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BRIEF COMMUNICATION

In vitro regeneration of Norway maple (*Acer platanoides* L.)

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Abstract

Regenerants were produced from axillary buds, but not from petiole segments, greenwood cuttings and leaf discs. Petiole segments and greenwood cuttings responded by massive callus cell proliferation without adventitious shoot formation. The development of induced buds into shoots occurred on WPM medium containing kinetin. Vigorous shoots larger than 2.0 cm in length were successfully rooted in half strength WPM medium supplemented with 1.0 mg dm^{-3} indole-3-butyric acid.

Additional key words: auxin, callus formation, cytokinin, explant type, organ culture.

The utilization of *in vitro* culture in the propagation and genetic improvement of forest trees holds promise of many benefits including faster multiplication of selected genotypes, faster development of improved genotypes, and the transfer of genes between non-fertile parents (Berlyn *et al.* 1986). The Norway maple (*Acer platanoides* L.) is readily propagated from seed and is the best understock for all its cultivars and some species of the *Platanoidea* section (van Gelderen *et al.* 1994). Only a short report on *in vitro* propagation of *A. platanoides* is given by Chalupa (1987). The aim of this study was to develop *in vitro* regeneration procedure for the Norway maple and so trials with juvenile explants were undertaken. Experiments described in this paper resulted in successful *in vitro* regeneration of vigorous plantlets.

Axillary buds, leaf discs, petiole segments and greenwood cuttings were explanted from 1 - 2 year old seedlings of *Acer platanoides* L. and were used as primary explants. The material was treated with 0.4 % *Chinosol* fungicide solution for 30 min and then was surface sterilized in 0.1 % mercury chloride solution for

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Abbreviations: ANOVA - analysis of variance; BAP - 6-benzylaminopurine; IBA - indole-3-butyric acid; KIN - kinetin; NAA - α-naphthaleneacetic acid.
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15 to 30 min. Few drops of Tween 20 were added. After three successive rinses in sterile distilled water explants were placed on WPM (Lloyd and McCown 1980), and MS (Murashige and Skoog 1962) nutrient media. Media were supplemented with bacto agar (6 - 7 g dm^{-3}), saccharose (20 g dm^{-3}) and the combination of 1.0 mg dm^{-3} 6-benzylaminopurine (BAP) plus 0.05 mg dm^{-3} α-naphthaleneacetic acid (NAA), or with kinetin (KIN; 0.1, 0.5, 1.0, and 2.0 mg dm^{-3}). For rooting indole-3-butyric acid (IBA) was used in concentrations 0.3 and 1.0 mg dm^{-3}, respectively. Media were autoclaved for 20 min at 121 °C. Cultures were grown at day/night temperatures of 25/19 °C and 16 h photoperiod (cool white fluorescent lamps; irradiance of 37.5 μmol m^{-2} s^{-1}). Cultures were regularly sub-cultured each four weeks. Differences in callusing responses of explants were tested by three-factor ANOVA, and frequencies in shoot and root formation were tested by *G*-test (likelihood ratio χ^2 - test).

Leaf discs which were placed either with their lower or top surface uppermost on media proved as an inconvenient type of explants because no cell proliferation occurred. After 6 - 7 weeks in culture discs perished. Since shoot proliferation from axillary buds was associated with basal callus formation as a wounding reaction of explanted buds, and only this type of explants led to the whole plantlet recovery, axillary buds were excluded from recording the percentages of callus formation. The callusing responses of petiole segments were significantly higher that those of greenwood cuttings (Table 1). The maximal percentage of callus-forming explants

Table 1. Callus formation from petiole segments and greenwood cuttings (initial number of cultures was 30).

Explant type	Phytohormones [mg dm^{-3}]	Medium	Number of cultures established *in vitro*	Number of explants forming callus
Petiole segments	1.0 BAP + 0.05 NAA	WPM	27	25
		MS	27	22
	0.5 KIN	WPM	26	20
		MS	28	25
Greenwood cuttings	1.0 BAP + 0.05 NAA	WPM	26	18
		MS	27	12
	0.5 KIN	WPM	28	22
		MS	25	14

was observed in the case of petiole segments cultured on WPM medium when adding 1.0 mg dm^{-3} BAP plus 0.05 mg dm^{-3} NAA, and the minimum with greenwood cuttings on MS medium supplemented with 1.0 mg dm^{-3} BAP plus 0.05 mg dm^{-3} NAA. No significant differences in callusing responses could be distinguished between WMP and MS media and between phytohormones tested. Unfortunately, not a single adventitious shoot was induced from petiole segments and greenwood cuttings explants.

From all explants which were selected to establish *in vitro* cultures, shoots were produced only from axillary buds. Shoot formation (Fig. 1) occurred after 3 - 4

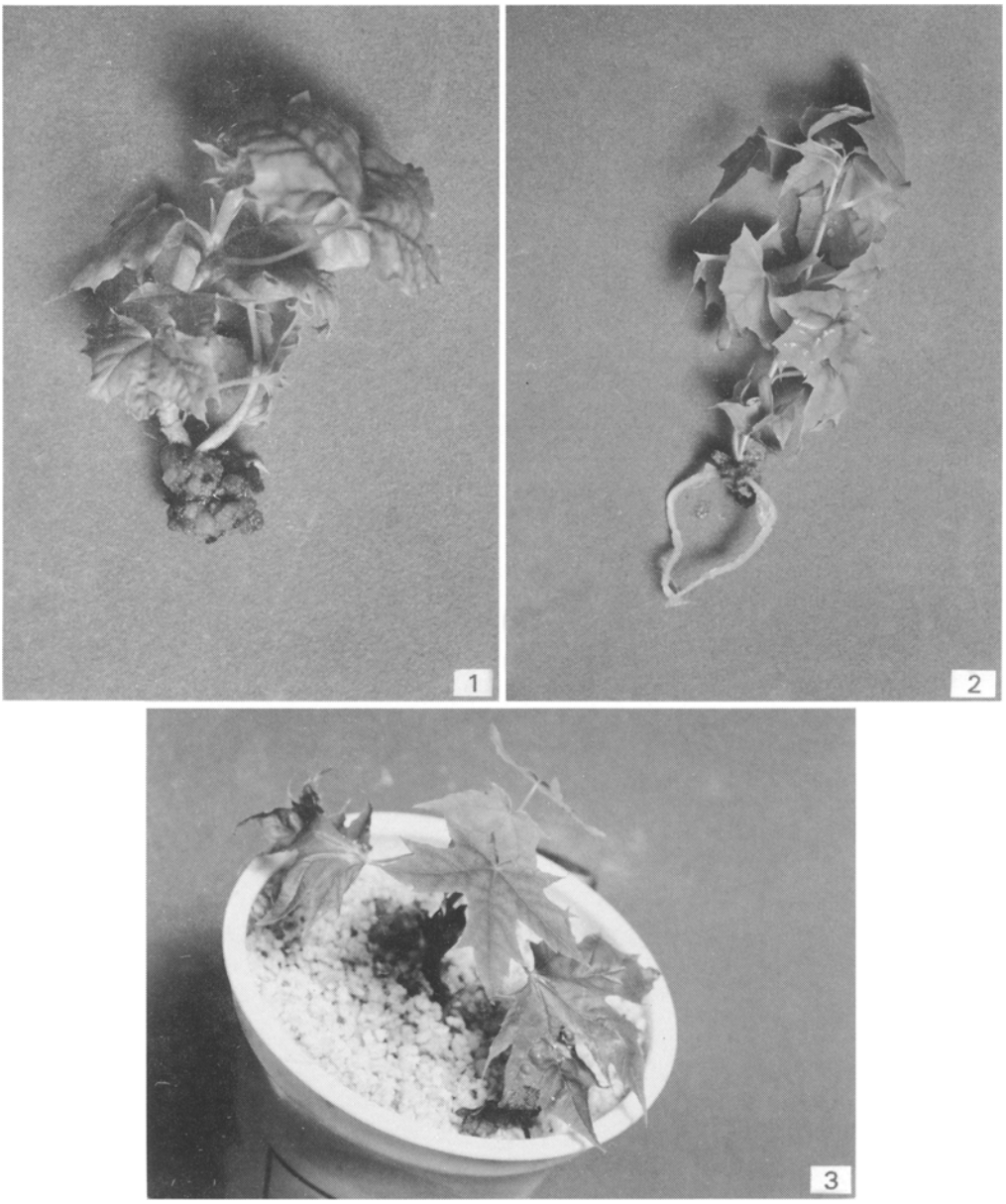

Fig. 1. Shoot proliferation from axillary bud.

Fig. 2. Root formation on excised shoot after 30 d.

Fig. 3. *In vitro* regenerated plantlet transferred into a perlite substrate which adapted to *in vivo* conditions.

weeks of cultivation on WPM medium supplemented with various concentrations of KIN (maximal percentage of shoot-forming explants with the concentration 0.5 mg dm^{-3} KIN - Table 2).

Table 2. Effect of various concentrations of KIN on shoot formation (initial number of cultures 30; means with the same letter are not significantly different according to *G*-test).

KIN [mg dm^{-3}]	Shoot-forming explants [number]	[%]
0.1	14 a	46.67
0.5	24 b	80.00
1.0	11 a	36.67
2.0	4 c	13.33

IBA (1.0 mg dm^{-3} or 0.3 mg dm^{-3}) was added into half strength WPM medium in order to stimulate root development on excised shoots. Significantly higher rooting percentage was obtained with the concentration of 1.0 mg dm^{-3} IBA (Table 3). Shoots produced 2 - 6 roots within 4 weeks (Fig. 2). Plantlets rooted in agar medium were transferred into a perlite substrate (Fig. 3) and were grown under high relative humidity for 4 - 6 weeks.

Table 3. Effects of IBA concentrations on root formation (initial number of cultures 20; * - significantly different at $P < 0.05$ according to *G*-test)

IBA [mg dm^{-3}]	Root-forming shoots [number]	[%]
0.3	7	35.00
1.0	14 *	70.00

The use of axillary buds proved as the most suitable way to induce shoot growth. Chalupa (1983, 1987) described shoot formation of *A. platanoides* which was achieved using BAP and IBA, and in the case of *A. pseudoplatanus* using BAP solely. However, significantly better results in shoot growth of *A. pseudoplatanus* were obtained when adding rather KIN into medium than BAP (results not published). Therefore KIN was selected to stimulate shoot development in these experiments (Table 2) finding the optimal concentration of 0.5 mg dm^{-3}. Higher auxin concentration promoted root formation with higher percentage frequency (Table 3). Rooted plantlets were transferred into the field conditions.

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