Eradication of Potato Spindle Tuber Virus by Thermotherapy and Axillary Bud Culture

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ABSTRACT

Potato spindle tuber virus (PSTV) was eradicated from infected potato (*Solanum tuberosum*) by nutrient culture of axillary buds excised from heat-treated plants. Plants infected with severe PSTV were subjected to air temperatures which alternated daily from 33 to 36 C. Fluorescent lights provided a 16-hr photoperiod. At intervals during treatment, axillary buds 0.5-1.0 mm long were excised for nutrient culture. Severe PSTV survived in 62 of the 66 plantlets that developed. The remaining 4 plantlets showed no symptoms, but indexing revealed that all were infected with mild PSTV. Rooted tip cuttings of 1 plant infected with mild PSTV were again subjected to the combined treatment of thermotherapy and nutrient culture of axillary buds. Mild PSTV survived in 242 of the 248 plantlets that developed. In the remaining 6 plantlets, PSTV could not be detected, either by direct inoculation to tomato (*Lycopersicon esculentum*) or by the challenge-inoculation technique. The low incidence of virus-free plantlets may be associated with the fact that PSTV appears to be free nucleic acid with no protein coat. Phytopathology 60:1857-1858.

Some viruses that are widespread among cultivars of potato (*Solanum tuberosum* L.) can now be eradicated from infected plants. Potato spindle tuber virus (PSTV) is one of the few that has resisted treatment. A single attempt to eradicate PSTV was reported by Fernow et al. (3), who subjected tubers of nine infected cultivars to 35 C for varying periods. PSTV survived up to 39 days, although similar treatment eradicated potato leaf roll virus.

Heat treatment alone seldom eradicates potato viruses from tubers or plants, but it greatly increases the effectiveness of bud culture as a means of exploiting local eradication within the plant. By nutrient culture of axillary buds excised from heat-treated plants, we have recently eradicated potato virus X and potato virus S from 18 potato cultivars (5). Success with these two viruses prompted our attempts to eradicate PSTV by the same technique.

The isolate of PSTV used in this study originated from a tuber kindly supplied by W. B. Raymer. Mechanical transmission to seedlings of tomato (*Lycopersicon esculentum* Mill. 'Rutgers') caused the severe epinasty, rugosity, and leaf necrosis described by Raymer & O'Brien (6). To provide material for eradication studies, the virus was rub-transmitted from infected tomato to virus-free potato, cultivar White Rose. Rooted tip cuttings of infected potato were heat-treated by growing them in a chamber in which air temp alternated daily from 33 to 36 C, soil temp from 30 to 32 C. Fluorescent lights provided a 16-hr photoperiod. At intervals during treatment, shoots were removed and axillary buds, 0.5-1.0 mm long, were excised for nutrient culture. The techniques have been described (4, 7). Buds that developed into plantlets in culture were transferred to soil and, when established, indexed for PSTV. For indexing, a leaf from each plantlet was ground in a well of a porcelain spot plate. For each leaf, a separate rounded glass rod was used, first as a pestle, and then to apply the leaf macerate to the cotyledons of small Rutgers tomato seedlings. Four seedlings, 3-4 cm tall, were inoculated from each potato plantlet. Diagnostic symptoms usually appeared within 2-3 weeks, but final readings were taken 1 month after inoculation. When reactions were doubtful or negative, inoculations were repeated.

TABLE 1. Eradication of severe potato spindle tuber virus (PSTV) from infected potato by heat treatment followed by axillary bud culture

Treatment period (weeks)	Plantlets developed	Plantlets with	
		Severe PSTV	Mild PSTV
2	6	6	0
4	6	6	0
6	5	4	1
8	34	31	3
12	5	5	0
14	10	10	0
Totals	66	62	4

Nearly all of the potato plantlets that developed were weak, with thin spindly shoots. Although obviously infected with PSTV, they were routinely indexed. In all instances, they induced typical symptoms of severe PSTV in tomato seedlings. A few plantlets were vigorous and, when established in soil, developed into apparently normal plants. Transmission from these plants to tomato seedlings did not cause symptoms of severe PSTV but, 3-4 weeks after inoculation, necrotic flecks developed on one or more leaves of the inoculated plants. These symptoms indicated that, although severe PSTV had been eradicated, a mild strain persisted. Of 66 plantlets that developed, severe PSTV survived in 62, mild PSTV in 4 (Table 1).

For further studies of PSTV eradication, one of the

TABLE 2. Eradication of mild potato spindle tuber virus from infected potato by heat treatment followed by axillary bud culture

Treatment period (weeks)	Plantlets developed	No. plantlets	
		Infected	Virus-free
2	11	11	0
4	27	27	0
6	56	53	3
8	74	73	1
10	52	50	2
12	28	28	0
Totals	248	242	6

potato plants that retained a mild strain of the virus was chosen as a virus source plant. Rooted tip cuttings of this plant were again subjected to 12 weeks' heat treatment, during which buds were excised at 2-week intervals. Excised buds were smaller than those previously taken, 0.3-0.8 mm long. Of 248 plantlets that developed, mild PSTV was detected in 242. The remaining six plantlets induced no symptoms on any tomato seedlings in two or more separate tests (Table 2). Also, PSTV was not detected in any of these six plantlets by the challenge-inoculation technique of Fernow (2). Tomato seedlings inoculated from these plantlets were not protected against subsequent infection by severe PSTV, indicating that the plantlets did not carry a latent PSTV that was too mild to detect in tomato. The plantlets from which mild PSTV was eradicated developed from the smallest excised buds.

The low incidence of virus-free plantlets indicates that PSTV is far more difficult to eradicate than any potato virus so far investigated. The reason is unknown, but may be associated with the fact that, unlike other viruses of potato, PSTV has no protein coat and the infective unit appears to be free nucleic acid (1). A possible mechanism may be that thermotherapy denatures the protein sheath, a component which is essential to most plant viruses. Another possibility is that the meristematic cells which are usually not invaded by viruses having a protein coat are invaded by viruses that exist as infectious nucleic acid.

LITERATURE CITED

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