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Original article

In vitro study on the effect of cytokines and auxins addition to growth medium on the micropropagation and rooting of *Paulownia* species (*Paulownia hybrid* and *Paulownia tomentosa*)

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ABSTRACT

This study represents an efficient preliminary protocol for *in vitro* mass production of two *Paulownia* species (*Paulownia hybrid* and *Paulownia tomentosa*) seedlings by using seed explant. Different concentrations of benzyladenine (BA) or Kinetin (Kin) (0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L) were tested during multiplication stage. The number of shoots/explants was significantly increased with increasing either BA or Kin concentration; however, the shoot length significantly decreased. Data show that media fortified by BA (10 mg/L) combined with indole butyric acid (IBA) at 1.0 or 1.5 mg/L recorded the highest number of shoots/explant (9.13 and 9.25, respectively). After six weeks during the multiplication stage, data cleared that media fortified by benzyladenine (10 mg/L) combined with IBA at 0.5 mg/L recorded the highest shoot length (3.23 cm). The inclusion of indole butyric acid (IBA) or naphthalene acetic acid (NAA) at 1.0–1.5 mg/L to the medium significantly increased the number of roots/plantlets and the highest root length. The results indicated that IBA supplementation was more effective than NAA for *in vitro* rooting of both *Paulownia* species. The best treatment for multiplication was 10 mg/L and 8.0–10 mg/L BA for *P. hybrid* and *P. tomentosa*, respectively. Peat moss and sand (1:1, v/v) or peat moss and sand (1:2, v/v) were investigated as soil mixture during the adaptation stage. The results referred that *Paulownia* species plantlets were successfully survived (100 %) in soil mixture contained peat moss: sand (1:2, v/v). This mixture recorded the highest values of plantlet height and number of leaves/plantlets.

1. Introduction

Several *Paulownia* species are planted in various temperate zones worldwide due to their fast growth and timber market value (Yadav et al., 2013). The *Paulownia* wood can avail a kindly material for furniture making, chests, boxes, lumber, composting, and coal (Rafighi and Tabarsa, 2011). *Paulownia* seed propagation is not dependable due to seed-borne pathogens and pests, bad seed germination, and changed growth habits. In addition, seedling growth is slow acting compared to originated root cutting from plants. Propagation by root cuttings shows limitations as these can be the source of potential pathogens (San Jose et al., 2014).

The impact of cytokinins is most remarkable in tissue cultures where they are utilized to promote the growth of axillary buds and minimize apical dominance in broad-leafed plants shoot cultures. Most studies focused on using cytokinins with shoot cultures; they are utilized in many species (George et al., 2007).

The modulation of auxin in the rooting medium was pointed out to increase the number of roots in the *Paulownia* plant (Chang and Donald, 1992). Auxin plays a focal turn in the formation of adventitious roots (Haissig and Davis, 1994; Guan et al., 2019), and the interdependent physiological stages of the rooting

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process are supported by changes in concentrations of endogenous auxin (Heloir et al., 1996).

Auxins are diversified in their activity. This variation in performance of various auxin sources may be attributed to their differential relationship to auxin receptors involved in the rooting process, which may be resultant from cultivar type (Tereso et al., 2008; Olatunji et al., 2017). The objective of the present study was to establish a preliminary protocol for micropropagation of two *Paulownia* species (*Paulownia hybrid* and *Paulownia tomentosa*) by investigating some factors affecting the *in vitro* propagation such as; cytokinin type and concentration, auxin type, and concentration during the rooting stage and acclimatization medium type.

2. Materials and method

2.1. Experimental design

The culture medium was Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962). The medium was solidified with 0.6% (w/v) agar and the pH was adjusted to be 5.8 before autoclaving at 121 °C for 20 min in TOMY Sx-700 autoclave (Japan). Jars (60 × 120 mm) were utilized as culture vessels and each one was filled with 50 ml of murashige and skoog medium. The cultures were incubated at a temperature of 25 ± 2 °C under 16 h/day photoperiod which provided by cool white fluorescent lamps (light intensity 2000 Lux).

Seeds of both Paulownia species were sterilized by immersion Rizolex (2 g/L) for 30 min followed by soaking in 0.3% mercuric chloride solution for 20 min., then transferred to sodium hypochlorite solution (1.0 %) for 20 min. Seeds were thoroughly rinsed three times with sterile distilled water after each previous step. After six weeks, seedlings initiated from seeds were cut into nodal segments (about 2 cm lengths) to use as explant for multiplication experiment. The MS medium was supplemented with different concentrations (0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L) of benzyladenine (BA) or Kinetin (Kin). Since 10.0 mg/L BA provided to be the best treatment for enhancing shoot multiplication and growth, this treatment was investigated alone or combined with indole-3-butyric acid (IBA) or naphthalene acetic acid (NAA) at different concentrations (0.0, 0.5, 1.0 1.5 and 2.0 mg/L). After 6 weeks from culturing number of shoots/explant and shoot length (cm) were recorded for both experiments.

During rooting stage, shoots (about 5 cm length) obtained from multiplication stage were cultured on MS medium fortified with different concentrations (0.0, 0.5, 1.0 1.5 and 2.0 mg/L) of indole-3-butyric acid (IBA) or naphthalene acetic acid (NAA). After 6 weeks from culturing number of roots/shoot and root length (cm) were recorded (El-Saadony et al., 2021d)

During acclimatization stage, plantlets obtained from rooting stage were washed under running tap water and then disinfected by immersing the roots in Rizolex at 2 g/L for 20 min. The plantlets were transferred to plastic pots (8 cm diameter) contained peat moss, peat moss and sand (1:1, v/v) or peat moss and sand (1:2, v/v). Each treatment consisted of 12 pots and each one contained one plantlet. Pots were covered with polyethylene bags for six weeks before removing them. The plantlets were held in a greenhouse at 25 °C. After six weeks, survivability (%), plantlet height (cm) and number of leaves/plantlets were recorded.

2.2. Statistical analysis:

The experiments design was factorial completely randomized design. Collected data were analyzed by the analysis of variance (ANOVA) procedure using computer program of Statistix Version 9 (Analytical Software, 2008). Differences between means were compared by using Duncan multiple range test (Gomez and Gomez. 1984).

3. Results

3.1. Effect of BA and Kin concentration on shoot proliferation and growth of both Paulownia species

Data presented in Table 1 show the effect of BA and Kin concentration on shoot proliferation and growth of both *Paulownia* species during multiplication stage. The number of shoots/explants was significantly increased with increasing either BA or Kin concentration. The highest mean number of shoots/explants i.e., 8.92 for both species was achieved by fortifying the medium with 10 mg/L BA. No significant difference between both *Paulownia* species concerning the number of shoots/explants.

The interaction between BA, Kin concentration and *Paulownia* species clear that the number of shoots/explants reached its maximum value as *P. hybrid* cultured on medium fortified with BA at 10 mg/L since it recorded 9.08 shoots/explant. Table 1 illustrate that shoot length significantly decreased as either BA or Kin concentration increased. The maximum mean shoot length of 5.25 cm was obtained on medium free from growth regulators. The shoot length didn't affect by *Paulownia* species.

The interaction between BA, Kin concentration and *Paulownia* species (*P. hybrid* and *P. tomentosa*) clear that both *Paulownia* species shoot length reached maximum values of 5.30 and 5.19 cm, respectively in growth regulators free medium.

3.2. Effect of IBA and NAA concentration combined with 10 mg/l BA on shoot proliferation and growth of Paulownia species

Table 2 clear that auxin type and concentration significantly affected number of shoots/explants after six weeks during multiplication stage. Data show that medium fortified with 10 mg/L BA combined with IBA at 1.0 or 1.5 mg/L recorded the highest number of shoots/explant (9.13 and 9.25, respectively) without significant difference between both concentrations. Based on the obtained results, *P. tomentosa* performed and recorded the highest number of shoots/explant (8.95).

Regarding the interaction between growth regulators and *Paulownia* species, the obtained results demonstrate that there were no wide differences among different interaction treatments especially when IBA or NAA was used at higher concentrations over 0.5 mg/L in both species.

As presented in Table 2, data show that auxin type and concentration significantly affected shoot length after six weeks during multiplication stage. Data clear that medium fortified with 10 mg/L benzyladenine combined with IBA at 0.5 mg/L recorded the highest mean shoot length (3.23 cm). While, there was no significant difference between both *Paulownia* species in this regard.

Concerning the interaction between growth regulators and *Paulownia* species, the obtained results indicate that *P. hybrid* cultured on medium fortified with 10 mg/L benzyladenine combined with IBA at 0.5 mg/l recorded the highest shoot length (3.27 cm). It is worth to mention that however there was no significant difference between this treatment and some other treatments but this treatment is recommended from an economical point of view.

3.3. Effect of IBA and NAA concentration on root proliferation and growth of Paulownia species

As shown in Table 3, The addition of IBA or NAA to the growth medium significantly increased the number of roots/plantlets. The *Paulownia* species were generated highest roots numbers /plantlet

Table 1
Effect of *Paulownia* species (A), BA and Kin concentration (B) as well as their interaction treatments (A × B) on shoot proliferation and growth after six weeks during multiplication stage.

	Cytokinin conc. (mg/l)	*Paulownia* species (A)		Mean (B)
		P. hybrid	***P. tomentosa***	
Number of shoots/explant	Control	1.17 l	1.33 l	1.25 I
	BA at 2	4.00 h	3.83 hi	3.92F
	BA at 4	6.92 e	7.08 e	7.00C
	BA at 6	7.92 cd	7.75 d	7.83B
	BA at 8	8.42 bc	9.08 a	8.75 A
	BA at 10	9.08 a	8.75 ab	8.92 A
	Kin at 2	2.67 k	2.50 k	2.58H
	Kin at 4	3.08 jk	3.33 ij	3.21 G
	Kin at 6	4.83 g	4.92 g	4.88 E
	Kin at 8	5.17 fg	5.33 fg	5.25 DE
	Kin at 10	5.42 fg	5.58f	5.50 D
	Mean (A)	5.33 A	5.41 A	
Shoot length (cm)	Control	5.30 a	5.19 a	5.25 A
	BA at 2	3.13 bc	3.03c	3.08C
	BA at 4	2.81 d	2.78 de	2.79 D
	BA at 6	2.52 fg	2.56f	2.54 E
	BA at 8	2.01 i	2.01 i	2.01 G
	BA at 10	1.82 j-l	1.79 kl	1.80H
	Kin at 2	3.24b	3.13 bc	3.19B
	Kin at 4	2.74 de	2.65 ef	2.70 D
	Kin at 6	2.38 gh	2.33 h	2.35F
	Kin at 8	1.95 ij	1.92 i-k	1.93 G
	Kin at 10	1.78 kl	1.75 l	1.76H
	Mean (A)	2.70 A	2.65 A	

Table 2
Effect of Paulownia species (A), IBA and NAA concentration (B) as well as their interaction treatments (A × B) combined with 10 mg/l BA on shoot proliferation and growth after six weeks during multiplication stage.

	Auxin conc. (mg/l)	*Paulownia* species (A)		Mean (B)
		P. hybrid	***P. tomentosa***	
No. of shoots/ explant	**Control**	8.17 de	8.67b-e	**8.42 DE**
	0.5 IBA	8.08 e	8.42c-e	**8.25 E**
	1.0 IBA	8.92 a-d	9.33ab	**9.13 AB**
	1.5 IBA	9.00 a-c	9.50 a	**9.25 A**
	2.0 IBA	8.67b-e	8.42c-e	**8.54C-E**
	0.5 NAA	8.75b-e	9.00 a-c	**8.87 A-D**
	1.0 NAA	8.92 a-d	9.17 a-c	**9.04 A-C**
	1.5 NAA	8.50c-e	8.92 a-d	**8.71B-E**
	2.0 NAA	9.00 a-c	9.17 a-c	**9.08 AB**
	Mean (A)	**8.67B**	**8.95 A**	
Shoot length (cm)	**Control**	3.04 de	3.03 de	**3.03C**
	0.5 IBA	3.27 a	3.19 a-d	**3.23 A**
	1.0 IBA	3.12 a-e	3.04 de	**3.08C**
	1.5 IBA	3.25 ab	3.18 a-d	**3.22AB**
	2.0 IBA	3.13 a-e	3.09b-e	**3.11A-C**
	0.5 NAA	3.22 a-c	3.20 a-d	**3.21AB**
	1.0 NAA	3.09b-e	3.11 a-e	**3.10BC**
	1.5 NAA	3.05c-e	2.98 e	**3.02C**
	2.0 NAA	3.06c-e	3.03 de	**3.05C**
	Mean (A)	**3.14 A**	**3.10 A**	

were obtained when *Paulownia* shoots was cultured on medium supplemented with any concentration of IBA without significant differences among these concentrations.

Number of roots/plantlet did not affect by type of *Paulownia* species. The interaction effect between *Paulownia* species and both auxins at different concentrations on number of roots/plantlet after six weeks during rooting stage was illustrated in Table 3. Data clear that shoots of *P. tomentosa* cultured on media IBA at 1.5 mg/L recorded the maximum number of roots/shoots. Data in Table 3 show that addition of IBA or NAA at 1.0–1.5 mg/L recorded the highest root length. In this regard, there is no significant difference between both paulownia species. While the interaction between auxin type, concentration and *Paulownia* species revealed that culturing any of experimental species on media supplemented with NAA or IBA at 1.0–1.5 mg/L recorded the maximum root length values in most cases.

3.4. Effect of Paulownia species, soil mixture as well as their interaction treatments on plantlet survivability and growth during acclimatization stage

Data presented in Table 4 clear that there are no significant differences among different tested soil mixtures or *Paulownia* species on survivability (%). Furthermore, there were no significant differences among different interaction treatments between *Paulownia* species and soil mixture.

Table 3
Effect of *Paulownia* species (A), indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) concentration (B) as well as their interaction treatments (A × B) on number of roots/plantlet and root length (cm) after six weeks during rooting stage

	Auxin conc. (mg/l)	*Paulownia* species		Mean (B)
		P. hybrid	***P.tomentosa***	
number of roots/plantlet	**Control**	2.00 g	1.92 g	**1.96 E**
	0.5 IBA	10.00 a-c	10.33 ab	**10.17 A**
	1.0 IBA	10.25 ab	9.67b-d	**9.96 A**
	1.5 IBA	10.25 ab	10.50 a	**10.38 A**
	2.0 IBA	9.92 a-c	9.91 a-c	**9.92 A**
	0.5 NAA	9.00 d-f	8.50f	**8.75CD**
	1.0 NAA	9.08 d-f	9.08 d-f	**9.08 BC**
	1.5 NAA	9.42c-e	9.08 d-f	**9.25B**
	2.0 NAA	8.75 ef	8.33f	**9.54B**
	Mean (A)	**8.74 A**	**8.59 A**	
Root length	**Control**	1.46 h	1.49 h	**1.48 D**
	0.5 IBA	3.93b-g	3.89 e-g	**3.91C**
	1.0 IBA	4.00 a-d	4.00a-d	**4.00 AB**
	1.5 IBA	4.00 a-d	4.00 a-d	**4.00 AB**
	2.0 IBA	3.84 g	3.88 fg	**3.86C**
	0.5 NAA	3.91 d-g	3.86 fg	**3.88C**
	1.0 NAA	4.02 a-c	4.00 a-d	**4.01 AB**
	1.5 NAA	4.04 a	4.03 ab	**4.03 A**
	2.0 NAA	3.95 a-f	3.92c-g	**3.93 BC**
	Mean (A)	**3.68 A**	**3.67 A**	

Table 4
Effect of *Paulownia* species (A), soil mixture (B) as well as their interaction treatments (A × B) on survivability (%), plantlet height (cm) and number of leaves/plantlet after six weeks during acclimatization stage

Soil mixture (v/v)	*Paulownia* species		Mean (B)
	P. hybrid	*P. tomentosa*	
	Survivability (%)		
Peat moss	75.00 a	83.33 a	**79.17 A**
Peat moss: Sand (1:1)	91.67 a	100.00 a	**95.83 A**
Peat moss: Sand (1:2)	83.33 a	91.67 a	**87.50 A**
Mean (A)	**83.33 A**	**91.67 A**	
	Plantlet height (cm)		
Peat moss	11.98 ab	11.91b	**11.95 AB**
Peat moss: Sand (1:1)	11.86b	11.92 ab	**11.89B**
Peat moss: Sand (1:2)	12.11 ab	12.43 a	**12.27 A**
Mean (A)	**12.09 A**	**11.98 A**	
	Number of leaves/plantlet		
Peat moss	14.50 ab	14.67 ab	**14.58B**
Peat moss: Sand (1:1)	14.00b	14.17 ab	**14.08B**
Peat moss: Sand (1:2)	15.24 a	15.25 a	**15.25 A**
Mean (A)	**14.59 A**	**14.69 A**	

Concerning plantlet height and number of leaves/plantlets, data revealed that soil mixture consisted of peat or peat: sand (1:2) generated the highest values of both characters without significant difference between both treatments. On the other hand, *Paulownia* species had no significant effect on both parameters.

The interaction between soil mixture and *Paulownia* species showed that there were no wide variations among these interaction treatments. From an economical point of view, it could be recommended to use the soil mixture of peat moss: sand (1: 2) since it is less expensive than other soil mixture.

4. Discussion

Due to the economic value of *Paulownia* wood, it is propagated to take advantage of this wood in various industries, therefore, some countries invest in these tress to reduce poverty and fighting COVID 19 (Swelum et al., 2020). The multiplication of these trees faced many problems such as seed-borne pathogens and pests, bad seed germination, and changed growth habits. As well, seedling growth is slow compared to root cutting originated from plants. Nevertheless, the addition of auxins accelerates the rooting and obtaining high-quality roots (Zayova et al., 2014). In addition, adding cytokinins to the growth medium help to get good stems and generate adventitious roots (Olatunji et al, 2017; Guan et al, 2019). The number of shoots/explants of *Philodendron selloum* was significantly increased with increasing cytokinin concentration (Hassan and Abdallah, 2015) and *Paulownia hybrid* (Fahmy and Gendy, 2018).

The BA and IBA combinations were more efficient in shoot regeneration than the combinations between BA and NAA. Cultures maintained on MS medium supplemented with 3 mg/L BA combined with 0.1 mg/L IBA recorded the highest shoot induction (100%), mean shoot length and mean number of nodes per explant of *Cryptolepiss anguinolenta*. (Monney et al., 2016), *Paulownia tomentosa* (Al-Tinawi et al., 2010), *Ficus Anastasia* (Elmeer and Al Malki, 2010), and *Paulownia tomentosa* (Thunb.) (Bahri and Bettaieb, 2013; Krishna Vrundha et al, 2021). The medium selection, choice of plant growth regulators and their concentration affected the priming and multiplication stages, and carbon sources for *P. elongata* micropropagation. Plants derived through organogenesis exhibited vigorous growth. Furthermore, the regeneration protocol could be successfully applied for producing transgenic plants with desirable traits (Bajaj et al., 2021).

Saiju et al. (2018) used MS media supplemented with different NAA concentrations (0.5, 1.0 and 1.5 mg/L) in the growth medium of two *Paulownia* species, and proved that explants treated with 0.5 mg/L NAA showed the highest average root length and density for *P. tomentosa*, while explants treated with 1.5 mg/l showed the highest average root length and density for *Paulownia fortuneii*. In addition, Fahmy and Gendy (2018) on *Paulownia hybrid* (*P. elongata* × *P. fortunei*) reported that MS medium supplemented with 2.0 mg/l NAA produced the best root and shoot growth. In addition, hybrid of almond peach rootstock was increased (Yadollahi et al., 2014).

Fahmy and Gendy (2018) found that rooted plantlets of *Paulownia hybrid* (*P. elongata* × *P. fortunei*) were successfully acclimatized in peat moss or peat moss and sand (1:1 and 1:2, v/v) media. In addition, Hassan and Moubarak (2020) showed that rooted plantlets of yucca plants were successfully acclimatized with maximum

survivability percentage (100%) in a soil mixture of sand and peat moss (1:1, v/v).

Growth hormones, whether auxins or cytokinins improve plant growth, productivity, and quality, some natural substances acting the same role, i.e., microorganisms (Alagawany et al., 2021; Desoky et al., 2020) herbal extracts (Abdel-Moneim et al., 2021; El-Saadony et al., 2021c; El-Tarabily et al., 2021; Saad et al., 2021a; Saad et al., 2020a), peptides (El-Saadony et al., 2021a,b, Saad et al., 2021b,b), and phenolic compounds extracted from agricultural wastes (Saad et al, 2021c; Saad et al, 2021d). Conventional growth regulators can also be mixed with natural materials followed by conventional breeding (Hassanin et al., 2020) to maximize the yield of *Paulownia* trees.

5. Conclusion

The use of auxins in increasing rooting and cytokines in increasing the number and quality of stems is valuable in the propagation of *Paulownia* trees of economic value, and the choice of soil type is a precious factor in the success of propagation. This study is in vitro study in preparation for the application of this experiment on a field scale and investment in these trees where its wood is of economic value.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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