Elimination of Potato Spindle Tuber Viroid by Low Temperature and Meristem Culture

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ABSTRACT

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Since growing of potato plants at low temperature seems to adversely affect the replication of the potato spindle tuber viroid (PSTV), a study was undertaken to investigate the possibility of eradicating it from an infected clone. A severe strain of PSTV was successfully eliminated from a potato clone by a combination of low temperature treatment (5–8 C) of the infected plant and subsequent meristem culture. Seven of 13 plants, which developed from meristems of plantlets grown in vitro at 5–6 C for 6 mo, were found to be free of PSTV. From plantlets derived from infected tubers and grown at 8 C for 4 mo, 17 excised meristems grew to plants and five of them were free of PSTV. All plants grown from meristems of in vitro plants or of plants from infected tubers that had been cultivated at 22–25 C, were still infected. PSTV was detected by electrophoresis and tomato bioassay on plants raised under temperature conditions favorable for PSTV multiplication.

RESUMEN

Debido a que la replicación de PSTV en material infectado parece ser afectada por temperatura baja se realizaron experimentos con el fin de eliminarlo de plantas infectadas. Una variante severa del viroide del tubérculo ahusado de la papa (PSTV) fue eliminada de un clon de papa mediante una combinación de tratamiento con baja temperatura y cultivo de meristemas. Siete plantas de un total de 13 originadas de meristemas de plántulas crecidas 'in vitro' a 5–6 C por 6 meses fueron halladas libre de PSTV. De plantas originadas de tubérculos crecidos en suelo a 8 C por 4 meses cinco de 17 plantas originadas de meristemas se hallaron libres de PSTV. Todas las plantas originadas de meristemas de plántulas 'in vitro' o de plantas de tubérculos infectados crecidos a 22–25 C se hallaron infectadas. PSTV fue detectado por medio de electroforesis e inoculación en tomates en plantas mantenidas bajo condiciones favorables para la multiplicación de PSTV.

Potato viruses routinely are eliminated by a combination of heat treatment at 36 C and meristem culture at the Centro Internacional de la Papa (CIP) in Lima, Peru. Thus, the important potato viruses X (PVX) and S (PVS) and several others are easily eliminated (1). Successful eradication of PSTV has not been achieved so far. However, Stace-Smith and Mellor (8) reported that a small number of PSTV-free plantlets were obtained from excised axillary buds of infected plants incubated at 33–36 C.

By growing tomatoes infected with PSTV at temperatures between 15 and 35 C Sänger and Ramm (7) showed that PSTV-RNA starts to accumulate in concentrations discernible in gels as UV-absorbing peaks only at temperatures above 24 C and that above 30 C PSTV-RNA is synthesized at unusually high concentrations. Although a marked effect of illumination on PSTV concentration was not demonstrated, the results of Sänger and Ramm suggested higher viroid concentrations in plants grown under high light intensity. Morris and Smith (3) also found higher viroid concentration in potato and tomato plants growing at 30 C than at 25 C.

This paper reports our investigation of PSTV eradication by treatment at low temperature and low light intensity.

MATERIALS AND METHODS

A clone (BR 63.5) of *Solanum tuberosum* × *S. phureja* found to be infected with a severe strain of PSTV was used in this study. Eradication was attempted in plantlets developed in vitro from node segments containing axillary buds (5,6) and in plants evolved from tubers. The following treatments were compared: A, plantlets grown from infected nodal cuttings in culture medium at 5–6 C under diffused (500 lux) light for 6 mo; B, plantlets grown from infected nodal cuttings in culture medium at 25 C for 2 mo under 1,500 lux light intensity; C, plants grown from infected tubers at 8 C and 5,000 lux 16 hr/day for 4 mo; D, plants grown from infected tubers under normal greenhouse conditions for 4 mo.

Plantlets that received treatments A and B were cultivated on a modified Murashige-Skoog medium (4). From each of these four types of plants 48 meristems with no more than one leaf primordium were excised and cultured in medium No. 6 developed by Kao and Michayluk (2) containing 0.8% agar. Meristems were incubated at 25 C at a light intensity of 1,000 lux for 16 hr per day. Calli developed on this medium within 2 mo. These were transferred to a liquid medium that contained the inorganic salts and vitamin components of the medium of Murashige and Skoog (4) and the various hormonal supplements developed by Roca et al (6) for differentiation of multiple shoots in shake culture.

Plantlets which developed were transferred to small pots filled

TABLE 1. Effect of temperature on detection of potato spindle tuber viroid (PSTV)

Treatment[a]	Temperature (C)	Incubation period (mo)	Ratio[b] (positive sample/ total samples tested)
A	30/36	1	8/8
B	24	1	8/8
C	5	3	0/8
D	5	6	0/8
E	(5)→ 25	(3)→ 1	8/8

[a]Plantlets of clone BR 63.5 for all treatments were obtained from nodal cuttings infected with a severe strain of PSTV. For treatment E, nodal cuttings were obtained from negative plantlets of treatment D and grown to plants at 25 C for 1 mo.
[b]Tests were carried out by inoculation onto tomatoes.

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TABLE 2. Elimination of a severe strain of potato spindle tuber viroid (PSTV) from infected potato by low temperature treatment followed by meristem culture

Treatment	Origin of PSTV-infected plants[a]	Temperature (C)	Light Intensity (lux)	Incubation period (mo)	Ratio (PSTV-free plants/ total plants)
A	Nodal cuttings	5–6	500	6	7/13 (53%)
B	Nodal cuttings	25	1,500	2	0/16 (0)
C	Tubers	8	5,000	4	5/17 (30%)
D	Tubers	22	5,000	4	0/16 (0)

[a]Plants from which meristems were excised after treatment.

with a mixture of peat and coarse sand and grown at 25 C and 6,000 lux for 16 hr a day.

PSTV testing methods. Testing for PSTV was performed by inoculating tomatoes grown at temperatures >25 C under continuous fluorescent light (2,000 lux) for 2–4 wk according to Yang and Hooker (9). Gel electrophoresis was performed according to the method described by Morris and Smith (3) in acrylamide gel slabs by using a Pantha-Phor apparatus (Labor-Müller, Hann-München, Germany). Plants resulting from every treatment were tested at intervals until maturity by inoculation to tomatoes and by electrophoresis. Tubers from both healthy and diseased plants from each treatment were planted in the screenhouse and tested again by the two methods.

RESULTS

Effect of temperature on detection of PSTV. Infected plantlets of clone BR 63.5 were grown at different temperatures and tested for PSTV by inoculation to tomatoes. PSTV was not detected in plantlets grown at 5 C for 3 and 6 mo (Table 1, treatments C and D) whereas in those grown at 24 C or 30–36 C (treatments A and B) the viroid always was detected. Nodal cuttings were obtained from plantlets kept at 5 C for 6 mo and tested after 1 mo of growth at 25 C (treatment E). In all cases the assay for PSTV was positive which suggested that the viroid was not eliminated from this material, but more likely it was present in such a low concentration that its detection was not possible.

Elimination of PSTV from infected plants. Although we excised 48 meristems from infected plants in treatments A and C (low temperature and low light intensity) only 13 and 17 plantlets, respectively, survived. One-half to one-third of those produced PSTV-free plantlets whereas no PSTV-free plants resulted from those grown at 22 or 25 C (treatment B and D, Table 2). The resulting PSTV-free plants were repeatedly propagated by stem cuttings grown in a glasshouse at 25 C and retested by electrophoresis and by inoculation to tomatoes. All plants remained free of PSTV, which suggested that PSTV had been eliminated. Special care was taken to determine the possible existence of mild strains of PSTV but no evidence of their presence was obtained by electrophoresis of extracts from either the potato plants or the inoculated tomatoes.

Tubers from PSTV-free plants as well as from some infected ones were sprouted and planted at 25 C. Extracts from plants which developed were tested by inoculation onto tomatoes and by gel electrophoresis. Complete agreement with the results of previous testing was obtained; all tubers from PSTV-free plants remained free of PSTV and all tubers derived from infected mother plants were infected.

DISCUSSION

Little work has been published on the eradication of potato spindle tuber viroid (PSTV). Stace-Smith and Mellor (8) obtained a low percentage (2.4–6.0%) of PSTV-free plants following culture of axillary buds derived from mother plants which had been incubated at 33–36 C. They also reported elimination of a severe strain in four of 66 plantlets, but they found these plantlets to be infected with a mild strain. Our results indicate that PSTV can be more successfully eliminated by growing infected mother plants under conditions of low temperature and low light intensity. It is felt that testing two generations by a combination of the two most reliable methods for diagnosis of PSTV, should ensure that the material is free of PSTV. The percentage of plants free of PSTV was higher in plantlets derived from nodal cuttings kept at 5–6 C (~53%) than in those developed from infected tubers at 8 C (~30%). We do not know the reason for this difference, but because small numbers of plants were involved this difference may be more apparent than real. There is a possibility, however, that low light intensity plays an important role in eradication which would correlate well with the findings of Sänger and Ramm (7) who found higher viroid concentration in tomato plants kept under high light intensity.

The incubation period at low temperature seems also to be an important factor for elimination of PSTV. In infected plantlets kept at 5–6 C for 3–6 mo PSTV was not detected in different sections of the plantlets, but it was detected in nodal cuttings grown at 25 C for 1 mo. On the other hand, recent results indicate that in plants treated at 5–6 C for only 2 mo no PSTV-free meristems were obtained. These results suggested that under low temperature conditions the multiplication rate of PSTV is lowered, thus permitting the development of viroid-free meristems whose numbers increase with time.

The low number of plantlets obtained from 48 meristems excised from plants in each treatment may be due to our intention of excising only the apical dome of the meristem. Whether this is also a factor contributing to our success in elimination of PSTV is not known. However, Stace-Smith and Mellor (8) indicated that the viroid-free plantlets they obtained were derived from the smallest excised axillary buds. Work in progress with several potato cultivars should clarify the role of each factor and may confirm our results. Even though an extended period of time is needed to eradicate PSTV by this procedure, it provides a potentially useful method of obtaining PSTV-free plants from infected plants with valuable germplasm.

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