MICROPROPAGATION OF FOREST TREES[1]

D.F. Millikan and Teresa Gowin[2]

Micropropagation of forest trees is a promising approach for solving one of our most critical problems. Our need for increased tree production and for the protection of the environment competes with the need for food and fiber crops, highways and urban settlements. Options for altering these demands are so limited that an increased supply is our most attractive solution. Genetic improvement of tree clones is an obvious method for increasing forest productivity but vegetative propagation of superior genotypes by conventional use of cuttings or grafting has not proven economically feasible. Therefore, alternative methods of in vitro propagation or micropropagation are under intensive study.

Four types of micropropagation can be used to propagate higher plants. Currently two of these, pollen and protoplast culture, are more useful for laboratory investigations but offer exciting possibilities for obtaining new and superior clones. Callus can be aseptically established from many plant species and induced to undergo organogenesis and plantlet development. Presently the usefulness of callus cultures to produce forest trees is limited by ploidy problems. On the other hand, if shoot tip meristem explants of many woody plant species are placed on the appropriate medium and exposed to the proper light regime and correct temperatures they will form plantlets of the same parental genotype.

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[1] Journal Series Paper No. 6030. Approved by the Director.
[2] Respectively Professor and Visiting Professor, Department of Plant Pathology, University of Missouri - Columbia, 65211

The differentiation of an explant into a plantlet rarely occurs on a single nutrient medium and under a single set of environmental conditions. Different demands at different stages of plantlet development from the explant are contributing factors. Three main stages in this differentiation have been recognized (Murashinge, 1974) and include: 1) establishment of a continuous culture, 2) morphogenesis leading to plantlet formation and 3) transfer of plantlets to soil. In the first stage it is essential that the explant be free from contamination and be exposed to low light intensities. The basal medium for sustained growth must be determined. The MS medium (Murashige & Skoog, 1962) can generally be appropriately modified. After the culture has been established morphogenesis can be induced by adding the correct amount or balance of the proper auxins and cytokinins to the medium. Gibberellins may be helpful in the first stage but generally are not necessary in the second stage as they tend to suppress root and shoot development. The major factor in the third stage is the prevention of desiccation since the plantlets have developed in a saturated environment. They have little or no protective cutin and possess poorly functional chloroplasts. It is necessary, therefore, to reduce the light intensity and gradually reduce the humidity from the saturated in vitro condition to the ambient humidity of the in vitro environment. During this period the chloroplasts will become functional and the cutin will thicken to form a protective layer on the leaf surfaces.

MICROPROPAGATION OF CONIFERS

Although species of Cryptomeria and Picea are routinely propagated by cuttings in Japan and Germany (Rediske, 1979) and cuttings of Douglas-fir and western hemlock have been rooted in Rediske's facilities, micropropagation

offers advantages of economy and quantities lacking in the more conventional methods of vegetative propagation. The potential of micropropagation was recognized in the 60's with the development of plantlets from aspen tissue cultures (Mather, 1964; Winton, 1968; Wolter, 1968) and since then this practice has been extended to conifers. Some of the conifer species which have responded to tissue culture are listed in Table 1.

Sequoia sempervirens (D Don) Endl was the first gymnosperm to be successfully maintained in continuous culture. Significantly, in some of the Sequoia subcultures the calli showed internal organization and a few produced buds. Fifteen years later, Konar and Oberoi reported the in vitro development of embryoids in the cotyledons of Biota orientalis Endl. When these embryoids plus some cotyledonary tissue were subcultured they produced shoots but no roots. Romberger et. al. obtained a 10 - 30 fold increase in dry weight of Picea abies (L) Karst from shoot tip meristem explants containing the dome (ca 200 p tall) without primordia. None of the 5000 meristems cultured produced any basal callus, indicating that shoot tip meristem could be used for in vitro conifer propagation. In India Konar initiated callus from the hypocotyl tissue of Pinus gerardiana Wall which in turn was induced to form roots and shoots. Subsequently, the in vitro formation of adventious buds and roots on the hypocotyl tissues of Cryptomeria japonica D. Don was reported by Isikawa. Later, Bonga excised embryonic shoots from 15 - 20 year-old Abies balsamea (L) Mill and after soaking them in water with and without certain growth regulators and caffeic acid, was able to induce organogenesis. Non-soaked embryonic shoots elongated and occasionally developed callus but never showed organogenesis. Plantlets have been obtained from Pinus palustris Mill, P. radiata D. Don, P. sylvestris L, P. taeda L, Psuedotsuga mensiesii (Mirb) Franco, Tsuga

heterophylla (Raf) Sarg and Thuja plicata Donn. Only from Thuja plicata, however, were these plantlets developed from adult tissues.

MICROPROPAGATION OF NON-CONIFERS

The favorable response of Populus species to micropropagation (Winton, 1970; 1971) and the popularity of Populus species or hybrids for biomass and other wood products has stimulated investigations on micropropagation of this genus as well as other hardwood species (Table 2). Plantlets have been produced from callus cultures of eight different Populus species or hybrids, and with the rapid introduction of newer clones possessing superior economic characteristics this list will surely grow. Plantlets also have been obtained from Castanea sativa Mill. Shoots were induced when isolated embryonic axes from C. sativa seed were cultured on a chemically defined medium containing BAP. Limited rooting was obtained when these shoots were excised and exposed to 2 mg/1 IBA. Earlier, Cresswell and Nitsch induced the formation of plantlets from explants of Eucalyptus grandis L seedlings which were later transplanted to the field. Somatic embryogenesis and plantlet formation has been reported in Santalum album L by Bapar and Rao. They found that shoot buds could be induced on hypocotyl explants and these shoot buds could be induced to form rooted plants. They also were able to induce embryos on the hypocotyl callus tissue which in turn could be induced to form complete plants.

All of the above plantlets were derived from juvenile tissues whose genotypes are unkown. If the desired genotypes of superior clones are to be increased by micropropagation then the explant must be fully differentiated tissue from mature trees. The recent reports of plantlets induced from explants of terminal or lateral buds from mature trees of Eucalyptus citriodora Hook,

Platanus x acerifolia Willd and Tetona grandis L, however, indicate that the problems associated with growth of plantlets from mature tissues are resolvable. Furthermore, the simple medium and unsophisticated facilities used for plantlet production of Platanus indicates that only conditions which minimize contamination and provide reasonable temperature control are essential.

MICROPROPAGATION OF BLACK WALNUT

Black walnut, Juglans nigra L, is a popular forest tree in Missouri. The fruit or nut is in great demand by the confectionary and dairy interests and the wood is highly prized by the furniture and veneer industries. Productive cultivars of high quality fruit have been available for over 50 years and recently several fast growing timber types have been patented. The genotypes of these selections can be propagated by grafting but poor stands result in high production costs and a fluctuating supply of salable trees. Successful micropropagation of black walnut would reduce these costs and insure a stable supply of trees.

In 1977 a modified MS medium was developed (Lenartowicz & Millikan) which appeared to support the growth of shoot tip meristems. Shoot tip meristem explants cultured early in the growing season generally grew well but rarely differentiated shoots. Explants from dormant material quickly turned brown and died. We then modified the medium by reducing the KNO3 by one half and doubling the CaC12.5 H2O (Makinen & Millikan, 1979) and this satisfies the explant's requirement for the first stage although browning remains a problem with the dormant material.

Presently we are investigating additional techniques to reduce or eliminate the browning of the explants. Soaking the tip or lateral but

(Cresswell & Nitsch, 1975) in 0.01 M sodium diethyldithiocarbamate = DIECA (James & Garten, 1952) overnight prior to sterilization is superior to soaking in an aqueous solution containing ascorbic acid (100 mg/1) and citric acid (150 mg/1). Charcoal (Fridborg & Eriksson, 1975) and polyvinylpyrrolidone = PVP (Jones et. at., 1965) are being tested as additives to the medium after sterilization.

DISCUSSION

Tree improvement programs can be accelerated by micropropagation. Using the shoot tip meristem explant, great numbers of trees genetically identical with the parent can be obtained in a relatively short period of time. Early work was largely confined to seed embryos or seedling explants whose economical values and silvicultural characteristics are largely unknown. Until recently, however, explants from adult trees with known genotypes would not form plantlets. The development of plantlets from shoot tip meristem plantlets of Thuja (Coleman & Thorpe, 1977), Eucalyptus (Gupta et. al., 1980), Platanus (Krzewski et. al., 1980) and Tectona (Gupta et. al., 1981) suggests that the difficulties associated with plantlet development from adult tissues are resolvable. Gupta et. al. (1981) list 5 requirements for obtaining viable plantlets with a high multiplication rate from mature trees of Eucalyptus. This information should be helpful in inducing plantlets from explants of adult trees of other forest tree species.

Pathogen-free plantlets can be obtained from explants of clones from tree species which are completely infected. Berbee et. al. (1972) cultured a shoot tip meristem from a virus infected clone of Populus and was able to obtain a healthy tree. Other pathogens, including bacteria, fungi and mycoplasma, also can be eliminated by micropropagation.

Micropropagation of elite clones of black walnut by shoot tip meristem explants needs additional study. The modified MS medium with reduced O^{-} and increased Ca^{++} appears to be adequate for the first stage but the proper balance of auxin and cytokinin for the second stage for consistent shoot growth and the formation of roots have not been precisely identified. Soaking the buds or tips containing the explants in water containing DIECA (0.01 M) prior to placing on the medium reduces the browning reaction but additional treatment appears necessary for explants from dormant material.

Although explants from trees with known genotypes are useful in tree improvement programs, explants from embryos or seedlings can provide valuable information. Since an individual explant can provide up to 100,000 trees/year with subcultures every 60 days (Gupta et. al., 1981), micropropagation of explants from seedlings or embryos can be useful in progeny testing of certain crosses. This has immediate application in southern pine improvement programs where sources of resistance to fusiform rust have been identified. Progenies from all possible crosses can be rapidly and statistically evaluated for resistance prior to plantation planting. By regulating the exposure to different sources and quantities of inocula the disastrous effects of monculture can be minimized.

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Table 1. ORGANOGENESIS IN TISSUE CULTURE OF CONIFER SPECIES

Abies balsamea (L) Mill	1977	Bonga, In Vitro 13:14
Biota orientalis L	1965	Konar & Oberoi, Phytomorphology 15:137
Cryptomeria japonica D. Don	1974	Isiwaka, Bot Mag, Tokyo 87:73
Picea abies (L) Karst	1971	Romberger & Tabor, Amer J Bot 58:131
P. glauca (Moench) Voss	1975	Campbell & Durzan, Can J Bot 53:1652
Pinus gerardiana Wall	1972	Konar, USDA PL 480
P. palustris Wall	1975	Sommer et. al., Bot Gaz 136:199
P. radiata D. Don	1977	Reilly & Washer, NZJ For Sci 7:199
P. sylvestris L	1980	Borman & Jansson, Z Pflenzenphysiol 96:1
P. taeda L	1979	Leach, Tappi 62:59
Pseudotsuga menziesii (Mirb) Franco	1975	Cheng, Plant Sci Lett 5:97
Sequoia sempervirens (D. Don) Endl	1950	Ball, Growth 14:295
Tsuga heterophylla (Raf) Sarg	1976	Cheng, Plant & Cell Physiol 17:1347
Thuja plicata Donn	1977	Coleman & Thorpe, Bot Gaz 138:298

TABLE 2. PLANTLET DEVELOPMENT FROM TISSUE CULTURES OF HARDWOOD SPECIES

Castanea sativa Mill	1980	Vieitez & Vieitez, Physiol Plant 50:127
Eucalyptus citriodora Hook	1981	Gupta et. al., Plant Sci Lett 20:195
E. grandis Hill ex Maiden	1975	Cresswell & Nitsch, Planta 125:87
Plantus x *acerifolia* Willd	1980	Krzewski et. al., J Arborculture 6:250
Populus canescens Smith	1977	Whitehead & Giles, NZJ For Sci 7:40
P. euroamericana (Dode) Guinier cv *robusta*	1974	Chalupa, Biol Plant 16:316
P. x "Flevo" (*P. deltrodes* x *P. Nigra*)	1977	Whitehead & Giles, NZJ For Sci 7:40
P. nigra L cv Italica	1977	Whitehead & Giles, NZJ For Sci 7:40
P. nigra var *typica* Schneider	1974	Chalupa, Biol Plant 16:316
P. tremula L.	1971	Winton, For Sci 17:348
P. tremuloides Michx	1970	Winton, Am J Bot 57:904
P. yunnanensis L.	1977	Whitehead & Giles, NZJ For Sci 7:40
Santalum album L.	1979	Bapat & Rao, Ann Bot 44:629
Tectona grandis L.	1981	Gupta et. al., Plant Sci Lett 20:259

TABLE 3. NUTRIENT MEDIUM FOR EXPLANTS OF BLACK WALNUT

Macroelements	mg/l	Microelements	mg/l
	Mineral Constituents		
NH_4NO_3	1650	KI	0.83
KNO_3	950	H_3BO_3	6.2
$MgSO_4 \cdot 7\ H_2O$	370	$MnSO_4 \cdot\ H_2O$	22.3
$CaCl_2 \cdot 2\ H_2O$	880	$ZnSO_4 \cdot 7\ H_2O$	8.6
KH_2PO_4	170	$Na_2MoO_4 \cdot 2\ H_2O$	0.25
		$CuSO_4 \cdot 5\ H_2O$	0.025
		$CoCl_2 \cdot 6\ H_2O$	0.025
		$Na_2 \cdot$EDTA	37.3
		$FeSO_4 \cdot 7\ H_2O$	27.8
	Organic Constiuents		
Inositol	100	NAA	0.1
Nicotinic acid	0.5	BAP	1.0
Pyridoxine·HCl	0.5	GA_3	0.05
Thiamine·HCl	0.1	sucrose	30000