Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
2015, volume 100, livre 4, pp. 223-230
First National Conference of Biotechnology, Sofia 2014

MICROPROPAGATION OF *PAULOWNIA* SPECIES AND HYBRIDS

ATANAS CHUNCHUKOV*, SVETLA YANCHEVA

Department of Genetics and Plant Breeding, Plant Biotechnology Laboratory, Agricultural University Plovdiv, Bulgaria
**Corresponding author: muad@abv.bg*

Keywords: micropropagation, *Paulownia*, MS, DKW, QL, McC, N6

Abstract: The present research shows the possibility for micropropagation of three different genotypes of *Paulownia* (*P. elongata*; *P. tomentosa* x *P. fortunei* hybrid and (*P. elongata* x *P. tomentosa*) x *P. elongata* complex hybrid) and some preliminary results concerning the effect of the main factors influencing the growth characteristics and propagation efficiency.

Basal media MS, DKW, QL, McC and N6 enriched with BAP (0.5 mg/l) and IBA (0.01 mg/l) were studied for multiplication efficiency. MS basal salt composition induced higher multiplication coefficient in *P. tomentosa* x *P. fortunei* hybrid (3,9) (*P. elongata* x *P. tomentosa*) x *P. elongata* complex hybrid (2) and *P. elongata* (1,8). Additionally, the effect of cultural vessels was determined towards optimization of the propagation efficiency. The reaction of the genotypes differed generally in the mean number of shoots per explant, number of internodes and mean shoot length. The obtained plants were rooted (100%) on MS basal medium enriched with 0,1 mg/l IBA and successfully adapted *ex vitro* with surviving up to 96%.

INTRODUCTION

Paulownia is a genus belonging to family *Paulowniaceae* (*Scrophulariacae*) indigenous to China and including nowadays over 20 species (Barton. I. L., 2007). They are deciduous trees 12–15m tall, with large, heart-shaped leaves 15–40 cm across, arranged in opposite pairs on the stem. The flowers are produced in early spring on panicles 10–30 cm long, with a tubular purple corolla resembling a foxglove flower. The fruit is a dry capsule, containing thousands of minute seeds.

Paulownia is very adaptable, widely distributed and extremely fast growing. Under optimum conditions, a 5-year old *Paulownia* tree can measure 30-40 cm diameter at breast height and will have a timber volume of 0,3-0,5 m^3. The timber is

light yet strong, dries fast, has a beautiful grain, does not warp or crack, or deform easily. The wood is easy to work with, and has excellent insulation properties. The trees can be used for reclamation of mined areas and especially for afforestation purposes, for making furniture, wooden details for planes and ships and other purpose useful for the industry (Zhu, 1986).

The high adaptation ability of *Paulownia* and the easy distribution of the seeds to huge distances make the genus very invasive in many areas in Bulgaria. Therefore for energy purposes the use of sterile species which do not produce viable seeds and fruits is needed.

The application of micropropagation techniques in agro-forestry was essential because it offers a rapid way of producing genetically uniform cloned stock with high quality (Jagannathan, 1986). Some authors have already reported the way of micropropagation technique of *Paulownia* sp. It was achieved through the shoot bud formation from intermodal explants of *P. kawakamii* (Lobna S., 2008) and *P. elongata* (Ipekci Z., 2003). The effect of explant source on *in vitro* propagation of *Paulownia* tomentosa has been studied (Ozaslan M., 2005). Callus induction from leaves of *P. elongata* (Rao, 1996, Nguyen, 2005) has been reported and for *P. tomentosa* x *P. fortunei* for shoot regeneration (Fan, 2001). Therefore, an efficient vegetative propagation is an essential element for *Paulownia* clonal forestry and has many advantages over seedling production. Furthermore a vegetative propagation system based on adventitious shoot production could be integrated with genetic engineering (Ipekci Z. and N. Gozukirmizi, 2003).

Results, presented from our study could be useful for the optimization of the rapid clonal propagation and further development of regeneration techniques aiming creation of genetic diversity.

MATERIALS AND METHOD

The study was carried out at the Laboratory of Plant Biotechnology, Agricultural University, Plovdiv in 2013 and 2014 years. Plant material was kindly provided by "Velboy" Ltd – Plovdiv.

Plant material. Young plants of *Paulownia elongata* (P9), *Paulownia tomentosa* x *Paulownia fortunei* hybrid (PTF) and (*P. elongata* x *P. tomentosa*) x *P. elongata* complex hybrid (PT4) with single formed stems were grown in pots. Single node segments were used as initial explants. Sterilization procedure was performed by washing in tap water with liquid detergent for 20 minutes and subsequently treated with a solution of mercuric chloride (0,1%) for 3 minutes followed by rinsing 3 times for 5 minutes with sterile distilled water. Explants were blotted on sterile filter paper and cultured individually in tubes.

Culture media and growth conditions. Initial explants were cultivated on basal medium MS, (Murashige and Skoog, 1962) supplemented with

BAP (0.5 mg/l), IBA (0.01 mg/l), sucrose (20 g/l), solidified with plant agar (5 g/l) and adjusted to pH = 5,8 before autoclaving.

In multiplication stage the effect of five different basal media was compared: MS (Murashige and Skoog, 1962), DKW (Driver, J. A. and A. H. Kuniyuki, 1984), QL (Quoirin & Lepoivre, 1977) medium, N6 (Chu CC, 1978) and McC (Lloyd, G. and B. McCown. 1980) with addition of growth regulators (Table 1).

For induction of roots well-developed single plants were put on MS basal medium, enriched with 0,1 mg/l IBA, 20 g/l sucrose, 5 g/l plant agar and pH=5,8 before autoclaving (Table 1).

The subculture period was 28 days in growth chambers with permanent temperature 22±1°C, white fluorescent light with intensity 2500 lx and 16/8 h photoperiod.

Table 1. Composition of culture media used for *in vitro* propagation of *Paulownia*.

Multiplication stage				
Basal medium	BAP (mg/l)	IBA (mg/l)	Sucrose (g/l)	Agar (g/l)
MS	0,5	0,01	20	5
DKW				
QL				
N6				
McC				
Rooting stage				
MS		0,1	20	5

Explant source and cultural vessels. Apical (Ap) and axial (Ax) stem cuttings with 1-2 nodes and length 15±1 mm were collected from micropropagated plantlets of the studied genotypes and cultivated in five variants of proliferation media.

The effect of culture vessels was compared using big (0,5 l) and small (0,18 l) glass jars, each containing 20 or 5 plants respectively on proliferation medium with basal medium MS and addition of growth regulators (Table 1).

Adaptation and acclimatization *ex vitro*. Rooted plants were rinsed with tap water to remove the medium from the roots and transferred to the substrate of peat and perlite (3:1), treated with fungicide (0.2%) and cultivated for 30-40 days in growth chambers with gradual decrease of the atmospheric humidity.

Data analysis. The following indexes were calculated: multiplication coefficient, number of internodes per shoot and mean shoot length. Presented results are based on data from minimum two independent experiments with five replications and analyzed by standard biometric methods (IBM® SPSS® Statistics).

RESULTS AND DISCUSSION

Multiplication stage

To study the effect of the basal salt composition plants of all three genotypes were cultivated on five culture media. The influence of basal media on growth characteristics of the plants are represented in Table 2.

Data analysis showed that the multiplication coefficient was higher on media with MS and McC salts for all genotypes. Maximum shoot proliferation (3.05) was achieved for PTF on McC, followed by P9 on MS (2.5) and PT4 on MS (2.15). Lower multiplication was counted for the three genotypes on medium N6.

As characteristics of the obtained *in vitro* plants the number of internodes and mean shoot length followed the same influence by the basal medium – MS and McC were the best.

Apical (Ap) and axial (Ax) cuttings were used for studying the effect of explant source on proliferation of the plantlets (Table 3). The apical segments of PTF demonstrated the highest multiplication coefficient (2,4) but we have established that for the other genotypes the proliferation of the plants derived from the axial cuttings was better than apical.

Regarding the number of internodes the best results were obtained from axial segments of PT4 and the both type explants of P9 (4,6). The lowest value was counted for the explants of PTF (3,12 and 3,29 respectively for Ax and Ap).

Analyzing the index mean shoot length we established that P9 demonstrated higher results for both types of explants. The hybrid PTF presented lowest vegetative growth (1,95cm for Ap and 1,67cm for Ax).

Table 2. Effect of basal media on the proliferation of three genotypes *Paulownia*.
Data are statistically significant at P 5% (LSD test).

Genotype	PT4					P9					PTF				
	MMS	DDKW	MMcC	QQL	NN6	MMS	DDKW	MMcC	QQL	NN6	MMS	DDKW	MMcC	QQL	NN6
Multiplication coefficient	22,15 ± 0,16	11,45 ± 0,11	22,05 ± 0,15	11,80 ± 0,18	11,35 ± 0,10	22,50 ± 0,35	11,75 ± 0,14	22,05 ± 0,13	22,35 ± 0,15	11,80 ± 0,11	22,30 ± 0,23	22,20 ± 0,22	33,05 ± 0,57	22,05 ± 0,21	11,8 ± 0,20
Number internodes	44,67 ± 0,30	44,40 ± 0,19	55,30 ± 0,21	44,90 ± 0,20	33,97 ± 0,16	55,20 ± 0,19	44,40 ± 0,20	44,47 ± 0,19	44,85 ± 0,21	33,90 ± 0,17	33,54 ± 0,22	33,48 ± 0,22	33,27 ± 0,27	22,95 ± 0,11	33,00 ± 0,16
Mean shoot length	33,86 ± 0,10	33,02 ± 0,15	33,77 ± 0,17	33,6 ± 0,22	22,52 ± 0,10	33,60 ± 0,11	33,04 ± 0,10	33,14 ± 0,10	33,45 ± 0,15	33,01 ± 0,11	22,38 ± 0,15	11,84 ± 0,10	11,51 ± 0,07	11,86 ± 0,09	11,45 ± 0,07

Table 3. Effect of explant type on proliferation of apical (Ap) and axial (Ax) segments. Data are statistically significant at P 5% (LSD test).

Genotype	PT4		P9		PTF	
	Ap	Ax	Ap	Ax	Ap	Ax
Multiplication coefficient	1,68±0,83	1,84±0,11	1,98±0,09	2,2±0,26	2,4±0,26	2,16±0,12
Number internodes	3,63±0,15	4,6±0,14	4,6±0,14	4,5±0,25	3,29±0,15	3,12±0,10
Mean shoot length	2,82±0,10	3,3±0,14	3,26±0,09	3,25±0,12	1,95±0,08	1,67±0,06

The effect of cultural vessel on proliferation of the plants of the three genotypes was studied only on proliferation medium with MS basal salts. Results presented in Table 4 show that the containers with volume 0,5 l (B) are more suitable for mass propagation than the small ones. All three indexes for all the genotypes have higher values when cultivation is performed in big containers.

Table 4. Effect of cultural vessels on proliferation. B* – Big cultural vessel (0,5 l); S** – Small cultural vessel (0,18 l). Data are statistically significant at P 5% (LSD test).

	PT4		P9		PTF	
	B*	S**	B	S	B	S
Multiplication coefficient	3,4±0,65	2,0±0,15	3,1±0,33	1,8±0,26	2,5±0,23	2,30±0,41
Number internodes	5,78±0,16	4,7±0,52	5,75±0,46	5,4±0,28	6,55±0,22	3,63±0,36
Mean shoot length	3,8±0,19	3,81±0,12	5,42±0,29	3,61±0,19	4,5±0,24	2,82±0,14

Interesting fact we have established was that the explants of P9 developed plants with maximal shoot length (5,42cm) that could be explained with genotypic propensity of the species *P. elongata* to form plants with strong apical dominance.

Complex evaluation of the efficiency of micropropagation protocol we have applied showed that our results are better in comparison with reported 1.5 coefficient of multiplication of *P. kawakamii* (Lobna, 2008) and 2.6 for *P. tomentosa* (Bahri, 2013). Moreover, the proliferation medium we used was enriched with 0.5 mg/l BAP as a cytokinine which is twice less than previously used by the authors cited above.

Rooting stage

We established that all studied genotypes demonstrated an efficient rooting (100%) on MS medium with 0,1 mg/l IBA (Fig.1) that could be explained with suggested high content of endogenous auxin. Similar results for rooting for *Paulownia elongata* were found by Bergman B. A. (1997). The here applied

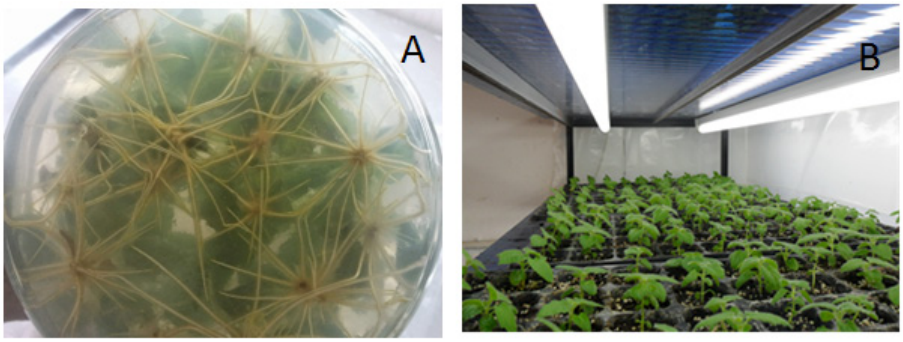

Figure 1. *Paulownia* plants following stages of rooting (A) and adaptation (B).

rooting approach with low exogenous auxin addition is a prerequisite for realizing economic effect in the commercial *in vitro* production of plant material.

Adaptation stage

Ex vitro adaptation of the rooted plants was performed in a growth chamber with a gradual decrease of the atmospheric humidity and the average survival rate 96% was achieved. In individual experiments, it ranged between 85 and 100%. The high average efficiency of the adaptation reported may be explained by both - genotypic characteristics, as well as the obtaining of a well-hardened plants during the rooting process, leading finally to improved adaptation efficiency.

CONCLUSIONS

Following the applied experimental protocol, the studied *Paulownia* genotypes were propagated *in vitro* and significant multiplication was achieved.

Application of the propagation medium with MS salts and addition of BAP (0.5 mg/ l) and IBA (0.01 mg l^{-1}l) and cultivation in big vessels resulted in high proliferation and induced the development of uniform plants as a prerequisite for effective rooting and quality material production.

Successful *in vitro* rooting was achieved when plants were cultivated on MS medium with IBA (0.1 mg/ l).

High average efficiency of adaptation (96%) was obtained.

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