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DEVELOPING TISSUE CULTURE SYSTEMS FOR INCREASING THE DISEASE RESISTANCE OF ASPEN

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ABSTRACT.--In vitro techniques are being developed for clonal propagation of selected clones of superior aspen and hybrid poplars. We have increased the efficiency of rooting tissue culture-derived aspen and are developing rapid screening systems for the identification and recovery of aspen that are resistant to Hypoxylon canker.

Recent forecasts indicate that demand for aspen pulpwood and particleboard will continue to increase (Blyth and Smith 1989). Some projections point to a shortage of aspen in the next 20-30 years in the Lake States due to the age structure of our existing stands. Plans for planting and managing aspen more intensively have been mentioned in response to the expected shortfall. Planting genetically improved clones of aspen that grow fast and resist disease can increase the supply of quality aspen in the near future. However, efficient, economical systems for screening trees for disease resistance and clonally propagating selected genotypes are needed.

Tree improvement involves identifying, recovering, and multiplying unique, useful gene combinations that provide the desired growth, quality, and stress resistance traits. The long generation time of trees, lack of knowledge about juvenile-mature trait correlations, and difficulty in obtaining the transfer and expression of desirable genes at high frequencies, seriously limit the use of the classical selection, breeding, and testing methods for forest trees. Manipulation of trees using various cell and tissue culture techniques provides a great potential advantage in tree improvement (Haissig et al. 1987).

Efficient in vitro regeneration of most commercially important forest trees is presently difficult or impossible. One exception has been the numerous successful in vitro techniques developed for members of the genus Populus (Ahuja 1987). The first complete plantlet to be regenerated from unorganized callus of a woody plant species and established in the field was obtained from trembling aspen (Populus tremuloides Michx.) (Winton 1970).

Our objectives are to develop rapid methods of identifying aspens resistant to Hypoxylon canker caused by Hypoxylon mammatum (Wahl.) Mill. and to develop in vitro systems for the recovery and clonal propagation of improved aspen genotypes.

CLONAL PROPAGATION

The conventional method of propagating selected aspen genotypes using root suckers in a greenhouse is labor- and time-intensive and inefficient in providing large numbers of plants. Trembling aspen, like most members of the Leuce section, does not root easily from stem cuttings. Previous research has

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demonstrated that in-vitro propagation of aspen is feasible; however, successful rooting of microshoots was variable (Wolter 1968, Christie 1978, Chalupa 1979, 1981, Ahuja 1983, Barocka et al. 1985, Noh and Minocha 1986).

We have developed techniques that have increased the efficiency of in vitro and in vivo rooting of tissue culture-derived shoots of a selected clone of trembling aspen. Roots were collected from a 76-yr-old ramet of a superior disease-resistant clone of aspen growing in northern Minnesota. Stock plants were produced by excising suckers from these roots and rooting them in the greenhouse.

Nodal segments (Rutledge and Douglas 1988) from actively growing shoots were surface sterilized and placed on WPM + 12 mM calcium gluconate + 20 mg l^{-1} adenine sulfate + 0.5 mg l^{-1} BAP (Christie 1978, Lloyd and McCown 1980, Ahuja 1983) and transferred every 7-10 days onto fresh medium under 16-hr photoperiods at 25°C in a growth chamber. After 4 months, 30-60 microshoots per nodal segment had formed.

To induce rooting in vitro, elongating microshoots were excised and transferred to WPM minus myoinositol and glycine + 12 mM calcium gluconate + 0.5 mg l^{-1} IBA and 0.1 mg l^{-1} NAA. Microshoots then were placed in growth chambers and grown at 25°C for 7 days in the dark followed by 7 days under a 16-hr photoperiod. After roots had formed, microshoots were transferred to a (3:1) vermiculite:perlite mixture and then placed in a growth chamber under 24 hrs continuous light at 25°C with frequent misting to maintain high humidity. Plants were transferred to the greenhouse after 2-3 weeks. Using this technique, 92 percent of the cultured microshoots rooted.

In vivo rooting of microshoots was accomplished by giving them a 12-hr pulse treatment in 50 or 100 mg l^{-1} IBA. The pulsed shoots were transferred to vermiculite:perlite mixture and grown in a growth chamber under 24 hrs continuous light at 25°C with frequent misting. After 4 weeks 94-100 percent of the shoots formed roots and were transferred to the greenhouse. These plants are now being field-tested in Minnesota and Wisconsin for growth and disease resistance.

Using these techniques, we have also cultured and rooted several other aspen clones with varying degrees of success. Such clonal differences were also noted by Ahuja (1983) and indicate that methods developed for one genotype may have to be modified to obtain maximum efficiency for other genotypes.

Zeldin and McCown (1986) demonstrated that excised roots of poplars can be grown in vitro and shoots can be differentiated from these cultured roots. We have successfully regenerated and transferred plants of hybrid poplar and aspen clones to soil in the greenhouse from excised root cultures. Sterile excised roots from shoot cultures were placed in liquid WPM supplemented with NAA, placed on a roller culture apparatus in the dark, and rolled at low RPM. Large masses of roots developed within 1-2 months. Shoots were induced by placing segments of roots onto solid WPM supplemented with BA. Thus far, 5-10 shoots per cm segment of aspen root have been produced. Preliminary results with aspen root cultures thus far are encouraging.

We have regenerated shoots from root cultures of 11 different hybrid poplar clones. Poplar clones vary in their regeneration response with some clones producing 10 or more shoots per cm root segment. Clonal propagation via in vitro root culture is a promising system for efficiently obtaining planting stock of superior aspen and hybrid poplars.

SCREENING FOR HYPOXYLON CANKER RESISTANCE

Recently, tissue culture systems have been developed to screen aspen for resistance to infection by H. mammatum. Einspahr and Wann (1985) screened aspen plantlets for resistance to H. mammatum toxin by regenerating plantlets from cotyledon explants on toxin-containing media. Valentine et al.

(1988) screened clones of P. tremuloides for resistance to H. mammatum. Callus cultures were exposed to fungal culture filtrates and tissue-cultured plantlets were inoculated with ascospores of the fungus. There is evidence that clones resistant to Hypoxylon canker have the ability to rapidly produce callus which closes wounds that could otherwise be invaded by the fungus (Ostry and Anderson 1983). This rapid callus development may be responsible for limiting canker expansion. We are evaluating in vitro systems to assess the ability of aspen clones to produce callus as an indicator of resistance to infection by H. mammatum.

FUTURE APPLICATIONS

Applying cell and tissue culture techniques to aspen offers promise for clonal propagation and screening for disease resistance. Tissue culture may also be used to produce useful somaclonal variation (Larkin and Scowcroft 1981). Somatic variation in the resistance of hybrid poplars to the pathogen Septoria musiva Peck was recently identified and recovered in hybrid poplars derived from tissue culture (Ostry and Skilling 1988). Some of these plants have remained disease-free after 3 years in the field. Similar somaclonal selection techniques could be used for developing aspen with resistance to Hypoxylon canker once reliable bioassay techniques are developed.

Many biotechnological strategies using tissue culture for tree improvement have been applied to poplars (Haissig 1986). Although the practical use of these techniques still need demonstration, the success of several model systems illustrates their potential to complement traditional tree breeding and reduce the time required to develop desirable traits in forest trees.

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