Detection of Potato Spindle Tuber Viroid in the Pollen and Various Parts of Potato Plant Pollinated with Viroid-Infected Pollen

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ABSTRACT

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Potato spindle tuber viroid (PSTVd) was detected in pollen grains from PSTVd-infected potato cultivar Katahdin by return polyacrylamide gel electrophoresis. Pollination of the flowers of healthy Katahdin plants with pollen from PSTVd-infected plants resulted in infection of leaves at the base of inflorescences, apical leaves, and tubers. Electrophoretic analysis of fruits indicated sporadic PSTVd infection of sepals, fruit skin, and fruit pulp. True potato seed from each fruit was 35–66% infected with PSTVd. Percentage of seed infection varied with individual plants and was not affected by the location of an inflorescence or by the number of fruits produced on the same inflorescence.

Potato spindle tuber viroid (PSTVd) is present in pollen and true potato seed (TPS) of infected potato plants (2,4,5, 8,9). The percentage of infected seedlings obtained from infected parents (selfed or cross-pollinated) is variable (2,8,12), but infection as high as 100% of the progeny seedlings has been observed in certain cultivars (2,4,8,12). Thus, the cultivar may be important (2), but the factors governing seed transmission of the viroid are not known.

Seed transmission of PSTVd in TPS has special significance for potato breeding programs, because infected seedlings are a particularly insidious source of inoculum. Pollen obtained from an infected plant may inadvertently be used to pollinate many flowers and thus contaminate a large number of seeds and seedlings. The role of infected pollen in PSTVd seed transmission is not known. Thus, the objectives of this study were 1) to develop a method to determine infection of pollen grains by PSTVd prior to pollination, 2) to determine if mother plants become infected after pollination with PSTVd-infected pollen, and 3) to determine if the number of inflorescences and the number of fruits per plant influence the rate of PSTVd seed transmission.

MATERIALS AND METHODS

Viroid strain and test procedure. A mild strain of potato spindle tuber viroid (PSTVd-FM) (10) was used in all of the experiments. Virus and viroid-free plantlets of potato cultivar Katahdin were obtained from the Plant Propagation Center (New Brunswick Department of Agriculture, Fredericton) and grown in a greenhouse with optimal environmental conditions for potato growth. Plants were inoculated when they were 10–15 cm high with viroid-enriched nucleic acid extracts. After inoculation, test plants were maintained in a greenhouse equipped with a device that sprayed water at regular intervals to provide high (55–65%) relative humidity. Fruit set in potato is optimal under higher than average relative humidity. Four weeks after inoculation, plants were assayed individually for PSTVd infection by return polyacrylamide gel electrophoresis (R-PAGE) (10).

Nucleic acid extraction from pollen. Pollen was removed either by streaking anthers longitudinally a few times or by vibrating the anthers with a mechanical device. Pollen was weighed and stored at 4 or −70 C. Nucleic acids were extracted by grinding 10–20 mg of pollen by glass rod in a microcentrifuge tube (1.5 ml) with or without Carborundum powder (350 mesh). The tubes contained 300 µl of nucleic acid extracting buffer (0.53 M NH_4OH; 0.013 M disodium ethylenediaminetetraacetate [EDTA] adjusted to pH 7.0 with Tris; 4 M LiCl; 1% purified bentonite [3]) and 400 µl of Tris-buffered saturated phenol containing 0.1 g of 8-hydroxyquinoline per 100 ml of solution. The suspension was centrifuged (10 min at 11,000 rpm) in a microcentrifuge at 22–25 C, and the aqueous layer was removed. Nucleic acids were precipitated from the aqueous layer with 2.5 vol of ethanol and 25 µl of 4 M sodium acetate (pH 5.5) and incubated at −20 C for 30 min. The extraction step was repeated once more, and nucleic acids were precipitated from the pooled aqueous layer. The precipitate (viroid-enriched nucleic acid fraction) was recovered by centrifugation, washed once with absolute alcohol, and air-dried. Total nucleic acids were dissolved in 12 µl of TBE buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA; pH 8.3) containing 40% glycerol. For comparison of viroid concentrations in pollen and leaf tissues, equivalent weights of pollen or leaves were subjected to the same extraction procedure, and 6 µl of nucleic acid extract was loaded onto the gel.

R-PAGE. Nucleic acid samples were fractionated as described previously (10,11). Samples were loaded in each well, and the first cycle of electrophoresis was carried out under nondenaturing conditions on 5% slab gels (140 × 160 × 1.5 mm) in TBE buffer for 2.5 hr at 46 mA and at 20–25 C. The return cycle of electrophoresis was carried out under denaturing conditions by filling the chambers with diluted TBE buffer (1:8) heated to 87–90 C after reversing the polarity and running it at 70 C for 2 hr at 46 mA. To make the RNA bands visible, all gels were stained with silver nitrate (10,11).

Pollination and detection of viroid in plant parts. Flowers from PSTVd-free Katahdin plants were emasculated with sterile forceps 16–24 hr before pollination. Pollen from PSTVd-free or PSTVd-infected Katahdin plants, stored for a few days at 4 C, was used for pollination. Pistil ends of emasculated flowers were pollinated with the bulked pollen. To avoid contact transmission of the viroid, disposable paper or gloves were used while handling flower parts. Flowers were tagged and observed regularly for fruit set.

Fruits were collected 10–12 wk after pollination, when fruit skin became dull green. Seeds were extracted by blending the fruits in a large amount of water and separating the seeds from fruit debris by repeated washing with excess water. Seeds were dried at room temperature for 1 wk and then stored at 4 C in paper envelopes labeled with the fruit location and the inflorescence position. TPS was tested for PSTVd content as described previously (11,12).

To determine infection of pollinated plants, leaves located near each fruit-bearing inflorescence, as well as the apical leaves, were sampled for PSTVd detection 6 wk after pollination. At 10 and 12 wk after pollination, additional plant parts, e.g., sepals attached to fruit,

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fruit skin, and fruit pulp, were sampled for nucleic acid extraction. Tubers were also sampled from each plant. Nucleic acid was extracted from 0.5-g tuber samples made up of pieces from all the tubers of each plant. The nucleic acid was analyzed for PSTVd by R-PAGE. Samples derived from plants pollinated with PSTVd-free pollen were used as controls.

Statistical analysis. Data from TPS tested for PSTVd were analyzed by the chi-square (χ^2) test of goodness of fit (1). First, observed values from different numbers of fruit were analyzed with the χ^2 test; secondly, the total number of TPS from each inflorescence position was analyzed with the χ^2 test.

RESULTS

Detection of PSTVd from pollen. PSTVd bands were easily observed in preparations from 20–23 mg of infected pollen stored at 4 and −70 C. Extraction with or without Carborundum was identical in regard to viroid band intensity. When nucleic acid samples were diluted 1:2 and analyzed by R-PAGE, PSTVd bands were visible in electrophoretograms. When proteinase K (50 μg/ml) was used to extract nucleic acids from 20-mg pollen samples, faint PSTVd bands were also visible on the electrophoretograms of undiluted and diluted (1:2) extracts.

To determine the viroid concentration in pollen and its relation to that in leaves, nucleic acids extracted from 58 mg of pollen or leaf were analyzed with R-PAGE at dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. Strong bands of viroid were detected in pollen extracts diluted 1:8 and in leaf extracts diluted 1:32; faint PSTVd bands were detected in pollen extracts diluted 1:32 and in leaf extracts diluted 1:64. Thus, leaves contained approximately twice as much viroid as pollen per unit fresh weight.

Distribution of PSTVd in pollinated plants. At 6 wk after pollination, PSTVd was detected in leaves located near the inflorescences of two plants (Table 1). In one plant, viroid was detected in leaves at the base of an inflorescence as well as apical leaves. PSTVd was detected only in the apical leaves of one additional plant (Table 1).

At 10 wk after pollination, PSTVd was detected in leaves at the base of the inflorescences in two additional plants. No additional PSTVd infections of apical leaves were detected (Table 1). When tubers were tested for viroid at 12 wk after pollination, PSTVd was detected in three plants previously identified as infected with PSTVd on the basis of apical leaves and inflorescence infection, as well as from one additional plant. Similarly, sepals and fruit skin from the fruit of five plants, representing six inflorescences, contained PSTVd. A larger number of fruit pulp samples were PSTVd-infected. Six of these fruits originated from plants previously known to be infected; the other four fruits were from plants in which PSTVd had not been detected before. TPS from 25 fruits was infected with PSTVd.

Inflorescences, fruits, and presence of PSTVd in TPS. Of the 20 potato plants pollinated, 16 developed fruit. Seven of these plants had fruit on two inflorescences, and two had fruit on three inflorescences (Table 2). The number of fruits ranged from one to 10 per inflorescence. A total of 1,563 TPS were individually tested, and 51.72% contained PSTVd. TPS bulked from fruit of each inflorescence exhibited PSTVd infection ranging from 35 to 66%. The lowest PSTVd incidence was associated with 10 fruit from the first inflorescence of one plant, and the highest incidence was detected on another plant in five fruit from the second inflorescence. However, the difference in percent PSTVd content between inflorescences was not significant (χ^2 = 6.83, 0.93, and 1.77). The average PSTVd incidence in TPS from multiple inflorescences ranged from 46 to 56%. However, there were no significant differences between plants with one to 10 fruits.

Table 1. Detection of potato spindle tuber viroid (PSTVd) in different parts of pollinated potato plants[a]

Plant parts tested	Viroid detection after pollination (wk)		
	6	10	12
Inflorescence	2/25[b]	4/25	NT[c]
Apical leaves	2/20	2/20	NT
Sepals on fruit[d]	NT	NT	5/25
Fruit skin[d]	NT	NT	6/25
Fruit pulp[d]	NT	NT	10/25
True potato seed[e]	NT	NT	25/25
Tubers[f]	NT	NT	4/20

[a] Twenty potato plants of cv. Katahdin were pollinated with pollen from PSTVd-infected plants.
[b] Fruit set occurred on 25 inflorescences distributed on 16 potato plants. No fruit set occurred on four plants.
[c] NT = not tested.
[d] Sepals, fruit skin, and pulp from all fruit of each inflorescence were combined and treated as one sample.
[e] True potato seeds were tested individually.
[f] Pieces from all tubers of each plant were combined and treated as one sample.

Table 2. Effect of inflorescence and fruit numbers on potato spindle tuber viroid (PSTVd) transmission through true potato seed of cv. Katahdin

Inflorescence No. of plants	No. of fruit	True potato seed		Percent infection[a]
		No. tested	No. PSTVd-infected	
First				
4	1	282	134	47.5
2	2	133	67	50.3
1	3	60	34	56.7
4	4	242	131	54.1
2	5	120	63	52.5
1	6	60	27	45.0
1	7	60	38	63.3
1	10	60	21	35.0
Second				
2	1	122	61	50.0
2	2	120	70	58.3
2	3	120	62	51.7
1	5	62	41	66.7
Third				
1	1	60	23	38.3
1	4	62	34	54.8

[a] The difference in percent infection between inflorescences was not significant. χ^2 = 6.83 for the first inflorescence, 0.93 for the second inflorescence, and 1.77 for the third inflorescence.

DISCUSSION

Although it has been demonstrated that plants can be infected with viruses through infected pollen (7), this is the first report of potato plants becoming infected through pollination with pollen from a PSTVd-infected plant (Table 1). However, this form of transmission is variable, ranging from infection of a few infected plant parts (inflorescences, apical leaves, and tubers) to about 40% of the fruit pulp. Although TPS obtained from all infected plants contained PSTVd, infection of other plant parts was irregular (Table 1). One explanation for sporadic infection of plants could be contamination during pollination; however, this would not explain the presence of PSTVd in sepals, fruit skin, and fruit pulp (Table 1). Infection of these fruit parts must be a result of pollination, be-

cause some of the mother plants showed no infection of PSTVd in any other plant parts but still produced PSTVd-infected TPS. In spite of the variable and low infection rates of certain plant parts, any viroid passed on to tubers could contaminate the resulting crop.

Pollen contains a lower concentration (per unit fresh weight) of PSTVd than leaf tissue. This may explain the variable infection of potato plant parts through pollination. In one earlier study, all tomato plants (five were used) became infected through pollination with three viroids (PSTVd, chrysanthemum stunt viroid, and cucumber pale fruit viroid), but no information on the viroid content of the pollen was given (6). Alternatively, the variable infection from pollination may indicate that a low percentage of pollen grains contain PSTVd. This is supported by the less than 100% TPS infection resulting after PSTVd-infected pollination (Table 2).

Pollination of a large number of flowers on a single plant was expected to yield higher percentages of PSTVd-infected TPS. However, percentage of seed infection with PSTVd in cv. Katahdin was not influenced by the number of inflorescences inoculated with infected pollen or the number of resulting fruits (Table 2). Therefore, percent seed transmission must be controlled by infected pollen alone.

Previous studies on PSTVd seed transmission utilized pollen of unknown viroid content. In general, a fraction of pollen was inoculated to indicator plants for the determination of viroid content, which required 2–3 wk to yield the needed information (8,9). The method described in this study yields information within one day, and pollen grains stored at 4 or −70 C remain viable; therefore, viroid content can be determined prior to pollination. Although the method of viroid detection requires a minimum of 20 mg of pollen to detect PSTVd, larger amounts of pollen (20–60 mg) were easily collected from potato cultivar Katahdin on each pollination date. In some potato cultivars, however, pollen production may be considerably reduced and PSTVd not be detectable (R. P. Singh, *unpublished data*). In a typical experiment, 20–25 mg of pollen was collected from 20–35 flowers; 20 mg was used for viroid detection, and the remaining pollen was used to pollinate 20–35 flowers. Thus, a relatively small amount of pollen was needed for pollination.

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