# A Fast-Reacting Bioassay for the Tobacco Veinal Necrosis Strain of Potato Virus Y ($PVY^N$)

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**ABSTRACT**

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*Solanum brachycarpum* Plant Introduction 498021 reacted with necrotic symptoms when inoculated with various isolates of the tobacco veinal necrosis strain of potato virus Y ($PVY^N$). Symptoms consisted of necrotic local lesions, wilting of leaflets, collapse of petioles, and death of entire plants within 7–10 days after inoculation. Inoculating *S. brachycarpum* plants with isolates of the common strain, $PVY^O$, produced mosaic symptoms within 13–16 days. Potato viruses M, S, and X did not cause any visible symptoms in *S. brachycarpum* plants, but potato virus A produced mosaic symptoms. Since the presence of potato virus X (PVX) in *S. brachycarpum* plants or simultaneous inoculation with PVX and PVY did not change the PVY symptomatology, there is no need to remove PVX from the sample, as is required for $PVY^N$ detection with the tobacco bioassay. Necrotic symptoms caused by $PVY^N$ isolates appeared at a temperature range of 20–35 C and at a light intensity of 48–72 to 140–196 $\mu E \cdot s^{-1} \cdot m^{-2}$. Therefore, this plant is suitable for bioassay under a range of environmental conditions. *S. brachycarpum* developed symptoms typical of either $PVY^N$ or $PVY^O$ isolates when inoculated with samples from potato foliage, sprouts, or tubers infected with those strains.

Additional keywords: dot-immunobinding assay, indicator host for PVY, PVY strains

The classification of potato virus Y (PVY) isolates that infect potato systemically into groups of common ($PVY^O$) or necrotic ($PVY^N$) strains is based on the reactions of tobacco (*Nicotiana tabacum* L. 'Samsun' or 'White Burley'). Isolates of $PVY^O$ cause vein-clearing followed by mild mosaic symptoms in tobacco, whereas $PVY^N$ isolates cause vein-clearing and veinal necrosis (4). A few PVY isolates that do not infect potato systemically can cause interveinal necrosis of tobacco, but these do not belong to the $PVY^N$ group (8). Thus, the presence of veinal necrosis symptoms in tobacco, in the absence of potato virus X (PVX), constitutes a definite diagnosis on indicator species of the tobacco veinal necrosis strain of PVY (5,7,8,13). Specific monoclonal antisera to distinguish these strains are now also available (10,16).

The appearance of $PVY^N$ symptoms in tobacco cv. Samsun may take 10–21 days, depending on the environmental conditions (R. P. Singh, *personal observation*). In addition, PVX and $PVY^O$, when present together in the inoculum, can mimic the veinal necrosis symptomatology caused by $PVY^N$ in tobacco (6,15). Thus, the removal of PVX from the test samples is an essential step for definite diagnosis of $PVY^N$ (17). This latter step may delay the identification of $PVY^N$ by an additional 2–3 wk. Because the Canada/USA $PVY^N$ Management Plan (1) requires testing of all generation II seed every year, there is a need for a fast-reacting, reliable bioassay host for the definite diagnosis of $PVY^N$. This study reports that *Solanum brachycarpum* Correll Plant Introduction (PI) 498021 is such a bioassay host. Additionally, it can be used with the test sample that may contain PVX without jeopardizing $PVY^N$ diagnosis.

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## MATERIALS AND METHODS

**Potato virus Y isolates and test plants.** All $PVY^N$ isolates, irrespective of their origin, were mechanically inoculated to potato (*S. tuberosum* L.) cultivar Jemseg, which is immune or highly resistant to potato viruses A, S, and X but susceptible to PVY (14). Jemseg-derived virus cultures were transferred to and maintained in tobacco cv. Samsun. The $PVY^N$ isolates were N-27, P-579, Q-9, TU-619, TU-654, and TU-660, as described earlier (16), plus TU-78 from potato cv. Norland from Manitoba and obtained from J. G. McDonald (Central Plant Health Laboratory, Nepean).

Eight $PVY^O$ isolates were collected from eastern Canada that differed in their symptom severity in potato cv. Jemseg, with isolate 1 being the least and isolate 8 the most severe (16). Five $PVY^O$ isolates—Excetrona, No. 2391, TGB-Scotland, MA-927, and MA-667—collected from other countries and British Columbia, Canada, were obtained from P. J. Ellis (Agriculture and Agri-Food Canada, Vancouver, BC), and isolates Y-136 and L-56 were obtained from J. G. McDonald. Potato plants showing mosaic symptoms in the field were also included in some experiments.

Tubers of *S. brachycarpum* PI 498021 were obtained from the NRSP-6 Potato Introduction Project (formerly IR-1), Sturgeon Bay, Wisconsin. Plantlets were multiplied by cuttings and used when the plants were 8–10 cm tall. True potato seed were obtained from berries as a result of self-pollination. The seed were germinated after treatment with 1,500 ppm of gibberellic acid for 24 hr at room temperature, rinsed with distilled water, and planted in vermiculite. Seedlings were used when they had five to six well-developed leaves.

**Symptom development using potato foliage, sprouts, and tuber extracts as inoculum.** *S. brachycarpum* plants were inoculated with extracts prepared from potato foliage, sprout, or tuber tissues. The plant tissues containing $PVY^O$ or $PVY^N$ were ground with a tuber slicer. Plant saps were diluted 1:1 with buffer (0.01 M sodium phospshate and 0.4% sodium sulfite, pH 7.5) prior to inoculation.

**Effect of PVX in the inoculum or in the test host.** *S. brachycarpum* plants were multiplied by cuttings from mother plants infected with PVX. These plants were compared with PVX-free seedlings grown from true potato seed for a $PVY^N$ reaction. In addition, PVX-free seedlings were used to determine the effect of the simultaneous presence of PVX in the inoculum with PVY.

**Symptoms following aphid inoculation.** Five healthy *S. brachycarpum* plants were exposed to 100 winged aphids (*Myzus persicae* (Sulzer)) in an insect-proof cage with $PVY^N$-infected tobacco plants in the center of the cage. Aphids were allowed to feed for 48 hr, then were killed by fumigation. Plants were observed for symptom development for up to 2 wk.

**Immunological detection.** The presence of PVY was verified in test plants by enzyme-linked immunosorbent assay (ELISA) or dot-immunobinding assay (DIBA) when the concentration of the virus was too low for detection by ELISA (16). The ELISA was carried out with polyclonal antibody F for $PVY^O$ and monoclonal antibody 4E7 for $PVY^N$ (10,16).

In ELISA, Immulon I plates were coated with 1 μg/ml of polyclonal or 0.5 μg/ml of monoclonal antibodies in coating buffer (0.05 M sodium carbonate + sodium bicarbonate, pH 9.6) and incubated at 37 C for 4 hr. Samples (200 μl) of healthy or infected plants (sap diluted 1:20 in phosphate buffered saline–Tween 20 and 2% polyvinylpyrrolidone) were loaded and incubated overnight at 4 C. Enzyme-conjugated antibodies were used at 1:600 and 1:2,000 dilutions for polyclonal and monoclonal antibodies, respectively. After addition of 1 μg/ml of the substrate *p*-nitrophenyl phosphate, absorbance was measured at 405 nm.

For $PVY^N$ detection in DIBA, 10-μl samples were spotted on a nylon membrane (Magnacharge, MSI, Westboro, MA), dried for 1 hr, blocked with 1× Tris-buffered saline (TBS) (20 mM Trisbase, 500 mM NaCl, pH 7.5) + 3% BSA for 2 hr, and washed in TBS. Membranes were then incubated overnight in mouse anti-$PVY^N$ (1:500,000 dilution) containing 1× TBS + 2% PVP + 0.2% BSA, rinsed briefly, washed three times, and then incubated with anti-mouse IgG-alkaline phosphatase conjugate (1:4,000 dilution) in the TBS-PVP-BSA solution. A combination of 5-bromo-4-chloro-2-indolyl phosphate and 4-nitro blue tetrazolium chloride dye was used for detection (16).

**Reaction to common potato viruses.** *S. brachycarpum* plants were tested for their susceptibility to mechanically transmitted potato viruses A, M, S, and X. Five plants were inoculated with each virus and observed for symptom development for 4 wk. Indicator plants were *Physalis angulata* L. (12) for potato virus A, *Datura metel* L. (2) for virus M, *N. debneyi* Domin (2) for virus S, and *Gomphrena globosa* L. (3) for PVX.

Infection of test plants with the various viruses was verified by either ELISA or DIBA. All tests were carried out at least three times during a 12-mo period.

## RESULTS

**Potato virus Y isolates and test plants.** Of 329 PIs of tuber-bearing *Solanum* species evaluated for $PVY^N$ reaction (*unpublished*), four were *S. brachycarpum*. In the first test, all 10 plants of PIs 498021 and 498249 inoculated with $PVY^N$ died within 10–12 days postinoculation, whereas plants of PIs 230459 and 243344 reacted with mosaic symptoms. In PIs with necrotic reactions, local, nearly circular, necrotic lesions developed within 5–6 days, followed by veinal necrosis, wilting of the leaflets, and dying of the entire apical part of the plant (Fig. 1, right). In subsequent tests, *S. brachycarpum* PI 498021 plants were inoculated with seven $PVY^N$ isolates, 13 known $PVY^O$ isolates, and two PVY isolates (Y-136 and L-56) of doubtful strain status. All $PVY^N$ isolates caused necrotic symptoms and all other isolates caused mosaic symptoms (Fig. 1, left). Necrotic symptoms developed within 7–10 days and mosaic symptoms usually took 13–16 days postinoculation. Symptoms developed more slowly in TU-78 than in the other $PVY^N$ isolates (Table 1), but eventually symptoms were the same.

**Symptom development using potato foliage, sprouts, and tuber extracts as inoculum.** *S. brachycarpum* plants developed necrotic symptoms with $PVY^N$ and mosaic symptoms with $PVY^O$, irrespective of whether inoculum was prepared from foliage, sprouts, or tubers. Generally, symptom development was 2–3 days slower in plants inoculated with tuber extracts than in those inoculated with foliage or sprout extracts.

**Symptoms following aphid inoculation.** When *S. brachycarpum* plants were exposed to winged aphids and a $PVY^N$ source in a cage, all five became infected. Symptom development was similar to that following mechanical inoculation, except that no local lesions were observed. Most of the leaves showed veinal necrosis and wilting, and most of the plants died. Symptoms appeared 8–10 days after aphid inoculation.

**Reaction to common potato viruses.** *S. brachycarpum* plants inoculated with PVA showed mosaic symptoms 18 days later. No symptoms were observed after

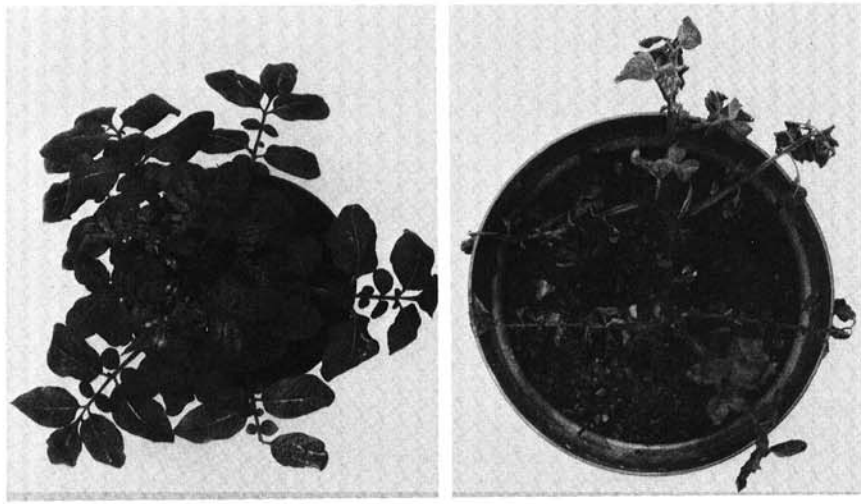

**Fig. 1.** Symptoms on *Solanum brachycarpum* PI 498021 2 wk after inoculation with (left) $PVY^O$, the common strain of PVY, and (right) $PVY^N$, the tobacco veinal necrosis strain of PVY.

**Table 1.** Reaction of various $PVY^N$ and $PVY^O$ isolates to plants of *Solanum brachycarpum* PI 498021

| Isolates | Symptoms[a] | | Symptom appearance (days) | |
|---|---|---|---|---|
| | Without PVX | PVX-infected | Without PVX | PVX-infected |
| $PVY^N$ | | | | |
| N-27 | LL,N,D | LL,N,D | 7–10 | 7–10 |
| Q-9 | LL,N,D | LL,N,D | 7–10 | 7–10 |
| P-579 | LL,N,D | LL,N,D | 7–10 | 7–10 |
| TU-78 | N,D | N,D | 10–13 | 10–13 |
| TU-619 | LL,N,D | LL,N,D | 7–10 | 7–10 |
| TU-654 | LL,N,D | LL,N,D | 7–10 | 7–10 |
| TU-660 | LL,N,D | LL,N,D | 7–10 | 7–10 |
| $PVY^O$ | | | | |
| No. 1 | M,NS | M,NS | 13–16 | 13–16 |
| No. 2 | M,NS | M,NS | 13–16 | 13–16 |
| No. 3 | M,NS | M,NS | 13–16 | 13–16 |
| No. 4 | M,NS | M,NS | 13–16 | 13–16 |
| No. 5 | M,NS | M,NS | 13–16 | 13–16 |
| No. 6 | M,NS | M,NS | 13–16 | 13–16 |
| No. 7 | M,NS | M,NS | 13–16 | 13–16 |
| No. 8 | M,NS | M,NS | 13–16 | 13–16 |
| Excetrona | M,NS | M,NS | 13–16 | 13–16 |
| No. 2391 | M,NS | M,NS | 13–16 | 13–16 |
| Y-136 | M,NS | M,NS | 13–16 | 13–16 |
| MA-927 | M,NS | M,NS | 13–16 | 13–16 |
| MA-667 | M,NS | M,NS | 13–16 | 13–16 |
| TGB-Scotland | M,NS | M,NS | 13–16 | 13–16 |
| L-56 | M,NS | M,NS | 13–16 | 13–16 |

[a] LL = local lesions, N = veinal necrosis, D = plant death, M = mosaic, NS = necrotic spots.

inoculation with PVX, PVM, or PVS, but all viruses were detected in inoculated and new leaves of *S. brachycarpum* plants by ELISA.

Since PVX was carried symptomlessly in *S. brachycarpum* plants, it was of interest to determine whether such plants would alter the $PVY^N$ or $PVY^O$ symptomatology. When inoculated with $PVY^N$ or $PVY^O$ isolates, *S. brachycarpum* plantlets multiplied by cutting from a PVX-infected mother plant developed the same symptoms as observed without PVX (Table 1). Simultaneous inoculation of PVX with $PVY^N$ or PVX and $PVY^O$ in a PVX:PVY ratio of 1:5, 1:20, and 1:50 did not change the typical symptoms observed with PVY.

**Effect of temperature on symptom development.** *S. brachycarpum* plants inoculated with $PVY^N$ developed symptoms at 20–25 C as well as at 25–35 C. Light intensity in the range of 48–72 to 140–196 $\mu E \cdot s^{-1} \cdot m^{-2}$ also did not affect symptom development.

**Evaluation of field samples.** Potato plants infected with $PVY^N$ are not permitted to be grown in the field in Canada or in the United States because $PVY^N$ is a quarantined plant pathogen. Therefore, potato plants showing mosaic symptoms in the field were collected and their virus content (PVA, PVM, PVS, PVX, and PVY) was determined serologically. $PVY^N$-infected leaves were mixed in various proportions with mosaic leaves, and the combined inoculum was rubbed onto *S. brachycarpum* plants. Necrotic symptoms typical of $PVY^N$ were observed only where $PVY^N$ was deliberately added (Table 2). None of the test plants inoculated with the field collections developed any necrotic symptoms (Table 2).

**Composite sampling.** When single 1-cm disks from $PVY^N$-infected leaves were combined with 4, 9, 49, 99, 249, or 499 disks from healthy leaves and inoculated onto *S. brachycarpum* plants, all inoculated plants showed local lesions and systemic necrosis and died within 10–12 days. Symptoms slowly developed at a 1:500 dilution.

## DISCUSSION

This study demonstrates that: 1) isolates of $PVY^N$ can cause necrotic symptoms in wild potato (*S. brachycarpum*) as well as in tobacco; 2) the necrotic symptoms in *S. brachycarpum* are caused by $PVY^N$ and not by other commonly occurring potato viruses (Tables 1 and 2); 3) the presence of PVX in *S. brachycarpum* plants or in inoculum does not alter the development of necrotic symptoms caused by $PVY^N$; and 4) symptoms are caused under greenhouse conditions at a wide temperature range of 20–35 C. Tobacco has been used exclusively as the necrotic indicator plant since the first observation of the tobacco veinal necrosis strain of PVY in the 1940s (9,11,18) until today (13,19), but *S. brachycarpum* appears to be a better indicator plant for $PVY^N$ testing.

As a bioassay host, *S. brachycarpum* is superior to the extensively used tobacco because it is fast-reacting; develops symptoms with inoculation of extracts from leaves, sprouts, or tubers; and shows definite symptoms within 7–10 days compared with 10–21 days for tobacco. *S. brachycarpum* did not develop necrotic symptoms when inoculated with a mixture of PVX and $PVY^O$, a situation that confounds the $PVY^N$ diagnosis with tobacco and thus requires removal of PVX prior to tobacco bioassay for $PVY^N$.

*S. brachycarpum* is self-pollinating and produces numerous berries under normal greenhouse conditions during winter as well as summer months. Thus, seed production is easy. Seedlings can be produced from true potato seed immediately after harvest if seeds are treated with gibberellic acid, thus providing a regular supply of suitable seedlings. Over a thousand seedlings of *S. brachycarpum* have been grown and used for the $PVY^N$ test. All have developed typical symptoms of $PVY^N$. In addition, *S. brachycarpum* appears to be a suitable indicator plant for assays when aphids are used as vectors.


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**Table 2.** Detection of $PVY^N$ using *Solanum brachycarpum* inoculated with sap of field-grown potato leaves mixed with $PVY^N$-infected tissue

| Sample no. | Viruses present | Symptoms[a] Test 1: Without $PVY^N$ | Test 1: Added $PVY^N$ Concn. | Test 1: Added $PVY^N$ Symptom | Test 2[b]: Without $PVY^N$ | Test 2[b]: With $PVY^N$ |
|---|---|---|---|---|---|---|
| 1 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 2 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 3 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 4 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 5 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 6 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 7 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 8 | PVA+PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 9 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 10 | PVS+PVX+$PVY^O$ | M | – | – | M | LL,N,D |
| 11 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:250 | N,D | – | – |
| 12 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:250 | N,D | – | – |
| 13 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:250 | N,D | – | – |
| 14 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:250 | N,D | – | – |
| 15 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:250 | N,D | – | – |
| 16 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:500 | N,D | – | – |
| 17 | PVS+$PVY^O$+$PVY^N$ | | 1:500 | N,D | – | – |
| 18 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:500 | N,D | – | – |
| 19 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:500 | N,D | – | – |
| 20 | PVS+PVX+$PVY^O$+$PVY^N$ | | 1:500 | N,D | – | – |

[a] M = mosaic, LL = local lesions, N = veinal necrosis, D = plant death.
[b] In test 2, the first 10 samples used in test 1 were inoculated or not inoculated with $PVY^N$ in a ratio of 1:5 ($PVY^N$:virus-containing sap); – = no tests performed.

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