

EMBRYO EXPLANT INFLUENCE ON CALLUS INITIATION
FREQUENCY FOR LOBLOLLY PINE

F.H. Huang, H.Y. Yan, J.M. Al-Khayri and X.Y. Li¹

Department of Horticulture and Forestry, University of
Arkansas, Fayetteville, AR 72701

Abstract. --The objective of this study was to compare callus initiation frequency in response to different culture methods of loblolly pine (*Pinus taeda*) embryo explants. Cones were collected at the end of August and September, 1992, from trees in Arkansas. The seeds were isolated, disinfected in 70% ethanol for 30 s and in 30% Clorox for 15 min, and rinsed in sterilized water. Seed coat was removed, and the embryos were inoculated either attached to or separated from the megagametophytes. The separated megagametophytes were also cultured to test their potential to produce callus. These explants were cultured on half-strength modified MS medium adjusted to pH 5.8 and supplemented with 11 mg/L 2,4-D, 4.5 mg/L kinetin, and 4.5 mg/L BAP. The cultures were maintained in the dark at 20+3°C for 4 weeks, after which callus formation was recorded. Callus initiation frequencies from seeds collected in August and in September, respectively, were 78.3% and 92.5% for embryos, 13.7% and 27.2% for megagametophytes, and 29.9% and 48.6% for embryos attached to the megagametophyte. These differences indicate that callus initiation frequency from isolated embryos was higher than the frequency obtained from the other explants. These results suggest that the presence of megagametophyte tissue may restrict callus initiation from the embryo.

Keywords: *Pinus taeda* L., megagametophytes, somatic poly embryogenesis, embryogenic callus.

INTRODUCTION

Loblolly pine is an economically important coniferous species in the southeastern United States. It is the main species on about 29 million acres of plantations (Brender et al. 1981). It accounts for half of the nation's volume of southern yellow pine and supports a huge and growing forest products industry.

¹Associate Professor, Graduate Assistant, Research Associate and Graduate Assistant, Department of Horticulture & Forestry, University of Arkansas, Fayetteville, AR 72701

Breeding and biotechnology are being integrated into genetic improvement program. Such integration may be more important in programs for woody than for herbaceous species because of the time required for sexual maturity in most woody species (Riemenschneider et al. 1988). Use of asexual propagation could yield gains more than twice as fast as those from sexual propagation, with gains of 18 to 32% in volume per acre for first generation seed orchards alone (Talbert 1982, McKeand et al. 1984). The principal advantage conferred by micropropagation is that plants can be selected for superior performance that results from both additive and non-additive genetic effects. Conventional seeding-oriented methods can effectively utilize only additive genetic gain (Ammirato 1986, Van Buijtenen and Lowes 1989).

Many ongoing loblolly pine improvement programs would benefit from capturing of genetic gain through somatic polyembryogenesis (SPE) of loblolly pine. Somatic polyembryogenesis is a multiplication process in which cells of an embryonal suspensory mass (ESM) differentiate into new embryos according to true-to-type development without excision of cells or tissues from the differentiated body or from the post-proembryonal stages of development. The multiple embryos that normally degenerate during seed development are rescued as an ESM and regenerated new plantlets (Durzan 1988a and 1988b). Compared with organogenesis, SPE produced large numbers of somatic embryos. Economies are expected not only for mass and rapid propagation of elite trees but also for eventual automated, large scale in vitro production (Gupta et al. 1993).

To realize these enormous advantages of SPE in loblolly pine tissue culture, the process must be well defined. Only a few groups have reported some progress on SPE of loblolly pine (Becwar and Feirer 1989, Becwar et al. 1988, 1990, 1991, Gupta and Durzan 1987a and b). The repetitive polyembryogenesis cycle can be induced; however, conversion of the immature somatic embryos to the mature somatic embryo and the recovery of plantlets remains difficult (Beckwar and Feirer 1989). Beckwar et al. (1990) stated that the explant developmental stages are the most important factor for the initiation of embryogenic cultures and somatic embryo development in loblolly pine. Al-Khayri et al. (1992) reported that the optimum periods to collect loblolly pine in our areas are June, July, and August. However, there has been no report on the effects of culture methods of immature loblolly pine embryos on callus induction.

The objectives of this study was to compare different explants from immature seeds of loblolly pine relative to their frequency of callus initiation.

MATERIALS AND METHODS

Cone Collection

Immature cones of loblolly pine were collected from six trees grown at the Arkansas Agriculture Experimental Station, Fayetteville, AR and at Hope, AR. Two collections were made at the end of August and of September during 1992. Four cones were collected from each tree, providing 24 cones at each collection.

Seed Extraction, Disinfection, and Embryo Removal

Following each collection date, scales were removed by peeling away from the axis of the cone with a knife. After being exposed the seeds were removed, placed in a plastic bag, and kept in a refrigerator for several days until all the seeds from the samples had been extracted. The seeds were surface disinfected in 70% ethanol for 30 seconds, emersed for 15 min in 30% Clorox solution containing 0.01% Tween 20, and rinsed 4 times in sterile water. With a scalpel, seed coats were removed exposing the immature embryos.

Explant Tissue Selection

Three types of explant were cultured depending on **their** isolation methods. They were: 1) The entire immature **seeds** including the embryo and megagametophyte (EG), 2) the embryo **alone** (E), and 3) the separated megagametophyte alone (G).

Callus Induction and Initiation Frequency

The explants were all cultured individually in culture tubes (5x100mm) containing 5 ml of half-strength modified MS medium (275mg/L, NH_4NO_3 , 233/mg/L KNO_3 , 220 mg/L $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 185 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 85 mg/L KH_2PO_4 , 18.65 mg/L Na_2EDTA , 13.90 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 11.15 mg/L $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 4.3 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3.1 mg/L H_3BO_3 , 0.415 mg/L KI, 0.125 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.0125 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.0125 mg/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1,000mg/L myo-inositol, 0.25mg/L nicotinic acid, 0.25 mg/L Pyridoxine Hcl, 1.0 mg/L thiamine. Hcl, 1.0 mg/L glycine, 450 mg/L L-glutamine, 500 mg/L Casein hydrolysate) adjusted to pH 5.8 and supplemented with 11 mg/L 2, 4-D, 4.5 mg/L kinetin, and 4.5 mg/L BAP. The cultures were maintained by transfer to fresh mediums at 4-week intervals in the dark at 20+3°C and the callus initiation frequency was recorded.

Cell suspension establishment and maintenance were performed according to Beckwar et al. (1988). Redifferentiation of callus and cytochemical staining procedures were used after Gupta and Durzan (1987a).

RESULTS AND DISCUSSION

Callus initiation frequency in response to the explants culturing method was evident. Cultured embryos alone produced the highest number of calli. The cultured embryo with the megagametophytes included was second high, and the cultured megagametophytes alone was the lowest (Table 1).

Table 1. Frequency of callus initiated from explants of 6 loblolly pine.

Tree	E	G	EG
F1	66.7	20.0	26.3
F2	76.7	22.0	44.4
F8	81.8	14.9	50.0
H2	91.0	28.9	33.3
H4	97.7	22.7	63.0
H15	97.9	25.5	20.0

E-embryo

G-Megagametophytes

F=Fayetteville, AR.

H=Hope, AR.

The frequency of callus initiation was different among six trees and between two locations (Table 1).

Frequency of callus initiation differ between collection made in August and September for the E and G explants (Figure 1).

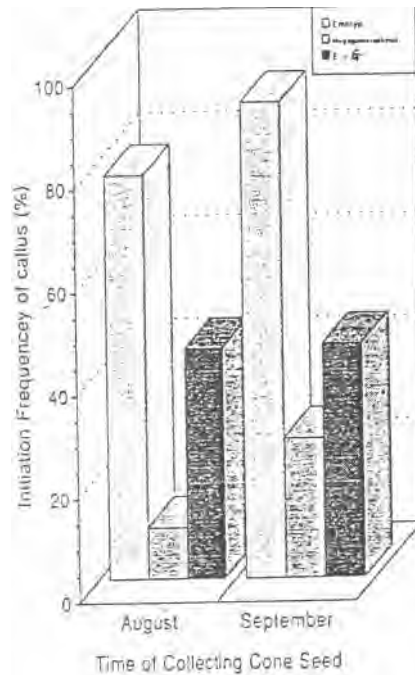


Figure 1. Initiation frequency of callus compared with the time of cone collection and three culture methods.

CONCLUSIONS

These differences indicated that callus initiation frequency from isolated embryos was higher than the frequency obtained from the other explants. This suggested that the presence of megagametophyte tissue may restrict callus initiation from the embryo.

Although the percentage callus initiation showed an indication of the ability of the immature embryos to produce callus, the relationship among cone collecting period, the ability to produce callus, and the capacity to regenerate plantlets have not been established. Further research is needed to identify these relationships.

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