Adventitious Shoot Production from a Vireya Hybrid of Rhododendron

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Abstract. An efficient adventitious shoot production protocol has been developed for Rhododendron laetum × aurigeranum. Shoot tips taken from greenhouse-grown plants were cultured on Anderson’s medium supplemented with 74 µM 2iP. Axillary shoots were excised and cultured on medium containing 23 µM IAA and 74 µM 2iP. After 6 months, brown callus developed at the cut surfaces of the shoot-tip explants. This callus produced many adventitious shoots (up to 70 per explant). Clusters of adventitious shoots were divided, subculture, and continued to proliferate shoots. An estimated 1600-fold increase in the number of shoots could be readily achieved in 6 months. In vitro rooting of adventitious shoots was accomplished in 4 weeks. Seventy-three percent of shoots rooted on 1/4 strength Anderson’s medium supplemented with 28 µM IAA. Plantlet survival was 100% 3 weeks after transfer to soil. Chemical names used: 1-H-indole-3-acetic acid (MA); N-(3-methyl-2-butenyl)-1H-purine-6 amine (2iP).

The genus Rhododendron, section Vireya, comprises ≈ 300 species endemic to Southeast Asia (Sleumer, 1966). Almost half of the 300 Vireya species, including the species R. laetum J.J. Smith and R. aurigeranum Sleumer, are native to New Guinea, which lies between lat. 2° and 12°S. According to the Royal Horticultural Society (Leslie, 1980), Vireya rhododendrons are classified as “normally requiring greenhouse protection during the winter months”. Those species native to low altitudes in New Guinea are irreparably damaged if exposed, even for a short time, to temperatures below 0°C (Withers, 1984). Since Vireya rhododendrons do not require a resting period, they may flower at any time of the year. Therefore, they may have potential as greenhouse-grown ornamental plants. Moreover, they represent a source of breeding material for the improvement of hardy rhododendron flower quality. Vireya flowers range from < 1 cm to 10 cm in length and have a variety of colors, from white to yellow, from orange to deep red; the flower has diverse shape, from tubular to semi-open bells (Williams and Rouse, 1983). Within the section Vireya, the hybrid R. laetum × aurigeranum deserves particular attention because of its long-lasting golden yellow flowers that are not found in most common cultivated hardy rhododendrons.

Many species of Rhododendron are currently propagated by tissue culture (Chee, 1985). However, there are no reports concerning the micropropagation of Vireya species. The development of a micropropagation system would facilitate mass propagation of these species. The objective of this research was, therefore, to develop a protocol for the propagation of the Vireya hybrid R. laetum × aurigeranum.

Establishment of shoot-tip cultures. Stock plants of the Vireya hybrid R. laetum × aurigeranum were grown in 7.7-liter plastic pots containing a 1 perlite : 1 peat : 1 sandy soil (by volume) mixture. The greenhouse was at 26 to 32°C. Plants were watered as needed to keep the medium moist.

Actively growing apical shoot tips, 7 to 8 cm in length, were collected from the stock plants. All leaves, except those immature leaves enclosing the apical meristem and the leaf primordia, were removed. Shoot tips were rinsed under running tap water for 20 min. The explants were submersed for 2 min in 70% ethanol, followed by soaking for 40 min with gentle agitation in 1.05% NaOCl containing 20 drops/liter of Tween 20. After being rinsed three times in sterile distilled water, the basal ends were removed and shoot tips (≈ 5 cm long) were placed horizontally on
mens were replicated three times over time. The data were analyzed using analysis of variance (ANOVA).

The elongation of axillary shoots. Growth regulators were added to the media before autoclaving. After adjusting the pH to 4.5 and adding agar, media were autoclave at 121°C for 20 min. Fifty milliliters of medium supplied with 23 µM IAA and 74 µM 2iP. This procedure resulted in the greatest average shoot proliferation per callus (≈ 25 shoots per cluster) (Fig. 1C). Similar observations were made by McCown and Lloyd (1983) on R. canadense, R. × ‘Boule de Neige’, and R. × ’PJM’. We estimated that once organogenic callus is induced, a 1600-fold increase in shoot count in 6 months can be obtained. This protocol represents the most successful method of micropropagating the hybrid R. laetum × aurigeranum. However, uniformity and genetic stability of the plant material must be carefully evaluated before such a propagation method can be adopted.

Shoot proliferation from adventitious shoots. Individual adventitious shoots ranging from 2 to 10 mm were excised from callus clusters and placed into 10 × 60-mm petri dishes containing 10 ml of Anderson’s medium supplemented with various concentrations of IAA (11 to 34 µM) and 2iP (49 to 172 µM) concentrations. A completely randomized design was used, with five replicates per treatment. A replication was defined as a petri dish containing four single shoots. Explants were subculture every 2 months, and the number of newly formed shoots per individual shoot explant was recorded after 6 months of culture. ANOVA was performed to evaluate the effects of each main effect, as well as interactions.

A small amount of callus and green shoot primordia developed from the base of individual adventitious shoots after 2 months in culture. The primordia differentiated into small leaves. New shoots were distinguished as they emerged from the mass of leaves after 6 months of culture. There was no significant difference among 2iP treatments, nor was there any interaction between the growth regulators. However, 34 µM IAA with all concentrations of 2iP was most effective for inducing new shoot formation from the basal end of single shoots (7.4 shoots per single shoot), compared to lower levels of IAA (data not shown). The average number of shoots per single shoot obtained in this experiment (about seven shoots per cultured shoot) was considerably smaller than that obtained when clumps of shoots with small amounts of callus were routinely subculture (≈ 40 shoots per cultured shoot clump).
Rooting and establishment in the soil. To determine the optimum conditions for in vitro rooting of microshoots, four concentrations of the salts in Anderson’s medium (1, 1/2, 1/4, and 1/8 strength) with five IAA concentrations (0, 0.6, 6, 28, and 57 µM) in a factorial experiment were used. A completely randomized design was used with five replicates. A replication consisted of a petri dish containing nine adventitious shoots. Data were recorded as percentage of shoots rooted per petri dish. Rooting and establishment in the soil was recorded 3 weeks after transplanting.

In vitro rooting of excised shoots was accomplished in = 1 month. Ionic concentration of Anderson’s medium in combination with concentrations of IAA influenced the rooting of shoots (Fig. 2A). Given the significant ionic strength × IAA concentration interaction, valid comparisons among IAA concentrations can be made only within ionic strengths. Absent or low levels of IAA (<28 µM) in combination with full-strength Anderson’s medium resulted in no rooting. Using 1/4 ionic strength, the percentage of rooted plants ranged from 7.8% with 0 µM IAA to 73% with 28 µM IAA. About 60% rooting was obtained using 1/8 ionic strength and 0.6, 28, or 57 µM IAA. The percentage of rooted plants was significantly increased by increasing IAA concentrations and lowering the ionic concentrations. The highest value of in vitro-rooted shoots (73%) was obtained on medium with 1/4 strength supplemented with 28 µM of IAA.

The number of roots per shoot was also affected by the treatments tested, and again a significant interaction was found between IAA concentrations and ionic strength (Fig. 2B). The highest value, six roots per plant, was recorded using 1/4 ionic strength and 28 µM IAA.

The low-salt medium developed by Anderson has been recognized as a suitable medium for several Rhododendron spp. (Anderson, 1984; Ettinger and Preece, 1985; Fordham et al., 1982; Harbage and Stimart, 1987). Anderson’s medium was also good for establishing and maintaining in vitro cultures of the Vireya hybrid *R. laetum × ariigeranum*. Anderson (1984) observed that lowering the ionic strength of modified Anderson’s medium to 1/4 strength resulted in increasing the percentage of in vitro rooting with several cultivars of *Rhododendron*. In our experiment, the low ionic salt concentration of the medium and the IAA concentrations interacted significantly to induce in vitro root formation in *R. laetum × ariigeranum*. It is also evident that a critical level of IAA must be reached before root induction can occur.

All rooted plantlets transferred to soil survived regardless of the medium used to induce rooting (Fig. 1F). Plants on soil generated new roots from the root system that had developed in vitro and, later, from the base of the plantlets.

In summary, a successful in vitro multiplication protocol has been developed for *R. laetum × ariigeranum*. Among the three types of shoot multiplication, adventitious shoot multiplication from organogenic callus, induced at the cut surfaces of the axillary shoot-tip explants, gave the best results.

**Literature Cited**


