ERADICATION OF POTATO VIRUS Y AND S FROM POTATO BY CHEMOTHERAPY OF CULTURED AXILLARY BUD TIPS[1]

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Abstract

Ribavirin (virazole) treatment of cultured axillary bud tips (3-4 mm) was tested as a method of eradicating potato virus Y (PVY) and potato virus S (PVS) from two potato cultivars, Norchip and Desiree.

Ribavirin treatment was phytotoxic at all concentrations tested, but cultivars treated at 5 mg/l were visually similar to the nontreated control cultures after 20 weeks. The buds treated with ribavirin at 20 mg/l had a survival rate of only 30-40%. Virus assays indicated that 2, 74, 82, and 89% of the plants were free of PVY, and 1, 71, 83 and 90% were free of PVS, 20 weeks following treatment with 0, 5, 10, and 20 mg/l, respectively. Virus assays indicated that 2, 74, 82, 89% of the plants were free of PVY, and 1, 71, 83 and 90% were free of PVS, 20 weeks following treatment with 0, 5, 10, and 20 mg/l ribavirin treatment respectively for cultivar Norchip. Desiree cultivar assayed 2, 69, 80 and 86% free of PVY and 2, 74, 85 and 93% free of PVS, 20 weeks following treatment with 0, 5, 10, and 20 mg/l ribavirin treatment respectively.

Introduction

Potato (*Solanum tuberosum* L.) serves as a host for a number of viruses. Two of the most commonly encountered in seedstock development in North Dakota and elsewhere are potato virus Y (PVY) and potato virus S (PVS). These viruses cause significant yield losses and the processes of elimination, maintenance and renewal of virus-free stock plants are costly (1).

Stace-Smith and Mellor (9) successfully combined thermotherapy of mother stock plants with axillary bud culture to obtain PVS-free potato clones. Thomson (11) successfully eradicated PVY from several potato cultivars by use of combined heat treatment and shoot-tip cultures. Even though thermotherapy and shoot-tip culture methods have been used as a means of eradicating virus with considerable success, they have not received routine adoption due to the minute amount of meristematic tissue (<0.05

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mm) that must be excised to achieve virus eradication, which can result in low survival rates and a prolonged plant regeneration period (9).

Chemotherapy has not been generally recognized as an effective method of eradicating viruses from infected plants. However, a synthetic riboside, ribavirin (1-B-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), also known as virazole (8, 10) has been reported to have antiviral activity against several plant viruses (1, 2, 3, 4, 7) including some in potato. Cassels (1) reported successful eradication of virus complexes Y, X, S and M from explant culture of potato cultivars by incorporation of 205 μM ribavirin in culture media. Klein and Livingston (3) reported 80% PVX eradication from meristem shoot-tip cultures of two potato cultivars by incorporation of 10 mg/l ribavirin in culture media. Shepard (7) reported eradication of PVY from tobacco protoplast calli using 10 mg/l ribavirin in culture media during the onset of shoot morphogenesis.

The objective of this research was to investigate the effects of ribavirin in culture media as an eradicant of potato virus Y (PVY) and potato virus S (PVS) from infected potato shoot-tip (3-4 mm) cultures rather than meristematic domes. Shoot tips are the preferred explants for rapid multiplication schemes because they are larger than meristems, grow faster, and have a higher survival rate.

Materials and Methods

Potato (*Solanum tuberosum* L.) cultivars Norchip and Desiree were grown in pots in the greenhouse. Enzyme linked immunosorbent assay (ELISA) tests were conducted on all plants to insure freedom from PVS and PVY. When plants were approximately 20 cm tall they were separated into three groups: Group I was maintained virus-free (not inoculated), Group II was inoculated with PVY and Group III was inoculated with PVS. Infected leaf tissue was ground in 0.05 M phosphate buffer pH 7.0 and mechanically inoculated to test plants. PVY was provided by Dr. E. Bantarri, University of Minnesota, and maintained in Burley 21 tobacco. PVS was provided by Dr. S.A. Slack, University of Wisconsin and maintained in potato selection B41956. Approximately one month after inoculation, plants were tested for PVY or PVS using ELISA kits (Boehringer-Mannheim Biochemicals, Indianapolis, IN). Non-infected plants from groups II and III were discarded, and infected plants were maintained for use as explant sources. Apical tips were removed to promote axillary shoot formation.

Lateral shoots with several axillary buds were removed from the parent potato plants and cut into short segments, each bearing one axillary bud. The segments were surface sterilized by sequential washes in sterile distilled water containing several drops of Tween 20 detergent, 70% ethanol soak for 3 minutes, 10 minute soak in 1% sodium hypochlorite with periodic shaking, and rinsing 4 times in sterile distilled water. Axillary bud tips approxi-

mately 3-4 mm in length, hereafter called bud tips, were excised with a razor blade and transferred to the surfaces of filter paper wicks in culture tubes containing liquid culture medium or directly to solid media.

The culture medium (M/S) for potato bud tip cultures was prepared from stock solutions as described by Murashige and Skoog (5), supplemented with 0.25 mg/l gibberellic acid (GA_3). The culture medium was autoclaved and cooled before adding filter sterilized ribavirin to avoid heat degradation. Ribavirin (Virazole) was obtained from ICN Nutritional Biochemicals, Cleveland, OH. Culture medium was used within one week after preparation.

Ribavirin treatments were done on each group of plants (uninfected, PVS infected, PVY infected) for both cv Norchip and Desiree (a total of six groups). Each group was treated with ribavirin at concentrations of 0, 5, 10, and 20 mg/l. Forty-four bud tips were used for each treatment.

All *in vitro* cultures were maintained in a growth chamber in a completely randomized design under a 16 hour photoperiod at 4000 Lux and temperature of 24±1C. After 10 weeks of culture, surviving bud tip plantlets were transferred to fresh M/S medium containing 0.8% agar in culture tubes without ribavirin, to promote shoot and root growth. After two weeks of growth on the medium without ribavirin (12 weeks after initial excision of the buds) measurements were taken on 10 uninfected (Group I) plants per ribavirin treatment for shoot length, root length, number of internodes, and fresh weight. Tuber yield per plant was taken from the average tuber weight measurements of harvested tubers three months after transplantation. These measurements were used to describe the phytotoxic effects of ribavirin on growth. All plantlets, after a total of 12 weeks of culture (10 weeks on ribavirin plus 2 weeks without ribavirin), were transplanted into plots containing a synthetic soil mixture, acclimatized under artificial light in the laboratory by gradually removing a plastic bag covering the transplants, and transferred to the glasshouse. The plantlets were tested for virus at 14, 16, and 20 weeks from the date of initial culture, which was 2, 4 and 8 weeks since transfer to the greenhouse. ELISA tests were conducted using sap dilutions of 1:20 and 1:200. Boehringer-Mannheim ELISA kits were used except for the 20 week PVY test which utilized Potascreen (Agdia, Inc., Granger, IN).

Values obtained by growth measurements were analyzed by analysis of variance and a least significant difference (LSD) at $P=0.05$ was used to compare ribavirin treatments to the control.

Results

The effects of ribavirin on plantlet growth after the 12 week culture period are summarized in Table 1. Ribavirin treatment reduced growth rate in culture for both cultivars, but bud tips that had been treated with 5 mg/l ribavirin recovered from the phytotoxic effects and visually resembled the

TABLE 1. — *Growth data of uninfected potato plantlets cv Desiree and Norchip after 12 weeks culture.*[1]

Ribavirin Conc Mg/l	Plant Length (cm)		Root Length (cm)		Number of Internodes		Fresh Weight (gm)		Tuber Yield[3] (gm)	
	Nor	Des	Nor	Des	Nor	Des	Nor	Des	Nor	Des
0	11.6a[2]	7.7a	12.0a	8.3a	12.0a	11.0a	1.9a	1.6a	122	90
5	9.4b	7.2a	6.4b	8.0a	9.0b	10.0a	1.0b	0.6b	97	67
10	8.2b	6.8a	2.3c	5.2b	6.0c	7.0b	0.8b	0.5b	52	58
20	3.0c	3.9b	0.6c	0.4c	3.0d	5.0c	0.4b	0.4b	21	20
LSD 0.05	1.9	1.3	2.3	1.9	2.0	1.2	0.8	0.8	ND[4]	ND

[1]Culture period included 10 weeks with ribavirin and 2 weeks without ribavirin on M/S medium.
[2]Each data point is the average of 10 plantlets. Means followed by the same letter do not differ significantly from the control (P=0.05).
[3]Tuber yield calculated three months after transplanting plantlets to pots in the greenhouse.
[4]Not done.

nontreated control cultures in the greenhouse at 20 weeks. Bud tip cultures treated with 20 mg/l ribavirin showed severe growth abnormalities which included chlorosis, stunting, root inhibition and base callus formation. In addition, there was a reduced survival rate (Table 2) and reduced tuber yield (Table 1) which corresponded with increased ribavirin concentration. Greater but tip mortality and phytoxicity were detectable earlier in cultures treated with the higher (10 and 20 mg/l) ribavirin concentrations. Shoot tip cultures of the cultivar Desiree appeared to be more sensitive to ribavirin treatment than those of Norchip. The results of PVY and PVS assays and survival rate of plantlets are shown in Table 2. Generally the 0, 5, 10, and 20

TABLE 2. — *Survival and percentage of developed plantlets of two potato cultivars that assayed PVY- and PVS-free following treatment of bud tip cultures with ribavirin at three concentrations and testing dates.*

Cultivar treated	Ribavirin Conc. mg/l	No. of shoot tips cult./group	% Survival of shoot tips		% Regenerated plantlets PVY-free			% Regenerated plantlets PVS-free		
			Gr. II	Gr. III	14 wks.	16 wks.	20 wks.	14 wks.	16 wks.	20 wks.
Norchip	0	44	93	100	1	2	2	0	1	1
	5	44	86	95	72	74	74	72	72	71
	10	44	75	82	81	82	82	82	83	83
	20	44	41	45	88	88	89	90	90	90
Desiree	0	44	95	98	0	2	2	3	2	2
	5	44	82	77	65	68	69	73	74	74
	10	44	57	61	81	80	80	84	85	85
	20	44	32	36	87	86	86	90	92	93

mg/l ribavirin treatments gave average survival rates of 97, 85, 70 and 40% and average of virus-free assays of 2, 72, 83 and 90% respectively, across the two potato cultivars and viruses. Similar results were obtained with ELISA whether sap was diluted 1:20 or 1:200.

The plantlets that developed from shoot tip cultures grown on solid nutrient medium containing ribavirin demonstrated abnormal growth attributable to ribavirin, but when assayed, were PVY and PVS positive for all ribavirin treatments for both cultivars.

Discussion

Phytotoxicity due to ribavirin treatment was evident at concentrations comparable to those reported to cause phytotoxicity in potato (3) and other plant host-virus combinations (6, 7). The results of Klein and Livingston (3) indicated that ribavirin treatment reduced growth in both cultivars, but shoot tip cultures treated with 1 and 10 μg/ml eventually equalled growth levels of the controls, while 100 μg/ml cultures failed to develop into plantlets. Their PVX eradication assay showed 0% for control, and 80% for 10 μg/ml treatments. These results are similar to the results of our control and 10 mg/l ribavirin treatment for both phytotoxic responses and virus eradication. If ribavirin treatment of plant tissues is developed for commercial use, it may become a routine method of virus eradication. This would be especially true if large bud tips (3-4 mm) are used rather than meristematic domes (0.2-0.5 mm), which are apparently more susceptible to the phytotoxic effects of ribavirin (3). An added advantage is that the regeneration time needed for larger bud-tips is much shorter than that required for meristematic domes under similar conditions. A regeneration time of 4-12 months is needed for meristematic domes under ribavirin treatment (1, 3), whereas our larger bud-tips were generally ready for transfer into the greenhouse after 3 months with a high percentage of virus eradication and explant regeneration. Stace-Smith and Mellor (9) reported that PVX and PVS were eradicated from excised axillary buds (0.3-1 mm) using heat therapy 51 and 11% of the time, respectively. Their procedure of heat therapy, from initial heat treatment to explant regeneration took approximately six months. This system, which combines chemotherapy and larger shoot-tips, has an advantage over other systems of virus eradication previously reported.

Virus eradication was not obtained when shoot-tips were placed on solid media containing ribavirin. This was probably due to the inability of the chemical to diffuse rapidly enough into the developing tissues. Therefore, it is imperative that ribavirin be used in liquid media in a virus-free seedstock program.

These results indicated that ribavirin exerts considerable inhibitory effects on virus replication (72% virus eradication) even at low concentrations (5 mg/l) with minimal phytotoxic effects to the plants.

Even though ribavirin affected the growth of the explants, it appeared that the effects were diminished by time, since ribavirin-treated mature plants were not noticeably depressed compared to control plants. It remains to be seen whether the phytotoxic effect of ribavirin persists during subsequent multiplication of explants via nodal cuttings.

We observed excessive phytotoxic responses of ribavirin-treated virus infected plants compared to ribavirin-treated uninfected plants as reported by others (3, 6). Schuster (6) reported that combined use of abscisic acid and ribavirin considerably reduced phytotoxic responses of the plants to ribavirin. A combination such as this may contribute to making chemotherapy of virus infected plants even more practical.

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