISOLATED FROM NATURALLY INFECTED PHILODENDRON SELLOUM

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ABSTRACT

A virus isolate was obtained from naturally infected philodendron plants grown under field conditions at Giza Governorate, A.R. E. According to symptomatology, host range differential hosts, physical properties, mode of transmission, serological reaction and electron microscopy, the isolated virus was identified as a strain of cucumber mosaic virus (CMV).

The isolated strain of CMV induced chlorotic spots and yellowing on the leaves acompanied with stunting of philodendron plants.

The virus has a wide host-range and out of the tested plants, only Chenopodium amaranticolor, C. album and C. quinoa showed local infection.

Thermal inactivation point of this strain was between 60-65°C, dilution-end point was found to be between 10^{-4}-10^{-5} and longivity in vitro was 48-60 hours.

The virus was easily transmitted by sap (90-100%), Myzus persicae (about 80%) in non-persistant manner as well as through cuttings (85%), corms (65%) and cormels (50%).

The infectious sap reacted positively with CMV antiserum, but not with the healthy one.

Electron micrographs showed numerous isometric particles, 30 nm in diameter.

INTRODUCTION

Cucmber mosaic virus was first isolated by Doolittle in 1920 After that several investigators reported CMV in cowpea (kuhn et al 1966 Volvas and Avgelis, 1972 and Ficher and Lockhart 1979). Ornamental plants suffered also from CMV This virus was first isolated from gladiolus plants by Wade 1948 (Berkeley 1953) and Rizkalla et al.(1978).

Infection of philidendron plants with CMV was first reported by Hearon in 1979.

The aim of this work is to isolate and identify viruses involved in these diseases of philodendron plants grown under field conditions at Giza Governorate, A. R. E.

MATERIALS AND METHODS

1- Preparation of inoculum and isolation of the virus:

The isolate used in this study was isolated from naturally infected philodendron plants growing under field conditions at Groppi and El Zuhria orchards and philodendron nurseries at Kaphr Hakem, El Giza Governorate. Diseased plants were stunted & there leaves showed chlorotic spots and yellowing.

The inoculum was prepared by grinding young infected leaves in sterilized mortar and pestle using 0.2 M phosphate buffer. The homogenate was pressed through cheesecloth and the obtained crude sap was used for mechanical inoculation. of 600 mesh carborundum-dusted leaves of Chenpodium qiunoa and C. amaranticolor (as diagnostic host plants) under an insect-proof greenhouse. Chlorotic local lesions were observed after about 10 days incubation period. Single local lesions were cut out and separately macerated with few drops of buffer on a glass slide and inoculated onto the diagnostic hosts. This procedure was repeated three times. Finally, extracts from the well developed local lesions were used to inoculate Nicotiana tabacum L. cv. White Burely plants. Plants showing mosaic symptoms were kept in the greenhouse as a source of virus inoculum throughout this study.

2- Host range:

To study the host range of this virus isolate, different plant species were rested. Ten seedlings, in two different trials, (2 weeks old) from each tested host plants were mechanically inoculated and observed 3-4 weeks under green house conditions. Back inoculations were made on Nicotiana glutinosa or C. amaranticolor in order to checck the absence of virus in symptomless plants.

3- Physical properties:

Thermal inactivation point, dilution end point and longivity in vitro of the present virus isolate were studied as described by Hill (1984). Chenopodium amaranticolor was used as local lesion host. For each determination, 9 plants, in three replicates (3 plants each) were inoculated.

4- Aphid transmission:

Aphid transmission tests were done with non-viruliferous apterous Myzus persicae. Sulz and Aphis craccivora Koch reared on turnip. Insect inoculation was carried out as recommended by Hill (1984) for the non-persistent viruses. In each trial, each N. glutinosa seedling received 10 insects from either Myzus persicae or Aphia craccivora. Control plants received the same number of non-viruliferous aphids which were fed on healthy plants. At the end of infection feeding, 0.15% Malathion (V/V) was sprayed to kill the aphids. Symptoms and percentage of transmission were recorded up to the end of the experiment. This study was repeated three times.

To study the transmission of the isolated virus by cuttings, corms and cormels, three hundred of each were obtained from philodendron plants previously infected by the virus isolate and planted in autoclaved soil in the green house.

5- Serological typing:

Serological typing was carried out using Immunodiffusion test described by Gooding and Bing (1970).

6- Electron micrographs:

For electron microscopy, purified virus suspension were prepared according to the modified method of Allam et al., (1984). Preparations were negatively stained with 2% phosphotungestic acid, pH 7.0 (Uyeda et al., 1975).

RESULTS AND DISCUSSION

1- Symptomatology and Host range:

Two weeks after mechanical inoculation, chlorotic spots and yellowing were appeared on the leaves of stunted philodendron plants (Fig. 1).

Results of the host range study of this virus isolate are presented in Table (1). Response of different hosts to virus infection might be summarized as follows:

(1) Test plants reacted with local lesions only:

Chenopodim amaranticolar, C. album and C. quinoa showed numerous reddish local lesions on inoculated leaves 3-5 days after inoculation (Fig.2). Newly developed leaves of C. quinoa were small, curved downward, and became necrotic.

Table (1) Host range of tested virus isolate.

Host plant	Symptoms
Solanaceae:	
Nicotiana glutinesa	M
N. rustica	M
N. tabacum cvs.	
White Burley	M
Xanthia	M
Samson	M
Solanum tuberosum cv. Alfpha	-
Capsicum annum cv. California Wander	M
Lycopersicon esculentum cv. Pearl Harbaur	M
Datura stramonium	L.L + SN
Petunia hybrida	M
Physalis floridana	M
Nicandra physaloides	M
Leguminosae:	
Phaseolus vulgaris cvs.	
Giza 3	M
Suisse Blan	-
Contender	M
Vigna sinensis cvs	
Azmerly	L.L. + SN
Fetriat	L.L. + SN
Black Eye	L.L. + SN

L.L. = Lecal lesion L.L.+SN = Local lesion+systemic necrosis
M = Mosaic - = No symptoms
Y.S = Yellow spots.

Table (1) Continoued

Host plant	Symptoms
Pisum sativum cv. Little Marvel	-
Vicia faba cvs	
Giza 1	-
Giza 2	-
Rebaya 40	-
Fam 401	-
Trifolium alexandrinum	-
Cucurbtaceae:	
Cucumis sativus cv. Balady	M
Cucurbita pepo cv. Eskandarani	M
Chenopodiaceae:	
Chenopodium album	L.L.
Ch. amaranticolor	L.L.
Ch. quinoa	L.L.
Compositae:	
Zinnia elegans	-
Amaranthaceae:	
Gomphrena globosa	-
Aradiaceae:	
Gladiolus grandiflorus var.	
Rose Suprem	M
Jackson Fill	M
Peter Pears	M
Araceae.	
Dieffenbachia maculata var Tropic Snow	YS
Philodendron selloum	YS

(2) Plants reacted with severe mosaic:

One week after inoculation, vein clearing was developed on the new leaves of N. glutinosa, N. rustica, N. tabacum cvs. White Burley, Xanthia and Samson, Capsicum annum cv. California Wander, Lycopersicon esculentum cv. Pearl Harbaur, Petunia hybrida, Physalis floridana, Nicandra physaloides, Phaseolus vulgaris cvs. Giza 3 and Contender ,Cucumis sativus cv. Balady, Cucurbita pepo cv. Eskandarani and Gladiolus grandiflorus cvs. Rose Supreme. Jackson Fill and Peter Pears. Ten days later, dark green mosaic was noticed on the young leaves (Figs. 3,4 and 5).

(3) Plants reacted with yellow spots:

Diffenbacia maculata var. Tropic Snow and Philodendron selloum showed chlorotic yellow spots on inoculated leaves. New leaves developed faint systemic mottling.

(4) Plants reacted with local lesion and systemic necrosis:

Datura stramonium and Vigna sinensis cvs Azmerly, Fetriat and Black Eye showed yellowish local lesions on inoculated leave, six days after inoculation. New leaves developed systemic spots or rings.

(5) No symptoms appeared on the following hosts:

Solanum tuberosum cv. Alpha, Phaseolus vulgaris cv. Suisse Blan, Pisum sativum cv. Little Marvel, Vicia faba cvs. Giza 1, Giza 2, Rebaya 40 and Fam 401, Trifolium alexandrinum, Zinnia elegans and Gomphrena globosa.

The isolated virus was found to have a wide host range including different species of Solanaceae, Leguminosae, Cucurbitaceae, Chenopodiaceae, Aradiaceae and Araceae. This came in agreement with the results given by Ruddy and Nariani (1963), Salama (1967) and Rizkalla et al. (1987) working with CMV on other hosts. Out of 36 host plant, only Chenopodium species reacted with local lesions. These results confirm the findings of Albouy (1966), Salama (1967), Tomaru and Udagowa (1970) and Rizkalla et al., (1987). These results were also found by Sabik (1973) working with CMV on gladiolus plants.

2- Physical Properties:

Thermal inactivation point, dilution end point and ageing in vitro were determined separately for the virus and the obtained results are recorded in Table (2)

Fig.1: Chlorotic spots on philodendron leaves

Fig.2: Local lesion on *Chenopodium amaranticoloor* leaves.

Fig.3: Severe mosaic on gladiolus leaves.

Fig.4: Severe mosaic on cucumber leaves.

Fig. (5): Severe mosaic on *N. glutinosa* leaves.

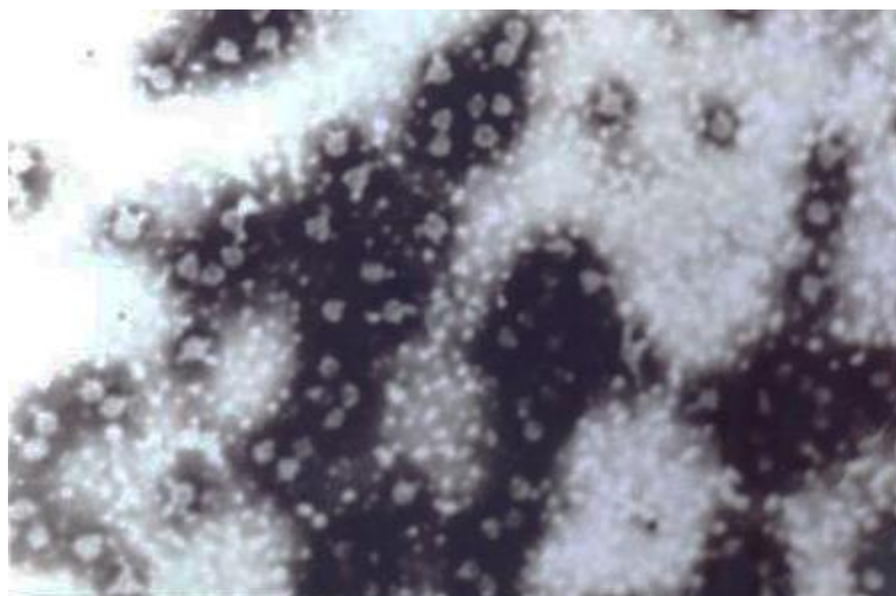

Fig. (6): Electron micrograph of negatively stained purified virus suspension showing isometric virus particles. Magnification 80,000 X.

Obtained results indicated that, this virus insolate had thermal inactivation point between 60-65°C, dilution end-point between 10^{-4} and 10^{-5} and longivity *in vitro* between 48-60 hours.

The isolated virus was found to be infectious at 60°C but not at 65°C. This agreed with the thermal inactivation point of CMV reported by Bridgmon and Walker (1952), Ruddy and Nariani (1963), Salama (1967) and Rizkalla *et al*. (1987). On the other hand, Sabik (1973), working with CMV on gladiolus plants, recorded 70°C as the temperature at which CMV was not infectious. The tested virus withstood dilution at 10^{-4} but not 10^{-5}. This verified the finding of Salama (1967), Sabik (1973) and Rizkalla *et al*. (1987). However the dilution of 10^{-6} was recorded by Bridgmon and Walker (1952). The isolated virus stayed infectious for 48 hrs but not for 60 hrs at room temperature. The longivity *in vitro* had been reported as 12-16 hs. by Ruddy and Nariani (1963), 12 hrs by Sabik (1973) and 6 days by Bridgmon and Walker (1952).

3- Mode of transmission:

The virus was easily transmissible by sap of *N. tabacum* cv. white Burely to other healthy plants. Aphid transmission of the present virus isolate from infected to healthy *N. tabacum* cv. White Burely seedlings was assayed using *Myzus persicae* as vector. The virus was easily transmitted through cuttings, corms and cormels. The percentage of transmission are tabulated in Table (3).

Data in Table (3) reveal that the highest percentage of virus transmission was by sap inoculation (90-100%), followed by cuttings (75-85%), insect transmission (65-80%) and cormes (45-65%). On the other hand, transmission by cormels showed the lowest percentage of virus transmission (30-50%).

Similar results on the transmission by sap and through the corms and cormels of philodendron plants were also stated by Berkeley (1951), Pinney and Hildebrandt (1968), Sabik (1973), and Rizkalla *et al*. (1987) working with CMV on other hosts.

The virus was easily transmitted by *M. persicae* Sulz. This was in agreement with data recorded by Bridgmon and Walker (1952), Swenson (1957), Sabik (1973), and Rizkalla *et al*. (1987).

Table (2): Physical properties of the isolated virus.

Physical Properties	*Average number of local lesion/leaf							
Thermal inactivation	40	45	50	55	60	65	70	^{o}C
Point	80	60	42	35	15	0.0	0.0	
Dilution-end	10^{-0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
Point	120	100	55	38	18	0.0	0.0	
Longivity	12	24	36	48	60	72		hours
	100	80	45	10	0.0	0.0		

* Average of 3 trials.

Table (3): Percentages of transmission of the isolated virus by different modes.

Mode of transmission	Percentage
Sap inoculation	90-100
Insect transmission	65-80
Cuttings	75-85
Corms	45-65
Cormels	30-50

4- Serological typing:

Results of the immunodiffusion test indicated that the infectious sap positively reacted with CMV antiserum but not with the healthy one. It is well known fact that serological studies are very useful and accurate method to detect and identify various plant viruses including CMV (Scott, 1963; Gerolo et al., 1965; Tremaine 1966; Sabik, 1973; Bouwen et al., 1978; Gamal El-Din et al., 1980 and Rizkallah et al., 1987).

5- Electron micrographs:

Electron micrographs showed numerous isometric particles ranged from 28-31 nm with an average of 30 nm in diameter. These results are in agreement with those reported by Scott (1963), Gerola et al.(1965), Boumen et al. (1978) and Gamal El-Din et al. (1980).

According to the previously mentioned characteristics this virus isolate was considered as a strain of cucumber mosaic virus (CMV).

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تعريف سلاله من فيروس موزايك الخيار
عزلت من نباتات فيلودندرون سيلويم مصابه طبيعيا

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تم الحصول على عزله فيروسيه من نباتات فيلودندرون مصابه طبيعيا ومنزرعه تحت ظروف الحقل في محافظة الجيزة ، جمهوريه مصر العربيه . وطبقا لدراسة مظهر الاصابه ، المدى العوائلي العوائل المشخصيه ، الخواص الطبيعيه ، طرق النقل ، التفاعل السيرولوجي وصور الميكروسكوب الالكتروني ، فقد تم تعريف العزله تحت الدراسه كسلاله من فيروس موزايك الخيار.

أظهرت نباتات الفيلودندرون المصابه بفيروس موزايك الخيار بقعا واصفرارا على الأوراق وتقـــزم النباتات :

تضمن المدى العوائلي للفيروس نباتات الخيار،الكوسه ، الفلفل ، الدخان ، الطماطم ، البيتونيا الفيزاليس : بعض أصناف الفاصوليا ، اللوبيا ، الداتورة ، الجلاديولس ، الفيلودندرون ، سيلويـــم وأظهرت تجارب اعادة الحقن على العوائل الحساسه أن النباتات التى لم تظهر عليها أعراض اصابـه كانت خاليه من الفيروس . وهناك أنواع قليله أخرى مثل الشينوبوديم امرانتيكولـــــــور ، الشينوبوديوم‌ألبم . الشينوبوديوم كينوا أظهرت اصابه موضعيه على شكل نقط محليه.

وجد أن درجة الحرارة الفاقده للتأثير الباثولوجي لفيروس موزايك الخيار كانت بين ٦٠ ، ٦٥ م° ، وأن نقطة التخفيف النهائيه كانت بين $١٠^{-٤}$ ، $١٠^{-٥}$ وأن مدة بقاء الفيروس نشـــطـا في العصير المعزى على درجة حرارة المعمل كانت بين ٤٨ ، ٦٠ ساعه .

أمكن نقل الفيروس عن طريق الحقن بالعصير حيث بلغت النسبه المئويه للانتقال ٩٠-١٠٠٪ كمـا وجد أيضا أن الفيروس يمكن نقله بحشرات من الخوخ الاخضر (أثبتت الدراسه أنه يتبع ا لفيروســـات الفيرياقيه) من نباتات الدخان المصابة الي السليمه حيث كانت النسبه المئويه للنقل ٨٠٪ وبلغت النسبه المئويه لانتقال الفيروس عن طريق الحقل و الكوزمـــــات والكريمات ٨٥ ، ٦٥ ، ٥٠٪ على التوالـــــى.

للتأكد من التعريف فقد تم عمل دراسات تأكيديه بواسطة الاختبارات السيرولوجيه والفحـــص بالميكروسكوب الالكتروني .

تفاعل العصير المعدي ايجابيا مع السيرم المضاد لفيروس موزايك الخيار ، بينما كان التفاعـــل سلبيا مع عصير النباتات السليمه .

أظهرت صور الميكروسكوب الالكتروني وجود جزئيات عديده كرويه قطرها ٣٠ نانومتر.