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Mass Production of *Paulownia tomentosa* Trees by Micropropagation

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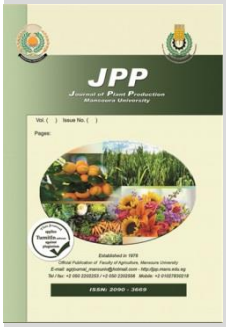

ABSTRACT

This study was carried out in the Tissue Culture Lab (TCL), in Al-Zahriya Garden at the Horticultural Institute of Agricultural Research Center in Giza, Egypt between 2018 to 2020. This study indicates the effect of different water sources and cytokinins during the proliferation stage of *in vitro* propagation *Paulownia tomentosa* plant. In this study, we do that by culturing tissues on epiphytic plants obtained from the same laboratory in the Zohriya garden. the first factor was the water source (tap and distilled water) Two different types of water sources were used: distilled water and tap water. Chemical analysis of tap water was carried out from pH TDS, TSS, EC NO3 and Ca, Na, and the second factor was the cytokinin treatments where three different types of cytokinins BA, Kin, and TDZ were used at different concentrations, 1mg/L, 2 mg/L 3mg/L 4mg/L and 5mg/L. At control concentration [0mg/L], The experiment was conducted by this study to find out the best protocol for *in vitro* micropropagation of *Paulownia tomentosa*. The results showed that the BA at 2mg/L got the highest rank for shoot length and shoot number and BA at 3mg/L got the highest rank of leaves number and shoot fresh weight while using Kin at 3mg/L got the highest rank for shoot length and total chlorophyll content. Therefore, we recommend using 2mg/L from BA with Kin at 3mg/L to get the highest rate of shoot length and shoot number and the highest rate of chlorophyll content.

Keywords: *Paulownia tomentosa*, water sources, cytokinins

INTRODUCTION

Paulownia tomentosa (Thunb.) Steud. is a very fast-growing hardwood tree known as Empress tree which belongs to the family *Paulowniaceae*. It is planted as an ornamental tree and as a source of renewable energy as well as paper pulp, electric poles, construction materials, plywood, and furniture (Barton *et al*. 2007). Paulownia wood is of high quality and convenient for making musical instruments, boxes, chests, lightweight skis, furniture, moldings, doors and windows. (Rafighi and Tabarsa, 2011). In addition, Paulownia species are among the most important forestry crops in the world. Traditional methods of vegetative propagation are slow and cannot cover the increasing demand every year. Tissue culture is the only tool that helps in producing high-quality planting material in large quantities. Also, is a sophisticated technique that involves different stages which must be performed carefully to successfully produce the planting material (Chesha *et al*., 2015). The propagation of *Paulownia tomentosa* was achieved mainly by using either seed (Ozaslan *et.al*., 2005) or nodal explants (Rout *et.al.* 2001). The conventional methods are not recommended because of the low number produced, susceptibility to pests & diseases as well as poor germination and slow growth (Bergmann and Moon,1997). However, *in vitro* propagation encouraged the production of huge numbers of healthy, homogenous, free from bacterial & fungal diseases, and with great resemblance to the mother trees. Thus, the application of tissue culture is greatly recommended for enhancing the scope and potentiality of mass propagation by exploiting regeneration behavior in a wide range of selected horticultural plants (Bonga,1987, and Durzan, 1987). The ultimate goals of this investigation are to find out the possibilities of the alternatives to conventional procedures for propagation *Paulownia tomentosa* L. plant to cover the progressive demand of this plant. Also, production of higher numbers in a short time with fewer expenses.

MATERIALS AND METHODS

This study was carried in the Tissue Culture Laboratory, Zohriya, Ornamental plants and landscape gardening res. Department of Horticultural Research Institute, Agricultural Research Center, Giza, Egypt from 2018 to 2020, to study the effect of water source and some cytokinins during the multiplication study of *in vitro* propagation *Paulownia tomentosa* plant through tissue culture on *in vitro* grown explants obtained from the same lab in Zohriya Garden.

plant material: shoot tips *In vitro* propagation of *Paulownia tomentosa* plant through tissue culture on *in vitro* grown micro-shoots explants obtained from the same lab in Zohriya Garden.

Culture medium:

Murashige and Skoog (1962) basal medium (MS) in all later experiments were autoclaved and dispensed in jars as mentioned later. Supplied with 30g/l sugar and 7g/1 agar. adjusted at 5.7+0.1 pH were autoclaved under 121 °C and 1.2 Kg/cm^2 pressure for 20 min. were poured in Jars of 250 cm^3 size were filled with about 40 cm^3 medium were used at all stages. The medium was left for 1 week to make sure that no contamination. The hormonal supplements differed according to the requirement of the specified experiments as will be mentioned later.

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Glassware:

Jars of 250 cm^3 size were filled with about 40 cm^3 medium were used at all stages.

Culture incubation conditions:

Cultures of all experiments were incubated at 28±2°C under fluorescent illumination of 2000-2500 lux at 16/8 day/night using fluorescent lamps (2 lamps per shelf). Each treatment comprised 3 (jars), with 3 micro-shoots for each replicate in each plant. And Each treatment comprised has 6 replicates

Medium type and strength experiment:

The medium used in this experiment was one type of medium in a completely randomized design experiment, namely Murashige and Skoog (1962) (MS). The medium was prepared at the concentrations mentioned before.

Proliferation stage:

The explants were inoculated singly in each jar and incubated in the previously mentioned conditions. Half strength MS medium supplemented with one of the three types of cytokinins, i.e., 6-benzylaminopurine (BAP), 6-Furfuryl-aminopurine (Kinetin or Kin) and 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (thidiazuron or TDZ), at 0, 1,2,3,4 and 5 mg/L were prepared and autoclaved as mentioned before. Bud explants were inoculated singly in each 250 cm^3 jars containing 40 cm^3 of the medium. These vessels were kept in the incubation room under the conditions. Two factors were studied in this experiment, namely cytokinin type and cytokinin concentration,The second one was water source, which was used to prepare the medium either distilled water or tap water.

In addition to their interaction accordingly, a factorial design in completely randomized experiment was adopted in this case. Three subcultures were consummated at 30 days intervals. Parameters studied in each subculture were the number of shoots,the number of leaves, shoot length (cm) total chlorophyll (mg/g FW), and shoot fresh weight (g), according to Saric *et al.*, (1967).

Statistical analysis:

These data were statistically analyzed using analysis of variance as described by Snedecor and Cochran (1989) and means were compared by Duncan critical range at a probability level of 5% (Duncan, 1955) by means of the SAS 1995 computer program.

Data recorded:

Parameters studied were number of shoots, number of leaves, shoot length (cm), shoot fresh weight (g). and total chlorophyll (mg/g FW) (Spectrophotometer reading) according to Saric *et al*. (1967).

RESULTS AND DISCUSSION

Effect of water source, cytokinin treatment and their interaction on shoot length, Table (1) and Figs. (1.a, 1.b and 1.c):

Effect of water source:

Water source affected shoot length significantly. Using distilled water gave rise to longer shoots compared to tap water (6.35 and 4.6 cm, respectively).

Effect of cytokinin treatment:

The effect of cytokinin treatment was significant. In general, shoots subjected to BAP were shorter than the other 2 cytokinins. However, as the cytokinin level increased, shoot length increased to a certain limit, where higher concentration affected shoot length negatively.

The longest shoots resulted when kinetin at either 2.00 or 3.00 mg/L, in addition to TDZ at 3.00 mg/L were applied (7.67, 7.93and 7.73 cm, respectively). The shortest ones were noticed when TDZ at 3.00 mg/L was used (2.90 cm).

Effect of the interaction between water source and cytokinin treatment:

This interaction was significant. The longest shoots were obtained when kinetin at either 2.00 or 3.00 mg/L combined with distilled water used (9.10 and 10.47 cm, respectively). The shortest shoots resulted when TDZ at 5.00 mg/L was used with tap water (1.93 cm).

Table 1. Effect of water source and cytokinin treatment on shoot length (cm) of *Paulownia tomentosa* plant.

Cytokinin types(B)mg/L	Water source (A)		Mean of (B)
	Distilled water	Tap water	
BAP 0.00 (control)	4.40 k-n	3.50 mn	3.95 fg
BAP 1.00	7.17 c-f	5.70 g-k	6.43 b
BAP 2.00	8.03 b-e	4.87 j-m	6.45 b
BAP 3.00	7.03 d-g	3.80 mn	5.42 c-e
BAP 4.00	6.80 e-h	3.73 mn	5.27 de
BAP 5.00	6.23 f-j	3.20 no	4.72 ef
Kin 0.00 (control)	4.40 k-n	3.50 mn	3.95 fg
Kin 1.00	5.83 f-j	3.50 mn	4.67 ef
Kin 2.00	9.10 ab	6.23 f-j	7.67 a
Kin 3.00	10.47 a	5.40 i-l	7.93 a
Kin 4.00	6.97 d-g	4.00 mn	5.48 b-e
Kin 5.00	3.93 mn	3.03 no	3.48 gh
TDZ 0.00 (control)	4.40 k-n	3.50 mn	3.95 fg
TDZ 1.00	8.27 b-d	4.23 l-n	6.25 bc
TDZ 2.00	6.77 e-i	5.47 h-l	6.12 b-d
TDZ 3.00	6.43 f-i	9.03 b	7.73 a
TDZ 4.00	4.20 l-n	8.43 bc	6.32 bc
TDZ 5.00	3.87 mn	1.93 o	2.90 h
Mean of (A)	6.35 a	4.61 b	

Means followed with the same letter (s) within each column are not significantly different at 1% level

Kin 3.00 mg/L Distilled water **Kin 3.00 mg/L Tap water**

Photo 1. Shows the difference in growth as affected by the water source

Effect of water source and cytokinin treatment and their interaction on the number of shoots, Table (2) and Figs. (2.a, 2.b and 1.c):

Effect of water source:

The effect of water source was significant. Distilled water gave rise to a greater number of shoots than did tap water (6.11 and 3.43 shoots, respectively).

Effect of cytokinin treatment:

cytokinin treatment affected the number of shoots significantly. The highest value in this concern was obtained when TDZ at 4.00 mg/L was used (20.83 shoots). The lowest number resulted when no cytokinins were used at all (1.17 shoots), besides shoots induced when TDZ was applied at 1.00 mg/L (1.17 shoots).

Effect of the interaction between the water source and cytokinin treatment:

The effect of the interaction is insignificant. The highest number of shoots was induced when applying TDZ at 4.00 mg/L combined with distilled water (24.33 shoots), followed in the second position by shoots produced when BAP at 3.00 mg/L and TDZ at 4.00 mg/L was applied (15.00 and 17.33 shoots, respectively). However, it should be noticed that these large numbers of shoots were not a privilege, as it they look abnormal. The lowest number of shoots (1 shoot only) was produced when distilled water was applied together with 5.00 mg/L kin, 1.00 and 2.00 mg/L TDZ, in addition to tap water deprived of any cytokinin, besides tap water combined with kin at 1.00, 2.00 or 3.00 mg/L (1.00 shoot for all).

Table 2. Effect of water source and cytokinin treatment on shoots number of *Paulownia tomentosa* plant 2020.

Cytokinin types (B)mg/L	Water source (A)		Mean of (B)
	Distilled water	Tap water	
BAP 0.00 (control)	1.33 kl	1.00 l	1.17 h
BAP 1.00	5.00 e-i	2.33 j-l	3.67 ef
BAP 2.00	14.33 c	2.33 j-l	8.33 b
BAP 3.00	15.00 bc	2.67 i-l	8.83 b
BAP 4.00	6.67 ef	5.67 e-g	6.17 cd
BAP 5.00	4.33 f-j	5.33 e-h	4.83 de
Kin 0.00 (control)	1.33 kl	1.00 l	1.17 h
Kin 1.00	2.00 j-l	1.00 l	1.50 gh
Kin 2.00	3.00 h-l	1.00 l	2.00 f-h
Kin 3.00	2.33 j-l	1.00 l	1.67 gh
Kin 4.00	2.00 j-l	1.00 l	1.50 gh
Kin 5.00	1.00 l	5.00 e-i	3.00 fg
TDZ 0.00 (control)	1.33 kl	1.00 l	1.17 h
TDZ 1.00	1.00 l	1.33 kl	1.17 h
TDZ 2.00	1.00 l	3.67 g-k	2.33 f-h
TDZ 3.00	10.33 d	7.00 e	8.67 b
TDZ 4.00	24.33 a	17.33 b	20.83 a
TDZ 5.00	13.67 c	2.00 j-l	7.83 bc
Mean of (A)	6.11 a	3.43 b	

Means followed with the same letter (s) within each column are not significantly different at 1% level

Photo 2. Shows effect of benzyl aminopurine (BAP) treatment on shoots number of Paulownia *tomentosa* plant.

Effect of water source and cytokinin treatment and their interaction on the number of leaves, Table (3) and Figs. (3.a, 3.b and 1.c):

Effect of water source:

water source affected the number of leaves significantly. Applying distilled water gave rise to a higher number of leaves compared to tap water (47.54 and 31.76 leaves, respectively).

Effect of cytokinin treatment:

The effect of cytokinin treatment was significant. Applying TDZ at 4.00 mg/L resulted in the highest number of leaves (174.50 leaves). The lowest record was obtained when no cytokinins were used at all (11.33 leaves), in addition to using 1.00 mg/L of either kinetin or TDZ (12.67 and 13.50 leaves, respectively).

Effect of the interaction between the water source and cytokinin treatment:

The effect of the interaction was significant. Using TDZ at 4.00 mg/L either with distilled water or tap water induced the highest number of leaves (179.33 ad 169.67 leaves, respectively). However, this huge number of leaves was distorted. The lowest ones resulted when kin at 5.00 mg/L with distilled water or kin at 1.00 pm with tap water was applied (8.33 and 8.67 leaves, respectively).

Table 3. Effect of water source and cytokinin treatment on leaves number of *Paulownia tomentosa* plant.

Cytokinin types(B)mg/L	Water source (A)		Mean of (B)
	Distilled water	Tap water	
BAP 0.00 (control)	11.33 hi	11.33 hi	11.33 f
BAP 1.00	45.00 de	11.67 hi	28.33 e
BAP 2.00	77.67 c	24.67 g-i	51.17 cd
BAP 3.00	112.67 b	27.33 e-h	70.00 b
BAP 4.00	55.33 d	43.33 de	49.33 cd
BAP 5.00	49.33 d	39.00 d-g	44.17 d
Kin 0.00 (control)	11.33 hi	11.33 hi	11.33 f
Kin 1.00	16.67 hi	8.67 i	12.67 f
Kin 2.00	25.33 f-i	10.00 hi	17.67 ef
Kin 3.00	21.33 g-i	17.33 hi	19.33 ef
Kin 4.00	18.33 hi	16.00 hi	17.17 ef
Kin 5.00	8.33 i	38.67 d-g	23.50 ef
TDZ 0.00 (control)	11.33 hi	11.33 hi	11.33 f
TDZ 1.00	13.67 hi	13.33 hi	13.50 f
TDZ 2.00	10.33 hi	43.00 d-f	26.67 e
TDZ 3.00	95.00 bc	47.67 d	71.33 b
TDZ 4.00	179.33 a	169.67 a	174.50 a
TDZ 5.00	93.33 c	27.33 e-h	60.33 bc
Mean of (A)	47.54 a	31.76 b	

Means followed with the same letter (s) within each column are not significantly different at 1% level

TDZ 4.00 mg/L **TDZ 2.00 mg/L**

Photo 3. Shows impact of TDZ at 2.00 and 4.00 mg/L with distilled water on leaves number of *Paulownia tomentosa* plant.

4. Effect of water source, cytokinin treatment and their interaction on shoot fresh weight (g), Table (4)

Effect of water source:

The effect of water source was not significant. However, it could be noticed that using distilled water resulted in heavier fresh shoots compared to using tap water (3.31 and 3.23 g, respectively).

Effect of cytokinin treatment:

The effect of cytokinin treatment was significant. TDZ at 5.00 mg/L gave rise to the heaviest fresh shoots (11.23 g). Values in the second position were the outcome of using TDZ at either 3.00 or 4.00 mg/L (5.96 and 5.55 g, respectively). The lowest records resulted when no cytokinins at all (1.22 g).

Effect of the interaction between the water source and cytokinin treatment:

The effect of this interaction was significant. The heaviest fresh shoots resulted when 5.00 mg/L TDZ with tap water was used (19.91 g) while the lightest fresh shoots were the outcome of using 1.00 mg/L kinetin with tap water (0.67 g). Values in the second position were confined to using 4.00 mg/L TDZ with tap water (6.79 g).

Table 4. Effect of water source and cytokinin treatment on shoot fresh weight (g) of *Paulownia tomentosa* plant.

Cytokinin types (B)mg/L	Water source (A)		Mean of (B)
	Distilled water	Tap water	
BAP 0.00 (control)	1.40 h-j	1.04 h-j	1.22 f
BAP 1.00	2.46 e-j	1.42 h-j	1.94 d-f
BAP 2.00	3.34 d-i	1.56 h-j	2.45 c-f
BAP 3.00	6.19 bc	1.34 h-j	3.77 c
BAP 4.00	2.07 f-j	3.40 d-h	2.73 c-f
BAP 5.00	1.98 f-j	1.94 g-j	1.96 d-f
Kin 0.00 (control)	1.40 h-j	1.04 h-j	1.22 f
Kin 1.00	2.67 e-j	0.67 j	1.67 ef
Kin 2.00	4.33 c-f	3.33 d-i	3.83 c
Kin 3.00	4.67 b-e	1.33 h-j	3.00 c-e
Kin 4.00	6.00 bc	1.02 ij	3.51 cd
Kin 5.00	4.33 c-f	1.36 h-j	2.85 c-f
TDZ 0.00 (control)	1.40 h-j	1.04 h-j	1.22 f
TDZ 1.00	2.27 f-j	1.42 h-j	1.84 d-f
TDZ 2.00	2.85 e-j	2.88 e-j	2.86 c-f
TDZ 3.00	5.34 b-d	6.58 bc	5.96 b
TDZ 4.00	4.30 c-g	6.79 b	5.55 b
TDZ 5.00	2.54 e-j	19.91 a	11.23 a
Mean of (A)	3.31 a	3.23 a	

Means followed with the same letter (s) within each column are not significantly different at 1% level

Effect of water source and cytokinin treatment and their interaction on total chlorophyll content (mg/g FW), Table (5):

Effect of water source:

The Effect of water source was not significant. However, it could be observed that using tap water resulted in higher total chlorophyll content than distilled water (2.07 and 1.76 mg/g FW, respectively).

Effect of cytokinin treatment:

The effect of cytokinin treatment was significant. Using 1.00 mg/L TDZ resulted in the highest content (4.08 mg/g FW), followed without significant difference by contents resulted when 2.00 or 3.00 mg/L of either kinetin (2.45 and 2.49 mg/g FW, respectively) or TDZ (2.45 and 2.48 mg/g FW, respectively) were applied. values in the second and lowest position were observed when 4.00 and 5.00 mg/L kinetin were used (2.38 and 0.56 mg/g FW, respectively).

Effect of the interaction between the water source and cytokinin treatment:

The effect of the interaction was not significant. However, it could be noticed that applying 1.00 mg/L TDZ and 5.00 mg/L kinetin gave rise to the highest and lowest contents (6.58 and 0.54 mg/g FW, respectively).

Table 5. Effect of water source and cytokinin treatment on total chlorophyll content (mg/g FW) of *Paulownia tomentosa* plant.

Cytokinin types (B)mg/L	Water source (A)		Mean of (B)
	Distilled water	Tap water	
BAP 0.00 (control)	1.74 a	1.59 a	1.67 b-d
BAP 1.00	1.55 a	1.59 a	1.57 b-d
BAP 2.00	1.60 a	1.69 a	1.64 b-d
BAP 3.00	2.33 a	2.29 a	2.31 bc
BAP 4.00	2.23 a	2.47 a	2.35 bc
BAP 5.00	0.83 a	0.88 a	0.86 b-d
Kin 0.00 (control)	1.74 a	1.59 a	1.67 b-d
Kin 1.00	1.88 a	1.96 a	1.92 b-d
Kin 2.00	2.35 a	2.55 a	2.45 ab
Kin 3.00	2.38 a	2.60 a	2.49 ab
Kin 4.00	2.51 a	2.25 a	2.38 b
Kin 5.00	0.54 a	0.58 a	0.56 d
TDZ 0.00 (control)	1.74 a	1.59 a	1.67 b-d
TDZ 1.00	1.59 a	6.58 a	4.08 a
TDZ 2.00	1.94 a	2.92 b	2.43 ab
TDZ 3.00	2.87 a	2.10 a	2.48 ab
TDZ 4.00	1.24 a	1.26 a	1.25 b-d
TDZ 5.00	0.69 a	0.71 a	0.70 cd
Mean of (A)	1.76 a	2.07 a	

Means followed with the same letter (s) within each column are not significantly different at 1% level

Sawsan and Seham (2007) found that the highest amount of chlorophyll "a" was recorded by adding 4 mg/l BA to shoots of *Cotoneaster horizontals*. On the other hand, the highest amount of chlorophyll "b" was recorded with 2 mg/l kin.

Our findings were compared with those of many researchers. Nasr El-Din and Madkour (1994) mentioned that *Philodendron sellum* shoot number was significantly enhanced by the addition of BA to the medium. There was no significant difference between 1.00 or 5.00 mg/l treatments. Zaghloul *et al.* (1996) found that the highest number of shoots of *Philodendron domesticum*/explant was obtained with 0.2 mg/l BA. Zeinab (1997) found that the addition of 5 or l0 mg/l BA significantly increased the number of *Philodendron erubescens* cv. Red Emerald and P. *scandens* "Crimson Giant" shoots. Evaldsson and Welander (1985) found that the largest number of *Cordyline terminalis* shoots/explant was obtained with 2 mg/l BA. Koriesh and AI-Manie (2000) found that medium supplemented with 2 mg/l BA significantly increased the number and length of shoots of *Philodendron scandens*. Dahab (2007) stated that BA at 2 mg/l resulted in the highest number of shoots /explant of *Hydrangea macrophylla*. Sawsan and Gabr (2007) reported that BA at 2 mg/1 increased the number of *Deutzia scarba* shoots. Sawsan and Seham (2007) found that for shooting behavior, adding 3 mg/l BA to MS medium increased shoot number/explant of *Cotoneaster horizontalis*. ChunQing *et al.* (2008) reported

that with BA at 0.5 mg/l the regeneration rate of Hanfu apple reached 35.7%, while with BA at 2.5 mg/l this trait rose to 90% and the number of regenerated buds per explant was the maximum. Radmann *et al*. (2011) stated that using BAP up to 4 mg/1 gave the highest shoot number of Prunus rootstock 'Flordaguard'. Ríos-Ramírez *et al*. (2017) observed that the highest number of shoots of Agave angustifolia was found in a medium with a BAP of 4 mg/L.

However, Kale *et al.* (2004) remarked that the multiple shoot formation of sugarcane (Co.740) decreased with the increase in the concentration of BAP over 0.5 mg/l. Ghatas. (2020) stated that BAP is more effective than both 2-ip and kinetin in increasing

proliferation parameter. The aforementioned results summarized that lower concentration (0.5 mg L) is recommended for Growth and Necrosis while 1.0 mg L from BAP induced the highest. Economou and Spanoudaki (1984) reported that at 2.5 mg/l, Kin and BA had a similar beneficial effect on *in vitro* Gardenia jaminoides explants. However, BA produced more shoots than did Kin. Mujib and Pal (1995) at 0.5 mg /l obtained a higher number of carnation shoots in the presence of BA than did Kin. Nayak *et al*. (1998) noticed that shoot bud differentiation was induced in the apical portions of *Cymbidium aloifolium* rhizomes on an MS medium containing Kin or BAP. However, the highest frequency of shoot regeneration and the highest number of shoots formed were recorded with BAP at 1.0 mg/l. Ghatas. (2016) that using 2.0 mg/L BAP is recommended for maximizing proliferation of *Paulownia tomentosa and to improve the growth stipulates its use* 1.0 mg /L of kinetin. Ning *et al*. (2006) deduced that the effect of BAP was better than that of Kin on shoot multiplication of *Cypripedium flavum*. Medium supplied with BAP (0.05 mg/L) was the most effective, providing the highest shoot multiplication frequencies associated with the highest number of shoots. RongZhe *et al*. (2007) noticed that in nodal and shoot tip cultures of *Anoectochilus formosanus*, BAP at 2 mg/l produced more shoots than did Kin. Shadang *et al*. (2007) reported that when axillary buds of *Ascocentrum ampullaceum* were cultured in a medium supplemented with various levels of BAP and Kin (0.5-2.0 mg/l), BAP at 0.5 mg/l produced the highest number of shoots. George *et al.* (2008) mentioned that BA was superior to kinetin in terms of the number of shoots produced per explant of *Baliospermum montanum* (Fam. Euphorbiaceae). Ružić and Vujović (2008) studied the effect of BA, Kin and TDZ on cherry cv. Lapins. They declared that the highest multiplication index of shoots was obtained on medium with BA at 0.45 mg/L. Very poor multiplication was achieved on medium with Kin or TDZ. Buah *et al*. (2010) declared that medium supplemented with 4.5 mg/l BAP induced the highest number of shoots in two cultivars of plantain (Oniaba and Apantu pa). BAP had the highest shoot induction response, followed by Kin. Asghar *et al*. (2011) indicated that axillary buds of orchid *Dendrobium nobile* var. Emma White was proliferated by using a medium supplemented with 0.5-3.0 mg/l of BAP or Kin. However, the maximum number of shoots was obtained at 2 mg/l BAP. Thakur and Dongarwar (2013) stated that BAP showed multiple shootings of *Oberoniare curva* in 1-5 mg/L, the best response was at 5 mg/L BAP that showed higher shoot number as compared with Kin. Gonbad *et al*. (2014) assessed the effect of BAP and TDZ, on nodal segments of tea clone Iran 100. They showed that the best treatment in terms of the number of shoots was obtained using 3 mg/l BAP. TDZ was found to be inappropriate. Ling *et al.* (2013) found that kinetin at 1 mg/l was better than kinetin at 3-7 mg/l in inducing the greatest number of shoots of *Labisia pumila* var. alata, (Fam. Primulaceae).

Kin is more effective:

On the contrary, Abu-Romman *et al*. (2015) examined *in vitro* shoot multiplication of cucumber from nodal explants using MS medium supplemented with different concentrations (0.5-3 mg/l) of cytokinins (BAP, Kin and TDZ). They stated that the maximum number of shoots was obtained with 1 mg/l Kin. The lowest culture responses were recorded for BAP.

TDZ is more effective:

Sreelatha *et al.* (2007) investigated rapid micropropagation of *Cassia siamea* with various PGR such as Kin and TDZ. They reported that the maximum shoot length was observed on medium with TDZ 0.5 mg/l. Aasim *et al*. (2009) showed that frequency of cowpea (*Vigna unguiculata*) shoot regeneration increased with the increase in TDZ concentration. The maximum number of shoots was recorded on an MS medium containing 0.25 mg/l TDZ. Kahia *et al*. (2016) cultured explants of anchote (*Coccinia abyssinica*) on a medium with various levels of BAP, Kin and TDZ. They reported that the highest number of microshoots was recorded on medium swith TDZ 0.01 mg/L. Park *et al*. (2019) placed explants of *Mertensia maritima* on a medium with 0-3.60 mg/L BA, 0-3.44 mg/L Kin and 0-3.52 mg/L TDZ. They stated that TDZ at 0.88 mg/L gave the maximum number of shoots.

CONCLUSION

Consequently, it is preferable to obtain an integrated protocol for *in vitro* multiplication of *Paulownia tomentosa* preferably use BA at 2 mg/L. got the highest rank for shoot length and shoot number BA at 3 mg/L got the highest rank of leaves number and shoot fresh weight. While using Kin at 3 mg/L got the highest rank for shoot length and total chlorophyll content. Finally, TDZ at 4 mg/L.**:** achieved the highest grade for shoot length and total chlorophyll content.at half strength Murashige and Skoog.

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الإنتاج الكمى لأشجار بولونيا تومينتوزا عن طريق الإكثار الدقيق

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تم تنفيذ هذا العمل في مختبر زراعة الأنسجة ، حديقة الزهرية ، معهد بحوث البستنة ، مركز البحوث الزراعية ، الجيزة ، مصر من 2018 إلى 2020 ، . لما لنبات البولونيا تومنتوزا من اهمية تجارية وبستانية حيث انها واحدة من أسرع الاشجار النامية والأكثر تكيفا على هذا الكوكب وتم البحث من أجل دراسة تأثير مصادر المياه المختلفة والسيتوكينينات خلال مرحلة التضاعف على نباتات البولونيا التي تم الحصول عليها من نفس المعمل في حديقة الزهرية. العامل الأول هو مصدر المياه (صنبور وماء مقطر) تم استخدام نوعين مختلفين من مصادر المياه: الماء المقطر ، ماء الصنبور. تم إجراء التحليل الكيميائي لمياه الصنبور من الأس الهيدروجيني TDS و TSS و EC NO3 و Ca و Na ، والعامل الثاني هو معاملات السيتوكينين حيث تم استخدام ثلاثة أنواع مختلفة من السيتوكينين BA و Kin و TDZ بتركيزات مختلفة ، 1 مجم / لتر ، 2 ملجم / لتر 3 ملجم / لتر 4 ملجم / لتر و 5 ملجم / لتر. عند 0 ملجم / لتر [كنترول] ، أجريت التجربة من خلال هذه الدراسة لاكتشاف أفضل بروتوكول للتكاثر الدقيق المعملى للبولونيا تومينتوزا. أوضحت النتائج أن تركيز (2 ملجم / لتر) من البنزيل ادينين حصل على أعلى مرتبة من حيث طول الافرع وعدد الافرع ، بينما حصل تركيز (3 ملجم / لتر) على أعلى مرتبة من حيث عدد الأوراق والوزن الطازج للنبات أثناء واستخدام Kin عند تركيز (3 ملجم / لتر): حصل على أعلى رتبة لطول المجموع الخضرى و إجمالي محتوى الكلوروفيل للنبات . لذلك ، نوصي باستخدام تركيز 2 mg / L BA مع 3 mg / L Kin للحصول على أعلى معدل طول للنبات و عدد الافرع وأعلى معدل لمحتوى الكلوروفيل