WARREN ROBERTS: This question is for Larry Landauer. Can you name any rhododendron cultivar that can be grown under alkali conditions, such as we have in the central valley of California?

LARRY LANDAUER: There are rhododendrons that will grow under alkali conditions, not well — but they will grow. However, when you combine alkaline soil with high temperatures, rhododendrons will not make it. They can tolerate one or the other of these conditions, but not both together.

ED SCHULTZ: How early in the season do you start taking rhododendron cuttings?

LARRY LANDAUER: We start June first, starting with the dwarf types — on through the end of December. You can root a rhododendron cutting anytime of the year the wood is hard enough to stick into the rooting medium. We have rooted 12 months of the year.

MICROPROPAGATION OF FILBERTS, *CORYLUS AVELLANA*[1,2]

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Abstract. A micropropagation system is described for shoot multiplication and root initiation followed by a successful transfer of filbert plantlets to soil in a greenhouse environment. Essential factors beneficial for shoot multiplication were the combination of two cytokinins, BAP and 2iP, and the incorporation of Anderson's inorganics, a low salt medium. Shoot proliferation arose primarily from lateral bud break. Proliferated shoots were subcultured on shoot elongation/rooting medium, then planted into greenhouse soil and placed into a humidity tent. The survival of the micropropagated filberts was 93%.

INTRODUCTION

The primary objectives of this research was to develop commercially feasible micropropagation techniques for filberts. Micropropagation of filberts may be an attractive alternative propagation method because of reductions in both costs of production and time required to introduce commercial quantities of new cultivars to the industry.

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In order to have a commercially feasible micropropagation system for filberts, three major objectives must be met: there should be an economic yield of useable propagules per subculture; the propagules should be successfully transferred to greenhouse conditions with minimial loss; and the system must maintain the genetic integrity of the cultivar or breeding line propagated. This paper address the first two objectives: adequate rates of shoot multiplication and successful establishment of the micropropagated plantlets in the greenhouse environment.

METHODS AND MATERIALS

Seedling Source of Explants. Recently harvested nuts were cracked and kernels placed on a tray. Kernels were then incubated 4 hrs in a 50 mg per liter (GA-3) solution one cm deep at 20°C. The kernels were planted in plastic flats and sprouted in the greenhouse. Softwood shoots were excised from the seedlings after they were 10 to 15 cm high, surface disinfested for 15 to 20 minutes with diluted bleach (0.53% sodium hypochlorite), containing 1 ml per liter Tween 20. Disinfestation was stopped by rinsing with sterile water. Lateral buds with about 0.5 cm stem section were excised and planted on semi-solid culture medium.

Source of Explants of cv. Daviana. Trees were grown in the greenhouse and the shoots were allowed to complete the first flush of growth. Softwood shoots were then removed from the trees, surface disinfested, and explanted like seedling explants. Contamination rates for tree explants, however, were over 90% indicating an alternative explanting technique must be developed for micropropagation of cultivars.

Basic Culture Medium and Environment. The initial culture medium was that of Anderson, (1,2) which is a modification of the Murashige and Skoog (5) formula with approximately 75% reduction in the concentration of ammonium nitrate and potassium nitrate and with other modifications of the medium involving phosphate, iron, and iodine. The organic constituents and concentrations (Table 5) were primarily those of Linsmaier and Skoog (e.g. inositol and thiamine). Sucrose and adenine sulfate concentrations followed the guidelines of Murashige (6).

The basal medium contained (per liter): sucrose (30 g), i-inositol (100 mg), adenine sulphate dihydrate (80 mg), thiamine HCl (0.4 mg), IAA (1 mg), kinetin (1 mg), and Phytagar (6 g). The pH was adjusted to 5.7 $\pm$ 0.1 with NaOH and HCl. The medium was dispensed 20 ml per 25 $\times$ 150 mm culture tube and autoclaved at 125°C and 1.05 kg/cm^2 pressure for 15 min-

utes. The standard culture conditions were 1,000 lux light, cool white fluorescent, 16 hr per day, and a constant 20°C. Standard length of time between subcultures was one month.

Description of Experimental Plant Materials and Treatment Design Used in These Experiments. Shoot tips (7 to 10 mm long), and generally with 3 to 4 expanded leaves, were harvested from existing multiplying cultures. These shoots, which were the initial propagules for most experiments, were placed upright with the basal cut portion pushed down into the semi-solid medium. A minimum of 10 replicate cultures were utilized for each treatment. Standard error of the mean was calculated to document variation within each treatment. (7).

RESULTS AND DISCUSSION

Experiments defining culture conditions were done with seedling source plant material; the cultures of the cultivar, Daviana, have been established and successfully propagated utilizing the system developed through the research presented here.

Initial Cytokinin Auxin Test. Cytokinins tested were kinetin, 6-benzylaminopurine (BAP), and N_6-(2-isopentenyl)-adenine (2iP) at 1.0, 2.5, and 5.0 mg per liter. Kinetin was ineffective in causing shoot multiplication. BAP at all concentrations produced healthy shoots but only caused some shoot multiplication for the highest concentration. The most effective 2iP treatment for shoot multiplication was 5.0 mg per liter.

Auxins tested were indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and β-naphthaleneacetic acid (NAA), at 1.0, 2.5, and 5.0 mg per liter with 1 mg per liter 2iP being the basal cytokinin. NAA caused callus to form on the basal portion of the shoot that was in contact with the media. IBA and IAA were both about equal for root initiation except IBA had greater phytotoxic effects at 2.5 and 5.0 mg liter. Combining IAA and 2iP in a single treatment resulted in leaf and stem necrosis.

Combination of Cytokinins, BAP and 2iP. Combinations of BAP and 2iP were tested when it was observed that cultures coming directly from a medium of BAP and then cultured on 2iP had very good shoot multiplication. The first experiment indicated that combinations of 1 mg per liter 2iP and 4 mg per liter BAP were best for shoot multiplication. Subsequent reculturing on that medium, however, was phytotoxic. Further factorial testing showed that combinations of 2 mg per liter BAP and 1 mg per liter 2iP were optimal (Table 1). Addition of IAA at 1 mg per liter had no beneficial effect in any of the cyto-

kinin combination treatments (Table 1). The growth regulator concentration rates finally adopted after numerous recultures and experiments was 1 mg per liter 2iP, 2 mg per liter BAP, and no auxin. Shoot multiplication utilizing this hormone combination causes lateral bud breaking followed by vigorous shoot growth.

Table 1. Effect of BAP and 2iP concentrations on numbers of shoots proliferated in a one month incubation period starting from shoot tips.[1]

BAP mg/l	0 mg/liter IAA			1 mg/liter IAA		
	2iP (mg/liter)			2iP (mg/liter)		
	0	1	2	0	1	2
0	1.0±0	1.0±0	1.3±0.2	1.0±0	1.0±0	1.0±0
1	1.2±0.2	1.4±0.2	1.2±0.2	1.1±0.1	1.1±0.1	1.1±0.1
2	1.3±0.2	2.2±0.7	2.2±0.7	1.3±0.2	1.2±0.2	1.4±0.2
3	1.8±0.6	2.1±0.6	2.1±0.6	1.6±0.2	1.5±0.2	1.5±0.2

[1] The basal medium contained either no or 1 mg per liter IAA.

Inorganic Formulas and Shoot Multiplication. The three inorganic formulas compared (Table 2) were Murashige & Skoog (MS)(6), Lloyd & McCown (LM) and Anderson (A)(1). Shoot multiplication rates were followed for three consecutive subcultures. Shoots produced in the MS medium had foliage that was pale yellow similar to that expected from salinity toxicity. The shoots in the LM medium showed greater variability. The number of shoots produced in the subcultures of the LM medium was approximately 75% of those grown on the MS formula. The A medium, however, was the most consistent and had the greatest shoot multiplication, producing 150% of the shoots of the MS medium.

Table 2. Comparison of inorganic formulas on the number of shoots proliferated per incubation in three consecutive subcultures[1].

Inorganic Formula	Subcultures		
	1	2	3
Anderson	4.5±0.6	5.4±1.0	14.2±2.4
Murashige & Skoog	2.0±0.2	3.5±0.7	10.7±2.9
Lloyd & McCown	2.3±0.3	2.7±0.6	7.3±2.2

[1] The first subculture was initiated with shoot tips while the second and third cultures were derived from shoot bases and recultured on the same inorganic treatment.

Comparison Between Shoot Tips and Shoot Bases for Shoot Multiplication. The growth of shoot tips was characterized by greater internodal space ranging up to 3 to 6 mm. In contrast, recultured shoot bases had many compressed nodes. Comparing shoots produced from shoot bases resulted in sig-

nificantly greater shoot proliferation rates than shoot tips, especially during the second and third recultures (Table 3).

Table 3. Number of shoots proliferated per subculture from shoot tips and basal stems in three consecutive subcultures.

Subculture	Shoot Tips	Basal Portions of stem
1	3.0±0.3	4.5±0.3
2	1.9±0.2	6.4±1.1
3	2.5±0.2	14.6±0.8

Shoot Elongation and Rooting. Preliminary experiments with auxins indicated IAA and IBA were effective in root initiation. The data from one test utilized 0.5× strength Anderson inorganics and organics at the following rates per liter, sucrose (30 g), inositol (100 mg), thiamine HCl (0.4 mg), IBA (0.5 mg), and Phytagar (6 g). After 5 weeks incubation, 65% of the cuttings rooted and all shoots elongated at least 1 cm.

Greenhouse Care. The micropropagated plantlets were planted in 1:1 mixture of Redi-earth and horticultural perlite. A fungicide mixture of 150 mg Benlate and 150 mg Captan per liter was sprayed after planting and at three to five day intervals. The plantlets were placed under a humidity tent for three weeks during which time all started new shoot growth. Survival of 250 plantlets after one month was 93 percent.

The media we used for filbert micropropagation are summarized in Table 4 and include the appropriate plant growth regulators and their concentrations and the most effective inorganic formula tested for both shoot multiplication and rooting. Shoot multiplication is primarily from lateral bud breaking. Consequently, the best multiplying propagules are from the basal portions of recultured stems. (Figure 1).

Table 4. Composition of filbert media for shoot multiplication and shoot elongation/rooting.

	Shoot multiplication (amount per liter)	Shoot elongation and rooting (amount per liter)
Sucrose	30 g	30 g
Inorganics	Anderson	Anderson 0.5×
Organics		
i-inositol	100 mg	100 mg
adenine sulfate dihydrate	80 mg	----
thiamine HCl	0.4 mg	0.4 mg
Growth Regulators		
IAA	----	0.5 mg
2iP	1-2 mg	----
BAP	2 mg	----
pH Adjusted		
with NaOH or HCl	5.7±0.1	5.7±0.1
Phytagar	6 mg	6 mg

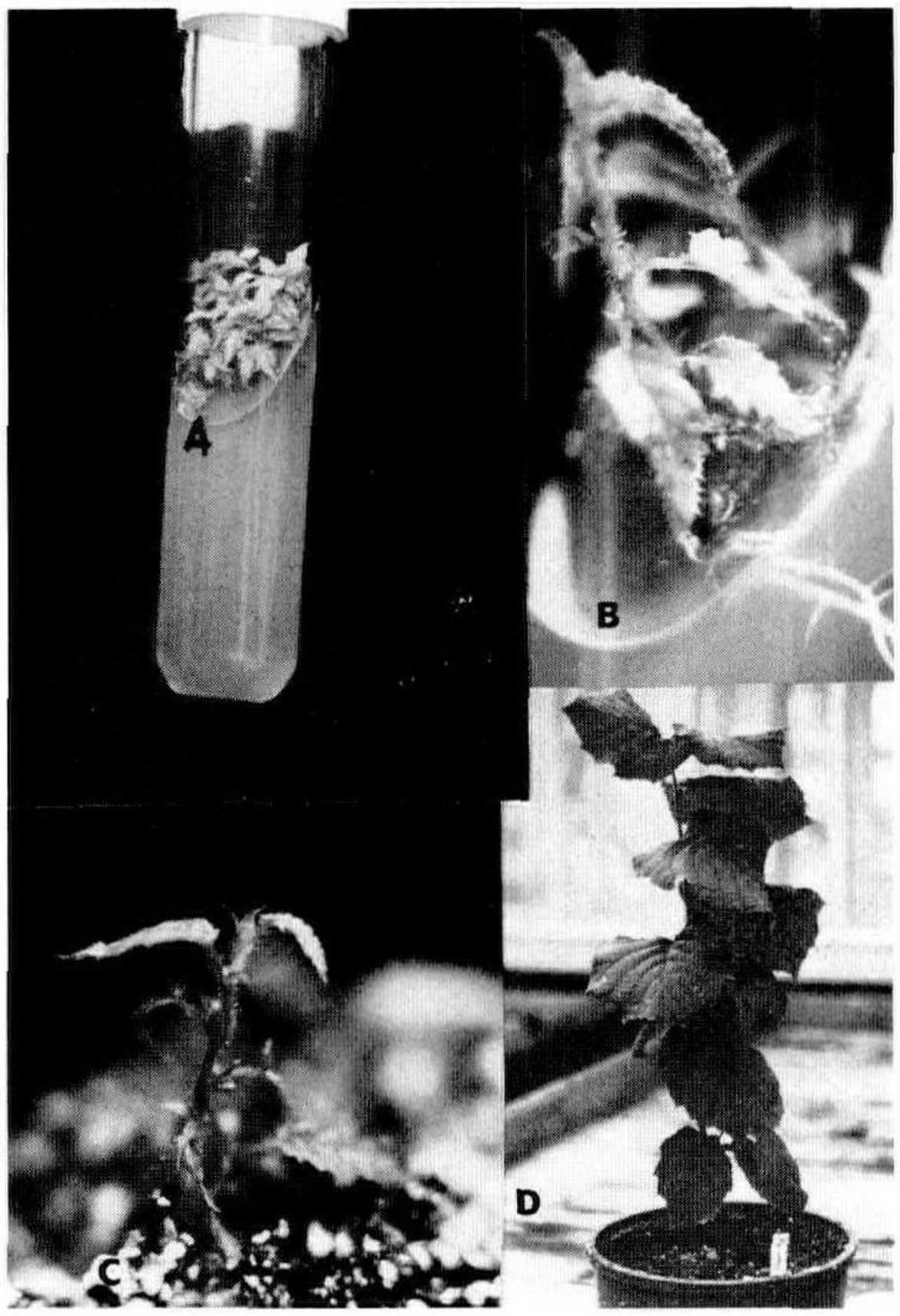

Figure 1. A. (upper left). Shoot multiplication culture arising from a subcultured basal portion of a shoot; B. (upper right). Plantlet derived from the shoot elongation/rooting medium five weeks after subculturing; C. (lower left). Plantlet three weeks after planting in soil and acclimatization in humidity tent; D. (lower right). Filbert tree three months after tissue culturing.

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