Biolife 3(4):910-916
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

In vitro growth behavior of *Paulownia kawakamii* hybrid under nutrient media and plant growth regulators effect

Abd El-Kader E. M[1] and Hanan. M. Abou El-ghit[2]*

[1]*Horticultural Research Institute, Agricultural Research Center, Giza, Egypt*
[2] *Department of Botany and Microbiology, Faculty of Science, University of Helwan, Cairo, Egypt.*

**Email: hanan8760@yahoo.com*

ABSTRACT

The goal of this study was to detect the most suitable treatment for multiplication of *Paulownia kawakamii* hybrid (101) trees to get rapid, healthy and normal plants. Shoot proliferation was induced using stem nodal explants cultured on MS, WPM or B5 medium containing different concentrations of BAP or Kin ± IBA. BAP was more suitable for inducing new shoots than Kin. The maximum numbers of the formed shoots per explant (8.3, 5.6 and 5.5) during the three successive subcultures were recorded for MS medium augmented with 1.0 mg/L BAP+0.2 mg/L IBA, 0.5 mg/L BAP+0.1 mg/L IBA and 0.5 mg/LBAP+0.1 mg/L IBA for the first, second and third subculture respectively.

Key words: *Paulownia*, *in vitro* culture, plant growth regulators.

Abbreviations: BAP (benzyl adenine purine), Kin (kinetin) and IBA (indole butyric acid). PGR (Plant growth regulator)

INTRODUCTION

Paulownia is a genus of between 6-17 species of plants in the family Paulowniaceae. They are native to much of China and have been naturalized in other parts of the world such as Europe and USA. They are fast growing deciduous trees, with large leaves arranged in opposite pairs on the stem. The trees are used for re-forestation, roadside planting and as ornamental trees. It grows well in a wide variety of soil types, notably poor ones, and yields a multiple-purpose wood of potential medicinal uses, and because of its wide-spreading root system, it may be used for phytoremediation of contaminated soils (Zhu *et al.* 1988).

How to cite this article:

Abd El-Kader E. M and Hanan. M. Abou El-ghit. (2015). *In vitro* growth behavior of *Paulownia kawakamii* hybrid under nutrient media and plant growth regulators effect. Biolife, 3(4), pp 910-916. DOI :10.17812/blj.2015.3426

Published online: 21 December 2015

The genus is receiving increasing attention as an extremely fast growing, short-rotation woody crop plant (Bergmann, 1997; Ipekci and Gozukismici, 2003). However, the potential invasive character of the species growing out of its natural range has recently been pointed out by Ding *et al.*(2006) and Essl, (2007). The *Paulownia* tree adapts easily to a wide range of climatic conditions. It grows well in tropical climates with abundant sunshine and rainfall. It can also be grown in regions as far north as 40 degrees latitude in the northern hemisphere with as little rainfall as 20 inches per year. In general, *Paulownia* tree grows well on sandy and clay soils. It adapts so easily, hence it can grow just as quickly on a wide variety of soils. *Paulownia* tree can also adapt to a wide range of temperatures. In Japan, *Paulownia* is commonly found in regions where the maximum temperature is 38-40 °C with a minimum temperature of -10 to -5 °C, and a mean temperature of 15-20 °C during the year so it would be a good plant to be introduced in Bangladesh for timber production and re-forestation.

Paulownia wood is used in house construction, for paper pulp, furniture making, farm implements and musical instruments (Ayan *et al.,* 2003). The *Paulownia* tree grows 5-6 m tall during the first

growing season and adds 3 to 4 cm in diameter annually if optimal growing conditions are present (El-Showk and El-Showk, 2003). However, other reports have suggested that *Paulownia* grew 4.9 m after 4.5 months growth in Maryland (Preston, 1983). Under certain conditions *Paulownia* trees can grow up to 55 cm (1.8 feet) diameter and to a height of up to 15 - 20 meters (40-50 feet) in 3 to 5 years.

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 internodal explants of *P. kawakamii* (Lobna, 2008) and *P. elongata* (Ipekci, 2003). The effect of explant source on *in vitro* propagation of *Paulownia* tomentosa has been studied (Ozaslan, 2005). Callus induction from leaves of *P. elongata* (Rao, 1996, Nguyen *et al.,* 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 and 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 to produce normal plantlets.

Material and Methods

This study has been carried out in the Tissue Culture Res. Lab., Hort. Res. Institute, Agri. Res. Center (A.R.C.), Egypt, during the period from 2014 to 2015.

Plant Material:

Nodal explants were taken from *Paulownia kawakamii* tree at Faculty of Agriculture, Ain Shams Univ., Shobra El-Khyema, Egypt.

Explant Disinfection:

Explants were washed in soapy water using septol soap, then agitated in disinfectant solution of Savlon (3%) for 40 minutes and rinsed with running tap water for one hour. Thereafter, explants were surface disinfected under aseptic condition in safety cabinet using ethanol 70% for one minute, followed by a further sterilization by Clorox (NaOCl 5.25%) 20% (v/v) with a few drops of Tween-20 as emulsifier for 10 minutes, followed by mercuric chloride at 0.1 % (w/v) with a few drops of Tween-20 as emulsifier for 10 minutes. Three rinses with distilled water were adopted after disinfection.

Culture Media and Incubation Condition:

Explants were cultured on the following medium types:

- MS-medium (Murashige and Skoog, 1962)
- WPM-medium ``Woody Plant Medium`` (Lloyd and McCown, 1980)
- B5-medium (Gamborg *et al.,* 1968)

Media was supplemented with 3% sucrose and 0.7% agar (W/V). MS medium was supplemented with the following plant growth regulators: BAP or Kin at 0.5, 1.0, 1.5 and 2.0 mg/L ± IBA 0.1 or 0.2 mg/L, compared to control (plant growth regulators free media). Cultures were incubated at 24+2 °C and illumination condition of 1500Lux using fluorescent lamp and 16/8 photoperiod. At this stage, each explant has been expanded into a cluster of shoots. Multiple shoots were separated and transplanted to new culture medium. Shoots were subcultured three times with 4 weeks intervals.

Statistical Analysis:

Mean separation was made using least significant differences (L.S.D.) at 5% level of significance. Test was applied for the comparison among means as described by Steel and Torrie (1980).

Results & Discussions

Shoot multiplication on different media type (MS, WPM and B5) with various concentrations of BAP or kin ± IBA, nodal segments showed bud break and growth after two weeks of culture in media supplemented with all concentrations of BAP and kin with or without IBA in the first cycle of subculture. Although variations were observed in the response of explants to plant growth regulator treatment at the earlier subculture, the results reported here reflect the status of multiplication after three subcultures, reported on the performance per shoot basis and not as cumulative output after three subcultures. In this experiment, the medium type with different concentrations of BAP or kin ± IBA shows significant effect on bud sprouting during the three cycles of subcultures. During the first cycle, bud sprouting ranged from 70 to 100% of cultured explants. WPM was more suitable and kin was more effective. Using IBA enhanced bud break, especially with low concentrations of BAP. Data takes the same trend during the second and the third cycles, but the bad effect of high concentrations of BAP increased especially when added to MS medium (Table 1).

Reduced number of shoots could be attributed to inhibition of adventitious meristem elongation by the use of higher BAP concentration as stated by Borchetia *et al.* (2009). Also, Lobna *et al.* (2008) stated that supplementation of the culture medium by 1.0 mg/l of BA favored shootlets initiation of *Paulownia kawakamii* compared with control. As for the number of the formed shoots per explant, MS

Table-1. Effect of medium type and plant growth regulators on bud sprouting (%) during three successive subcultures

(PGR) (mg/l)			Medium type								
			Subculture I			Subculture II			Subculture III		
BAP	Kin	IBA	MS	WPM	B5	MS	WPM	B5	MS	WPM	B5
--	--	--	90	85	80	85	80	80	85	80	80
0.5			95	90	90	90	100	80	90	90	85
1.0	--	--	80	85	80	82	100	80	80	82	80
1.5	--	--	80	80	75	60	85	73	70	75	80
2.0	--	--	80	80	70	55	75	70	40	70	70
0.5	--	0.1	95	95	90	90	100	85	40	100	80
0.5	--	0.2	95	95	90	90	100	80	60	100	80
1.0	--	0.1	95	95	80	80	100	80	60	80	80
1.0	--	0.2	90	95	80	80	100	80	60	80	80
1.5	--	0.1	82	90	80	75	100	70	55	60	80
1.5	--	0.2	82	90	80	75	100	70	50	60	80
2.0	--	0.1	70	85	70	40	95	50	50	65	70
2.0	--	0.2	60	85	70	40	92	55	50	65	70
--	0.5	----	90	95	90	100	100	90	100	100	80
--	1.0	--	90	95	90	100	100	90	100	100	80
--	1.5	--	90	95	90	100	100	90	100	100	80
--	2.0	--	90	95	90	90	100	85	100	100	80
--	0.5	0.1	100	100	90	100	100	85	100	100	90
--	0.5	0.2	100	100	90	100	100	90	100	100	90
--	1.0	0.1	100	100	90	100	100	90	100	100	90
--	1.0	0.2	100	100	90	100	100	90	100	100	90
--	1.5	0.1	90	100	90	90	100	90	100	100	90
--	1.5	0.2	90	100	90	90	100	90	100	100	90
--	2.0	0.1	85	100	90	85	100	90	80	100	90
--	2.0	0.2	90	100	90	80	100	90	80	100	90
L.S.D. at 5%			16.286			15.321			18.513		

Table-2. Effect of medium type and plant growth regulators on shoot number/explant during three successive subcultures

PGR (mg/l)			Medium type								
			Subculture I			Subculture II			Subculture III		
BAP	Kin	IBA	MS	WPM	B5	MS	WPM	B5	MS	WPM	B5
--	--	--	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0.5	--	--	4.1	2.2	1.6	4.4	2.2	1.1	4.1	2.1	1.0
1.0	--	--	6.2	2.1	1.2	4.1	2.3	1.2	2.2	2.3	1.2
1.5	--	--	4.2	2.5	1.2	2.3	2.2	1.2	1.2	1.2	1.6s
2.0	--	--	3.3	3.2	1.1	2.2	3.3	1.3	1.3	1.3	1.5
0.5	--	0.1	6.1	3.2	1.3	5.6	2.2	1.2	5.5	3.0	1.0
0.5	--	0.2	6.3	3.3	1.1	5.1	2.1	1.1	5.4	2.2	1.0
1.0	--	0.1	4.6	2.3	1.2	3.0	2.2	1.2	3.1	2.3	1.0
1.0	--	0.2	8.3	2.6	1.5	3.2	3.5	1.2	2.2	2.2	1.0
1.5	--	0.1	5.2	2.2	1.1	4.0	2.2	1.5	2.3	2.0	1.0
1.5	--	0.2	5.8	2.1	1.6	4.5	2.2	1.4	2.5	2.1	1.0
2.0	--	0.1	4.1	2.4	1.1	2.0	2.1	1.1	1.7	2.1	1.0
2.0	--	0.2	4.5	2.2	1.2	2.1	2.1	1.2	1.8	2.0	1.0
--	0.5	--	3.1	2.1	1.1	3.2	2.2	1.1	2.2	2.5.	1.0
--	1.0	--	3.3	2.2	1.1	3.5	2.2	1.6	2.3	2.7	1.0
--	1.5	--	3.6	3.5	1.2	3.2	2.1	1.2	2.1	2.2	1.0
--	2.0	--	4.1	3.3	1.2	3.2	3.3	1.1	2.4	2.1	1.0
--	0.5	0.1	3.2	2.2	1.2	3.2	2.6	1.2	2.2	1.5	1.0
--	0.5	0.2	4.3	2.3	1.2	3.1	2.1	1.3	2.3	1.3	1.0
--	1.0	0.1	4.1	3.4	1.2	2.3	2.5	1.1	2.1	1.3	1.0
--	1.0	0.2	4.5	3.3	1.1	3.6	2.0	1.2	2.2	1.4	1.0
--	1.5	0.1	3.4	3.4	1.2	3.4	2.0	1.1	2.3	1.1	1.0
--	1.5	0.2	4.3	3.2	1.2	2.1	2.1	1.5	2.4	1.0	1.2
--	2.0	0.1	3.1	2.1	1.1	3.2	2.2	1.1	2.1	1.0	1.6
--	2.0	0.2	3.1	2.1	1.1	2.1	2.1	1.2	2.1	1.0	1.2
L.S.D. at 5%			0.871			0.692			0.653		

medium was more effective for inducing more shoots compared with WPM and B5 (Table 2). The number of the formed shoots during the first subculture was greater than those of the second and the third ones, may be due to the explants during the first subcultures were under their physiological state of mother plant. Plant growth regulators were found to be necessary for multiplication as medium with 0.0 mg/L cytokinins did not show any multiplication except for growth of single shoot. BAP was more suitable for inducing new shoots than Kin. The maximum numbers of the formed shoots/explant (8.3, 5.6 and 5.5) during the three subcultures were recorded for MS medium augmented with 1.0 mg/L BAP+ 0.2 mg/L IBA, 0.5 mg/L BAP+0.1 mg/L IBA and 0.5 mg/L BAP+0.1 mg/L IBA for the first, second, third subcultures, respectively. Whereas, the lowest ones (1.0–1.6) were recorded for B5 medium and growth regulators free media.

Shoot length was significantly affected by treatments under investigation, either medium composition or growth regulator type (Table 3). Augmented media with IBA either 0.1 or 0.2 mg/l led to increases in shoot length compared to those of IBA free media. The maximum shoot lengths (6.2-6.3 cm) were attained at 0.5 mg/l BAP plus 0.1 or 0.2 mg/L IBA on MS medium. Increased concentration of BAP decreased the increased length of shoots produced by augmenting media with IBA. Also, Kin with all used concentrations increased shoot length compared with control growth regulator free media. Using B5 medium reduced the multiplication rate in terms of shoot number and shoot length. Previous studies were carried out to investigate micropropagation of *paulownia*, in this concern Rout *et al*. (2001) reported that shoot proliferation of *paulownia* was carried out on MS medium containing BAP or Kin as well as auxins were used. The combination of BAP (4.44 µM) and NAA (0.53 µM) was optimal.

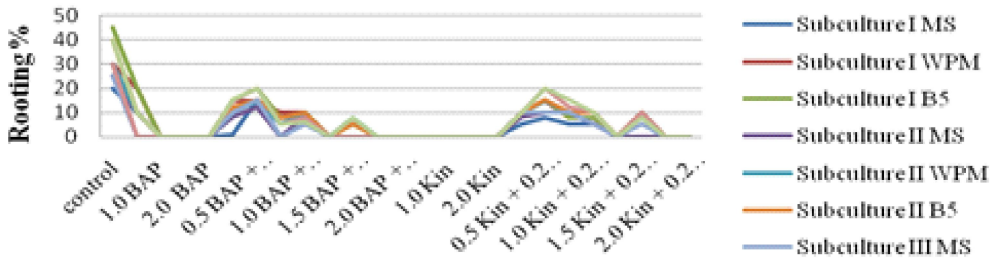
Undesirable influence of different concentrations of BAP (mg/l) on shoot multiplication

As shown in Fig. (1) and Plate (1), cytokinin type and concentration suitable for micropropagation of woody plants have to be carefully optimized. The data reported in this study are based on observation made after three subculture cycles. The observations show that continuous subculture on medium with BAP concentration of above 1.0 mg/L resulted in hyperhydricity (vitrification). Furthermore, increased

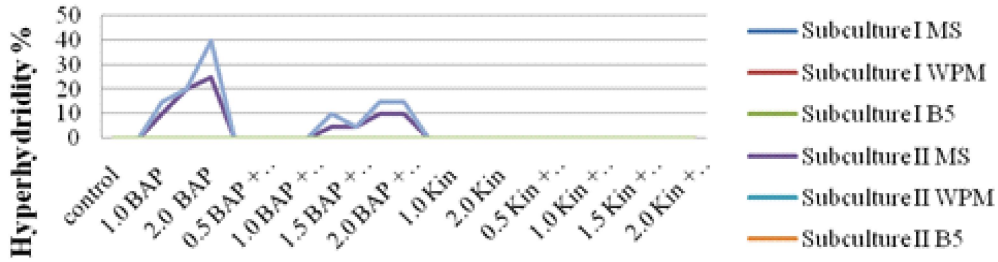
Table-3. Effect of medium type and plant growth regulators on shoot length (cm) during three successive subcultures

PGR (mg/l)			Medium type								
			Subculture I			Subculture II			Subculture III		
BAP	Kin	IBA	MS	WPM	B5	MS	WPM	B5	MS	WPM	B5
--	--	--	4.0	3.0	2.0	2.0	2.0	1.4	1.5	1.0	1.0
0.5	--	--	6.7	5.9	3.6	3.3	5.1	3.6	4.3	3.2	2.4
1.0	--	--	4.6	4.8	3.6	3.1	4.2	3.2	3.7	3.5	2.6
1.5	--		4.1	3.9	3.4	2.8	3.1	3.1	2.7	2.9	2.1
2.0	--	--	3.7	3.7	3.2	2.7	2.9	3.0	2.5	2.1	1.4
0.5	--	**0.1**	6.2	6.1	4.1	5.8	5.4	3.6	5.9	3.3	2.7
0.5	--	**0.2**	6.3	6.3	4.4	6.0	5.6	2.8	5.7	3.5	2.8
1.0	--	**0.1**	6.0	5.4	4.1	4.2	4.6	2.7	4.0	3.1	2.6
1.0	--	**0.2**	6.2	5.5	4.2	4.3	4.3	2.1	3.1	3.2	2.5
1.5	--	**0.1**	4.3	4.8	3.5	2.9	3.7	2.6	2.8	3.2	2.0
1.5	--	**0.2**	4.5	4.9	3.4	2.8	3.1	2.0	2.9	2.8	1.9
2.0	--	**0.1**	3.2	4.5	3.6	2.5	2.4	2.2	2.2	2.2	1.9
2.0		**0.2**	3.1	4.5	3.8	2.6	2.2	2.1	2.3	2.1	1.7
--	**0.5**	--	5.9	6.1	5.7	4.2	3.6	3.1	4.9	3.3	2.6
--	**1.0**	--	6.4	5.2	4.1	4.3	3.2	3.2	4.1	3.5	2.8
--	**1.5**	--	6.3	5.1	3.5	3.6	3.3	3.0	3.8	3.5	2.7
--	**2.0**	--	6.1	5.1	3.1	3.1	3.5	2.8	3.9	3.3	2.6
--	**0.5**	**0.1**	6.8	6.1	4.2	5.2	3.7	3.7	5.2	4.3	3.1
--	**0.5**	**0.2**	6.7	6.2	4.5	5.4	4.6	3.9	5.1	4.1	3.5
--	**1.0**	**0.1**	6.3	6.0	3.2	4.1	4.5	4.1	4.7	4.2	2.8
--	**1.0**	**0.2**	6.2	5.9	3.4	4.2	4.3	3.1	4.7	3.5	2.7
--	**1.5**	**0.1**	5.3	5.4	3.2	3.6	4.1	3.0	4.6	3.2	2.7
--	**1.5**	**0.2**	5.1	4.3	3.1	3.4	4.1	2.5	4.9	2.2	2.6
--	**2.0**	**0.1**	4.8	4.2	2.8	2.7	3.3	2.1	4.3	2.2	2.5
--	**2.0**	**0.2**	4.9	4.4	2.7	2.9	3.7	2.1	4.1	2.0	2.5
L.S.D. at 5%			1.089			0.934			0.873		

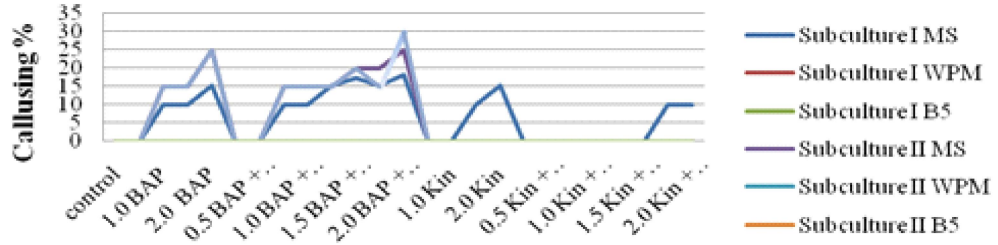
Figure-1. Influence of different concentrations of BAP (mg/l) on shoot multiplication



A. Root formation as a result of medium type & plant growth regulators

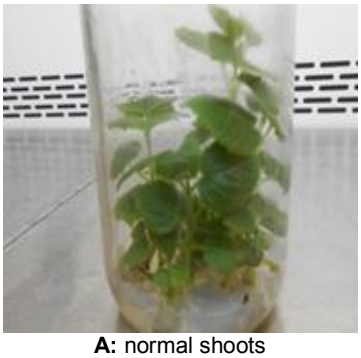


B. Hyperhydridity as a result of medium type & plant growth regulators

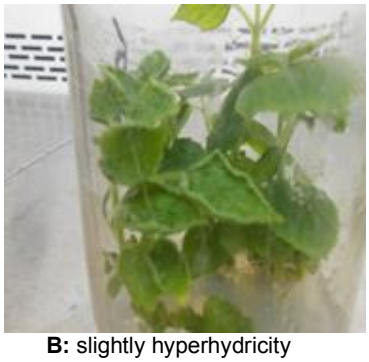


C. Callus formation as a result of medium type & plant growth regulators

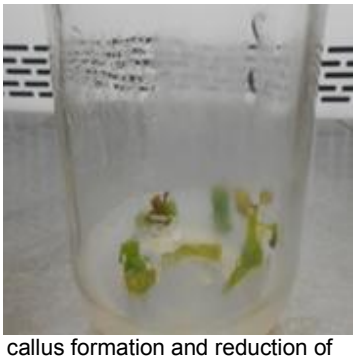
Plate.1. Undesirable influence of different medium type and concentrations of BAP (mg/l) on shoot multiplicatio



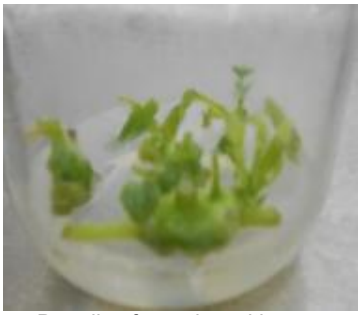
A: normal shoots



B: slightly hyperhydricity



C: callus formation and reduction of bud sprouting



D: callus formation with hyperhydritity

E: root formation, growth reduction with low bud sprouting

F: normal shoot with base callus formation and rooting.

callus formation and hyperhydricity at high concentrations of BAP were prominent. Constant presence of BAP at optimal concentrations was necessary at different subcultures and there seemed to be accumulative gain over subcultures in both the number as well as the sturdiness of the shoots.

Neither WPM nor B5 media caused hyperhydridity or callusing for the in vitro propagated Paulownia but they lead to less multiplication rate compared to MS medium. Our investigation is in agreement with those of Clapa *et al*. (2014) who suggested that the media supplemented with BAP insured intense proliferation of axillary buds but it caused negative physiological effects (abundant callus growth at plantlets base). Therefore, cytokinin type and concentration suitable for micropropagation of woody plants have to be carefully optimized.

Conflict of Interests:

Authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Ayan, S.; Gercek, V. and Sahin, A. (2003). Performation of nursery stage of *Paulownia* Sieb.& Zucc. Species and Origins, Review of Faculty of Forestry University of Suleyman Demirel, Seri. A, 2: 41-56, Isparta, Turkey.
2. Bergmann, B.A.and Moon, H.K. (1997). *In vitro* adventitious shoot production in *Paulownia*. Plant Cell Reports, 16(5): 315–319.
3. Borchetia, S.; Das, S.C.; Handique, P.J. and Das, S. (2009). "High multiplication frequency and genetic stability for commercialization of the three varieties of micropropagated tea plants (*Camellia* spp.)," Scientia Horticulturae, 120 (4): 544–550.
4. Clapa, D.; Alexanru, F.I.R.A.; Manuela, S.I.M.U. and Laura, B. (2014). Improved in vitro propagation of *Paulownia elongata*, *P. fortunei* and its interspecific hybrid *P. elongata* x *P. fortune*. Bulletin UASVM Horticulture, 7 (1): 6 – 14.
5. Ding, J.Q.; Reardon, R.; Wu, Y.; Zheng, H. and Fu, W.D. (2006). Biological control of invasive plants through collaboration between China and the United States of America: a perspective. Biological Invasions, 8: 1439-1450**.**
6. El-Showk, S., and El-Showk, N. (2003). The *Paulownia* tree – An alternative for sustainable forestry. The Farm. http://www.cropdevelopment.org/paulownia/Brochure. pdf.
7. Essl, F. (2007). From ornamental to detrimental? The incipient invasion of Central Europe by *Paulownia tomentosa.* Preslia, 79: 377-389.
8. Fan Guo-qiang;, Zhi Xiao-qiao, Zhai Cui-juan, BI Hui-tao, (2001). Callus induction from laves of different Paulownia species and its plantlet regeneration. Journal of forestry Research, 12 (4): 209-214.
9. Gamborg, O.L.; Miller, R.A. and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res. 50: 151-158.
10. Ipekçi, Z. and Gozukirmizi, N., (2003) Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. Plant Cell Reports, 22(1): 16–24.
11. Jagannathan. (1986). Phenotypic and ploidy status of Paulownia tomentosa trees regenerated from culture hypocotyls. Plant cell, Tissue and organ culture 7: 227-236.
12. Lloyd, G. and McCown B., 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use of shoot tip culture. *Proc. Intl. Plant Prop. Soc.*, 30: 421-427.
13. Lobna S., 2008, A micropropagation protocol of *Paulownia kawakamii* through *in vitro* culture technique. *Australian Journal of basic and applied sciences*, 2 (3): 594-600
14. Murashige, T. and Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473-497.
15. Nguyen M. U., Thai Xuan Du, Bui Van Le, 2005. Effect of plant growth regulators on callus induction and shoot regeneration of *Paulownia fortunei*. *Tap chi Cong nghe Sinh hoc* 3 (4): 479-485.
16. Ozaslan M. (2005). Effect of explant source on *in vitro* propagation of *Paulownia tomentosa* Steud. *Biotechnol. & Biotechnol. Eq*. 19 (3): 20-26.
17. Preston, D. (1983). Paulownia: a miracle tree or passing fancy? *Am. Forests*. 89(5): 15-19, 47-52.
18. Rao, C.D.; Goh, C. J. and Kumar, P.P. (1996). High frequency adventitious shoot regeneration from excised leaves of *Paulownia Spp*. cultivated *in vitro*. Plant Cell Reports, 16: 204-209.
19. Steel, R.G.D. and Torrie, J.H. (1980). Principle of Statistics. A Biometrical Approach 2nd, MC Graw-Hillkogakusha, Ltd.
20. Zhu, Z.H.; Chao, C.J.; Lu, X.Y. and Xiong, Y.G., (1988). *Paulownia* in China: Cultivation and utilization. Singapore: Asian Network for Biological Science and International Development Research Centre of Canada.

سلوك الباولونيا كاواكامياي تحت تأثير البيئة الغذائية و منظمات النمو بمزارع الأنسجة

تهدف هذه الدراسة الى تحديد أنسب معاملة لاكثار هجين الباولونيا كاواكامياي (101) للحصول على نباتات صحيحة وطبيعية بصورة سريعة. تم الحصول على براعم متكشفة باستخدام العقد الساقية كمنفصل نباتي على بيئات MS, WPM , B5 تحتوي على تركيزات مختلفة من البنزيل أمينو بيورين أو الكينيتين منفردين أو مضافا معهما حمض اندول بيوتريك . تم الحصول على أكبر قيم لعدد الفروع المتكونه لكل منفصل نباتي خلال الثلاث نقلات (8.3و 5.6 و 5.5) عند استخدام بيئة MS مضافا اليها 1.0 ملجم/لتر بنزيل أمينو بيورين + 0.2 ملجم /لتر اندول أسيتك أسيد ، 0.5 ملجم/لتر بنزيل أمينو بيورين + 0.1 ملجم /لتر اندول أسيتك أسيد، 0.5 ملجم/لتر بنزيل أمينو بيورين + 0.1 ملجم /لتر حمض اندول أسيتك أسيد للثلاث نقلات على التوالي.

***