

PROPERTIES OF A HEAT RESISTANT STRAIN OF TOBACCO MOSAIC VIRUS ISOLATED FROM TOMATO IN EGYPT

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ABSTRACT

A severe disease of tomatoes *Lycopersicon esculentum* Mill. cv. Money marker was observed in a tomato field in the Faculty of Agriculture, Shibin El-kom, Menoufia, Egypt. The virus induced mosaic mottling and severe abnormalities on leaves of infected plants. The virus was sap-transmissible. Boiling the crude and purified virus for 10 min at 100 °C did not cause complete destruction of virus infectivity. In addition, it resisted heating at 100 °C for 31 min. Dilution endpoint was $1:2 \times 10^{-16}$; and longevity in vitro was more than 26 months at 25-32 °C. The virus withstood storage at 0 °C for more than 26 months and in air-dry infected leaves for more than 14 months. Hundred different plant species (spp.), varieties (vars.) and cultivars (cvs.) were inoculated mechanically with the virus. The virus induced mosaic mottling and severe abnormalities on 11 hosts. These are : *Lycopersicon esculentum* Mill. cvs. Ace, Early pack, E 6203, Money marker, Pritchard's, Super marmande, UC 97 - 3, V.F.N. 8, and *Lycopersicon pimpinellifolium*, *Nicotiana tabacum* cv. Samsun NN and *Solanum nigrum*. It induced necrotic local lesions on 19 hosts. These are : *Capsicum frutescens*, *Chenopodium murale*, *Datura innoxia*, *Datura metel*, *Datura stramonium*, *Gomphrena globosa*, *Nicotiana* spp. clevelandii, debneyi, glutinosa and rustica, and *Nicotiana tabacum* var.angustifolia and cvs. White Burley, white gold and xanthi nc, *Petunia*

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hybrida, Phaseolus vulgaris cv. Pinto, phytolacca americana, Solanum melongena cvs. Abyed and Eswed and Solanum tuberosum. Seventy plants did not react with the virus among which was Nicotiana glauca. The virus induced the formation of cytoplasmic inclusions in leaf hairs and epidermal cells of Money marker tomato leaves and in parenchyma cells of root, stem and leaf petiole. Its rod-shaped particles (300 x 18 nm) were typical of a tobamovirus and were serologically closely related to the Egyptian and dahlemense strains of tobacco mosaic virus.

Key words : tomato cv. Money marker; Datura stramonium; Datura metel; heat resistant strain of TMV.

INTRODUCTION

Tomato plants are often affected by viruses, either in the field or in the greenhouse. The list of viruses isolated from this species is fairly long and includes well - known and wide - spread viruses. Several have been recorded to produce mosaic mottling and different abnormalities (DUBEY *et al.*, 1981; ESKAROUS and HABIB 1970; ESKAROUS *et al.* 1982; HOLMES 1941; JENSEN 1933; KHATRI and SINGH 1977; LINNASALMI 1980; MAJ and BEDNAREK 1982; MAMULA *et al.* 1974; MAZYAD *et al.* 1969; RAMALLO *et al.* 1977; SMITH 1972).

Tomato plants cv. Money maker have been found to be naturally infected by a severe virus disease at Shibin El-kom, Menoufia, Egypt. The virus induced mosaic mottling and severe abnormalities on infected leaves. Preliminary studies showed that the virus isolate is most probably a strain of tobacco mosaic virus with amazingly high resistance to heat (loosing infectivity only after 32 min at 100

°C. Dilution endpoint is also high about $1 : 2 \times 100^{-16}$.

As tomato plants are of great economic importance to Egypt and this tomato virus isolate seems to be new, it was thought useful to study and identify this virus although research on strains of tobacco mosaic virus (TMV) is extensive.

MATERIALS AND METHODS

Studies on the virus were done with expressed sap from infected Money maker tomato leaves and included; host range, thermal inactivation point, dilution endpoint, resistance to ageing, serology and electron microscopy. Thermal inactivation point, dilution endpoint and electron microscopy were also done with purified preparations of virus obtained by alternative low (3000 rpm for 15 min) and high speed centrifugation (35,000 rpm for 1 h) carried out two or three times till the virus was sufficiently purified.

Host range and symptomatology

Leaves of healthy Money maker tomato plants dusted with 600 - mesh Carborundm were rubbed with virus - saturated cheesecloth pads. Comparative host range studies were carried out using 100 plant species, varieties and cultivars belonging to 20 families amongst which were : Solanaceae, Chenopodiaceae and Amaranthaceae.

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Physical properties of virus

Thermal inactivation point, dilution endpoint and resistance to ageing were performed. The effect of ageing was determined at freezing point (0 °C), at room temperature (25 - 32°C) and in air-dry infected leaves stored at room temperature. *Datura stramonium* and *Datura metel* as test plants were used in pots and detached leaves.

When inoculation was carried out in plants in pots, 3 plants each with at least 5 leaves of 50 - 60 day - old *D. stramonium* and *D. metel* were used. When inoculation was carried out on detached leaves, three 50 - 60 day - old *D. stramonium* and *D. metel* plants were selected so that each plant carried at least 5 suitable leaves. The leaves were detached and arranged in Petri dishes over a wet filter paper, following the Latin square design. Each treatment was inoculated into 3 healthy *D. Stramonium* and *D. metel* plants and into 15 detached leaves of both plants. The number of local lesions produced on the inoculated leaves was counted, compared to that on control plants and the percentage of virus infection was then calculated. Thus the results were confirmed by carrying out the experiments in two ways : inoculation on plants in pots and on detached leaves.

Serological tests

Serological studies were done using the Ouchterlony double diffusion test. The antisera to 4 tobacco mosaic virus strains were used. These are : Dahlemense, Holmes' rib grass, Vulgare and an

Egyptian strain of TMV isolated from *Nicotiana glauca* (ESKAROUS et al. 1982).

Electron microscopy

Electron microscope studies were done using a JEM 100 S electron microscope (Jeol, Japan) after negative contrasting of virus with 2 % potassium phospho - tungstate solution at a pH of 6.1.

RESULTS

Host range and symptomatology

Out of the 100 tested plant species, varieties and cultivars which were mechanically inoculated with the virus, 11 hosts showed mosaic mottling and different forms of abnormalities at both low and high temperatures. These are the 8 cvs. of tomato : Ace, Early pack, E 6302, Money maker, Pritchard's, Super marmande, UC 97 - 3, V.F.N. 8, *Lycopersicon pimpinellifolium*, *Nicotiana tabacum* cv.Samsun NN and *Solanum nigrum* var. *induratum*. Mosaic mottling was observed to be accompanied by other symptoms such as alteration of leaf symmetry and leaf apex, crinkling and rolling of the blade, blistering and reduction of the leaf lamina so that a number of filiform leaflets were produced. The midrib of some infected leaflets were either zigzagged or extended freely. A second leaf might have developed on the upper or lower surface of the original leaf (Fig. 1).

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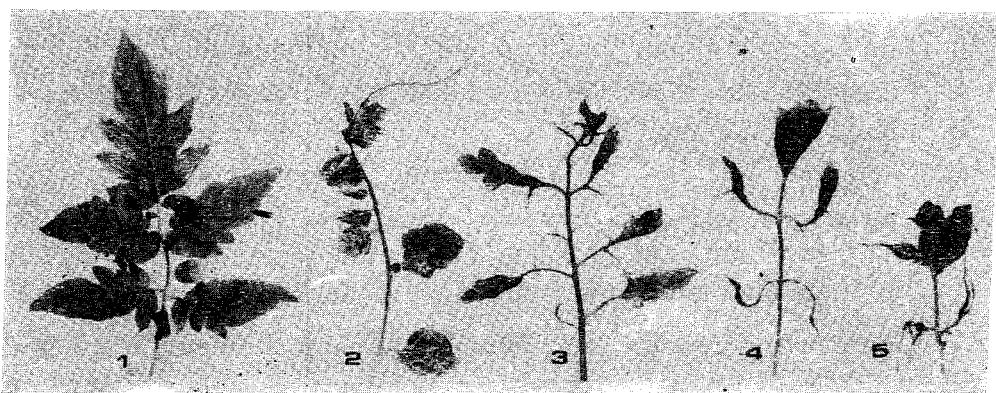


Fig. 1 : *Lycopersicon esculentum* cv. Money maker leaves infected with virus under test
 1 - healthy leaf
 2 - 5 leaves showing symptoms produced by virus

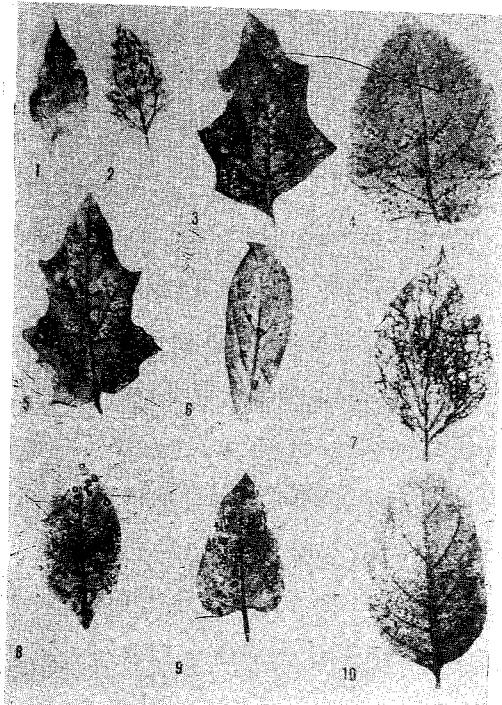
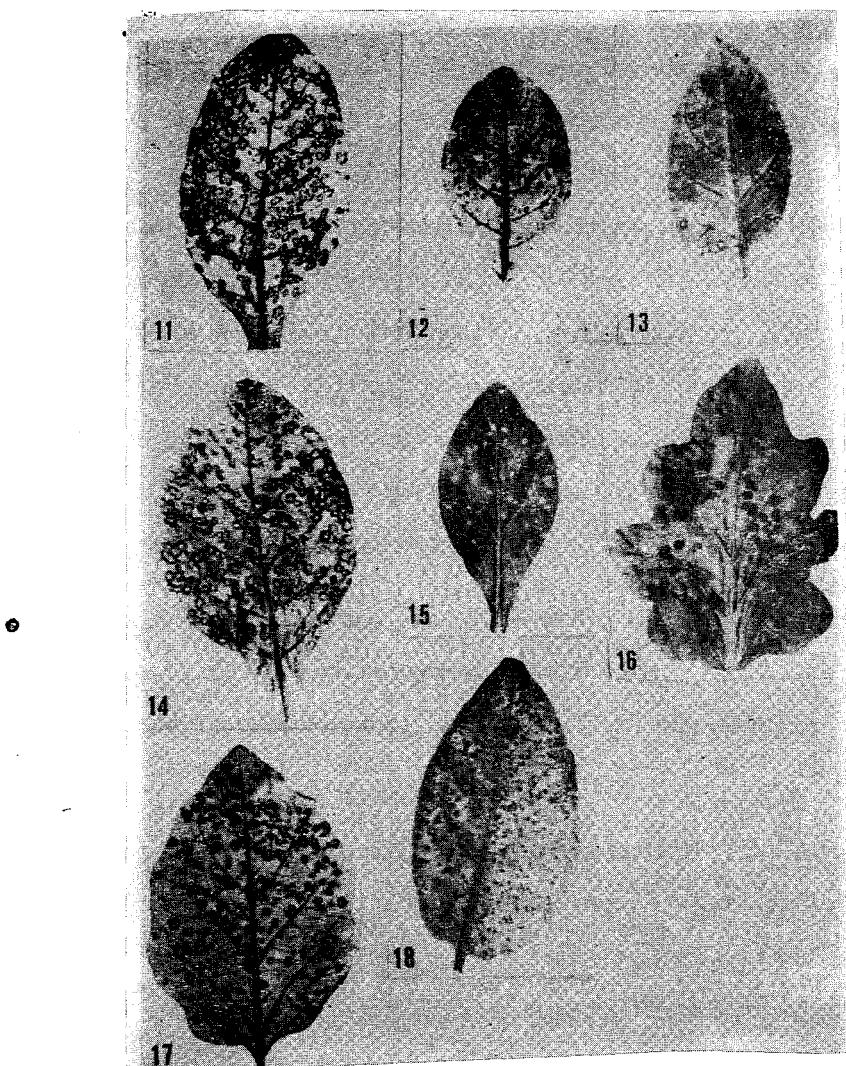


Fig. 2: Necrotic local lesions produced by virus on :
 1- *Capsicum frutescens* 6-*Gomphrena globosa*
 2- *Chenopodium murale* 7-*Nicotiana celevelandii*
 3- *Datura innoxia* 8-*Nicotiana debneyi*
 4- *Datura metel* 9-*Nicotiana glutinosa*
 5- *Datura stramonium* 10-*Nicotiana rustica*



- 11- *Nicotiana tabacum* cv. White Burley
12 -*Nicotiana tabacum* cv. White gold
13 -*Nicotiana tabacum* cv. Xanthi nc
14 -*Nicotiana tabacum* var. angustifolia
15 -*Petunia hybrida*
16 -*Solanum melongena* (with white elongated fruit, Abyed)
17 -*Solanum melongena* (with black round fruit, Eswed)
18 -*Solanum tuberosum*.

Hibiscus cannabinus, *Hibiscus esculentus*, *Hibiscus - rosa-sinensis*, *Hordeum vulgare*, *Ipomoea batatas*, *Lactuca sativa*, *Lathyrus odoratus*, *Lens esculentus*, *Luffa aegyptiaca*, *Lupinus angustifolius*, *Lupinus termis*, *Malva parviflora* cv. Cristate, *Matthiola incana*, *Medicago sativa*, *Mentha piperita*, *Nicotiana glauca*, *Ocimum basilicum*, *Pelargonium peltatum*, *Pelargonium zonale*, *Petroselinum sativum*, *Phaseolus vulgaris*, cv. Seminole, *Phlox drummondii*, *Pisum sativum*, *Plantago major*, *Portulaca oleracea*, *Raphanus sativus*, var. *aegyptiaca* (radish), *Ricinus communis* var. *aegyptiaca*, *Salvia splendens*, *Sisymbrium irro*, *Sonchus oleraceus*, *Spinacia oleracea*, *Trifolium alexandrinum*, *Trigonella foenum graecum*, *Triticum vulgare*, *Tropaeolum majus*, *Vicia faba* cv. Deltae (Broad bean), *Vigna sinensis* cv. Black (Cowpea), *Vinca rosea*, *Viola tricolor*, *Withania somnifera* and *Zea mays*.

On carrying out experiments to determine whether the plant saps contained only one virus, the following results were obtained :

- (a) Necrotic local lesions appeared when necrotic lesions cut out from *D. metel* leaves infected with plant sap, were used as fresh inocula to healthy *D. metel* plants.
- (b) No symptoms appeared on *D. metel* plants inoculated with tissue areas surrounding the necrotic lesions.
- (c) Mosaic symptoms appeared on the newly formed leaves of Money maker tomato plants inoculated with virus- infected sap

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heated at 90, 95 and 100 °C for 10 min and at 100°C for 31 min.

Physical properties of virus

Thermal inactivation point

When the stability of the infective particles in crude sap proved to be amazingly high, it was important to confirm these results using purified preparations. Almost the same results were reached. Boiling the curde and purified sap containing virus for 10 min at 100°C did not cause complete destruction of virus infectivity but only 99.4% in *D. stramonium* and 99.5% in *D. metel*. In addition, the virus resisted at 100°C for 31 min and was completely inactivated after 32 min. The above results were confirmed when two additional local lesion hosts *N.tabacum* cv.White Burley and *N.rustica* were used (Fig. 3). *Solanum nigrum* var. *induratum* was used as a mosaic host. The result was confirmed and clear mosaic mottling appeared after 15-18 days.

Dilution endpoint

The virus was completely inactivated in both *D. Stramonium* and *D. metel* when diluted to $1 : 2 \times 10^{-16}$.

Resistance to ageing

The longevity of virus *in vitro* was more than 26 months at room temperature (25 - 32°C). The virus withstood storage at 0°C for more than 26 months and in air - dry infected leaves for more than 14 months (Fig. 4).

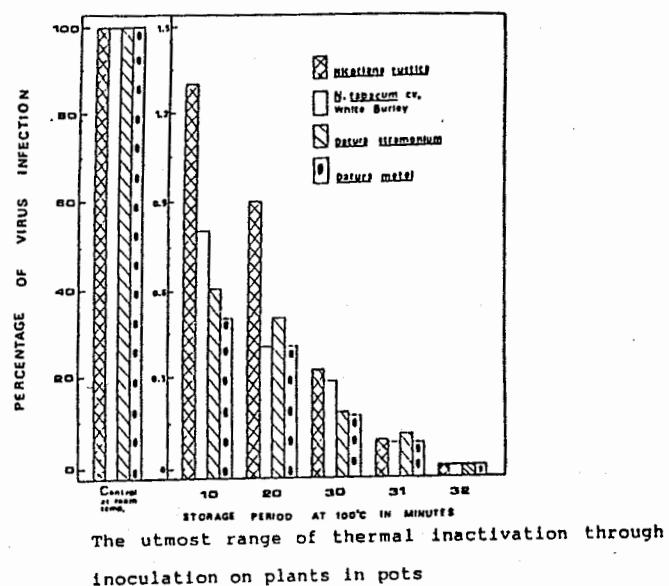


Fig. 3 : The utmost range of thermal inactivation through inoculation on plants in pots.

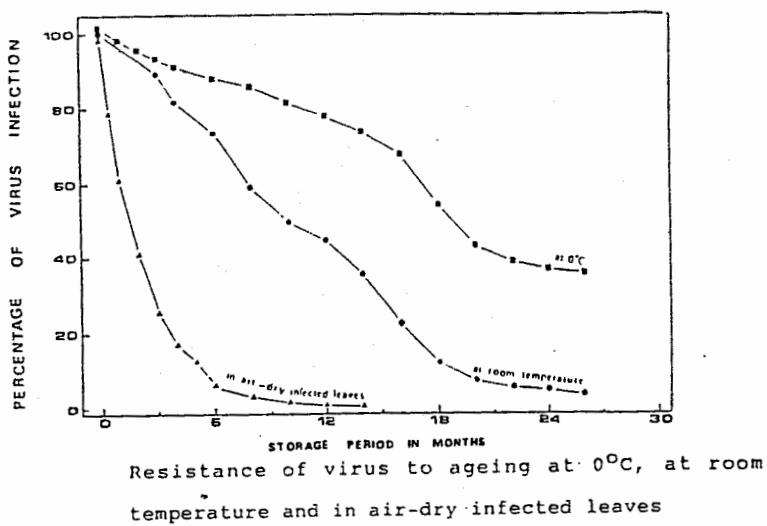


Fig. 4 : Resistance of virus to ageing at 0°C, at room temperature and in air - dry infected leaves.

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Electron microscopy

Examination of negatively stained preparations with 2% PTA of the virus diluted 1 : 500 revealed typical TMV - like rods (300 x 18 nm).

Serological tests

In Ouchterlony double - diffusion test, the Egyptian strain and dahlemense antisera showed reaction against the tested virus. Vulgare and Holme's rib grass antisera did not show any apparent reaction.

Inclusions associated with virus

The virus induced the formation of cytoplasmic inclusions in various sizes and shapes in leaf hair and epidermal cells of Money maker tomato leaf and in parenchyma cells of root, stem and leaf petiole (Fig. 5).

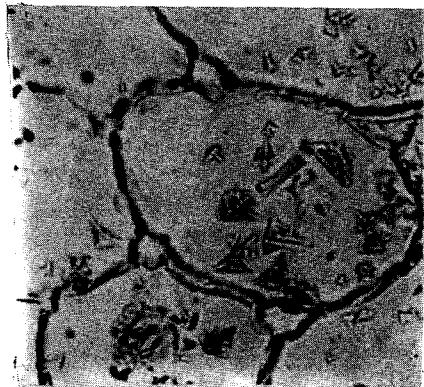


Fig. 5 Transection of leaf petiole infected with the virus under test showing crystals in various sizes and shapes in cells of parenchyma of cortex.

DISCUSSION

Tomato plants *Lycopersicon esculentum* Mill. cv. Money maker have been found to be naturally infected by a virus disease in a farm in Menoufia, Egypt. The virus induced mosaic mottling, blistering and distortion of the leaves.

Comparative studies between the virus under test and other viruses that were recorded to infect tomato plants and to produce mosaic mottling and different abnormalities viz. :

- 1- A new strain of TMV in Tucuman province.
- 2- A tobamovirus isolate from naturally infected *Nicotiana glauca* in Egypt.
- 3- Holmes' rib grass strain of TMV.
- 4- P. 5 and S. 1 strains of TMV in UAR.
- 5- S. 12 strain of TMV in the Punjab.
- 6- Tobacco mosaic virus (TMV) types from tomato in Finland.
- 7- Tomato mosaic virus in Hungary
- 8- Tomato streak virus.
- 9- Typical tobacco mosaic virus.
- 10- Wild tobacco mosaic virus.

revealed the following :

Aside from the big difference in the thermal inactivation point and dilution endpoint and although there are some properties in common, the virus under test has still its own unique characteristic

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host range and symptomatology.

The new strain of TMV recorded in tobacco plantations in Tucuman Province produces mosaic mottling on Burley tobacco plants (RAMALLO *et al.* 1977). On the contrary the virus under test gives necrotic local lesions on Burley tobacco plants.

Although the virus under test reacts with the antiserum of the Egyptian strain of TMV isolated from *Nicotiana glauca* in Egypt (ESKAROUS *et al.* 1982), *N. glauca* plants are immune to the virus under test.

Holmes' rib grass strain of TMV produces local lesions on inoculated tomato leaves. On *Plantago major*, the virus gives chlorotic streaks along the veins and systemic chlorotic mottling (HOLMES 1941). Contrary to this, the virus under test shows no symptoms on *Plantago major* and do not show any apparent reaction with Holmes' rib grass antiserum.

P. 5 and S. 1 strains of TMV isolated by MAZYAD *et al.* (1969) in Egypt produce mosaic mottling on *Lycopersicon esculentum* cvs. Ace, Money maker and Pritchard's. P. 5 strain produces local lesions on *Capsicum annuum* and does not react with *N. rustica*, *Solanum tuberosum*, *Capsicum frutescens* and *Gomphrena globosa*. S. 1 strain produces mosaic mottling on *N. tabacum* cv. White Burley, *Capsicum frutescens* and *Petunia hybrida*. It produces local lesions on *Capsicum annuum* and *Vicia faba* and does not react with *Solanum tuberosum*. On the contrary, the virus under test

produces necrotic local lesions on *N. rustica*, *Solanum tuberosum*, *Capsicum frutescens*, *Gomphrena globosa*, *N. tabacum* cv. White Burley and *Petunia hybrida* and does not react with *Capsicum annuum* and *Vicia faba*.

S. 12 strain of TMV on tomato plants in the Punjab produces mosaic mottling on *Capsicum annuum* and local lesions on *Chenopodium album* and *Phaseolus vulgaris* (KHATRI and SINGH 1977). Contrary to this, *Capsicum annuum* and *Chenopodium album* are immune to the virus under test.

Three types of TMV from tomato in Finland were characterized by the mild green mosaic, yellow mosaic and severe green mosaic. On *N. tabacum* cv. Samsun, the three types produce green mosaic, yellow mosaic and severe green mosaic, respectively followed by necrotic local lesions in the first type only. On *N. rustica*, the first and second types produce \pm necrotic local lesions while the third type produces severe green mosaic, \pm necrotic local lesions (LINNASALMI 1980). On the contrary, the virus under test produces faint mosaic mottling on samsun tobacco plants and very large number of necrotic local lesions followed by systemic necrosis on *N. rustica*.

Tomato mosaic virus isolated in Hungary produces brown necrotic lesions followed by mosaic mottling on *N. tabacum* L. cv. White Burley. On *Plantago major*, the virus produces local and systemic symptoms (MAMULA et al 1974). On the contrary, the

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virus under test produces only necrotic local lesions on Burley tobacco plants and *Plantago major* is immune to the virus.

Tomato streak virus isolated from tomato plants cv. Marmande in UAR produces local lesions on tobacco plants accompanied by a mottle. It produces local lesions on *Withania somnifera* and is systemic in *Hibiscus cannabinus* and *Spinacia oleracea*. Temperature greatly affects the symptoms produced by the tomato streak virus on the inoculated tomato plants. During summer (30 - 40°C), very clear mosaic mottling appear. During winter (16 - 25°C), the leaves show necrotic spots and patches which subsequently enlarge causing the leaves to shrivel, dry out and wither (ESKAROUS and HABIB 1970). Contrary to this, the virus under test induces only mosaic mottling on tobacco plants. On tomato, the virus produces mosaic mottling at both high and low temperatures. *Hibiscus cannabinus*, *Spinacia oleracea* and *Withania somnifera* are immune to the virus.

As a rule no local lesions are formed on *N. tabacum* leaves inoculated with typical TMV. However, when temperature and light intensity are high small circular, faintly chlorotic spots may appear on the rubbed leaves followed by mosaic mottling and distortion. On *Spinacia oleracea* the virus produces yellowing and mottling of the leaves (SMITH 1972). Contrary to this, the virus under test produces mosaic mottling on tobacco plants at both high and low temperatures. *Spinacia oleracea* is immune to the virus.

Although the virus under test reacts with the dahlemense

antisera, the dahlemense strain of TMV isolated from *Physalis franchetti* produces on tomato very clear necrotic spots followed by systemic necrosis. Newly formed leaves show mosaic mottling after 17-20 days (MAJ and BEDNAREK 1982). On the contrary the virus under test shows different systemic symptoms on tomato.

Finally, although some of the general characteristics of the virus isolated from naturally infected Money maker tomato plants appear to be related to TMV, its 100 °C temperature stability for 31 min makes it reasonable to conclude that it is a new heat resistant strain of TMV in Egypt.

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دراسة صفات سلالة مقاومة للحرارة لفيروس تبرقش الدخان ومعزولة

من نبات الطماطم في مصر

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لوحظ وجود مرض فيروسي شديد الضراوة يصيب الطماطم (مونى ماكر) في أحد حقول الطماطم التابعة لكلية الزراعة بشبين الكوم بالمنوفية بمصر ، محدثا برقشة وتشوهات شديدة لأوراق نبات الطماطم المصابة بالفيروس . ويدراسة صفات هذا الفيروس ، وجد أنه ينتقل ميكانيكيا عن طريق العصير ، وأن الفيروس الخام والنوى يقاوم درجة الغليان (100°C) لمدة نصف ساعه وأن نشاطة يتوقف عند تعرضه لهذه الدرجة لمدة ٣٢ دقيقة ، وأن النقطة النهاية لتخفيض الفيروس $1 : 2 \times 10^{-16}$. وقد ثبتت الدراسة كذلك أن الفيروس يحتفظ بقدرته على اصابة نبات الداتوره لفترة تزيد على ٢٦ شهرا عند حفظة في درجة حرارة الغرفة أو في درجة الصفر المئوي ولفتره تزيد على ١٤ شهرا في الأوراق المجففة عند درجة حرارة المعمل ، ويدراسة المدى العائلى للفيروس والاعراض التي يحدوها وجد أنه من بين مائة نبات حقن بالفيروس ظهرت الاعراض على شكل تبرقش وبعض التشوهات في الأوراق في احدى عشرة منها كما ظهرت بشرات موضعية على تسعه عشرة أخرى وأن من بين السبعون نباتا التي لم تتفاعل مع الفيروس كان نبات المصاص (النيكتوباتا جلاوكا) واحدا منها .

كما أنه وجدت محتويات خلوية ذات اشكال واحجام مختلفة في شعيرات وخلايا بشره ورقة نبات الطماطم المصابة ، وتظهر هذه المحتويات أيضا داخل الخلايا البارنشيمية في الجذر والساق وعنق الورقة .

وقد أوضحت دراسات المجهر الالكتروني أن جزيئات هذا الفيروس عصوية ذات ابعاد تطابق تلك الخاصة بفيروس تبرقش الدخان .