

Effect of Plant Growth Regulators on *In Vitro* Direct Organogenesis of *Paulownia tomentosa* plant

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ABSTRACT

In vitro propagation of *P. tomentosa* (Thunb.) Steud. was performed in the Tissue Culture Laboratory of the Department of Agricultural Botany to find out the effect of plant growth regulators (PGRs) on the ability of stem internodes of *Paulownia* Plant to regenerate direct organs as well as on their morphological characteristics. *Paulownia* stem internodes obtained from seeds planted *in vitro* were cultured on Murashige and Skoog (MS) medium with supplements of different combinations of α -naphthalene acetic acid (NAA) with Benzyladenine (BA), and NAA with 2-isopentenyl adenine (2ip). The results showed that the combinations of 0.5 mg L⁻¹ NAA with 2 mg L⁻¹ 2ip, as well as 0.5 mg L⁻¹ NAA with 4 mg L⁻¹ BA gave the highest regeneration percentage, shoot length (cm), shoot fresh and dry weights (g). For root regeneration, the obtained shoots were excised and cultured on a rooting medium containing half-strength MS salts with different concentrations of indole-3-butyric acid (IBA) and NAA (each individually). It was observed that either the concentration of 1.0 mg L⁻¹ NAA or 2.0 mg L⁻¹ IBA resulted in the highest values of rooted shoots percentage, No. of roots per shoot, average root length (cm), and fresh and dry weights (g).

KEYWORDS: Direct organogenesis, growth regulators, morphological characteristics, *Paulownia tomentosa*, stem internodes.

1. INTRODUCTION

Paulownia is a genus belonging to family Paulowniaceae (Scrophulariaceae) indigenous to China and including nowadays over 20 species (El-Homosany and Noor El-Deen, 2019). It is a deciduous tree, up to 15 m tall, with heart-shaped leaves arranged in opposite pairs on the stem. *Paulownia* is very adaptable and extremely fast-growing under optimum conditions. It is famous for ornamental use, timber production. Also, different plant parts are used in Chinese herbal medicine (Puxeddu *et al.*, 2012; Ben Bahri and Bettaieb, 2013).

Propagation of *Paulownia* tree can be carried out through seeds, seedlings, stem and root cuttings, however, it is very difficult and time consuming (Zayova *et al.*, 2013). For that purpose, micropropagation techniques provide promising tools in the mass production of many important plant species as they offer rapid methods for the production of high quality stocks (Ben Bahri and Bettaieb, 2013). In that concern, many researchers reported the procedure of *Paulownia* micropropagation through shoot bud regeneration from leaf explants (Rao *et al.*,

1996; Ozaslan *et al.*, 2005), root explants (Ozaslan *et al.*, 2005), nodal and internodal explants and/or shoot tips (Castillo-Martinez *et al.*, 2012; Ben Bahri and Bettaieb, 2013; Zayova *et al.*, 2013; Ghatas 2016).

For the multiplication stage, axillary shoot proliferation was performed on MS medium containing cytokinins (BAP) and auxins (NAA) as PGRs (Zayova *et al.*, 2013). It was found that the best *in vitro* culture conditions for plant regeneration of *Paulownia elongata* through internodes as obtained when applying a protocol for direct organogenesis that could use the basal MS medium with addition of different concentrations of BA and NAA, where, internodal segments showed a better induction by producing 83% explants with shoots and 1.52 shoots per explant with the 0.20 mg /l NAA and 4.00 mg L⁻¹ BA (Castillo-Martinez *et al.*, 2012). For root regeneration, addition of IBA to half strength MS medium was more effective than NAA and IAA achieving the highest percentage of rooting and the maximum number of roots/plant for *P. elongata* (Zayova *et al.*, 2014).

This study aims to clarify the effect of PGRs on the ability of stem internodes of *Paulownia* plant to

regenerate direct organs as well as on their morphological characteristics.

2. MATERIALS AND METHODS

This study was carried out on *Paulownia tomentosa* plant during the period from 2017 to 2019 in the Tissue Culture Lab., Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.

2.1. Establishment of sterile intact seedlings

Ripe *Paulownia tomentosa* seeds (were secured from Orman Botanic Garden, Giza governorate, Egypt) were surface-sterilized through flooded in ethyl alcohol 70% for 1 min, soaked in 0.1% (w/v) mercuric chloride (HgCl_2) solution for 3 minutes, then rinsed for 15 min in sterile distilled water (3 times).

2.2. Culture media and culture conditions

The seed germination medium was tested to check its effectiveness in promoting germination and subsequent development of seeds. Basal MS (Murashige and Skoog, 1962) medium was supplemented with 30 g L⁻¹ sucrose and 2 g L⁻¹ gelrite. pH of medium was adjusted to 5.7 before autoclaving at 1.1 kg/cm² and 121 °C for 20 min

2.3. Shoot regeneration

Segments from stems derived from 45-days-old aseptic seedlings were used for shoot regeneration. Stems were cultured on MS medium supplemented with different combinations of NAA (0.0, 0.2, 0.5, and 1.0 mg L⁻¹) and BA (0.0, 2.0, 4.0, and 8.0 mg L⁻¹), as well as various formulations of NAA (0.0, 0.2, 0.5, and 1.0 mg L⁻¹) and 2ip (0.0, 2.0, 4.0, and 8.0 mg L⁻¹). After 3 weeks of culture, the explants began to regenerate shoots, and after 5 weeks of culture, the frequency of explants producing shoots was recorded and used to calculate the regeneration ratio.

2.4. Root regeneration

For root formation, *in vitro* regenerated well-developed and elongated shoots were excised and cultured on rooting medium containing half-strength MS salts with different concentrations of each of IBA (0.0, 0.5, 1.0, and 2.0 mg L⁻¹) and NAA (0.0, 0.5, 1.0, and 2.0 mg L⁻¹) individually. All cultures were incubated under controlled environmental conditions (26±2°C, 3000 lux light intensity using white inflorescence tubes, 16/8 h light/dark, 60% relative humidity).

2.5. Data record

Number of explants regenerated shoots per treatment was recorded and used to calculate the regeneration ratio as follows:

$$\text{Regeneration \%} = \frac{\text{No. of explants regenerated shoots/treatment}}{\text{No. of explants/treatment}}$$

After shoot regeneration, the following data were recorded: Shoot length (cm), Shoot fresh weight (g), Shoot dry weight (g), and after regeneration root, the following data were recorded: No. of rooted shoots, No. of roots/shoot, Average root length (cm) and Root fresh and dry weights (g).

2.6. Acclimatization of plantlets

For hardening, plantlets with developed roots were removed from jars, washed thoroughly and gently with tap water to remove the traces of agar sticking, then transplanted into polyethylene bags containing a mixture of clay, sand, and peat moss in 1:1:1 ratio, covered with transparent bags. The bags were constantly punctured and sprayed with water through a sprinkler when needed in order to gradually adapt to the external environment. This process continued for a month, after which the plants are transferred to an open field.

2.7. Statistical Analysis

Experiments were conducted in completely randomized block design; thirty explants were used for each treatment in 3 replicates. Calculated means were compared using least significant difference test (L.S.D) at 5% level as described by Snedecor and Cochran (1972).

3. RESULTS AND DISCUSSION

3.1. Shoot regeneration

Results of shoot regeneration from stem internodes cultured on MS medium supplemented with different concentrations and combinations of NAA (0.0, 0.2, 0.5, and 1.0 mg L⁻¹) and 2ip (0.0, 2.0, 4.0, and 8.0 mg L⁻¹) are shown in Table (1). Data clarified that the absence of growth regulators or the addition of only auxin had led to the failure of direct shoot regeneration process, while using different combinations of the cytokinin (2ip) and the auxin (NAA) together, or the use of different concentrations of the cytokinin only, led to different ratios of direct shoot regeneration, with significant differences in most cases.

The concentration of 0.5 mg L⁻¹ NAA and 2 mg L⁻¹ 2ip (auxin: cytokinin ratio=1:4) recorded the

Table 1. Effect of MS medium supplemented with NAA (0.0, 0.2, 0.5, and 1.0 mg L⁻¹) and 2ip (0.0, 2.0, 4.0, and 8.0 mg L⁻¹) on direct shoot regeneration of *Paulownia* stems.

NAA (mg L ⁻¹)	2ip (mg L ⁻¹)	Regeneration (%)	Shoot length (cm)	FW (g)	DW (g)
0.0	0.0	0	0	0	0
0.0	2.0	85	9.0	7.2	0.81
0.0	4.0	80	8.2	6.5	0.71
0.0	8.0	43	5.5	4.5	0.42
0.2	0.0	0	0	0	0
0.2	2.0	85	8.4	7.6	0.75
0.2	4.0	77	8.0	6.3	0.65
0.2	8.0	77	7.5	6.1	0.62
0.5	0.0	0	0	0	0
0.5	2.0	95	10.3	8.1	0.84
0.5	4.0	88	8.5	7.0	0.72
0.5	8.0	82	8.2	6.5	0.66
1.0	0.0	0	0	0	0
1.0	2.0	77	8.0	6.3	0.71
1.0	4.0	75	7.6	6.0	0.65
1.0	8.0	50	6.1	5.8	0.55
LSD_{0.05}		3.3	0.8	0.5	0.03

highest regeneration percentage, shoot length (cm), shoot fresh and dry weights (g); 95%, 10.3 cm, 8.1 g and 0.84 g, respectively (Fig. 1 a). While the concentration of 8 mg L⁻¹ 2ip only recorded the lowest

regeneration percentage, shoot length (cm), shoot fresh and dry weight (g); 43%, 5.5 cm, 4.5 g, 0.42 g, respectively (Fig. 1 b).

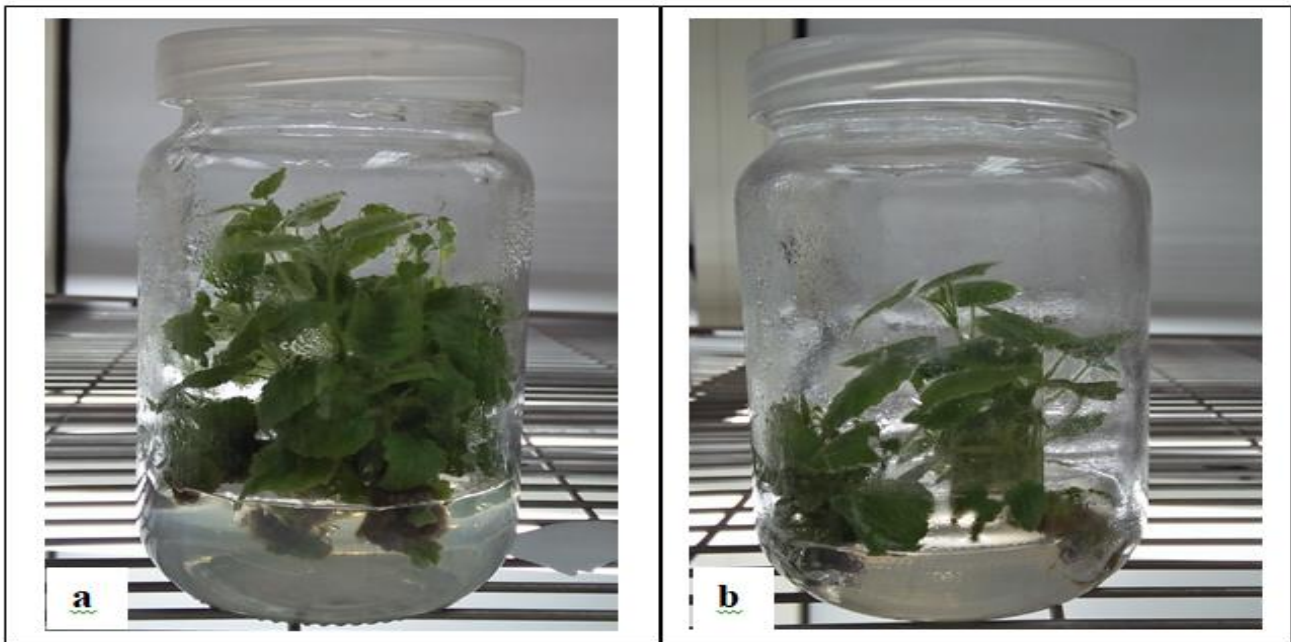


Fig. 1. Shoot regeneration from stem explants of *Paulownia* cultured on MS medium supplemented with (a) 0.5 mg L⁻¹ NAA + 2 mg L⁻¹ 2ip, (b) 8 mg L⁻¹ 2ip.

Table (2) presents data on stems cultured on MS medium supplemented with different concentrations and combinations of NAA (0.0, 0.2, 0.5, and 1.0 mg L⁻¹) and BA (0.0, 2.0, 4.0, and 8.0 mg L⁻¹) to regenerate shoots. It was observed that the hormone free-medium or that containing only auxin did not regenerate any adventitious buds whereas various concentrations of BA alone or in combination with NAA mostly led to significant different ratios of direct regeneration of adventitious buds. The combination of 0.5 mg L⁻¹ NAA and 4 mg L⁻¹ BA (auxin:cytokinin ratio=1:8) significantly scored the highest values of regeneration percentage, shoot length (cm), shoot fresh and dry weights (g); 90%, 9.2 cm, 9.2 and 1.0 g, respectively (Fig. 2a). While, the concentration of 2 mg L⁻¹ BA alone clearly recorded the lowest values of regeneration percentage, shoot length (cm), shoot fresh and dry weight (g); 60%, 7.1 cm, 6.5 and 0.66 g, respectively (Fig. 2b).

From the previous results (Tables, 1 and 2), it was found that auxin to cytokinin ratio played an important role in the ability of stem internodes of

Paulownia plant to regenerate shoots directly. The above mentioned results were in agreement with the results reported by Perez- and Ochoa-Alejo (1997) on *Citrus reticulata*, Taha *et al.* (2008) on *Paulownia*, Samantaray and Maiti (2011) on *Chlorophytum arundinaceum* and Castillo-Martinez *et al.* (2012) on *Paulownia elongata*. According to Samantaray and Maiti (2011), the cytokinin-auxin balance is essential for the regulation of apical dominance and some morphogenetic processes in plants *e.g.* multiplication and shoot formation. Moreover, interaction between endogenous PGRs and those present in the culture media is another factor influencing the above-mentioned processes during *in vitro* culture. The *in vitro* induction of adventitious shoots is affected by various factors such as type and composition of basal medium, as well as concentration and combinations of PGRs (Feng *et al.*, 2010). Besides, auxin-cytokinin combinations were proved to be essential in enhancing the rate of shoot bud regeneration in plants (Samantaray *et al.*, 2009; Samantaray and Maiti, 2011).

Table 2. Effect of MS medium supplemented with NAA (0.0, 0.2, 0.5, and 1.0 mg L⁻¹) and BA (0.0, 2.0, 4.0, and 8.0 mg L⁻¹) on direct shoot regeneration of Paulownia stems.

NAA (mg L ⁻¹)	BA (mg L ⁻¹)	Regeneration (%)	Shoot length (cm)	FW (g)	DW (g)
0.0	0.0	0	0	0	0
0.0	2.0	60	7.1	6.5	0.66
0.0	4.0	80	8.0	7.9	0.80
0.0	8.0	62	7.5	7.5	0.70
0.2	0.0	0	0	0	0
0.2	2.0	80	8.5	7.5	0.75
0.2	4.0	87	8.6	8.2	0.80
0.2	8.0	83	7.5	7.1	0.72
0.5	0.0	0	0	0	0
0.5	2.0	85	8.4	7.6	0.74
0.5	4.0	90	9.2	9.2	1.00
0.5	8.0	80	9.0	8.8	0.85
1.0	0.0	0	0	0	0
1.0	2.0	67	7.5	6.5	0.68
1.0	4.0	72	7.8	7.3	0.75
1.0	8.0	71	7.0	6.8	0.70
LSD _{0.05}		3.8	0.5	0.2	0.05

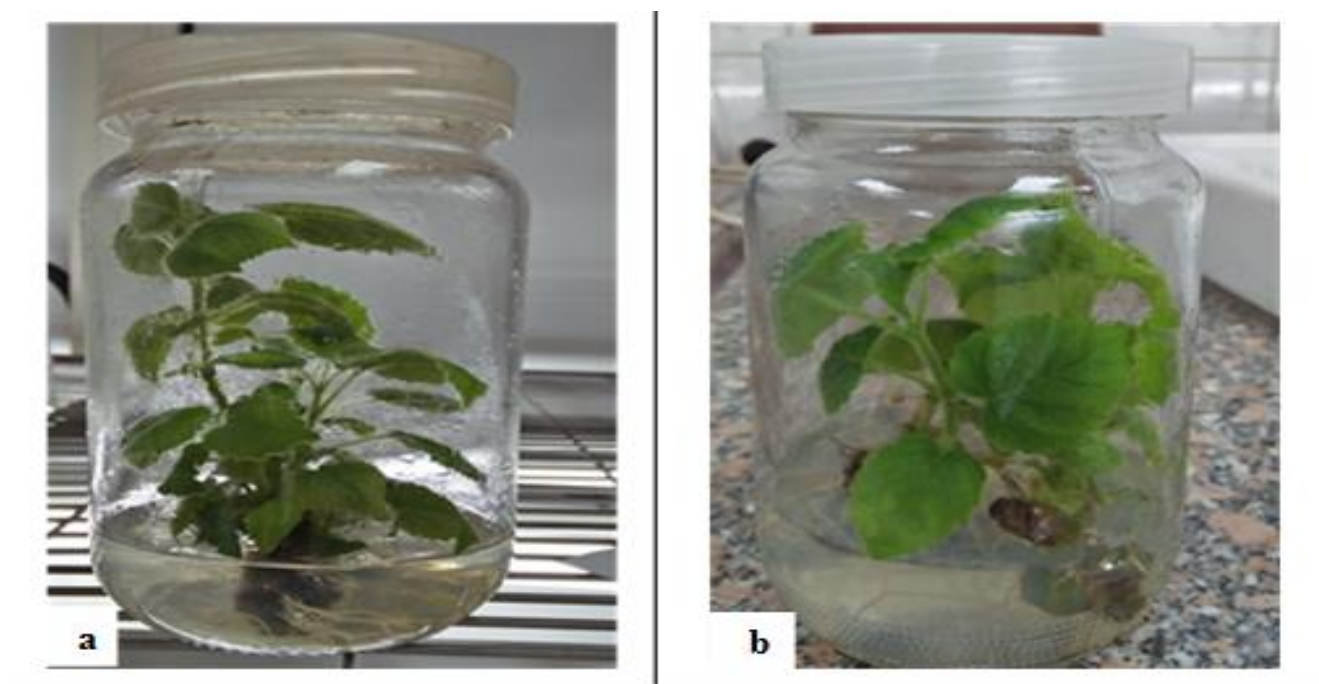


Fig. 2. Shoot production from stem explants of *Paulownia* cultured on MS medium supplemented with (a) 0.5 mg L⁻¹ NAA + 4 mg L⁻¹ BA, (b) 2 mg L⁻¹ BA.

3.2. Root regeneration

Table (3) shows the data of root formation on the shoot initiated on MS medium supplemented with a combination of 0.5 mg L⁻¹ NAA and 2 mg L⁻¹ 2ip (best shoot regeneration ratio) and cultured in half-strength MS medium with different concentrations of NAA (0.0, 0.5, 1.0, and 2.0 mg L⁻¹) or IBA (0.0, 0.5, 1.0, and 2.0 mg L⁻¹). It was observed that concentration of 1.0 mg L⁻¹ NAA or 2.0 mg L⁻¹ IBA gave the highest values of rooted shoots percentage,

no. of roots per shoot, average root length (cm), fresh and dry weights (g) as follows; (89%, 82%), (5.0, 5.0), (4.7, 4.2 cm), (0.95, 0.90 and 0.10, 0.08 g), respectively. Whereas, using of 0.5 mg L⁻¹ NAA or IBA recorded the lowest values of rooted shoots percentage, no. of roots per shoot, average root length (cm), fresh and dry weight (g) as follows; 75%, 4.0, 3.8 cm, 0.50 and 0.04 g, respectively, with 0.5 mg L⁻¹ NAA or 66%, 3.0, 3.2 cm, 0.50 and 0.04 g, respectively with 0.5 mg L⁻¹ IBA.

Table 3. Effect of auxins in half-strength MS medium on root formation of regenerated shoots of *Paulownia* (Shoot regeneration medium contained 0.5 mg L⁻¹ NAA and 2.0 mg L⁻¹ 2ip).

Auxin Type	Concentration (mg L ⁻¹)	% of rooted shoots	No. of roots per shoot	Average root length (cm)	Root FW (g)	Root DW (g)
NAA	0.0	0.0	0.0	0.0	0.00	0.00
	0.5	75	4.0	3.8	0.50	0.04
	1.0	89	5.0	4.7	0.95	0.10
	2.0	80	5.0	4.5	0.80	0.09
IBA	0.0	0.0	0.0	0.0	0.00	0.00
	0.5	66	3.0	3.2	0.50	0.04
	1.0	71	4.0	3.8	0.73	0.06
	2.0	82	5.0	4.2	0.90	0.08
LSD _{0.05}		3.1	1.2	0.2	0.08	0.02

Data in Table (4) clarifies the root formation initiated on shoots by using MS medium with addition of a combination of 0.5 mg L⁻¹ NAA and 4.0 mg L⁻¹ BA (second best shoot regeneration ratio) and cultured in half-strength MS medium with different concentrations of NAA (0.0, 0.5, 1.0, and 2.0 mg L⁻¹) or IBA (0.0, 0.5, 1.0, and 2.0 mg L⁻¹). The results obtained showed that the concentration of 1.0 mg L⁻¹ NAA or 2.0 mg L⁻¹ IBA gave the highest records of rooted shoots percentage, no. of roots per shoot, average root length (cm), fresh and dry weights (g) as their values were; (78%, 73%), (4.0, 3.0), (3.9, 3.8 cm), (0.86, 0.77 and 0.09, 0.08 g), respectively. On the other side, using of 0.5 mg L⁻¹ NAA or IBA recorded the lowest records of rooted shoots percentage, no. of roots per shoot, average root length (cm), fresh and dry weight (g) as their values were; 70%, 3.0, 3.2 cm, 0.45 and 0.04, respectively, with 0.5 mg L⁻¹ NAA or 65%, 3.0, 3.4 cm, 0.65 and 0.06 g, respectively, with 0.5 mg L⁻¹ IBA.

From the results of Tables 3 and 4, it is clear that the ratio of auxin to cytokinin in the direct shoot regeneration medium had a clear effect on its response to the type and level of auxin used in the rooting medium. The previous results were in agreement with those obtained by many researchers. Most authors indicated that IBA was the best auxin in root induction of *Paulownia* (Rout *et al.*, 2001; Ben Bahri and Bettaieb, 2013; Shtereva *et al.*, 2014; Zayova *et al.*, 2014) with a similar result concluded by Samantaray and Maiti (2011) on *Chlorophytum arundinaceum*. On the other hand, Taha *et al.* (2008) reported that NAA at 0.5 and 1 mg L⁻¹ were more effective in terms of rooting percentage and number of roots per shootlet of *Paulownia kowakamii*, while Feng *et al.* (2010) deduced that half strength MS medium supplemented with NAA was optimal for rooting of jujube ‘Huizao’. In conclusion, the presence of an auxin in the culture medium is essential for root induction.

Table 4. Effect of auxins in half-strength MS medium on root formation of regenerated shoots of Paulownia (Shoot regeneration medium contained 0.5 mg L⁻¹ NAA and 4.0 mg L⁻¹ BA).

Auxin Type	Concentration (mg L ⁻¹)	% of rooted shoots	No. of roots per shoot	Average root length (cm)	Root FW (g)	Root DW (g)
NAA	0.0	0.0	0.0	0.0	0.00	0.00
	0.5	70	3.0	3.2	0.45	0.04
	1.0	78	4.0	3.9	0.86	0.09
	2.0	75	3.0	3.5	0.65	0.06
IBA	0.0	0.0	0.0	0.0	0.00	0.00
	0.5	65	3.0	3.4	0.65	0.06
	1.0	67	3.0	3.5	0.75	0.07
	2.0	73	3.0	3.8	0.77	0.08
LSD _{0.05}		4.3	0.9	0.2	0.10	0.02

3.3. Acclimatization of plantlets

The well rooted shoots of *Paulownia* (Fig. 3a) were transferred *ex vitro* to acclimate to the natural weather conditions in polyethylene bags containing a mixture of clay, sand, and peat moss in 1:1:1 ratio (Fig. 3b), more than 90 % of the acclimatized plants successfully survived (Fig. 3c). Peat-soil mixtures were found effective for the acclimatization of *Paulownia* rooted shoots (Ben Bahri and Bettaieb, 2013; Zayova *et al.*, 2014).

4. CONCLUSION

Due to difficulty of propagating *Paulownia* by seeds, it is possible to obtain it through tissue culture

after acclimatization. It could be concluded from the previous results that, *Paulownia* can be regenerated via *in vitro* culture using MS-medium supplemented with a combination of 0.5 mg L⁻¹ NAA and 2 mg L⁻¹ 2ip or 0.5 mg L⁻¹ NAA and 4 mg L⁻¹ BA. *In vitro* root regeneration was achieved at 1.0 mg L⁻¹ NAA or 2.0 mg L⁻¹ IBA, each individually.

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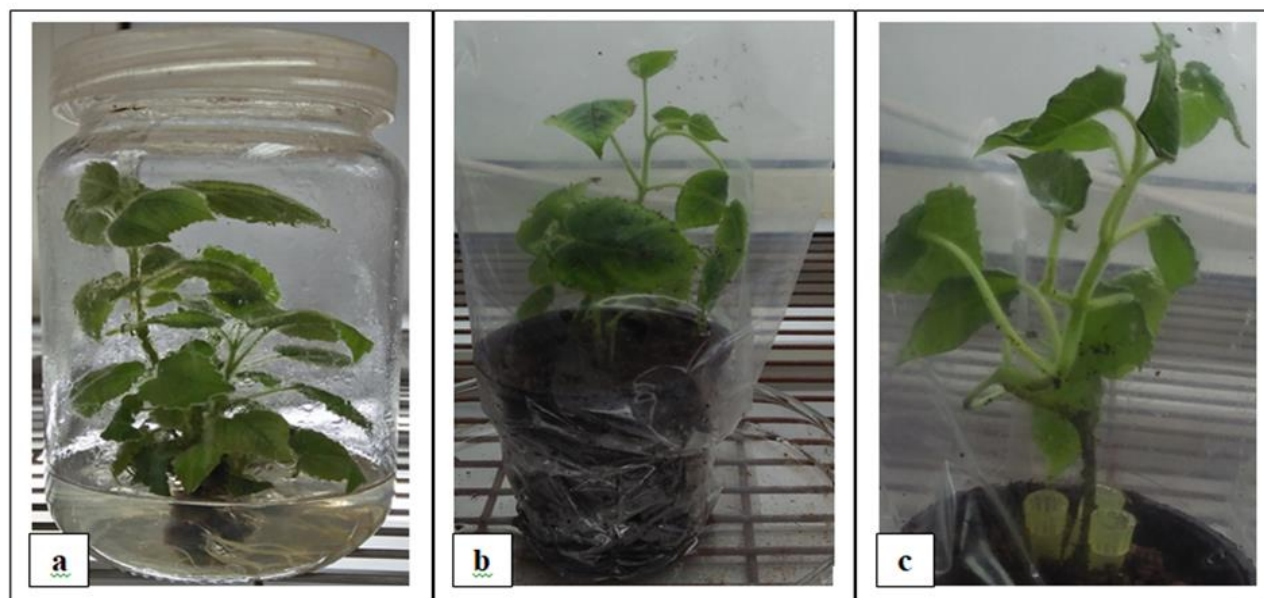


Fig. 3. Acclimatization stages of plantlets, (a) Root formation, (b) *ex vitro* acclimatization, (c) *Paulownia tomentosa* plantlets under natural conditions.

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الملخص العربي

تأثير منظمات النمو النباتية على التخلق المباشر للأعضاء معمليا في نبات البولونيا

زينب قاسم طه و انجي علاء سليم

قسم النبات الزراعي، كلية الزراعة، جامعة القاهرة، جيزه، مصر

تم إكثار نبات البولونيا في معمل زراعة الانسجة التابع لقسم النبات الزراعي لمعرفة تأثير منظمات النمو على قدرة سلاميات ساق النبات على التجدد المباشر للأعضاء وعلى خصائصها المورفولوجية. تم زراعة سلاميات ساق النبات الناتج من زراعة البذرة داخل المعمل على بيئة MS في وجود توليفات مختلفة من منظمات النمو (نفتالين حامض الخليك مع البنزويل أدينين وأيضا مع ٢ أيزو بنتينايل أدينين). وقد أظهرت النتائج أن التركيز ٠.٥ مجم/لتر من نفتالين حامض الخليك مع ٢ مجم/لتر من ٢ أيزو بنتينايل أدينين والتركيز ٠.٥ مجم/لتر من نفتالين حامض الخليك مع ٤ مجم/لتر من البنزويل أدينين قد أعطت أعلى نسبة تجدد مباشر للأعضاء وكذلك طول المجموع الخضري (سم) والوزن الطازج والجاف للمجموع الخضري (جم). أما بالنسبة لتكوين الجذور فقد تم زراعة المجموع الخضري على بيئة MS نصف تركيز تحتوي على تركيزات مختلفة من نفتالين حامض الخليك و إندول البيوتريك (كلا على حده). وقد لوحظ أن استخدام تركيز ١.٠ مجم/لتر من نفتالين حامض الخليك أو تركيز ٢ مجم/لتر من إندول حامض البيوتريك قد نتج عنه أعلى نسبة لتكوين الجذور وعدد الجذور وطول الجذور (سم) والأوزان الطازجة والجافة للجذور (جم).

الكلمات المفتاحية: التخلق المباشر للأعضاء، منظمات النمو النباتية، الخصائص المورفولوجية، نبات البولونيا، سلاميات الساق