Isolation and Identification of Potato Virus X From Potatoes at Ain-Zara, Libya

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ABSTRACT

A virus was isolated from naturally infected potatoes growing in a potato field at Ain-Zara, Libya. The young leaves showed mild mosaic, whereas the older ones developed general yellowing. The virus was mechanically transmitted to 19 out of 24 tested plant species belonging to six families. It has TIP of 70-75°C, DEP of 10^{-5} – 10^{-6} and LIV of 60 days. The virus was purified by differential and density gradient centrifugation. Its particles are slightly flexuous. The virus is immunogenic and has a serological relationship with potato virus X (PVX) but not with eight other potexviruses and two carlaviruses. It is concluded that the virus under study is PVX.

INTRODUCTION

Several fields of potato (*Solanum tuberosum* L.) plants at Ain-Zara around Tripoli were inspected during the winter of 1984. Various types of virus-like symptoms were observed, the young leaves showed different kinds of mottling and mosaic patterns, whereas the older ones developed general yellowing.

Potato plants were reported to be infected naturally with many viruses (4). Among those that are most serious and wide-spread is potato virus X (PVX) (2, 3, 5). Based on symptomotology, PVX was first observed in 1963 in a potato field in Tripoli area (7).

The present work was undertaken to identify and study the virus or viruses involved in naturally infected potato plants showing the above described symptoms.

MATERIALS AND METHODS

Source of Virus Isolate:

The original source of a potato virus isolate was obtained from a potato field at Ain-

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Zara, a suburb of Tripoli. Thereafter, mechanically inoculated tobacco (*Nicotiana glutionsa* L.) plants were used as a source of virus throughout the study.

Mechanical Transmissiom:

Inocula were prepared by grinding fresh young infected leaves exhibiting the characteristic symptoms of the disease in a 0.1 M phosphate buffer, pH 7.0 or distilled water with a mortar and pestle. Inoculations were made by rubbing carborundum-dusted leaves of test plants with the freshly extracted juice using the forefinger, and the inoculated leaves were rinsed with tap water 5 minutes after inoculation.

Host Range and Symptomatology:

Twenty four plant species and cultivars representing six families were used to check the host range of the virus. Three to six seedlings of each species were sap inoculated. Back inoculations were made 20-25 days after inoculation.

Properties in the Crude Sap:

Standard procedures as described by Ross (8) were followed. Fresh sap was extracted from young infected *N. glutinosa* leaves 15-21 days after inoculation by grinding them in distilled water with a mortar and pestle. *Gomphrena globosa* seedlings were inoculated for bioassay. Thermal inactivation point (TIP), dilution end-point (DEP), and longevity *in vitro* (LIV) experiments were performed.

Purification:

Six hundred grams of infected leaf tissues were homogenized in 0.1 M phosphate buffer, pH 7.0 in a Waring blender. The homogenate was strained through double layers of cheese cloth. Two clarification methods were followed:

a) Organic solvents: Ten ml of n-butanol were added to 100 ml of leaf homogenate under continuous stirring.
b) Heating: Leaf homogenate was heated at 50-55°C for 10 min. in a water bath.

In both methods, the homogenate was subjected to a low speed of 8,000 rpm for 10 minutes and to a high speed centrifugation of 27,000 rpm for two hours using Beckman L5-50 ultracentrifuge. Final pellets were resuspended in 2ml of phosphate buffer. The virus suspension was then recentrifuged at 8,000rpm for 10 minutes, and a part of the virus preparation was centrifuged in linear sucrose gradients (10-40%) at 20,000 rpm for 90 minutes. The virus bands formed were collected with a syringe and ultracentrifuged at 40,000 rpm for 90 minutes. The pellets were resuspended in 2 ml of buffer, and then subjected to low speed centrifugation at 8,000 rpm for 10 minutes.

The infectivity of the purified virus was tested in *G. globosa* seedlings.

Electron microscopy:

One drop of the virus preparation was placed on a filter paper (Whatman-phase separators silicon treated). An electron microscope grid (EM-grid) was placed on the virus drop for 2-3 minutes, washed with 15 drops of distilled water, stained by adding 15 drops of 2% uranyl acetate on the EM-grid, and excess stain was removed by touching the edge of the grid with a piece of filter paper. The preparation was examined under the electron microscope (Philips, 201C).

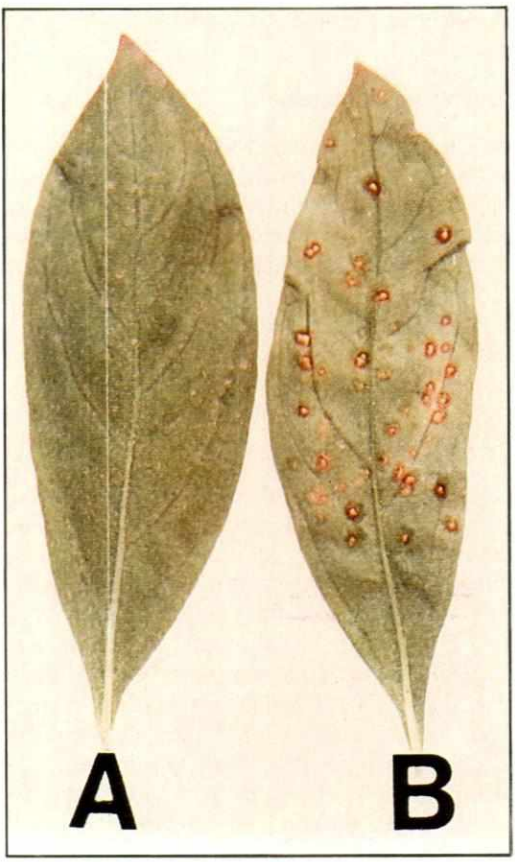

Fig. 1 Necrotic local lesions induced by the potato virus isolate in *Gomphrena globosa*. (A) healthy, (B) Diseased.

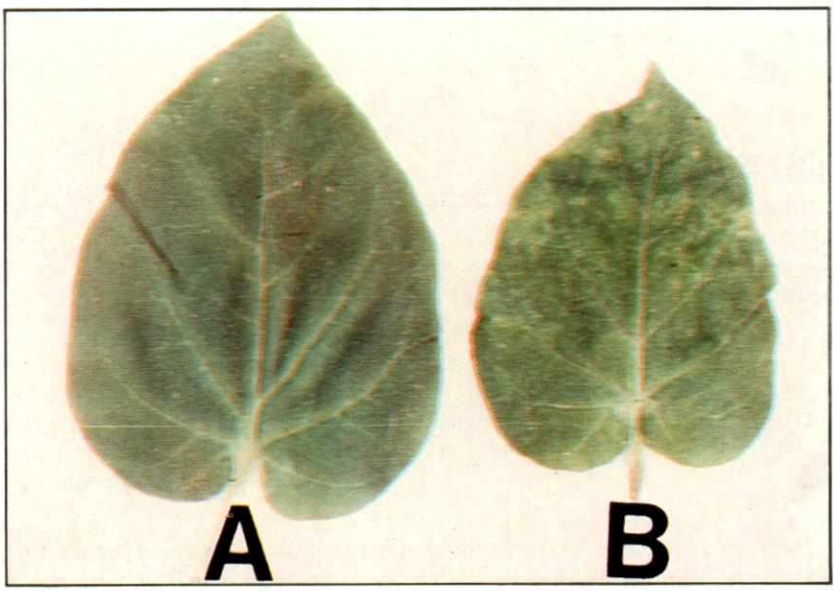

Fig. 2 Mosaic symptoms induced by the potato virus isolate in *Nicotiana glutinosa* leaves. (A) healthy, (B) diseased.

Serology:

a) Antiserum preparation:

One ml of a highly purified virus suspension was mixed with 1 ml of 0.85% saline solution (NaCl). This preparation was intravenously injected into the external marginal ear vein of a New Zealand white female rabbit using 5 ml syringe. This was followed by three intramuscular injections through the femur at weekly intervals with an emulsion of 1 ml of virus suspension and 1 ml of Freunds incomplete adjuvant oil. Blood was collected one week after the final injection. The blood was left at room temperature for one hour and then kept in a refrigerator for an overnight. The blood clot was removed, and the antiserum was separated from the remaining blood cells by low speed centrifugation at 3,000 rpm for 15 minutes.

b) Serological Tests:

Three types of serological tests were employed: the microprecipitin, agar double diffusion, and electron microscopic serology. The microprecipitin and agar double diffusion tests were made as described by Ball (1). In the electron microscopic serology the following steps were followed; one drop of the purified virus preparation was placed on a silicon-treated filter paper. Drops of the normal serum, homologous antiserum, and antisera of the following viruses were placed on another filter paper: Cactus X (CaVX), Clover yellow mosaic (CLYMV), Cymbidium mosaic (CybMV), narcissus mosaic (NaMV), Pepino mosaic (Pep MV), Potato sieg, Potato M (PVM) Potato S (PVS), Viola mottle (VioMV) and White clover mosaic (WCLMV). An EM-grid was placed on the virus drop for 2-3 minutes, washed with 15 drops of 0.1 M phosphate buffer, pH 7.2. The same grid was placed on a drop of the normal serum for 15 minutes, then removed and washed with 20 drops of distilled water. The EM-grid was placed on a drop of 2% uranyl acetate for 2-3 minutes. The same steps were repeated with each drop of the above mentioned antisera. The preparations were examined under the electron microscope.

RESULTS

Host Range and Symptomatology:

The potato virus isolate was easily mechanically transmissible. Out of 24 plant species or cultivars tested, 19 belonging to the families Amaranthaceae, Chenopodiaceae, Compositae and Solanaceae were found to be susceptible to the virus infection. Results of the host range and symptomatology are summarized in Table 1.

Properties in the Crude Sap:

The experiments showed that, the thermal inactivation point (TIP) of the virus was between 70 and 75°C, the dilution end point was $10^{-5} - 10^{-6}$ and the longevity *in vitro* at room temperature (23-25°C) was 60 days.

Purification:

Heat clarificatin followed by differential centrifugation was more successful in producing clean preparations containing slightly flexuous virus particles than clarification by n-butanol followed by differential centrifugation when examined with the electron microscope.

Table 1 — Host range and symptom studies using test plants mechanically inoculated with the potato virus isolate.

Test plants	Response of test plants*	Back inoculation
Capsicum annuum L.	NLL, 7-10 DAI	–
Chenopodium amaranticolor Coste & Reyn.	CLL, 5-7 DAI	+ve
C. quinoa Willd	CLL, 5-7 DAI	+ve
Citrullus lanatus (Thumb) Matsum. & Nakai	NVR	–
Cucumis sativus L.	NS	–ve
Cucurbita pepo L.	NVR	–
Datura metel L.	SM, 14 DAI	+ve
D. stramonium L.	SM, 7 DAI	+ve
Glycine max (L.) Merr.	SL	+ve
Gomphrena globosa L.	NLL, 4-5 DAI (Fig. 1)	+ve
Lycopersicon esculentum Mill	SMM, 7-10 DAI	+ve
Nicotiana debneyi Domin	NLL, SM, LD, 5-15 DAI	+ve
N. glutinosa L.	SM, 7-10 DAI (Fig. 2)	+ve
N. rustica L.	SL	+ve
N. sylvestris Speg & Comes	SM, 10 DAI	+ve
N. tabacum L. cvs. 'White Burley'	VC, SM, LNS, 10-14 DAI	+ve
& 'Xanthi-nc'	VC, LNS, RS, 10-14 DAI	+ve
Petunia hybrida Vilm	SL	+ve
Phaseolus vulgaris L.	NS	–ve
Physalis floridana Rybd.	Y. SM, ST, 10-14 DAI	–
Solanum melongena L.	SM, 10 DAI	–
Vicia faba L.	NS	–ve
Vigna sinensis (Torner) Savi	SL	+ve
Zinnia elegans Jacq	SL	+ve

*CLL = Chlorotic local lesions, DAI = Days after inoculation,
LD = Leaf deformation, LNS = Local necrotic spots, NLL = Nectoric local lesions, NS = Not susceptible, NVR = No visible reaction, RS = Ring spots, SL = Symtomless, SM = Systemic mosaic, SMM = Systemic mild mosaic, ST = Stunting, VC = Vein clearing, and Y = Yellowing.
+ve = Positive response, –ve = Negative response, – = Not tested.

Electron Microscopy:

Electron micrographs of the virus show slightly flexuous broken virus particles which were difficult to measure (Fig. 3).

Serological Tests:

Microprecipitin, agar double diffusion and the electron microscopic serology tests (Fig. 4), show that the potato virus isolate is immunogenic and is serologically related to PVX. No serological reactions were observed with the antisera of the following viruses using the electron microscopic serology: CaVX, CLYMV, CybMV, NaMV, PepMV, Potato sieg, PVM, PVS, VioMV and WCLMV.

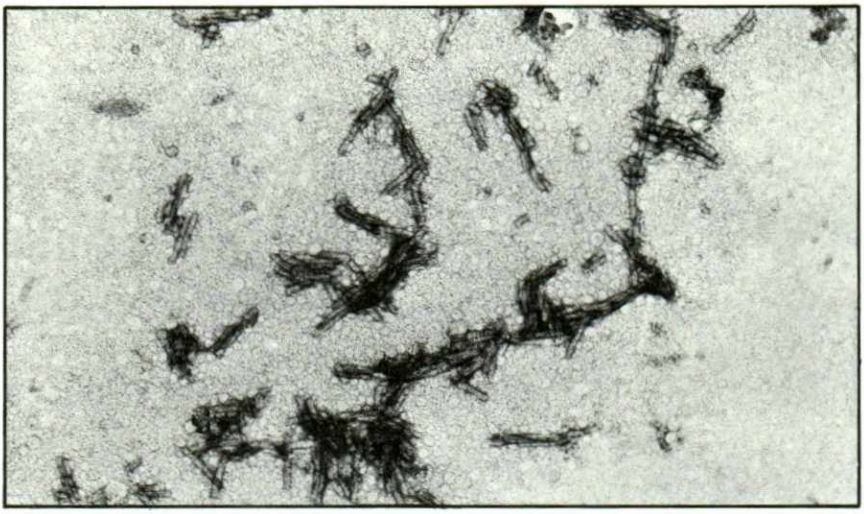

Fig. 3 An electron micrograph of the potato virus isolate negatively stained with 2% uranyl acetate showing slightly flexuous broken particles (X 70,000).

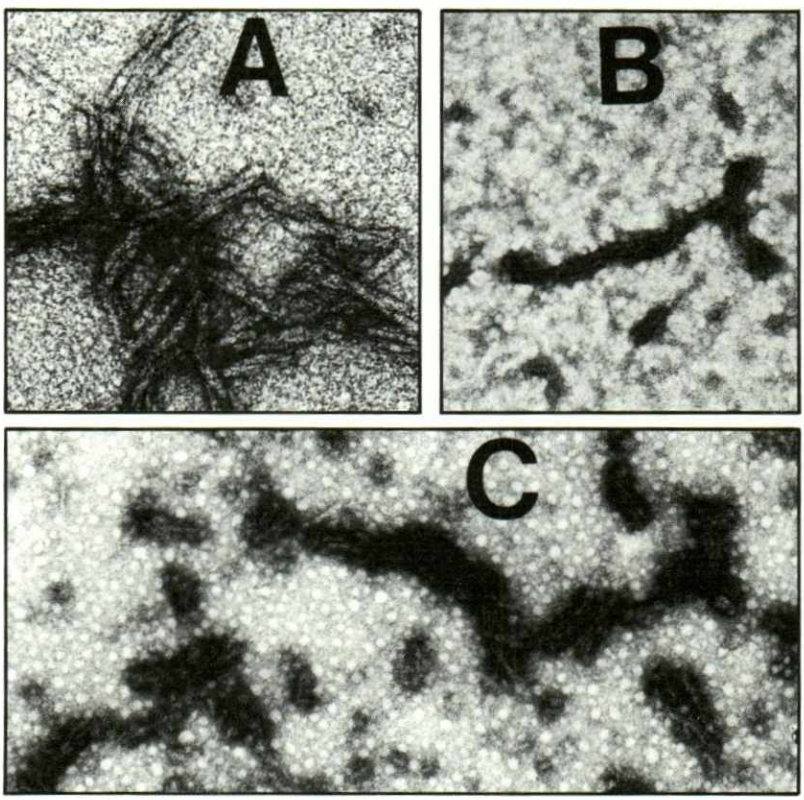

Fig. 4 Electron micrographs showing serological reactions between: (A) potato virus isolate and its normal serum (X 70,000), (B) Potato virus isolate and its homologous antiserum (X 70,000), and (C) Potato virus isolate and PVX antiserum (X 70,000).

DISCUSSION

The potato virus isolate induced mild mosaic and other symptoms more or less similar to those reported for PVX in the following test plants: *C. amaranticolor* (9), *D. stramonium* (2, 3, 6), *G. globosa* (3, 5, 10), *N. debneyi* (3, 5), *N. glutinosa* (2, 9) and *N. tabacum* (2, 3). It was found that the potato virus isolate is readily mechanically transmitted and its properties in the crude sap were in agreement with those reported by Bercks (3) for PVX. The electron microscope revealed the presence of slightly flexuous broken virus particles, which were difficult to measure. The virus had a serological relationship with PVX, but none with the following viruses: CaVX, CLYMV, CybMV, NaMV, PepMV, Potato sieg, PVM, PVS, VioMV and WCLMV.

Based upon host range, symptomatology, properties in the crude sap, virus particle morphology and serological reactions, it is concluded that the potato virus isolate under study is PVX.

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LITERATURE CITED

1. Ball, E.M. 1974. Serological tests for the identification of plant viruses. *Am. Phytopath. Soc. USA*, 31pp.
2. Beemster, A.B.R. and A. Rozendaal. 1972. *Potato viruses: Properties and symptoms.* pp. 115-143 in: Viruses of potatoes and seed potato production (de-Bokx, J.A. ed.). Poduc. Wageningen, The Netherlands, 233pp.
3. Bercks, R. 1970. Potato virus X. CM1/AAB. Descriptions of Plant Viruses. No. 4, England.
4. Horvath, J. 1967. Separation and determination of viruses pathogenic to potatoes with special regard to potato virus Y. Acta. *Phytopath. Acad. Sci. Hung.* 2: 319-360.
5. Jones, R.A.C., C.E. Fribourg and S.A. Slack. 1982. Potato virus and virus-like diseases. *Set No. 2,* in: Plant virus slide series (Barnett, O.W. and S.A. Tolin, eds.). Clemson University, Clemson, Sc. USA, 59 pp.
6. Munro, J. 1961. *The importance of potato virus X.* Am. Potato J. 38: 440-447.
7. Pucci, E. 1963. Actual situation of major plant disease in Libya. *Ministry of Agriculture,* Tripoli, Libya, 7pp.
8. Ross, A.F. 1964. *Identification of Plant Viruses: Properties in crude juice* pp. 77-80 in: Plant Virology (Corbett, M.K. and H.D. Sisler, eds.). University of Florida Press, Gainesville, 527pp.
9. Smith, K.M. 1972. *A test book of plant virus diseases* (3rd ed.). Academic Press, New York, 684 pp.
10. Wikinson, R.E. and F.M. Blodgett. 1948. *Gomphrena globosa* a useful plant for qualitative and quantitative work with potato virus X. *Phytopathology* 38: 28.

عزل وتعريف فيروس البطاطا من نباتات بطاطا بمنطقة عين زاره بليبيا

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المستخلص

عزل فيروس من نباتات بطاطا بمنطقة عين زاره حيث لوحظ ظهور أعراض الموزيك على الأوراق الحديثة واصفرار عام على الأوراق الأكبر سناً. ينتقل الفيروس ميكانيكياً الى 19 نوعاً من مجموع 24 نوعاً تتبع ست عائلات نباتية، وتقع درجة الحرارة المميتة للفيروس بين 70-75° م، ودرجة التخفيف النهائية بين $10^{-5} - 10^{-6}$، ومدة استمرارية نشاطه بالعصارة الخام ستون يوماً. تمت التنقية الأولية للفيروس عن طريق التسخين مع استعمال الطرد المركزي التفاوتي، ثم أجريت التنقية العالية باتباع طريقة الكثافة المتدرجة لمحلول السكروز المصحوبة بالطرد المركزي. وبفحص عينات منقاة من الفيروس تبين وجود جسيمات خيطية مرنة نوعاً ما ولكنها متكسرة. كما ثبت أن الفيروس مناعي وله علاقة سيرولوجية مع فيروس البطاطا ×، بينما لا توجد علاقة سيرولوجية بينه وبين ثمانية فيروسات أخرى من مجموعة فيروسات بوتيكس وفيروسين آخرين من مجموعة فيروسات كارلا. وبناءً على ما تقدم يتضح أن الفيروس الذي جرى عزله هو فيروس البطاطا ×.