Effectively Using Tissue Culture for Ericaceous Plants

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INTRODUCTION

Briggs Nursery is a pioneer in the field of plant tissue culture. Nearly 25 years ago Bruce Briggs cooperated closely with Dr. Wilbur Anderson on research focused to develop a micropropagation system for rhododendron. At that time progress was slow and sometimes disappointing—but a system was developed to effectively micropropagate rhododendron. In 1976, Dr. Anderson (Anderson, 1976) published an outstanding paper presenting a new medium that could be used to micropropagate rhododendron.

Currently Briggs Nursery produces yearly over 3 million ericaceous plants, including rhododendron, using tissue-culture methods. Over the past 25 years, we have built and used three different laboratories, and grown on over 20 million tissue-cultured rhododendrons.

The heath family or ericaceae is comprised of nearly 70 genera with over 1900 species (Bailey, 1976). This family is composed of mostly shrubs, perennial herbs, small trees, or occasionally vines. Many genera in the ericaceae are important ornamental and fruit crops, being widely grown throughout the world. Several members of the ericaceae have been successfully micropropagated (Table 1). We have yet to discover an ericaceous genus that is recalcitrant to tissue culture.

Table 1. Genera in the Ericaceae that have been micropropagated.

Andromeda	×*Gaulnettya*
Arbutus	Kalmia
Arctostaphylos	Kalmiopsis
Calluna	Leucothoe
Daboecia	Oxydendrum
Enkianthus	×*Phylliopsis*
Erica	Pieris
Gaultheria	*Rhododendron*

Nearly all genera in the Ericaceae can be micropropagated using the following protocol.

INITIATION

Cultures can be initiated from a variety of explants. Tissues including: vegetative buds, meristems, and shoots at a variety of stages of development can be used. Floral pedicels and ovary bases have also been reported to be successful as explants to initiate cultures of *Rhododendron* cultivars (Meyer, 1982).

At Briggs Nursery, shoots are the preferred explant to initiate cultures with ericaceous plants. Shoots that are somewhat stiff and mature are removed and the

foliage is stripped off. These leafless stems are then placed into a soap (Tween 20) water rinse. This solution is agitated for 3 to 5 min at 60 to 80 rpm on a rotary shaker. This process may be repeated if the stems are particularly dirty. Once rinsed thoroughly with tap water, the washed stems are placed into 10% laundry bleach (0.5% NaOCl) for 15 to 60 min, at 60 to 80 rpm on a rotary shaker. The exposure time to the sodium hypochlorite solution is dependent upon the number of shoots, maturity of the stems, and the concentration of the bleach solution.

After sterilization or disinfection of the explants is complete, shoots are transferred into sterile water to free the stem tissue of bleach. Sterilized shoots or shoot pieces may be transferred to a liquid or semisolid medium.

Sterile vegetative buds or meristems may also be used as a explant. Using a binocular microscope, scalpel, and fine-tipped forceps, vegetative buds are dissected to remove the outer bud scales. These dissected buds are then either used as an explant or dissected further to remove the meristem. The terminal meristem can be quite small, approximately 0.2 to 0.4 mm in diameter in several different *Rhododendron*. Growth of the dissected buds or meristems is quite rapid, with green primordial leaves appearing within 2 to 4 weeks.

SHOOT MULTIPLICATION

When shoot cultures have stabilized, a typical multiplication rate of 2.0 to 4.0× is sought. Higher multiplication rates can be achieved, but at the expense of adventitious shoot formation. Shoot quality is critical. Shoots should have good caliper with expanded leaves and no hyperhydricity.

Ericaceous shoots are proliferated on a low-salt medium, such as those proposed by Anderson (1976) or McCown and Lloyd (1983). Shoots respond to cytokinins: 2iP, [N^6-(-2-isopentenyl)-adenine]; zeatin, 4-CPPU, [N-(-2-chloro-4-pyridyl(-N-phenylurea)]; and thidiazuron. The optimum 2iP concentration for shoot multiplication with ericaceous plants varies from 0.4 to 16.0 μM.

ROOTING

Microshoots may be rooted in vitro as suggested by Anderson (1978) or rooted out of culture. Microshoots are rooted ex vitro using microcuttings approximately 1.0 cm in length that are stuck into a peat : perlite (1 : 1, v/v) soil mix. No rooting hormones are required. Microcuttings are placed in a humid, warm environment such as: under open mist with bottom heat or in a closed plastic tent. Rooting occurs within 10 to 14 days.

Rooted microcuttings are later transplanted into a porous but moisture-retentive, acidic soil mix. Ericaceous shrubs are sheared from one to four times during the growing season to enhance quality producing a multi-branched liner. Ericaceous trees are normally raised to a single- or few-stemmed liner.

CONCLUSION

The Ericaceae is a family rich with important ornamental and fruit crops. Micropropagation has revolutionized the propagation and introduction of superior and often difficult-to-root new selections. With further research we should expect to see many more new and exciting members of the ericaceae available in the near future.

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