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Regeneration of 'Frontier' Elm from Leaf Explants[1]

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Abstract

This report describes the regeneration of a hybrid elm from leaf explants. Surface sterilized leaf explants from *Ulmus* X 'Frontier' were cultured on a medium containing combinations of cytokinins used previously to regenerate plants from other elms. Leaf strips cultured on a solid medium containing thidiazuron (TDZ), with or without 6-benzyladenine (BA), demonstrated organogenesis from 95% of the explants, with an average of approximately 6–8 shoots being produced per productive explant. Shoots elongated following transfer of the explant to a medium containing gibberellic acid. These elongated shoots were then transferred to soil, where about one-third of them successfully rooted.

Index words: Elm, organogenesis, thidiazuron, tissue culture, woody plants.

Growth regulators used in this study: Thidiazuron (TDZ), N-phenyl-n'-1,2,3-thiadiazol-5-yl urea; 6-Benzyladenine (BA), 6-Benzylaminopurine.

Significance to the Nursery Industry

Genetic engineering of trees is a reality. Foreign DNA has been introduced using the *Agrobacterium* binary vector system into a number of woody species, including apple (7, 13), larch (6), peach (11), poplar (3), plum (9), sugar pine (8) and walnut (10). In addition, regeneration conditions have been determined for a number of other tree species (4). However, individual regeneration conditions must be determined for each species which is targeted for genetic manipulation through the introduction of foreign DNA. Therefore, this report describes the first step toward the transformation of *Ulmus* x 'Frontier', a hybrid elm developed by the U.S. Arboretum and currently being grown for sale by landscape nurseries across the U.S. One potential benefit which genetically-engineered elms could provide for the nursery industry is improved resistance to insect pests. These new elms could be capable of defending themselves against such predators as the elm leaf beetle (*Xanthogaleruca luteola* (Müller)) or the elm bark beetle (i.e., *Scolytus mulitstriatus* (Marsham)), the vector which carries the Dutch elm disease-causing fungus, *Ophiostoma ulmi*.

Introduction

Since the near removal of the American elm (*Ulmus americana* L.) from the North American urban landscape, applied elm research has moved in two basic directions. First, a quest has been ongoing to replace the American elm through the development of new (non-American) elm cultivars which have highly desirable properties. Second, investigators have worked on identifying or developing a Dutch elm disease (DED)-resistant American elm. Each avenue of investigation has produced promising results, although the pursuit of superior landscape elms is far from over.

One of the new elm varieties which has been released is the 'Frontier' elm, a hybrid between Chinese elm (*Ulmus parvifolia* Jacq.) and European elm (*Ulmus carpinifolia* Gleditsch) (12). 'Frontier' elm is resistant to DED and elm yellows, but shows only moderate resistance to the elm leaf beetle (5). One possible application of biotechnology toward making the 'Frontier' elm an even better landscape tree would be the improvement of its elm leaf beetle resistance. One of the first steps in genetic engineering of any plant species is the development of an efficient regeneration system. The following report describes conditions used to successfully regenerate 'Frontier' elm.

Materials and Methods

'Frontier' elm trees were provided for research purposes by the U.S. National Arboretum and by Microplant Nurseries, Inc. Tissue culture techniques were based on previously published experiments (1) involving Chinese elm (Ulmus parvifolia Jacq.), one of the parents of 'Frontier' elm. A brief description of methods follows: Fully expanded leaves (second leaf from apical meristem) were surface sterilized (with stirring) for 12 min in a solution of 15% bleach (0.79% sodium hypochlorite), with 0.05% Tween 20. Leaves were then rinsed twice in autoclaved deionized water, allowed to drain, then cut into strips (6 strips per leaf) and transferred to Magenta GA-7 tissue culture vessels containing Murashige and Skoog basal medium supplemented with 30 g/l sucrose and selected growth regulators. The pH of each medium was adjusted to 5.6, to which 6 g/l of Phytagar was added.

This basic medium was modified by the addition of 0.1 μM thidiazuron (TDZ) and/or 1 μM 6-benzyladenine (BA). After an appropriate length of time for the initiation of shoots, cultures were transferred to the basic medium containing 3 μM gibberellic acid to promote shoot elongation. Explants were transferred to fresh medium every four weeks. Cultures were initiated and maintained at 25°C (77°F) under a 16 hr photoperiod with an average irradiance of 60 μmol/m^{-2}s^{-1} provided by cool white fluorescent lamps. Expanded shoots were transferred to a 1:1 (by volume) mixture of Pro-

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Mix and perlite in covered plastic deli containers to maintain an appropriate humidity level until a new set of leaves had emerged.

Results and Discussion

Regeneration parameters were selected from within a fairly narrow range based on previous success with the regeneration of plants from leaf explants from Chinese elm (Ulmus parvifolia) (1). In those experiments, Chinese elm leaf explants produced large numbers of shoots on a medium containing 0.1 μM TDZ, which was also successful for regeneration from leaf explants from American elm. Therefore, preliminary experiments were initiated to test the possibility of regeneration from explants of 'Frontier' elm using this same medium. A preliminary experiment was conducted using 0.1 μM TDZ with or without 1 μM BA (which appears to enhance regeneration from American elm; P.A. Herman, D.A. Stone, and M.G. Bolyard, unpublished observations).

The basal medium (no TDZ or BA) or a medium containing only 1 μM BA showed very little callus formation and virtually no organogenesis. However, media containing 0.1 μM TDZ promoted shoot organogenesis from 87% (20 out of 23) of the explants, while 52% (11 out of 21) of the explants cultured on a combination of TDZ and BA produced shoots. However, more shoots per explant appeared to be produced on the medium containing TDZ and BA than on a medium a containing TDZ alone, but this observation was not verified statistically.

A second experiment was then undertaken to determine the average number of shoots per explant produced from explants cultured on a medium containing TDZ alone vs. TDZ supplemented with BA. Once again, no shoots were produced from explants cultured on the basic medium or the medium containing BA alone. In contrast to the preliminary experiment, the percent of regeneration from explants containing TDZ (95.6%; n = 23) was nearly identical to those containing TDZ and BA (95%; n = 20). The difference in the percent of shoots undergoing organogenesis on medium containing TDZ and BA between the first two experiments could be attributed to the health of the explants at the time of culture, or perhaps to slight variations in sterilization procedures.

There was a similar number of shoots produced from explants cultured on a medium containing TDZ and BA (8.21

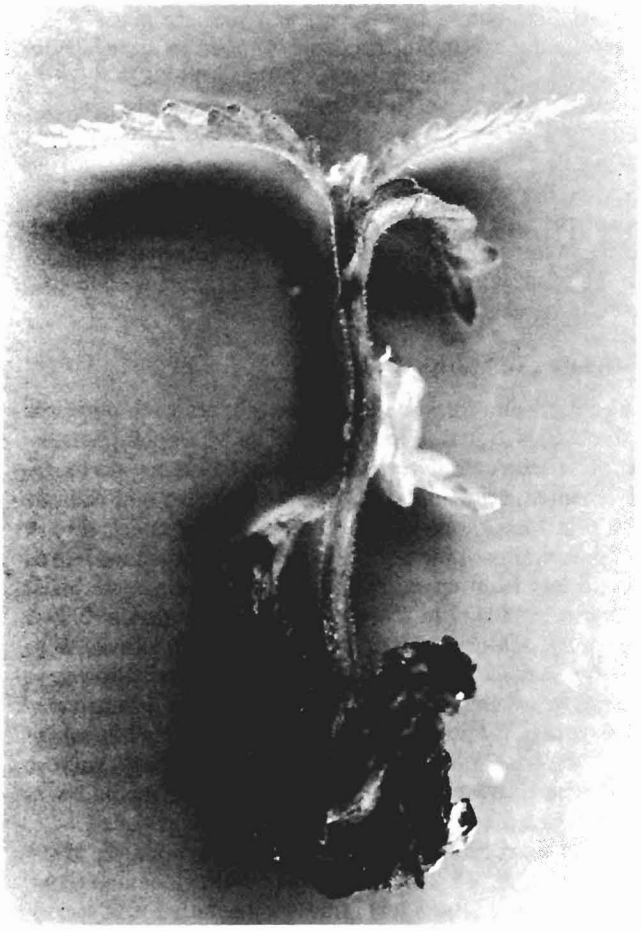

Fig. 1. 'Frontier' elm shoots after five months in culture (two months on a medium containing TDZ and BA, three months on a medium containing GA). Other shoots were produced from similar cultures in a shorter period of time, although there was not a uniform maturing of shoots from each explant. Overall size of this explant was 2.2 cm.

Fig. 2. 'Frontier' elm shoot, regenerated on a medium containing TDZ and BA, following transfer to soil after six months in culture. Height of the shoot at this stage was 3.5 cm.

± 6.73 shoots per explant) when compared to explants on TDZ alone (6.18 ± 3.28 shoots per explant). It appears that either medium can be used to successfully regenerate plants from 'Frontier' elm leaf explants.

In a third experiment, shoots were elongated on the basic medium supplemented with 3 μM GA (Fig. 1). Elongated shoots with at least two sets of leaves (>2 cm) which had been regenerated on a medium containing TDZ or TDZ and BA were transferred to soil. Of 11 shoots regenerated on TDZ alone, 4 rooted in soil (36.3%), whereas 12 of the 35 shoots (34.2%) regenerated on TDZ and BA rooted following transfer to soil, indicating no difference in shoot survival relative to the medium from which the shoots originated (Fig. 2). This survival rate probably could be elevated by transferring more mature shoots to soil, although fewer shoots will survive in vitro for extended periods of time. In addition, treatment with various auxins may improve rooting, as was published for *U. procera* (2). These results indicate that 'Frontier' elm responds to treatment with TDZ, although not as favorably as Chinese elm (in terms of shoots per productive explant). It would be interesting to determine the shoot regeneration response of European elm (the other parent of 'Frontier' elm) to a medium containing TDZ.

Shoot morphology was not unusual in the regenerated shoots, although it is difficult to determine "normal" morphology by comparison to seedlings, because none of the 'Frontier' elms now in existence have produced flowers or seeds (personal communication, Ms. Susan Bentz, U.S. National Arboretum). In fact, because 'Frontier' elm is an interspecific hybrid between spring-flowering and fall-flowering species, it may be sterile. This would provide an ideal situation for genetic engineering, in that the "danger" of outcrossing introduced genes would be eliminated due to the sterility of the recipient plants.

These results indicate that thidiazuron appears to be a potent growth regulator for regeneration of 'Frontier' elm from leaf tissue. The regeneration procedure described in this paper may be a useful starting point for genetic engineering of a large number of economically important elms. A very interesting research challenge would be to determine whether the regeneration potential of hybrid elms can be predicted on the basis of their pedigree.

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