In Vitro Culture and Micrografting of White Pine Meristems

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INTRODUCTION

Our long-term research goal is to develop a vegetative propagation method for mature eastern white pine (*Pinus strobus* L.) trees. Unfortunately, rooting from cuttings of white pines, like most other conifers, decreases with increasing plant age. Rejuvenation, that is restoration of rootability to mature tissue, may be possible by tissue culturing or grafting shoot apical meristems. Recently, rejuvenated shoots have been obtained via meristem culture from Sierra redwood, *Sequoiadendron giganteum,* (Monteuuis, 1991) and via meristem micrografting from maritime pine, *Pinus pinaster*, (Dumas et al., 1989). If rejuvenated shoots can be obtained from mature white pines, they will be used as a stock block source of rootable cuttings to clonally propagate superior genotypes. In this paper we describe our efforts to develop methods for tissue culturing and micrografting of white pine meristems.

MERISTEM CULTURE

We have conducted experiments with juvenile (from 4- to 6-week-old seedlings) and mature (from 90-year-old trees) meristems in tissue culture. For dissection, we remove the apical dome and one to several closely appressed leaf primordia, but no primordia which have undergone significant enlargement. Factors investigated include medium mineral salt concentration, growth hormones in the medium, type of sugar in the medium, type of gelling agent in the medium, and addition of complex organic additives, such as coconut water, to the medium. In these experiments survival was generally poor. In a few cases, juvenile meristems grew into shoots and rooted to form plantlets. Survival of mature meristems was even more limited, and made it difficult to reliably assess treatment effects. We then undertook a study to test if inserting a cellulose acetate filter (Romberger, 1970) between the meristem and the medium would improve survival. Use of these filters dramatically improved survival of both juvenile and mature meristems. Shoots developed normally from juvenile meristems, but new leaf production has been

quite limited from mature meristems dissected from branches collected in the spring prior to bud flush. We are currently conducting an experiment to determine if mature meristems from flushing buds initiate new leaves more readily, since spring is the season in which meristems produce leaves for the following year's growth (Owston, 1969). Preliminary results indicate a beneficial effect of bud-forcing on meristem growth and development.

MERISTEM MICROGRAFTING

We have attempted micrografts of meristems onto three stock types: (1) epicotyls of 12-week-old seedlings grown in vitro, (2) epicotyls of 10- to 12-week-old seedlings grown in a greenhouse, and (3) dissected zygotic embryos in vitro. With the in vitro-grown seedlings, no meristem survival was observed and extensive browning and drying of the wounded area of the stock occurred. The greenhouse-grown seedling stock allowed for transitory survival of meristems and less, but still problematical, browning of the wound area. The zygotic embryos appear to be the most promising stock type. We have conducted an experiment to determine the best location of the graft site for juvenile and mature meristems. For both meristem types, grafting onto the embryo hypocotyl at a point midway between the radical and the base of the cotyledons was superior to grafting the meristem just below the base of the cotyledons. To date, we have obtained mature meristems which have survived and exhibited limited growth up to 12 weeks after grafting, and juvenile meristems which are actively growing 14 weeks after grafting. We are currently examining some of these grafts histologically to determine if a graft union has been established. Another experiment currently underway will refine the best site on the embryo for performing the graft, and determine the optimal time to remove the top of the stock plant to permit scion growth.

FUTURE RESEARCH

We intend to use the information from these experiments in both meristem culture and micrografting to develop a method for producing shoots from mature trees. We are optimistic that dissecting meristems from forced branches will provide us with explants that have the potential for vigorous growth and development, and that when micrografting techniques are perfected, zygotic embryos or very young seedlings will provide an excellent stock on which to grow those shoots. Future research will focus on testing the rootability and performance of cuttings from meristem-generated shoots, and developing methods for keeping rejuvenated shoots juvenile so that many rooted cuttings can be produced from each meristem-derived shoot.

ACKNOWLEDGEMENTS

This research has been conducted with financial support from the University of Minnesota Landscape Arboretum, the U.S. Forest Service North Central Station, the International Plant Propagator's Society, and the Horticultural Research Institute. We thank Jennifer Feist, Joan Schneider, and Michelle Steiner for excellent technical assistance, and Kathryn Louis for aiding in the development of meristem dissection techniques. All plant material was generously provided by the U.S. Forest Service Oconto River Seed Orchard.

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