Evaluation of procedures for the detection of potato spindle tuber viroid by polyacrylamide gel electrophoresis'

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Two procedures for the extraction of nucleic acids from tissues infected with potato spindle tuber viroid (PSTV) were evaluated. A 'I-day' procedure consisted of homogenization in buffer-phenol-lithium chloride solution followed by precipitation of the nucleic acids with ethanol. A '2-day' procedure involved homogenization of tissues in buffer-phenol mixture, stirring in chloroform-amyl alcohol, lithium chloride precipitation, overnight dialysis and precipitation of nucleic acid with ethanol. The final detection of PSTV was by polyacrylamide gel electrophoresis of preparations using both extraction procedures. Both procedures were equally sensitive; however, band intensity was much stronger with the '2-day' procedure. The band intensity in the 'I-day' procedure was improved by varying the tissue to buffer ratio and by reducing the amount of water in which nucleic acids were dissolved prior to electrophoresis. Using this procedure, the following conclusions were made: 1) PSTV was detected more reliably from foliage than from sprouts, 2) PSTV was detected more reliably from potato plants grown at 25°C than at 15°C, 3) PSTV detection was unreliable from 2 to 3 month-old plants.

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Deux procedures pour l'extraction d'acides nucleiques à partir de tissus infectés par le viroide de la filosité des tubercules de pomme de terre (PSTV) ont ete evaluees. La premiere procedure dite "1 jour" consiste en l'homogeneisation des tissus dans une solution de tampon-phenol-chlorure de lithium suivie par la precipitation des acides nucleiques dans de l'éthanol, la seconde dite "2 jours" implique l'homogeneisation des tissus dans un melange tampon-phenol, agitation dans un melange chloroforme-alcool amylique, precipitation au chlorure de lithium, dialyse au cours de la nuit et precipitation de l'acide nucleique dans de l'éthanol. La détection du PSTV est effectuee par electrophorese sur gel de polyacrylamide des extraits obtenus à l'aide de chaque procedure. Les deux procedures ont montre une sensibilite égale toutefois, l'intensité de la bande était beaucoup plus forte avec les extraits "2 jours". L'intensité de la bande obtenue avec la procedure "1 jour" a ete améliorée en changeant le rapport tissu-tampon et en reduisant la quantité d'eau utilisee pour dissoudre les acides nucleiques avant l'electrophorese. A l'aide de cette procedure, les conclusions suivantes ont été tirees: 1) Le PSTV est detecte plus surement à partir du feuillage que des pousses, 2) PSTV est detecte plus surement chez des plants de pommes de terre cultive à 25°C au lieu de 15°C. 3) La detection du PSTV n'est pas constante chez les plants ages de 2 à 3 mois.

Introduction

The report that potato spindle tuber viroid (PSTV) was a free-ribonucleic acid of low molecular weight (1, 15) and separated as a distinct band on polyacrylamide gel electrophoresis (PAGE) (2) resulted in the development of a PAGE procedure for its routine detection from potato plants (7, 8, 12). The PAGE procedures initially took 2-3 days to complete, but recently the procedure has been modified and can be completed in one day (10).

Potato spindle tuber viroid is carried through the pollen and seeds of potato (3, 6, 13, 14) and has been noted to occur in the major potato germ plasm collections, e.g., the Commonwealth Potato Collection in Scotland (4, 5), the International Potato Center in Peru (5), and the United States Department of Agriculture Potato Collection at Sturgeon Bay, Wisconsin (5, 9). Thus, there is apparent danger of its spreading through the exchange of potato germplasm, either in the form of tuber or as true seed. There is a need for routine testing of large numbers of potato breeding lines and the modified procedure of PAGE (10) appeared worth evaluating. However, when compared with the 2-day procedure the PSTV band appeared weaker. The improvements of this procedure and its suitability for testing sprouts and tubers are the subject of this paper. A preliminary report has appeared elsewhere (16).

Materials and methods

Potato (*Solanum tuberosum* L. cv. Russet Burbank) plantlets infected with a mild strain of PSTV (17) and field-infected tubers of different cultivars and seedlings were used. Tubers were stored at 5°C for 3 months, then sprouted at 25°C for 1 to 3 weeks. The sprouts were either used from one tuber or from three tubers combined to make the desired weight. The 1-day procedure consisted of homogenization of tissues in buffer-phenol-lithium chloride solution (1.0 ml of distilled H_2O, 0.4 ml of 4 M NH_4OH, 0.4 ml of 0.1 M ethylenedinitrilotetraacetic acid disodium salt (adjusted to pH 7.0 with Tris), 1.2 ml of 10 M LiCl, and 4 ml of water saturated phenol containing 0.1% 8-hydroxyquinoline), followed by precipitation of the nucleic acids with ethanol (10). The 2-day procedure consisted of homogenization of the tissues in buffer-phenol solution [0.5 ml of buffer (0.2 M glycine, 0.1 M $Na_2HPO_4.7H_2O$, 0.6 M NaCl, pH 9.6), 0.1 ml of 10% sodium dodecyl sulphate and 2 ml of phenol], stirring the

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aqueous layer in chloroform-amyl alcohol (2 ml), fractionation with lithium chloride, overnight dialysis and final precipitation of the nucleic acids with ethanol (7). The tissues were homogenized with a PT-35 polytron equipped with a PT10-ST microgenerator (Brinkman Instruments, Rexdale, Canada) for 30 sec at full speed. The PAGE was performed on 5% gels [acrylanide: N,N' methylene-bis-acrylamide 40:1 W/W] in 0.04 M tris, 0.02 M sodium acetate, 0.001 M disodium EDTA pH 7.2 (7). Electrophoresis was performed in cylindrical gels (0.6 × 9 cm) at room temperature for 2.5 hr at 6-8 nA/gel. Gels were stained with Toluidine Blue O (7) and, after destaining, were scanned at 550 nm with a Beckman DU-8 spectrophotometer.

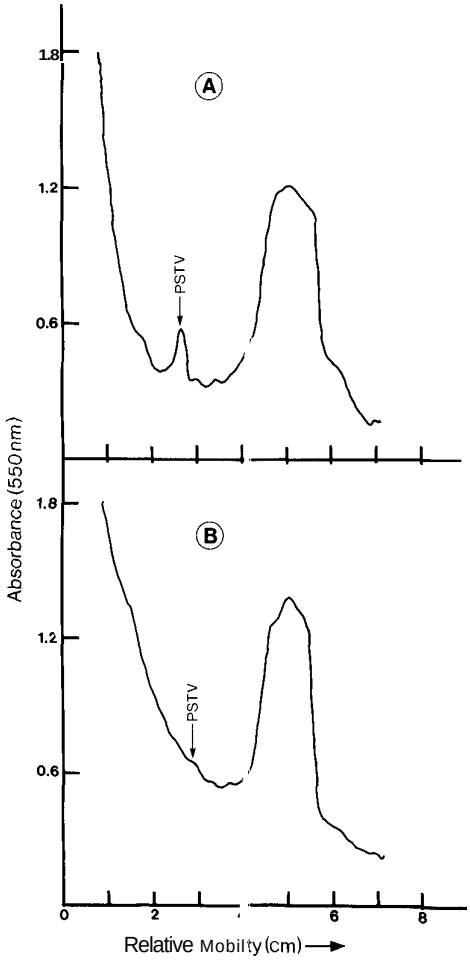

Fig. 1 Scanning profile of stained polyacrylamide gels (5%) containing nucleic acid extracted by 1-day procedure. A) potato plants grown at 25°C, B) potato plants grown at 15°C.

Results

In an initial test, 89 potato cuttings derived from a potato plant infected with a mild PSTV strain (17) and 32 cuttings from a healthy plant were evalcated by the I-day procedure using the published procedure (10). The tests were done by two persons and the indentity of the samples was coded.

Table 1. Comparision of 1-day and 2-day procedures for the detection of potato spindle tuber viroid in potato sprouts.

Test no.	No. of samples	No. positive PSTV	
		I-day procedure	2-day procedure
1	16*	15	16
2	16*	15	15
3	16**	9	9

* Sprouts from three different tubers were combined to make one sample.

** Individual tubers were suspected to be infected with potato spindle tuber viroid.

The results indicated that all 89 PSTV samples were positive while the 32 healthy cuttings were negative on the basis of the PSTV gel banding. However, the PSTV bands were fainter in some cases and repeated testing of such samples often gave PSTV bands of variable intensity. Since the I-day procedure was reliable, efforts to improve the PSTV band were made by manipulating buffer to tissue ratio and final volume of water to dissolve the precipitated nucleic acid. After several tests it was observed that when the tissue to extracting buffer ratio was doubled (0.5 g: 7 ml) the PSTV peak was more pronounced, compared to the single volume (0.5 g:3.5 ml) as used by Pfannenstiel *et al.* (10). Similarly, the 50, 100, and 150 µl of water used to dissolve the nucleic acid gave varying degrees of peak height. Use of 100 ul appeared suitable while those of 50 µl and 150 µl gave broader peaks. Further, we tested 72 samples of potato sprouts, 36 using modifications discussed above and 36 by the published procedure (10). Both methods detected the same number of PSTV positives (24 each), but the band's intensity was more than double of the published procedure in 20 of the 24 samples by improved procedure described here.

Table 2. Effect of potato plant parts on the detection of potato spindle tuber viroid by polyacrylamide gel electrophoresis.

Plant parts	No. samples*	No. PSTV positives
Leaves	31	30
Sprouts	31	28
Sprouts – stem end	16	15
Sprouts – mid tuber**	16	16
Sprouts – bud end	16	16
Tuber tissue***	31	24

* About 0.5 g of tissue was used for extraction of nucleic acid.

** Sprouts in the central part of the tuber, away from stem or bud ends.

*** Tuber tissue from the bud end without any sprouts.

Comparison of I-day and 2-day procedures. Etiolated sprouts from field-infected tubers of genotypically different seedlings were combined (3 tubers/sample) for the extraction by the I-day and 2-day procedures. The results (Table 1) showed that both procedures were equally sensitive. In the first and second test the sprouts from the same tubers were used a week apart, while in the third test suspect potato tubers were used. In contrast to Pfannenstiel *et al.* (10) we noticed again that PSTV bands were more distinct in the 2-day procedure than the I-day published procedure (10). The seven negative sprouts from the third test were retested after growing the plants. They remained healthy.

Suitablity of I-day procedure for testing potato plant parts. About 0.5 g of tissue from sprouts of 36 tubers were tested individually by the I-day procedure. Thirty-one were found positive for PSTV. One eye from each of these 31 tubers was planted and leaves were tested about 1 month after emergence. The remaining tuber was used to collect 0.5 g of sprouts from various tuber parts (near stem end, mid-part and bud-end); and also tuber tissues from the bud-end were tested. The results (Table 2) showed that 30 were detected from the leaves, but only 28 from the sprouts. However, sprouts taken from various parts of the tuber were all positive and it appears that PSTV was equally distributed throughout the tuber. The PSTV detection was poor from the tuber tissues (Table 2). In addition to poor detectability of PSTV from tuber tissue, several faint bands were encountered in the vicinity of the PSTV band, which also made identification difficult.

Table 3. Effect of air temperature on the potato plants and the detection of potato spindle tuber viroid by polyacrylamide gel electrophoresis.

Age of plants	No. of samples*	15°C	25°C
1 week	32	31	29
4 weeks	32	16**	32
6 weeks	32	13	30
8 weeks	31	8	26
12 weeks***	30	9	21

* Only the top young growing tips were used in all the tests.

** Potato spindle tuber viroid bands were very faint.

*** The top growth was senescent and leaves were chlorotic.

Effect of air temperature on PSTV detection in potato by PAGE. The concentration of PSTV in tomato plants has been shown to increase at high temperature (4,11), but no information is available on PSTV concentration in potato plants and its effect on detectability of PSTV by PAGE. To determine this, 32 known PSTV-infected tubers were cut in two and planted one at 15°C and the other 25°C. The temperature range (15-25°C) selected was that which is encountered in the greenhouses during winter-indexing of potatoes for mosaic diseases. The results (Table 3) showed that within one week of emergence 31 and 29 samples were indexed PSTV positive at 15" and 25°C, respectively. However, the PSTV detection improved at 25°C up to six weeks and then declined slowly (Table 3), while at 15°C the PSTV detection declined sharply after the first week. When the PSTV bands of the same plants from both temperatures were compared by scanning, after four weeks of growth, it was noticed that PSTV peaks from 25°C grown plants were very distinct while those at 15°C were barely visible (Fig. 1A,B). The difficulty in PSTV detection could be due to low concentration of viroid present, as observed by the infectivity test (Table 4).

PSTV detection by PAGE from potato plants inoculated at various stages of growth. Twenty-four virus-free cuttings of equal age were grown at 25°C and were inoculated with a mild strain of PSTV at 1, 2 and 3 months apart. Plants inoculated at 1 month developed symptoms within 4 weeks of inoculation and PSTV was detected from apical leaves of each plant. However, no symptoms developed in plants inoculated at 2 and 3 months growth, and only 2 of the 6 plants inoculated at 2 months growth stage were found PSTV positive, while none of the 6 plants inoculated at 3 months growth stage yielded any PSTV by PAGE.

Table 4. Effect of air temperature on viroid infectivity at various periods of arowth.

	Average number of lesions""	
Age of plants"	25°C	15°C
1 week	376	321
3 weeks	276	162
6 weeks	143	16
9 weeks	40	0.6

* Potato cuttings 10–15 cm in height infected with mild strain of potato spindle tuber viroid were transferred to growth cabinet at specified temperatures.

** Composite samples from 5 plants were ground in glycine phosphate buffer (0.05 M glycine + 0.03 M K_2HPO_4, pH 9.2) and inoculated to 10 leaves of *Scopolia sinensis* plants.

Discussion

This study confirms that the shortened procedure of Pfannenstiel *et al.* (10) for PSTV detection is as reliable as the 2-day procedure of Morris & Smith (7), except that band intensity was variable prior to the improvements made in this study. The shortened procedure enables one worker to complete 60 to 80 tests per week using cylindrical gels and Toluidine Blue O staining. The efficiency could be improved further with slab-gel and ethidium bromide staining and photography. However, caution must be exercised in putting too much emphasis on the number of tests performed, rather than on the quality of nucleic acid extraction. It has been observed that steps of tissue homogenization, tissue to buffer ratio, drying of nucleic acid precipitate, and resuspension of precipitated nucleic acid could affect the results. It

was noticed that when nucleic acid precipitates were air dried instead of vacuum drying (10), the precipitates did not dissolve and PSTV bands did not separate from other nucleic acid. However, drying with N_2 gas had similar effect as vacuum drying. Strict adherence to the prescribed steps are needed for reproducible results. The negative results should be retested, if the material is for use in pollinations for potato breeding or for multiplication.

From the experiments with temperature, it is obvious that for the best results potato tubers should be sprouted or grown at 25°C rather than at low temperatures, and plants should be tested while green and young, rather than very mature plants.

Since recovery from plants inoculated at maturity was poor and such plants did not develop symptoms, there is some indication that mature plant resistance may be operative in viroid diseases also.

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