Meristem Culture Frees Strawberries of Mild Yellow Edge, Pallidosis, and Mottle Diseases

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ABSTRACT

Apical meristem culture freed strawberry plants of strawberry pallidosis, strawberry mottle, and strawberry mild yellow edge (SMYE) viruses. However, heat therapy in combination with apical meristem culture produced 33-75% more SMYE-free plants. Meristems cut from heat-treated SMYE-infected Fresno strawberries developed into plants faster than meristems from infected control plants held under ambient conditions. Progeny of the meristem-cultured cultivars has remained free of detectable graft transmissible diseases in a greenhouse and in a screenhouse for 7 yr.

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Clean planting stocks are essential for the control of virus diseases of strawberries. Traditionally, such stocks have been maintained by propagation of plants identified as virus-free in nurseries which are isolated with respect to fruit-producing areas and aphid vector populations. Certain cultivars appeared to be universally virus-infected, and various therapeutic methods have been applied to cure individual plants. These methods included heat therapy utilizing hot water (6, 13) or hot air (2, 4, 5, 7, 12, 14, 19, 20), propagation from small tip cuttings and from apical meristems (1, 15, 16), rooting 0.5- to 1.0-cm-thick crown disks sliced from heat-treated plants (12, 21), rooting axillary buds in sand during or shortly after heat therapy (12), and heat therapy in combination with apical meristem culture (11, 12, 21, 23). At various times, such treatments were applied to strawberry mottle (SM), strawberry mild yellow edge (SMYE), strawberry crinkle (SC), strawberry vein banding (SVB), strawberry latent C (SLC), and strawberry necrotic shock (SNS) viruses. Although unequivocal evidence for the viral nature of all these diseases has not been presented, they are referred to as virus diseases throughout the literature.

Apical meristem culture has been highly successful for freeing carnations and chrysanthemums of viruses (8), and it is applied commercially by these industries in California. Virus-free potatoes (22), dahlias (18), and

cymbidiums (17) have also been produced by this method.

Over the years, SMYE was perhaps the most widespread virus in California strawberries. Recently, "virus-free" planting stock of a number of cultivars was available, but the important cultivars Fresno, Solana, Tufts, and J.6 carried SMYE, and Shasta carried strawberry pallidosis (SP). In 1967, we began to investigate ways of ridding infected stocks of these diseases. The meristem culture technique for strawberries described by Vine (23) was evaluated, but we were unable to adapt her procedure to our conditions. In the present paper, we describe a chemically defined nutrient medium suitable for our cultivars and conditions, and report the successful application of this system to the production of virus-free strawberry planting stock.

MATERIALS AND METHODS.—Cultivated strawberry field plants carrying SMYE were heat-treated for 6 wk in a growth chamber maintained at 36 C air temp. The day length was 18 h, and the light intensity at the leaf surface was 2,100 lux. Meristems were cut from such plants within a few days after the completion of heat treatment. Meristems were also taken from control plants which had been held in the greenhouse under ambient conditions. Apical and axillary bud meristems were cut from both crowns and runners. The knives used for cutting the meristems were made by attaching small chips of razor blade to applicator sticks and were sterilized by overnight exposure to ultraviolet light. One knife was used to expose the meristem tip and a second knife to remove and place it in the culture tube. The meristem tips averaged 0.3 - 0.8 mm in ht and included a leaf primordium. They were excised under ×30 magnification through a binocular dissecting microscope.

The culture media, developed by Vine and by ourselves, include a medium (A) for the first 4 wk of culture and a medium (B) for subsequent use. The two sets of media are compared in Table 1. The most important differences are that our media are chemically defined, containing indole acetic acid and kinetin, but no sucrose or coconut milk. Media were prepared in bulk, adjusted to pH 5.7 - 5.8, and stored in 100 ml aliquots in a freezer. When needed, the appropriate medium was thawed and 2-ml aliquots were pipetted into 10 ml (13 × 100 mm) glass tubes containing Whatman No. 1 filter paper bridges 2.9 cm high to support the meristem tips. The pH of our media after sterilization dropped to approximately 4.5, and a small amount of white precipitate was present in the tubes. Studies on the effect of pH showed that the meristem tips did not survive at a pH lower than 4.5.

Freshly cut meristem tips were placed on the filter paper bridges in tubes containing our medium A. Cultures were grown at approximately 27 C in the laboratory under two banks of cool-white fluorescent lamps mounted 31 cm above the cultures. This lighting arrangement did not measurably increase the temp inside the tubes. After 4 wk, and every 4 wk thereafter, cultures were transferred to fresh medium B until they formed tops about 0.6 cm high and roots at least 1.3 cm long (Fig. 1). Contaminated cultures and cultures which did not grow into plants within 6 mo were discarded.

TABLE 1. Comparative formulation of two sets of meristem culture media used to grow plants from strawberry meristem tissue

Components	Vine's Medium A (10, 23)	Vine's Medium B (10, 23)	Medium A	Medium B
Knop's[a]	---	1 liter	1 liter	1 liter
Major elements[b]	1 liter	---	---	---
Minor elements[c]	1.0 ml	1.0 ml	---	---
Berthelot's[d]	---	---	0.5 ml	0.5 ml
Iron-EDTA[e]	---	---	0.5 ml	0.5 ml
Dextrose	---	40 g	30 g	30 g
Sucrose	20 g	---	---	---
Na-EDTA	0.02 g	0.2 g	---	---
Thiamine·HCl	0.1 mg	---	1.0 mg	1.0 mg
Nicotinic acid	0.5 mg	---	---	0.5 mg
Pyridoxin·HCl	0.5 mg	---	---	0.5 mg
Glycine	2.0 mg	---	---	2.0 mg
Meso-Inositol	100 mg	100 mg	---	100 mg
Indole acetic acid	---	---	1.0 mg	2.5 mg
Indole butyric acid	1.0 mg	---	---	---
Kinetin	---	---	---	0.1 mg
Coconut milk	100 ml	---	---	---

[a]Knop's Solution: $Ca(NO_3)_2 \cdot 4H_2O$, 1.0 g/liter; KNO_3, 0.25 g/liter; $MgSO_4 \cdot 7H_2O$, 0.25 g/liter; KH_2PO_4, 0.25 g/liter.

[b]Major Element Solution: NH_4NO_3, 1.65 g/liter; KNO_3, 1.9 g/liter; $CaCl_2 \cdot 2H_2O$, 0.44 g/liter; KH_2PO_4, 0.17 g/liter; $MgSO_4 \cdot 7H_2O$, 0.37 g/liter.

[c]Minor Element Solution: H_3BO_3, 6.2 mg/liter; $MnSO_4 \cdot 4H_2O$, 22.3 mg/liter; $ZnSO_4 \cdot 7H_2O$, 8.6 mg/liter; KI, 0.83 mg/liter; $Na_2MoO_4 \cdot 2H_2O$, 00.25 mg/liter; $CuSO_4 \cdot 5H_2O$, 0.025 mg/liter; $CoCl_2 \cdot 6H_2O$, 0.025 mg/liter.

[d]Berthelot's Solution: $Fe_2(SO_4)_3$, 50 g/liter; $MnSO_4 \cdot 7H_2O$, 2.0 g/liter; H_3BO_3, 0.05 g/liter; KI, 0.5 g/liter; $NiCl_2 \cdot 6H_2O$, 0.05 g/liter; $CoCl_2 \cdot 6H_2O$, 0.05 g/liter; $ZnSO_4 \cdot 7H_2O$, 0.1 g/liter; $CuSO_4 \cdot 5H_2O$, 0.05 g/liter; H_2SO_4 (32 N), 1.0 ml/liter.

[e]Iron Solution: Na-EDTA, 24.7 g/liter; $FeSO_4 \cdot 7H_2O$, 26.6 g/liter; $Na_2CO_3 \cdot H_2O$, 16.0 g/liter (9).

TABLE 2. Heat therapy of the source plants increases the number of meristems which grow into plants and the number of the resulting meristem plants which are free of strawberry mild yellow edge disease (SMYE)

Cultivar	Heat-treated: Number of meristems: Cut	Which grew to plants	Indexed	SMYE-free	Controls: Number of meristems: Cut	Which grew to plants	Indexed	SMYE-free
Fresno	112	22	12	9	405	22	4	0
Solana	43	15	8	8	44	6	3	2
J.6	74	17	7	4	16	0	---	---
Tufts	164	33	7	7	97	1	1	0

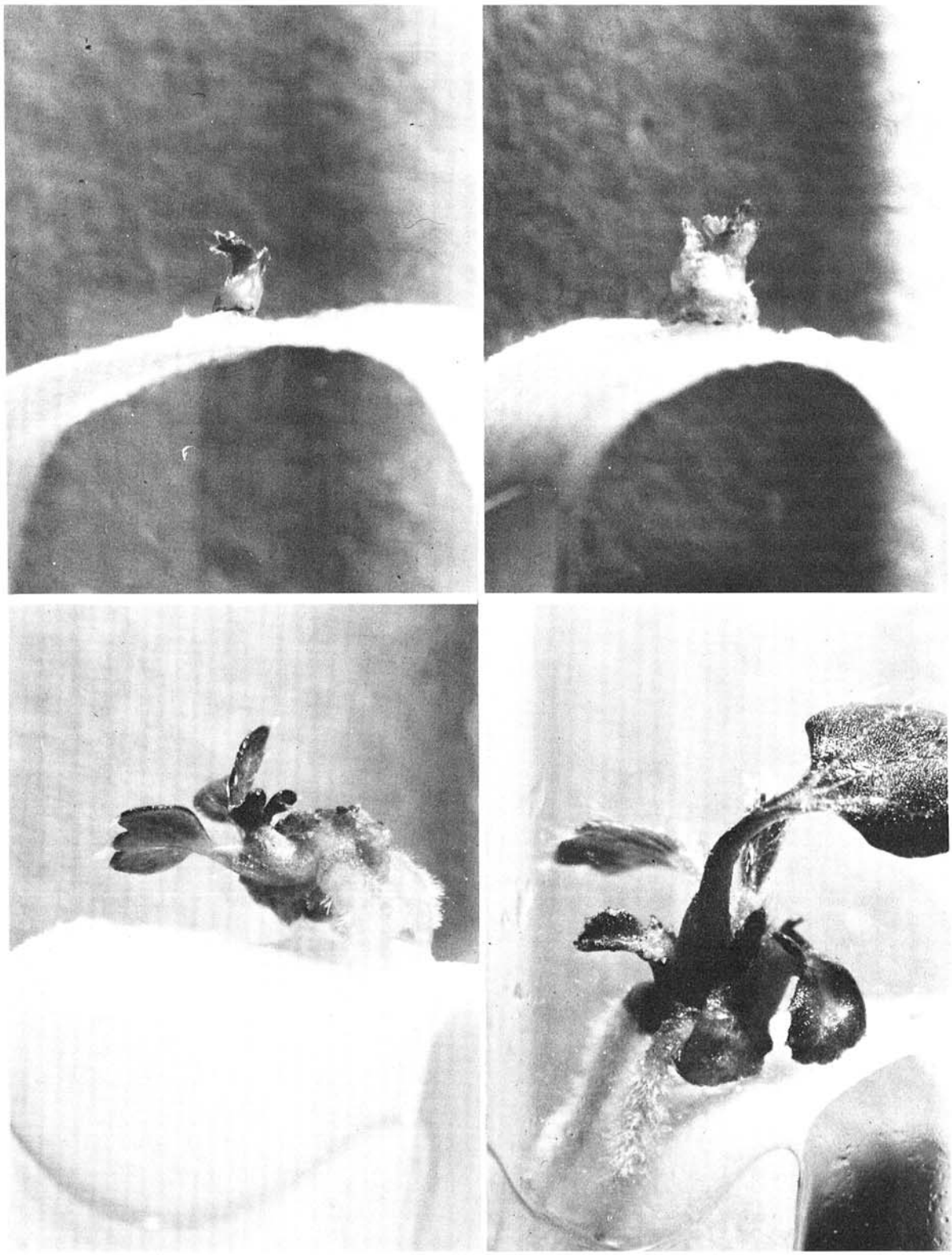

Fig. 1. Middle and late stages of strawberry meristem growth. First, a small amount of callus tissue is produced, followed by simultaneous growth of the tops and roots with numerous root hairs. The plant at lower right is ready to transfer to soil. (Photographs by C. J. Krass.)

The young meristem-derived strawberry plants were transplanted into a mulch which had been partially sterilized by fumigation with chloropicrin. The young plants were very fragile at this time and care was taken to prevent sudden temp and humidity changes. They were enclosed in a plastic container covered with clear plastic wrap on the laboratory bench for 1-2 wk, gradually hardened off, and then transferred to a greenhouse. Once mature, they were indexed every year by leaf insert grafts to *Fragaria vesca* L. and *F. virginiana* Duchese indicator plants.

Progeny of meristem-derived plants which indexed virus-free were moved to a screenhouse approved by the Nursery and Seed Services of the California Department of Agriculture and located in the northern California strawberry nursery area. The plants were increased on raised benches in boxes of soil fumigated with methyl bromide. A representative plant from each box was indexed in the same manner as the greenhouse plants. After inspection by the Nursery and Seed Services, they were released to the California Strawberry Nurserymen's Association as certified meristem foundation planting stocks.

RESULTS AND DISCUSSION.—This study indicated that the combination of heat therapy and apical meristem culture was more reliable than apical meristem culture alone for ridding infected strawberries of SMYE (Table 2). The relative ease with which SMYE was eliminated from the Solana strawberries may be due in part to cultivar differences and/or to SMYE strain differences. For us, SMYE was more difficult to eliminate from plants than SP or SM, and only the combination of extended heat therapy and culture of apical and lateral bud meristems was reliable to successfully eliminate SMYE from cultivars.

Meristem culture without heat therapy resulted in SM-free *Fragaria grandiflora* Ehrhart (source: Jardin des Plantes, Paris), *F. vesca* clone 3C16, and the White Carolina and Ettersburg 121 cultivars. SP-free clones of the Shasta cultivar were also produced in this way.

Meristems cut from heat-treated SMYE-infected Fresno strawberries grew to plants faster than comparable meristems from nonheated plants. In contrast, the growth rate of Solana meristems was not affected by heat treatment (Table 3). Preliminary results of subsequent experiments with SP, SM, SNS, and SC virus-infected strawberry cultivar Hood indicate that meristems from plants infected with these viruses also grow faster after heat therapy. We have not yet determined if meristems from heat-treated healthy plants also grow faster than meristems from nonheated healthy controls, but this point needs study.

The main objective of the meristem culture program, to develop nuclear stocks of California strawberry cultivars free of virus diseases, has been accomplished, and progeny of the nuclear mother plants has been grown in the strawberry nurseries for 6 yr. During that time, performance tests indicated that meristem-derived Fresno planting stock produced 15-24% more fruit than the original SMYE-infected stock, even after growing side by side in a nursery for several years (3).

All meristem clones of Fresno, Solana, Tufts, J.6, and Shasta strawberries have continued to index free of detectable viruses in the greenhouse and in the screenhouse for up to 7 yr. Consequently, we feel that the clones are truly virus-free. Each year, the most promising new University of California strawberry selections are entered into the program, so that meristem-derived planting stock will be available to nurserymen when the selections are released as new cultivars.

TABLE 3. The effect of heat therapy on the growth rate of meristems of four strawberry mild yellow edge (SMYE)-infected cultivars

	Heat treated		Controls	
	Number of:		Number of:	
Cultivar	Meristem plants	Days	Meristem plants	Days
Fresno	22	106[a]	22	122
Solana	15	85	6	81
Tufts	33	99	1	119
J.6	17	79	0	---

[a]Days to transplanting age.

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