FIELD PERFORMANCE OF LOBLOLLY PINE TISSUE CULTURE PLANTLETS

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Abstract.--Loblolly pine tissue culture plantlets of cotyledon origin were compared to seedlings from the same half-sib families after three growing seasons in the field. Early growth in the field was slower for the plantlets than the seedlings although the plantlets appeared more resistant to fusiform rust. Morphologically, the plantlets appear more mature than the seedlings. Further studies to understand and manipulate these differences are underway.

Additional Keywords: Pinus taeda, vegetative propagation, tree improvement.

INTRODUCTION

A major potential benefit of tissue culture to forestry operations is its use as a method of vegetative propagation of elite genotypes from tree improvement programs. Seed for planting stock produced from currently applied seed orchard technology captures only additive genetic effects; however, commercial propagation of planting stock via tissue culture techniques could utilize all (additive and nonadditive) genetic effects. In loblolly pine (Pinus taeda L.), such technology could conservatively increase genetic gains by one-third to one-half (McKeand 1981). Based on published estimates of genetic variance components (McKeand et al. 1985), genetic gains for some traits such as volume growth and disease resistance may double by employing tissue culture technology.

In addition to greater genetic gains, a large decrease is expected in the length of time between selection of improved individuals and production of planting stock from tissue culture as opposed to seed orchard propagation. Loblolly pine seed orchards require a minimum of eight to ten years from grafting until large-scale seed production begins. Hopefully, tissue cultured propagules from select trees could be mass-produced one or two years following selection. Therefore, tissue culture technology offers not only the opportunity to capture greater genetic gains, but also to utilize this genetic gain earlier than with conventional seed orchard technology (McKeand and Frampton 1984, Amerson et al. 1985).

These and other (Durzan and Campbell 1974, Mott 1981, Sommer and Brown 1979) benefits of tissue culture have great potential, but the technology

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necessary for operational propagation of loblolly pine via tissue culture is not yet available. Ultimately, embryogenesis or organogenesis from callus or cell suspensions are desired to facilitiate mechanization of propagation for mass-production as well as integration with molecular genetic biotechnology. Currently, organogenic propagation of loblolly pine from needle fascicles (Mehra-Palta et al. 1977), cytokinin-treated winter dormant buds (Abo El-Nil 1982) and cotyledon explants (Mott and Amerson 1981) is possible. While the technology to propagate from other sources is less reliable, clonal propagation of loblolly pine from cotyledon explants on a research scale is routine. The use of cotyledons as starting material offers less genetic advantage than propagation from older trees of proven genetic value. However, until more reliable methods of propagation from older trees are available, studies of the field performance of tissue culture plantlets from cotyledon origin will be useful in identifying and understanding general problems associated with tissue culture propagation and facilitate amelioration of these problems when other propagation systems are employed.

For this reason, the Project on Tissue Culture of the Southern Forest Research Center at North Carolina State University has established a series of field plantings containing tissue culture propagules of cotyledon origin. This paper compares the growth and development of these plantlets with seedlings after three growing seasons in the field.

METHODS

Laboratory

The propagation system used to establish these studies was reported in 1981 (Mott and Amerson) and involves the timely application and removal (i.e., pulsing) of growth regulators to progress from shoot initiation through rooting. Although this sequence has been and continues to be improved, a summary of the process follows. Basal media used in this study was BLG (Brown and Lawrence 1968) with glutamine (10 mM) substituted for NH_4 and NO_3 and 10 mM Kcl added, or GD (Gresshoff and Doy) based media diluted by one-half (Mott and Amerson 1981).

Seeds scarified at the micropylar end were partially germinated in hydrogen peroxide (typically three days in 1% aqueous H_2O_2 followed by one to two days in 0.03% H_2O_2 at 28-30°C). Subsequent to seed coat removal and surface sterilization, the embryos were aseptically excised from the female gametophyte. Next the cotyledons were surgically removed and planted horizontally on a shoot initiation medium (BLG) which was cytokinin-rich [typically 44µm benzylaminopurine (BAP)]. Cotyledons were maintained on this medium for 14 to 28 days. On this medium, cell divisions occurred in the peripheral areas of the cotyledons producing a warty, meristematic surface. Cotyledons were removed from this medium prior to the actual observance of shoots and placed on a hormone-free (GD_1 · 1/2) medium containing charcoal to further aid cytokinin removal. On this medium, shoot apices became recognizable on the cotyledons and the shoots began to elongate. Shoot growth continued during further monthly subcultures on hormone-free (BLG) medium. The multiple shoots crowded on the cotyledons were individually excised and separated from the cotyledon for further growth.

Following growth, shoots about 1-2 cm in length were transferred to auxin-rich (GD_1 · 1/2) medium [typically,o(-Naphthaleneacetic acid (NAA) at

2.5µM]. Shoots were freshly cut at the base, implanted upright and pulsed for six to nine days on the auxin medium. Pre-root cell divisions formed near the cambial region at the stem base, resulting in a swollen, callusy region. To facilitate organization and rapid root growth, the shoots were transferred to hormone-free (GD_1 1/2) medium. Plantlets typically were ready for transfer to greenhouse soil three to five weeks after the root initiation treatment.

Greenhouse

Plantlets were transferred from the agar medium to the greenhouse when their total shoot lengths (including needles) exceeded 1-2 cm and their individual root lengths exceeded 3-4 mm. Plantlets meeting these requirements were carefully removed from the agar medium and planted in 164 cc RL Super Cells containing a fine textured mix of peat, vermiculite, and perlite (2:2:1). The plantlets were grown in a mist bench the first three to six weeks in the greenhouse. After the first week, they were fertilized three to five times weekly with Peters 15-30-15 mixed at 30 ppm N. Plantlets in the mist bench were sprayed weekly with a fungicide, Captan, to reduce damping off and other disease problems. When necessary, the photoperiod of the plantlets was extended to 16 hours using incandescent lights (approximately 4 Wm^{-2}) to prevent dormancy.

Although the initial growth of the plantlets in the greenhouse was very slow, after about six weeks, new vigorous growth appeared. At this time, plantlets were removed from the mist bench and fertilization was changed to Peters 20-19-18 mixed at 40 ppm N applied three to five times weekly. After removal from the mist bench, plantlets were watered as needed with pH 5.5 water. Generally, plantlets reached a suitable size for field planting (about 20-30 cm in height and 3-5 mm in caliper) after six months in the greenhouse.

Using similar procedures, seedlings were also grown in the greenhouse to use in field tests for comparison purposes. Seedlings generally required only four months to attain plantable size so that it was often necessary to manipulate the watering and fertilization regime of the plantlets and seedlings in order to coordinate their growth.

Before field planting, both the plantlets and seedlings were gradually adapted to conditions outside the greenhouse. The succulent growth was hardened-off by first stopping fertilization and reducing watering. Next, the trees were transported outside for two to four weeks in order to adapt to direct sunlight, natural photoperiod and outdoor temperatures.

Field

The field tests were carefully site-prepared, hand-planted and intensively managed. At the time of establishment, a soil analysis was conducted and any nutrient deficiencies were corrected. Additionally, approximately 50 g N usually in the form of ammonium nitrate was applied to every tree during the spring to enhance growth. Weeds in the plantings were controlled either by periodic mowing or with herbicides. Nantucket pine tipmoth (Rhyacionia frustrana Comst.) which often kills young loblolly pine shoot tips was controlled with Furadan applications. Spacing was typically 3.05 x 3.05 m.

The North Carolina State University Project on Tissue Culture has established 16 field plantings across the Southeast (Figure 1). Over 3000 trees each of plantlets and seedlings have been planted representing over 25 half-sib families of loblolly pine. Results discussed in this paper will be limited to the first series of eight plantings that were established in 1981 with brief mention of results from two of the plantings established in 1982.

All of the 1981 field plantings contain paired row-plots of plantlets and seedlings from several half-sib families. The plantlets in a plot represent one clone produced from the cotyledons of a single embryo. The trees of the seedling plots were grown from seed of the same half-sib family from which the plantlets were derived. The 1981 field plantings contained from 16 to 49 plots. Plot size varied from two to 46 trees depending on the number of plantlets produced in a clone. Planting size varied from 158 to 324 trees. Several of the field plantings established after 1981 in addition to row-plots contain clonal block plots of 16 or 25 plantlets compared to block plots of seedlings from the same half-sib family.

Total height and the incidence of fusiform rust (caused by Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme (Cumm.) Burds. et Snow) were recorded annually in all field tests. Additional measurements of morphological characteristics were also made in some of the plantings.

Figure 1. Location of the North Carolina State University Tissue Culture Project's loblolly pine field plantings.

Data Analysis

Analyses of variance were utilized to determine differences between plantlets and seedlings at each location. Although the field design necessitated a different model for some plantings, height measurements at most locations were analyzed using the following sources of variation: 'plant type' (plantlets verus seedlings), 'family', ' plant type x family', 'plot(plant type x family)' and 'tree(plot(plant type x family))'. Incidence of fusiform rust was analyzed on plot means employing a similar model. Plant type differences in the analysis of variance were tested using the 'plant type x family' interaction as the error term. Plant type differences across all locations were tested by a paired t-test where the difference between plant type means at each location was weighted by its number of observations.

RESULTS AND DISCUSSION

Height Growth

Average second year survival exceeded 94 percent for both the plantlets and seedlings in the eight 1981 field studies. Third year height averaged 2.72 and 3.38 m, respectively, for the plantlets and seedlings (Table 1). The plantlet height averaged 74 to 84 percent of the seedling height and except for one location was statistically (P 0.05) shorter than that of the seedlings.

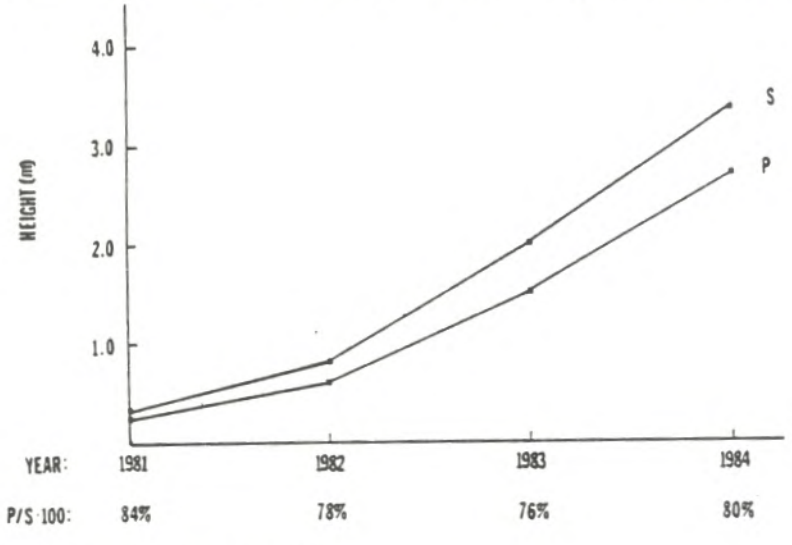
Table 1.--Mean third year heights of the eight field plantings established in 1981 by the North Carolina State University Tissue Culture Project.

Organization	Location	Total Height(m) Year 3 P		S	P/S*100
Federal Paper Board Co., Inc.	Lumberton, NC	2.96	*	3.95	75
Brunswick Pulp Land Co.	Jesup, GA	4.21	*	5.14	82
Westvaco Corp.	Summerville, SC	3.45		3.98	87
Scott Paper Co.	Monroeville, AL	3.31	*	3.99	83
N. C. State University	Raleigh, NC	1.38	*	1.80	77
ITT Rayonier, Inc.	Yulee, FL	2.99	*	3.72	80
Champion International Corp.	Newberry, SC	1.60	*	2.16	74
Crown Zellerbach Corp.	Bogalusa, LA	3.49	*	4.40	79
Weighted Mean		2.72	*	3.38	80

1/ P = plantlet, S = seedling, * = significantly different at $p \leq .05$ level.

Figure 2 compares the height growth curves of the plantlets and seedlings averaged over the eight 1981 field plantings. Within each year, the seedling height significantly exceeded the plantlet height (McKeand and Frampton 1984, Amerson et al. 1985). Although every effort was made to establish the plantlets and seedlings at similar heights and stages of development, the plantlets averaged only 84 percent of the seedling height at the time of field planting. The plantlet height fell further behind the seedling height during the first and second growing seasons. However, after the third growing season, plantlets had gained to only a net loss of four percent from the time of establishment.

Figure 2.--Mean height growth curves of tissue culture plantlets and seedlings in the eight field plantings established in 1981 by the North Carolina State University Tissue Culture Project.



1/ P = Plantlet, S = Seedling

Thus, the difference between the third year height of the plantlets and seedlings is largely related to differences in initial planting size and a first year lag in plantlet height growth in the field. In these studies, the seedlings will most likely remain taller than the plantlets in absolute height through rotation age. Since plantlet growth rates are similar to that of seedlings during the third year in the field, cultural treatments which overcome the plantlets' initial slow growth should yield plantlets of comparable size as seedlings in future studies. Measurements will continue in these tests to monitor long-term growth trends.

Fusiform Rust Resistance

After three growing seasons in the field, the plantlets had less fusiform rust incidence than the seedlings in all eight of the 1981 field plantings (Table 2). Overall, the plantlets averaged 27.7 percent infection while the seedlings averaged 47.6 percent, a statistically significant difference ($P < 0.05$). In some high hazard regions of the Southeast, such reductions in fusiform rust incidence would be of great economic benefit and could offset initial slower growth. The nature of the plantlets' relative resistance is not yet understood.

Table 2.--Mean third year incidence of fusiform rust in the eight field plantings established in 1981 by the North Carolina State University Tissue Culture Project.

Organization	Location	Fusiform Rust Infection (%) Year 3 1/	
		P	S
Federal Paper Board Co., Inc.	Lumberton, NC	1.8	8.4
Brunswick Pulp Land Co.	Jesup, GA	56.0	71.0
Westvaco Corp.	Summerville, SC	54.4 *	83.5
Scott Paper Co.	Monroeville, AL	29.8 *	73.5
N. C. State University	Raleigh, NC	1.7	7.3
ITT Rayonier, Inc.	Yulee, FL	34.1 *	57.8
Champion International Corp.	Newberry, SC	4.1	18.0
Crown Zellerbach Corp.	Bogalusa, LA	39.4	61.7
Weighted Mean		27.7 *	47.6

1/P = plantlet, S = seedling, * = significantly different at $p \leq .05$ level.

Morphological Characteristics

Many differences between plantlet and seedling shoot morphology have been observed in the field plantings. Although the plantlets originated from embryonic material, their morphology appears more mature-like than seedlings. Older loblolly pine generally has larger needles, fewer branches per unit of height, fewer growth cycles per season and slower growth rates than younger material (Greenwood 1984). A detailed measurement of these and other characteristics (after two growing seasons) at the 1981 field planting in Jesup, Georgia, has verified quantitatively that the plantlets are expressing more mature-like morphology than the seedlings (McKeand 1985). Examples from that study are presented in Table 3.

Additionally, early production of female stobili has been observed on many plantlet clones. One clone which is represented by 75 plantlets at each of two of the 1982 field plantings, located at Rincon, Georgia, and Cantonment, Florida, represents the extreme of this phenomenon. After one full growing season in the field, 85 percent of the plantlets of this clone had produced female strobili. Only five percent of the seedling plots from the same half-sib family had produced female strobili at the Cantonment, Florida, study while no trees in the seedling plots had produced strobili at the Rincon, Georgia, study (McKeand 1985).

Table 3.--Second year measurements of the loblolly pine tissue culture planting established in 1981 at Jesup, Georgia (McKeand 1985).

Trait	Plantlets		Seedlings
Terminal Bud Length (cm)	3.4	NS[1/]	3.1
Terminal Bud Diameter (mm)	6.3	*	5.4
Needle Length (cm)	19.1	NS	18.6
Needle Dry Weight (g)	0.16	*	0.12
Percent Fusiform Rust Infection	47.4	NS	68.8
Number of Cycles	4.6	*	5.4

1/ * = significantly different at $P \leq 0.05$ level.

The cause of this apparent early maturation of loblolly pine tissue culture plantlets relative to seedlings is unknown.

CONCLUSION

Early results from field trials of loblolly pine tissue culture plantlets have identified differences in growth, fusiform rust resistance and morphology between plantlets and seedlings. In an attempt to better understand the nature of these differences, new studies have been initiated. These include: (1) exploring alternative treatments for producing shoots and roots in vitro, (2) investigating the effect of cultural practices such as root pruning on subsequent plantlet development, (3) establishment of field plantings to compare seedlings having tissue culture–produced root systems, plantlet shoots grafted onto seedling roots and seedling shoots grafted onto plantlet roots and (4) excavation of both plantlet and seedling root systems after several growing seasons in the field. Forthcoming results from these and other studies will not only provide knowledge necessary to improve tissue culture propagation but also provide some insight into the control processes of development in loblolly pine.

The use of trade names throughout this paper does not imply endorsement of these products, nor criticism of products not named.

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