ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLIII, nr 4
1974

Detection of potato virus X in potato leaves by means of polyacrylamide gel electrophoresis

MARTA CZUPRYN and MARIA BŁASZCZYK

Institute of Biochemistry, Warsaw University, Poland

(Received: July 5, 1974).

Abstract

A rapid method of detection and evaluation of potato virus X (PVX) infection in potato leaves is described. The method is based on: 1) discarding of major part of cytoplasmic proteins from leaf homogenate by precipitation with polyethylene glycol in final concentration of 2%, 2) precipitation of virus containing fraction by increasing the concentration of polyethylene glycol to 3.5% and electrophoretic identification of virus coat protein extracted from this fraction. The procedure allows early detection of PVX in plants inoculated with virus as well as grown from infected tubers.

INTRODUCTION

Sensitive and reliable methods for detection of plant viruses are essential for practical purposes. The two most commonly used techniques for detection of potato virus X (PVX) in potato leaves and tubers are microprecipitin tests and inoculation of indicator plants. The more accurate that microprecipitin test a bentonite flocculation test (Scott et al. 1964; Bercks 1967; Kahn et al. 1967) and immunodiffusion tests in agar gel (Shepard, Secor 1969) are laborious and time-consuming. Electron microscopy technique described by Sampson and Taylor (1968) is difficult to develop in routine tests.

In the present paper a rapid method for PVX detection in potato leaves is decribed. The method is based on separation from the leaf homogenate the PVX containing fraction and electrophoretic identification of virus coat protein.

MATERIAL AND METHODS

Material

The experiments were performed with two groups of potato plants. First was cv. Flisak inoculated in stage of 7-8 leaves with the suspension of PVX. Second was cv.

Bintje in the same stage of development grown from tubers infected with PVX. In the first group both old inoculated and young noninoculated leaves were used for assays. Samples were collected from 2 to 10 day after infection in two-day intervals. In both groups healthy plants grown in the same conditions were used as a control.

Material was obtained from Institute for Potato Research, Research Unit Młochów.

METHODS

Isolation of the PVX containing fraction

2.0 g of leaves were twice frozen and thawed and then ground in a mortar with 2 ml of 0.1 M sodium citrate buffer, containing 1% sodium hydrosulphite, pH 7.4. The homogenate was centrifuged at 10.000 g for 10 min (Janetzki T 24). The supernatant was collected and the pellet ground with 2 ml of the same buffer. After low speed centrifugation the pellet was discarded. To the pooled supernatants (5 ml) 1.0 g of NaCl and 3 ml of 5% polyethylene glycol (PEG 6000, Maybux Products Ltd.) were added (final PEG concentration of about 2%). After 30 min the suspension was centrifuged at 10,000 g for 10 min. The pellet was discarded and 2 ml of 10% PEG were aded to the supernatant (final concentration of 3.5%). After 30 min the precipitate ("virus containing fraction") was collected by low speed centrifugation, yielding 0.5 — 2.0 mg of protein.

All procedures were carried out at 4C.

Protein was determined by the method of Lowry et al. (1951).

Extraction of proteins with acetic acid

For extraction a slightly modified method of Fraenkel-Conrat (1957) was used. The pellets obtained by precipitation with 3.5% PEG were suspended in 0.01 M sodium citrate buffer (0.1 — 0.4 ml depending on the amount of protein). To resulting suspension double volume of glacial acetic acid was added and extraetion was carried for 1 hr in ice bath with occasional stirring. The suspension was clarified by centrifugation at 10.000 g for 10 min. The pellet was discarded and the supernatant was used for electrophoretic analysis.

Preparation of standard PVX coat protein

Potato virus X used for extraction of standard PVX coat protein was obtained from tobacco leaves (cv. Samsun) by precipitation with 10% PEG and additional filtration on a Sepharose 2B column.

Electrophoresis

The gel system pH 3.8, containing 8 M urea was prepared according to Duesberg and Rueckert (1965) but electrophoresis was performed in 8% gel in 5-fold diluted electrophoretic buffer. 60 mm gels of 6 mm diameter were used.

Preelectrophoresis was carried for 1 hr at 150 V, and 20 µl of protein extracts were applied on each gel. Electrophoresis was conduced for 2 hr at 200 V (about 2 mA/gel). Gels were stained for about 4 hr with 0.1% solution of Coomassie Brilliant Blue in water: methanol: acetic acid mixture (6 : 3 : 1) and destained overnight in 7% acetic acid.

Densitometer tracings of gels were obtained in ERI-65 densitometer (Carl Zeiss Jena).

RESULTS AND DISCUSSION

The addition of PEG to 10% concentration to the homogenate from healthy tobacco leaves results in precipitation of 0.5 mg protein/g fresh weight, whereas the same concentration of PEG yields about 10-fold greater amount of protein from potato leaves. Discarding of the fraction precipitated with 2% PEG allows the removal of 60 — 75% of soluble leaf proteins without precipitation of the virus. Fraction from 2.0 to 3.5% PEG contains about 20% of soluble leaf proteins and practically the total amount of the virus.

Fig. 1 shows densitometer tracings of electrophoretic patterns of proteins of "virus containing fraction" from healthy and PVX infected potatoes cv. Flisak. Fraction from healthy leaves (A) appears as one band different from coat protein band (B). Virus coat protein band is not visible 2 days after infection (C) but makes distinct band 4 days after infection (D).

As the disease develops (8 — 10 days after infection) the amount of virus coat protein increases. The concentration of PVX is always greater in young noninoculated than in old inoculated leaves.

Fig. 2 shows densitometer tracings of electropherograms of proteins of "virus containing fraction" from leaves of potato cv. Bintje. Plants were grown from healthy and PVX infected tubers. Distinct band of PVX coat protein is visible in fraction from infected plants.

The method described allows a detection and identification of PVX in the amount of 2—5 µg/g fresh weight, and together with the densitometric scanning of gels it makes enable the quantitation of the virus. As compared with the immunodiffusion and infectivity tests the method is less time consuming.

We wish to thank Professor I. Chmielewska for her interest and discussions.

This work was partly supported by the Ministry of Agriculture within the project 09.1.2.

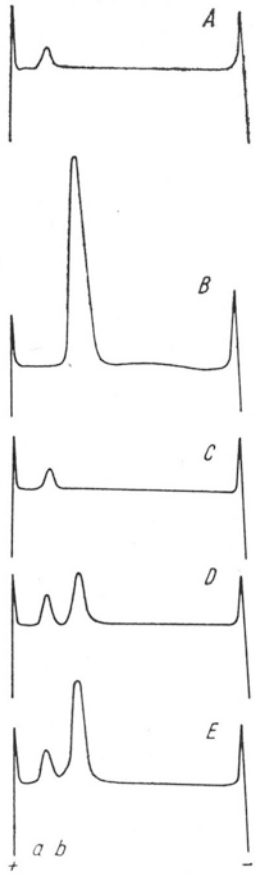

Fig. 1

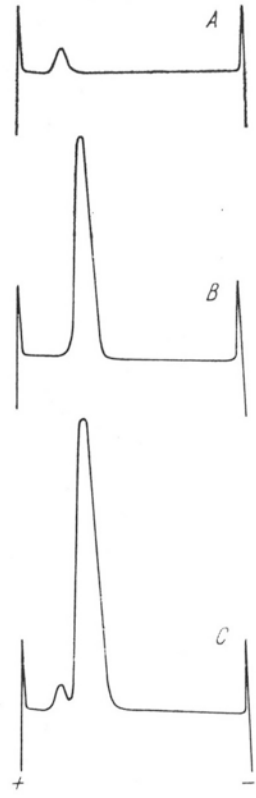

Fig. 2

Fig. 1. Densitometer tracings of electrophoretic patterns of proteins extracted from "virus containing fraction" from leaves of Flisak. A- healthy plants, B- PVX standard, C- plants 2 days after inoculation, D- plants 4 days after oniculation, E- plants 6 days after inoculation. a/ cytoplasmic protein, b) virus coat protein. C, D, E - material from tip leaves.

Fig. 2. Densitometer tracings of electrophoretic patterns of proteins extracted from "virus containing fraction" from leaves of Bintje. A, B as in Fig. 1., C- plants from PVX infected tubers.

REFERENCES

Bercks A., 1967. Methodische Untersuchungen über den serologischen Nachweis pflanzenpathogener Viren mit dem Bentonit-Flockungtest, dem Latex-Test und dem Bariumsulfat--Test, Phytopathol. Z. 58: 1—17.

Duesberg P. H. and Rueckert R. R., 1965. Preparative zone electrophoresis of proteins on polyacrylamide gels in 8M urea, Anal. Bioch. 11: 342—361.

Fraenkel-Conrat H., 1957. Degradation of tobacco mosaic virus with acetic acid, Virology 4: 1—4.

Kahn R. P., Scott H. A., Bozicevich J. and Vincent M. M., 1967. Detection of potato viruses X, M, and S in dormant potato tubers by the bentonite flocculation test, Phytopathology 57: 61—65.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J., 1951. Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193: 265—275.

Sampson P. J. and Taylor R. H., 1968. A comparison of the electron microscope, microprecipitin tests, and indicator plants for the detection of potato viruses S, X and Y, Phytopathology 58: 489—493.

Scott H. A., Kahn R. P., Bozicevich J. and Vincent M. M., 1964. Detection of potato virus X in tubers by the bentonite flocculation test, Phytopathology 54: 1292—1293.

Shepard J. F. and Secor G. A., 1969. Detection of potato virus X in infected plant tissue by radial and double-diffusion tests in agar. Phytopathology 59: 1838—1844.

Author's address:
Mgr Marta Czupryn and
Mgr Maria Błaszczyk
Institut of Biochemistry,
Warsaw University,
ul. Żwirki i Wigury 93,
02-089 Warszawa, Poland

Wykrywanie wirusa X w liściach ziemniaka metodą elektroforezy na żelu poliakrylamidowym

Streszczenie

Opisano szybką metodę wykrywania wirusa X w liściach ziemniaka i oceny stopnia zakażenia tym wirusem. Metoda polega na: 1) oddzieleniu większości białek cytoplazmatycznych homogenatu liści przez wytrącanie glikolem polietylenowym o końcowym stężeniu 2%, 2) wytrąceniu frakcji zawierającej wirus przez zwiększenie stężenia glikolu polietylenowego do 3.5% i elektroforetycznej identyfikacji białka płaszcza wirusa ekstrahowanego z tej frakcji. Metoda pozwala na wczesne wykrywanie wirusa X w liściach ziemniaków inokulowanych tym wirusem, jak również wyhodowanych z bulw zakażonych.