RAPID PROPAGATION OF POPLARS BY TISSUE CULTURE METHODS

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ABSTRACT

A rapid method for the propagation of poplars by tissue culture has been developed. In comparison with conventional practices very large numbers of rooted plants can be rapidly formed from small explants and the potting mix can be manipulated to give establishment advantages to the tree when planting out. The technique also gives a method for the international exchange of poplar material under sterile conditions, to eliminate the danger of disease introduction, in a form that can be quickly bulked up at any time of year.

INTRODUCTION

Because of the die-back and death of many varieties of poplars in New Zealand due to the introduction of the rust diseases *Melampsora laricini-populina* and *M. medusae,* large numbers of poplars planted for soil conservation purposes and timber must be replanted with varieties resistant to these diseases. Large numbers of rust-resistant trees have been needed, therefore, to keep up with current planting programmes and replacement plantings. Many of these trees are newly introduced disease-resistant or tolerant overseas varieties, so there is likely to be a continuing need for large numbers of poplars of diverse origin for planting in many parts of New Zealand over the next few years.

Winton (1968), Berbee and Hildebrandt (1972) and Venverloo (1973) have shown that some species of poplar can be differentiated from callus cultures. The methods they describe do not offer a ready method for rapid propagation since the differentiation is slow and only limited numbers of shoots were formed on callus. This paper describes a method for the very rapid micropropagation of poplars by tissue culture.

EXPERIMENTAL

Axillary buds of *Populus nigra* "Italica", *P. "Flevo"* (*P. deltoides* × *P. nigra*) and *P. yunnanensis* were taken from either leafed or dormant branches. The buds were surface sterilised by dipping them in ethanol and flaming them, and then by submersion in a 0.2% hypochlorite solution followed by several washes in sterile water. The outer bracts were dissected from the buds which were placed on medium 1 (Table 1). Cultures were maintained at 25°C with a 16 h photoperiod and a total radiant flux density of 20 W/m^2.

TABLE 1—Composition of medium used for rapid micropropagation of poplar species. Modified from Murashige and Skoog (1962). Weights in mg/litre

Inorganic nutrients				Organic supplements	
NH_4NO_3	1650	KNO_3	1900	Nicotinic acid	0.5
$CaCl_2.2H_20$	440	$MgSO_47H_20$	370	Pyridoxin-HCl	0.1
KH_2PO_4	170	H_3BO_3	6.2	Thiamin-HCl	0.1
$MnSO_4.4H_20$	22.3	$ZnSO_4.4H_20$	8.6	Inositol	100
KI	0.83	$Na_2Mo0_42H_20$	0.25	Lysine	100
$CuSO_4.5H_20$	0.025	$CoCl_2$ $6H_20$	0.025	Sucrose	2000
FeEDTA	65.1			Adenine sulphate	20

	Growth substances	
	Benzyl adenine	Naphthalene acetic acid
Medium 1	0.2	0
Medium 2	0.1	0.02
Medium 3	0.01	0.01

Agar 1500

Bud break occurred within 2-3 weeks on medium 1, and within 4 weeks the axillary buds on the initial shoot had started to lengthen. The shoots were cut into 0.5-cm sections and replaced on medium 1. Adventitious bud formation and proliferation occurred on both cut and uncut surfaces, and existing axillary buds grew out (Fig. 1a, b, c). Once proliferation had started tissue was transferred to medium 2 on which proliferation and growth continued for 6-8 weeks, after which time subculturing was necessary. Within this time 120-220 shoots had formed from each original bud explanted and some shoots had attained lengths of 6-8 cm. These shoots were then either rooted in pumice and peat or rooted under sterile conditions on medium 3 (Table 1). Root initiation under sterile conditions took place within 1-2 weeks (Fig. 1d). Alternatively these shoots could be cut into 0.5-cm sections and replaced on medium 1 to initiate another round of bud proliferation.

The results indicated that more than 10^6 plantlets per year could be produced from one bud of any of the clones used. *P. yunnanensis* gave more shoots than the other two clones used, and *P. nigra* "Italica" produced the least number of adventitious buds.

The plantlets produced initially exhibited juvenile leaf shape, but after transplanting into pumice : peat the mature leaf shape became established. After rooting, the plants were transferred to polythene sleeves containing the growing mix (Fig. 1e). The plants received half strength Hoagland's nutrient solution whilst growing in pumice and peat. Within 3 months the trees were 1 to 1½ m tall (Fig. 1f).

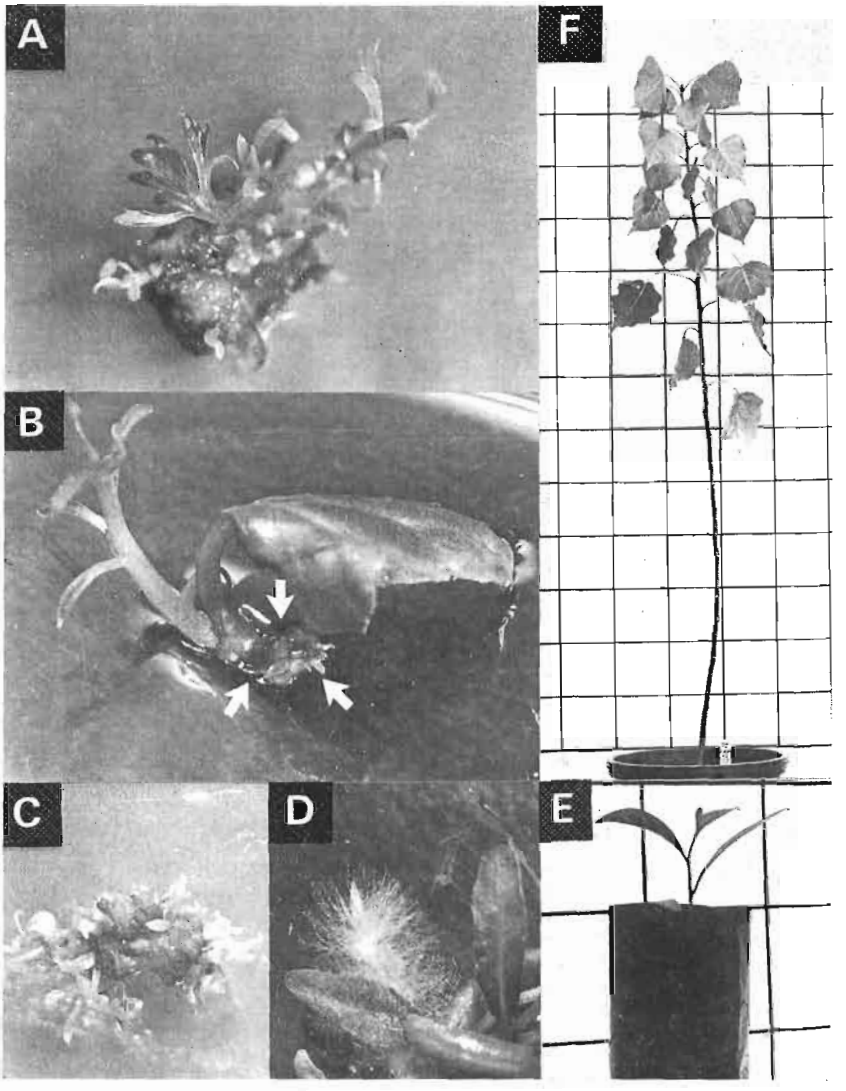

FIG. 1—(a) Stem explant from a bud of **P. "Flevo"** proliferating shoots and buds on medium 1 (× 10). (b) Leaf of **P. yunnanensis** taken from a proliferating shoot replaced on medium 1 showing the growth of the axillary bud and further proliferation of buds at the base of the petiole (arrows) (× 15). (c) Stem explant from a proliferating shoot of **P. nigra "Italica"** showing both proliferation and growth of buds on medium 2 (× 4). (d) Root with root hairs growing from the stem of **P. "Flevo"** after 1 week on medium 3 (× 10). (e) Rooted plantlet of **P. yunnanensis** growing in pumice : peat (50 : 50) in a 10 cm diam. polythene tube 1 month after potting-up. (Squares are 10 cm × 10 cm). (f) Rooted plant of **P. "Flevo"** three months after potting-up in pumice, peat (Squares are 10 cm × 10 cm).

DISCUSSION

The method of micropropagation described was rapid and gave large numbers of shoots from small explants of tissue. It has been shown to be effective with members of the genus *Populus* section Aigeiros and section Tacamahaca. Attempts are underway to see whether it can also be used to propagate members of the section Leuce. The method would be ideal for the rapid multiplication of new varieties introduced from overseas since very large numbers can be quickly made available to catchment authorities and to those concerned with timber production. It has the further advantage that material can be exchanged internationally under sterile conditions reducing the risk of transmitting disease, yet maintaining the tissue in a state that allows rapid clonal propagation to begin immediately irrespective of the season.

The final product of propagation using this method is a rooted tree in growing medium. The method allows for the manipulation of this growing medium to assist establishment in difficult areas. The height reached in a 3 month period after potting up, 1 to 1½ m, is ideal for the production of barbatelles. In this method the top is cut back to near ground level at planting and this allows for the establishment of a better root to shoot balance. The method is commonly used in France and Italy and has been found to be extremely good in wind-prone areas, since the growth of the barbatelle *in situ* allows it to adapt to wind without the danger of wind throw.

Poplars for timber production are usually planted as 0/1 rooted cuttings, and equivalent specimens can be produced using the technique described here, provided a full season is available for growth prior to planting out. Production can be regulated so that rooted plantlets are potted up at the beginning of the growing season to ensure a supply of trees by the next winter.

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