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DETECTION OF TOBACCO MOSAIC TOBAMO VIRUS (TMV) ON SAFFLOWER IN EL-MANSOURA – DAKAHLIA, EGYPT

El-Mazaty, M.A.
Plant Pathology Department, Faculty of Agric., Mansoura Univ., Egypt.

ABSTRACT

Tobacco mosaic virus (TMV) was isolated from safflower cv. Giza 1 plants showing mosaic symptoms in the field of El-Mansoura region, Dakahlia, Egypt. Identification of virus isolate was based on symptoms developed on diagnostic hosts, host range, physical properties, serological techniques, including precipitin, agglutination, ouchterlony double diffusion and Rocket immunoelectrophoresis assay by using the specific antiserum to TMV and electron microscopy.

Safflower TMV isolate induced systemic mosaic symptoms on *Nicotiana tabaccum* cv. Turkish and chlorotic local lesion without systemic spread on *Chenopodium amaranticolor* and *C. qunoa*.

Safflower TMV isolate was inactivated at 85°C - 90°C with stood dilution at 10^{-5} – 10^{-6}, longevity in vitro more than 4 months. Safflower TMV virus isolate was not transmitted by *Myzus persicae*, but transmitted mechanically. Transmission electron microscopy (TEM) indicate that, negative stained partially purified virus particles were rigid rod shaped with 300 – 310 nm in length and 18 nm in width. Protein subunit molecular weight was 18000. All serological tests with sap containing the virus gave strong reaction with TMV antiserum.

Keywords: Safflower; tobacco mosaic virus (TMV); identification, serology; electron microscopy.

INTRODUCTION

Safflower (*Carthamus tinctorius*) is becoming an important fatty oil and flowers crop in many countries (Klisiewicz, 1983 and Khalil & Abu Hazaza 1990).

The cultivated areas of this crop in Egypt still very limited however, safflower crop area has rabidly increased during the last few years throughout the new reclaimed land because of its resistance to both drying and salinity.

Certain plant viruses are known to infect safflower crop in most cultivated areas around the world including Egypt. Such viruses are: Tobacco mosaic virus (Lockhart and Goethals 1977); Cucumber mosaic virus (Nitzany, 1960 and Klisiewicz, 1962) and Turnip yellow mosaic virus (Klisiewicz, 1981 and 1983).

Therefore, this study was undertaken to isolate, identify and characterize the causal pathogen of safflower mosaic disease in Egypt.

MATERIALS AND METHODS

Safflower cv. Giza 1 plants grown in the farm of Faculty of Agriculture, El-Mansoura University, Egypt, were checked for the natural infection of virus diseases.

Samples showing mosaic symptoms were collected from diseased safflower plants in growing season of 2001 for identification purposes.

Indicator plants were lightly dusted with 600-mesh carborandum. Inoculum was prepared by grinding infected leaves in phosphate buffer (pH 7.0) with mortar and pestle. The virus isolated from safflower was maintained on *Nicotiana tabacum* cv. Turkish, which served as virus source, for subsequent inoculation (Noordam, 1973).

For host range studies, different host plants were grown in 20 cm plastic pots containing sandy – lome soil under insect proof greenhouse conditions. Ten to twelve plants were inoculated with sap extracted from infected virus source plants (Holmes, 1946 and Zaitlin & Israel, 1975).

In vitro physical properties of the obtained pathogen were determined. The thermal inactivation point, dilution end-point, and *in vitro* longevity of the isolated virus were studied, by using *N. tabacum* cv. Turkish, three weeks after inoculation as virus source plant and *N.glutinosa* as test plant (Noordam, 1973).

Insect transmission: *Myzus persicae* (Sulz.) was used in this studies using infected Turkish tobacco as source plants for the virus isolated from safflower and also as a test plant.

Aphids were starved for one hour, allowed half an hour acquisition feeding period, then transferred with a hair brush to test plants (5 aphids/test plant). After a feeding period of 24 hour, the aphids were killed by spraying with 0,15% malathione (Noordam, 1973)..

Microprecipitin, agglutination, ouchterlony double diffusion, and Rocket immunoelectrophoresis tests (RIET) were used to confirm the identification of the isolate virus. The immunoelectrophoresis test was conducted as described by (Ball, 1974), performed in 0.8 – 1.0% agarose containing 0.6% NcL and 0.02% NaN_3 + TMV specific antiserum (was kindly provided by Dr. Mathur – Danish Government Institute of Seed pathology fro Developing Countries, Thorvaldsensvej, Frederiksbergc., Denmark). Concentration was 1: 300 (v/v) with 0.05 phosphate buffer – pH 8.6. Boiled agarosa was layered on microscopic slides. After gel solidification, wells (5 mm in diameter) were punched into the agarosa layer.

Leaves of Turkish tobacco were packed up in small piece, 8 – 10 days after virus inoculation then its sap was pressed out. Exactly 20 µL of undiluted samples of crude sap was immediately dropped into the well. The same mentioned phosphate buffer was used for electrophosphesis running at 2MA for one slide then left overnight 12 – 16 h. in running. Slides were air dried and stained by commassie Brilliant blue R-250 (Merk.).

To determine the molecular weight of viral protein subunit *N. tabacum* cv. Turkish plants were inoculated with the identified tested virus. Infected leaves were harvested 2 weeks after inoculation and frozen at -20°C. The frozen tissue was ground in a warning blender with addition of sodium phosphate buffer 0.02 M pH 7.2 (200 ml/100 g. tissue). The juice was expressed through cheesecloth and then clarified by centrifugation for 20 min. at 13000 rpm (Noordam, 1973).

Virus from clarified sap was precipitated by adding half of its volume of saturated ammonium sulphate solution followed by incubation for 2 h. at room temperature. The precipitate was sedimented by centrifugation at 10000 rpm for 20 min. at 4°C. Prepared virus was Taken up in resuspention prosses, then dialyzed against several changes of distilled water at 4°C and centrifuged at 10000 rpm for 20 min. to remove the non virus material. This technique was repeated once more.

The pellet was dissolved in phosphate buffer pH 7.0 and again reprecipitated as described above (Allam *et al.*, 1988). Concerning the electronmicroscopy study according to (Siqurigrsson and Stanley 1947), partially purified virus preparations were stained with 2.0 phosphotungistic acid (PTA) then viewed in Jule Transmission Electronmicroscope.

The relative Molecular weight of viral protein subunits of isolated virus was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using gel-system (5% stacking gel and 12% separating gel). Purified virus-isolate was diluted in 0.05 M phosphate buffer, pH 7.5, to A 260 = 0.5 – 0.6 and stored frozen at –40°C. For electrophoretic analysis, the purified virus was mixed with an equal volume of treatment buffer (0.125 M Tris-Cl, pH 6.8, 40 g/L SDS, 20% (v/v) glycerol, and 10% (v/v) 2- mercaptoethanol) then degraded in boiling water for 1 min., the molecular weight markers were bovine serum albumin, ovalbumin carbonic anhydrase, soybean trypsin inhibitor and lysozyme (Bio-Rad Co. Richmond. CA. USA) gels were run at constant 30 mA for 4.5 h. and stained with coomassie blue R-250 according to the method of Huang *et al.* (1989).

RESULTS

Isolation and identification:

An isolate of tobacco mosaic virus (TMV) was isolated from naturally infected safflower cv. Giza1 plant showing mosaic symptoms.

The developed symptoms on the diagnostic host, host range, properties in infectious sap, modes of virus transmission, electronmicroscopy and serological reaction using precipitin test, double diffusion test and Rocket immunoelectrophoresis test were the identification means used in the present study.

Symptomatology:

The naturally infected safflower plants showed variable systemic mosaic symptoms ranged from mild to dark green mosaic as well as yellowing. There are also mottle, blisters and leaf malformations, which were associated with the natural symptoms. The reduction of the safflower growth of foliar and flowers were more pronounced on the infected safflower plants Photo (1).

Diagnostic plants:

The reactions of several plant species belonging to different families to the tested TMV-El-Mansoura isolate were illustrated in Table (1).

Sap and aphid transmission:

The tested virus was mechanically transmitted to *N. tabacum* L. cv. Turkish plants but not by aphid *M. persicae*.

Table (1): Plant reactions to cucumber mosaic virus (TMV) isolate on safflower plants from El-Mansoura, Egypt.

Host plant	Symptoms
- **Amaranthaceae**	
Gomphrena globosa L.	Necrotic local lesions
- **Asteraceae**	
Helianthus annus	No reaction
Lactuca sativa L.	No reaction
- **Chenopodiaceae**	
Chenopodium amaranticolor Cost & Reyn.	Necrotic local lesions
C. quinoa willd	Necrotic local lesions
- **Cucurbitaceae**	
Cucumis sativus L. Beita Alpha?	No reaction
Cucurbita pepo L. cv. Eskandrani?	No reaction
Citrullus lanatus cv. Giza 1	No reaction
Cucumis melo L. cv. ananas	No reaction
- **Solanaceae**	
Dature stramonium L. (Tprr)	Necrotic local lesions
Lycopersicon esculentum Mill cv. Strain B	Mosaic
Capsicum annuum L.	Mosaic –leaf distortion
Nicotiana glutinosa L.	Necrotic local lesions
N. rustica L.	Mosaic –leaf distortion
N. tabacum L. cv. Xanthi NC	Necrotic local lesions
N. tabacum L. cv. white Burley	Necrotic local lesions - wilting
N. tabacum L. Samsun	Mosaic
N. tabacum L. Samsun LL.	Necrotic local lesions
N. tabacum L. Turkish	Mosaic
Petunia hybrida Hort. Vilm.	Mosaic
Physalis floridane Rydberg	Mosaic
- **Compositae**	
Carthamus tinctorius *cv. Giza-1*	Mosaic
Zinnia elegans	Mosaic
- **Leguminosae**	
Vicia faba L. Giza 2	No reaction
Vigna sinensis L. cv. Browen eye	No reaction

Physical properties in sap:

Thermal inactivation point of the present TMV-isolate was 85 – 90°C and dilution end point 10^{-5} - 10^{-6}. However; longevity in vitro was more than 4 months at room temperature.

Virus protein:

TMV isolate had a single polypeptide band of molecular weight of 18000 (average of two separated electro SDS-poly-acrylimid gel electrophoresis runs).

Electron microscopy:

Negative stained TMV-virus particles of partially purified virus preparations revealed rigid Rod Shaped particles of 300 – 310 nm long and 18 nm wide (Photo 2).

Serology:

All used serological techniques (precipitin, agglutination, double diffusion and Rocket immune electrophoresis tests gave positive strong reaction with TMV-specific antiserum when tested with infected plant and no reaction against healthy extracts were noticed (Photo 3).

Photo 1. Safflower naturally infected with TMV El-Mansoura isolate

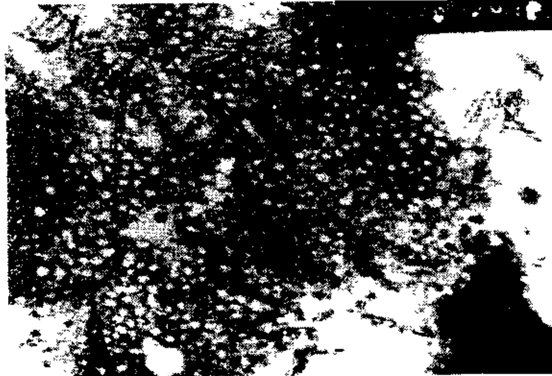

Fig (2)Electron micrograph of purified TMV safflower isolate after stained with 2% phosphotungastic acid (%30000)

DISCUSSION

The previous results indicate that an isolate of tobacco mosaic "tobamovirus (TMV) was isolated from El-Mansoura, Egypt from naturally infected safflower plants which were expressing mosaic disease symptoms. Such symptoms appeared on indicator plants were closely similar to those previously obtained by other investigators for TMV. The isolated virus induced systemic mosaic on *N. tabacum* cvs. Turkish and white burley, necrotic local lesions without systemic symptoms on *Datura stramonium*, *N. glutinosa* and *N. tabacum* cv. Xanthi-nc. and necrotic local lesions with systemic wilting and drying in *N. rustica* (Holmes, 1946 and Hollings & Hutlinga, 1976).

Data concerning physical properties in sap and transmission were very similar to those reported in for TMV by (Holmes, 1946).

However, the positive serological reactions with specific TMV antiserum in all serological procedures conformed the identification of TMV virus. Electron microscopy revealed that the isolated safflower virus isolate had a rigid rod shaped particles (320 X 18 nm). Such results are in agreement with those obtained by (Zaitlin and Israel, 1975). Obtained value of molecular weight of protein subunits (18000) of tested TMV-safflower isolate is similar to the value of type TMV member.

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تشخيص فيروس تبرقش الدخان (TMV) على نباتات القرطم والمنزرعة فى المنصورة – دقهلية – مصر ٠

محمد أحمد على المزاتى
قسم أمراض النبات – كلية الزراعة – جامعة المنصورة

١- تم عزل فيروس تبرقش الدخان TMV من نباتات القرطم صنف جيزه (١) والمصابة طبيعية فى حقول مدينة المنصورة بمحافظة الدقهلية بجمهورية مصر العربية، وتم تعريف عزلة الفيروس باستخدام كل من الأعراض التى تتكشف على العوائل الدالة والمدى العوائلى والخصائص الفيزيائية فى العصير المصاب والطرق السيرولوجية (اختبار الترسيب والتجميع والانتشار المزدوج لاشترلولى والاختبار المناعى الصاروخى خلال التيار الكهربائى باستخدام المصل المضاد للفيروس وكذلك المجهر الإليكترونى٠

٢- عزلة فيروس تبرقش الدخان TMV أعطت أعراض التبرقش على كل من نباتات الدخان نوع تاباكم *N. tabaccum* والنصف التركى٠ وبقع موضعية باهتة بدون أعراض جهازية على كل من نوعى الزربيح أمرانتكلر وكينوه٠

٣- عزلة فيروس تبرقش الدخان TMV فقدت قدرتها المرضية عند درجة حرارة ٨٥ – ٩٠ °م، وكانت درجة التخفيف النهائية للفيروس هى ١٠^-٥ – ١٠^-٦ ومدة التعمير أطول من ٤ شهور على درجة حرارة الغرفة٠

٤- لم تنتقل عزلة فيروس تبرقش الدخان TMV المعزولة من القرطم بحشرة من الخوخ ولكن إنتقلت بالعدوى الميكانيكية٠

٥- دراسات المجهر الإليكترونى النافذ أثبتت أن الجزيئات الفيروسية المصبوغة كانت عصوية طويلة صلدة بطول ٣٠٠ – ٣١٠ نانوميتر وعرض ١٨ نانوميتر٠

٦- كان الوزن الجزيئى لوحدة تحت البروتين الفيروسى المعزولة 18000٠

٧- كل الاختبارات السيرولوجية أعطت تفاعل موجب قوى للعصير المحتوى على الفيروس مع المصل المضاد للفيروس