Etiology

Detection of the Mild Strains of Potato Spindle Tuber Viroid from Single True Potato Seed by Return Electrophoresis

R. P. Singh, A. Boucher, and J. E. A. Seabrook

Agriculture Canada Research Station, P.O. Box 20280, Fredericton, New Brunswick, Canada E3B 4Z7.
We thank L. K. Douglas and T. H. Somerville for valuable technical assistance.
Accepted for publication 24 November 1987 (submitted for electronic processing).

ABSTRACT

Singh, R. P., Boucher, A., and Seabrook, J. E. A. 1988. Detection of the mild strains of potato spindle tuber viroid from single true potato seed by return electrophoresis. Phytopathology 78:663-667.

A modified procedure of return polyacrylamide gel electrophoresis (R-PAGE) was used to detect mild strains of potato spindle tuber viroid (M-PSTV) from single true potato seeds (TPS). Seed lots obtained from field-grown PSTV-infected potato plants varied in their PSTV content. The viroid was detected in both dormant and germinated individual TPS. Dormant TPS contained approximately 0.8–10 ng of viroid RNA per seed. PSTV was detected by R-PAGE in nucleic acid extracts of single seed, diluted to 1:16 (about 500 pg). Germinated seed and TPS seedlings grown in vitro at 19 C showed similar rates of seed transmission. There was no change in PSTV detection in TPS seedlings grown for 4–10 wk. In composite samples of infected and healthy TPS, PSTV was detected when one infected TPS was combined with 90–100 healthy seeds. PSTV detection by R-PAGE was comparable or superior to that obtained by nucleic acid hybridization.

Additional key words: dot-blot hybridization, routine indexing, *Solanum tuberosum*, viroid strains.

True potato seed (TPS) has gained prominence as planting material (1) in developing countries because of its ease of transportation and storage and freedom from potato pathogens, with the exception of a few Andean potato viruses (8) and potato spindle tuber viroid (PSTV) (14). True seed has been used for potato production in the People's Republic of China since 1972.

Potato spindle tuber viroid is highly seed transmissible in several host plants including the cultivated potato (*Solanum tuberosum* L.) (2,4–7,14,17). PSTV has been encountered in TPS of several potato germ plasm collections and potato breeding materials (3,5,6). Therefore, the exchanges of TPS between various potato breeders or the commercial production of potatoes through the use of TPS requires a reliable and rapid means of detecting PSTV in TPS. Potato seedlings from batches of TPS known to be 100% PSTV-infected have not all had detectable PSTV symptoms (6,11). In some cases, PSTV may be detected only from 2–3-wk-old TPS seedlings but not from older plants (6). Therefore, diagnosis of PSTV on the basis of symptoms alone may be difficult.

It has been difficult to detect PSTV in a single TPS. Previously, detection of PSTV required inoculation of extracts from 2–3-wk-old seedlings onto tomato (*Lycopersicon esculentum* Mill.), followed by polyacrylamide gel electrophoresis (PAGE) testing 8 wk later (6). This restricts the application of the method to a limited number of samples. Nucleic acid hybridization can detect PSTV in a single seed in a combined sample of 16 TPS or from an extract equivalent to a sample containing one seed from an infected plant plus 80 seeds from healthy plants (11).

A sensitive return-polyacrylamide gel electrophoresis (R-PAGE) (12) method has been described and modified to distinguish between mild and severe strains of PSTV (16). We report here on the detection of mild strains of PSTV (M-PSTV)

from a single TPS by the use of modified R-PAGE and compare the method with nucleic acid hybridization (dot-blot) (10).

MATERIALS AND METHODS

PSTV-infected and healthy seeds. Potato cultivars Cariboo, Hindenberg, Katahdin, and Tobique were mechanically inoculated with a mild strain of PSTV, grown in an experimental plot at the Fredericton Research Station, and indexed for PSTV infection during the growing season of 1986. Berries, which were a result of the open pollination of infected female parents, were collected and the seeds extracted. Viroid-free TPS used for comparative testing were obtained from the National Potato Breeding Program at the Fredericton Research Station.

Nucleic acid extraction from TPS. Grinding TPS with a pestle and mortar or a polytron (15) resulted in poor homogenization of seeds or loss of extracts in transfers from the mortar to centrifuge tubes. Grinding individual dormant or germinated seeds or groups of seeds in microtubes (Eppendorf, Brinkmann Instrument Division) with a glass rod in 300 μl of extracting buffer (16) and 400 μl of phenol provided the best results. The homogenate was centrifuged at 15,000 rpm for 10 min in a microcentrifuge (Eppendorf 5414, Brinkmann Instrument Division, Sybron, Canada). Three hundred microliters of the aqueous layer was removed, and nucleic acids were precipitated with ethanol. The nucleic acids were dissolved in 10 μl of high salt buffer (89 mM Tris, 89 mM boric acid, 2.5 mM ethylene diaminetetraacetate, pH 8.3), of which 6 μl was used for R-PAGE and 3 μl for dot-blot hybridization.

Viroid detection procedures. Bioassays were performed using *Solanum berthaultii* Hawkes (15) and tomato cultivar Sheyenne (16) to verify the nature of viroid strains and to assess the infectivity of the nucleic acid extracts from TPS seedlings and gels. Nucleic acid hybridization (10) or R-PAGE (16) tests were used to detect PSTV from nucleic acid extracts of infected TPS. Healthy TPS were used as controls. Nitrocellulose membranes from PSTV-Prep Kit (Agdia Inc., Mishawaka, IN) were spotted with 3 μl of nucleic acid extracts and returned to Agdia for hybridization with ^{32}P-labelled $_{p}$DC-29 recombinant DNA prepared by nick translation as described (11). The R-PAGE procedure was that of Schumacher et al (12) as modified (16). Six microliters of nucleic acid extracts dissolved in high salt buffer containing 40% glycerol was mixed with 4 μl of 2% dye solution (xylene cyanol FF and bromophenol blue) and applied to each well on a slab gel (14 × 16 × 0.15 cm). After the first electrophoresis (5% nondenaturing), the buffer was replaced with a heated (87–90 C) low salt buffer (1:8 dilution of the high salt) and electrophoresis was carried out at 70–71 C (denaturing condition with reverse polarity). Viroid bands on the gel were detected by staining with 0.2% silver nitrate (12) and photographed with a Polaroid B/W-type film using a 4 × 5 Land Camera.

Estimation of PSTV concentration in single TPS. To estimate the PSTV concentration in single TPS, nucleic acid extracts from individual seeds were diluted twofold (1:2, 1:4, 1:8, and 1:16) and compared with nucleic acid standards. Average PSTV concentration was determined from 10 replicates of single seed. The nucleic acid standards were purified by electrophoresing a seed extract on a 5% nondenaturing gel, excising the viroid band, and electroeluting in a dialysis tube. Concentration of purified PSTV RNA was calculated with an extinction coefficient 20 $(mg/ml)^{-1}$ cm^{-1} at 258 nm. Various dilutions (15, 7.5, 3.75, 1.88, 0.94, 0.47, and 0.24 ng) were assayed by electrophoresis along with seed extracts. Intensity of bands was compared visually. For confirmation of PSTV detection, an aliquot of the same RNA was used for the dot-blot hybridization test.

Germination of TPS and growth of seedlings. TPS were placed in cheesecloth bags, soaked in a 2,000 ppm solution of gibberellic acid (GA_3) (Eastman Kodak) for 24 hr at 25 C, washed with distilled water three or four times, and germinated on a filter paper in a petri plate placed in the dark.

For in vitro culture the GA_3-treated seeds were surface sterilized in 0.1% mercuric chloride for 7 min followed by three rinses with sterile distilled deionized water. The seeds were transferred to germination media under sterile conditions, i.e., instruments were dipped in 1% sodium hypochlorite for 30 sec between transfers and were then rinsed with distilled deionized water and flamed with 95% ethanol. The germination medium consisted of 1/2 strength Murashige and Skoog salts (9), 0.5 mg/L of thiamine, 1.0 mg/L of pyridoxine, 0.5 mg/L of nicotinic acid, 2.5 mg/L of pantothenic acid, 50 mg/L of *i*-inositol adjusted to pH 5.8 ± 0.1 (13). After dispensing 75 ml of autoclaved medium per vessel, seven seeds were planted in each vessel (Magenta GA7, Magenta Corporation, Chicago, IL) and placed in a growth room at 19 C with 20 $\mu E \cdot sec^{-1} \cdot m^{-2}$ light intensity. Seven days later, when the majority of the seeds had germinated, the seedlings were moved to a shelf lighted by standard cool white (F40 Sylvania xl) and agrolite fluorescent bulbs (F40/AGO Westinghouse) with 120 $\mu E \cdot sec^{-1} \cdot m^{-2}$.

The seedling shoot tips were harvested by using sterile techniques at 4, 6, 8, and 10 wk after planting, and nucleic acid was extracted as described above.

RESULTS

Detection of M-PSTV in TPS of potato cultivars. A preliminary experiment was done to determine the percent infection of TPS collected from four field-grown, PSTV-infected potato cultivars. TPS of each cultivar were ground in groups of 5, 10, 15, 20, 25, 30, 35, 40, and 60 seeds (about 2–25 mg) and the nucleic acid extracts were tested for PSTV by R-PAGE and by the dot-blot test. Rates of infection of three cultivars were similar by both methods, but R-PAGE detected more infection in Tobique than did dot-blot hybridization (Table 1). A bioassay on *S. berthaultii* plants of stained PSTV bands from an electrophorogram caused PSTV infection, indicating that infectious viroids can be isolated by R-PAGE. Hindenberg contained the highest rate of seed infection (Table 1) and was used for the development of a seed-testing methodology.

PSTV detection in dormant and germinated TPS. TPS of two lots (A and B) differing in seed transmission rates were used. TPS, either dormant or germinated for a week, were ground in groups of 1, 2, 3, 4, 5, 6, 8, or 10. Lower numbers of seeds were replicated at least three times and others at least once in each test. PSTV detection by R-PAGE (Table 2) indicated that single TPS, either dormant or germinated, could be used for PSTV detection with high seed transmission rates. Distinct PSTV bands were observed on the electrophorogram from dormant single seeds, with variable intensity (Fig. 1). With lower rates of seed transmission, use of single TPS would not be adequate for diagnosis and composite samples would be needed (Table 2).

TABLE 1. Detection of potato spindle tuber viroid from true potato seeds of open-pollinated potato cultivars by two methods

Seeds from infected plants (no.)	Potato cultivars							
	Cariboo		Hindenberg		Katahdin		Tobique	
	R-PAGE[a]	Dot-blot[b]	R-PAGE	Dot-blot	R-PAGE	Dot-blot	R-PAGE	Dot-blot
Infected plants								
5	–[c]	–	+	+	–	–	–	–
10	–	–	+	+	–	–	–	–
15	–	–	+	+	–	–	–	–
20	–	–	+	+	–	–	+	–
25	–	–	+	+	–	–	+	–
30	–	–	+	+	–	–	+	–
35	–	–	+	+	+	–		
40	+	±	+	+	–	–	+	±
60	NT	NT	NT	NT	–	–	+	–
Healthy plants								
30	–	–	–	–	–	–	–	–

[a] Return-polyacrylamide gel electrophoresis (R-PAGE).
[b] Nucleic acid hybridization (dog-blot) using Agdia Kits.
[c] – = Negative for potato spindle tuber viroid, + = positive, ± = doubtful, and NT = not tested.

In a comparative test, six single dormant TPS were tested by R-PAGE and the dot-blot test. All six were found to be positive for PSTV by R-PAGE, but only four by the dot-blot test.

PSTV content of single TPS. PSTV bands from single TPS varied in intensity (Fig. 1, the bands in lanes 4, 6, 7, 9, 10, 12, and 15 vs. bands in lanes 3, 8, and 11). The intensity of the bands depended on the viroid concentration as shown in Figure 2. In this test, bands containing nanogram amounts of PSTV were more intense than those containing picograms (lanes 3–6 vs. lanes 7 and 8). The intensity of PSTV band in lane 9 was so faint that it was not visible in the photograph, although it was visible by eye on the illuminated electrophorogram. Two dot-blot tests detected PSTV up to a dilution of 0.94 ng.

Comparison of PSTV band intensities of single TPS (more than 100 samples) with those of nucleic acid standards indicated that variation in PSTV content of individual seed could range from 0.8 to 10 ng per seed. Therefore, further twofold dilutions of individual seed extracts were tested to find the lower limits detectable by R-PAGE. PSTV concentrations of three TPS extracts out of the 10 tested, depicted the range (Fig. 3). PSTV was detected up to a dilution of 1:16 (about 500 pg) from one TPS extract (lane 6, Fig. 3), but only up to a dilution of 1:8 (1 ng) with the other two (lanes 10 and 14, Fig. 3). Similar dilutions of seed extracts from healthy TPS did not show any band (lanes 15–18, Fig. 3). In this experiment, the dot-blot tests were as sensitive as R-PAGE (data not shown).

Determination of seed transmission rate by germinated TPS and in vitro-grown TP seedling. Because PSTV was detectable from single seeds, either dormant or germinated, both forms can be used for PSTV detection. However, the PSTV band intensity was greater in germinated TPS, and this was, therefore, used in determining the rate of seed transmission. Use of germinated seed for PSTV detection is destructive because PSTV-free TPS cannot be saved, and thus in vitro-grown seedlings were also evaluated. Individual testing of the germinated seeds on filter paper indicated a 45.8% infection, while the percentage for a sample of in vitro-grown individual TP seedlings from the same lot of seed was 50.0% (Table 3). Of four TPS that failed to germinate, one was also found positive for PSTV by R-PAGE. Similarly, of the 98 TP seedlings germinated in vitro, four displayed abnormal germination or slow growth and one of these was found to be positive for PSTV by R-PAGE. There was no change in PSTV detection from the TP seedlings after 4, 6, 8, or 10 wk of growth (Table 3).

Seven seedlings per vessel were grown for a 10-wk period in the present study, and shoot tips were removed four times, thus providing chances for PSTV spread by contact and by dissecting tools. By 10 wk, many seedlings had lodged and were touching one

TABLE 2. Detection of potato spindle tuber viroid in various numbers of dormant and germinated true potato seeds by return polyacrylamide gel electrophoresis

True potato seeds (no.)	Dormant seed		Germinated seed	
	Lot A[a]	Lot B[b]	Lot A	Lot B
1	1/3[c]	0/3	5/8	0/3
2	3/3	1/3	5/6	1/3
3	3/3	1/3	3/3	2/3
4	1/1	0/3	2/3	2/3
5	1/1	2/3	3/3	0/3
6	1/1	0/3	1/2	0/3
8	1/1	3/3	1/1	2/3
10	1/1	2/3	1/1	1/3

[a] Lot A, with 45% seed transmission.
[b] Lot B, with 9% seed transmission.
[c] The numerator is the number of times PSTV was positively detected and the denominator is the number of replicates.

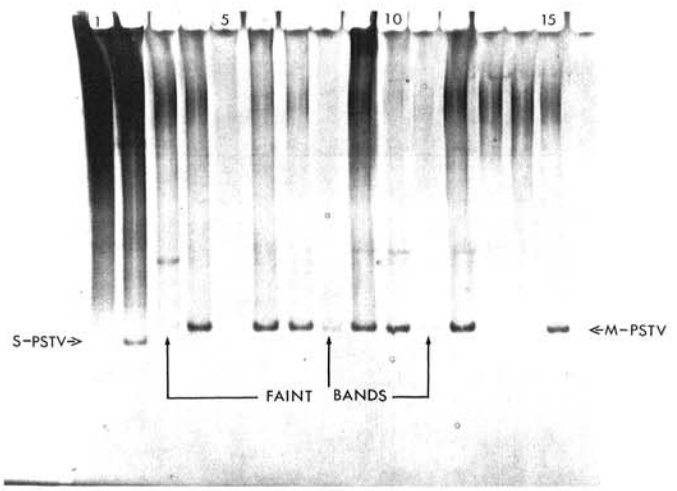

Fig. 1. Return polyacrylamide gel electrophoresis of dormant true potato seeds infected with the mild strain of potato spindle tuber viroid (M-PSTV). Lane 1, extract from healthy seeds; lane 2, severe PSTV; lanes 3–15, single TPS extracts; lanes 3, 8, and 11 contain faint M-PSTV band (arrow). Lanes 4, 6, 7, 9, 10, 12, and 15 contain strong M-PSTV band. Lanes 5, 13, and 14 do not contain a PSTV band. Electrophoresis was carried out at 70–71 C, and the second electrophoresis buffer was heated to 87–90 C. First electrophorsis was on 5% gels under nondenaturing conditions and the second under denaturing conditions at 46 m Amp for 2.5 and 2.0 hr, respectively.

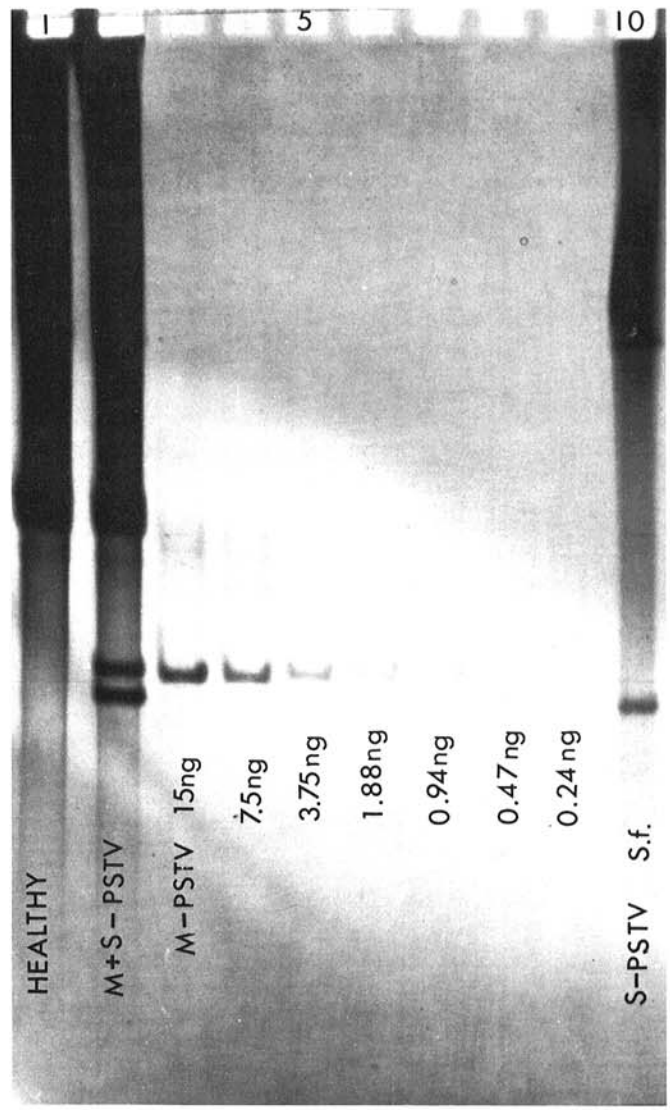

Fig. 2. Return polyacrylamide gel electrophoresis of purified nucleic acids standards. Lane 1, extract from healthy seed; lane 2, mild and severe PSTV mixture from leaves; lanes 3–9, with various amounts of purified nucleic acid (15, 7.5, 3.75, 1.88, 0.94, 0.47, and 0.24 ng) from seed. Lane 10, with S-PSTV from seeds of *S. fendleri*. The electrophoresis conditions were similiar to Figure 1.

another. However, there was no evidence of cross-contamination between the seedlings in the same vessel during the 10 wk of growth.

Detection of PSTV in mixtures of healthy and infected TPS. To determine if R-PAGE can be used to detect low percentages of PSTV infection from TPS, we combined a single TPS from the infected group with 1, 2, 4, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 healthy TPS before germination. The test was replicated three to eight times to increase the probability of getting infected TPS in most samples, and it was assumed that if PSTV infection would be detected in any sample, it would be from the single TPS from the infected group. The results (Table 4) indicated that PSTV can be detected by R-PAGE if one infected seed was mixed with 90–100 healthy seeds (also Lanes 19 and 20, Fig. 3). However, PSTV was detected by dot-blot only up to a 1:50 dilution (Table 4).

Application of R-PAGE method to various seed lots. To determine whether different seed lots affect viroid detection by R-PAGE, 11 TPS lots, stored 1–6 yr, were tested. Six of the TPS seed lots were from healthy potato plants, four were collected from PSTV-infected potato, and one from *Solanum fendleri* Wright (P.I. 458417) plants. Composites of 40 dormant TPS from each lot were tested.

Mild PSTV was detected in the four TPS lots from PSTV-infected plants, severe PSTV was detected in *S. fendleri* (Lane 11, Fig. 2) and PSTV was not detected in the six TPS seed lots from healthy plants. Two additional tests using 40 TPS from each of six lots from healthy plants (dormant or germinated) failed to indicate presence of PSTV.

TABLE 3. Detection of potato spindle tuber viroid in single germinated seed or in vitro-grown seedlings by return polyacrylamide gel electrophoresis

Test material	Seed planted (no.)	Seedling tested (no.)	PSTV infected (no.)	Infected (%)
Germinated seed[a]	102	96	44	45.8
Seedlings[b] tested at 4 wk	98	82	41	50.0
Seedlings tested at 6 wk	98	82	41	50.0
Seedlings tested at 8 wk	98	82	41	50.0
Seedlings tested at 10 wk	98	82	41	50.0
Seedlings[c] tested once during 4 to 10 wk	98	90	45	50.0

[a] Individual true potato seed, germinated on filter paper in a petri plate at 20 C in the dark for 1 wk, were used for nucleic acid extraction.
[b] Individual true potato seedling tips from in vitro culture were used for nucleic acid extraction at various periods after germination.
[c] Individual true potato seedlings tips tested at least once during a 4–10-wk period.

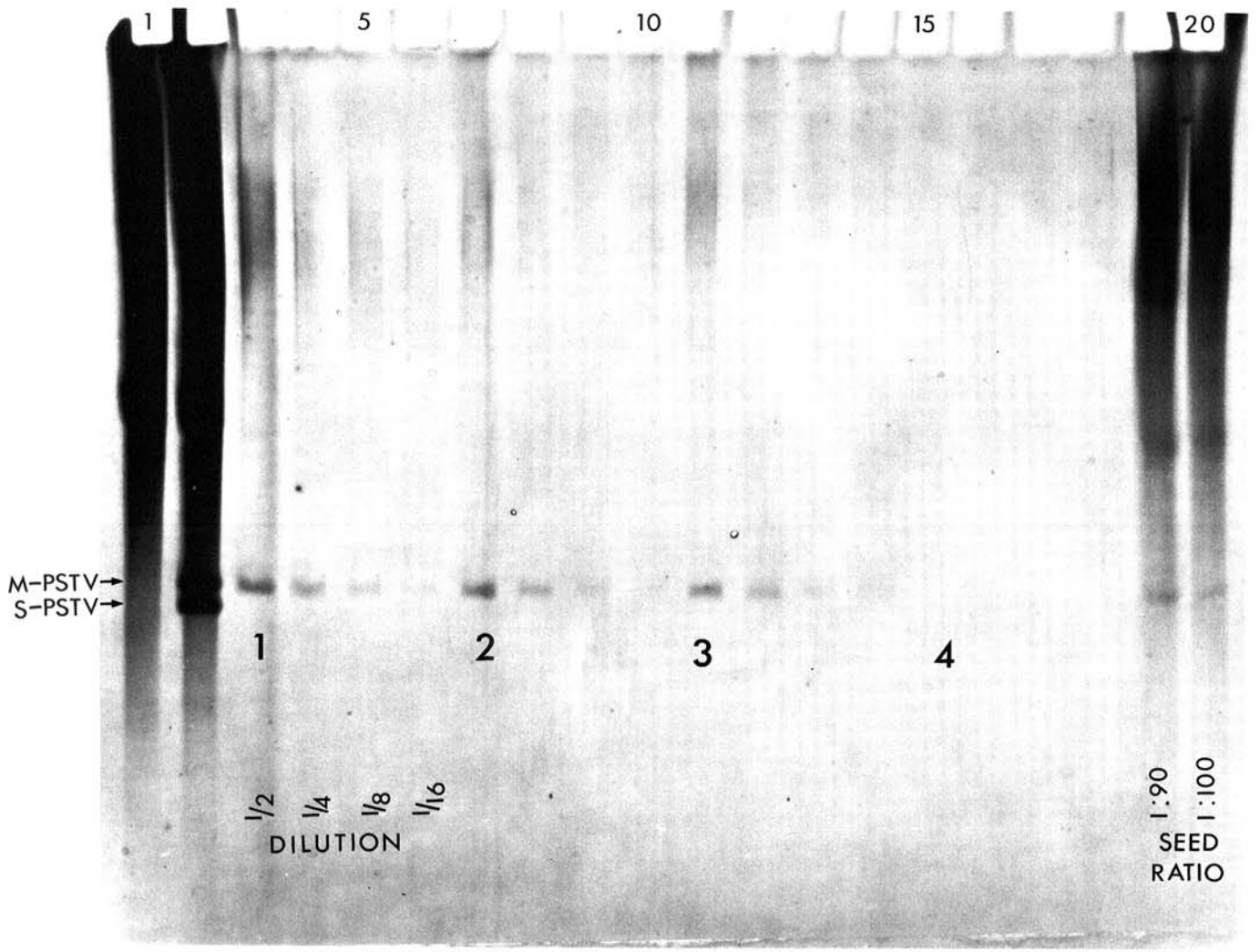

Fig. 3. Return polyacrylamide gel electrophoresis of various dilutions of single TPS extracts. Lanes 1 and 2 are similar to Figure 2. Lanes 3–6 with twofold dilutions of one M-PSTV-infected TPS (1:2, 1:4, 1:8, and 1:16); lanes 7–10, with second M-PSTV infected TPS dilutions; lanes 11–14 with third M-PSTV infected TPS dilutions, and lanes 15–18 with healthy TPS (No. 4) dilutions. Lanes 19 and 20 contain seed extracts from groups containing one infected and 90 healthy seed (lane 19), or one infected and 100 healthy seed (lane 20). The electrophoresis conditions were similar to Figure 1.

TABLE 4. Detection of potato spindle tuber viroid by return polyacrylamide gel electrophoresis or by dot-blot tests of composite true potato seed samples from infected and healthy potato plants

Infected:Healthy[a]	PSTV detection	
	R-PAGE	Dot-blot
1:1	1/3[b]	NT[c]
1:2	2/3	NT
1:4	2/3	NT
1:8	1/3	NT
1:10	4/8	3/5
1:20	4/8	3/5
1:30	3/8	0/5
1:40	2/8	0/5
1:50	4/8	1/5
1:60	2/8	0/5
1:70	6/8	0/5
1:80	2/7	0/5
1:90	4/7	0/5
1:100	4/7	0/5

[a]One dormant seed from an infected lot was combined with various numbers of seeds from healthy plants, nucleic acid extracts were prepared, dissolved in 6 μl of buffer, and assayed by R-PAGE or spotted on a nitrocellulose membrane (3 μl) for the dot-blot test.
[b]Numerator is the number of viroid positive identifications and denominator is the number of tests made.
[c]NT = not tested.

DISCUSSION

The findings reported here clearly demonstrate the feasibility of detecting PSTV in a single TPS by R-PAGE. Because mild strains of PSTV are more common in nature than the severe strains (3,18) and can be differentiated from a severe strain by modified R-PAGE (16 and Fig. 2), this study provides a method of PSTV detection from TPS and separation of strains at the same time. To date, detection of severe strains of PSTV from TPS has been possible only when 16 presoaked seeds (one infected in 15 healthy) were used in a dot-blot test (11) or five dormant seeds or 30 seedlings in bioassay on tomato followed by PAGE (6). Thus, the present method is a significant improvement over earlier detection procedures because of the saving of time and effort, as well as providing a greater sensitivity when used for very small samples, i.e., a single TPS.

It has been observed that tomato plants inoculated with PSTV-infected TPS extracts failed to develop symptoms, although containing PSTV (11), and it was suggested that the PSTV concentration in these samples, although sufficient to infect assay hosts, was not high enough to lead to recognizable symptoms in the test plants (11). Furthermore, a poor correlation exists between symptom expression in PSTV-infected TP seedlings and their bioassay or PAGE results (6). In view of our observation that concentration of PSTV in individual TPS could vary from less than 1 to more than 10 ng (Figs. 1 and 3), the absence of symptoms in inoculated tomato plants or the poor correlation from TP seedlings between PSTV symptoms and detection by bioassay or PAGE, may be a reflection of PSTV concentration. It is possible that in seed extracts with high viroid concentration PSTV could be detected by bioassay or PAGE, while those with a lower concentration may require more than one generation of viroid multiplication to reach the concentration detectable by bioassay or PAGE.

The reliability of PSTV detection in single TPS is indicated by the successful detection of the viroid in individual germinated seeds or in vitro-grown seedlings (Table 3). There was a 4% difference in PSTV infection between seeds and seedlings, and this could result from the expected variation between seed samples taken from the same seed source. The observation that rate of PSTV detection from in vitro-grown seedlings remained constant during the growing period, up to 10 wk, further supports the reliability of the method. Bioassays of seedlings grown beyond 2–3 wk showed a decline in the percent transmission (6).

The rate of seed transmission in PSTV-infected TPS lots is known to vary widely (6,7,14) depending on cultivar, age of seed, and method of detection. Therefore, to be of universal use, a method of PSTV detection that could detect PSTV in TPS of low seed transmission rate is necessary. Evidence that PSTV can be detected reliably from one infected seed mixed with 90–100 healthy seed, provides a basis for composite TPS testing by R-PAGE for low seed-transmitted samples (Fig. 3, Table 4) and could be of considerable value in mass testing regimes. Although the dot-blot test has been shown to detect PSTV in seed extract equivalent to one infected seed mixed with 80 healthy seeds (11), in our study it detected PSTV in composite samples of 1:50 and not beyond (Table 4).

In this study, the R-PAGE method is more reliable and sensitive for detecting PSTV from TPS than the dot-blot method. In R-PAGE no radioactive materials are employed, and the technique can be adopted easily in most developing countries. Samples do not have to be sent away for processing and R-PAGE can, therefore, be faster. Because of a relatively simple change from PAGE to R-PAGE, the latter method has been adopted for large-scale testing of seeds and tubers suspected to be infected with PSTV in Heilongjiang, China (R.C. Cui, *personal communication*). R-PAGE can be used to assess the PSTV content of various seed lots before planting either from seeds, when large numbers of seeds are available, or from in vitro seedlings, when germ plasm is valuable and in limited quantity.

LITERATURE CITED

1. Accatino, P., and Malagamba, P. 1982. Potato production from true seed. International Potato Center, Lima, Peru. 20 pp.
2. Benson, A. P., and Singh, R. P. 1964. Seed transmission of potato spindle tuber virus in tomato. (Abstr.) Am. Potato J. 41:294.
3. Cammack, R. H., and Harris, P. S. 1973. Potato spindle tuber in the Commonwealth Potato Collection. EPPO Bull. 3:117-118.
4. Fernow, K. H., Peterson, L. C., and Plaisted, R. L. 1970. Spindle tuber virus in seeds and pollen of infected potato plants. Am. Potato J. 47:75-80.
5. Grasmick, M. E., and Slack, S. A. 1986. Effect of potato spindle tuber viroid on sexual reproduction and viroid transmission in true potato seed. Can. J. Bot. 64:336-340.
6. Grasmick, M. E., and Slack, S. A. 1987. Detection of potato spindle tuber viroid in true potato seed by bioassay on Rutgers tomato. Am. Potato J. 64:235-244.
7. Hunter, D. E., Darling, H. M., and Beale, W. L. 1969. Seed transmission of potato spindle tuber virus. Am. Potato J. 46:247-250.
8. Jones, R. A. C. 1982. Tests for transmission of four potato viruses through potato true seed. Ann. Appl. Biol. 100:315-320.
9. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.
10. Owens, R. A., and Diener, T. O. 1981. Sensitive and rapid diagnosis of potato spindle tuber viroid disease by nucleic acid hybridization. Science 213:670-672.
11. Salazar, L. F., Owens, R. A., Smith, D. R., and Diener, T. O. 1983. Detection of potato spindle tuber viroid by nucleic acid spot hybridization: Evaluation with tuber sprouts and true potato seed. Am. Potato J. 60:587-597.
12. Schumacher, J., Meyer, N., Riesner, D., and Weidemann, H.L. 1986. Diagnostic procedure for detection of viroids and viruses with circular RNAs by "Return"-gel electrophoresis. J. Phytopathol. 115:332-343.
13. Seabrook, J. E. A. 1987. Changing the growth and morphology of potato plantlets in vitro by varying the illumination source. Acta Hortic. 212:401-410.
14. Singh, R. P. 1970. Seed transmission of potato spindle tuber virus in tomato and potato. Am. Potato J. 47:225-227.
15. Singh, R. P. 1984. *Solanum* × *berthaultii*, a sensitive host for indexing potato spindle tuber viroid from dormant tubers. Potato Res. 27:163-172.
16. Singh, R. P., and Boucher, A. 1987. Electrophoretic separation of a severe from mild strains of potato spindle tuber viroid. Phytopathology 77:1588-1591.
17. Singh, R. P., and Finnie, R. E. 1973. Seed transmission of potato spindle tuber metavirus through the ovule of *Scopolia sinensis*. Can. Plant Dis. Surv. 53:153-154.
18. Singh, R. P., Finnie, R. E., and Bagnall, R. H. 1970. Relative prevalence of mild and severe strains of potato spindle tuber virus in eastern Canada. Am. Potato J. 47:289-293.