[1.]A. FOKINA, [1,2.]T. SATAROVA, [2.]K. DENYSIUK, [3.]M. KHARYTONOV

OPTIMIZATION OF PAULOWNIA MICROPROPAGATION AT THE LATE CYCLES OF ASEPTIC CUTTING IN VITRO

[1.]Ukrainian State University of Chemistry and Technology, Dnipro, UKRAINE
[2.]Institute of Grain Crops of NAAS, Vdnipro, UKRAINE
[3.]Dnipro State Agrarian and Economic University, Dnipro, UKRAINE

Abstract: Paulownia is an important energy and decorative wood crop, which is still new and uncommon in Ukraine. Microclonal propagation of Paulownia elongata × P. fortune at late cycles of aseptic cutting in vitro is accompanied by a decrease in axillary buds activity as well as slow growth and development of cuttings on medium MS with 30 g / l sucrose, 2.5 g / l lysine, 7 g / l agar, and 2.0 mg / l 6-benzylaminopurine. Optimization of the phytohormonal composition of the given medium by adding 1.5 mg / l 6-benzylaminopurine, 1.0 mg / l of gibberellic acid GA_3, 0.5 mg / g adenine and 0.1 mg / l 3-indolylacetic acid allowed to increase the efficiency of in vitro micropropagation of paulownia in late cycles (from the sixth to the tenth) up to the level of early cycles of aseptic cutting.
Keywords: microclones, phytohormones, shoots, activation of axillary buds, nutrient medium

INTRODUCTION

Paulownia is an ornamental fast-growing woody plant (*Zhao et al., 2017; Yang et al., 2019*), which in 5-6 years can reach a height of 20 meters (*Pozoga et al., 2019; Linnik, 2020*). This property of *Paulownia* ensures the popularity of its utilization as a source of renewable energetic materials as well as a rapid restorer of forests, for land reclamation. It is possible to receive 240-350 m^3 of qualitative wood from 1 hectare for 7 years, while the bioethanol yield from 1 ton of dry wood can reach 0,5 t (*Linnik, 2020*). Moreover Paulownia is used in medicine due to antioxidant, anti-inflammatory, cytotoxic, enzyme-inhibitory properties of its flavonoids for both humans (*Cheng et al., 2019*) and animals (*Yang et al., 2019*). *Paulownia* leaves have been used as an admixture to the traditional diet of some farm animals (*Al-Sagheer et al., 2019*). *Paulownia* is also a honey plant with honey productivity of 700 liters per 1 ha (*Linnik, 2020*). For Ukraine, *Paulownia* is a new, uncommon plant that is gaining popularity.

Microclonal propagation of *Paulownia in vitro* provides a rapid rate of creation of pest-, disease-free genetically identical plants (*Rave et al., 2019*). Introduction into *in vitro* culture of nodular explants, petioles and shoot tips can often be used as explants (*Yadav et al., 2013*). The effectiveness of *Paulownia in vitro* cultivation depends on phytohormonal composition of a nutrient medium, in particular on the ratio of auxins and cytokinins (*Shtereva et al., 2014; Pozoga et al., 2019*) 6-benzylaminopurine (BAP), thidiazuron, 3-indolylacetic acid (IAA), kinetin are often used for successful micropropagation of Paulownia (*Yadav et al., 2013*). A positive effect of thidiazuron (0.5-1 mg/l) under the background of IAA (0.1 mg/l) on the frequency of shoot formation and shoot length for six genotypes of *Paulownia* was ascertained (*Shtereva et al., 2014*). The most effective concentration of BAP for regeneration via organogenesis from nodular explants of *P. tomentosa* × *P. fortunei* was 0.5 mg/l (*Pozoga et al., 2019*).

The best results on the proliferation and length of shoots from apical and axillary buds of *P. elongata x P. fortunei* were obtained with1.5 mg/l BAP and 0.1 mg/l indolylbutyric acid (*Rave et al., 2019*).

Most studies on *Paulownia* regeneration efficiency were conducted at the first cycles of microclonal cutting *in vitro* (*Shtereva et al., 2014; Pozoga et al., 2019*), although for prolonged industrial cultivation the production of clones not only at the early cycles of cutting, but also at the later ones seemed to be more rational. The aim of the work was to optimize the technology of *Paulownia* microclonal propagation at the late cycles of aseptic cutting *in vitro*.

MATERIALS AND METHODS

The hybrid *Paulownia elongata* S.Y.Hu ×*P. fortunei* (Seem.) Hemsl. was used as the material. Cuttings, 1 cm long, obtained from annual donor plants were used as explants. For their obnaining young unlignified 2-3-month-old branches were selected for explant obtaining. Explants included a stem section, a node with remnants of two opposite leaves and two axillary buds located in the leaf axils.

Explants were sterilized for 10 minutes in calcium hypochlorite solution and washed five times with sterile distilled water. For shoot formation by activation of axillary buds aseptic explants were put on the hormoneless nutrient medium: macro-, microsalts of MS medium (*Murashige and Skoog, 1962*), 2.5 mg/l lysine, 30 g/l sucrose, 7 g/l agar. Explants were cultured at a temperature of 25 °C, 16-hour photoperiod and light intensity of approximately 1500 lux for 30 days to induce the formation of new formed shoots (NFSs) from axillary buds.

NFSs were aseptically cut off into cuttings which were explanted on the fresh nutrient medium of the same composition and cultivated for 30 days (cycle I of aseptic cutting). NFSs from cycle II of cutting were used to obtain cuttings for the next cycle to continue multiplication. The process of aseptic cutting and the cultivation of NFSs were

continued until cycle X. Cutting cycles I-III were considered as early while cycles IV-X were examined as late cycles of cutting. The effect of growth regulators on the development of *P. elongata* × *P. fortune* at the late (VI) cycle of aseptic cutting *in vitro* was studied for prolongation of multiplication. Growth regulators were added according to the scheme (table 1) to the nutrient medium for cutting with macro-, microsalts MS, 30 g/l sucrose, 2.5 mg/l lysine, 7 g/l agar.

Table 1. The scheme of the investigation of the influence of growth regulators on the development of P. elongata × P. fortune cuttings at the late (VI) cycle of multiplication in vitro

Number of nutrient medium	Growth regulators, mg/l					
	BAP	GA3	Adenine	IAA	Kinetin	2iP
1	1.5	1.0	1.5	0.1	–	–
2	1.5	1.0	0.5	0.1	–	–
3	2.0	1.0	0.1	0.3	–	–
4	2.5	1.0	2.5	0.1	–	–
5	2.5	4.0	2.5	0.5	0.05	–
6	2.5	4.0	2.5	0.5	0.05	0.5

Note. Here and in tables 3-4 (Figure 2-3) the basic medium for cutting contained macro-, microsalts MS, 30 g/l sucrose, 2.5 mg/l lysine, 7 g/l agar; GA_3 – gibberellic acid 3, 2iP – 2-isopentenyladenine.

Forty cuttings were used for each variant of the experiment. The data are reported as x±SE (SE – standart error).

RESULTS

In our experiments on the medium MS with 30 g/l sucrose, 2.5 mg/l lysine, 7 g/l agar and 2 mg/l BAP the depression of axillary buds activity and the slowdown of the growth and development of *P. elongata* × *P. fortune* cuttings in late cycles of multiplication were observed. Then, the number of internodes per 1 explanted cutting at the late (V) cycle decreased 2.6 times and the shoot length – 2.3 times compared to the early (III) cycle (table 2). *P. elongata* × *P. fortune* cuttings at the 2nd and 5th cycles of aseptic cutting are shown in figure 1.

Table 2. Efficiency of *P. elongata* × *P. fortunei* micropropagation at early and late cycles of cutting *in vitro*

Indicator	Aseptic cycle of cutting	
	early (III)	late (V)
The number of internodes of newly formed shoots per 1 explanted cutting, pcs	6.9±0.4	2.7±0.4
The shoot length, cm	3.4±0.4	1.5±0.3

Note. Cultivation on medium MS with 30 g/l sucrose, 2.5 mg/l lysine, 7 g/l agar and 2 mg/l BAP.

At the late (VI) cutting cycle the addition of adenine (0.5 mg/l) gave the possibility to activate almost all *P. elongata* × *P. fortunei* buds with the formation of new shoots (table 3, Figure 2). Increasing the concentration of adenine up to 1.5 mg/l led to the depression of buds activity: only 1.4 buds per cutting were activated with shoot formation. A similar trend was observed in the formation of internodes on newly formed shoots. The number of internodes decreased 1.6 times (from 3.6 pcs. to 2.3 pcs. per shoot) when the concentration of adenine was increased from 0.5 mg/l to 1.5 mg/l. The enlargement of the BAP and adenine concentrations both to 2.5 mg/l did not have a significant effect on the formation of new shoots and their internodes.

Figure 1 – New formed shoots of P. elongata × P. fortune at early (II, left) and late (V, right) cutting cycles in vitro on medium with 2 m/l BAP

Table 3. Influence of growth regulators on the proliferation of shoots via axillary buds activation at the late (VI) cycle of *P. elongata* × *P. fortune* cutting *in vitro*

Number of nutrient medium	The content of growth regulators in the medium for cutting	Number of newly formed shoots per 1 explanted cutting, pcs.	Number of internodes per 1 newly formed shoot, pcs.
1	1.5 mg/l BAP+1.0 mg/l GA_3+1.5 mg/l adenine+0.1 mg/l IAA	1.4±0.4	2.3±0.4
2	1.5 mg/l BAP+1.0 mg/l GA_3+0.5 mg/l adenine+0.1 mg/l IAA	1.9±0.2	3.6±0.2
3	2.0 mg/l BAP+1.0 mg/l GA_3+0.1 mg/l adenine+0.3 mg/l IAA	1.6±0.2	2.9±0.3
4	2.5 mg/l BAP+1.0 mg/l GA_3+2.5 mg/l adenine+0.1 mg/l IAA	1.9±0.2	3.1±0.2
5	2.5 mg/l BAP+4.0 mg/l GA_3+2.5 mg/l adenine+0.5 mg/l IAA+0.05 mg/l kinetin	1.8±0.2	3.4±0.4
6	2.5 mg/l BAP+4.0 mg/l GA_3+2.5 mg/l adenine+0.5 mg/l IAA+0.05 mg/l kinetin+0.5 mg/l 2iP	1.9±0.5	3.1±0.2

Note. Numbers of nutrient media in table 3-4 correspond to the same numbers in table 1.

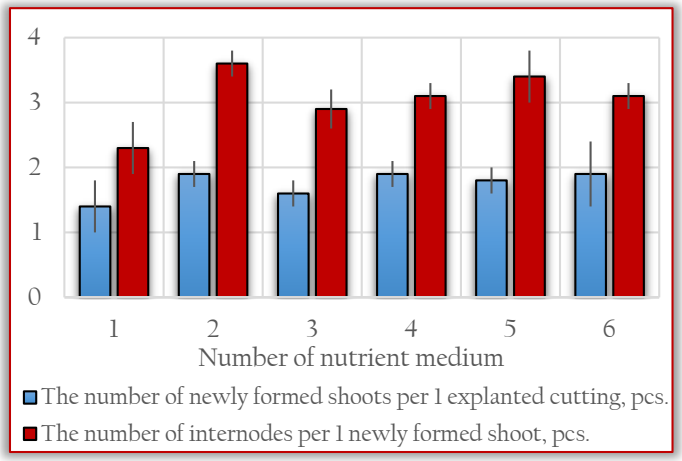

Figure 2 – The development of *P. elongata* × *P. fortune* shoots at the late (VI) cycle of cutting *in vitro* on media with different phytohormones (indicated in table 1)

The tendency to rise the values simultaneously with the complication of medium composition at the cycle VI was established (table 4, Figure 3). At the cycle VI the best result was obtained by reducing the content of BAP from 2.0 to 1.5 mg/l and supplementing the medium with 1.0 mg/l GA_3, 0.5 mg/l adenine, and 0.1 mg/l IAA. On this medium the shoot length increased on average to 3.7 cm, and the number of internodes per 1 explant reached 6.8 pcs. This result is similar to the result obtained at the 3rd cycle of cutting, where the shoot length was 3.4 cm, and the number of internodes of newly formed shoots per 1 explanted cutting was 6.9 pcs.

Table 4. Influence of growth regulators on the growth of P. elongata × P. fortune at the late (VI) cycle of cutting in vitro

Number of nutrient medium	The content of growth regulators in the medium for cutting	The length of newly formed shoots, cm	Number of internodes of newly formed shoots per 1 explanted cutting, pcs.
1	1.5 mg/l BAP+1.0 mg/l GA_3+1.5 mg/l adenine+0.1 mg/l IAA	2,5±0,3	3,3±0,3
2	1.5 mg/l BAP+1.0 mg/l GA_3+0.5 mg/l adenine+0.1 mg/l IAA	3,7±0,4	6,8±0,2
3	2.0 mg/l BAP+1.0 mg/l GA_3+0.1 mg/l adenine+0.3 mg/l IAA	2,0±0,3	4,7±0,5
4	2.5 mg/l BAP+1.0 mg/l GA_3+2.5 mg/l adenine+0.1 mg/l IAA	2,4±0,2	5,9±0,3
5	2.5 mg/l BAP+4.0 mg/l GA_3+2.5 mg/l adenine+0.5 mg/l IAA+0.05 mg/l kinetin	2,9±0,1	6,2±0,3
6	2.5 mg/l BAP+4.0 mg/l GA_3+2.5 mg/l adenine+0.5 mg/l IAA+0.05 mg/l kinetin+0.5 mg/l 2iP	3,3±0,4	5,9±0,5

The worst results at the cutting cycle VI were obtained by increasing the concentration of adenine up to 1.5 mg/l. In this way the number of internodes per explant was almost twice less than at 0.5 mg/l of adenine. The shoot length was the lowest on medium with 2.0 mg/l BAP, 1.0 mg/l GA_3, 0.1 mg/l adenine, and 0.3 mg/l IAA. *P. elongata* × *P. fortune* multiplication at the late (VI) cycle of aseptic cutting on media of studied compositions is shown also in figure 4.

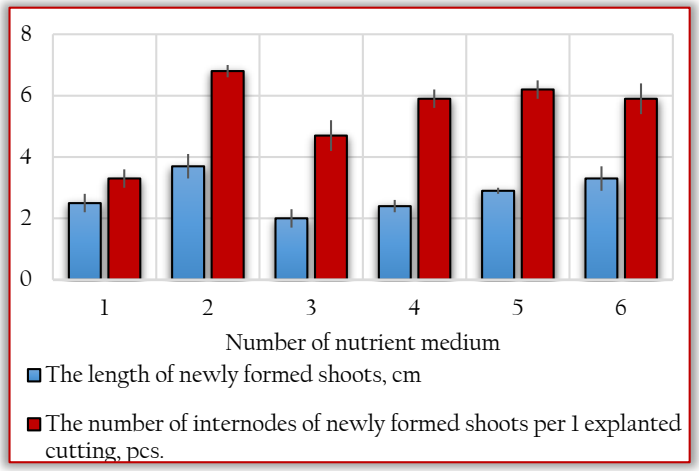

Figure 3 – The extension of *P. elongata* × *P. fortune* at the late (VI) cycle cutting *in vitro* on media with different phytohormones (indicated in table 1)

Thus, the results in tables 3-4 indicate the possibility of effective cultivation of *P. elongata* × *P. fortune* at the late cycles of aseptic cutting under increasing action of growth regulators. The medium MS with 30 g/l sucrose, 2.5 mg/l lysine, 7 g/l agar supplemented with 1.5 mg/l BAP, 1.0 mg/l GA_3, 0.5 mg/l adenine and 0.1 mg/l IAA was the most effective for the late (VI) cycle of cutting.

Figure 4 – P. elongata × P. fortune shoots multiplication at the late (VI) cycle of aseptic cutting, numbers of nutrient media from left to right: 5, 4, 3, 2, 6, 1 (see table 1)

Figure 5 – The exhaustion of P. elongata × P. fortunei after the cutting cycle X in vitro

In our experiments *P. elongata* × *P. fortune* in isolated culture was effectively microcloned up to the cycle X. It became possible due to the mentioned above optimized medium for late cycles of cutting. After cutting cycle X *in vitro* culture of *P. elongata* × *P. fortunei* began to wear out and die (Figure 5).

CONCLUSIONS

The depression of the axillary buds activity of *Paulownia. elongata* × *P. fortune* and slowing down the growth and development of shoots from them was proved to the late (V) cycle of cutting *in vitro* on nutrient medium MS with 30 g/l sucrose, 2.5 mg/l lysine, 7 g/l agar supplemented with 2.0 mg/l BAP. The optimization of the given nutrient medium with 1.5 mg/l BAP, 1.0 mg/l GA_3, 0.5 mg/l adenine and 0.1 mg/l IAA permitted to perform microclonal propagation of *P. elongata* × *P. fortune* in late (VI) cutting cycle on the level of early (III) one. On the given optimized nutrient medium multiplication of *P. elongata* × *P. fortune in vitro* was effective up to the tenth cycle of cutting.

Note:
This paper is based on the paper presented at ISB–INMA TEH' 2020 International Symposium (Agricultural and Mechanical Engineering), organized by Politehnica University of Bucharest – Faculty of Biotechnical Systems Engineering (ISB), National Institute of Research–Development for Machines and Installations Designed to Agriculture and Food Industry (INMA Bucharest), Romanian Agricultural Mechanical Engineers Society (SIMAR), National Research & Development Institute for Food Bioresources (IBA Bucharest), National Institute for Research and Development in Environmental Protection (INCDPM), Research-Development Institute for Plant Protection (ICDPP), Research and Development Institute for Processing and Marketing of the Horticultural Products (HORTING), Hydraulics and Pneumatics Research Institute (INOE 2000 IHP) and "Food for Life Technological Platform", in Bucharest, ROMANIA, 30 October, 2020.

References

[1] Al-Sagheer A.A., Abd El-Hack M.E., Alagawany M., et al., (2019), *Paulownia* leaves as a new feed resource: chemical composition and effects on growth, carcasses, digestibility, blood biochemistry, and intestinal bacterial populations of growing rabbits, *Animals (Basel)*, Vol.9(3), Issue 95, pp.1-13, Basel, Switzerland;

[2] Cheng C.L., Jia X.H., Xiao C.M., Tang W.Z., (2019), *Paulownia C*-geranylated flavonoids: their structural variety, biological activity and application prospects. *Phytochem Rev*., Vol.18, pp.549-570, Ed. Springer Nature, Switzerland;

[3] Linnik A., (2020), *Paulownia* as power culture, *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies,Series: Agricultural Sciences*,Vol.22, Issue 92, pp.19-22, Lviv, Ukraine;

[4] Murashige T., Skoog F., (1962), A revised medium for rapid growth and bioassays with tobacco tissue cultures,*Physiol. Plant*., Vol.15, Issue 3, pp.473–497, Lund,Sweden;

[5] Pozoga M., Olewnicki D., Jablonska L., (2019), *In vitro* propagation protocols and variable cost comparison in commercial production for *Paulownia tomentosa* × *Paulownia fortunei* hybrid as a renewable energy source, *Applied Sciences*, Vol.9, pp. 2272-2280, Basel, Switzerland;

[6] Rave M.I.D., Castro-Restrepo D., García J.J.D., (2019), Propagación clonal *in vitro* de *Paulownia elongata x fortunei*, *Latin American Journal of Biotechnology and Life Sciences*,Vol. 4(1). pp.775-778, Yachay, Ecuador;

[7] Shtereva L., Vassilevska-Ivanova R., Karceva T.,Kraptchev B., (2014), Micropropagation of six *Paulownia* genotypes through tissue culture, *Journalof Central European Agriculture*, Vol.15, Issue 4, pp.147-156, Zagreb, Croatia;

[8] Yadav N., Vaidya B., Henderson K. et al., (2013), A review of*Paulownia* biotechnology: A short rotation, fast growing multipurpose bioenergy tree, *American Journal of Plant Sciences*, Vol. 4, Issue 11,pp.2070-2082, Coulterville, USA;

[9] Yang H., Zhang P., Xu X. et al., (2019), The enhanced immunological activity of *Paulownia tomentosa* flower polysaccharide on Newcastle disease vaccine in chicken, *Biosci Rep*., Vol.39, Issue 5, pp.1-9, London, U.K.;

[10] Zhao Z., Li Y., Liu H., et al., (2017), Genome-wide expression analysis of salt-stressed diploid and autotetraploid *Paulownia tomentosa*, *PLoS One*, Vol.12(10), pp.1-23, San Francisco/ California, Cambridge/United Kingdom.

ISSN: 2067-3809