Eradication of Potato Viruses X and S from Potato Shoot-Tip Cultures with Ribavirin

R. E. Klein and C. H. Livingston

Graduate research assistant and plant pathologist, respectively, Department of Botany and Plant Pathology, Colorado State University, Fort Collins, CO 80523.
Supported by the Colorado State University Experiment Station and published as Scientific Series Paper No. 2780.
Accepted for publication 11 February 1983.

ABSTRACT

Klein, R. E., and Livingston, C. H. 1983. Eradication of potato viruses X and S from potato shoot-tip cultures with ribavirin. Phytopathology 73:1049-1050.

Ribavirin treatment of cultured potato shoot tips was tested as a means of eradicating potato virus X (PVX) and potato virus S (PVS). Doubly infected shoot tips were cultured on a liquid medium containing 10, 20, or 40 μg/ml ribavirin and a control medium without ribavirin. Cultures were evaluated periodically for relative growth rate, inviability, and the time required for plantlet regeneration. Developed plantlets were assayed for PVX by transmission tests to *Gomphrena globosa*, and serologically for PVS by the latex agglutination test. Ribavirin proved to be phytotoxic at all concentrations tested, and resulted in the inviability of all cultures treated with 40 μg/ml. Treatment delayed plantlet development by 106 and 127 days for the 10- and 20-μg/ml treatments, respectively. Virus assays indicated that 93 and 87% of the plantlets were free of PVX and PVS, respectively, after treatment with 10 μg/ml. All plantlets developed from the 20-μg/ml treatment were free of both viruses, whereas 10 and 0% of the controls were free of PVX and PVS, respectively.

Additional key words: chemotherapy.

The most common method of eradicating potato virus X (PVX) and potato virus S (PVS) from infected seed potato stocks has been heat treatment of rooted cuttings followed by axillary shoot-tip culture. This method has been particularly effective against PVX (14), but eradication of PVS by this method has proven to be more difficult and yields variable results (7–9).

A synthetic riboside, ribavirin, was reported to have antiviral activity against a wide range of plant viruses (2,3,5,6,10–13). The eradication of PVX from infected shoot-tip cultures by ribavirin treatment has been reported (4). No reports have been made of PVS-eradication studies involving chemotherapy. Therefore, this study was undertaken to determine the efficacy of ribavirin treatments of cultured potato shoot tips as a means of eradicating PVX and PVS from doubly infected potatoes.

MATERIALS AND METHODS

Liquid nutrient culture medium containing 10, 20, and 40 μg/ml ribavirin as well as a control medium lacking ribavirin was prepared as described by Mellor and Stace-Smith (9). The medium was sterilized by filtration. Aliquots of 3.5 ml of the sterile medium were pipetted into presterilized 16 × 100-mm culture tubes containing hooped filter paper wicks; tubes were capped to ensure sterility. This volume was sufficient to immerse all but the top surface of the wick.

Solanum tuberosum, 'Russet Burbank' plants, previously ascertained to be doubly infected with PVX and PVS, were grown in a greenhouse and served as a source of shoot tips. After surface disinfestation with 70% ethanol and 0.5.% sodium hypochlorite, the most terminal tissue of each axillary bud, 0.2–0.5 mm in length, was excised and transferred to the domed surface of a filter-paper wick. Shoot-tip cultures were maintained in a growth chamber under a 15-hr photoperiod of 30,000 lux provided by fluorescent lighting and a temperature of 25 C. At intervals of approximately 1 mo, the shoot tips were transferred to culture tubes containing freshly prepared liquid medium and a filter-paper wick. Regenerated plantlets were transplanted into plastic pots 10 cm in diameter containing potting mix for continued growth in a greenhouse.

When each plant was approximately 20 cm tall, several leaflets were removed and assayed for both PVX and PVS. PVX assays were performed by mechanical inoculation of the local lesion indicator host, *Gomphrena globosa* L. Several leaflets were triturated in a small amount of 0.1 *M* phosphate buffer, pH 7.2. The triturate was rubbed onto *G. globosa* leaves that had been dusted with 600-mesh carborundum. Local lesions commonly developed within 10 days of inoculation. PVS assays were performed serologically using the latex agglutination test (1). Plants testing positively for both viruses were discarded. Those testing negatively were periodically assayed over a period of several months.

Sufficient shoot tips were excised to provide three replications of 12 subsample cultures for each experimental ribavirin concentration and the nontreated control. Cultured shoot tips were maintained in a controlled-environment chamber according to a completely random design. Individual shoot tips were evaluated after each month of culture on a 0–5 relative growth scale (4).

Values determined by relative growth ratings (omitting zero scores) were averaged for each replication and analyzed by a one-way analysis of variance. When justified by a significant F value, treatment means were compared to the control mean with the least significant difference test of $P \leqslant 0.05$. Shoot-tip cultures were examined frequently to determine the time required for plantlet regeneration. Regeneration times were averaged across each replication and analyzed with a one-way analysis of variance. Treatment means were compared to the control mean with the least significant difference test at $P \leqslant 0.05$.

RESULTS

A comparison of mean relative growth scores indicates that all three ribavirin treatments inhibited shoot-tip growth after 1 mo of culture (Table 1). The extent of inhibition is directly related to the ribavirin concentration.

Ribavirin treatment resulted in a significant increase in shoot-tip inviability, which increased with increasing ribavirin concentration (Table 2). Even at the relatively low concentration of 10 μg/ml, ribavirin treatment resulted in a sixfold increase in culture inviability as compared with the nontreated control. Treatment with 40 μg/ml ribavirin resulted in the inviability of all cultures. A

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

TABLE 1. Average relative growth scores[a] of shoot-tip cultures of *Solanum tuberosum* 'Russet Burbank' exposed to each of three concentrations of ribavirin and the corresponding treatment period

Ribavirin concentration	Culture period (months)[b]									
	1	2	3	4	5	6	7	8	9	11
Control	1.9	2.5	4.1	4.6	4.8	4.9	4.9	5.0	5.0	5.0
10 μg/ml	1.6	2.2	3.2*	3.5*	3.7*	4.0*	4.2*	4.5	4.5	5.0
20 μg/ml	1.4*	2.0*	2.8*	3.2*	3.3*	3.9*	3.8*	3.6*	4.3*	5.0
40 μg/ml	1.3*	1.6*	2.5*	2.4*	2.8*	2.8*	2.5*	...[c]	...	...

[a] Growth scale: 0 = inviable; 1 = no evident change from originally excised tissues; 2 = one or two leaflets visible; 3 = three or more leaflets visible; 4 = stem present; 5 = stem and roots present and plantlet ready for transplanting.

[b] Asterisks within each column designate a significant difference at $P \leq 0.05$ from the control.

[c] All cultures treated with 40 μg/ml ribavirin were inviable, and the growth could not be analyzed.

comparison of mean times required for plantlet regeneration shows that ribavirin at 10 and 20 μg/ml significantly delayed regeneration and a concentration of 40 μg/ml was lethal (Table 2). Although the ranges of regeneration times overlap, treatment of shoot-tip cultures with 10-μg/ml ribavirin delayed plantlet regeneration by approximately 106 days, whereas treatment with 20 μg/ml ribavirin delayed regeneration by approximately 127 days when compared with the nontreated control.

Ribavirin was effective as an eradicant for both PVX and PVS at treatment levels of 10 and 20 μg/ml. Control plantlets exhibited 10% PVX eradication and 0% PVS eradication, whereas plantlets regenerated from cultures treated with 10 μg/ml ribavirin exhibited 93 and 87% eradication of PVX and PVS, respectively. All plantlets regenerated from cultures treated with 20 μg/ml ribavirin were free of both PVX and PVS.

Repeated virus assays detected one plant infected with PVX and a second plant infected with PVS that had previously escaped detection. Both plants were regenerated from shoot tips that had not been exposed to ribavirin. In both cases the infections were detected the second time the plants were assayed approximately 6 wk after the initial assay.

DISCUSSION

Treatment of cultured shoot tips with ribavirin resulted in growth inhibition, culture inviability, and delayed plantlet regeneration. The extent of growth inhibition and culture inviability was similar to that reported earlier (4) for a ribavirin treatment level of 10 μg/ml. The delay in plantlet regeneration was greater than that reported in the earlier experiments. However, the percentage of plantlets that developed from cultures treated with 10 μg/ml ribavirin and tested negatively for PVX was greater than previously reported. The difference is probably due to experimental variation, but may also be indicative of variance in PVX strains to treatment.

Ribavirin at 10 and 20 μg/ml was also effective as a PVS eradicant. Treatment with ribavirin at 10 μg/ml resulted in PVS eradication from 87% of the plantlets, and 20 μg/ml resulted in 100% eradication. Although PVS is considered to be one of the potato viruses most difficult to eradicate (9), it may be amenable to chemotherapy using ribavirin.

TABLE 2. Mean inviability of shoot-tip cultures with the mean and range of regeneration times for plantlets developed from shoot-tip cultures exposed to each of three concentrations of ribavirin

Ribavirin concentration	Mean inviability	Regeneration time (days)	
		Mean	Range
Control	6.9	123	79–213
10 μg/ml	41.6*[a]	229*[a]	179–313
20 μg/ml	68.4*[a]	250*[a]	201–313
40 μg/ml	100.0*[a]	...	...

[a] Asterisks designate a significant difference from the control at $P \leq 0.05$.

LITERATURE CITED

1. Aapola, A. I. E. 1974. Flocculation or adsorption precipitin test. Pages 9-11 in: Serological Tests for the Identification of Plant Viruses. E. M. Ball, ed. Am. Phytopathol. Soc., St. Paul, MN.
2. Campbell, R. N. 1980. Effects of benomyl and ribavirin on the lettuce big vein agent and its transmission. Phytopathology 70:1190-1192.
3. Fazio, G. de, Caner, J., and Vincente, M. 1978. Inhibitory effect of Virazole Ribavirin on the replication of tomato white necrosis virus: A brief report. Arch. Virol. 58:153-156.
4. Klein, R. E., and Livingston, C. H. 1982. Eradication of potato virus X from two cultivars by ribavirin treatment of cultured shoot tips. Am. Potato J. 59:359-365.
5. Kluge, S., and Oertel, C. 1978. Testing of virazole on multiplication of cucumber mosaic virus and carnation mottle virus. Prufüng von Virazol auf die Vermehrung des Gurkenmosaic-virus und des Nelkenscheckungs-virus. Arch. Phytopathol. Pflanzenschutz 14:219-225.
6. Lerch, B. 1977. Inhibition of the biosynthesis of potato virus X by ribavirin. Phytopathol. Z. 89:44-49.
7. Lozoya-Saldana, H., and Dawson, W. O. 1982. The use of constant and alternating temperature regimes and tissue culture to obtain PVS-free potato plants. Am. Potato J. 59:221-230.
8. Mellor, F. C., and Stace-Smith, R. 1970. Virus strain differences in eradication of potato viruses X and S. Phytopathology 60:1587-1590.
9. Mellor, F. C., and Stace-Smith, R. 1977. Virus-free potatoes by tissue culture. Pages 616-637 in: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. J. Reinert and Y. P. S. Bajaj, eds. Springer-Verlag, Berlin.
10. Schuster, G. 1976. Wirkung von 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazol) auf die Vermehrung systemischer Viren in *Nicotiana tabacum* 'Samsun.' Ber. Inst. Tabakforsch. 23:21-36.
11. Schuster, G. 1979. On some interactions of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole) and plant hormones in virus-infected plants. Phytopathol. Z. 94:72-79.
12. Secor, G. A., and Nyland, G. 1978. Rose ring pattern: A component of the rose-mosaic complex. Phytopathology 68:1005-1010.
13. Shepard, J. F. 1977. Regeneration of plants from protoplasts of potato virus X-infected tobacco leaves. II. Influence of Virazole on the frequency of infection. Virology 78:261-266.
14. Stace-Smith, R., and Mellor, F. C. 1968. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. Phytopathology 58:199-203.