Comparison of Tomato Bioassay and Slab Gel Electrophoresis for Detection of Potato Spindle Tuber Viroid in Potato

G. L. Schumann, H. D. Thurston, R. K. Horst, S. O. Kawamoto, and G. I. Nemoto

Graduate Research Assistant, Professor, Associate Professor, Research Technician, and Research Support Specialist, respectively, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

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ABSTRACT

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Potato plants from a randomly selected set of 146 Cornell University breeding lines were tested for the presence of potato spindle tuber viroid (PSTV) by both polyacrylamide gel electrophoresis and tomato bioassay. The electrophoretic assay was an adaptation of Morris and Smith's procedure to a slab gel apparatus that permitted testing of 25 samples at once. Results of both tests were in agreement in 123 cases. In 23 cases, results of the original tests were not in agreement. When 20 were tested a second time, results supported the original electrophoretic data in all but one case. Potato plants of four commercial cultivars and 45 breeding lines representing diverse germplasm were inoculated with mild or severe strains of PSTV. The electrophoretic assay accurately detected PSTV in the inoculated plants. Assay for PSTV by gel electrophoresis is more rapid and reliable than the tomato bioassay.

Because potato spindle tuber viroid (PSTV) is both seed- and pollen-borne in potatoes (*Solanum tuberosum* L.) (2) it is desirable to use only parent plants that are not infected with PSTV for potato breeding. A bioassay described by Fernow et al. (1) using tomato as a test plant has been used for over a decade to detect PSTV in potato breeding lines at Cornell University. Although the test has been useful, it has two main disadvantages: (i) 6-8 wk are required to complete the test, and (ii) it lacks accuracy under unfavorable environmental conditions. An assay described by Morris and Smith (4) which uses polyacrylamide gel electrophoresis is an appealing procedure because it requires days rather than weeks to perform. The accuracy of the electrophoretic assay was not known for testing a wide variety of potato lines infected with a range of isolates of PSTV of unknown titers. We made a comparison of the bioassay and a modified electrophoretic assay to determine their relative reliability and usefulness.

MATERIALS AND METHODS

Plant culture.—Tubers of Cornell University potato breeding lines to be assayed for PSTV were planted in pots in a polyethylene greenhouse maintained at about 25 C. Samples of young leaves were taken from 4- to 5-wk-old plants of a randomly selected set of 146 breeding lines for bioassay and electrophoretic assay. All tomato bioassay plants were grown in a glass greenhouse maintained at about 25 C with supplemental light during a 16-hr photoperiod.

A selected set of tubers of 45 breeding lines representing diverse germplasm was planted in pots in a glass greenhouse maintained at about 25 C with supplemental light to provide a 16-hr photoperiod. Each breeding line was represented by a set of three plants all grown from the same tuber. One plant of each set was not inoculated, one was inoculated with a mild strain of PSTV, and one was inoculated with a severe strain of PSTV. All inoculations were made as soon as the leaves began to expand by rubbing crushed PSTV-infected tomato leaves on the upper leaf surfaces of Carborundum-dusted potato plants. Samples from all three plants of each breeding line were assayed 4-5 wk postinoculation by the electrophoretic assay described below. Assay results were compared to a coded list to determine the accuracy of the electrophoretic assay.

A third set of potato plants of four commercial cultivars, Sebago, Katahdin, Kennebec, and Russet Rural, was grown. Eighteen plants of each cultivar were inoculated with a severe strain of PSTV, and eighteen were inoculated with a mild strain of PSTV. Inoculations were made as described previously. Samples of young leaves were assayed 4-6 wk postinoculation by the electrophoretic assay described below.

Tomato bioassay.—Bioassays were initiated with Rutgers tomato plants at the cotyledon stage (1). Cotyledons of two tomato plants were dusted with 0.15-μm (400-mesh) Carborundum, rubbed directly with crushed leaves from the potato plant to be tested, but not rinsed. If the potato plant was infected with a severe strain of PSTV, the tomato plant should develop severe symptoms. If no symptoms appeared, then the potato plant was either healthy or infected with a mild strain of PSTV.

After 14-16 days one of the tomato plants was challenge

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inoculated with a severe strain of PSTV. If the original potato plant was not infected with PSTV, the challenged plant should develop severe symptoms after 3-5 wk. If a mild strain was present, the challenged plant should be protected against the severe strain and not develop severe symptoms. Nonchallenged plants also were maintained for observation as controls.

Electrophoretic assay.—The detection of PSTV by polyacrylamide gel electrophoresis was accomplished by using the Morris and Smith procedure (4) modified for use on a slab gel apparatus. Samples consisted of young leaves (1 g fresh weight) taken from each potato plant to be assayed. The Morris and Smith procedure consists of an initial phenol extraction with the addition of chloroform-pentanol forming an emulsion. After centrifugation, the upper aqueous layer is removed and enough 10 M LiCl is added to make a 2 M LiCl solution which produces a heavy, white precipitate. After centrifugation the supernatant was dialyzed overnight. All centrifugations were done in a Sorvall SS-3 centrifuge with an SM-24 rotor in a cold room at 3 C.

Several changes were made from the Morris and Smith procedure for our samples. In the initial extraction, mercaptoethanol was found unnecessary for good results, and chloroform-butanol (1:1, v/v) rather than chloroform-pentanol (25:1, v/v) was used. Centrifugation for 20 min after the initial step produced a better phase separation in our samples. The second centrifugation was for 15 min as Morris and Smith described.

After dialysis, the sample was precipitated in two volumes of 95% ethanol in the freezer for 1 hr. The precipitate was resuspended in 50 μliter cold, boiled, distilled water with 1 drop of a 75% solution of RNase-free sucrose containing bromphenol blue (0.5 mg/ml). A 5% polyacrylamide gel (11.5 cm × 14 cm × 0.4 cm) was used in a slab gel apparatus (Aquabogue Machine and Repair Shop, Aquebogue, NY 11931) that accepted 25 samples per gel. A 50-μliter sample of each extract was applied to the gel. The electrophoretic buffer was 0.12 M Tris, 0.06 M sodium acetate, and 0.3 M disodium EDTA, adjusted to pH 7.6. Gels were run at 100-125 ma, 100 V until the bromphenol blue had moved 9 cm (about 2.25 hr). Gels then were stained with ethidium bromide (2 mg/100 ml 0.001 M EDTA) for 10-20 min and examined under shortwave UV light (UVSL-25, Ultraviolet Products, Inc., San Gabriel, CA 91778) (3). Infection by PSTV was indicated by the presence of the viroid RNA band described by Morris and Smith (4) (Fig. 1).

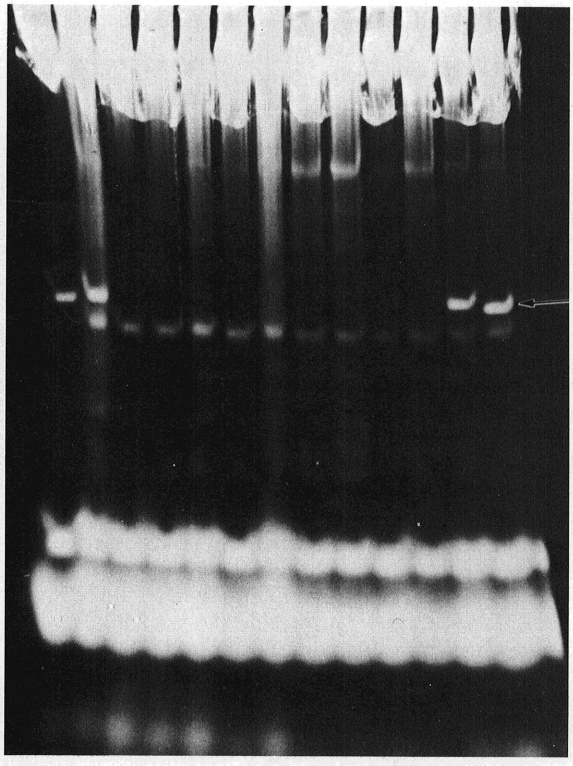

Fig. 1. Polyacrylamide slab gel (5%) showing the results of electrophoresis of 13 extracts of 1-g samples of potato leaves. The nucleic acids were electrophoresed at 100-125 ma, 100 V for about 2.25 hr and stained with ethidium bromide for 10-20 min. The photograph was taken while the slab was under shortwave UV light which causes the stain to fluoresce. Of the 13 samples, left to right 1, 2, 12, and 13 are from plants infected with potato spindle tuber viroid (PSTV) as indicated by the presence of the viroid RNA band near the center of the gel. The arrow locates the position of the PSTV-RNA.

RESULTS

Plants from 146 Cornell University potato breeding lines were tested for PSTV infection by both electrophoretic assay and tomato bioassay. Results were in agreement for 123 of the tests with 121 negative and two positive in each test. In 23 cases, however, there was disagreement between the assays (Table 1).

The bioassay indicated infection of the potato plants with a mild strain of PSTV in 15 cases since the challenged tomato plants did not develop severe symptoms. In three of these cases the results were questionable. No viroid RNA could be detected, however, by electrophoretic assay of leaves from the same 15 potato plants. In eight other cases the bioassay indicated that the potato plants were healthy since the challenged tomato plants did develop severe symptoms, but the results were questionable in six cases.

We thought that if mild strains existed that could not be detected by electrophoresis, but could be detected by bioassay, these mild strains might be present in the potato plants that gave differing results in the two assays. Because the original potato plants were quite old by the time the bioassay was completed, tubers from these plants were planted; leaf samples from the new plants were then used in a repetition of both assays. The assays were repeated for 20 of the original 23 potato plants for which disagreement between the first assays occurred. Results of the second assays are listed in Table 1. In all but one case the second electrophoretic assay and tomato bioassay confirmed the original electrophoretic results.

Detection of PSTV in Cornell University breeding lines of diverse germplasm by electrophoretic assay was accurate and reliable (Table 2). PSTV was detected in plants of all 45 breeding lines that had been inoculated with either a mild or severe strain of PSTV. The PSTV was not detected in noninoculated plants of 40 breeding

TABLE 1. Results of parallel tests for potato spindle tuber viroid (PSTV) infection of potato breeding lines by tomato bioassay and polyacrylamide gel electrophoretic assay

Results of first tests			Results of second tests of samples at left		
Number of samples	Bioassay	Gel electrophoresis	Number of samples	Bioassay	Gel electrophoresis
121	−	−			
2	+	+			
12	+	−	11[a]	−[c]	−
3	+?[b]	−	3	−[c]	−
2	−	+	1	+[c]	+
			1	−	−
6	−?[b]	−	4[a]	−	−

[a]Potato plants not available to repeat tests in all cases.
[b]In the first tests, if the bioassay result was difficult to interpret, the result was identified as +? or −?.
[c]In the second tests, if the bioassay results were difficult to interpret, the results were confirmed by electrophoretic assay of tomato test plants. This was necessary in five cases.

TABLE 2. Detection of potato spindle tuber viroid (PSTV), by polyacrylamide gel electrophoresis, in Cornell University potato breeding lines representing diverse germplasm after inoculation with a mild or severe strain of PSTV

	Detection of PSTV by gel electrophoresis[b]		
No. of breeding lines[a]	Noninoculated	Inoculated with PSTV mild strain	Inoculated with PSTV severe strain
40	−	+	+
5	+[c]	+	+

[a]Sets of three plants were grown from single tubers of each breeding line. One plant was not inoculated, one was inoculated with a mild strain of PSTV, and one was inoculated with a severe strain of PSTV.
[b]Samples of young leaves were assayed by electrophoretic assay 4-5 wk postinoculation.
[c]Five breeding lines were apparently infected with PSTV before the experiment began.

TABLE 3. Detection of potato spindle tuber viroid (PSTV) by polyacrylamide gel electrophoresis in commercial potato cultivars inoculated with a mild or severe strain of PSTV

	Detection of PSTV by gel electrophoresis[a]	
Cultivar	Inoculation with PSTV severe strain	Inoculation with PSTV mild strain
Katahdin	18/18	18/18
Sebago	18/18	18/18
Kennebec	18/18	18/18
Chippewa	18/18	18/18

[a]Samples of young leaves were assayed by electrophoretic assay 4-6 wk postinoculation.

lines. Tubers of five breeding lines were apparently infected with PSTV before the experiment began. The electrophoretic assay also accurately detected PSTV in inoculated plants of four commercial cultivars (Table 3). PSTV was detected in 36 plants of each cultivar inoculated with either a mild or severe strain of PSTV.

DISCUSSION

Our comparison of the electrophoretic assay and tomato bioassay for detection of PSTV suggests that the electrophoretic method can be used with confidence. The tomato bioassay is slow and requires much greenhouse space. Two tomato test plants are required for each potato plant, and 4-8 wk are needed before the final results are known. In a breeding program where individual plants must be assayed for PSTV, it is desirable to electrophorese as many samples as possible. However, the tube gel apparatus described by Morris and Smith (4) can only electrophorese 12 samples at one time. This paper describes a modification of their procedure for a slab gel apparatus that electrophoreses 25 samples at once. Toluidine blue O, the stain used by Morris and Smith,requires 2-3 days of destaining before the gel bands become visible. With the use of ethidium bromide, time for the electrophoretic assay has been shortened from four days to only one day after the sample is first taken. The short time required for electrophoretic assay also allows a rapid retest if, for some reason, the gel is not satisfactory.

The accuracy of the electrophoretic assay was determined by comparison with the bioassay described by Fernow et al. (1). However, this test is conservative in that a plant not properly challenge inoculated would be interpreted to be infected with a mild strain of PSTV since severe symptoms would not develop. In the test described in this paper, the bioassay results would have led to destruction of over 12% of the breeding lines because of PSTV infection. When the tests were repeated, the accuracy of the electrophoretic assay was substantiated, and 15 breeding lines were saved. Inoculations of commercial cultivars and breeding lines of diverse germplasm with either mild or severe strains have also demonstrated the accuracy of the electrophoretic assay. It is possible that PSTV might be present in a low titer that cannot be detected by electrophoretic assay, however, assays of over 300 additional leaf samples from naturally infected potato plants have shown that the viroid band has been either definitely present or absent (G. L. Schumann, *unpublished*). We have not seen faint,

questionable bands in any of our tests in a 2-yr period.

Since PSTV can be both seed and pollen borne (2), it is of particular importance to detect PSTV in plants before breeding crosses are made. The electrophoretic assay is more reliable and less time consuming than the bioassay. Use of the electrophoretic assay should facilitate the production of PSTV-free breeding lines.

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