COMMERCIAL MICROPROPAGATION OF RHODODENDRONS

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Abstract. Young shoot tips from rhododendron #704-792-1450 were excised from the mother plant two weeks after a flush of growth was initiated. This tissue proved quite productive in a modified Murashige and Skoog (MS) salt solution with added organic constituents and 5 mg/l of N^6-(Δ^2-isoentenyl)-adenine (2iP) and 1 mg/l indole-3-acetic acid (IAA). Plants rooted well and were transferred to soil with 90 percent survival rate.

The tissue culture industry in Florida is growing rapidly. Four years ago the laboratory at Oglesby Nursery was the only one in the state: now there are 21. Oglesby Nursery is also one of the pioneers in the propagation of hardwood species.

Plant tissue culture is a new adventure when it is used for propagating hardwood plants, which seem to be more difficult than most of the plants used previously. During the past four years we have been propagating primarily foliage plants. Producing hardwood plants will provide a new opportunity for tissue culture laboratories. We have had good success with rhododendron and have been able to develop our technique because of the excellent support of growers and other individuals not only in Florida but throughout the country. Clonal propagation using tissue culture as the tool for propagation of new superior rhododendron cultivars may save the rhododendron farmer 5 to 7 years in developing sufficient stock for large volume sales. This method also is an answer for propagating clones that are difficult to root by conventional methods.

The purpose of this work was: (1) to adapt rhododendron #704-692-1450 to tissue culture techniques; (2) to provide Rhododendron Farm with large numbers of these plants; (3) and to determine if this tissue-cultured cultivar will remain consistently true-to-type.

This rhododendron is a superior deep crimson red that has been grown successfully on Cape Cod. It is a selection made by Ted Richardson, Rhododendron Farm, Mountain Home, S.C. and is expected to receive an Hl hardiness rating from the American Rhododendron Society.

MATERIALS AND METHODS

Dr. Wilbur Anderson, N.W. Washington Research & Extension Unit, Mt. Vernon, Washington, has developed a workable general system for rhododendron tissue culture (1,2), and our purpose was to develop a workable system for this particular

rhododendron. We found that it was necessary to alter the hormone concentrations in the Anderson medium for this cultivar. The basic medium in which we grow all our tissue culture plants is a standard salt mix of macro- and micro- nutrients. This is no different from what might be used for any plants but quantities must be precise. Our most successful rhododendron mixture is somewhat lower in ammonium and potassium salts than the usual solutions. The Anderson medium and two hormone variations were tried: 25 mg/l 2iP with 5 mg/l IAA, 15 mg/l 2iP with 4 mg/l IAA (Anderson) and 5 mg/l 2iP with 1 mg/l IAA.

The rhododendron 704-692-1450 was tip-trimmed to stimulate lateral breaks and placed in a dry, air conditioned room with 16-8 hours light-dark cycles. By removing the plants from the humid Florida climate (which encourages development of fungal and bacterial disease) we were able to obtain clean tips suitable for culture. After a flush of growth, the tips were cut with a pen knife, placed in a mason jar with a screen lid and put under running water for 15 minutes. They were cut to a size of 3 mm × 5 mm by removing leaf and stem tissue, and agitated for 10 minutes in a 10 percent Clorox solution. The shoot tips were then cut to a 1 mm × 2 mm size, with a scalpel and forceps using 30× dissecting microscope under a laminar flow transfer hood. This is about the smallest size we can handle easily. The small size is more likely to be disease-free since we take the youngest portion of the tip and discard older portions each time the tissue is cut. The tiny shoot tips were then rinsed for 10 seconds in 10 percent Clorox, rinsed in sterile water and placed in the various stage I and II media (Table 1).

The media were prepared in 1 to 5 liter portions. The pH was adjusted to 5.7 with drops of 1 m solution of NaOH and HCl. The media for stage I and II were dispensed in 25 × 150 mm tubes (Bellco Co., Vineland, N. J.), 10 ml per tube, by means of a funnel, rubber tube, clamp and a plastic tube (40 cm) with holes of the appropriate size to fill 4 tubes at the same time. The tubes were closed with Kaput closures (Bellco Co., Vineland, N. J.). The tubes with medium were placed in stainless steel racks of 36 and autoclaved at 250°F for 15 minutes at 15 pounds of pressure.

The cultures were placed in a culture room at 27°C (81°F) and given 100 ft-candles of light on a 16-8 hour light-dark cycle. At 6-week intervals the plants were moved to fresh medium.

The stage III medium was prepared in 5-liter batches in a large stainless steel stock pot. It was then transferred to two 4,000 ml Kimax bottles which have an outlet at the bottom

Table 1. Composition and concentration comparisons between the Murashige-Skoog (MS) and revised rhododendron formulas for Stages I and II.

Inorganic salts (mg/l)	Murashige — Skoog	Rhododendron (Revised)	Rhododendron (Anderson)	Rhododendron (Revised)
NH_4NO_3	1650	400	400	400
KNO_3	1900	480	480	480
$MgSO_4.7H_2O$	370	370	370	370
$CaCl_2.2H_2O$	440	440	440	440
KH_2PO_4	170	—	—	—
$NaH_2PO_4.H_2O$	170	380	380	380
$FeSO_4.7H_2O$	27.84	55.7	55.7	55.7
Na_2EDTA	37.24	74.5	74.5	74.5
$MnSO_4H_2O$	16.9	16.9	16.9	
$ZnSO_4.7H_2O$	8.6	8.6	8.6	8.6
H_3BO_3	6.2	6.2	6.2	6.2
KI	0.83	0.83	0.83	0.83
$CoCl_2.6H_2O$	0.025	0.025	0.025	0.025
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025
$NaMoO_4.2H_2O$	0.25	0.25	0.25	0.25
Organic materials (mg/l)				
Sucrose		30,000	30,000	30,000
Bactoagar		10,000	10,000	10,000
i-Inositol		100	100	100
adenine sulfate		80	80	80
N^6-(Δ^2-isopentenyl)-adenine (2iP)		25	15	5
Indole-3-acetic acid (IAA)		5	4	1
Thiamine HCl		0.4	0.4	0.4

(#14605). Aluminum foil was placed over the opening at the top and 1 foot of amber tubing was attached to the bottom. A pinch clamp was placed at the dispensing end of the tube. Aluminum trays (EKCO Products, Inc. #705-30) were placed in a mylar bag and autoclaved. Both the medium and trays were autoclaved for 15 minutes at 250°F, 15 pounds pressure.

Clear polystyrene lids (EKCO Products, Inc.#9105-10) for the trays were soaked for 30 minutes in a bucket with a 10 percent Clorox solution. They were then rinsed with hot sterile water. This procedure was done under a laminar flow hood. The medium was then dispensed into the sterile trays under a laminar flow hood and covered with the sterile lids. The third stage container, now complete, was placed on an enclosed cart to cool.

The stage III area in the culture room was provided with 1000 ft-candles of light on a 16-8 hour light-dark cycle at 28°C (82°F).

RESULTS AND DISCUSSION

Of 30 initial explants cut and put in culture, 21 developed into clean, healthy cultures. The remaining 9 turned brown,

died or showed signs of contamination. All 21 cultures responded reasonably well regardless of the test medium. However, at the two highest concentrations no discernible plants developed. The higher concentrations produced a mass of green tissue resembling callus; but when the green clusters were placed on the medium with lower concentrations of hormones, tiny plants emerged within 3 weeks.

After 4 subcultures, each at 6 week intervals, the medium that proved best for #704-692-1450 contained 5 mg/l 2iP and 1 mg/l IAA. Subsequent culturings gave 22.9 plants per culture (tube) every 6 weeks. The tubes contained discernible plants in clusters with elongated stems. The tops were removed for stage III and the base, with multi-stems, was placed in a fresh stage II medium.

The tops, after being removed from the cluster, were rooted in the aluminum tray in stage III medium. Within 5 weeks 90 percent rooting was achieved and the plants were moved to the glasshouse to be planted in soil and acclimated for the outside environment.

We must now experiment with methods of acclimation to the outside as these plants are very succulent with a thin cutin layer and less than the usual amount of pigments to help protect them. We are presently using a humidity tent during the first few days of this period. The plants have all the desirable characteristics of any high quality plant. They are vigorous, healthy and may also have residual effects of high level hormones, which may give more branching. Sanitation is extremely important at this stage.

We must also watch carefully for possible aberrations of both leaves and flowers although, so far, reports from Dr. Anderson have been favorable. He has followed several clones through the entire growth and bloom cycle and has found few changes from the parent plants.

Our purpose was to develop a technique for providing this rhododendron in commercial quantities in a relatively short period of time. We feel confident that this is now possible on a commercial basis.

LITERATURE CITED

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FIELD PROPAGATION TECHNIQUES FOR CONIFEROUS EVERGREENS

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At Bush Ranch we produce about 5 million bare-root coniferous evergreen liners per year. We have several unique practices in our production system. I will discuss three of them this morning but first I will explain how we prepare our field beds for propagation.

Everything is done outside in open beds, each holding approximately 100,000 liners. We start preparation by tilling the soil. We then take a soil sample, make the necessary adjustments with fertilizer and lime, apply a nemataciде and a prophylactic dose of fungicide and, finally, fumigate the soil by injecting Brozone.[1] This is a form of methyl bromide, which we use because it is effective at low temperatures, and we do most of our fumigating during the cold season.

After soil preparation is completed, we install galvanized pipe mist lines. Eight 48-inch beds are irrigated by a single line, 4 beds on each side. Each of these 8-bed sections has a single time clock that controls both the mist and irrigation systems so that it is possible to irrigate, mist, or both at the same time.

As stated earlier, we follow three unusual practices that I believe will be of interest to you. The first of these is our practice of sticking these coniferous evergreen liners in the summer, in contrast to the usual recommendations. One reason for doing this is to help balance our labor requirements throughout the year, since most of our harvesting and shipping is done during fall and winter. We are able to achieve reasonable success by rooting at this season. However, I do not feel I can recommend widespread use of this practice. Watering is extremely critical; a small error can result in a tremendous loss. In most cases there is no real advantage to summer sticking; however, it can be done, and I encourage you to experiment with this idea.

Our second unique practice is sticking cuttings right in the field. Nothing special has been done to the soil except for the fumigation and fungicide treatments. As you well realize there

[1] Brozone: Dow Chemical Company