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USING EXPLANT, MEDIA, CYTOKININE TYPES AND GELLING AGENTS ON PAULOWNIA HYBRID (*Paulownia elongata* × *Paulownia fortunei*) TISSUE CULTURE

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ABSTRACT

These experiments were carried out at (Prof. Dr. Abdel Fattah Helmy Belal) plant tissue culture laboratory, Faculty of Environmental Agricultural Sciences - Arish University, North Sinai, Egypt, during the period from 2018 to 2021. The aim of this study was to investigate the effect of some factors affecting micropropagation of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)* to cover the cumulative demand on this plant. Different media (MS, DKW, WPM and B5), explant types (shoot tip and nodal segment), cytokinin types (Kin, BA and 2Ip), gelling agent types (guar gum, gellan gum, gelrite, corn starch, wheat starch, and locust bean), and IBA concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0) mgl^{-1}, were studied to identify the most effective treatments for *in vitro* micropropagation of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)*. Results clarified the superiority of nodal segment over shoot tip, and superiority of MS medium over all other tested media. The addition of BA (3 mgl^{-1}) to MS medium was the most effective treatment for shoot proliferation compared with all other tested cytokinins. At the multiplication stage, results showed that the most effective gelling agent were agar alone and its combinations with gillan gum at 2 – 6 gl^{-1}. During rooting stage, using of IBA at 1mgl^{-1} was more effective on increasing root number and root length (cm) compared with control treatment.

INTRODUCTION

Paulownia is a genus belonging to the family *Paulowniaceae (Scrophulariacae)* indigenous to China and including nowadays over 20 species such as *P. elongata*, *P. fortunei*, *P. tomentosa*, *P. kawakami*, *P. australis*, *P. albiphloea*, *P. fargesi*, *P. taiwaniana* and *P. catalpifolia* **(Barton *et al.*, 2007)**. Paulownia is very adjustable, distributed to a wide degree and very fast growing under perfect conditions (**Zhu *et al.*, 1988**). It has been demonstrated that these species are a good cumulative of heavy metals at soils which are contaminated (**Azzarello *et al.*, 2012; Miladinova *et al.*, 2014**). Paulownia wood was used for making high strength installed boards which used in buildings. It is used for making furniture, boxes, moldings, delivery containers, doors and windows, musical instruments, chests and lightweight skis. It is also suitable for the constructions of beehive, toys, picture frames, fishing net floats, particleboard, and for shoes making. **(Rafighi and Tabarsa, 2012; Khanjanzadeh *et al.*, 2012)**. Also, the woods of paulownia is good for paper pulp making **(Latibari *et al.*, 2012).** In comparison with Eucalyptus, Paulownia paper is of high quality **(Feria *et al.*, 2013)**. Because of these features, Paulownia is one of the most important forestry trees in the world.

Tissue culture is a group of mechanisms that allow the regeneration of cells, tissues or organs of plants, from plant tissues or

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organs segments, by using nutrient solutions in aseptic and controlled conditions **(Lima *et al.*, 2012).** Tissue culture is a method that helps to produce high numbers in high quality planting materials **(Chesha *et al.*, 2015).**

Selection of the promising explant is a significant step in starting any *in vitro* study. Many authors showed that shoot organogenesis and callus induction were conditional on the explant source **(Passey *et al.*, 2003; Debnath, 2005).**

Murashige and Skoog (1962) stated that the morphogenesis of tissues and perfect growth may change from one plant to another according to their nutritional requirements. Furthermore, tissues which are from different organs of plants may also have various requirements for optimal growth.

Growth regulators play an essential role for developing a definite mode of growth in the cultured tissues or cells, which may be because of an assemblage of definite contents of biochemical in them. The different hormones in the medium cause the conservation of balanced and definite organic and inorganic contents in the growing tissue. This leads the tissues or cells to develop either into roots or shoots or even death **(Ikram and Dahot, 2007).**

The type and concentration of gelling agent is one of the essential factors which affect the physical and chemical characteristics of the culture medium *in vitro*. Media are assorted as liquid, semi-solid and solid based on physical form. Gelling agents are added to culture media to increase stickiness in which explants are not flooded in the medium **(Prakash *et al.*, 2000).**

Schaller *et al.* (2015) showed that cytokinin and auxin act together functionally, with functions that can be antithetically supportive and antagonistic, to supply robustness to evolution processes and to consult distinct cell fates to precursor cells in close proximity which is yielding a whole that is greater than the sum of its parts.

Based on the above-mentioned advantages of paulownia trees and tissue culture technique, this study aime to investigate the effect of some factors affecting micropropagation of *Paulownia hybrid* (*Paulownia elongata*×*Paulownia fortunei*) to cover the cumulative demand on this plant.

MATERIALS AND METHODS

These experiments were carried out at (Prof. Dr. Abdel Fattah Helmy Belal) plant tissue culture laboratory, Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt, during the period from 2018 to 2021 to assess the most effective treatments for *in vitro* micropropagation (establishment, multiplication, rooting and acclimatization) of *Paulownia hybrid (Paulownia elongata*× *Paulownia fortunei)*.

Explant Source

In vitro plantlets of *Paulownia hybrid (Paulownia elongata*×*Paulownia fortunei)* were obtained from plant tissue culture laboratory, faculty of Agriculture, Zagazig University. These plantlets were used as a source of explants. Plantlets were cut into small cuttings included shoot tips and nodal segments with about 2 cm length.

Establishment Stage

Effect of explant types

Shoot tips as well as single node cuttings were used as explant source to examin which one will be the most suitable type. Shoot tips or single node segment with about 2 cm long were excised and transferred to culture jars containing **Murashige and Skoog (1962)** medium supplemented with sucrose (30 gl^{-1}) and Agar (7 gl^{-1}). The medium was complemented

with benzyladenine BA (2 mgl^{-1}) and naphthalene acetic acid NAA (0.1 mgl^{-1}). The pH of the medium was adjusted to 5.8 with HCl or NaOH before adding agar and then the medium was autoclaved (1.3 kg/cm^2 for 20 minutes at 120 °C). After four weeks, number of shoots/ explant, number of leaves/ shoot, shoots length (cm) and numbers of nodes/ shoot were recorded.

Effect of medium type

Shoots derived from nodal explants were cut into about 2.0 cm nodal segment and recultured on four media types *i.e.*; MS medium **(Murashige and Skoog, 1962)**, DKW medium **(Driver and Kuniyuki, 1984)**, WPM medium **(Lloyd and McCown, 1980),** or B5 medium **(Gamborg *et.al.*, 1968)**, to detect the most effective medium type for regeneration of paulownia *(Paulownia elongata×Paulownia fortunei).* Each medium was supplemented with NAA (0.1 mgl^{-1}), BA (2 mgl^{-1}) and sucrose (30 gl^{-1}) and gelled with agar (7 gl^{-1}). Four explants were cultured in each jar. Cultures were incubated in the growth room for one month at the temperature of 25 ± 2 °C under 2000 Lux light intensity.

Effect of growth regulators

For shoot proliferation, nodal segments derived from 30-days-old shoots were used as explant source. Explants were cultured on MS medium containing sucrose (30 gl^{-1}) solidified with agar (7 gl^{-1}) and supplemented with different concentrations of growth regulators.

Kinetin (Kin)**,** benzyladenine (BA), 2-isobentenyl- adenine (2-ip) at different concentrations (0, 1, 2, 3 and 4 mgl^{-1}) to select the best type of cytokinin and the suitable concentration that could promote the highest proliferation rate. Media pH was adjusted to 5.8. Number of shoots per explant, number of leaves/ shoot and shoot length (cm) were recorded after 4 weeks from culture.

Multiplication Stage

Nodal segment with about 1.5 cm length and had two nodes were used as explants during multiplication stage. Four explants were cultured per jar.

Effect of different gelling agents

Six types of gelling agents have been tested (guar gum, gellan gum, gelrite, corn starch, wheat starch, and locust bean) in combination with agar in order to identify the most suitable combination. Pills of corn, guar, wheat and locust bean were bringed and ground at laboratory to obtain its' flour. MS media were supplemented with BA (4mgl^{-1}), NAA (0.1mgl^{-1}) and different combinations of solidifying agents as follows:

- Medium supplemented with 8 gl^{-1} agar was considered as control.
- Gellan gum at (2, 4, 6 and 8 gl^{-1}) + Agar at (6, 4, 2 and 0 gl^{-1}) respectively.
- Locust bean at (2, 4, 6 and 8 gl^{-1}) + Agar at (6, 4, 2, 0 gl^{-1}) respectively.
- Corn starch at (2, 4, 6 and 8 gl^{-1}) + Agar at (6, 4, 2, 0 gl^{-1}) respectively.
- Guar gum at (2, 4, 6 and 8 $gl^{-1)}$ + Agar at (6, 4, 2, 0 gl^{-1}) respectively.
- Gelrite at (2, 4, 6 and 8 gl^{-1}) + Agar at (6, 4, 2, 0 gl^{-1}) respectively.
- Wheat starch at (2, 4, 6 and 8 gl^{-1}) + Agar at (6, 4, 2, 0 gl^{-1}) respectively.

All Media pH were adjusted at 5.8 pH before autoclaving. Number of shoots per explant, number of leaves / shoot and shoot length (cm) were recorded after four weeks from culture.

Rooting Stage

During rooting stage shoots with about 5.0 cm length were cultured on half-strength MS basal medium supplemented with different concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mgl^{-1}) of IBA. All Media were supplemented with sucrose (30

gl^{-1}) and agar ($7gl^{-1}$). Media pH was adjusted at 5.8 before autoclaving. This experiment was designed to detect the most effective concentration of IBA. After four weeks, number of roots/ explant, root length (cm), shoot length (cm), number of shoots/ explant and number of leaves/ shoot were recorded.

Acclimatization Stage

Rooted shoots (about 10 cm length) were successfully acclimatized by transferring them to plastic pots (8 cm diameter) containing a pre-sterilized combination of peat moss + sand (1:1). Pots were covered with clear polyethylene pages then kept in a plant growth chamber at about 25°C under diffuse light conditions at 16/8 hr photoperiod and 95% – 98% relative humidity. The humidity was reduced gradually and after 4 weeks the plantlets were transferred from the growth chamber to green house.

Experimental Design and Statistical Layout

The statistical layout of all above mentioned experiments were completely randomized design (CRD) with three replications. All collected data were analyzed with analyses of variance (ANOVA) procedure using MSTAT-C Statistical Software Package (**Michigan State University, 1983**). Differences between means were compared by using Duncan multiple range test (**Gomez and Gomez, 1984**).

RESULTS AND DISCUSSION

Establishment Stage

Effect of explant type on shoot proliferation of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)* during establishment stage

Regarding the effect of explant type, two types of explant were tested in this experiment, it's noticed from results presented in Table 1 that using of nodal segment explant increased number of shoots/explant (1.40), number of leaves/ shoot (11.63), plantlet length (cm) (6.82) and number of nodes/ shoot (6.10)**,** number of shoots/explant compared with shoot tip explant which recorded lower values of these parameters (1.17, 9.90, 4.41, 5.31 respectively). The superiority of nodal segments is in the same line of the findings of many researchers who came to similar results **(Ipekci *et al.*, 2001**; **Arockiasamy *et al.*, 2002; Rahman *et al*., 2004; Kassim *et al*., 2010; El-Sawy *et al*., 2015)**.

Effect of medium type on shoot proliferation during establishment stage of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)*

Results presented in Table 2 and illustrated in Fig. 1A show that MS medium was more effective of concerning number of shoots/ explant (1.63), number of leaves/shoot (15.21), shoot length (6.83 cm) and number of nodes/shoot (7.33) as compared with the other three media tested. While DKW medium achieved the lowest values of these parameters (1.00, 4.67, 3.52, and 3.25 respectively) after 4 weeks from culture date. These results were agreed with the results obtained by **Ghatas (2016)** who stated that Murashige and Skoog medium was premium in increasing of explant development, survival percentage and greening parameters of Paulownia species in comparison with the other media types.

Effect of cytokinin types and concentrations on shoot proliferation during establishment stage of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)*

Results presented in Table 3 and illustrated in Fig. 1B show that the most effective cytokinin was benzyladenine (BA) especially at 4 mgl^{-1} since it produced the maximum number of shoots/explant (3.33), number of leaves/shoot (30.33) and shoot length (cm) (7.10). Whereas 4 mgl^{-1} Kin gave

Table 1. Effect of explant type on number of shoots/ explant, number of leaves/ shoot, shoot length (cm) and number of nodes/ shoot of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)* plants

Explant type	Number of shoots/explant	Number of leaves/shoot	Shoot length (cm)	Number of nodes/ shoot
Shoot tip	1.17 **b**	9.90 **a**	4.41 **b**	5.31 **b**
Nodal segment	1.40 **a**	11.63 **a**	6.82 **a**	6.10 **a**

Mean values followed by different letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability.

Table 2. Effect of media type on shoot proliferation of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)*

Medium type	Number of shoots/explant	Number of leaves/shoot	Shoot length (cm)	Number of nodes/shoot
MS	1.63 **a**	15.21 **a**	6.83 **a**	7.33 **a**
B5	1.33 **b**	12.75 **b**	6.56 **ab**	6.54 **b**
WPM	1.17 **bc**	10.42 **c**	5.54 **b**	5.71 **c**
DKW	1.00 **c**	4.67 **d**	3.52 **c**	3.25 **d**

Mean values followed by different letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability.

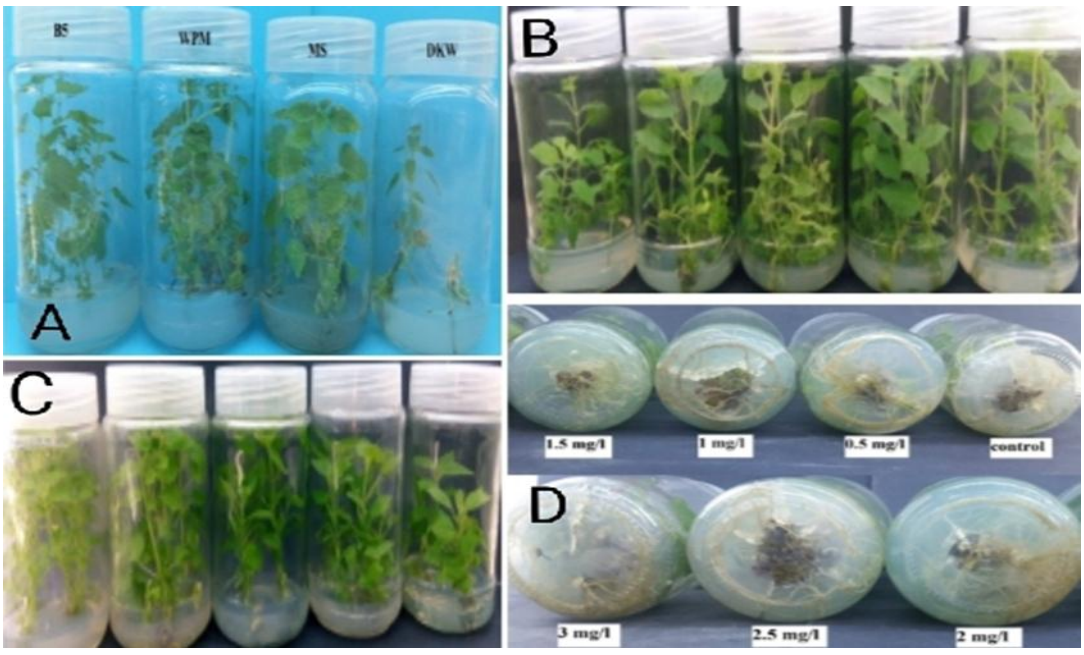

Fig. 1. Plantlet proliferation of *Paulownia hybrid* (*Paulownia elongata*×*Paulownia fortunei*)

A) Effect of different medium types on shoot proliferation of *Paulownia hybrid* (*Paulownia elongate* × *Paulownia fortunei*) using nodal segment. **B)** Shoot proliferation when benzyladenine (BA) at concentrations of (0, 1, 2, 3 and 4 mgl-1) was used as a growth regulator**. C)** Effect of addition of Gellan gum (Gellan gum at 2, 4, 6 and 8 gl^{-1} +Agar at 6, 4, 2 and 0 gl^{-1} respectively) to the plant medium**. D**) Effect of different concentrations of Indole-3-butyric acid (IBA) on root induction during rooting stage.

Table 3. Effect of cytokinin types and concentrations on shoot proliferation of *Paulownia hybrid (Paulownia elongata × Paulownia fortunei)*

Growth regulator (mgl^{-1})	Number of shoots/explant	Number of leaves/shoot	Shoot length (cm)
Control	1.33 **b**	15.00 **b-d**	4.75 **b**
1 BA	1.67 **b**	19.00 **bc**	4.83 **b**
2 BA	1.83 **b**	18.67 **bc**	4.66 **b**
3 BA	2.17 **ab**	23.33 **ab**	6.25 **ab**
4 BA	3.33 **a**	30.33 **a**	7.10 **a**
1 Kin	1.50 **b**	10.00 **cd**	5.42 **ab**
2 Kin	1.17 **b**	8.33 **d**	5.10 **ab**
3 Kin	1.83 **b**	12.00 **cd**	5.33 **ab**
4 Kin	1.33 **b**	8.00 **d**	5.67 **ab**
1 2Ip	1.67 **b**	9.83 **cd**	6.10 **ab**
2 2Ip	1.50 **b**	11.67 **cd**	5.17 **ab**
3 2iP	1.67 **b**	11.67 **cd**	5.83 **ab**
4 2ip	1.33 **b**	9.00 **d**	4.42 **b**

Mean values followed by different letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability.

the lowest values recorded with number of shoots/explant (1.33) and number of leaves/shoot (8.00) while the lowest shoot length (4.42 cm) was obtained with 4 mgl^{-1} 2ip after 4 weeks from culturing date. These results are agreed with those given by **Al-Tinawi *et al*. (2010)** on *Paulownia tomentosa* since they showed that the highest multiplication rate was achieved with BA (4.44 mgl^{-1}) + IBA (0.22 mgl^{-1}) + GA_3 (0.58 mgl^{-1}). Also, **Abd Elrazik (2012)** on *Paulownia tomentosa* found that the medium containing BA (4.44 mgl^{-1}) with NAA (0.5 mgl^{-1}) was the most effective treatment for raising shoot proliferation after 2 weeks from culture date. Also, **Ben Bahri and Bettaieb (2013)** found that the medium containing BA (1.00 mgl^{-1}) and IBA (0.25 mgl^{-1}) was the most effective treatment to promote shoot multiplication of *Paulownia tomentosa* (Thunb.). Moreover, **Fahmy and Gendy (2018)** on *Paulownia hybrid* (*P. elongata* × *P. fortunei*) obtained the best rate of shoot multiplication when the medium was supplemented with 8.0 mgl^{-1} BA. Similarly, **Hamza (2019)** reported that BA concentrations gave high number of proliferated shoots of *Paulownia tomentosa*. He obtained the maximum number of shoots per explant from the concentration of 3.0 mgl^{-1}.

Multiplication's Stage

Effect of different gelling agent types and concentrations on shoot proliferation during multiplication stage of *Paulownia hybrid (Paulownia elongata ×Paulownia fortunei)*

Results presented in Table 4 and Fig. 1C confirm that the most effective gelling agent

Table 4. Effect of different types and concentrations of gelling agents on shoot proliferation of *Paulownia hybrid (Paulownia elongate × Paulownia fortunei)*

Gelling agent	No. of shoots/explant	No. of leaves/shoot	Shoot hength (cm)
Control (8 gl^{-1} Agar)	1.67 a	8.67 a-e	6.63 a-f
Gellan gum 2 gl^{-1} + Agar 6 gl^{-1}	2.33 a	10.67 ab	9.67 a
Gellan gum 4 gl^{-1} + Agar 4 gl^{-1}	1.33 ab	9.33 a-d	6.33 a-f
Gellan gum 6 gl^{-1} + Agar 2 gl^{-1}	1.67 a	7.33 a-e	6.33 a-f
Gellan gum 8 gl^{-1} + Agar 0 gl^{-1}	1.33 ab	6.67 b-e	4.00 d-f
Guar gum 2 gl^{-1} + Agar 6 gl^{-1}	1.33 ab	6.00 c-e	5.33 b-f
Guar gum 4 gl^{-1} + Agar 4 gl^{-1}	1.00 ab	4.67 e	3.50 e-g
Guar gum 6 gl^{-1} + Agar 2 gl^{-1}	1.00 ab	4.67 e	3.00 fg
Guar gum 8 gl^{-1} + Agar 0 gl^{-1}	0.00 b	0.00 f	0.00 g
Locust bean 2 gl^{-1} + Aga 6 gl^{-1}	2.00 a	8.67 a-e	6.67 a-f
Locust bean 4 gl^{-1} + Agar 4 gl^{-1}	1.33 ab	7.33 a-e	6.00 a-f
Locust bean 6 gl^{-1} + Agar 2 gl^{-1}	1.00 ab	5.33 de	4.83 c-f
Locust bean 8 gl^{-1} + Agar 0 gl^{-1}	0.00 b	0.00 f	0.00 g
Corn starch 2 gl^{-1} + Agar 6 gl^{-1}	1.33 ab	11.33 a	7.33 a-d
Corn starch 4 gl^{-1} + Agar 4 gl^{-1}	1.00 ab	8.00 a-e	6.00 a-f
Corn starch 6 gl^{-1} + Agar 2 gl^{-1}	0.00 b	0.00 f	0.00 g
Corn starch 8 gl^{-1} + Agar 0 gl^{-1}	0.00 b	0.00 f	0.00 g
Wheat starch 2 gl^{-1} + Agar 6 gl^{-1}	1.67 a	8.00 a-e	5.67 b-f
Wheat starch 4 gl^{-1} + Agar 4 gl^{-1}	1.67 a	7.33 a-e	5.00 b-f
Wheat starch 6 gl^{-1} + Agar 2 gl^{-1}	2.00 a	8.00 a-e	5.67 b-f
Wheat starch 8 gl^{-1} + Agar 0 gl^{-1}	0.00 b	0.00 f	0.00 g
Gelrite 2 gl^{-1} + Agar 6 gl^{-1}	1.33 ab	9.33 a-d	4.67 c-f
Gelrite 4 gl^{-1} + Agar 4 gl^{-1}	2.00 a	8.67 a-e	8.00 a-c
Gelrite 6 gl^{-1} + Agar 2 gl^{-1}	2.33 a	10.67 ab	8.67 ab
Gelrite 8 gl^{-1} + Agar 0 gl^{-1}	1.67 a	10.00 a-c	7.00 a-e

Mean values followed by different letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability.

treatments were agar alone and its combinations with gellan gum at 2-6 gl^{-1}. these treatments resulted in the maximum values of all recoded parameters without significant differences among them. These results are in the same way with those found by **Jaime and Tanaka (2009)** who reported that gellan gum resulted in greater PLB (protocorm-like bodies) regeneration and callus formation of hybrid Cymbidium compared with all other gelling agents studied which included agar, corn starch, bacto agar, oatmeal agar, phytagel, guar gum, isubgol and potato dextrose agar.

Rooting Stage

Effect of different concentrations of Indole-3-butyric acid (IBA) on root proliferation during rooting stage of *Paulownia hybrid (Paulownia elongata × Paulownia fortunei)*

Results presented in Table 5 and illustrated in Fig.1D, show that the addition of IBA increased the number of roots and root length, while the rest of parameters did not significantly respond to supplementation the medium with IBA. Since there were no significant differences among IBA concentrations, from the economical point of view it is recommended to use the lowest concentration (0.5 mgl^{-1}) of IBA. These results are at the same side as those obtained by **Abd El-Kader (2004)** who demonstrated that the best rooting of *Taxodium distichum* was occurred when 1.00 mgl^{-1} IBA was used. Also, **Meftahizada *et al.* (2010)** reported that for *Melissa officanalis*, addition of IBA at 1.00 mgl^{-1} induced rooting in 64% of shoots. Moreover**, Asghar *et al*. (2016)** mentioned that rooting was obtained with the highest frequency in *Rosa damascene* when cultured on a medium included 1.00 mgl^{-1} IBA.

Table 5. Effect of different concentrations of Indole-3-butyric acid (IBA) on root proliferation of *Paulownia hybrid (Paulownia elongatea × Paulownia fortunei)*

IBA concentration (mgl^{-1})	No. of shoots/explant	No. of leaves/shoot	Shoot length (cm)	Number of roots/shoot	Root length (cm)
0.0	1.33 **a**	8.67 **a**	6.00 **a**	1.33 **b**	2.67 **d**
0.5	1.33 **a**	14.67 **a**	8.33 **a**	3.67 **a**	9.00 **ab**
1.0	2.00 **a**	16.67 **a**	2.00 **b**	4.67 **a**	11.00 **a**
1.5	2.33 **a**	13.33 **a**	6.67 **a**	3.33 **a**	6.33 **b-d**
2.0	2.00 **a**	14.00 **a**	7.00 **a**	3.67 **a**	7.33 **a-c**
2.5	1.33 **a**	12.67 **a**	8.33 **a**	1.67 **b**	4.00 **cd**
3.0	1.33 **a**	12.00 **a**	7.00 **a**	1.33 **b**	3.50 **cd**

Mean values followed by different letter(s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability.

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الملخص العربي

استخدام أنواع من المنفصلات النباتية، البيئات، السيتوكينينات وعوامل تكوين الحالة الجلية في زراعة الأنسجة لنبات الباولونيا

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أجريت هذه الدراسة بمعمل الأستاذ الدكتور/ عبدالفتاح حلمي بلال لزراعة الأنسجة النباتية، كلية العلوم الزراعية البيئية، جامعة العريش، شمال سيناء، مصر وذلك خلال الفترة من ٢٠١٨ م الى ٢٠٢١ م. الهدف من هذه الدراسة هو إنتاج عدد أكبر من نبات الباولونيا المهجن في وقت قصير وبتكلفة أقل، إلى جانب إيجاد إجراءات بديلة للإجراءات التقليدية لإنتاج هذا النبات لتلبية الطلب المتزايد عليه. في مرحلة التأسيس تم دراسة إستخدام أنواع مختلفة من البيئات الغذائية (بيئة موراشيجي وسكوج، بيئة النباتات الخشبية، بيئة جامبورج، وبيئة DKW) وأنواع مختلفة من الأجزاء النباتية (القمة النامية، السلامية ذات العقدة الواحدة) بالإضافة إلى أنواع وتركيزات مختلفة من السيتوكينينات (البنزيل أدينين، والكنتين، وأيزوبنتينيل أدينين) وتأثير ذلك على صفات طول النبيتة (سم) وعدد الأفرع وعدد الأوراق لكل نبات. أما في مرحلة التضاعف تم دراسة أنواع وتركيزات مختلفة من مواد تكوين الحالة الجلية (صمغ الجوار، صمغ الجيلان، الجيلريت، نشا الذرة، نشا القمح، مسحوق بذور الخروب)، ولتجذير النبات تم دراسة تأثيرإضافة تركيزات مختلفة من إندول حامض البيوتيريك (٠,٠ ، ٠,٥ ، ١,٠ ، ١,٥ ، ٢,٠ ، ٢,٥ و٣,٠ ملجم/لتر). كذلك تمت أقلمة النبيتات لإيجاد بروتوكول كامل لإنتاج نبات الباولونيا المهجن بإستخدام تقنية زراعة الأنسجة النباتية. وقد أظهرت النتائج تفوق إستخدام السلامية ذات العقدة الواحدة كجزء نباتي منزرع مقارنة بالقمة النامية، كما تفوقت بيئة موراشيجي وسكوج MS على جميع البيئات المستخدمة في الدراسة، وأدت إضافة البنزيل أدينين BA بتركيز ٤ ملجم/ لتر مع ١ ملجم /لتر من نفتالين حامض الخليك الأكثر تأثيرا على النمو الخضري أكثر من أي سيتوكينين آخر. في مرحلة التضاعف، كان أفضل خليط لتكوين الحالة الجلية والذي أعطى أفضل نتائج للتضاعف هو إستخدام ٦ جم/لتر جيلريت + ٢ جم/لتر آجار). أما في مرحلة التجذير، كان أفضل تركيز لأندول حامض الخليك والذي أعطى أفضل نتائج لعدد الجذور وطول الجذور وطول النبات وعدد الاوراق وعدد الأفرع هو (١ ملجم/ لتر) وذلك بعد ٤ أسابيع من تاريخ الزراعة.

الكلمات الإسترشادية: نبات الباولونيا، السيتوكينينات، الأوكسينات، عوامل تكوين الحالة الجلية.وجية والخسارة الاقتصادية.

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