DETECTION OF POTATO VIRUSES X AND S IN TISSUE CULTURE PLANTLETS

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Abstract

The latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) were evaluated in separate studies for their ability to detect potato virus X (PVX) and potato virus S (PVS) in tissue culture plantlets. Healthy and infected clonal lines of several potato cultivars were used. LAT was unsatisfactory because only low levels of agglutination were obtained with infected samples, and because variable and inconsistent results were obtained with both healthy and infected clones. ELISA, however, consistently gave high spectrophotometric readings and intense visual reactions for infected but not for healthy clones. The results indicate that ELISA can be used to detect PVX and PVS in tissue culture plantlets, and in programs where tissue culture is employed, early detection and elimination of infected plantlets is possible.

Resumen

En estudios separados se evaluaron la prueba de aglutinación con Latex (LAT) y la de inmunoabsorción con conjugados enzimáticos (ELISA) con el fin de determinar su capacidad para detectar los viruses X y S de la papa en plántulas de cultivo de tejidos. Se utilizaron líneas clonales sanas e infectadas de varios cultivares de papa. La prueba LAT no fue satisfactoria porque sólo se obtuvieron niveles de aglutinación bajos con las muestras infectadas y porque se obtuvieron resultados variables e inconsistentes con ambos clones, sanos e infectados. Sin embargo, ELISA dió consistentemente valores espectrofotométricos altos y reacciones intensas a simple vista para las muestras infectadas pero no para los clones sanos. Los resultados indican que ELISA puede usarse para detectar PVX y PVS en plántulas de cultivo de tejido y que en programas en los que se utiliza cultivo de tejidos es posible la detección temprana y eliminación de las plántulas infectadas.

Introduction

Tissue culture, specifically shoot-tip and meristem-tip culture, plays an important role in the production of nuclear potato seed stocks in New York

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State. In addition to providing pathogen-free material, it is also used for rapid multiplication (9). However, material produced by tissue culture is not necessarily pathogen-free, so consequently must be screened for potato pathogens.

Potato virus X (PVX) and potato virus S (PVS) commonly occur in potato stocks in North America (10). Yield losses from these viruses have been frequently reported (8, 15, 16, 20, 22, 23). Consequently, the elimination of PVX and PVS from seed stocks is a concern in many seed certification programs (10). Prior to this study, there were no data indicating whether existing virus assays were suitable for detecting PVX or PVS in tissue culture plantlets. While satisfactory in detecting PVS in mature plants, chloroplast agglutination is not sensitive enough to detect PVS in tissue culture plantlets (Jones, unpublished). Although chloroplast agglutination can detect PVX in plantlets, it is not as reliable as the inoculation of *Gomphrena globosa.* Inoculation of *Gomphrena globosa* appears to reliably detect PVX in plantlets (Jones, unpublished), but use of an indicator plant requires time and greenhouse space. In addition, a suitable indicator plant for PVS is not available (2).

Separate studies were undertaken during 1979-81 to determine whether the latex agglutination test (LAT) or the enzyme-linked immunosorbent assay (ELISA) could be used to reliably detect PVX and/or PVS in tissue culture plantlets of several potato cultivars. Each of these methods has previously been shown to detect these and other potato viruses in potato leaf and tuber tissue (1, 3, 5, 6, 11, 12, 14). Recently, McMorran and Allen (19) reported results of tests where ELISA successfully detected several viruses, including PVX and PVS, in tissue culture plantlets of a single cultivar, Russet Burbank; however, details of their ELISA procedure were not given.

Materials and Methods

Tissue culture plantlets produced by shoot-tip or meristem-tip culture (9), were obtained from the Henry Uihlein II Laboratory, Lake Placid, NY. These included plantlets free of both viruses (X^-S^-), infected with PVS only (X^-S^+), and plantlets infected with both viruses (X^+S^+). The virus content of each plantlet line was confirmed by testing mature greenhouse plants grown from each line, using chloroplast agglutination for PVS and *Gomphrena globosa* for PVX.

The LAT was performed basically as described by Khan and Slack (11), using the micro method of Marcussen and Lundsgaard (17) and Lundsgaard (13). The PVX and PVS antisera were purchased from Montana State University, and the procedure for conjugation of the antisera and latex particles was obtained from Dr. Steven A. Slack (Univ. of Wisconsin, personal communication).

In preliminary tests with LAT for PVS, only low levels of agglutination were obtained with infected plantlet tissue. Attempts were made to increase the amount of agglutination by varying conditions such as the antiserum concentration, sample dilution, reaction time, and ratio of latex preparation to sample preparation, but were not uniformly successful. However, based on the preliminary tests which gave optimal results, the following sample preparation and test procedures were used: tissue samples were ground with a mortar and pestle, and three drops (using Pasteur pipets) of the resulting sap were diluted in 1 ml of Tris buffer (0.1 M, pH 7.4, with 0.85% NaCl) to obtain the sample preparation. Equal volumes (15 ul) of the 1/25 sensitized latex preparation (11) and the sample preparations were used. Reaction time was 15 minutes.

Because of the low level of agglutination, a simple qualitative scale was used to score the results of individual tests: + indicated a distinct positive reaction; − indicated a distinct negative reaction; and ± indicated an indistinct or inconclusive reaction.

Entire plantlets, excluding roots, or leaves only were used as the tissue source. Several potato cultivars were included in the tests for PVS: Bake-King, C-13, Green Mountain, LaRouge, Rosa and Superior, with varying numbers of healthy and infected samples of each. In the tests for PVX, Chippewa and Katahdin plantlets were used; five samples each of the X^-S^- and X^-S^+ clones, and 10 samples of the X^+S^+ clones were used for each cultivar.

The double-sandwich ELISA procedure used closely followed that of Clark and Adams (4). Coating globulins and conjugates were incubated for 4 hrs at 37 C, while samples were incubated overnight at 4 C. Substrate was incubated 50 min at room temperature. Reactions were assessed first visually, then spectrophotometrically with a Micro-ELISA Auto Reader (Dynatech Laboratories, Inc., Alexandria, VA).

For ELISA, the PVX antiserum was again obtained from Montana State University, while the PVS antiserum was supplied by Dr. Slack. Three different combinations of coating globulin/conjugate concentrations were tested: A) coat with 2.5 μg/ml, conjugate with 1:400 dilution of stock; B) coat with 1.0 μg/ml, conjugate with 1:400 dilution of stock; and C) coat with 1.0 μg/ml, conjugate with 1:1000 dilution of stock.

A 1:9 tissue dilution was prepared by grinding a gram of tissue in 9 ml buffer with a mortar and pestle. The resulting sap was strained through two layers of cheesecloth. Entire plantlets, excluding roots, were used. Tests were performed on healthy and infected lines of 5 potato cultivars: X^-S^-, X^-S^+ and X^+S^+ clones of Chippewa, Katahdin and Sebago, X^-S^- and X^+S^+ clones of Green Mountain, and X^-S^+ and X^+S^+ clones of Kennebec. Tests for PVX and PVS were performed separately, but utilized the same sample

preparations. Five samples were prepared from each line, and each sample was replicated twice.

Results

The effectiveness of LAT assays for PVS in plantlets was limited by the low levels of agglutination obtained with known infected samples. Results with both healthy and infected samples were inconsistent. The LAT assays for PVS are summarized in Table 1. PVS-infected samples (X^-S^+) gave positive, negative and indistinct agglutination reactions; healthy samples (X^-S^-) yielded both negative and indistinct reactions. This was most pronounced when entire plantlets were used as the tissue source. When leaves only were assayed, the number of indistinct reactions was reduced but not eliminated.

TABLE 1. — *Summary of latex aggutination tests for PVS in plantlets.*

Sample	No. of samples	Test Results (No.)		
		+	−	±
Entire plantlet excluding roots				
C-13 X^-S^-	65	0	38	27
ROSA X^-S^-	14	0	8	6
ROSA X^-S^+	14	7	2	5
SUPERIOR X^-S^-	20	0	10	10
SUPERIOR X^-S^+	66	33	0	33
Leaves only				
BAKE-KING X^-S^+	14	12	2	0
C-13 X^-S^-	4	0	4	0
GREEN MOUNTAIN X^-S^-	6	0	6	0
LAROUGE X^-S^-	8	0	8	0
ROSA X^-S^-	26	0	26	0
ROSA X^-S^+	31	18	11	2
SUPERIOR X^-S^-	26	0	26	0
SUPERIOR X^-S^+	44	29	1	14

Table 2 summarizes the results of the LAT assays for PVX. These data are similar to those for PVS. Indistinct reactions did occur, but less frequently when leaves only were tested. In general, LAT appeared to perform better for PVX than PVS, giving more agglutination in known positives.

The results of the ELISA tests for PVS and PVX are summarized in Table 3. Visual detection occurred at a spectrophotometric reading of approximately 0.04-0.05. In general, the mean absorbance values for the healthy samples or buffer controls were low, and there was little, if any, visual reaction. Conversely, infected samples gave strong visual reactions and high absorbance values.

The greatest amount of nonspecific reaction in the ELISA tests with healthy samples occurred at the higher concentration of conjugate, and ap-

TABLE 2. — *Summary of latex agglutination tests for PVX in plantlets.*

Sample	No. of samples	Test Results (No.) +	−	±
Entire plantlet excluding roots				
CHIPPEWA X^-S^-	5	0	5	0
X^-S^+	5	0	4	1
X^+S^+	10	5	2	3
KATAHDIN X^-S^-	5	0	4	1
X^-S^+	5	0	3	2
X^+S^+	10	5	1	4
Leaves only				
CHIPPEWA X^-S^-	5	0	5	0
X^-S^+	5	0	5	0
X^+S^+	10	6	1	3
KATAHDIN X^-S^-	5	0	5	0
X^-S^+	5	0	5	0
X^+S^+	10	7	0	3

TABLE 3. — *Summary of ELISA tests for PVS and PVX in plantlets: Mean A_{405} values.*[1]

Sample	PVS Antiserum/Conjugate Dilution[2] A	B	C	PVX Antiserum/Conjugate Dilution[2] A	B	C
BUFFER (PBS)	0.02	0.02	0.00	0.02	0.01	0.00
CHECK[3]	1.5+[4]	1.5+	1.32	1.5+	1.20	0.53
CHIPPEWA X^-S^-	0.08	0.07	0.03	0.10	0.06	0.05
X^-S^+	1.5+	1.38	0.82	0.05	0.04	0.00
X^+S^+	1.5+	1.5+	1.04	1.5+	1.38	0.60
GREEN MOUNTAIN						
X^-S^-	0.28	0.24	0.11	0.18	0.17	0.02
X^+S^+	1.5+	1.5+	1.44	1.5+	1.5+	0.71
KATAHDIN X^-S^-	0.13	0.08	0.04	0.12	0.08	0.02
X^-S^+	1.5+	1.5+	0.92	0.12	0.06	0.03
X^+S^+	1.5+	1.5+	0.97	1.5+	1.35	0.53
KENNEBEC X^-S^+	1.5+	1.5+	0.99	0.40	0.34	0.09
X^+S^+	1.5+	1.5+	0.92	1.5+	1.5+	0.69
SEBAGO X^-S^-	0.02	0.00	0.00	0.08	0.11	0.03
X^-S^+	1.5+	1.43	0.84	0.13	0.12	0.03
X^+S^+	1.5+	1.5+	0.95	1.5+	1.34	0.57

[1]Means of five samples, two wells per sample

[2]A = 2.5 μg/ml coating antiserum, 1:400 dilution of conjugate; B = 1.0 μg/ml coating antiserum, 1:400 dilution of conjugate; C = 1.0 μg/ml coating antiserum, 1:1000 dilution of conjugate

[3]Greenhouse Katahdin X^+S^+ plant

[4]Maximum spectrophotometer reading of 1.500

peared to be more prevalent with certain cultivars. For example, when Kennebec X^-S^+ plantlets were tested for PVX or Green Mountain X^-S^- plantlets

tested for PVS, relatively high absorbance values occurred when the conjugate dilution was 1:400. These values were greatly reduced and essentially all visual reactions for healthy tissue were eliminated at a conjugate dilution of 1:1000. This was true for all cultivars. However, at this lower conjugate concentration, the absorbance values and visual reactions in the infected samples remained strong (≥ 0.82 for PVS and ≥ 0.53 for PVX).

Discussion

Tissue culture plays an important role in the production of potato seed stocks in New York (9) and in other states and provinces in North America (7, 21, 24) and very likely will increase in use and importance. Appropriate techniques for detecting viruses and other pathogens in tissue culture plantlets are necessary to ensure maximum efficiency and effectiveness in the development of nuclear stocks.

In separate studies reported here, two serological techniques, the latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) were tested for their ability to detect potato viruses X (PVX) and S (PVS) in tissue culture plantlets of several potato cultivars. These methods have previously been shown to detect these and other potato viruses in potato leaf and tuber tissue (1, 3, 5, 6, 11, 12, 14). Recently, McMorran and Allen (19) reported on the use of ELISA to detect several viruses in tissue culture plantlets of a single cultivar, Russet Burbank.

Results indicated that LAT, as performed here, was not a suitable method for testing tissue culture plantlets for PVX or PVS infection. Only very low levels of agglutination were achieved with infected samples, and this contributed to the frequent occurrence of indistinct or negative results. The low level of agglutination was likely due to low titer of the viruses in plantlet tissue. LAT, therefore, lacked the sensitivity and consistency to warrant its use in the routine screening of plantlets.

Work with ELISA began after some of the limitations with LAT had been identified. The rapid success with ELISA was a major reason there was no further attempt to modify LAT to increase its sensitivity in this system. Additional efforts might have improved the performance of LAT.

ELISA was very sensitive and consistent in these tests. When a low concentration of conjugate (1:1000) was used, low readings were obtained for healthy samples while maintaining strong reactions with infected samples of all the cultivars. This clear separation between healthy and infected samples should make the routine testing of unknown samples possible.

ELISA is a relatively simple procedure to perform, requiring only 1.5 days for the results. It can be used to detect PVX and PVS in tissue culture plantlets of different potato cultivars, and its use in programs employing tissue culture should result in early detection and, consequently, facilitate elimination of PVX and PVS in nuclear potato seed stocks.

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