DETECTION ON POLYACRYLAMIDE GEL OF A DIAGNOSTIC NUCLEIC ACID FROM TISSUE INFECTED WITH POTATO SPINDLE TUBER VIROID[1]

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Abstract

A diagnostic procedure for potato spindle tuber disease is described which allows detection of the causal viroid in small amounts of potato and tomato tissue. The method involves extraction of cellular nucleic acids, their separation by polyacrylamide gel electrophoresis and staining with toluidine blue O. The procedure has been used to index greenhouse and field potatoes for mild and severe strains of the pathogen.

Resumen

Un procedimiento para diagnosticar la enfermedad del "tubérculo ahusado" (PSTV) es descrito, el cual permite detectar el viroide cau sante en pequeñas cantidades del tejido de la papa y del tomate. Los métodos incluyen extracción de ácidos nucléicos celulares, su separación por geles de polyacrylamida, electroforesis y tincción con azul de toluideno O. El prodedimiento ha sido usado para indexar papas tanto en el invernadero como en el campo para los "strains" sauve y severo del pa tógeno.

Introduction

Potato spindle tuber has been recognized as an important potato disease for many years. It is incited by a replicating ribonucleic acid (RNA) of low molecular weight, termed a viroid by Diener (2). What may be the same pathogen was shown recently by Semancik, Magnuson and Weathers (5) to be the cause of citrus exocortis disease. Potato spindle tuber viroid (PSTV) can be spread in the field by contact or by leaf chewing insects. It is very heat tolerant (8), is seed borne (4), and is not easily eradicated by thermotherapy and axillary bud culture (10).

Mild strains of PSTV, which may be quite prevalent (9), are difficult to diagnose because symptoms are obscure or lacking. Fernow (3) used the cross protection test on tomato to diagnose mild PSTV but the possibility of inoculation failure and the 6 to 8 weeks required to complete a test make

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this method unsatisfactory. We attempted to use the local lesion host, *Scopolia sinensis* Hemsl. (7), but no lesions developed.

Because of the difficulty of diagnosing mild PSTV by biological assays we undertook to develop a method to detect the infectious agent in diseased tissue. We modified the techniques of RNA isolation and polyacrylamide gel electrophoresis used by Semancik and Weathers (6) to achieve visual detection of PSTV. This paper presents the procedure and the results of analysing potato and tomato tissues, some of which were infected with potato viruses X and S as well as PSTV.

Materials and Methods

A severe strain of PSTV obtained from Dr. W. B. Raymer, Beltsville, Md., and a mild strain isolated locally were available. Both were maintained in potato *(Solanum tuberosum* L.) and tomato *(Lycopersicon esculentum* Mill., cv. Rutgers). Potato plants were grown from infected tubers; tomato seedlings were rub-inoculated at the 4- to 6-leaf stage. Infected tomato leaves were ground in GPS buffer (0.1 M glycine, 0.1 M Na_2HPO_4, O.3 M NaCl, pH 9.5). Celite was used as an abrasive and the plants were washed after inoculation. Tests were made on tubers, stems petioles and leaves. Tomato seedlings were tested at least 2 weeks after inoculation.

Plant tissues (5 to 10 g per sample), either freshly harvested or from deep-freeze storage were treated according to the following procedure:

1) Homogenize the tissue in 8 ml GPSS buffer (0.2 M glycine, 0.1 M Na_2HPO_4, 0.6 M NaCl, 0.2 M Na_2SO_3, pH 9.5), 1 ml 10% sodium lauryl sulfate, 8 ml chloroform and 8 ml butanol at room temperature in a Virtis homogenizer for 1 minute. Note: in all subsequent steps keep the RNA extracts cold.
2) Centrifuge the homogenate at 4°C at 8000 rpm for 20 min in a Sorvall RC2 centrifuge.
3) Remove the upper aqueous phase and extract by emulsification with 10 ml water-saturated phenol with 0.1% 8-hydroxyquinoline added. Hold the solution on ice for 20 min, then centrifuge as in step 2.
4) Remove the upper aqueous phase and mix with 2 volumes of cold 95% ethanol. Stand the mixture on ice for 20 min to precipitate the nucleic acids, then centrifuge as in step 2.
5) Discard the ethanol supernatant and suspend the nucleic acid pellet in 3 ml TKM buffer (0.1 M tris hydroxymethylamine, 0.01 M KCl, 10^{-4} M $MgCl_2$, pH 7.4) by rapid agitation. Dialyze the suspension against TKM buffer for at least 1 h.

6) After dialysis, mix the nucleic acid solution with an equal volume of 4 M LiCl and place on ice overnight.
7) Centrifuge the solution as in Step 2 and carefully decant the supernatant containing LiCl-soluble nucleic acids. Discard the pellet. Precipitate the nucleic acids with ethanol as in step 4.
8) Separate the ethanol precipitate from the LiCl supernatant by centrifugation as in step 2, and resuspend the pellet in 0.5 to 1.0 ml TKM buffer. Dialyze against TKM buffer for at least 2 h and store frozen until required for electrophoresis.

Electrophoretic separation of LiCl-soluble nucleic acids was performed on 5 or 8% polyacrylamide gels in 0.04 M tris hydroxymethylamine, 0.02 M sodium acetate, 0.001 M disodium ethylenediamine-tetraacetate at pH 7.2 (1). Gels 9 cm long were poured in plexiglass tubes (6 mm i.d.) and prerun for 30 min at 75 V. A 0.2 ml sample of nucleic acid was applied to the top of each gel with a drop of 50% sucrose and electrophoresed for 2¼ or 3½ h at 75 V, 6 mA per gel, at room temperature. The gels were then removed from the tubes and stained overnight in 0.01% toluidine blue 0 in water. Excess stain was removed by submersion in water for several hours and the gels were stored in 0.01% sodium azide.

Quantitative estimation of PSTV requires preparations of nucleic acid free of dark brown discoloration. These were obtained by making the initial extraction with water-saturated phenol (8 ml) instead of chloroform-butanol (16 ml) or by the addition of 0.1% sodium diethyldithiocarbamate to the extraction buffer. The concentration of PSTV was estimated by scanning unstained gels at 280 nm in a quartz tube with an ISCO model 659 gel scanner. The area of the unknown was related to a standard curve prepared against known concentrations of RNA.

Results

Fig. 1 shows the separation of LiCl-soluble nucleic acids extracted from tomato plants infected with mild and severe strains of PSTV. The plants used in this analysis, photographed just before processing, are shown in Fig. 2. The extra RNA band (Fig. 1), present only in infected tissue, is the viroid. The size of the band and the density of staining indicate that both infected plants contained about equal quantities of PSTV.

The detection of PSTV in a dormant potato tuber infected with a mild strain of the viroid is illustrated in Fig. 3.

Potato plants grown from tubers infected with a mild strain of PSTV, alone or with potato viruses X and S, failed to develop diagnostic symptoms in a field planting. However, diagnosis was possible by polyacrylamide gel electrophoresis (Fig. 4) and the viroid in infected plants was reliably detected whether tests were made soon after emergence or at later

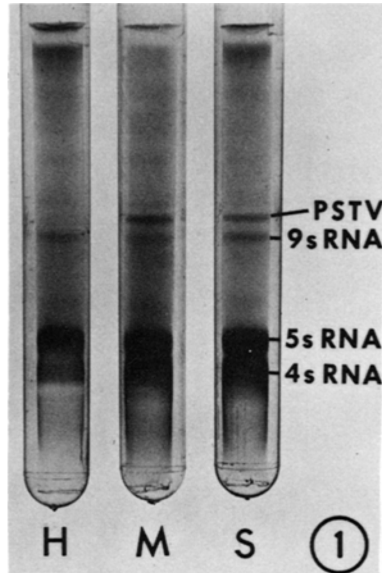

FIG. 1.—Polyacrylamide gels (5%) showing, midway in tubes, RNA extracted from healthy tomato (H), and tomato infected with mild (M) and severe (S) PSTV, separated by electrophoresis at 75 V, 6 mA/gel for 2¼h and stained with 0.01% toluidine blue 0 in water.

FIG. 2.—Rutgers tomato 2 months after inoculation with extracts from healthy potato (H) and potato infected with mild (M) and severe (S) PSTV.

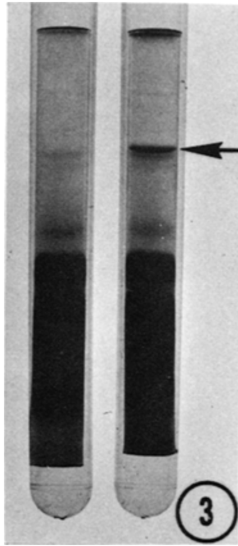

FIG. 3.—Polyacrylamide gels (8%) showing, midway in tubes, RNA extracted from healthy (H) and mild PSTV-infected (M) potato tubers separated by electrophoresis at 75 V, 6 mA/gel for 3½h. Arrow indicates the PSTV RNA.

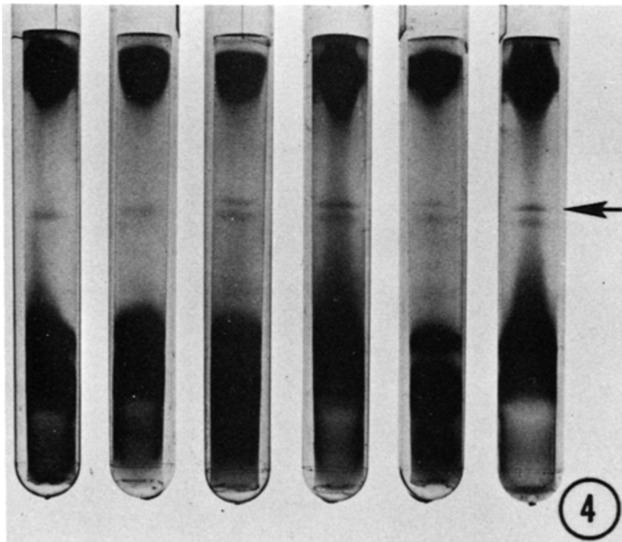

FIG. 4.—Polyacrylamide gels (5%) showing, midway in tubes, RNA extracted from field-grown potato tissue and separated by electrophoresis at 75 V, 6 mA/gel for 2¼h. The extracted plants were infected with the following viroids and viruses, left to right: none, none, mild PSTV, mild PSTV + PVX, mild PSTV + PVS and mild PSTV + PVX + PVS. Arrow indicates the PSTV RNA.

stages of growth (Table 1). The presence of the other virus did not interfere with the test for PSTV.

Concentration of PSTV in potato plants grown from tubers infected with a mild strain of the viroid was highest in the terminal shoots (Table 2) and in the midveins of young leaves. The concentration of PSTV was relatively lower in tubers, petioles, and mature stems and leaves.

TABLE 1.—*Detection of mild PSTV in field grown potatoes by the polyacrylamide gel procedure.*

Virus content of[1] planted tubers	Presence of PSTV in Polyacrylamide Gel[2]				
	June 5	June 18	July 2	July 16	July 29[3]
Virus free	0/4	0/6	0/6	0/12	0/2
Potato virus S (PVS)	—	—	0/2	0/2	0/2
Potato virus X (PVX)	—	—	0/2	0/2	0/2
Potato virus X + S	0/2	0/3	0/2	0/2	0/2
Mild PSTV	2/2	2/2	2/2	3/3	2/2
Mild PSTV + PVS	—	—	2/2	—	2/2
Mild PSTV + PVX	—	—	2/2	—	2/2
Mild PSTV + PVS + PVX	2/2	2/2	2/2	2/2	2/2

[1] Tubers planted May 1.
[2] Gels with PSTV band over number of plants tested.
[3] Date of sampling.

TABLE 2.—*Distribution of mild PSTV in potato tissue.*

Type of tissue	Micrograms of PSTV per gram of tissue[1]		
	EXPT 1[2]	EXPT 2[2]	EXPT 3[3]
Terminal shoot	1.20	1.13	1.40
Mature leaves	0.33	0.41	0.32
Leaf midveins	1.25	0.90	0.95
Petioles	0.42	0.47	0.60
Stem	0.28	0.31	0.27
Tuber	0.10	0.11	0.49

[1] Determined from planimetry of gels scanned at 280 nm.
[2] Greenhouse grown potato and mature tuber.
[3] Field grown potato and immature tuber.

Discussion

The demonstration of a disease-specific, pathogenic RNA of low molecular weight which was readily identified in a polyacrylamide gel was made first by Semancik and Weathers (6) for the citrus exocortis pathogen. Using

a modification of their procedure we detected severe and mild strains of PSTV in greenhouse- and field-grown potatoes and in greenhouse-grown tomato plants. The complexity of the procedure makes it impractical for large scale field testing but it should be a valuable diagnostic aid for indexing elite or basic seed stocks in certification programs.

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