IN VITRO DISEASE RESISTANCE FOR EXPRESSION
OF SOMACLONAL VARIATION IN LARIX

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ABSTRACT

Somaclonal variation expressed by scleroderris canker resistance or susceptibility was examined using Larix decidua micropropagules generated from caulogenic calli. Callus had been initiated from adventitious shoots showing persistent resistance to the pathogen. Inoculation responses by these propagules were compared to those by adventitious propagules initiated from juvenile tissues, and presumed to be canker-susceptible. Inoculation of those hosts developed on callus from one ostensibly resistant plantlet showed no resistance. Colonization by the pathogen was complete on all shoots.

INTRODUCTION

Opportunities to circumvent economic losses due to microbial diseases of important plants have been primarily found through development of disease-resistant plant genotypes. Genotype "development" implies selection of apparently resistant individuals from an otherwise infected, naturally or artificially inoculated population.

In the case of the tree host, traditional tree improvement strategies require years and large acreages, both to generate new host genotypes and to challenge them with the pathogen. In contrast, the advantages of tissue culture micropropagative systems and in vitro challenge with the pathogen have been shown for the larch/scleroderris canker system (1) and with others (3).

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However, the most promising source of novel genotypes of plants appears to be via somaclonal variation from cell culture. Not only may variants be generated at high frequency, but since genotype variation is random, the potential multiplicity of genotypes may be limited only by the size of the genome. Such multiplicity has obvious advantages for subsequent natural propagation, over monoclones or systems generating only a small number of clones.

Methods have been developed for shoot organogenesis using callus generated from short shoot buds of *Larix* (5). Calli/cell cultures are those in which genetic variation may be expected to occur most frequently. We may thus anticipate such variation in calli grown from short shoot buds of plantlets expressing either resistance or susceptibility to inoculation with *Gremmeniella abietina* (Lagerb.) Morelet, the causative agent of scleroderris canker disease. This would result in resistant shoots from an otherwise genetically susceptible cell population, and vice versa. The objective of this study was to examine for somaclonal variants among populations of callus-initiated *L. decidua* Mill. propagules.

**MATERIALS AND METHODS**

Short shoot buds were excised from two-year-old *Larix decidua* rooted tissue culture plantlets in a greenhouse. These plantlets included nine elongated from propagules which earlier and repeatedly (4x) had resisted *in vitro* inoculation with conidia of *G. abietina* isolate 18-46 from northern Wisconsin (unpublished). Buds were surface-sterilized, scales removed, and shoot primordia placed on a modified Schenk and Hildebrandt medium (5). Tissue was subcultured monthly. A second group of propagules was initiated from juvenile tissues as described (1). Propagules from both groups were elongated to shoots of approximately 15 mm stem height, on a growth regulator-free Litvay medium containing glutamine as the sole source of amino nitrogen (4). To each shoot was then applied a single 0.01 ml aqueous drop suspension of 1750 viable conidia of isolate 18-43 from northern Wisconsin prepared as described by Abdul Rahman et al. (1). Nine 15 mm adventitious shoots from juvenile tissues were also inoculated, as were non-caulogenic callus cultures, grown on a modified Brown and Lawrence medium (1) from cambial explants of each member of the two groups of rooted plantlets. Two replicates of each callus genotype were inoculated. Shoots and calli were examined during 30 days for colonization by the pathogen.

**RESULTS**

Callus developed slowly from only 6 of 211 (3%) cultured shoot primordia. Isolated, small needles and a few bud-like structures eventually appeared on several calli. However, only three buds on callus from one canker-resistant plantlet elongated to shoots. Within two weeks following inoculation with conidia, all three showed extensive colonization by the pathogen, as did the nine inoculated adventitious shoots. All non-caulogenic callus cultures were similarly colonized.