Survival and growth of eastern white pine shoot apical meristems in vitro

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Received 11 October 1995; accepted in revised form 6 August 1996

Key words: juvenility, maturation, Pinus strobus, propagation, tissue culture

Abstract

Shoot apical meristems of seedling and mature eastern white pine trees were excised and grown in vitro. Placing the meristems on filters instead of directly on agarose-solidified nutrient medium enhanced survival of both juvenile and mature meristems. Applying forcing treatments to mature branches improved survival and growth of dissected meristems compared with meristems from non-forced branches in experiments conducted over two years. No consistent differences were observed among 2-, 4-, and 6-week forcing treatments. Including 5.37 nM (0.001 mg l⁻¹) 1-naphthaleneacetic acid in the culture medium did not affect meristem survival or growth. Some meristems from seedlings grew rapidly, produced primary leaves, underwent internode elongation, and in three cases, produced adventitious roots. Meristems from mature trees did not grow as rapidly as seedling meristems. The leaves produced by mature meristems appeared to be scale leaves and a few of these had brachyblast primordia in the axils. The shoots derived from mature meristems did not produce adventitious roots.

Abbreviations: BA – 6-benzyladenine; NAA – 1-naphthaleneacetic acid

Introduction

The shoot apical meristem gives rise to all aerial plant parts (reviewed in Sussex, 1989; Medford, 1992). In perennial plants, the developmental characteristics of shoots can vary according to a process known as maturation (reviewed in Greenwood and Hutchison, 1993; Lawson and Poethig, 1995). Leaf morphology is one characteristic that changes with plant age. Another important maturational change is the decline in the ability of stem cuttings to form adventitious roots as the donor plant matures (reviewed in Hackett, 1988). It has been hypothesized that the maturational state of a tissue is determined by the meristem from which it was derived (Bonga, 1987). The maturation state of a meristem, in turn, may be affected by the signals received as a result of its location on the plant. It has been observed that removing meristems from the mature surrounding tissues of older plants can release the meristems juvenile potential (Monteuuis, 1988).

For example, an excised shoot apical meristem of a 100-year-old Sequoiadendron giganteum tree yielded shoots that displayed juvenile organogenic, morphological, (Monteuuis, 1991) and biochemical (Bon and Monteuuis, 1991) characteristics.

Because of the importance of the shoot apical meristem for woody plant development, its potential importance in determining maturation, and the difficulty of asexually propagating mature eastern white pine (Pinus strobus L.), it would be advantageous to have a system for culturing shoot apical meristems of this species in vitro. This system could potentially be used to study the internal and external factors controlling development and for testing for rejuvenation by meristem excision and culture.

In preliminary experiments, cultured meristems from both mature trees and seedlings showed very poor survival. We tested a number of medium components including sugar type and concentration, inorganic nutrient formulation, gelling agent, auxins and
cytokinins; but none of these factors substantially improved survival. As a result of these preliminary experiments, we chose to investigate whether culturing the meristems on a filter placed upon the agarose-solidified medium improved survival. Obtaining higher survival would allow us to test the effects of forcing treatments on meristem survival and growth and for possible rejuvenation of excised meristems.

The objectives of these studies were to determine if:
- meristem survival is improved by culturing meristems on filters,
- meristem survival and growth is affected by applying forcing treatments to the donor branches, and
- removing mature meristems from surrounding tissues results in rejuvenation in eastern white pine meristems.

**Materials and methods**

**Filter experiment**

Mature meristems were obtained from branches in the upper half of the crowns of four ramets of one eastern white pine clone (H-130). The ramets had been grafted into the U.S. Forest Service, Oconto River Seed Orchard near White Lake, Wisconsin. H-130 was originally selected in 1966 and was estimated to be 50 years from seed at that time. Thus, by the time we first collected branches from the ramets (in 1992), the material was approximately 76 years from seed. Branches up to 2 m long were collected, cut into sections no longer than 1 m, and placed in plastic bags with moist paper towels. Branches were collected on January 6, 1992 and stored for six months at -20 °C. Two weeks before dissection, branches were transferred to 4 °C. Terminal meristems of primary, secondary and tertiary branches were excised and cultured.

Meristems from young seedlings were excised and cultured for comparison with the mature meristems. Open-pollinated seeds from another tree were stratified at 4 °C for 6 to 8 weeks. Seeds were sown in plastic tubes (“Pine Cells,” Stuewe & Sons, Inc., Corvallis, OR) containing a mix of composted pine bark, peat, vermiculite, and perlite (“Universal Mix,” Strong Lite Horticultural Products, Seneca, IL) and placed in a greenhouse at a temperature of 23-26 °C. Ambient light was supplemented with high-pressure sodium lamps (Sylvania LU400) at a photosynthetic flux of 100 μmol m⁻² s⁻¹ to provide a 16-h photoperiod.

Meristems were excised from the seedlings six weeks after sowing.

Surface disinfestation procedures were not used on buds, shoots or seedlings before meristem dissection. Dissecting tools were sterilized in 70% ethanol between the removal of external structures such as needles or bud scales and the actual meristem excision to prevent contamination of the meristems with microorganisms present on the external structures. Excisions were performed in a laminar-flow hood using a dissecting microscope. The meristematic dome plus the 4-7 smallest leaf primordia were separated from the shoot using a cut transverse to the shoot axis. Excisions were made with carbon steel razor blade shards mounted in a blade holder. Excised meristems were placed either directly on nutrient medium (approximately 4.5 ml per dish) or on filters upon the same medium in 35-mm diameter, disposable petri dishes (Falcon 1008, Becton Dickinson Labware, Lincoln Park, New Jersey). The filters were 25 mm in diameter, had a 5-μm pore size, and were made of mixed cellulose esters (MicronSep, Micron Separations Inc., Westboro, Massachusetts). The medium consisted of 1/2-strength Schenk and Hildebrandt (SH) (1972) salts, full-strength SH vitamins and 30 g l⁻¹ sucrose. It was adjusted to pH 5.75 prior to adding 5 g l⁻¹ agarose (Ultrapure, Life Technologies Inc., Gibco/BRL, Gaithersburg, Maryland) and autoclaving.

At the time of excision, meristems ranged from 0.3 to 0.5 mm in diameter at the base. Juvenile meristems were consistently smaller than mature ones with a diameter of approximately 0.3 mm, whereas mature meristems ranged from 0.4 to 0.5 mm. Plates containing the meristems were placed in a growth chamber at 23±1 °C. Light was provided for 16 h per day from GTE Sylvania GroLux fluorescent tubes at a photosynthetic flux of 40 to 50 μmol m⁻² s⁻¹. Three weeks after excision, the meristems, or the filters supporting the meristems, were transferred to new plates containing fresh medium of the same composition. After six weeks in culture, the meristems were scored for survival using a dissecting microscope. Meristems were considered alive if they had become green and had exhibited some growth since dissection. Meristems contaminated with microorganisms were considered as missing data.

The experiment was a two by two factorial. One factor was age of the source material for the meristems (seedling or mature tree) and the other factor was the presence or absence of filters between the meristem and the nutrient medium. Thirty meristems were excised