Detection and Distribution of Latent Viruses in the Potato Cultivar Atlantic

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ABSTRACT

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Enzyme-linked immunosorbent assay (ELISA) and an infectivity assay using the potato virus X (PVX) indicator plants, *Gomphrena globosa* and *Datura stramonium* var. *tatula*, were used to assay individual tubers and plants from 10 lots of *Solanum tuberosum* cv. Atlantic collected in Maine, Colorado, and Nebraska in a effort to determine presence of PVX, potato virus S (PVS), and potato virus M (PVM). PVX was not detected in any sample. All inoculation experiments to infect cultivar Altantic with PVX were unsuccessful. PVS was present in all plants of all samples. PVM was detected only in samples collected in Maine. The incidence of PVM ranged from 20% in foundation material to 90% in one commercial seed lot.

Most cultivars of potato (*Solanum tuberosum* L.) are susceptible to one or more of the following latent viruses: potato virus X (PVX), potato virus S (PVS), and potato virus M (PVM). Under most environmental conditions, these viruses do not cause symptoms but can reduce yield as much as 15% and also decrease the specific gravity of the tuber (1,6,11,13). Because of their latent nature, reactions of indicator plants and serological tests are commonly used to detect and identify these viruses (1,7,9,11,13).

Because of its sensitivity, reliability, amenability for large-scale testing, and adaptability for automation, the recently developed enzyme-linked immunosorbent assay (ELISA) is now used extensively and in many instances exclusively to detect these and other potato viruses (4,5,8,15,16). Currently, this procedure is used to assay for these viruses in potato seed certification programs, in potato germ plasm enhancement programs, and in the development of nuclear material for virus-tested stem-cutting programs (2,10,14).

In 1976, the cultivar Atlantic was released as a PVX-immune cultivar with good processing characteristics and adapted for growth in diverse areas of the world (18). Since its release, this cultivar has shown adaptation for commercial production and has been grown in many areas of the United States, Canada, Central America, Pakistan, and Australia. It has been assayed by ELISA by several state certification agencies, germ plasm enhancement programs, and virus-tested stem-cutting programs. Throughout these repeated ELISA evaluations, Atlantic retained its PVX immunity status until 1983, when Tavantzis and Southard (15), also using standard ELISA procedures, reported that 85% of the tubers from some commercial stocks of this cultivar were infected with PVX.

The objectives of this study were to determine whether the reported PVX infection in Atlantic was caused by a new ELISA-detectable virus strain capable of infecting this cultivar, whether the PVX infection was restricted to tubers of Atlantic grown in specific areas, and what the incidence was of three latent viruses in this cultivar.

MATERIALS AND METHODS

Plant materials. Tubers of potato cultivar Atlantic were obtained from 10 sources immediately after the 1983 harvest. Because tubers of Atlantic assayed by Tavantzis and Southard (15) were collected from sites in Maine exclusively, seven of the 10 lots used in this study were also collected in Maine. The foundation tuber lot of cultivar Atlantic used in this study was obtained from the same foundation seed farm operated by the Maine Seed Potato Board as the foundation seed lot assayed by Tavantzis and Southard (15). Of the three non-Maine lots, two were from Nebraska and one from Colorado.

The certification classification of the seed lots used in this study ranged from foundation to commercial. The term "foundation" is used to designate tubers from plants 1 yr after being pathogen-tested. The commercial seed was propagated on seed-potato farms operated by individual growers. The terms "generation 1, generation 2," and "certified seed" refer to classes of seed potatoes increased for 1, 2, and 3 yr, respectively, beyond the foundation stage.

In this study, each tuber was numbered and stored at 4–6 C for 6 wk, then two seed pieces each containing a "single eye" were removed from each tuber. One seed piece from each tuber was planted in an 8-in. pot containing Jiffy Mix and placed in a greenhouse where the ambient temperature ranged from 15 to 20 C. High-pressure sodium lights with intensity of 8,600–10,700 lux were used to maintain a 16-hr light period. The other seed piece was planted in a field plot at Beltsville, MD. At various intervals, foliar, stem, root, and tuber tissue from both the greenhouse-grown and field-grown plants were harvested and assayed immediately for PVX, PVS, and PVM by ELISA. The remaining portions of the original tubers collected from Maine, Nebraska, and Colorado were treated as follows: A tissue sample from the apical end of each tuber was used for the original ELISA. After this evaluation, each tuber was placed on a greenhouse bench where temperatures ranged from 19 to 24 C. The tubers were covered with two layers of burlap and allowed to sprout. Twenty-one days later, tuber, sprout, and sprout-tuber tissues were used for ELISA and indicator plant assays.

PVX-infected scions approach-grafted to Atlantic stock were of two types: Green Mountain naturally infected with PVX and Chippewa infected with a virulent strain of PVX. Before being grafted, all Atlantic plants were tested for PVX. Approach-grafted plants were cut 21 days after the initial graft, producing a plant with a single scion and a single stock. At intervals after the graft, ELISA was done to detect PVX from grafted stocks of Atlantic. The roots, foliage, and tubers grown on stocks of grafted plants were assayed by ELISA.

PVX-infected tubers and plants of cultivars Chippewa, Green Mountain, Irish Cobbler, and Katahdin were used as infected controls for PVX. PVM-infected tubers and plants of cultivar Saco were used as infected controls for PVM. Tubers and plants of line S41956 from the Schultz (17) cage collection were used as the infected controls for PVS. Nuclear seed of Atlantic that was used as healthy "negative" controls throughout this study was derived from a meristem tip-culture plantlet (14) obtained from Edward Jones of Cornell University in 1981. Nuclear seed of the heat-treated Katahdin potato was obtained from N. S. Wright of Agriculture Canada.

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Sample preparations and ELISA evaluations. Tuber, root, stem, and foliage tissues were assayed. Tissues used for tuber assays were 0.25 g of tuber, sprout, and sprout with adjacent tuber tissue from the apical section of the tuber. Tissue was macerated with a mortar and pestle in 1.25 ml of sample extract buffer, PBS2T (i.e., 8.0 g of NaCl, 0.2 g of KH_2PO_4, 2.9 g of $Na_2PO_4 \cdot 12H_2O$, 0.2 g of KCl, 20 ml of Tween 20 in 1 L of distilled water, pH 7.4). The same procedure was used to prepare stem and root samples. Foliar samples (i.e., leaf and stem) were assayed when plants were about 16 cm tall. Leaf samples consisted of the terminal leaflet from the compound leaf at the third node from the soil line. Leaf and petiole samples were processed in a roller-type leaf press, and the expressed sap was immediately added to PBS2T at a dilution of one part sample to five parts buffer (v/v). All samples were placed in borosilicate culture tubes (10 × 75 mm) and kept at 4 C overnight before their use in ELISA. POTA-SCREEN (Agdia Inc., Mishawaka, IN), a commercial test kit using peroxidose conjugate was used to test all the viruses studied. All ingredients and incubation times used were those recommended by the supplier. We measured absorbance (492 nm) with a Titertek Multiskan ELISA plate reader (Flow Laboratories, McLean, VA). To check for possible interference from plant peroxidases, a second check without enzyme conjugate was tested for each sample. Buffer, healthy, and known PVX-, PVS-, and PVM-infected controls were included in each microplate. We considered a reaction positive when the mean absorbance of the sample exceeded the mean absorbance plus four standard deviations of healthy controls. Results were subjected to analysis of variance, and means were compared by Duncan's multiple range test. The reasonableness of analysis was confirmed by histograms of the data indicating distinct bimodal populations of negative and virus-containing samples.

Gomphrena globosa L. and *Datura stramonium* var. *tatula* (L.) Torr. were used as indicator hosts for PVX. Leaves of each test plant were sprayed with distilled water, dusted with 600-mesh Carborundum, and rubbed with cotton gauze saturated with inoculum. After rubbing, we allowed inoculum to dry on leaves. Leaves were washed later with tap water. Plants were placed in a greenhouse chamber where the ambient temperature ranged from 20 to 24 C. A 16-hr day length was maintained with supplemental light from high-pressure sodium vapor lights with an intensity of 8,600–10,700 lux.

RESULTS

Detection of viruses. *PVX.* No PVX was detected with ELISA in either tuber or sprout tissue of any of the 105 original tubers of cultivar Atlantic collected in 1983. This virus also was not detected in leaf, stem, root, tuber, and tuber-sprout tissue of the 105 plants grown from the original ELISA-tested tubers in the greenhouse and tested with ELISA immediately after harvest. Similarly, PVX was not detected in any of the plants derived from 105 seed pieces taken from the original tubers that were planted 6 mo later in a Beltsville field plot (Table 1). We were unable to detect PVX by ELISA in leaves, stems, roots, and tubers from stocks of cultivar Atlantic that were grafted with PVX-infected scions of cultivars Chippewa and Green Mountain. PVX was readily detected by ELISA in all leaf, stem, root, tuber, and sprout-tuber tissue of PVX-infected control cultivars Chippewa, Green Mountain, Irish Cobbler, and Katahdin and stocks of heat-treated Katahdin that had been grafted to PVX-infected scions.

PVS. Using ELISA, we detected PVS in all the leaf, stem, root, and sprout-tuber samples of six lots. In the remaining four lots, PVS was detected in 77.6% of the sprout-tuber samples (Table 2). Those sprout-tuber tissue samples from which we detected no PVS were taken from tubers with sprouts less than 5 mm long as opposed to sprouts 1.5–2.0 cm long on tubers from which we readily detected PVS.

PVM. PVM was detected from both the sprout-tuber tissue and leaf tissue derived from tuber of samples collected in Maine. The incidence of PVM detected in the Maine samples by ELISA ranged from 30 to 90%. PVM was not detected in samples received from Colorado or Nebraska (Table 2).

Throughout this study, plant peroxidases did not interfere with our ELISA evaluations.

Table 1. Absorbance (492 nm) values of an enzyme-linked immunosorbent assay for potato virus X in leaf, stem, root, and tuber tissue of the cultivars Atlantic, Chippewa, Green Mountain, and Katahdin

	Tissue			
Cultivar	**Leaf**	**Stem**	**Root**	**Tuber**
Atlantic	0.030[a]	0.041[a]	0.011[a]	0.111[b]
Chippewa[c]	1.023[b]	1.041[b]	0.284[b]	0.657[b]
Green Mountain[c]	1.057[b]	1.339[b]	0.389[b]	0.853[b]
Irish Cobbler[c]	1.186[b]	0.421[b]	1.161[b]	0.077[b]
Katahdin[c]	1.063[b]	0.983[b]	0.870[b]	0.086[b]
HT Katahdin[d]	0.061[b]	0.071[b]	0.087[b]	0.093[b]
MT Atlantic[e]	0.057[b]	0.037[b]	0.021[b]	0.097[b]

[a]Each number represents mean absorbance of three experiments with 105 test samples in each experiment using Agdia system; all measurements made with a Titerek Multiskan plate reader.
[b]Each number represents mean absorbance of three experiments, with seven test samples in each experiment.
[c]Chippewa, Green Mountain, Irish Cobbler, and Katahdin obtained from Schultz potato virus collection (17).
[d]Progenies of heat-treated meristem plant obtained from N. S. Wright, Agriculture Canada.
[e]Progenies of meristem plant obtained from E. Jones, Cornell University.

DISCUSSION

PVX. Results of this study using both comparable ELISA procedure and infectivity hosts failed to confirm the previously reported high incidence of PVX in tissue of Atlantic tubers collected at various locations in Maine (15). Our initial failure to detect PVX in the original tubers may have been caused by a decrease in PVX titer in tubers between harvest and our ELISA assay. However, subsequently, we were unable to detect PVX in either developing tubers or mature tubers harvested from greenhouse-grown and field-grown plants and assayed within 2 hr of harvest and/or after 14 days of storage at 6 C. Therefore, a decrease in titer of PVX during storage could not account for our not detecting PVX. Also, a decrease in titer did not account for the nondetectability of PVX in tubers from stocks of Atlantic grafted with PVX-infected scions. Because none of the tubers used in this study were obtained from growers who had taken any drastic actions such as heat treatment or meristem tip culture to eliminate PVX and some samples were collected from the same foundation seed farm as those tested by the previous workers (15), the probability of selecting only PVX-free tubers from seed lots that contained a high incidence (85%) of PVX 2 yr earlier is extremely remote. Unfortunately, we were unable to make any direct comparative assays of our samples and the earlier-reported PVX-infected material because we were unable to acquire either the tubers or progenies of the earlier-tested seed lots (15). Therefore, we could not directly confirm the existence of a strain or strains of PVX in cultivar Atlantic that were earlier reported to occur and could be detected with standard ELISA procedures (15).

PVS. PVS was detected in leaf samples of all cultivars tested, which indicates that the cultivar Atlantic is uniformly infected with PVS. In this study, we missed detecting PVS in 9.5% (11 of 105 samples) in the sprout-tuber samples. The sprouts in which PVS was not detected were shorter than 5 mm, whereas the sprout length in the other tubers ranged from 1.0 to 1.5 cm.

Table 2. Incidence of potato viruses M, S, and X in sprout-tuber tissue and leaf-plant tissue of the potato cultivar Atlantic grown in various geographic locations

Sample source	Seed class and generation	Potato virus M		Potato virus S		Potato virus X	
		Tuber	Plant	Tuber	Plant	Tuber	Plant
Maine M-1	Foundation[a]	4/20[b]	4/20	15/20	20/20	0/20	0/20
Maine M-2	G-2	4/10	4/10	8/10	10/10	0/10	0/10
Maine M-3	G-3	5/9	7/9	9/9	9/9	0/9	0/9
Maine M-4	G-3	5/10	6/10	8/10	10/10	0/10	0/10
Maine M-5	G-3	6/10	6/10	10/10	10/10	0/10	0/10
Maine M-6	G-4	3/10	3/10	10/10	10/10	0/10	0/10
Maine M-7	G-4	9/10	9/10	10/10	10/10	0/10	0/10
Colorado C-1	G-7	0/9	0/9	9/9	9/9	0/9	0/9
Nebraska N-1	G-7	0/9	0/9	7/9	9/9	0/9	0/9
Nebraska N-2	G-7	0/8	0/8	8/8	8/8	0/8	0/8
MT Atlantic[c]	− Control	0/5	0/5	0/5	0/5	0/5	0/5
Inoculated Atlantic[d]	+ Control	5/5	5/5	5/5	5/5	0/5	0/5
Chippewa[e]	+ Control	5/5	5/5	5/5	5/5	5/5	5/5

[a] From state seed farm; G-2, G-3, G-4, G-5, G-6, G-7 refer to classes of seed increased 2, 3, 4, 5, 6, 7 yr, respectively, beyond foundation stage.
[b] Number of samples infected/number of samples tested.
[c] Atlantic/negative control = progenies of meristem tip plantlet from E. Jones, Cornell University.
[d] Inoculated Atlantic/negative control Atlantic inoculated with sap from the Chippewa positive control.
[e] Chippewa = infected control.

PVM. We do not know why PVM infection of the cultivar Atlantic was restricted to tubers collected in Maine. However, we found that the percentage of PVM-infected samples from the Maine-grown tuber lots was quite similar to the percentage of PVX infection reported in this cultivar by previous workers (15). We were able to readily detect PVM in foliage, stem, root, and sprout tissue of the infected plants. Unfortunately, because we were not able to obtain either the antiserum or the antigen used by these workers, we could not perform direct serological comparison of our sample tissue and tissue of the earlier-reported samples.

Conclusion. Results of this study suggest that the cultivar Atlantic, released in 1976 as a PVX-immune cultivar (18), continues to have functional immunity to prevailing strains PVX. We use the term functional immunity to designate those cultivars in which the virus fails to become established in contrast to hypersensitive reaction in which tip necrosis develops after virus inoculation (3).

Results of this study indicate that the cultivar Atlantic is uniformly infected with PVS. These results also suggest that PVS can be readily and reliably detected in sprouts longer than 5 mm. However, PVS was not accurately and reliably detected from sprouts shorter than 5 mm.

Results of this limited sampling show that PVM is prevalent in all samples collected in Maine but not detected in samples from Colorado and Nebraska.

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