IN VITRO PROPAGATION OF FOREST TREES BY TISSUE CULTURE

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ABSTRACT.--Many plantlets were produced from the axillary shoots through micropropagation and regenerated from calli by tissue culture in two poplar species, but only one plantlet was regenerated from callus which was derived from cotyledon in white spruce. Young leaf pieces were the best material for callus induction and adventitious shoot production in poplars while cotyledons were best for white spruce because of their embryonic characteristic and the amount of material available.

Clonal propagation is one of the most important aspects of tissue culture technology. A large number of plants of a genotype can be produced in a short period of time. The success of tissue culture application to herbaceous and some woody plants has brought great attention to this technology as a possible means for propagation of forest tree species.

Substantial progress has been made in tissue culture of tree species. Wolter (1968) reported either shoots or root initiation on calli in trembling aspen (Populus tremuloides). Two years later, Winton (1970) succeeded in producing plantlets from white callus tissue of triploid trembling aspen. Berbee et al. (1972) regenerated plantlets from calli which were derived from 0.5 to 1 mm long shoot tips in P. x euramericana. Whitehead and Giles (1977) described a rapid micropropagation technique of poplars by tissue culture. In conifers, adventitious shoots have been produced from many species but rooting of the shoots is successful only in a few species. Rooting of the adventitious shoots has remained a real problem not only in conifers but also in hard-to-root broadleaf species.

The present report is on in vitro propagation of 4 poplar species and white spruce (Picea glauca) by using overwinter buds, and explants of young shoots and seedlings.

Material and Methods

Populus species

Four clones each of P. alba, P. grandidentata, P. tremula and P. nigra were used in the experiment (Table I). All trees were about 20 years old and growing in the poplar arboreta at Maple, Ontario. Overwinter buds were sampled in December and the outerbud scales were removed before sterilization. They were

then dipped in 70% ethanol for a few seconds and flamed; this was followed by sterilizing in 10% Javex bleach solution for 15 minutes and washing 3 or 4 times in sterile water. Five buds were incubated on each of 10 plates per clone in two sets. The MS basal medium (Murashige and Skoog 1962) was supplemented with various concentrations of BAP (benzylaminopurine) and 0.3% agar (Table II). Cultures were maintained at 25°C during the day and 15°C at night with a photoperiod of 16 hours. When the shoots had emerged from the bud scales, they were cut into 5 mm pieces and placed on the basal medium containing 0.1 mg/1 of BAP and 0.05 mg/1 of NAA (naphthaleneacetic acid) for callus induction and for further proliferation. To compare the efficiency of callus formation from different explants in P. nigra, young stems, leaves and petioles were sampled from a branch in water culture in a greenhouse and transverse sections were incubated on the callus-inducing medium. The adventitious shoots developed in these media were transferred to a rooting medium which consisted of the basal medium with only 1/2 of the macronutrients, 0.002 mg/1 of NAA, 2% sucrose and 0.4% agar. After root development, the plantlets were transplanted into pots containing an equal mixture of sand, peat and loam.

White spruce

White spruce seeds were germinated on sterile sand. After cotyledons were fully emerged from the seed coats, they were surface-sterilized as described for poplar buds. The cotyledons and hypocotyls were cut into 3 mm pieces and placed 8 to 10 pieces per dish on agar medium. The epicotyls with the cut ends of cotyledons and hypocotyls were also incubated. Explants from two age classes of seedlings were tested for the efficiency of callus formation (Table IV).

The MS basal medium with 0.3 mg/1 of BAP and 0.05 mg/1 of NAA was used for callus induction. Calli were transferred onto the organ-inducing media for adventitious shoot development. These contained 1/2 of the macronutrients and full micronutrients of the MS basal medium, with or without vitamins, and 1 or 2% sucrose (Table V). The shoots were then rooted in rooting media which consisted of the MS basal medium supplemented with different growth regulators - IAA (indoleacetic acid), IBA (indolebutyric acid), NAA and coumarin.

Results

Populus species

The results of micropropagation by incubating the overwinter buds are shown in Table II. Bud break occurred in 3 to 4 weeks in all species on all levels of BAP except for P. tremula on 0.1 mg/1 where none occurred. The percentage of bud break was best at 0.4 mg/1 and declined at 0.6 mg/1. After the break, the shoots grew very rapidly in P. nigra and P. alba (Plate I, Figs.1 and 2) but very slowly in P. grandidentata and P. tremula.

Sections of shoots with leaves of the first 2 species proliferated and the axillary shoots started to elongate after

transferring onto the basal medium containing BAP and NAA (Plate I, Fig. 3). The shoots grew to 3 to 4 cm in height in 3 to 4 weeks. Adventitious shoots were also regenerated from the proliferated shoot sections. The shoots were excised and rooted in 2 weeks in the rooting medium (Plate I, Figs. 6 and 7).

The percentage of callus formation from different explants in P. nigra is shown in Table III. About 60% of the leaf pieces and stem sections proliferated and formed calli while only 8% of petioles developed into calli (Plate I, Figs. 4 and 5). The time required for callus formation and organogenesis was shorter for calli derived from leaf pieces than for those derived from stem and petiole sections. However, no difference was found in the time required for rooting of the adventitious shoots.

White Spruce

Explants from one-week-old seedlings appeared to be superior to those from seedlings freshly emerged from the seed coats in callus formation (Table IV). Epicotyls responded best to callus induction followed by cotyledons and hypocotyls (Plate II, Figs. 1 and 2). 88% of the epicotyls and 83% of the cotyledons from one-week-old seedlings proliferated and produced calli. Only 5% of the hypocotyls in each age class of seedlings responded to the treatments.

The number of calli with organogenesis and the average number of primordia per callus after transferring onto the organ-inducing medium are shown in Table V. The medium containing 1/2 macronutrients, full micronutrients and vitamins of the MS basal medium plus 1% sucrose gave the best results. Shoot primordia were formed in 42% of the calli and as many as 40 primordia were produced on a callus. Calli incubated on the other 3 media showed some shoot primordium formation but browning occurred in the calli.

Shoot primordia grew slowly and it took about two months for a shoot to grow 2 to 3 cm high (Plate II, Fig. 3). Out of 100 shoots planted in various rooting media, only one shoot developed roots in the medium containing 0.1 mg/1 of NAA and 2% sucrose (Plate II. Fig. 4).

Discussion

Different species responded differently to the media in bud break. Overwinter buds of P. nigra responded favourably to the concentrations of BAP used while low and high concentrations of BAP were inhibitory to bud break in P. tremula. Although bud break occurred in the 4 species studied, shoot elongation was present only in P. alba and P. nigra. It appeared that different combinations of plant growth regulators would be required for the elongation of shoots in P. grandidentata and P. tremula.

In Pinaceae, excised embryos and explants from cotyledon, epicotyl and hypocotyl of young seedlings have all been successfully used for callus induction and initiation of adventitious shoots in in vitro culture (Sommer and Brown 1979). Campbell and Durzan (1975) used the hypocotyl segments of white spruce seedlings before the cotyledons emerged from the seed coats and produced shoot primordia on each segment. Many of the primordia developed into adventitious shoots. A year later, they reported the production of plantlets by using the hypocotyl explants (Cambell and Durzan 1976). Explants from the hypocotyl were used in the present study but cotyledons were the best material for induction of adventitious shoots. Epicotyl was superior but there is only one epicotyl per seedlings, whereas there are 5 to 7 cotyledons and each could be cut into 3 to 4 segments. Because of the embryonic characteristic and supply, cotyledons would be most suitable for clonal multiplication. Aitken et al (1981) also reported that the cotyledons from germinated seed in radiata pine (Pinus radiata) were best for the formation of large numbers of adventitious shoots.

Rooting of the adventitious shoots was much more difficult in white spruce than in the poplars. Success in producing significant numbers of plantlets capable of growth in soil has been scanty in conifers. Loblolly pine (P. taeda) appears to be the only one in which sufficient plantlets have been produced for clonal evaluation (Leach 1979). Further investigation in rooting of adventitious shoots from in vitro culture is imperative.

TABLE I. List of trees from which buds were collected for micropropagation.

Clonal No.	Species	Origin
G1	P. grandidentata	Petawawa, Ont.
G8	"	Blacksburg, VA
G9	"	Mountain Lake,VA
G42	"	Waltan Twp., Ont.
A209	P. alba	Germany
A214	"	"
A219	"	"
A321	"	"
E18	P. tremula	Finland
E44	"	USSR
E81	"	USSR
E116	"	Polland
N168	P. nigra	Hungary
N169	"	Hungary
N170	"	Hungary

TABLE II. Percentage of bud break after 4 weeks on different concentrations of benzylaminopurine (BAP).

Species	BAP mg/1			
	0.1	0.2	0.4	0.6
P. grandidentata	7.3	27.5	32.5	32.5
P. tremula	0	23.3	23.3	16.6
P. alba	43.0	30.0	40.0	36.6
P. nigra	40.0	66.6	70.0	43.3

TABLE III. Performance of different explants in P. nigra.

Types of explants	No. of explants incubated	Percentage of explants developed into callus	No. of days required for			
			Callus formation	Organogenesis	Growth of shoots to 3 cm size	Rooting
Leaf pieces	100	59	60	45-50	40	8-10
Stem sections	100	60	60	60-90	50	8-10
Petioles	50	18	60-70	60-80	-	-

TABLE IV. Performance of different explants from two age classes of seedlings in callus formation in white spruce.

Explants of seedlings	Age classes of seedlings					
	Soon after emergence of cotyledons			One week after emergence of cotyledons		
	No. of incubation	Callus development		No. of incubation	Callus development	
		No.	%		No.	%
Cotyledons	80	40	50	120	100	83
Epicotyl	15	10	66	25	22	88
Hypocotyl	40	2	5	80	4	5

TABLE V. Performance of white spruce calli on different media.
36 calli were incubated for each medium.

Media	Sucrose conc. %	No. of callus with shoot primordia	Avg. No. of shoot primodia per callus	Condition of calli
1/2 MS (macro) + vitamins	2	9	1.5	Brown
1/2 MS (macro) + vitamins	1	15	10	Well developed
1/2 MS (macro) + no vitamins	2	11	3	Brown
1/2 MS (macro) + no vitamins	1	10	5	Brown

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ILLUSTRATIONS

Plate I

Fig. 1 Bud break and shoot elongation of overwinter buds after 3-week incubation in *P. alba*.

Fig. 2 A close-up of the elongating shoot in *P. alba*.

Fig. 3 Axillary shoot development in *P. alba*.

Fig. 4 Adventitious shoot formation on leaf callus in P. *nigra*.

Fig. 5 A close-up of adventitious shoot on leaf callus in P. *nigra*.

Fig. 6 A plantlet of *P. nigra*.

Fig. 7 A plantlet of *P. alba*.

Plate II

Fig. 1 Shoot primordia and adventitious shoots on a callus derived from cotyledon in white spruce.

Fig. 2 Adventitious shoots on a callus.

Fig. 3 Elongation of an adventitious shoot.

Fig. 4 Rooting of an adventitious shoot in white spruce.

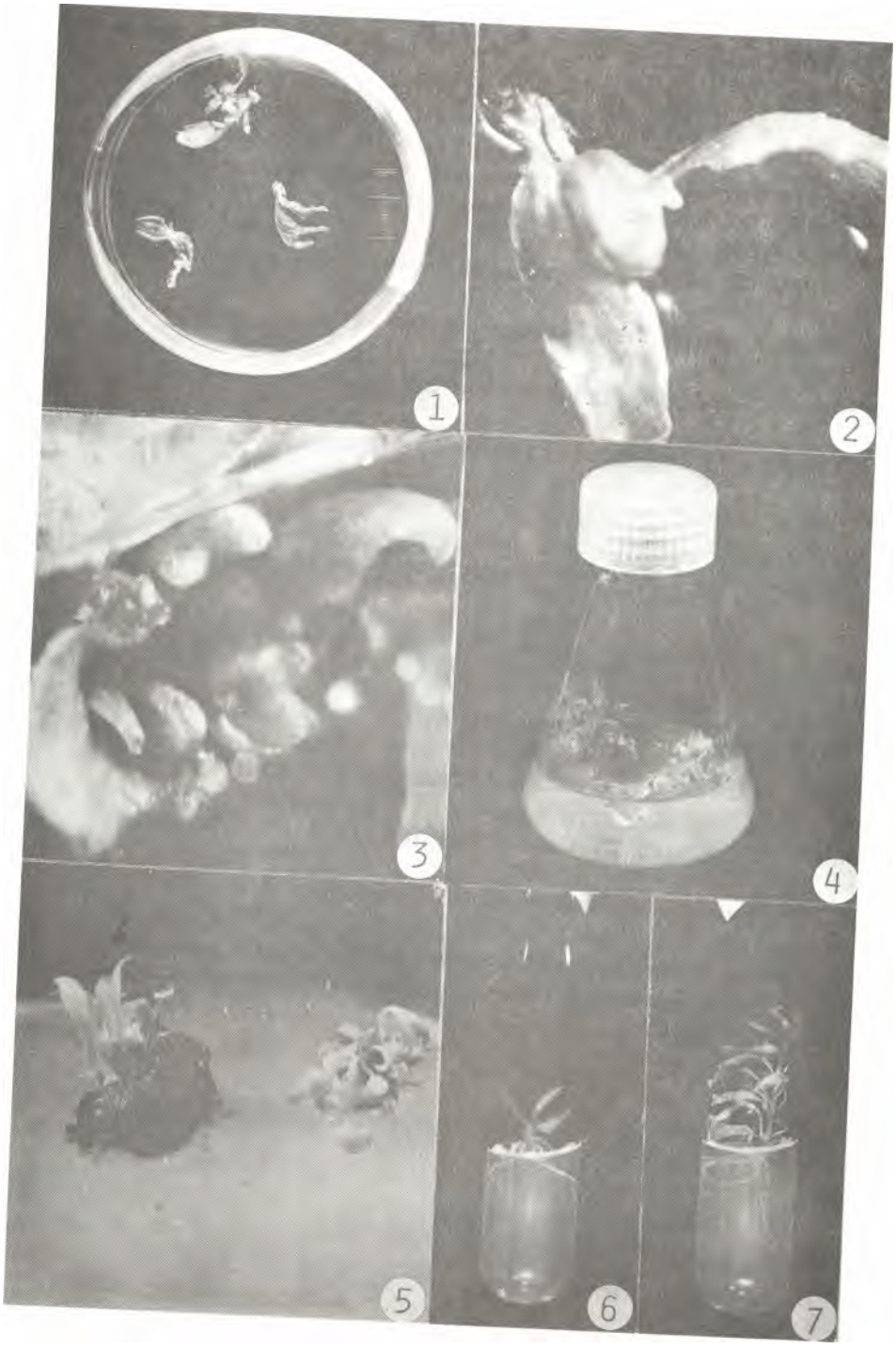

PLATE I

PLATE II