PROPAGATION OF SWEETGUM BY TISSUE CULTURE

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Abstract.--Clones of sweetgum (Liquidambar styraciflua L.) for research purposes are difficult to obtain using conventional means of vegetative propagation, i.e., rooting cuttings. We have developed tissue culture methods for the vegetative propagation of sweetgum using hypocotyl sections as explant tissue. Multiplication rates of 2 to 30X are obtained from 30-75% of the explant sources. Plantlets have been hardened off, transferred to the lathe house and are ready for field planting. The lapse time from culture to planting stock can be 9 months.

Additional keywords: Organogenesis, adventitious buds, plantlets.

It is evident that there is much research to be done on the tissue culture of forest trees and the role of tissue culture in forestry research and operational forestry. Our current primary interest is in the culture of hardwood species, particularly those being tested for use in short rotation coppice plantations in the Southeast. This is a particularly attractive management option to attempt to integrate with tissue culture propagation. The trees are juvenile throughout the rotation, thus phenotypic selections should be readily brought into culture and differentiation obtained. The species used for the study that is reported here is sweetgum (Liquidambar styraciflua). It is difficult to propagate from cuttings. Also problems have been reported with its propagation from seed in the nursery (Kormanik et al., 1977).

METHODS

The general methods used have been described previously (Sommer and Brown 1980, Birchem et al., 1981, and Sommer 1981). Half-sib seedlots, designated as from upland or bottomland sites, from the U.S. Forest Service Falling Creek seed collection area were used for these experiments.

Media used are those of Murashige and Skoog (MS) (1962), Blaydes (BL) (Witham et al., 1971), and modified Risser and White (RW) (1964). Modifications made to these media have been described (Sommer and Brown 1980, Sommer 1981).

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RESULTS

Preliminary Results

While the results reported here were obtained using seedlings, preliminary results indicate some methods are applicable to stump sprouts and young tree explants.

Earlier we have reported on embryogenesis (Sommer and Brown, 1980). The only comment needed at present is that the process is still sporatic. However, we have grown a number of trees to 2-3 feet in height. Some have survived a summer and a winter in the lathe house and leafed out this spring.

Several years previous to the start of the work to be reported here, callus was obtained from some 50 species of hardwoods on a MS media; however, sweetgum did not produce any callus (Brown, unpublished). Likewise during our initial experiments sweetgum explants did not proliferate on MS based media. Since we had seen callus and roots in anther cultures of sweetgum on BL media we switched to BL based media for initial screening experiments.

Using BL basal medium, the concentrations of NAA (a-napthalene acetic acid) and BA (6-benzyl adenine) were varied. After about 6-8 weeks the hypocotyl sections formed a callus. Some calluses differentiated shoots or roots. In general the effect of the hormones on morphogenesis was similar to the classical results from tobacco, a high ratio of NAA to BA favoring root development, while a low ratio favored bud differentiation. At some intermediate ratios no organogenesis was noted. No combination of auxin and cytokinin used caused all calluses on that treatment to produce organs.

Intermediate Results

A series of experiments was then established in an attempt to optimize conditions for organogenesis or embryogenesis. For this experiment seedlings from 3 upland and 3 bottomland half-sib seed lots were used. Medium consisted of one of 2 basal media, BL and modified RW, with one of 20 combinations of NAA and BA for a total of 2,400 cultures. In 4-8 weeks buds differentiated directly on some of the hypocotyl sections. Most of the hypocotyl sections had produced only callus. Upon analysis of the results, we found buds had differentiated on only one of the 40 media used i.e., modified RW with 0.01 ppm NAA and 0.5 ppm BA. Analysis based on seed source revealed that for all bottomland sources, hypocotyl sections had differentiated buds with a frequency of 40-70%, while the sections of only one upland source hypocotyl section had differentiated buds with a frequency of 40%.

Current Research

These results immediately raised two additional questions. Were the cultures that had not produced **buds** in this experiment capable of producing buds if transferred to either a basal medium or a medium with additional factors favoring bud inititation such as adenine, and if so, can buds be differentiated directly on hypocotyl sections grown under the above conditions? Transfer to basal medium usually led only to the differentiation of roots. The results for

the enriched media have been reported elsewhere (Sommer 1981). Just as an example, when hypocotyl sections were cultured on a medium using 5 ppm 2-iP (2-isopentenyl adenine) as the cytokinin and 1 ppm IAA (indole acetic acid) was also added, in 1 month 75% of the cultured sections had differentiated buds. Thus improvements in yield, speed and synchrony may be possible.

The second question raised was could the buds and shoots formed from bottomland sources on modified RW medium with 0.01 ppm NAA and 0.5 ppm BA be rooted and grown to planting stock size. To answer this question five bottomland seedlots were chosen, and germinated under aseptic conditions.

As before cultures were started from 3 mm hypocotyl sections placed modified RW medium with 0.01 ppm NAA and 0.5 ppm BA. The results are given in Tables IA and IB. It is obvious from the results that the yield of buds varies greatly from seed lot to seed lot. Multiplication rates can be calculated either on total number of seedlings used for explants or only on seedlings that gave buds. In the latter case it gives an idea of potential yield if the shoots from the buds are subcultured. The overall multiplication rate based on buds transferred to RW basal medium for growth was positive. Shoots grown from buds on this medium generally rooted spontaneously. In the case of this experiment no attempt was made to stimulate rooting or shoot growth; the plantlets in the RW medium were transferred to a potting mix and covered with a plastic bag, test tube or beaker. Hardening off to laboratory conditions was done either by gradually cutting away the bag or removing the covering for progressively longer time periods over a week. The relatively low percentage of plantlets recovered (Table IB) was the result of loss due to buds not forming shoots, shoots not rooting, loss during hardening due to uncontrolled relative humidity, and some loss after hardening. However, 94 plantlets have been turned over to Dr. K. Steinbeck and have been planted this spring. Forty-three others are still too small to plant out, so will probably be healed out in the nursery for planting next winter.

Table IA.--Bud differentiation from hypocotyls

Seed lot[a]	# of Seedlings used (A)[b]	# of Hypocotyls forming buds (B)[c]	% Seedling forming buds B/A x 100	Total # of buds (C)	Multiplication rates C/B	C/A
76-1	80	22	28	64	2.9	0.8
76-5	93	52	56	143	2.8	1.5
76-7	50	40	80	156	3.9	3.1
76-10	53	21	40	44	2.1	0.8
78-1	23	6	26	13	2.2	0.6
SUM	299	141	(47)[d]	420	(3.0)[d]	(1.4)[d]

a. All seed lots - half - sibs from bottom landsites.
b. Differences in number of seedlings used due to differences in germination rate.
c. Hypocotyls cut into 3 mm sections and cultured on modified RW with 0.01 ppm NAA, and 0.5 ppm BA.
d. Based on sum of all seed lots.

Table IB.--Plantlets from buds

Seed lot[a]	Total # of buds (C)	# of plantlets[e] (D)	% Plantlets per seedling (D/A x 100)	% Buds yielding plantlets (C/D x 100)
76-1	64	23	29	36
76-5	143	56	60	39
76-7	156	43	86	28
76-10	44	12	23	23
78-1	13	3	13	23
SUM	420	137	(46)d	(33)d

a-d. See Table IA.
e. See text for details on path from buds to plantlets.

We are currently studying these steps in the culture process using empirical methods. In addition Dr. Hazel Wetzstein is using electron microscopy to follow changes in general anatomy, cuticle and chloroplast development during the hardening off process.

At present we have no data on the survival of sweetgum tissue culture plantlets in the field. However in December 1979, we planted 25 black locust (Robinia pseudoacacia L.) and 46 paulownia (Paulownia tomentosa (Thumb.) Siev. & Zucc. plantlets. This April 96% of the black locust plantlets and 94% of the paulownia had leafed out. However 57% of the paulownia had regenerated from the roots only. No weed control was used and the trees were not watered even during the extremely hot dry summer we had last year.

CONCLUSIONS

Based on the results reported here we can conclude that the regeneration of sweetgum plantlets in tissue culture from juvenile tissue is possible. Field survival of the plantlets is expected.

Our work with tissue cultures from sweetgum seedling is nearly completed except for hardening off and shoot growth studies. The methods developed for culturing are now being applied to explants from stump sprouts and older trees.

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