Tissue Culture for Challenging Woody Plants©

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INTRODUCTION

Successful micropropagation is dependent upon a well-trained staff, employing a repeatable protocol, to produce consistent results with the finished product. Micropropagation techniques continue to evolve as new ideas are explored and new products are incorporated into the process.

ESTABLISHMENT IN VITRO

Before micropropagation can begin plants must first be successfully initiated into culture. The traditional sterilization protocol at Briggs was a 3-step process of first rinsing the explants in a soapy water solution, followed by a 10% Clorox (active ingredient NaOCl) treatment, finishing with a 1% Clorox rinse to remove the stronger Clorox solution prior to placing the plant piece into culture. At Briggs Nursery we often encountered plant tissue damage and death when using the Clorox solution. We wanted to find a gentler and more reliable method for surface sterilizing plants for tissue culture.

Sodium salt of dichloroisocyanuric acid (NaDCC) has proved to be much more effective at surface sterilizing plant material. Our current protocol is a 2-step process of first rinsing the explants in a soapy water solution to remove loose contaminants and break surface tension on the plant tissue. We drain off the soap solution and then immerse the plant pieces in a 5 $mg \cdot L^{-1}$ solution of NaDCC. Plants are gently rotated on an orbital shaker while they are being treated with NaDCC. Because NaDCC is not as toxic as NaOCl, no rinse is required prior to placing the plant piece into culture.

Both NaDCC and NaOCl, when diluted in water, form hypochlorous acid (HOCl). Dichloroisocyanuric acid has a more potent sterilant action since the compound dissociates to maintain a constant level of HOCl in solution as it is used. Dichloroisocyanuric acid solution has a pH of 6.8 as compared to 10 for NaOCl, so it has a more plant physiologically friendly pH. The low toxicity of NaDCC permits culture of shoots without rinsing, so higher levels of the sterilant are in contact with the plant material for a longer period of time, this is the major advantage of using this product instead of NaOCl. Dichloroisocyanuric acid is also very stable in solution and has a long shelf-life when stored in a sealed container at room temperature.

NOVEL NUTRIENT SALT FORMULATION

Once plants have been successfully established in culture the multiplication process can begin. Medium is comprised of a nutrient salt formulation amended with plant growth regulators to manipulate growth. Two published nutrient salts formulations used for woody plant micropropagation have been used at Briggs Nursery. Woody Plant Medium (WPM) was developed by Lloyd and McCown in 1980 for micropropagation of *Kalmia latifolia.* Driver–Kuniyuki Walnut (DKW) media was developed for micropropagation of *Juglans* by Driver and Kuniyuki in 1984. A novel

nutrient salt formulation now widely used at Briggs Nursery came about in a passing conversation with Dr. John Preece (Southern Illinois University) many years ago at an IPPS meeting. Dr. Preece suggested trying a nutrient salt mix that was half WPM and half DKW. We've found that many woody plants, and non-woodies too, seem to have better color and vigor on this nutrient salt mix. It's been coined "Preece Media" in our lab. Plants that respond well to this mix include: *Actaea, Anemone, Arbutus, Betula, Fothergilla, Heuchera, Hydrangea, Kalmia, Liquidambar, Nandina, Pieris, Rhododendron, Ribes, Syringa*, and *Vaccinium*.

NEW CYTOKININ

Benzyladenine (BA) is a widely used cytokinin in micropropagation systems, but can result in root inhibition. A new BA analogue called meta-topolin (mT) results in good multiplication rates in vitro and does not inhibit root formation in vitro or post vitro. Benzyladenine is an inexpensive cytokinin compared to mT, but for certain plants (*Cotinus, Nandina*) mT has a definite advantage in our lab.

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