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IN VITRO PROPAGATION OF *Paulownia hybrid* (*P. elongata x P. fortunei*) TREE

Asmaa A. Fahmy* **and A.S.H. Gendy**

Hort. Dept., Fac. Agric., Zagazig Univ., Egypt

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ABSTRACT: This study was carried out in Plant Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt throughout the period of 2016 to 2018. An efficient protocol was achieved for *in vitro* mass propagation of *Paulownia hybrid* (*P. elongata x P. fortunei*) by using seeds. The seeds were germinated onto different liquid Murashige and Skoog (MS) medium strength (¼, ½, ¾ and full) free from growth regulators. The highest germination percentage (53.33%) was obtained with full MS. Different concentrations (0.0, 0.5, 1.0, 2.0, 4.0 or 8.0 mg/l) of benzyladenine (BA) or isopentenyladenine (2iP) were tested during multiplication stage. The best treatment for shoot multiplication (31 shoots/explant) was 8.0 mg/l BA. Multiplied shoots were transferred to MS medium containing different concentrations (0.0, 1.0, 2.0, 3.0 and 4.0 mg/l) of indole-3- acetic acid (IAA) or naphthalene acetic acid (NAA). NAA was more effective than IAA for *in vitro* rooting of Paulownia. All tested concentrations of NAA gave (100%) rooting percentage. MS medium supplemented with 2.0 mg/l NAA produced the best root and shoot growth. Rooted Plantlets were successfully acclimatized in peat moss or peat moss and sand (1:1 and 1:2, *V/V*) media. On the other hand, prefer using peat moss and sand (1:2, *V/V*) because its was economic method.

Key words: Paulownia hybrid, BA, 2iP, IAA, NAA, *in vitro*, MS.

INTRODUCTION

Paulownia, a hardwood tree, which belongs to family Paulowniaceae (Scrophulariaceae) is characterized with extremely fast-growing and short-rotation plant with large leaves arranged in opposite pairs on the stem. It grows under several weather conditions and in different types of soil even poor ones. It is growing found in several parts of the world including China, Japan and southeast Asia, Europe, north and central America, and Australia (**Rahman *et al.*, 2013**).

Nowadays, Paulownia species are considered the most important forestry crops in the world. There are nine species of Paulownia but the most important of them include *P. kawakamii, P. australis, P. catalpifolia, P. elongata, P. fargesii, P. fortunei, P. albiphloea, and P. tomentosa.* Many Paulownia species are cultivated in several temperate zones worldwide due to their rapid growth and value in the timber market (**Zhu *et al.*, 1986 ; Yadav *et al.*, 2013**). The wood of Paulownia can serve as a good material for composting, lumber, firewood and coal. Also suitable for making musical instruments, boxes, chests, lightweight skis and furniture making (**Rafighi and Tabarsa, 2011**).

Paulownia trees are conventionally propagated either by seeds or root cuttings. Seed propagation has many difficulties due to the presence of seed borne pathogens and pests, poor seed germination and altered growth habit. Propagation by root cuttings posses' limitations as these can be a potential source of pathogens (**Bergmann and Moom, 1997**). However, tissue culture propagation technique normally gives a high number of healthy and homogenous plants which free from microbial diseases in a short period of time and all over the year (**Lobna *et al.*, 2008**). A high demand for planting material in domestic and international markets for a forestation and

* **Corresponding author: Tel. :** +201007606041
E-mail address: shaker8873@gmail.com

bioenergy production has necessitated the development of efficient micropropagation protocols for rapid and mass propagation of Paulownia (**San Jose *et al.*, 2014**). Although, protocols for adventitious shoot regeneration in other different Paulownia species and hybrids have been defined (**Rao *et al.*, 1996; Yang *et al.*, 1996; Bergmann and Moom, 1997**) only one attempt (**Clapa *et al.*, 2014**) has been done to propagate Paulownia hybrid *(P. elongata x P. fortunei) in vitro*.

The inclusion of auxin in rooting medium was reported to increase root number of *P. elongata* plant (**Chang and Donald, 1992**).

Auxin plays a central role in the process of adventitious root formation (**Haissig and Davis, 1994**) and the interdependent physiological stages of the rooting process are associated with changes in endogenous auxin concentrations (**Heloir *et al.*, 1996**). Auxins are varied in their effectiveness. This variation in effectiveness of different auxin sources may be attributed to their differential affinity to auxin receptors involved in the rooting process, which may be depend on cultivar type (**Tereso *et al.*, 2008**).

The effect of cytokinins is most noticeable in tissue cultures where they are used to encourage the growth of axillary buds, and reduce apical dominance in shoot cultures of broadleafed plants. Most demonstrations of a requirement for a particular cytokinin have been made with shoot cultures; they are dispersed over many species (**George *et al.*, 2008**).

The present study was conducted to improve the *in vitro* propagation protocol of Paulownia hybrid (*P. elongata x P. fortunei*) by investigating some factors affecting the *in vitro* propagation of this important woody tree species.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt throughout the period of 2016 to 2018.

Plant Material Disinfection

Paulownia seeds were rinsed in soapy water for 10 min., then washed under running tap-water for 30 min., and immersed for 30 min. in fungicide solution of Rizolex at a concentration of 2 g/l. Under aseptic conditions in a laminar air-flow cabinet, the seeds were soaked in 0.3 % mercuric chloride solution for 10 min., followed by immersion in commercial Clorox solution (NaOCl, 5.25% free chlorine) at 3% for 5 min. The seeds were rinsed three times with sterile distilled water after each previous step.

Culture Media and Growth Conditions

The culture medium consisted of **Murashige and Skoog (1962)** basal medium and vitamins with 30 g/l sucrose. The medium was solidified whenever needed with 0.6% (*W/V*) agar and the pH was adjusted to 5.8 with NaOH or HCl before autoclaving at 120°C for 20 min under a pressure of 1.2 kg/cm². Jars (60 × 120 mm) were used as culture vessels and each one was filled with 50 ml medium. The cultures were incubated at 23 ±1°C under 16/8 hr., (light/dark cycle) photoperiod with 2000 Lux.

Shoot Induction

Sterilized seeds were cultured on liquid MS medium at different strengths (¼, ½, ¾ and full) free from growth regulators. The distilled water was served as control. Each treatment consisted of three jars and each jar contained 50 ml of MS medium or distilled water. Twenty seeds were cultured in each jar. Cotton was used as supporting material for cultured seeds. After three weeks from culture, seed germination percentage, shoot length (cm), number of leaves/ shoot, number of roots/shoot and root length (cm) were recorded.

Shoot Multiplication Induction

The best establishment treatment (full MS medium) was repeated to obtain enough shoots for multiplication stage experiments. Obtained shoots (about 1.23 cm length with 6.1 leaves/ shoot without roots) were separated and transferred to MS medium contained different concentrations (0.0, 0.5, 1.0, 2.0, 4.0 or 8.0 mg/l) of isopentenyladenine (2iP) or benzyladenine (BA). The best concentration (8.0 mg/l BA) which determined from the previous experiment was used combined with different concentrations (0.0, 0.25, 0.5, 1.0 or 2.0 mg/l) of naphthalene

acetic acid (NAA). Number of shoots/explant, shoot length (cm) and number of leaves/shoot were recorded after six weeks from inoculation date.

In vitro Rooting and Acclimatization

The best multiplication treatment (8.0 mg/l BA) was repeated to obtain enough shoots for rooting stage experiment. These shoots were transferred to MS medium supplemented with different concentrations (0.0, 1.0, 2.0, 3.0 or 4.0 mg/l) of naphthalene acetic acid (NAA) or indole-3- acetic acid (IAA). Data (*i.e.;* rooting (%), No. of roots/shoot, root length No. of shoots/explant, shoot length and No. of leaves/shoot, of plantlet were recorded after six weeks from culture. Each treatment of the above mentioned experiments consisted of five jars, each jar contained three explants and plugged with polypropylene closure.

During acclimatization stage, the rooted plantlets obtained from rooting stage were washed under running tap water, then transferred to plastic pots (9 × 7cm) containing peat moss, peat moss and sand (1:1, *V/V*) or peat moss and sand (1:2, *V/V*) and covered with polythene bags for six weeks to ensure high humidity. Each treatment consisted of 20 pots, and each one contained one plantlet. The plantlets were hold in greenhouse at about 25°C. The survival percentage plant height (cm) and No. of leaves/shoot were recorded after eight weeks.

Statistical Analysis

The statistical layout of all experiments was simple complete randomized design. All data were analyzed with analysis of variance (ANOVA) procedure by using the 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

Effect of MS Strength on Seed Germination and Growth

This experiment was conducted as an attempt to enhance the germination process of Paulownia seeds by using different liquid MS medium strengths. As shown in Table 1 and Fig. 1, germination percentage attained the highest germination percentage (53.33%) by using full MS strength, while the lowest value (30%) was obtained with water. Shoot length was affected by addition of salts to the medium where, 1/4 MS gave the tallest shoot length (2.0 cm). Using of water recorded the lowest value (0.5 cm) in this regard. It is clear that all applied MS strengths significantly increased the number of leaves/shoot compared with control (distilled water). There were no significant differences among these strengths in most cases. No. of roots/shoot followed similar trend to No. of leaves/shoot without significant differences among 1/2, 3/4 and full MS strengths. Only quarter strength had enhanced root length (3.6 cm) compared with distilled water, while other MS strengths caused significant decrease in root length compared with control. This enhancing effect of MS medium on seed germination compared with water was previously mentioned by **Thakur *et al.* (1998)** on *Melia azedarach* and demonstrated here.

Effect of 2iP Concentration on Shoot Proliferation and Growth

Results in Table 2 clear the effect of different concentrations of 2iP on shoot proliferation and growth. Concerning shoot number, increasing of 2iP concentration up to 2.0 mg/l did not significantly increase the number of shoots per explant, while higher concentration (4.0 mg/l) significantly enhanced this character which reached to its maximum value (17.0). On the other side, increasing this concentration to be 8.0 mg/l, significantly decreased the number of shoots (5.80) compared with control treatment. Supplementation the medium with 2iP significantly decreased the shoot length. The longest shoot (5.60 cm) was belonged to control treatment. Number of leaves per shoot followed similar trend to shoot length, since the highest number of leaves (10.40 cm) was recorded with control treatment.

Abovementioned results are in harmony with those obtained by **Taha *et al.* (2001)** who reported that the shoot bud proliferation ability of date palm shoot tips was strongly enhanced by low concentration of 2iP. Similarly, **Vejsadova (2008)** found that the highest shoot multiplication rate of Rhododendron was amended on MS medium supplemented with 2iP. Also, **Rahimia *et al.* (2013)** on *Rhododendron indicum* mentioned that 2iP was the most effective cytokinin to induce shoot bud regeneration.

Table 1. Effect of MS strength on germination of *Paulownia hybrid* (*P. elongata* × *P. fortunei*) after three weeks during establishment stage

Treatment	Seed germination (%)	Shoot length (cm)	No. of leaves/shoot	No. of roots/shoot	Root length (cm)
Distilled water	30.00	0.50 c	2.00 c	1.00 c	2.00 b
1/4 MS	40.00	2.00 a	6.40 ab	1.50 b	3.60 a
1/2 MS	36.66	1.60 b	5.40 b	2.20 a	1.57 c
3/4 MS	36.66	1.26 b	6.90 a	2.40 a	0.75 d
Full MS	53.33	1.23 b	6.10 ab	2.20 a	0.85 d

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

Table 2. Effect of isopentenyladenine (2iP) concentration on shoot proliferation and growth of *Paulownia hybrid* (*P. elongata* × *P. fortunei*) after six weeks during multiplication stage

2iP conc. (mg/l)	No. of shoots/explant	shoot length (cm)	No. of leaves/shoot
Control	1.40 c	5.60 a	10.40 a
0.5	2.00 c	2.79 b	8.40 b
1.0	2.00 c	3.00 b	7.50 b
2.0	3.60 bc	2.39 b	7.53 b
4.0	17.00 a	2.95 b	7.60 b
8.0	5.80 b	3.60 b	7.52 b

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

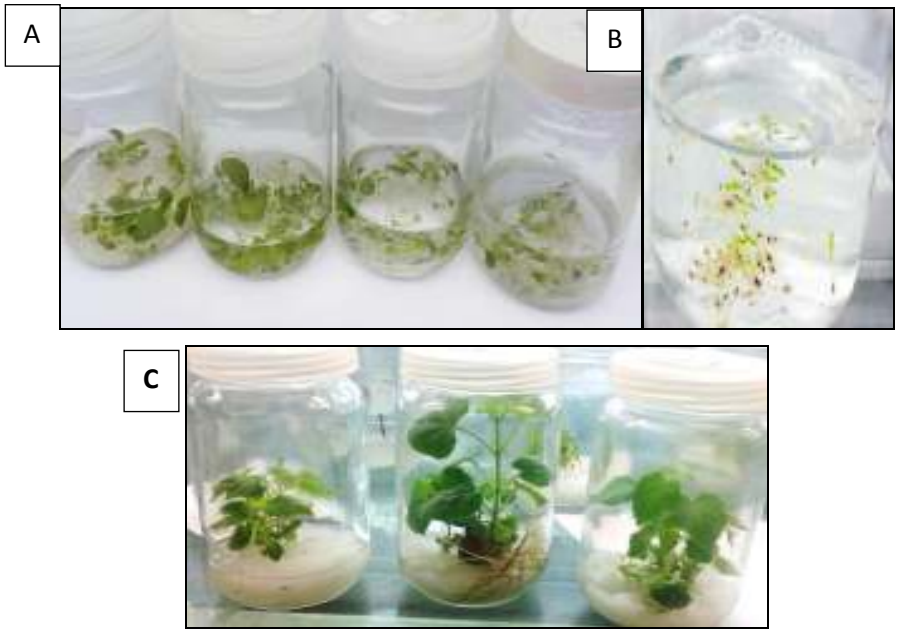

Fig. 1. Seed germination of *Paulownia hybrid* (*P. elongata* × *P. fortunei*): A) on liquid MS medium, B) on water and c) on MS with cotton as supporting material

Effect of BA Concentration on Shoot Proliferation and Growth

Results in Table 3 show that number of shoots/explant was gradually increased with increasing BA concentration. This increase reached its maximum value (31.60 shoots/ explant) with the highest concentration of BA (8 mg/l). As for shoot length and number of leaves per shoot, the results demonstrated that control treatment produced the tallest shoot (5.6 cm) and the highest number of leaves per shoot (10.4) while supplementation the medium with BA at any concentration decreased these characters values. In most cases there were no significant differences among BA concentrations in this regard.

This result indicates the efficiency of high concentration (8.0 mg/l) of BA in enhancing shoot multiplication compared with lower concentrations and control treatments. Similar findings were recorded by **Abdi *et al.* (2013)** on *Aloe vera*, **Hang *et al.* (2013)** on *Philodendron xanadu* and **Krishnan *et al.* (2018)** on *Ophiorrhiza mungos*.

These results could be explained by the fact that cytokinins have important physiological effects, as they have been shown to stimulate cell division as well as cell elongation, to activate RNA synthesis and to stimulate protein synthesis and enzyme activity (**Kulaeva, 1980**).

Effect of interaction between 8 mg/l BA and different concentrations of NAA on shoot proliferation and growth

Results in Table 4 show another attempt to enhance shoot proliferation by using different concentrations of NAA in combination with 8.0 mg/l BA which proved to be the best treatment from previous experiment. As for number of shoots, the highest significant value was obtained from 8.0 mg/l BA alone (31.33 shoots per explant). Augmentation the medium with any applied concentration of NAA, significantly and dramatically decreased the number of proliferated shoots. The lowest number of shoots (2.16) was recorded with the highest concentration of NAA (2 mg/l) combined with 8.0 mg/l BA. Concerning shoot length and number of leaves, it is clear that only the lowest concentration of NAA (0.25 mg/l) could enhance both characters values compared with BA alone. On the other side, higher concentrations of NAA in most cases were not significantly differed than control treatment (8 mg/l BA).

While supplementation the medium with 8 mg/l BA filed to induce rooting, enriching the medium with NAA at any investigated concentration success to induce rooting on shoot base. The highest concentration of NAA was the most effective treatment in this regard. Similar trend was recorded for number of roots/shoot without significant differences among different NAA concentrations. Root length did not significantly affected by the concentration of NAA added to the medium.

This result clearly confirms that BA alone is more effective than its combination with NAA concerning multiple shoot induction. This result agreed with those published by **Jun *et al.* (2001) and Vardja and Vardja (2001)** on different dieffenbachia species. On the other hand, the enhancing effect of NAA on root initiation which reported here, was previously demonstrated by **Abass *et al.* (2016)** on *Aglaonema commutatum* and **Dutta (2018)** on *Murraya Koenigii*.

Effect of Indole-3-acetic Acid (IAA) Concentration on Shoot Rooting and Growth During Rooting Stage

From results presented in Table 5, it could be, noticed that the treatment of 2.0 mg/l IAA gave the highest rooting percentage (75%) while, using the highest concentration (4 mg/l) prevented root initiation completely. As for number of roots per shoot, the highest significant numbers of roots (19.83 and 17.67) were obtained with 2.0 and 3.0 mg/l IAA, respectively without significant difference between them. Regarding root length, the results indicated that the tallest roots were recorded with 2.0 mg/l IAA (8.33 cm), while lower and higher concentrations were less effective in this regard. Number of shoots per explant was gradually increased as NAA concentration increased up to 3.0 mg/l IAA which gave 5.5 shoot/explant then decreased with higher concentration. However, shoot length didn't significantly affect by the increase in IAA concentration. Concerning number of leaves per shoot, the results cleared that the highest

Table 3. Effect of benzyladenine (BA) concentration on shoot proliferation and growth of *Paulownia hybrid* (*P. elongata* × *P. fortunei*) after six weeks during multiplication stage

BA conc. (mg/l)	No. of shoots/explant	Shoot length (cm)	No. of leaves/shoot
Control	1.40 c	5.60 a	10.40 a
0.5	3.60 c	3.08 b	9.33 ab
1.0	4.40 c	3.06 b	7.50 b
2.0	5.00 c	2.80 b	7.40 b
4.0	10.00 b	2.21 bc	7.80 b
8.0	31.60 a	1.90 c	7.60 b

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

Table 4. Effect of interaction between 8 mg/l BA and different concentrations of NAA on shoot proliferation and growth of *Paulownia hybrid* (*P. elongata* x *P. fortunei*) after six weeks during multiplication stage

BA (mg/l)	NAA (mg/l)	Root length (cm)	No. of roots/shoot	Rooting (%)	No. of leaves/shoot	Proliferated shoot length (cm)	No. of shoots/explant
8.0	**0.00**	0.00 c	0.00 b	00.00	7.75 c	1.92 b	31.33 a
	0.25	4.17 a	10.16 a	50.00	12.2 a	5.05 a	14.50 b
	0.50	3.33 ab	10.17 a	62.50	9.33 bc	2.77 b	12.33 b
	1.00	2.83 b	10.16 a	75.00	9.00 bc	2.71 b	10.66 b
	2.00	3.17 ab	11.50 a	87.50	10.00 b	2.44 b	2.16 c

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

Table 5. Effect of indole-3- acetic acid (IAA) concentration on shoot rooting and growth of *Paulownia hybrid* (*P. elongata* x *P. fortunei*) after six weeks during rooting stage

IAA concentration (mg/l)	No. of leaves/shoot	Shoot length (cm)	No. of shoots/explant	Root length (cm)	No. of roots/shoot	Rooting (%)
0.0	10.33 b	5.50 a	1.50 c	1.50 c	2.42 b	25.0
1.0	11.14 b	5.01 a	1.83 c	2.17 c	4.00 b	37.5
2.0	11.87 b	6.57 a	3.66 b	8.33 a	19.83 a	75.0
3.0	15.00 a	5.92 a	5.50 a	5.00 b	17.67 a	62.5
4.0	12.00 b	4.00 a	1.33 c	0.00 d	0.00 c	00.0

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

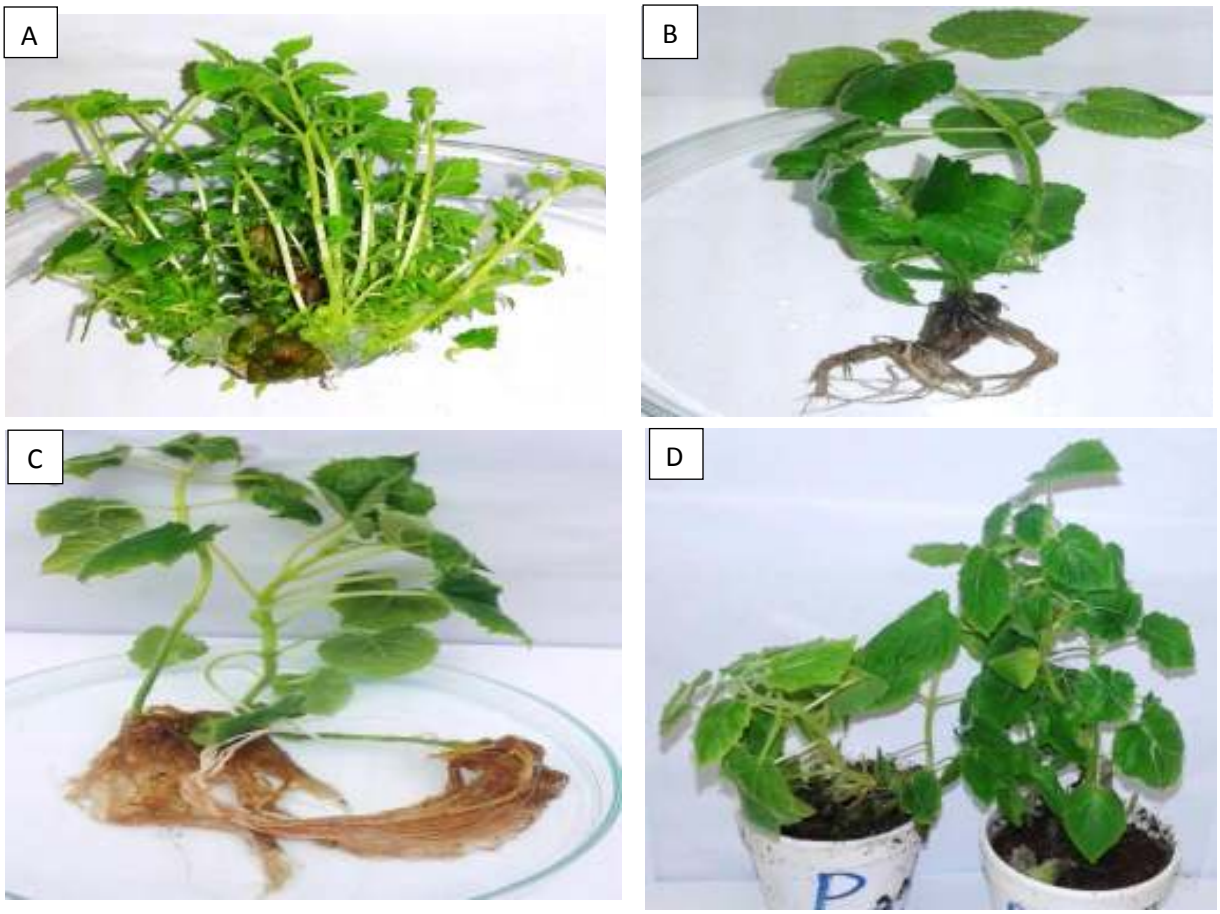

Fig. 2. ***Paulownia hybrid*** **(*P. elongata* x *P. fortunei*) micropropagation, A): Shoot multiplication on MS medium containing 8 mg/l BA, B): Paulownia rooted shoot on 3 mg/l IAA, C): Paulownia rooted shoot on 2 mg/l NAA and D): Acclimatized plantlets of Paulownia after 4 weeks from adaptation**

significant number of leaves per shoot (15) was obtained with 3.0 mg/l IAA. On the other side, there were no significant differences among other treatments. These results agreed with those published by **Mendi *et al.* (2009)** on *Gazania rigens* who mentioned that the best rooting percentage was observed on the media containing IAA.

Effect of Naphthaleneacetic Acid (NAA) Concentration on Shoot Rooting and Growth During Rooting Stage

Results in Table 6 show another attempt to enhance root initiation by using different concentrations of NAA. Results refer that rooting percentage was highly improved by addition of NAA to the medium without significant differences among different concentrations since all concentrations gave 100% rooting percentage. Number of roots per shoot was gradually increased with increasing the concentration of NAA and reached the maximum value (26.5 roots/shoot) with 3.0 mg/l NAA, while higher concentration (4.0 mg/l) was less effective (19.28 roots/shoot). The lowest value (2.43 roots/shoot) was obtained with control. Concerning root length, the results cleared that the longest roots (8.20 and 7.72 cm) were recorded by 2.0 and 3.0 mg/l NAA, respectively without significant differences between both treatments. On the other side, the lowest value (1.50 cm) was recorded by control.

Number of shoots per explant didn't significantly affected by addition of NAA at any

Table 6. Effect of naphthaleneacetic acid (NAA) concentration on *Paulownia hybrid* (*P. elongata* × *P. fortunei*) shoot rooting and growth after six weeks during rooting stage

NAA concentration (mg/l)	No. of leaves/ shoot	Shoot length (cm)	No. of shoots/ explant	Root length (cm)	No. of roots/ shoot	Rooting (%)
0.0	10.28 c	5.43 b	1.50 a	1.50 c	2.43 d	25
1.0	19.72 a	9.02 a	2.00 a	5.57 b	21.28 bc	100
2.0	19.43 a	9.92 a	2.14 a	8.20 a	25.14 ab	100
3.0	16.72 ab	12.07 a	1.57 a	7.72 a	26.50 a	100
4.0	14.28 b	11.72 a	1.14 a	5.43 b	19.28 c	100

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

applied concentration. Shoot length was improved by fortifying the medium with NAA without significant differences among different tested concentrations. Concerning number of leaves per shoot, it is clear that augmentation the medium with NAA significantly enhanced this character. There were no significant differences among 1.0, 2.0 and 3.0 mg/l NAA, while increasing of concentration to be 4.0 mg/l was less effective in this regard. From a commercial point of view it is better to use 2.0 mg/l NAA during rooting stage to obtain the maximum root and shoot growth.

Abovementioned results are in harmony with those obtained by **Burger *et al.* (1985), Lobna *et al.* (2008) and Roy (2015)** who reported that NAA was more effective than IAA and IBA for *in vitro* rooting of other Paulownia spices. Also, **Seyyedyousefi *et al.* (2013)** reported that the highest shoot length, root length, maximum root number and bud number of *Alstroemeria* were produced on MS medium supplemented with 1.0 mg/l NAA.

Effect of Planting Media on Survivability and Growth of Plantlets

Results in Table 7 illustrate the effect of different planting media on survivability (%) and growth of plantlets. It is clear that mixing sand with peat moss slightly increased survivability percentage (100%) compared with peat moss alone (90%). Plant height didn't significantly affected by using any of tested medium. On the other side, the highest number of leaves/plantlet (22) was observed with sand: peat (1:1, *V/V*) medium. These results agreed with those published by **Lobna *et al.* (2008)** on *Paulownia kowakamii* who reported that the highest percentage of survival was obtained by using a soil mixture of peat moss and sand.

Conclusion

The abovementioned results present an *in vitro* propagation protocol of Paulownia hybrid (*P. elongata x P. fortunei*) which could be summarized as follows: 1) During establishment stage, the highest germination percentage of seeds (53.33%) was obtained with liquid full MS free from growth regulators. 2) During multiplication stage, the most efficient treatment for shoot multiplication was 8 mg/l BA which produced the highest number of shoots (31 shoots /explant). 3) Multiplied shoots could be rooted (100%) when transferred to MS medium supplemented with NAA at 2.0 mg/l. 4) Obtained plantlets were acclimatized successfully (100% survivability) in peat moss and sand (1:1, *V/V*) with good shoot growth characters.

Table 7. Effect of different planting media on survivability (%) and growth of *Paulownia hybrid* (*P. elongata* × *P. fortune*) plantlets after eight weeks

Planting media	Number of leaves/plantlet	Plant height (cm)	Survivability (%)
Peat	18.25 b	14.62 a	90
Peat moss : Sand (1:1)	22.00 a	13.75 a	100
Peat moss : Sand (1:2)	18.00 b	14.25 a	100

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

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الإكثـــــــار المعـملي لشـــجرة الباولـــونيا

أسماء أحمد فهمى – أحمد شاكر حسين جندى

قسم البساتين - كلية الزراعة - جامعة الزقازيق – مصر

أجريت هذه الدراسة في معمل زراعة الأنسجة، قسم البساتين، كلية الزراعة جامعة الزقازيق، مصر في الفترة من ٢٠١٦ إلى ٢٠١٨، أمكن التوصل إلى بروتوكول فعال للإكثار المعملي الكثيف لهجين أشجار الباولونيا (*P. elongata x P. fortunei*) باستخدام البذور، حيث تم إنبات البذور على قوى مختلفة (٤/١ ، ٢/١ ، ٤/٣ قوة كاملة) لأملاح بيئة موراشيج وسكوج الخالية من منظمات النمو، وقد أدى استخدام بيئة موراشيج وسكوج بالقوة الكاملة للحصول على أعلى نسبة إنبات للبذور(٥٣,٣٣%)، و قد اختبرت تركيزات مختلفة (صفر ، ٠,٥ ، ١ ، ٢ ، ٤ أو ٨ ملليجرام/لتر) من البنزيل أدنين والأيزوبنتنيل أدنين خلال مرحلة التضاعف، فثبت أن أفضل معاملة لتضاعف الأفرخ (٣١ فرخ لكل منفصل نباتى) هى ٨ ملليجرام/لتر بنزيل أدنين، وقد تم نقل الأفرخ المتضاعفة لبيئة موراشيج وسكوج المحتوية على تركيزات مختلفة (صفر، ١، ٢ ، ٣ أو ٤ ملليجرام/لتر) من إندول حامض الخليك أو نفثالين حامض الخليك، فوجد أن نفثالين حامض الخليك أكثر فاعلية من إندول حامض الخليك لتجذير أفرخ الباولونيا، حيث أعطت جميع تركيزات نفثالين حامض الخليك المختبرة نسبة تجذير ١٠٠%، هذا و قد أعطى تركيز ٢ ملليجرام/لتر نفثالين حامض الخليك أفضل تجذير و نمو للفرخ، وقد أمكن أقلمة النبيتات الناتجة بنجاح (١٠٠% نسبة بقاء) بزراعتها في بيئة من البيت موس أو البيت موس و الرمل بنسب ١:١ أو ٢:١ كنسب حجمية/حجمية، ويفضل استخدام بيئة البيت موس و الرمل بنسب ٢:١ لأنها اقتصادية.

المحكمـــــون:

١- أ.د. صفاء مصطفى محمـد أستاذ الزينة – كلية الزراعة – جامعة بنها.

٢- أ.د. هشام عبدالعال الشامي أستاذ الزينة – كلية الزراعة – جامعة الزقازيق.