The Application of Tissue Culture Techniques to Various Problems in Forest Tree Improvement

CLAUD L. BROWN [1/]

Tissue culture techniques have become a, valuable tool in studies of plant grow th and development. Today, with our knowledge of plant nutrition it is possible to culture cells, tissues and organs of many plants for indefinite periods of ti me. The cells and tissues of some species are easily grown on a relatively simple synthetic medium, whereas those from other species may require combinations of complex growth factors, and still the tissues of other species are cultured with much difficulty or not at all.

To the physiologist and developmental morphologist, tissue culture techniques have been extremely useful in studying certain aspects of plant nutrition, the biogenesis of numerous cellular components, and various processes involved in tissue and organ differentiation. To the geneticist and cytologist, in vitro cultures have been useful from time to time to nurse excised hybrid em bryos to maturity which would otherwise have aborted because of their incompatibility with maternal tissues. In addition, cell and tissue cultures have proved of much utility in studies of chromosome morphology,ploidy, somatic mutations, and tumor formation.

I wish to limit this discussion on the use of sterile culture techniques to two major problems directly related to the progress of tree improvement: 1) the-vegetative propagation of clonal material, and 2) the production of homozygous diploids.

Propagation of Clonal Material

One of the most time consuming and costly procedures used in the establishment of clonal orchards is the process of grafting. The many problems associated with graft success and failure are well-known to tree improvement foresters. Fortunately some woody species can be propagated with ease from stem cuttings or juvenile stump sprouts. For these species clonal multiplication is no problem. But in other species, expecially many of the pines, rootability is so low that clonal lines can only be established through grafting.

Real progress is now being made in propagating difficuIt to root species, including conifers, through the use of tissue culture techniques, but the real breakthrough has not yet arrived. For example, in our laboratory we have spent considerable time during the past four years developing a synthetic medium for the rapid continuous growth of longleaf, slash, and loblolly pine callus. Only during the past year has a defined medium been developed that supports excellent growth of these species (Table 1). Since this was achieved, we have been able to culture successfully members of 15 genera of gymnosperms out of the 21 genera tested from various parts of the world.[2/]

The reason we are so enthusiastic about the establishment of rapidly growing callus cultures of pine tissues lies in the fact that those of us engaged in this type of work have often observed a high correlation between species "rootability" and ease of establishing tissue cultures. Stated differently, given a species that roots easily, e. g. willow, aspen, red maple, etc. it is likely that its cells will also culture with ease. Conversely, those species that root with difficulty, usua l ly culture poorly or not at all. Such was the case with the southern pines, but now that we know more about their cultural requirements in vitro it is probable that we will soon be able to induce bud and root formation in callus cultures.

The classical work of Skoog and Miller (1957) showing the effects of a proper balance of indole-3-acetic acid (IAA) and cytokinin (kinetin) on root and bud formation in tobacco callus can now be used with modifications to produce similar effects in certain hardwood cultures (cottonwood and red maple), but so far we have not yet worked out the proper conditions to induce either bud or root formation in the callus cultures of pine. This is not too discouraging, however, because we know that achieving the precise balance of growth factors for producing pine plantlets is no simple undertaking. It is only a matter of time before such can be accomplished.

1/ Professor of Forestry, School of Forestry, University of Georgia, Athens.

2/ Unpublished results of C. L. Brown and J. Mustoe, University of Georgia.

Table 1. Composition of medium used for culturing longleaf pine callus. Modified from Murashige and Skoog (1962).

Inorganic Nutrients	mg/liter	Organic Supplements	mg/liter
NH_4NO_3	1650		
		Nicotinic acid	0.5
KNO_3	1900	Pyridoxin-HC1	Oil
		Thiamin -HC1	0.1
$CaC1_2.2H_20$	440		
$MgSO_4.7H_20$	370		
		Kinetin	.5
KH_2PO_4	170	2, 4-D	5.0
		Inositol	100.0
		Asparagine	100.0
		Sucrose (2%)	20000
H_3B0_3	6.2	Agar (0.8%)	8000
$MnSO_4.4H_20$	22.3		
		($_p$H 5.7-5.8)	
$ZnSO4.4H_20$	8.6		
KI	0.83		
$Na_2MnO_4.2H_20$	0.25		
$CuS0_4.5H_20$	0.025		
$CoC1_2.6H_20$	0.025		
$FeSO_4.7H_20$	27.8*		
Na_2-EDTA	37.3*		

*5 m 1 of a stock solution containing 5:57 ·g $FeSO_4$. 7H20 and 7.45 g Nat -EDTA per liter of H2O.

In attempts to carry this work further, i. e. , the mass production of diploid plantlets in laboratory flasks, we have used the approach of Steward and his colleagues (Steward **et al,** 1958) where they were successful in growing whole carrot plants from small aggregates of cells, or even from single cells, in liquid suspension cultures. Although we have only recently (within the past 3 months) been able to establish liquid suspension cultures of longleaf pine, we can now grow h u n dreds of thousands of small cell colonies all with the same genotype in a few small flasks under controlled laboratory conditions. It seems only a matter of time before one will be able to propagate, by the thousands, many of the difficult to root species with considerable facility and little expense. This possibility is real, and these techniques will undoubtedly be used more and more in forest genetics, agronomy, and horticulture. The production of a few thousand plantlets from a single mass of cultured body cells from superior pines, or from hybrid orchids wou ld be equally exciting and economically rewarding to foresters and horticulturists.

Culture of Haploid Tissues and the Development of Homozygous Diploid Lines

In addition to mass propagating selected heterozygous clones, it is now possible to grow haploid tissues (callu s cultures) of higher plants. It is significant and encouraging that the gametophyte cells of a woody plant species and especially a gymnosperm (Ginkgo), was the first to be cultured on artificial media. Tulecke (1957) successfully established pollen cultures of Ginkgo on a fairly complex medium, and more recently, he has been able to grow the female gametophyte of the same species under similar conditions (Tulecke, 1964). So far in our laboratory we have been unable to establish pollen cultures of any native pine on media similar to that used by Tulecke for Ginkgo. Neither have we been able to establish pollen cultures from any woody or herbaceous angiosperm although we have attempted to grow numerous species from a wide array of plant families beginning with members of the primitive **Magnoliaceae** through the highly advanced **Scrophulriaceae.** There is still hope, however, because over three years ago one of my students was able to obtain excellent proliferation of the female gametophyte of longleaf pine under certain conditions. Since that time we have been able to keep this haploid tissue growing fairly well for about 5 transfers (six months) before finally losing it.

Thus, we have not given up on the possibility of growing haploid tissues of pines and hardwoods because of the extremely important application of these procedures to forest genetics. With the production of haploid tissues, followed by chromosome doubling, and the formation of plantlets (roots and buds), a homozygous line would be effectively produced. This alone would by-pass a century or more of inbreeding in forest trees, and in the case of exceptionally desirable superior hybrids permit the forest geneticist to establish a true-breeding line almost overnight. Although I am well aware of the pitfalls in the widespread use of the monocultures, and the fact that some of our fellow foresters might be quite perturbed over the commercial use of homozygous lines, many of us working in tree physiology and genetics would be more than elated to nave such material available for experimental purposes. Although it is hazardous for the researcher to make predictions or attempt to out-guess the

plants with which he works, I would like to emphasize the fact that the scientific know-how for producing haploid tissues, and the control of plantlet formation is real. Logically it is only a ma tter of time (probably 3-5 years) before the production of homozygous lines in forest trees, horticultural plants, or agronomic crops becomes a reality through the use of tissue culture techniques.

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