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MICROPROPAGATION AND EVALUATION OF GENETIC STABILITY OF FOXGLOVE TREE *(Paulownia tomentosa)*

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Hassan, K.M.[1,*], A.M. Hosni[1], M. Hewidy[1] and A.B. Abd El razik[2]

1- Horticulture Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt

2- Genetics Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt

*Corresponding author: karim_hassan84@yahoo.com

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ABSTRACT

This reasearch work aimed to fine-tune micropropagation of *Paulownia tomentosa* in addition to assessing the genetic stability of *in vitro* raised clones from it. *Paulownia tomentosa* explants were surface sterilized using clorox (commercial bleach 5.25% sodium hypochlorite) at 10, 20, 25 and 30% + 0.5 g/l mercuric chloride ($HgCl_2$) at different duration times, i.e. 10, 15, 20 and 25 min. In the multiplication stage, shoots were transferred to MS medium at ¾ strength containing BAP and Kin each at (0, 0.5, 1, 2, and 4 mg/l). Whereas, the rooting medium was MS medium at ¾ strength with IBA and NAA treatments each at 0, 0.5, 1, 2, and 4 mg/l. Sterilized explant with 30% Clorox for 20 min recorded highest survival percentage. The treatment of Kin at 4 mg/l gave higher significant shoot length. Whereas BAP application at 2 and 4 mg/l gave highest significant value of both shoot number and leaf number. Both IBA and NAA at 0.5 or 1 mg/l gave highest significant root number/shoot. Whereas, auxin at 4 mg/l gave highest significant root lengths. Young plantlets resulted from *in vitro* were acclimitized successfully in a mixture of peat moss: perlit (2: 1) by volume that showed 85.93% survival.

The genetic stability of *in vitro* raised *Paulownia tomentosa* clones was assessed by using inter-simple sequence repeats (ISSRs) markers. All of the three ISSR primers screened, produced clear, reproducible and scorable bands.

The molecular size of Polymerase Chain reaction (PCR) products generated 22 fragments by these ISSR ranged from ≈460 to18660 bp. All banding profiles from micropropagated plants were monomorphic and similar to those of the mother plant, indicating 100% similarity. This confirmed the true to type nature of the *in vitro* raised clones.

Keywords: *Paulownia* (foxglove tree - princess tree), Micropropagation, Genetic stability.

INTRODUCTION

The genus *Paulownia* from the family *Scrophulariaceae* includes 9 species of trees indigenous to China and East Asia **Zhu et al 1986**. Most species of *Paulownia* are fast growing as the harvesting of wood begins within 8-10 years and can continue yearly for as long as is desired. The tree is extremely hardy and its plantation requires only minimal inputs from the grower. Recently, *Paulownia* intercepted large interest due to variable uses and applications of different parts of it. The value of *Paulownia* as a short-rotation woody crop plants was reported by **Bergmann and Moon, 1997** in afforestation by **Zhu et al 1986** and in mine site reclamation by **Carpenter, 1977** The wood of *Paulownia* is soft, lightweight with excellent machining and finishing properties **Akyildiz and Shahin, 2010**. Its bark has been used in Chinese herbal medicine as a component for remedies of some infectious diseases. Aside from their timber products, some Paulownia species have also an ornamental use **Puxeddu et al 2012; Ben Bahri and**

Taoufik, 2013. Propagation of *Paulownia* tree was limited to seed and root cuttings. Both methods represent low production rates. The use of *in vitro* propagation techniques provides healthy, homogeneous planting stock for afforestation and woody biomass production as in the case of *Paulownia*. Efficient vegetative micropropagation protocols have been established over seedling production of *Paulownia spp*. **Bergmann and Moon, 1997; Bergmann, 1998; Rout et al 2001.**

Markovic et al (2013) mentioned that *in vitro* culture was successfully established using disinfection of nodal segments with 0.1% $HgCl_2$ for 10 min. Immersion in 30% solution of sodium hypochlorite for 15 minutes to sterilized *Paulownia* seeds removed surface contamination. **Shtereva et al (2014). Chunchukov et al (2015)** ascertained that sterilization procedure was performed by washing in tap water with liquid detergent for 20 minutes and subsequently treated with a solution of mercuric chloride (0.1%) for 3 minutes followed by rinsing 3 times for 5 minutes with sterile distilled water reduce contamination and increase survival rate. **Rout et al (2001)** sterilized nodal explants by washing them in 0.1% (v/v) detergent "Teepol" (Qualigen, India) solution for 15 min and subsequently rinsed in running tap water. Further, the explants were disinfested in 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 15 min and then rinsed three times in sterile distilled water.

Shoot proliferation is induced by application of cytokinins at the multiplication stage. **Taha et al (2008)** reported superiority of BAP over other cytokinins for multiple shoots formation in *Paulownia*. **Lobna et al (2008)** tested the effect of BAP and various light intensities upon *P. kawakamii* in vitro proliferation. The medium with 1 mg/l BAP was the best for *in vitro* proliferation. **Ben Bahri and Taoufik (2013)** found that Shoot proliferation was induced by the mean of MS medium containing different concentrations of BAP (1or 2 mg /l). Shoot proliferation was effectively induced using cytokinins in combination to different type of auxins.

Ipekci et al (2001) and Litwinczuk and Bochnia (2012) reported that MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA was optimum to regenerate multiple shoots in *Paulownia* from shoot tip explant. Also, **Rahman et al (2013**) showed that 2.5 mg/l BAP + 0.5 mg/l NAA showed highest shoot induction. At same year, **Markovic et al (2013)** mentioned that MS medium supplemented with 6 mg l-1 BA and 0.5 mg l-1 IBA gave the best multiplication rate.

At rooting stage of *Paulownia*, **Taha et al (2008)** observed that microshoots transferred to half strength MS with 1.0 mg/l NAA gave best rooting rate. **Roy (2015**) Shoots rooted well in half strength MS supplemented with 2.0 mg/l NAA. **Markovic et al (2013**) reported that rooting percentage was 95% on MS medium with 0.8 mg /l of IBA or NAA. **Lobna et al (2008)** stated that microshoots derived from multiplication stage were separated and transferred to a rooting medium having half strength MS medium supplemented with two concentrations (1.0 and 0.5 mg/l) of IAA, IBA or NAA.

For acclimatization plantlets were transferred to small plastic pots containing a mixture of peat and perlite 2:1 v/v by **Shtereva et al (2014). Zayova et al (2011)** reported that the highest survival percentage (100%) existed in the plants grown on a mixture of peat:perlite in a 2:1 ratio in a greenhouse. They were characterized by rapid growth and development.

All banding profiles from micropropagated plants were monomorphic and similar to those of the mother plant were previously reported by **Martin et al (2004)** in almond, **Joshi and Dhawan (2007)** in *Swertia chirayita* and **Abd elrazik (2012)** in *Paulownia tomentosa.*

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory, Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB), Faculty of Agriculture, Ain Shams University, Egypt during the period of 2013-2018.

- Culture media: Murashige and skoog (1962) medium (MS) was used in the different *in vitro* growing stages. Carbon and energy source was 3% of sucrose. Also, 8 g/l agar was added as a solidified agent of media at all stages. The medium was adjusted to pH 5.7±0.1 and autoclaved at 121°C and 1.2 Kg/ cm^2 for 20 min before being used. The hormonal supplements were differed according to the requirement of the specified experiments as will be mentioned later. Jars 150 cm^3 size filled with about 25 cm^3 medium were used at all stages.

-Culture incubation conditions: Culture of all experiments were incubated at 28±2°C and exposed to 2000-2500 lux for 16 hours light using fluorescent lamps (2 lamps per shelf) alternated with 8 hours dark. Each treatment of *Paulownia tomentosa* comprised three replicates, with three explants per jar for each replicate.

- **Experimental plants**: Shoot tips from *Paulownia tomentosa* tree from a three years old *Paulownia* tree, grown at Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB), Faculty of Agriculture Ain Shams University, were chosen as a source of explants for the experiments.

- **Establishment stage:** Healthy terminal vegetative growth part of 2.5-3 in length containing the shoot tip and 2-3 lateral buds were collected as explants. Then, the collected explants were defoliated and cleaned with liquid soap and rinsed with a continuous flow of tap water for an hour. Afterwards, they were surface-sterilized using clorox commercial bleach (5.25% of sodium hypochlorite NaOCl) at 10, 20, 25 and 30% + 0.5 g/l mercuric chloride ($HgCl_2$) for 10, 15, 20 and 25 min. After four weeks survival percentage was calculated.

- **Multiplication stage:** Microshoots resulting from the establishment stage containing 3-4 nodes were taken for the multiplication experiment. Defoliated microshoots were cultured in the multiplication medium which was supplemented with cytokinins either BAP or Kin each at 0, 0.5, 1, 2 and 4 mg/l.

The proliferated shoot length, number of proliferated shoots and number of leaves/shoot in the first, second and third subcultures were calculated separately. Reculture was done every four weeks for three times.

- **Rooting stage:** Microshoots resulted from the multiplication stage were transferred to the rooting medium that was supplemented with either IBA or NAA at 0, 0.5, 1, 2 and 4 mg/l. Rooting percentage, average of root length and root number /plantlet were calculated after four weeks.

- **Acclimatization stage:** *Paulownia tomentosa* young plantlets resulted from *in vitro* culture were washed with a flow of tap water to get rid of medium remains then dipped in a fungicide solution (Rhizolex) 0.5 g / l for 30 min. Afterwards, the plantlets were cultured in plastic pots filled with a mixture medium of peat moss: perlit (2:1 v/v) and irrigated with water only. For that sake, plantlets were kept inside a plastic tunnel and incubated in a plastic house. After six weeks survival percentage was recorded.

- **DNA extraction:** DNA was extracted from 250 mg fresh leaves derived from *in vitro* raised plants by the CTAB method of **Bousquet et al 1990** where DNA quantity is estimated spectrophotometrically.

- **Polymerase Chain reaction (PCR) technique: Mullis (1990)** discovered the PCR as an enzymatic assay which allows for amplification of a specific DNA fragment from a complex pool of DNA.

- **Inter Simple Sequence Repeats (ISSR) technique:** ISSR was carried out according to the protocol discovered and performed by **Meyer et al (1993).**

- **Experimental design:** A complete randomize design was adopted. In it, each treatment comprised three replicates with three explants per jar for each replication.

- **Statistical analysis:** All result data were statistically analyzed using the analysis of variance method as reported by **Snedecor and Cochran (1980)** The differences between means were differentiated by using Duncans range test. **(Duncan, 1955).**

RESULTS AND DISCUSSION

- In the Establishment stage

Results in **Table (1)** showed that application of clorox at 25 % and 30 % gave higher survival percentage than in other concentrations. Also, sterilization duration periods at 20 and 25 min gave higher survival percentage than other duration periods. Use of Clorox at 30% with 20 min duration recorded the highest significant survival percentage.

Table 1. Effect of Colorx concentration and duration period and their interaction during *in vitro* establishment stage on survival percentage of *Paulownia tomentosa* explants.

Clorox concentration (%)+ 0.5 g/l ($HgCl_2$)	Duration period (min)				
	10 min.	15 min.	20 min.	25 min.	Mean
10	35.00g	41.67fg	43.33fg	50.00f	**42.50E**
15	45.00fg	63.33e	63.33e	73.33de	**61.52D**
20	68.33e	81.67cd	86.67bc	91.67abc	**82.08C**
25	83.33cd	90.00abc	95.00ab	98.00a	**91.75B**
30	90.00abc	98.76a	100.00a	95.76ab	**96.08A**
Mean	**64.33C**	**75.07B**	**77.67B**	**81.87A**	

Means having the same letter(s) in each group or interaction had insignificant differences at 5% level.

These results are in harmony with those found by **Ben Bahri and Taoufik (2013)** who reported that *Paulownia* explants were rinsed in sterile distilled water and soaked in 0.1% (w/v) $HgCl_2$ for 5 min. **Shtereva et al (2014)** immersed them in a 30% solution of NaOCl for 15 minutes to sterilize *Paulownia* seeds.

- In the Multiplication stage

Results in **Table (2)** showed that Cytokinin type, concentration and their interaction had significant effects on shoot length at all three subcultures carried out. Kin gave significant shoot length (7.76, 7.57 and 7.14 cm) in respect order of the three subcultures when compared to BAP (5.40, 5.15 and 4.76 cm). Free cytokinin media showed the tallest shoot length when compared to different concentrations of both BAP and Kin at the different subcultures. Evidently, Kin application at 4 mg/l gave significant shoot length (8.50, 8.26 and 4.76 cm) in respect order of the three subcultures when compared to other Kin concentrations and all BAP concentrations.

Table 2. Effect of type and concentration of cytokinin and their interaction on shoot length (cm) of *Paulownia tomentosa* during *in vitro* multiplication stage for three individual consequtive subcultures.

Cytokinin Type	Concentration (mg/l)					
	0	0.5	1	2	4	Mean
Subculture1						
BAP	7.23d	5.38e	5.41e	4.53f	4.46f	**5.40B**
Kin	7.23d	7.61c	7.41cd	8.03b	8.50a	**7.76A**
Mean	**7.23A**	**6.49B**	**6.41BC**	**6.28C**	**6.48BC**	
Subculture 2						
BAP	7.24c	5.12d	5.12d	4.11e	4.16e	**5.15B**
Kin	7.24c	7.39c	7.22c	7.73b	8.26a	**7.57A**
Mean	**7.24A**	**6.26B**	**6.17B**	**5.92C**	**6.21B**	
Subculture 3						
BAP	6.47d	4.70e	4.59e	3.81f	3.80f	**4.67B**
Kin	6.47d	6.97c	6.90c	7.50b	7.87a	**7.14A**
Mean	**6.47A**	**5.83B**	**5.74B**	**5.66B**	**5.83B**	

Means having the same letter(s) in each group or interaction had insignificant differences at 5% level.

Results in **Table (3)** highlighted that Cytokinin type, concentration and their interaction possessed significant effects on shoot length at all three subcultures. BAP gave significant leaf number (15.20, 15.87 and 17.53) in respect order of the three subcultures when compared to Kin (12.69, 12.64 and 13.16). However, BAP application at 2 and 4 mg/l gave significant leaf number (17.33, 18.89 and 20.44) and (18.67, 21.00 and 22.67) in respect order of three subcultures when compared to almost all other BAP concentrations and all Kin concentrations.

Table 3. Effect of type and concentration of cytokinin and their interaction on leaf number of *Paulownia tomentosa* during *in vitro* multiplication stage for three individual consequtive subcultures

Cytokinin Type	Concentration (mg/l)					
	0	0.5	1	2	4	Mean
Subculture 1						
BAP	11.78c	13.67c	14.56bc	17.33ab	18.67a	**15.20A**
Kin	11.78c	13.56c	12.44c	12.78c	12.89c	**12.69B**
Mean	**11.78C**	**13.61B**	**13.50BC**	**15.00AB**	**15.78A**	
Subculture 2						
BAP	11.22c	13.67bc	14.56b	18.89a	21.00a	**15.87A**
Kin	11.22c	12.67bc	12.33bc	12.89bc	14.11bc	**12.64B**
Mean	**11.22C**	**13.17B**	**13.44B**	**15.89A**	**17.50A**	
Subculture 3						
BAP	11.67d	16.44b	16.44b	20.44a	22.67a	**17.53A**
Kin	11.67d	13.44cd	11.11d	13.44cd	16.11bc	**13.16B**
Mean	**11.67D**	**14.94C**	**13.78C**	**16.94B**	**19.39A**	

Means having the same letter(s) in each group or interaction had insignificant differences at 5% level.

Results in **Table (4)** indicated that Cytokinin type, concentration and their interaction manifested significant effects on shoot number at all three subcultures. BAP gave significant shoot number (2.73, 2.91 and 3.58) in respect order of the three subcultures when compared to Kin (1.33, 1.40 and 1.64). However, BAP application at 2 and 4 mg/l gave significant shoot number (3.56, 3.89 and 5.00) and (4.22, 4.67 and 5.56) in respect order of three subcultures when compared to the remaining BAP concentration and all Kin concentrations.
These results are in harmony with those found by **Taha et al (2008)** who reported superiority of BAP over other cytokinins for multiple shoots formation in *Paulownia*. Similarly**, Ben Bahri and Taoufik (2013)** found that shoot proliferation was induced by the mean of MS medium containing different concentrations of BAP (1or 2 mg /l) in combination to different type of auxins.

- In the Rooting stage

Data in **Table (5)** indicated that effect of auxin type, concentration and their interaction on rooting percentage of *Paulownia tomentosa* had no show significant difference at all.

Table 4. Effect of type and concentration of cytokinin and their interaction on shoot number of *Paulownia tomentosa* during *in vitro* multiplication stage for three individual consequtive subcultures

Cytokinin Type	Concentration (mg/l)					
	0	0.5	1	2	4	Mean
Subculture 1						
BAP	1.33cd	2.11bc	2.44b	3.56a	4.22a	**2.73A**
Kin	1.33cd	1.22d	1.11de	1.44cd	1.56cd	**1.33B**
Mean	**1.33B**	**1.67B**	**1.78B**	**2.50A**	**2.89A**	
Subculture 2						
BAP	1.11d	2.44b	2.44b	3.89a	4.67a	**2.91A**
Kin	1.11d	1.11d	1.33cd	1.44cd	2.00bc	**1.40B**
Mean	**1.11D**	**1.78C**	**1.89C**	**2.67B**	**3.33A**	
Subculture 3						
BAP	1.44d	2.89b	3.00b	5.00a	5.56a	**3.58A**
Kin	1.44d	1.44d	1.33d	1.67cd	2.33bc	**1.64B**
Mean	**1.44D**	**2.17C**	**2.17C**	**3.33B**	**3.94A**	

Means having the same letter(s) in each group or interaction had insignificant differences at 5% level.

Table 5. Effect of auxin type, concentration and their interaction after three subcultures on rooting percentage of *Paulownia tomentosa* from *in vitro* rooting

Conc.(mg/l) / Auxin Type	0	0.5	1	2	4	Mean
IBA	100 a	100 a	100 a	100 a	100 a	**100A**
NAA	100 a	100 a	100 a	100 a	100 a	**100 A**
Mean	**100 A**	**100 A**	**100 A**	**100 A**	**100 A**	

Data in **Table (6)** indicated that auxin type, concentration and their interaction showed significant effects on root length after three subcultures. NAA gave significant root length (4.33 cm) in respect order when compared to IBA (4.04 cm). However, NAA application at 4 mg/l gave significant root number (7.26 cm) in respect order when compared to other NAA concentrations and all IBA concentrations without exception.

Table 6. Effect of auxin type, concentration and their interaction after three subculture on root length of *Paulownia tomentosa* from *in vitro* rooting

Conc.(mg/l) / Auxin Type	0	0.5	1	2	4	Mean
IBA	2.53h	3.77ef	3.88de	4.24d	5.80b	**4.04B**
NAA	2.53h	3.47fg	3.22g	5.16c	7.26a	**4.33A**
Mean	**2.53D**	**3.62C**	**3.55C**	**4.70B**	**6.53A**	

Means having the same letter(s) in each column, row or interaction had insignificant differences at 5% level

Data in **Table (7)** illustrated that auxin type, did not exhibit significant difference. With regards to concentration, at 0.5 mg/l recorded highest significant root number (13,83). Concerning the interaction between auxin type and concentration, NAA and IBA at 1, 0.5 mg/l gave highest significant values (14.00, 13.89, 13.78 and 12.56) in respect order.

Table 7. Effect of auxin type, concentration and their interaction after three subculture on root number of *Paulownia tomentosa* from *in vitro* rooting

Conc.(mg/l) / Auxin Type	0	0.5	1	2	4	Mean
IBA	10.78b	13.78a	12.56ab	12.78ab	12.11b	**12.40A**
NAA	10.78b	13.89a	14.00a	11.00b	12.11b	**12.36A**
Mean	**10.78D**	**13.83A**	**13.28AB**	**11.89CD**	**12.11BC**	

Means having the same letter(s) in each column, row or interaction had insignificant differences at 5% level.

Ben Bahri and Taoufik (2013) reported that the addition of 0.5 mg /l IBA to MS medium gave 100% rooting percentage of *Paulownia tormentosa* shoots. **Shtereva et al (2014)** stated that1 mg /l IBA gave 100 % rooting in *Paulownia tormentosa* and 99.4% in *Paulownia elongata*. **Roy (2015**) mentioned that *Paulownia* shoots rooted well in half strength MS supplemented with 2.0 mg/l NAA.

- In the Acclimatization stage

Paulownia tomentosa acclimatization of *in vitro* plantlets was highly successful with an 85.93% survival rate after four weeks when placed in a greenhouse **(Plate 1)**. Similarly, **Zayova (2011)** reported that the highest survival percentage (100%) was provided in plants grown on a mixture of peat: perlite in a 2:1 ratio in a greenhouse. **Roy (2015)** mentioned that regenerated plantlets were successfully acclimatized in poly bags containing a mixture of soil and compost in 2:1 ratio. About 90% plantlets survived under open field conditions.

- Assessment of genetic stability using ISSR markers

All three primers produced clear and reproducible bands **(Plate 2)**. All banding profiles from micropropagated plants were monomorphic. The three ISSR primers produced 22 distinct and scorable bands in the size range of 460 bp to 18660 bp **(Table 8)**. The number of scorable bands for each primer varied from 6 (ISSR-HB12) to 9 (ISSR-HB13). Similar results have been reported by **Martin et al (2004)** in almond, **Joshi and Dhawan (2007)** in *Swertia chirayita,* **Sreedhar et al (2007)** in *Vanilla planifolia* and **Abd elrazik (2012)** in *Paulownia tomentosa.* Earlier, **Reynoird et al (1993)** did not observe any phenotypic variations during vegetative and reproductive phases among the regenerates of *Gerbera*.

Plate 1. Acclimatization of *in vitro* produced plantlets of *Paulownia tomentosa*

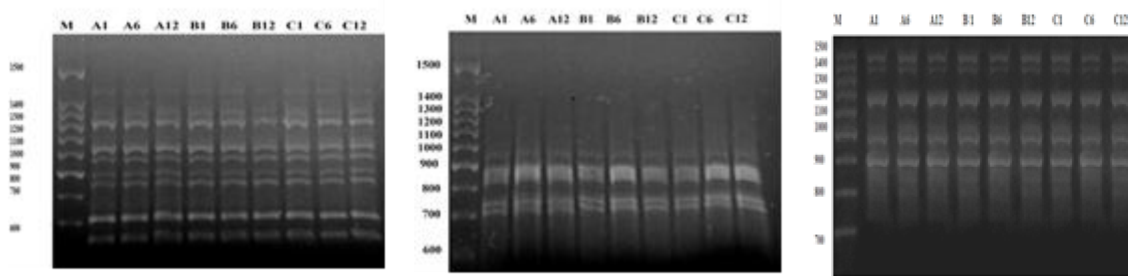

Plate 2. DNA bands generated for *Paulownia tomentosa* by ISSR primers (HB 11 - HB 12 - HB 13) with 9 samples (M= Marker, A1= Mother plant A , A6= explant after 6 subcultures , A 12= explant after 12 subcultures , B1= Mother plant B , B 6= explant after 6 subcultures , B 12= explant after 12 subcultures , C1= Mother plant C , C 6= explant after 6 subcultures , C 12= explant after 12 subcultures).

Table 8. Summary of number of band patterns resulting from ISSR electrophoretic of nine samples of *Paulownia tomentosa*

Primer	No. of bands	Lowest length (≈ bp)	Highest length (≈ bp)
HB 11	7	1350	11160
HB 12	6	850	3510
HB 13	9	460	18660

CONCLUSIONS

- Successful micropropagation through tissue culture of *Paulownia tomentosa* can be obtained from: use of clorox at 30% for 20 min. as a sterilization agent. Kin at 4 mg/l can be used to obtain tallest shoots, but greatest multiplication rate and highest number of leaves could be achieved using BA at 2 and 4 mg/l. NAA at 1 mg/l was appropriate to obtain highest root number per shoot and a mixture of peat moss: perlite (2: 1 v / v) under plastic tunnel was suitable to achieve high survival rate (85.93%) of resultant plantlets from the acclimatization stage.

-The genetic stability of *in vitro* raised *Paulownia tomentosa* clones was assessed by inter-simple sequence repeats (ISSRs) markers. All banding profiles from resultant micropropagated plantlets were monomorphic and similar to those of the mother plant, indicating 100% similarity.

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الاكثار الدقيق وتقييم الثبات الوراثي للباولونيا *(Paulownia tomentosa)*

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كريم محمد حسن[1] – عبدالعزيز محمد حسنى[1] – محمد هويدى محمود[1] – أشرف بكرى عبد الرازق[2]

1- قسم البساتين – كلية الزراعة – جامعة عين شمس – ص. ب68 – حدائق شبرا 11241 –االقاهرة– مصر

2- قسم الوراثـــة – كلية الزراعة – جامعة عين شمس – ص. ب68 – حدائق شبرا 11241 –االقاهرة– مصر

*Corresponding author: karim_hassan84@yahoo.com

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الموجـــــــز

تم اجراء التجربة بمعمل زراعة الانسجة بمركز الهندسة الوراثية – قسم الوراثة – كلية الزراعة جامعة عين شمس– مصر فى الفترة من 2013 الى 2018. هدفت الدراسة للوصول الى افضل المعاملات لأكثار الباولونيا بتقنية زراعة الأنسجة. عقمت البراعم المأخوذة بالكلوركس (مبيض تجاري 5.25% هيبوكلوريت الصوديوم) بتركيزات 10 و15 و20 و25 و30 % باستخدام فترات تعرض مختلفة (10 و15 و20 و25 دقيقة). زرعت البراعم المعقمة على بيئة ميوراشيج واسكوج الخالية من الهرمونات بمرحلة التأسيس. تم نقل الأفرخ بمرحلة الأكثار السريع الى بيئة ميوراشجي واسكوج المحتوية على تركيزات مختلفة لكل من البنزيل ادينين والكينيتين (0 و0.5 و1 و2 و4 ملجم/لتر). وكانت بيئة التجذير مكونة من 4/3 قوة بيئة ميوراشيج واسكوج التي امدت بتركيزات مختلفة لكل من اندول حامض البيوتريك ونفثالين حامض الخليك (0و0.5 و1 و2 و4 ملجم/لتر). أثبتت النتائج أن أقل نسبة تلوث وأعلى نسبة بقاء كانت مع المعاملة 30% كلوراكس لمدة 20 دقيقة. تم الحصول على أطول الأفرخ وأكبر عدد للأوراق مع المعاملة 2,4 ملج/لتر كينيتين بينما كان أكبر عدد للأفرخ مع المعاملة 2,4 ملج/لتر بنزيل ادينين. وكانت أطول الجذور مع المعاملة 4 ملجم/لتر نفثالين حامض الخليك. تمت اقلمة النباتات الناتجة من زراعة الانسجة عن طريق زراعتها فى بيئة تتكون من بيت موس : بيرليت بنسبة 2:1 تحت نفق بلاستيكى داخل صوبة بلاستيكية وبعد 6 اسابيع تم تقدير نسبة البقاء لهذه النباتات وكانت 85.93%.

وتم تقييم الثبات الوراثي للنباتات الناتجة من زراعة الانسجة بتقنية التتابعات البسيطة البينية المتكررة **(ISSR)** بعد 12 نقلة باستخدام 3 بادئات متخصصة (HB11, HB12 , HB13).وكانت النباتات الناتجة ثابتة وراثية ولم يحدث بها اى تغيرات وراثية على مدار 12 نقلة.

الكلمات الدالة: زراعة الأنسجة، الباولونيا، تقييم الثبات الوراثي

تحكيم: ا.د هشام هاشم عبد القادر
ا.د هشام عبد الرؤوف الشورى