Heat Therapy and Stolon Apex Culture to Eliminate Mild Yellow-edge Virus from Hood Strawberry

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ABSTRACT

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Strawberry mild yellow-edge virus (SMYEV) was eliminated from 4-mm-long stolon apex explants of Hood strawberry plants held at a constant 38 C in a growth chamber. Virus detection in each regenerated plant was by means of leaf graft analysis to a maximum of three indicator plants of *Fragaria vesca*. The percentage of virus elimination from these explants was directly related to the length of time that they had grown at 38 C. Equation I, which describes this linear relationship ($Y = 70.35 - 1.69X$ in which Y = arcsin of the square root of the fraction of plants infected with SMYEV and X = number of days at 38 C), predicts elimination of SMYEV from 50% of regenerated, 4-mm-long stolon apices excised from source plants after 15 days at 38 C and 100% after 42 days. SMYEV was also eliminated from regenerated stolon apices 0.25–4 mm in length from infected Hood plants grown at greenhouse temperatures of 20–25 C. Equation II, which describes this linear relationship ($Y = 4.90 + 17.96Z$ in which Y = arcsin of the square root of the fraction of plants infected with SMYEV and Z = explant length in millimeters) predicts elimination of SMYEV from 50% of explanted shoot apices that were 2.25 mm in length and 98.6% that were 100 μm in length. According to this equation, elimination of SMYEV does not occur from all explants from plants grown at ambient temperature regardless of their size. Heat therapy and stolon apex culture contribute independently to freeing explants of SMYEV and therefore can be combined to obtain increased reliability in developing strawberry clones free of SMYEV.

Strawberry mild yellow-edge virus (SMYEV) is a persistent, aphid-borne virus (probably a luteovirus) of cultivated strawberry in many parts of the world (5,6,8,9). Clones of many cultivars have been freed of SMYEV by the use of heat therapy and/or shoot apex culture (8–10,13,15,16). In the present study, we determined the incidence of SMYEV in regenerated, excised stolon apices after varying the length of time of heat therapy of plants from which stolon apices of constant length were then taken and after varying the size of stolon apices excised from plants grown in the greenhouse at temperatures of 20–25 C.

MATERIALS AND METHODS

Isolate 76-B of SMYEV in strawberry cultivar Hood from Aurora, OR, was used in these studies. The Hood clone was found to be free from other virus or viruslike agents detectable by leaf graft analysis (3) on clones UC-4 and UC-6 of *Fragaria vesca* (2). SMYEV-infected Hood daughter plants were propagated in a screened, insecticide-treated greenhouse. For heat therapy studies, Hood plants that had been grown in 13-cm wood-fiber pots for 3 mo were inserted into 15-cm wood-fiber pots. The space between the two pots was filled with peat moss, and over a 30-day period the plants were brought from greenhouse temperatures (20–25 C) to a constant 38 ±1 C by daily temperature increases of about 0.6 C in a growth chamber with fluorescent and incandescent light (11,000 lux) for 16 hr per day. The plants were watered only with 1/4-strength Hoagland's solution at 38 C. At intervals (Table 1) during 45 days, stolon tips 4 mm long were excised and grown in tissue culture (1,13) to regenerate whole plants.

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The influence of explant size on the incidence of SMYEV in stolon tips taken from Hood plants grown at temperatures of 20–25 C in the greenhouse was studied by taking explants ranging in size from 0.25 to 8.0 mm in length. Whole plants were regenerated from these explants as noted above.

Regenerated plants were grown on greenhouse benches and indexed by leaf grafting to UC-4 to identify those infected with SMYEV (2,3). Plants that indexed negative were reindexed on UC-4 and on *F. vesca* var. *semperflorens* (Duch.) Ser. 'Alpine' over a 9-mo period. A separate study (R. H. Converse, *unpublished*) has shown that the reliability of detecting SMYEV by leaf grafting is such that three separate indexings must be negative before concluding that the source plant is free of graft-detectable SMYEV with 95% confidence. Moreover, repeated virus testing over time is desirable because there are reports (4,8,12,14) that the titer of some viruses falls below detection threshholds in explants after therapy, but later rises to detectable levels.

RESULTS

SMYEV incidence decreased in regenerated 4-mm-long explants as the period of source plant growth at 38 C increased (Table 1). These data fit ($r = -0.970$ [$P = 0.05$], and F for regression mean square/residual mean square from analysis of variance was significant at $P = 0.05$) linear regression equation I:

$$Y = 70.4 - 1.69X \qquad \text{(I)}$$

in which Y = arcsin of the square root of the fraction of plants infected with SMYEV and X = number of days at 38 C. Calculated percentages of plants infected with SMYEV based on equation I are presented in Table 1. Equation I predicts 50% inactivation of SMYEV in 4-mm explants obtained after 15 days at 38 C.

As the length of excised stolon apices from source plants grown at greenhouse temperatures of 20–25 C decreased, the incidence of SMYEV in the resulting regenerated plants also decreased (Table 2). When data for apices 8 mm long are omitted, the data for apices

TABLE 1. Incidence of strawberry mild yellow-edge virus (SMYEV) in plants regenerated from excised stolon tips[a] of strawberry culitivar Hood grown for various periods of time at 38 C

Heat therapy period (days)	Number of explants		Explants infected with SMYEV (%)	
	Infected	Total	Observed	Predicted[b]
0	26	27	96	89
15	6	19	32	50
30	1	12	8	11
45	0	4	0	0

[a]Excised tips 4 mm long.
[b]Predicted by equation I.

TABLE 2. Incidence of strawberry mild yellow-edge virus (SMYEV) in plants regenerated from various lengths of strawberry cultivar Hood stolon tips grown at greenhouse temperatures of 20–25 C

Average length of excised stolon tips (mm)	Number of explants		Explants infected with SMYEV (%)	
	Infected	Total	Observed	Predicted[a]
0.25	1	20	5	3
0.5	0	12	0	6
1.0	6	14	43	15
2.0	4	15	27	43
4.0	26	27	96	95
8.0	11	12	92	100

[a]Predicted by equation II.

0.25–4.0 mm long ($r = 0.91$ [$P = 0.05$], and F for regression mean square/residual mean square from analysis of variance was significant at $P = 0.05$) fit linear regression equation II:

$$Y = 4.90 + 17.96Z \qquad \text{(II)}$$

in which Y = arcsin of the square root of the fraction of plants infected with SMYEV and Z = explant length in millimeters. Calculated percentages of regenerated explants infected with SMYEV based on equation II are shown in Table 2. Equation II predicts that 50% of the greenhouse explants 2.2 mm in length would be freed of SMYEV.

In this study, the overall reliability of leaf graft indexing on Alpine and UC-4 to detect this Hood isolate of SMYEV was 73%. This percentage was obtained by combining the indexing results from known positive plants tested from all three of the graft tests (43/62, 17/23, 12/14). Total numbers decreased with each succeeding graft test because only SMYEV-negative plants were retested each time.

DISCUSSION

Although there are no previous similar time studies on the elimination of SMYEV in strawberry plants by heat therapy, Posnette and Cropley (15) obtained a daughter plant free of SMYEV after 16 days at 37 C, while the mother plant remained infected. Vine (16) found no evidence that heat treatment helped to eliminate SMYEV from infected clones. Mellor and Fitzpatrick (8) found that mother plants remained infected after 6 mo at 38 C but that small axillary buds propagated from those plants after 3 mo at 38 C were often freed of SMYEV. In the present study, an inverse linear relationship was found between the length of time infected source plants were held at 38 C and the resulting incidence of SMYEV in explants from these source plants. Equation I predicts that 4-mm explants taken from infected source plants held 42 days at 38 C should be free of graft-detectable SMYEV.

It is widely accepted for that for many viruses, including SMYEV, the smaller the shoot apex excised the more likely it is to be free of the virus being studied (1,4,7,13,14). Although not relating size of the initial explant to the incidence of SMYEV in the resulting regenerated plants, several workers (10,11) found that excising shoot apices 0.3–8.0 mm in length from SMYEV-infected plants grown at greenhouse temperatures of 11–25 C gave rise to some healthy plants. Miller and Belkengren (10) found no evidence that brief (1–7-day) heat therapy assisted in eliminating SMYEV from explant sources; however Mullin et al (11) found that only 25% of 0.3 – 0.8 mm explants taken from normally grown infected strawberry plants were free of SMYEV, while 82% of such explants taken from plants grown for 42 days at 36 C were free of SMYEV. The present data show that the incidence of SMYEV decreases as explant length of stolon apices from infected plants grown at greenhouse temperatures of 20–25 C decreases. Equation II describing this relationship predicts that 98.6% of explants of 100 μm in length are likely to be free of SMYEV.

Analyses of the variance in Tables 1 and 2 attributable to regression and to residual error show that F is significant ($P = 0.05$) in each case. These analyses support the interpretation that both relations are linear. As judged by the dissimilar slopes of equations I and II, heat therapy and the excision of small stolon apices act independently to reduce the incidence of SMYEV in the resulting regenerated plants. A combination of both methods to eliminate this virus from strawberry clones is advantageous. In addition, combining heat therapy and stolon apex tissue culture to increase the likelihood of overcoming variations in cultivars, virus strains, and environmental influences on the elimination of SMYEV in strawberry clones is desirable.

This study also points out the unreliability of leaf graft indexing to detect SMYEV. The use of UC-4, a sensitive indicator for SMYEV detection (2), was only 73% reliable. Therefore, three separate negative indexing tests of a given strawberry plant are needed in order to state with a confidence level $\geq$99% that the plant is free from SMYEV detectable by leaf grafting to UC-4. Even so, preliminary data showed that 1 of 12 unheated 8-mm propagants indexed negative for SMYEV in three consecutive leaf graft tests on UC-4.

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