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In Vitro Propagation of Heat-treated Red Raspberry Clones[1]

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Abstract. Small explants from four clones of red raspberry (*Rubus idaeus* L.) obtained from heat treatment (8 weeks at 37°C) to eliminate viruses were successfully regenerated *in vitro* using major Anderson's medium with ¼ strength levels of Murashige and Skoog major salts.

The combination of heat treatment followed immediately by micropropagation is currently the best system known for obtaining raspberry clones free from viruses (4). It is an objective of the U.S. Department of Agriculture, Science and Education Administration, Agricultural Research to produce heat-treated, micropropagated (0.8 mm or smaller shoot apices), horticulturally evaluated clones of all the major *Rubus* cultivars grown commercially on the U.S. Pacific Coast. Mother plants of all these major *Rubus* cultivars are already being propagated in the USDA-SEA-AR screenhouses at Oregon State University from clones that have indexed free from known viruses and viruslike diseases by standard procedures (4). Heat treatment and shoot-apex propagation of these clones may eliminate some viruses that are not detectible by current methods. After evaluation for trueness to horticultural type, these clones can be introduced into various commercial raspberry nursery stock improvement programs.

The use of thermotherapy and micropropagation to eliminate viruses from various crops has been reviewed (5, 12). Raspberry plants have been successfully regenerated from explants in tissue culture (1, 6, 7, 17). *Rubus* cultivars that are free from known viruses have been obtained through tissue culture without heat treatment (13, 14, 15), or following heat treatment and standard propagation (2, 3, 9). In the present study, the red raspberry cultivars 'Willimette', 'Canby', 'Fairview', and the advanced selection OR-US 1835 from the USDA-SEA-AR breeding program at Corvallis conducted by Dr. F.J. Lawrence were subjected to heat treatment. We then sought to develop methods to propagate shoot apices from these heat-treated plants and regenerate plants from them to establish clonal lines.

USDA indexed stocks, which were free from known viruses at the outset of the study, were used in all cases. All clones developed during the study continued to index free from known viruses (4) at the end of the study. Well-established red raspberry plants were grown in 25 cm pots which were placed in larger pots lined with sphagnum peat moss. Plants were pruned and placed into a growth chamber for 16 hr/day of 10 klx, from a combination of incandescent and fluorescent lights. The temperature was slowly increased over a period of a week from ambient to 38°C and was kept at 38 ± 1° thereafter. During the ensuing heat treatment period, plants were watered with a 25% Hoagland's solution. After 8 weeks new shoots 1-3 cm in length were harvested directly from heat-treated plants that were still in the 38° growth chamber. These shoots were disinfested for a few seconds in 95% ethanol before exposing the explant tissue areas for excision. The apices and axillary buds of these shoots were used for tissue culturing. Explants ranged in size from 200-800 μm and consisted of the meristematic dome and 2-4 leaf primorida of new shoots produced during heat treatment.

Explants were placed in test tubes and kept in subdued light for 1-3 days before being placed under 5 klx flourescent light, 16 hr/day, 24°C conditions.

Heat-treated shoot apices in Stage I medium, terminology of Murashige (10), were evaluated at 4, 6, and 8 weeks or longer. Plants 5 mm or more in size or having at least 4 leaves were placed in a Stage III medium for *in vitro* rooting or rooted directly in pasteurized sand under mist in the greenhouse.

Several media were evaluated, and some degree of success was achieved in regenerating plants from excised shoot apices on media modified from those described by Anderson (1), Huth (6), based on Knudson's salts (8), and Putz (13), based on Murashige and Skoog's (M&S) salts (11.) Anderson's rhodendron medium (1), which utilizes ¼ strength M&S salts (Table 1), was the most satisfactory of the media tested for the State I and Stage III regeneration of the clones studied.

For Stage I regeneration 6-benzyl amino purine (BA), indolebutyric acid (IBA) and gibberellic acid A_3 (GA_3) were added to Anderson's medium at concentrations (mg/liter), respectively, of 2, 0.05, and 0.1 to provide a total of 51 successful Stage I regeneration of 109 attempted from all clones tested (Table 2). Levels of BA and IBA that promoted successful Stage I regeneration of 'Fairfiew' explants (8 of 11) on Huth's medium (6), were unsatisfactory for Stage I regeneration of 'Canby' or OR-US 1835. A few of BA, and whole 'Willamette' plants (Stage III) were subsequently developed on Anderson's medium with no BA and IBA at 1-2 mg/liter (Table 2).

Some Stage II proliferation of shoots (3-5 fold in 2 months) occurred on Anderson's medium at BA levels of 1-3.5 mg/liter and IBA at 0.05 mg/liter. However, our objective was to regenerate single plants with as little added hormones and as little callus and proliferation as possible in order to develop clones that were horticulturally true-to-type as well as possibly being free from viruses.

Rooting *in vitro* was unpredictable on

Table 1. Composition of modified Anderson's Rhododendron Medium used sucessfully for initial regneration (Stage I) of red raspberry explants immediately after heat treatment at 38°C.

Components of medium	Contents of medium[z] (mg/liter)
Inorganic	
Major salts	¼ x M&S[y]
$NaH_2PO_4 \cdot H_2O$	170
Minor salts	M&S
Iron solution	2 x M&S
Organic	
Thiamine HCl	0.4
m-Inositol	100
Adenine sulfate	80
Hormones	
BA	2
GA_3	0.1
IBA	.05
Sucrose	30,000
Agar	10,000

[z]Modified from Anderson (1).
[y]Murashige and Skoog (11).

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Table 2. *In vitro* propagation of red raspberry in modified Anderson's Rhodendron Medium.[z]

Cultivars and selections	Successful response/ total explants		
	Stage I[y]	Stage III[y]	Sand[y]
Canby	12/16	3/6	4/5
Fairview	8/9	2/8	4/4
OR-US 1835	26/58	0/13	2/4
Willamette	5/26[x]	3/7	–

[z]See Table 1 for composition of this medium.
[y]Stage I = initial regeneration and Stage III = rooting in tissue culture (10); "Sand" indicates that rooting was acomplished in sand in the greenhouse after explants about 5 mm high were removed from tissue culture.
[x]'Williamette' Stage I was grown on a modified Putz medium (13).

the media studied including Anderson's medium, where limited rooting (8 of 34 explants of all clones tested) was achieved with no BA and IBA in the 1-2 mg/liter range. Good rooting (10 of 13 explants of all clones tested) was obtained in the greenhouse by placing unrooted Stage I shoots, 5 mm or more in height, into pasteurized sand under intermittent mist (30 sec every 15 min during daylight hours) after dusting them with commercial rooting powder containing 0.1% IBA. Shoots developed vigorous root systems after 2-4 weeks. Rooted plants from tissue culture or from sand were planted in standard soil mixture and placed on the greenhouse bench for establishment, virus testing, increase, and maintainance.

Following the 8 week heat treatment period, the establishment stage *in vitro* for heat-treated shoot apices of red raspberry requires an average of 6-8 weeks, and up to several months for some cultivars. Rooting occurs in 2-6 weeks. Greenhouse establishment and propagation of daughter clones in sufficient numbers for field trails takes 3-5 months. Dormancy requirements would need to be met before subsequent outdoor planting. The entire process, including 2 years of field observation, could take a minimum of 3 years and more realistically, 4. If *in vitro* micropropagation were used to increase heat-treated shoot apices that were found to remain true to horticultural type, evaluation and commercial release could possibly be achieved in 3 years.

During these studies it became apparent that variations in Anderson's medium might be of value if a particular red raspberry cultivar responded poorly to the standard procedures just described for Stage I regeneration. Some useful variations in our own laboratory included a) increasing major salts to ½ the M&S (11) level; b) increasing major M&S salts to the 1 x level, changing NH_4NO_3 to 400 mg/liter, KNO_3 to 480 mg/liter, and omitting KH_2PO_4; and c) combining each of the above medium variations with BA:I-BA ratios (mg/liter) of 2:0.5; 1:0.1; and 0.5:0.1. Other factors influencing successful regeneration included seasonal periodicity of plant regeneration capacity (in our experience and as noted in the literature (16) shoot apices coming out of the heat chamber in the spring seemed to do best), and etiolation.

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In Vitro Propagation of *Grevillea rosmarinifolia*[1]

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Abstract. **Shoot segments of *Grevillea rosmarinifolia* A. Cunn. placed on solid half-strength Murashige and Skoog (MS) medium containing 0.5 mg/liter 6-benzylamino purine (BA) proliferated and formed shoots which were subcultured and rooted on paper bridges in Murashige and Skoog medium containing 0.1 mg/liter α-napthaleneacetic acid (NAA).**

Vegetative shoots, 10 cm long, of *Grevillea rosmarinifolia*, Proteaceae, were obtained from 1-year-old plants grown in the greenhouse. Shoots were washed in water with detergent, rinsed in distilled water, and surface sterilized for 15 min in 3.7% sodium hypochlorite solution. After 3 successive rinses in sterile distilled water, the top 2 cm of the shoots, which were very soft, were discarded and the remaining portion of each shoot was cut into 1 cm segments containing 2-4 leaves each and transferred aseptically into 100 x 25 mm glass culture tubes, each containing 10 ml half-strength MS (4), salt medium containing MS vitamin mixture (4), 100 mg/liter myo-inositol, 2% sucrose, 0.8% Bactoagar (Difco), and 0.5 mg/liter BA. The tubes were placed in a growth room at 25°C, with fluorescent light of 5 klx for 16 hr daily. Each node produced a shoot within 1 month and these shoots were transferred for further proliferation in the same medium under the same environmental conditions. After an additional month, each terminal shoot (1 cm) produced 5 or 6 new lateral shoots; these, in turn, were again subcultured for further proliferation (Fig. 1).

Rooting was obtained by subculturing 1 cm terminal shoots into a liquid medium with the shoots supported by paper bridges. The medium was similar to the

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