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Micropropagation of Platanus acerifolia in vitro

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Summary

The possibility for micropropagation from vegetative buds of *Platanus acerifolia* WILLD. collected from 4-years and 50 years plants was tested. The buds of the 4 years old plants showed significantly higher morphogenic activity. The induction processes occured best under the condition of GD (1972) medium supplemented with 0.3 mg/l BAP. The best multiplication rate was achieved at 0.5 mg/l BAP. The rooting of cultures on 1/2 GD with 0.5 mg/l IBA was 100% and adaptation in vivo was 70% after a month. This simple system for micropropagation is suitable for propagation of elite trees.

Key words: Platanus acerifolia, axial buds, in vitro propagation.
FDC: 165.442; 176.1 *Platanus acerifolia.*

Introduction

Platanus acerifolia is a valuable, fast growing woody species and the most popular tree in the cities and urban areas in Bulgaria, due in part to its resistance to air polution. Vegetative propagation methods have been described by DELKOV (1977) and VLACHOV (1982), however results from these experiments with cuttings and seedlings are variable. They demonstrate both main obstacles for its intensive propagation: the limited possibility for production of a sufficient number of seedlings, the distinct dependence of the rooting capability on humidity, the temperature during the conservation in coldhouse and the type of cutting — summer or winter.

A method for overcoming these problems is in vitro micropropagation wich can be used all year round. In addition, induction of the axillary buds is a useful method for mass clonal micropropagation especialy of decidious species which are known with their episodic growth (AHUJA, 1982, 1983, 1984; CHALUPA, 1983; EVERS, 1987; VIEITEZ, 1982, 1983, 1985, 1991). As a result of those investigations many protocols exist but results with *Platanus* species are limited (EVERS 1988).

In these study the effect of different culture conditions and plant hormones on the morphogenic response were investigated as well as the in vitro rooting of *Platanus acerifolia.*

Materials and Methods

Plant material

Cuttings of 4- and 50-years old plants of *Platanus acerifolia* grown outdoors in the Lulin nursery, Sofia, were taken and stored at 40 °C for several days. After sterilization treatment with 5% Ca-hypochlorite for 18 min and 3-fold rinsing with sterile distiled water for 15 min, 15mm microcuttings were established weekly in 3 different periods (15.9 to 20.10; 15.1 to 30·1; 15.5 to 30.6)

Culture conditions

Modifications and standard media for proliferation in vitro were used. These included the basal media MS (1962), SH (1972), GD (1972) and WPM (1981) supplemented with BAP from 0 mg/l to 3 mg/l with or without IBA from 0.001 mg/l up to 0.01 mg/l.

For rooting in vitro both liquid with sterile perlite and solidified with 7 g/l agar variants were used with the following media: halfstreng basal macroelements of Ms (1962) and GD (1972 supplemented with IBA, IAA or NAA from 0.0 up to 2.0 mg/l.

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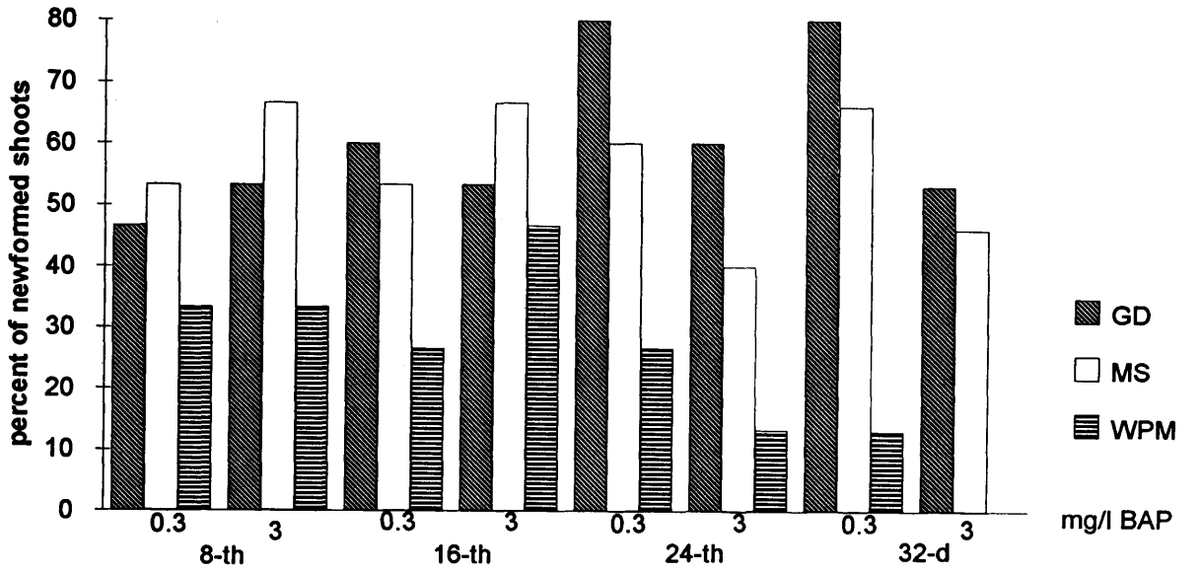

Figure 1. — Effect of different media and BAP concentration on the percent of newformed shoots during 32-days cultivation.

All media were brought to pH 5·5 to pH 5.6 before autoclaving at 1.2 kgf/cm² pressure for 20 min. Cultures were incubated in a growth chamber with day and night temperatures of 26 °C and 180 °C, respectively, and a 16-h photoperiod under 3000 lux provided by cool white fluorescent lamps.

Cytological analysis

Cytological studies were carried out with root tips of donor plants and regenerantes using squash and Feulgen techniques (500 microplants were tested).

Results

Only 20% of the axillary buds from 50 years od trees of *Platanus acerifolia* inoculated on GD medium with 3mg/l BAP formed shoots. In the induction 12-weeks phase the average length of the newformed shoot was 13 mm ±0.8 mm. There was a low rate of multiplication efficiency per explant in the subsequent experiments — 2.88±0.6 on GD medium with 1mg/l BAP. In all media tested root induction and extension was less abundant — 26.60% or absent Therefore, all further emphasis was placed on the in vitro culture of 4-years old donor plants.

Season had a signifficant effect on the induction and growth of buds on GD with 0.3 mg/l BAP. The best results were obtained in April and October — 86.60% and September — 80.00%. A considerable reduction in bud formation was observed on the July — 13.30%.

Using 2 different concentrations of BAP 0.3mg/l and 3 mg/l, the effect of different media on the percent of developing buds was compared (*Fig. 1*). The buds on MS medium showed the highest rate of bud swelling and bursting after 8 days in culture. However after 16 days or 24 days no significant difference between the induction capacity of the cultures on MS and GD media was observed. At the lower BAP level shootformation was high — about

Table 1. — Effect of different media and BAP concentration on the percentage of buds forming shoots, mean number buds forming per explant (Km) and mean length (L) of newformed bud per explant after 5 weeks culture.
Student test: ** significant at 1 % level; number explants per variant-126

Growth	0.0		0.3			0.5			0.7			1.0 mg/l BAP	
medium	%	%	Km	L	%	Km	L	%	Km	L	%	Km	L
GD	95.23	80.95	4.35	11.08	90.47	5.2**	9.88	76.19	4.80	11.41	71.41	4.16	10.40
/1972/			±0.2	±2.6		±0.7	±2.5		±0.4	±2.9		±0.4	±2.4
WPM	90.47	80.95	3.60	10.61	80.95	3.16	14.21	90.47	5.0	11.64	66.66	7.0	6.85
/1981/			±0.5	±1.6		±0.7	±4.9		±0.5	±4.2		±1.7	±2.7
mod.MS	52.38	66.66	2.98	10.83	57.14	4.8	6.70	52.38	4.5	9.44	38.09	4.21	8.75
			±0.2	±1.6		±0.3	±1.5		±1.2	±3.2		±0.9	±2.3

Table 2. — Effect of solid and liquid GD medium on the percentage of rooting, mean number of roots (R) and mean length of root (Lr) after 4 weeks cultivation.

Root medium	0	0.2	0.5	0.7	2.0
			mg/l IBA		
1/2 GD liquid					
% of rooting	-	80.00	100.00	100.00	26.66
R		4.50±0.1	5.96±0.9	5.08±0.6	6.28±1.2
Lr		11.62±3.9	14.79±4.1	9.26±3.1	5.06±1.9
					callus
1/2 GD solid with 7.2 g/l agar					
% of rooting	23.80	86.66	100.00	100.00	40.00
R		4.21±0.2	5.43±0.7	4.92±0.9	5.38±0.7
Lr		10.74±3.5	15.07±4.6	8.51±2.7	4.83±1.6
					callus

80%. The tendency of increased shootformation on GD with 0·3 mg/l BAP was especially evident — after 32 days. No response was observed on WPM medium with 3mg/l BAP after 32 days.

Based on the results obtained in the previous experiments, the effect of different media composition and concentration of BAP on the shootforming capacity of every newformed bud was examined further (*Tab. 1*). The percentage of buds forming after a shift decreased with the rainsing of BAP to 1.0 mg/l — from 80.95% at 0.3 mg/l BAP up to 71.41% at 1.0 mg/l BAP and 80·95% up to 66.66% in comparison to the control variant (without BAP) respectively on the GD and WPM media. This high percentage was rapidly lost in the same levels of BAP on the MS medium — from 66.66% up to 38.09%. At the followed second subculture on the MS supplemented with 0.3 mg/l BAP the newproduced buds became brown and died.

Shoot multiplication was the highest at 0.5 mg.l BAP on GD — 5.2±0.7 and with increasing the BAP up to 1.0 mg/l multiplication decreased. On WPM, shoot multiplication increased from 3.60±0.5 at 0.3 mg/l BAP up to 7.0±1.7 at 1.0 mg/l BAP. The difference was significant at p=1% for GD and p=0.1% for WPM medium according to Student test.

The mean length of newformed buds per explant was decreased with increasing of BAP up to 1mg/l independent on the type of medium. Further cultivation of Platanus acerifolia explants on WPM leads to partial necroses of leaf margins and to obstruction of plant development.

Replacing of $CaCl_2 \cdot 2H_2O$ with another sources of Ca^{2+} such as Ca-gluconate or Ca-nitrate in the range at up to 3mM did not give better growth or development.

As elongation of shoots was unsatisfactory on media free of cytokinin and auxin, it was considered nessesary to transfer buds to media supplemented with BAP and IBA. The development of virogous shoots was achieved within 5 weeks on WPM with the addition of 0.05 mg/l BAP, 0.01 mg/l IBA and 1000 mg/l Ca-nitrate.

After treatment with IAA up to 0.7 mg/l the percentage of rooting increased from 6.66% to 13.33% but remained the lowest compared with the same concentration of other auxins and the newformed roots were very small. Root production was not achieved at 2mg/l IAA. The same was true after treatment with NAA. At 2mg/l NAA only callus formation was occurred round the basal end of the shoots. The best result were obtained on GD medium supplemented with IBA at 0.5 mg/l and 0.7 mg/l where 100% of the cultures rooted. On the medium without IBA about 20% of the cultures demonstrated spontaneous rooting. Further detailed investigations on the type of medium-

Table 3. — Description of the micropropagation steps of *Platanus acerifolia.*

Steps	Time	Medium
1.In vitro establishment	5 weeks	GD, BAP 0.3 mg/l
		IBA 0.01 mg/l
2.Shoot multiplication	5 weeks	GD, BAP 0.5 mg/l
		IBA 0.01 mg/l
elongation	5 weeks	WPM BAP 0.05 mg/l
		IBA 0.01 mg/l
		1000 mg/l Ca-nitrate
3.Rooting in vitro	4 weeks	1/2 macrosalts of GD
		0.7mg/l IBA

Table 4. — Effect of different media and BAP concentration on the percent of newformed shoots during 32-days cultivation.

Media	BAP,	Percent of newformed shoots during			
	mg/l	8-th	16-th	24-th	32-d day
GD	0.3	46.60	60.00	80.00	80.00
	3.0	53.30	53.30	60.00	53.00
MS	0.3	53.30	53.30	60.00	66.00
	3.0	66.60	66.60	40.00	46.60
WPM	0.3	33.30	26.60	26.60	13.00
	3.0	33.30	46.60	13.30	–

Note: number explants per variant — 126

liquid or agar-solidified on the capability for root formation did not lead to the significant difference in the mean number of roots per shoot and the mean root length (*Tab. 2*).

The plants were subsequently transfered on a soil-sand-perlite mixture (1:2:2) in greenhouse. After 1 month adaptation to the lower humidity 70% of plants surived and remained in good condition.

No changes in chromosome number of the regenerants compared with the donor plant were observed (2n=42).

A summary of the propagation steps considered suitable for the commercial micropropagation of *Platanus acerifolia* is shown in *table 3*.

Discussion

According to the results reported here cuttings collected in the dormant stage (April and October) were better explant sources than non-dormant cuttings collected in July. These results are in agreement with those reported for other woody species (Bonga, 1985; Evers, 1987; Salonen, 1987; Welander, 1988).

Plant formation in vitro of *Platanus acerifolia* could be devided into 3 stages:1) establishment in culture — shoot induction and separation; 2) bud initiation, development and elongation; 3) rooting of shoots in vitro.

Each stage needs appropriate nutrient and hormonal regimes during the cultivation period and must be determined experimentally for every genotype tested. The most suitable medium tested for shoot induction was GD medium which confirm results from other authors (Noh, 1986; Vieitez, 1991) that the low concentration of macrosalts is better suited to woody species. At the same time the results obtained on the regeneration in vitro of *Platanus acerifolia* on MS medium show that in the beginning of the cultivation period there is a tolerance but in the next steps most of the cultures became brown. It is speculated that the high concentration of NH_4NO_3 in MS seems to limit the proliferation ability of explants in a particular stage of development. Similar conclusions were demonstrated by other authors (Chalupa, 1983; Vieitez, 1982, 83, 1985).

In the present investigation optimum BAP concentration was 0.5 mg/l for shoot multiplication on GD medium. Similar low BAP levels have been obtained for other woody species (Ahuja, 1982; Chalupa, 1983; Vieitez, 1985). Addition of very low level of BAP and IBA is suitable for elongation step as mentioned Evers (1988). The best rooting was achieved at 0.7 mg/l IBA — 100%.

In our investigation intensive callus production was observed on medium containing IAA. Evers (1988) obtained maximum effect of rooting on media with IAA at 6—9 · 10^{-6}mol. It is possible this difference is due to individual genetic differences of the species used. The results obtained here provide good basis for acceleration and improvement of forest selection programmes by micropropagation of valuable trees.

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