TISSUE CULTURE OF SWEETGUM (LIQUIDAMBAR STYRACIFLUA L.)

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Abstract.--An improved method for the tissue culture propagation of sweetgum (Liquidambar styraciflua L.) using a liquid culture stage is under development. This method produces more and larger shoots per culture than previous agar based methods. Plantlets from these shoots have been hardened off and grown in a nursery bed. The root collar diameters and heights of several clones after one season in the nursery are reported. Poor root form is the current problem limiting the use of these plantlets for field establishment. Photosynthesis, anatomy and alternate rooting methods have been studied for the evaluation of the efficiency and predictability of plantlets.

Liquidambar styraciflua is one of the major hardwood species in the Southeast United States. Once superior selections have been made, a method of propagation will be needed. Several alternatives include use of 1/2 sib families, conventional vegetative propagation and tissue culture. The latter two methods have the advantage of immediate genetic gain equal to the genotype of the selection through clonal replication. However, with Liquidambar, conventional vegetative propagation methods are inefficient. Thus alternative propagation methods using tissue culture are being investigated for this species. This report describes some of the refinements in tissue culture methods for Liquidambar.

MATERIALS AND METHODS

Agar Culture Methods

Seed was collected by the U.S. Forest Service from the Oconee National Forest and kept as half-sib lots. Seeds were surface sterilized and germinated under aseptic conditions on a modified Risser and White's basal medium (1,4,7). Hypocotyl sections were placed on a modified Risser and Whites medium with 1.0 ppm IAA and 5.0 ppm 2ip. After excision, the shoots were rooted on a modified Risser and White's rooting medium (4,7).

Liquid Culture Methods

Seed were prepared as described under agar culture methods. Shoots were initiated from hypocotyl sections on a modified Risser and White's medium with 0.1 ppm NAA and 0.5 ppm 2BA (4,7), multiplied

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on a modified Blaydes' medium with 0.01 ppm NAA and 0.5 ppm BA, and then placed on a modified Risser and White's basal medium for growth and rooting as previously described (6,7,8). Cultures were maintained at 25 ± 2°C with a 15 hr photoperiod, under cool white fluorescent lamps.

Nursery Bed Evaluations

Plantlets were removed from the agar rooting medium, and planted in Can Am pine tubes filled with a potting mix of vermiculite and sand (1:1 v/v). Plantlets were hardened off by gradually lowering the relative humidity (10,11), then maintained in a greenhouse or lathhouse prior to planting.

About 800 plantlets were planted on 4" centers in a cement block nursery bed between 30 May 1983 and 20 June 1983. Plantlets were lifted on 5 March 84. Stem length and root collar diameter were measured; root quality ratings were made.

Photosynthesis and Anatomy

Plantlets and seedlings were placed in a growth room maintained at 25 ± 2°C with a 16 hr photoperiod and placed under one of three quantum flux densities: 50 ± 5 (low light), 155 ± 10 (medium light), 315 ± 15 (high light) $pEm^{-2}s^{-1}$ (2). Net photosynthesis of seedlings and plantlets was determined using an infra-red CO_2 analyzer (9). Tissues were prepared for light and scanning electron microscopy as previously described (10,11).

RESULTS

Agar Culture

The results of the culture of sweetgum hypocotyl sections on agar have been reported (6). The yield of plantlets using the agar system is shown in Table 1. No net increase in plant number was obtained when considering initial seedling numbers. Even in terms of plants responding to culture, multiplication rates were low. Results were highly variable among seedlots This method was the optimum of an investigation involving a 2x5x5 factoral experiment with 2400 cultures (7). It was thus felt that the limiting factor was not nutritional, but some other factor in the system. Agar in the medium may cause water stress, thus a liquid medium step was incorporated into the culture protocol.

Table 1.--Yield of plantlets from agar cultures

Seedlot	# of Seedlings	% Seedlings Giving Successful Cultures	Average # Shoots	% Shoots Yielding Plantlets
76-1B	80	28	2.9	36
76-5B	93	56	2.8	39
76-7B	50	80	3.9	28
76-10B	53	40	2.1	23
78-1B	23	26	2.2	23

Hypocotyl sections from seedlings were cultured on Risser and White's medium. After excision, shoots were rooted in medium without hormones.

Liquid Culture

For liquid culture experiments, buds were first initiated from hypocotyl sections on a revised Risser and White's medium, solidified with agar (4,7,8). Eight to 12 weeks following initiation, hypocotyls were transferred to a liquid Blayde's medium (8,12). After 8 weeks, some of the cultures had proliferated and started to produce shoots of varying sizes. The yield of shoots using liquid culture proliferation is shown in Table 2. Shoot number varied with clone. There exists the potential of harvesting large numbers of shoots per culture. In addition, several harvests and subcultures can be taken from each flask, which would greatly amplify the multiplication potential of this system. Table 3 shows the rooting of shoots obtained from liquid culture, when placed on basal modified Risser and White's medium. Most clones exhibited well over 50% rooting of shoots.

Table 2.--Yield of shoots from liquid culture

Clone	Seedlot Origin	# of Shoots
5529	80-5B	28
5587	81-8U	17
5595	81-8U	32
5635	81-2U	52
5637	81-2U	45
5646	81-2U	0
5659	81-2U	77
5688	81-14B	70
5718	81-4U	15
5727	81-4U	10
5757	81-4U	40
5780	81-76-8U	16

Hypocotyl sections were placed on modified Risser and White's medium 26 August 1983, removed 27 February 1984 and transferred to modified Blaydes liquid medium. The above data is from the harvest of 10 June 1984.

Growth of Plantlets in Nursery Beds

Growth of the six major clones in nursery beds is given in Table 4 and in Figures 1 and 2. From 74 to 86% of the plantlets were recovered; average height and root collar diameters varied among clones (Table 4). Variation within a clone was large (Figs. 1, 2). Most of the plantlets were less than 85 cm high (Fig. 1), which is considered the maximum height desired for planting. The root collar diameter of most of the plantlets exceeded 6.25 mm (minimum diameter for planting), and few exceeded 9.5 mm (preferred minimum for planting) (Fig. 2).

Table 3.--Rooting of shoots from liquid culture

Clone	Seedlot Origin	Total # of Shoots	% Rooted
5529	80-5B	27	63
5531	80-5B	4	50
5587	81-8U	12	67
5635	81-2U	38	55
5637	81-2U	37	73
5659	81-2U	78	73
5670	81-14B	12	92
5688	81-14B	77	90
5724	81-4U	20	60
5757	81-76-8U	40	73
5780	81-76-8U	16	44

Shoots excised from liquid multiplication culture 5-10 May 1984 and placed on rooting medium. Observations were made 2-21 August 84.

When the plantlets were lifted from the nursery beds in March 1984, it was surprising to find a high incidence of plantlets with girdling roots (Table 5). The girdling was traced back to the placement of the plantlets into the Can Am plugs. Manipulation during transplanting pushed the roots into a position promoting girdling root growth. This could similarly have occurred during transplant into the nursery beds. Plantlets were found with bent, "S"-shaped stems under the surface, proposed to result from transplant of the extremely flexible cultured shoots.

Table 4.-- Growth of sweetgum plantlets in cement block nursery beds

Clone#	Seed Lot Origin	Number Planted	% Recovered	Height (cm)	Root Collar Diameter (mm)
3070	79-6B	96	84	66.3	7.3
3083	79-6B	138	86	57.2	8.0
3090	79-6B	106	83	63.7	8.2
3201	78-1B	51	78	43.7	6.3
3346	77-10U	34	74	56.4	8.0

Plantlets were planted between 30 May 83 and 20 June 83, and lifted on the week of 5 March 84.

Table 5.--Plantlets with girdling roots in the nursery

Clone	Plantlets	% Girdling
3070	81	68
3083	119	87
3090	88	93
3201	40	88
3346	25	80

Evaluation of roots of plantlets lifted the week of 5 March 1984 for girdling root systems.

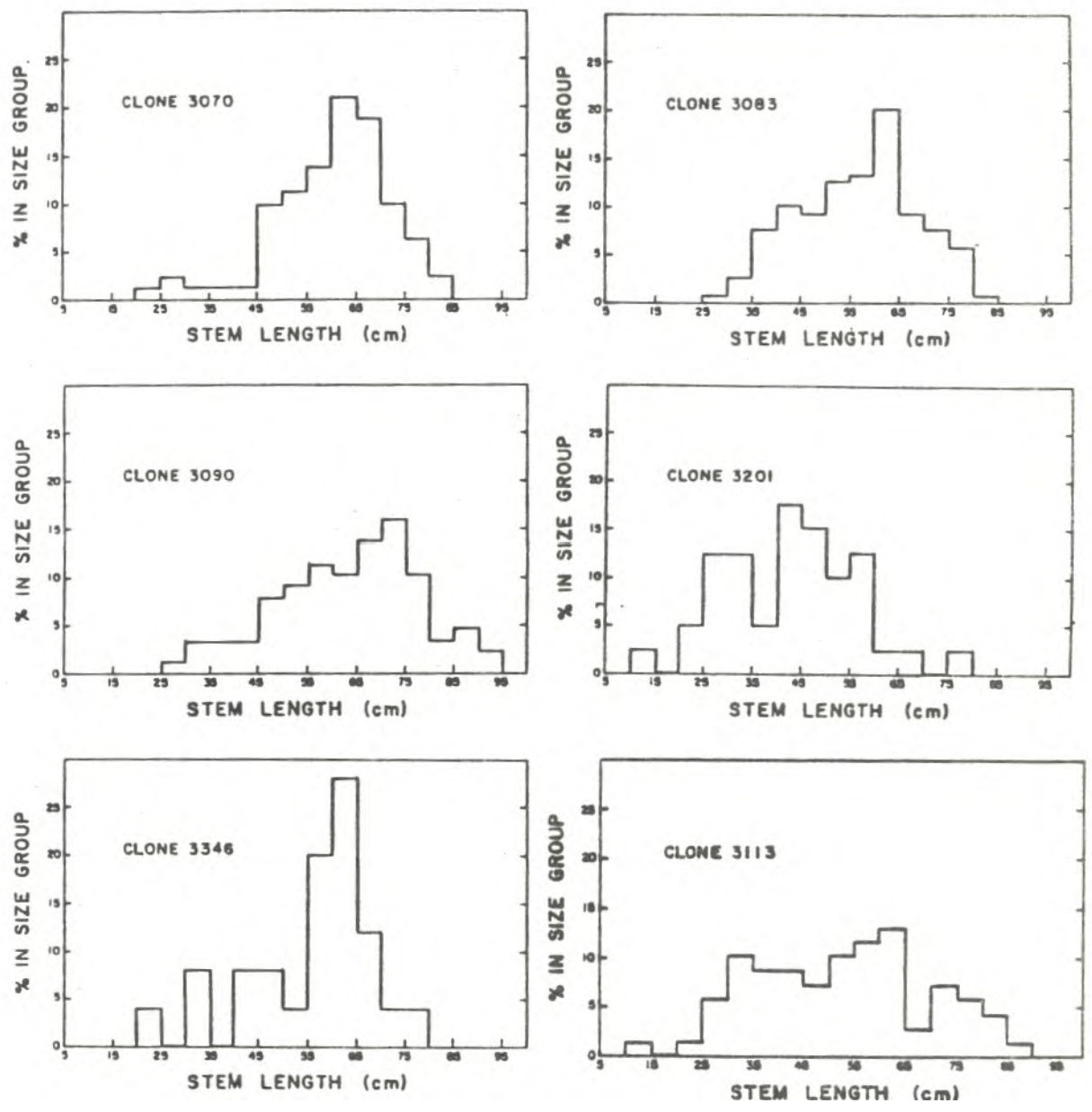

Figure 1. Stem length of 6 major clones grown in nursery beds.

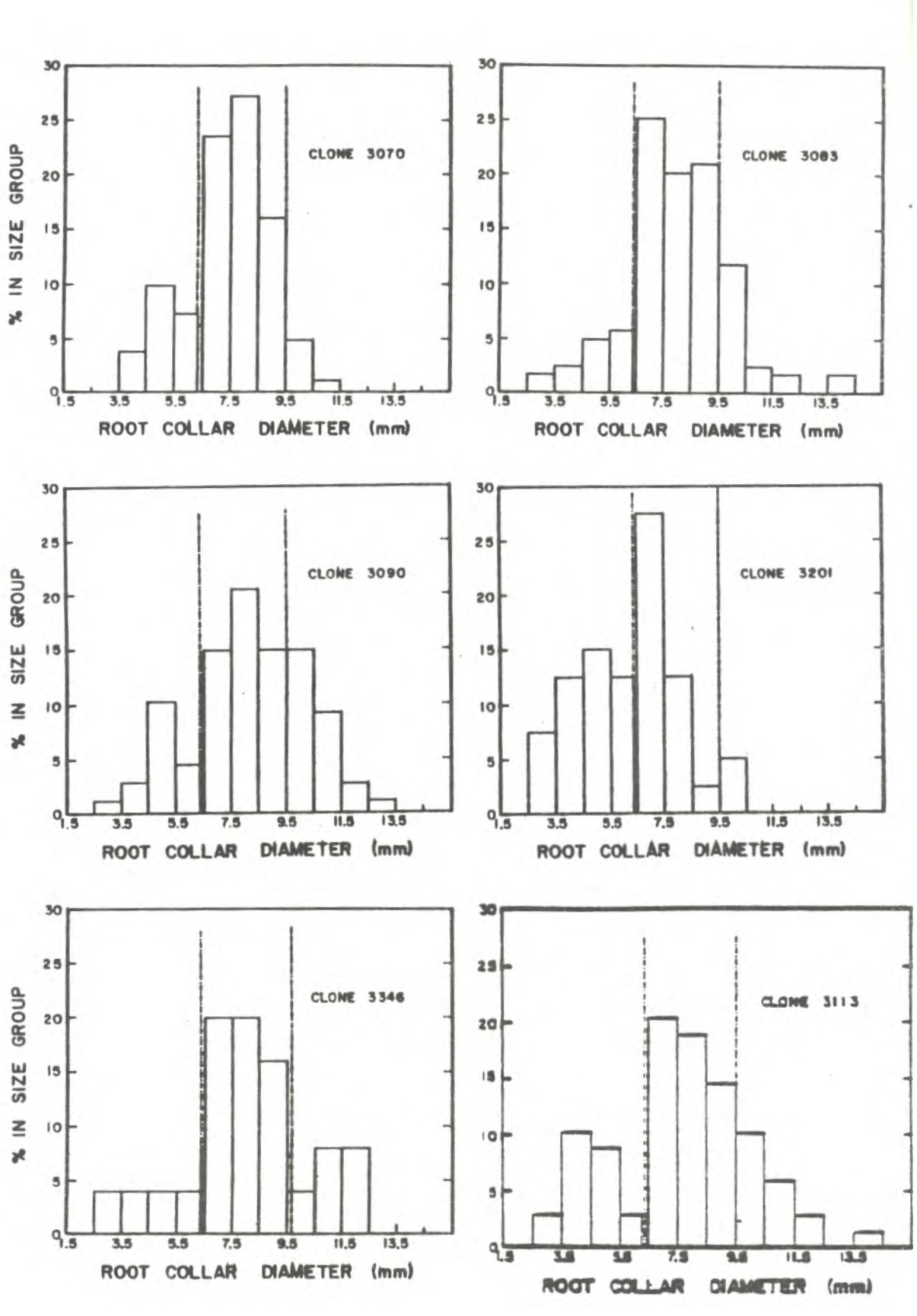

Figure 2. Root collar diameter of 6 major clones grown in nursery beds. Dotted lines indicate the minimum root collar diameter for planting and the preferred minimum root collar diameter for planting.

Photosynthesis and Anatomy

Net photosynthesis of seedlings and plantlets is shown in Figure 3. Seedlings showed typical light saturation curves when grown at the different light levels. High light seedlings had the highest maximum rate of photosynthesis. Photosynthesis of in vitro grown plantlets saturated at higher levels, with medium light plantlets attaining the highest photosynthetic rates. Plantlets exhibited much higher photosynthetic rates than seedling under all conditions.

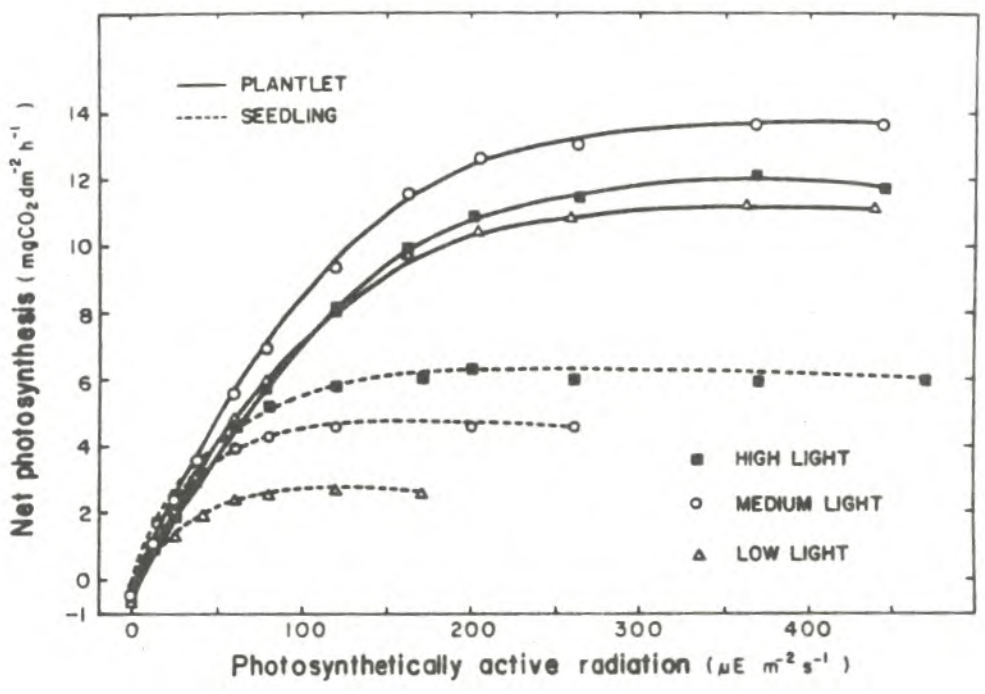

Figure 3. Net Photosynthesis of seedlings and plantlets grown under 3 quantum flux densities: 50 ± 5 (low), 155 ± 10 (medium) or 315 ± 15 (high) µEM $^{-2}$ S $^{-1}$.

Anatomical observations of plantlet leaves showed that chlorplast structure, leaf thickness, and mesophyll development were affected by quantum flux differences in culture (2). The higher stomatal densities and sizes found in cultured leaves were not affected by light levels. Factors other than light are responsible for the atypical stomatal configurations which contribute to water loss and wilting of cultured plants.

DISCUSSION

The inclusion of the liquid medium step has greatly increased the number of shoots obtainable in culture. However, more research is needed to increase the percentage of cultures that respond in the liquid step.

The other obvious problem that needs solution is that of root girdling. McKeand and Wisniewski (3) reported a similar problem with pines, which was solved by using shorter roots at planting and a ridged tube instead of a pot. This may not be feasible for Liquidambar, however, in that cuticular development and stomatal functioning are less developed in cultured sweetgum than pine (8). A well developed root system is expected to be important in maintaining good water relations (10,11). Rooting in a stationary medium such as foam or peat plugs may be a possible solution.

We have determined that plantlets developed in vitro are capable of significant levels of photosynthesis. However, it is unknown if culture conditions (i.e. CO_2 and light levels) promote photosynthesis. The efficienc of our culture system could be improved if this photosynthetic capability were utilized. Further research is needed in this area as are anatomical observations for evaluating and predicting plantlet growth and culture efficiency.

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