Nat. Volatiles & Essent. Oils, 2022; 9(1): 575-591

NVEO
Natural Volatiles &
Essential Oils

Micropropagation Of Paulownia Tomentosa (Thumb. Stud.) By Tissue Culture

Munaf A.Mohameed , Aqeel A.S Alkhalifa

College of Agriculture, University of Basrah.

Abstract

Paulownia trees are adapted to grow in different environments, where they grow in the tropics and temperate climates and withstand temperatures of 25-47 ° C. Given the importance of the plant and its widespread, this study was conducted in the Laboratory of Textile Agriculture at the College of Agriculture, University of Basra for the year 2020.To study the testing of different levels of plant growth regulators (auxin and cytokinin) in stimulating the formation of the primary callus tissue, the formation of for adventitious shoots from it, their multiplication, the formation of vegetative shoots, their rooting and their acclimatization. The results of the study showed Callus tissue was formed when auxin NAA at a concentration of 1 $mg.L^{-1}$ and cytokinin TDZ at a concentration of 5 $mg.L^{-1}$ were used with the least time period of 20 days and the highest fresh weight of 2.94 g and dry weight of 0.36 g in the MS medium with full strength, It was observed that the use of auxin NAA at a concentration of 0.5 $mg.L^{-1}$ and cytokinin TDZ at a concentration of 2 $mg.L^{-1}$ gave the shortest period of time for adventitious shoots to be 20 days and numbering 8.66 shoots .The use of auxin NAA at a concentration of 1 $mg.L^{-1}$ and cytokinin TDZ at a concentration of 5 $mg.L^{-1}$ resulted in the highest shoots multiply average of 22.3 shoots and an average length of 7.3 cm, The use of auxin IBA at a concentration of 1 $mg.L^{-1}$ resulted in the formation of roots with the highest average of 12 roots and a length of 6.6 cm, It was noted that the percentage of adapted plants increased to 90-100% than irrigation with MS salts at half and a quarter of the strength, respectively. The highest content of total chlorophyll pigment was 38.96%, carbohydrate content was 35.5 $mg.g^{-1}$, and the average of proline was 1.96 $mg.g^{-1}$

Keywords: Micropropagation - paulownia plants - plant growth regulators

Introduction

Paulownia tomentosa (thumb. Stud.) is an environmentally friendly tree native to China and belongs to the Paulowinaceae family. It is considered one of the widely spread trees in the world due to its rapid growth and the beauty of its flowers, as well as being one of the economic trees of many uses, where it is used in the production of good quality wood as well as the production of organic fertilizer (Icka et al., 2016).Its leaves are also a good source of carbohydrates and protein and can be used as animal feed or green manure (Yadav et al., 2013).Paulownia trees reproduce in different methods, including propagation by seeds, shoots and roots (Burger, 1989).It is also propagated by root cuttings of 1-2 years age plants at the end of winter and early spring (Salkić et al., 2018). As well as multiplying it with the technology of plant tissue culture (Petrus-vancea, 2018).Some studies have been conducted in the propagation of forest trees by plant tissue cultures with the aim of preserving difficult and desirable species as well as producing new cultivars and producing medicinal compounds, antioxidants, and disease-resistant plants (Matkowski, 2008).Some scientific research indicated the

propagation of the paulownia plant by the technique of plant tissue culture and for different cultivars, where it showed the possibility of propagating the paulownia by the plant tissue culture and gave good results in the production of plants corresponding to cultivar as well as the rapid production compared to using traditional propagation methods (Chunchukov & Yancheva, 2015; Venkateswarlu et al. , 2001). (Celik et al., 2008),showed that the role of plant growth regulators (BAP, Kin, TDZ, NAA) in the propagation of paulownia by plant tissue culture and the importance of choosing the type of plant part and plant growth regulator in the success of paulownia propagation histologically.(Toma, 2019) indicated the use of the MS nutrient medium in stimulating the formation of multiplying shoots on the plant parts of Paulownia, while it was shown that the WPM nutrient medium was better in stimulating the roots on the vegetative shoots in the presence of the growth regulator IBA. A study was conducted by (Idris, 2021) for the propagation of Paulownia by plant tissue culture, where it indicated the use of plant growth regulators BAP and Zeatine in stimulating lateral branch replication, while GA3 was used in branch elongation, and NAA and IBA were used in rooting shoots and forming plants ready for the acclimatization process due to the importance of the plant and its widespread, this study was conducted to achieve the following objectives Propagation of Paulownia tomentosa by plant tissue culture and several concentrations of MS organic salts. Study of multiple combinations of plant growth regulators (BAP, TDZ and NAA) in callus formation and vegetative branch multiplication.

Materials and methods

The current study was conducted in the plant tissue culture laboratory of the College of Agriculture - University of Basrah for the period from October 2020 to June 2021,The apical ends of the seedlings resulting from sowing seeds (Picture 1) on MS nutrient medium free of plant growth regulators, 1-2 cm long, were used and the following experiments were performed.

Picture (1) Plants resulting from seed cultivation on MS medium at 20 days age

Induction of primary callus.

Effect of plant growth regulators on callus induction.

The effect of cytokinin TDZ at a concentration of (7.5,2.5,0) $mg.L^{-1}$ was studied in the presence of NAA auxin at concentrations (1.5,1.0.5,0) $mg.L^{-1}$ in stimulating the formation of primary callus from plant parts. The plant parts were cultured in a solid nutrient medium consisting of MS salts and the materials mentioned in Table (1). The culture was done on a stratified airflow table that was sterilized before the culture date. After completion of the process of culture the plant parts (Explants), the plants were incubated in the Growth Chamber in the dark and

at a temperature of 27 ±1 ° C. Re-cultivation was conducted once every four weeks until the callus was formed (Millam, 2013).

Table (1) Concentrations of additives to the food medium

Material name	Amount (g/L)
Sucrose	30
Sodium hydrogen Ortho phosphate	0.170
Mesoinositol	0.100
Adenine Sulphate	0.040
Thiamine –HCl	0.0005
PVP Poly vinyl pyrolidone	1

Effect of different concentrations of MS salts.

The effect of different concentrations of MS salts (half strength, full strength, and half strength) on stimulating callus formation was studied. (1) mg/L of NAA auxin and (5) mg/L of cytokinin TDZ were added based on previous experiments. Add the substances listed in Table (1) to the nutrient medium. The following were calculated

1- The time period for the formation of the primary callus/day

2- The fresh and dry weight of the callus (g).

The formation of the adventitious shoot on the callus

The effect of plant growth regulators

The effect of slytokinin TDZ at a concentration of (0, 1,2,3) $mg.L^{-1}$ was studied in the presence of NAA auxin at a concentration of (1.0.5,0) $mg.L^{-1}$ in stimulating the formation of an adventitious shoot on the callus. The callus was cultured on a solid nutrient medium consisting of MS salts.100 mg of callus were grown with ten replications for each treatment on the stratified air flow table that was sterilized before the culture date and after the completion of the process of culture the plant parts (Explants)The Cultures were incubated at a temperature of 27 ± 1 °C and the intensity of illumination was 1000 lux for 16 hours daily. The duration of the first emergence of the adventitious shoot was recorded and the results of the formed buds were collected and prepared, as they were replanted every six weeks. (Millam, 2013).

multiplication

The effect of plant growth regulators.

The effect of cytokinin TDZ was studied at a concentration of (7.5,5,2.5,0) mg.L^{-1} in the presence of NAA auxin at concentrations (1) mg.L^{-1}, based on the previous experiment in stimulating the formation of adventitious shoot. The formed shoots were cultured at an average of 3 shoots in a solid nutrient medium consisting of MS salts at full strength and the materials mentioned in Table (2).The Cultures were incubated at a temperature of 27 ± 1 °C and a light intensity of 1000 lux for 16 hours daily. Replanting them every four weeks

rooting stage

The rooting stage is an important stage of propagation by tissue culture, in which a root system was formed on the vegetative shoots resulting from the previous stages. Accordingly, some experiments were conducted to encourage the formation of roots. The rooting experiments were as follows:

Effect of different concentrations of IBA auxin

The effect of concentrations (3,2,1,0) mg.L^{-1} of auxin IBA was studied on the rooting of vegetative shoots resulting from tissue culture. Full strength MS medium was used and ten replicates were cultured for each treatment. It was incubated at a temperature of 27 ± 1 °C and the intensity of illumination was 1000 lux for 16 hours a day. The results represented by the number and length of roots and the number of leaves were collected, as they were replanted every four weeks.

Acclimatization

Plants with integrated vegetative and root growth were selected, washed with distilled water several times to remove the remnants of the nutrient medium, and then dipped in the fungicide Elsa at a concentration of 100 mg.L^{-1} for 10 minutes, Then it was placed in a glass bottle containing a nutrient solution of MS salts only at a quarter of the strength and covered with plastic caps for 10 days, after which it was a culture in two pots measuring 15 * 15 cm, container on a medium consisting of peat moss. The plants were incubated in the acclimation room at a temperature of 23-25 m 0 and the intensity of illumination 3000 lux averaged (12 hours of illumination and 8 hours of darkness) (Pospíšilov et al., 2007; Ziv, 1995).

Statistical Analysis

1- The experiments of plant growth regulators were conducted as factorial experiments and according to a completely randomized designs. Factorial experiment conducted (C.R.D) and the significance between the means was tested according to the Revised least significant differences test (R.L.S.D) with a probability level of 1%

2- The MS experiment and the multiplication, rooting and Acclimatization experiments were conducted separately as simple experiments according to The Completely Randomized Design (C.R.D) and the significance between the means was tested according to the Revised least significant differences test (R.L.S.D) with a probability level of 1%.

Results and discussion

The effect of plant growth regulators on the induction of primary callus

The effect of auxin NAA and cytokinin TDZ

The time period for the formation of the primary callus

Results in Table (2) showed that effect of auxin growth regulators NAA and cytokinin TDZ on the time period of primary callus formation. The effect of adding the auxin growth regulator NAA to the MS culture media led to the early formation of primary callus, where the shortest period of time was recorded when treated with the concentration (1 mg.L^{-1}). It was 27 days. Which differed significantly from all other NAA concentrations treatments. While the period of 52.66 days was recorded for the (control) treatment. The addition of the cytokinin growth regulator TDZ to the MS food media led to the early induction of primary callus, and the shortest period of time for the appearance of callus was recorded when the concentration (7.5 mg.L^{-1}) treatment was 36.58 days, with a non-significant difference from the concentration (5 mg.L^{-1}) treatment. It was 36.74 days. Which differed significantly from the other two treatments and no significant value appeared when the (control) treatment. As for the interaction between the NAA auxin treatments and the cytokinin TDZ treatments, which led to the early and the shortest time for the appearance of the primary callus, the shortest time for the appearance of the callus was recorded when the treatment was at the concentration (1 mg.L^{-1}).) of NAA with concentration (5 mg.L^{-1}) of TDZ and it reached 20.33 days. Followed by the treatment with concentration (1.5 mg.L^{-1}) of NAA with the treatment with concentration (5 mg.L^{-1}) of TDZ and it amounted to 27.33 days. which was significantly similar with the treatment with concentration (1 mg.L^{-1}) of NAA with the treatment with concentration (7.5 mg.L^{-1}) and reached 29 days (Picture 2).which differed significantly from the rest of the interaction treatments. While the value recorded 61.33 days and 44 days. When treating (control) the interaction treatments.

Table (2) The effect of the growth regulator auxin NAA and cytokinin TDZ on the time period for the formation of the primary callus (day)

NAA concentration mg.L^{-1}	TDZ concentration mg.L-1				NAA average
	control	2.5	5	7.5	
control	--	--	61.33 g	44.00 f	52.66 D
0.5	--	46.66 e	38.00 d	39.00 d	41.22 C
1	--	34.00 c	20.33 a	29.00 b	27.77 A
1.5	--	39.33 d	27.33 b	34.33 c	33.66 B
TDZ average	--	39.99 B	36.74 A	36.58 A	
R.L.S.D	NAA*TDZ=2.27		TDZ=3.31	NAA=3.26	

fresh weight of primary callus

The statistical analysis table (3) shows the effect of adding the growth regulators NAA and cytokinin TDZ to the MS medium on the fresh weight of the formed primary callus. Where the addition of NAA concentrations to the MS medium led to an increase in the percentage of fresh weight of the primary callus, and the highest percentage of the weight of the callus was recorded when treated with the concentration (1 mg.L^{-1}) and it amounted to (2.25 g). With a significant difference from the treatment with concentration (1.5 mg.L^{-1}) and it was (1.95 g), while the control treatment gave the lowest weight of callus. The addition of the cytokinin growth regulator TDZ to the MS medium led to an increase in the fresh weight of the primary callus formed, and the highest percentage was achieved when treated with concentration (5 mg/L), which amounted to (2.66 g), which was significantly similar

to treatment with concentration (7.5 mg \L^{-1}) amounted to (2.48 g), which differed significantly from the concentrations of the remaining treatments. No concentration value was observed when using the control treatment. As for the interaction between NAA auxin treatments and cytokinin TDZ treatments, which led to an increase in the fresh weight of the primary callus formed, where the highest increase in the fresh weight of the primary callus was recorded when treated with concentration (1 mg/L^{-1}) of NAA with treatment with concentration (5 mg/L^{-1}) of TDZ and it amounted to (2.94 g). It was followed by the values of the treatments in concentrations (1 mg/L^{-1}) of NAA with (7.5 mg/L^{-1}) TDZ, which amounted to 2.78 g. and (1.5 mg/L^{-1}) of NAA with (7.5 mg/L^{-1}) of TDZ and it amounted to (2.49 g). and (1.5 mg/L^{-1}) of NAA with (5 mg/L^{-1}) of TDZ and it amounted to (2.44 g). As well as (1 mg/L^{-1}) of NAA with (5 mg/L^{-1}) of TDZ, which amounted to (1.70 g). These values differed significantly from the rest of the interactions treatments values.

Table (3) The effect of the growth regulator auxin NAA and cytokinin TDZ on the fresh weight of the primary callus (g)

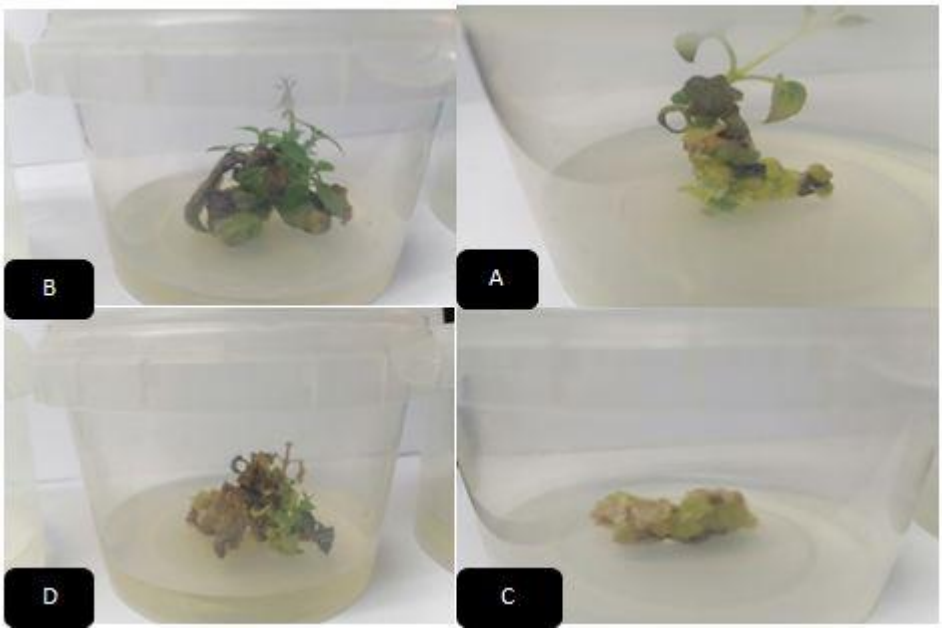
NAA concentration mg.L^{-1}	TDZ concentration mg.L-1				NAA average
	control	2.5	5	7.5	
control	--	--	B0.94	B0.96	E 0.95
0.5	--	B0.88	A1.70	B1.25	C 1.27
1	--	B1.05	A2.94	A2.78	A2.25
1.5	--	B0.94	A2.44	A2.49	B 1.95
TDZ average	--	B 0.95	A 2.66	A2.48	
R.L.S.D	NAA*TDZ=0.155		TDZ=0.22	NAA=0.22	

Dry weight of primary callus

The results in Table (4) show the effect of adding auxin growth regulators NAA and cytokinin TDZ to the MS medium on the dry weight of the primary callus. The addition of the auxin growth regulator NAA to the MS medium resulted in full strength. There was an improvement in the dry weight of the primary callus, where the treatment with concentration (1 mg.L^{-1}) gave the highest average weight of 0.27 g with a significant difference from the rest of the concentrations, while the concentration (1.5 mg.L^{-1}) gave 0.19 g, and the control treatment recorded the lowest weight of callus It was 0.06 g. The addition of the cytokinin growth regulator TDZ to the MS food media led to obtaining the highest dry weight of the primary callus, as the treatment with the concentration (5 mg.L^{-1}) gave a weight of (0.29 g).Which was significantly similar to the concentration (7.5 mg.L^{-1}) which was 0.27 g. No significant value was observed when the (control) treatment of TDZ concentrations treatments was observed. As for the interaction treatments between auxin NAA and cytokinin TDZ treatments, which gave excelled in recording the highest average dry weight of primary callus. Where, treatment with concentration (1 mg.L^{-1}) of NAA and treatment with concentration (5 mg.L^{-1}) of TDZ gave the highest dry weight of callus and it was (0.36 mg) with an insignificant difference from treatment (1 mg.L^{-1}) of NAA with concentrated treatment (7.5 mg.L^{-1}) of TDZ. It was followed by treatment with concentration (1.5 mg.L^{-1}) of NAA with treatment with concentration (7.5 and 5 mg.L^{-1}) of TDZ, which amounted to (0.26 and 0.24g), respectively.

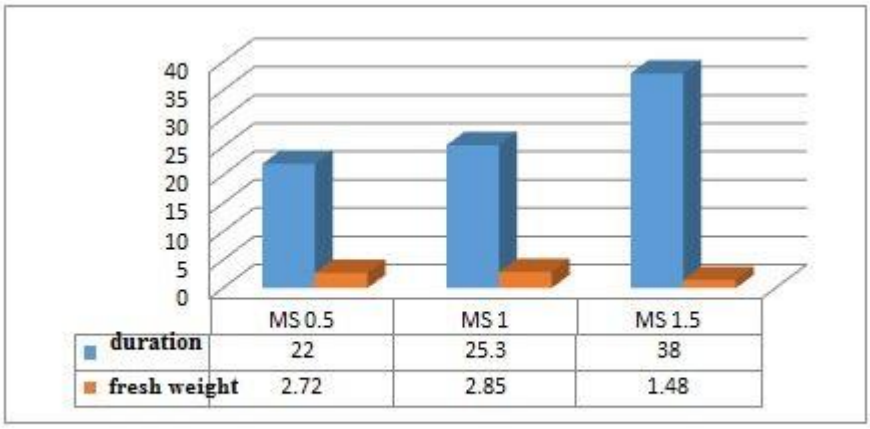
Table (4) The effect of the growth regulator auxin NAA and cytokinin TDZ on the dry weight of the primary callus (g)

NAA concentration mg.L^{-1}	TDZ concentration mg.L-1				NAA average
	control	2.5	5	7.5	
control	--	--	e0.09	d0.11	C0.10
0.5	--	e0.09	c0.18	c 0.15	BC0.14
1	--	d 0.13	a0.36	a0.33	A0.27
1.5	--	d0.11	b0.24	b 0.26	B0.19
TDZ average	--	B 0.1	A0.29	A0.27	
R.L.S.D	NAA*TDZ=0.03		TDZ=0.05	NAA=0.05	



Panel (2) The primary callus tissue formed on the edges of the vegetative shoots by the influence of growth regulators

A(1 NAA,5 TDZ) B(1 NAA,7.5 TDZ) C(1.5 NAA, 5 TDZ) D(1 NAA,2.5 TDZ(Effect of different levels of MS salts on primary callus formation



LSD= 4.39 for duration LSD= 0.62 for fresh weight

Figure (1) Effect of different levels of MS salts on primary callus formation

Figure (1) shows the effect of different levels of Murashige and Skoog MS salts on primary callus formation on the tips of vegetative shoots in the presence of plant growth regulators (NAA at a concentration of 1 mg.L^{-1} and TDZ at a concentration of 5 mg.L^{-1}),It is noted that the use of MS salts at half strength led to the formation of calluses in the shortest period of time, which amounted to 22 days, with an insignificant difference from the level of full strength, which gave callus with a period of 25.3 days, while the level gave strength and half callus with a relatively longer period of 38 days. It is also noted that MS salts in the same figure at the full strength level recorded the highest average of fresh weight of callus, which was 2.85 g, with an insignificant difference from the half-strength level, which amounted to 2.72 g, while the level of strength and half recorded the lowest average fresh weight of 1.48 g.

Effect of auxin NAA and cytokinin TDZ

The time period for adventitious shoots to formed

Shows the table in statistical analysis (5). Effect of auxin growth regulator NAA and cytokinin TDZ on the time period for the formation of adventitious shoots of callus tissue. The effect of adding the auxin growth regulator NAA to the MS food media led to the early emergence and formation of adventitious shoots of the callus. Where it gave the shortest period of time when treated with concentration (0.5 mg.L^{-1}) of NAA. It was 24 days. This was significantly similar with the treatment that followed it with concentration (1 mg.L^{-1}) of the growth regulator NAA and it amounted to 26.44 days, while shoots formed after 35.3 days when the (control) treatment. with regard to the interaction between auxin growth regulator NAA treatments and cytokinin TDZ treatments, it gave the shortest time for the appearance of adventitious shoots of these treatments when treated with concentration (0.5 mg.L^{-1}) of NAA with treatment with concentration (2 mg.L^{-1}) of TDZ, which reached 20 days Which did not differ significantly with the concentration (1 mg.L^{-1}) of NAA with the treatment with concentration (2 mg.L^{-1}) of TDZ, which reached 20.33 days. which differed significantly with the other treatments, while the formation of adventitious shoots of callus tissue was not observed at the concentrations of the control treatment except for the treatment (0 mg.L^{-1}) of NAA with the treatment (3 mg.L^{-1}) of TDZ, which amounted to 35.3 day plate (3).

Number of adventitious shoots

Table (6) shows effect of adding the growth regulator auxin NAA and cytokinin TDZ to the MS medium and the effect of that addition on the number of adventitious shoots resulting from callus tissue

Where the addition of concentrations of NAA to the medium of MS led to an increase in the number of adventitious shoots, and the highest percentage of the number of adventitious shoots when treated with concentration (0.5 mg.L^{-1}) of NAA was 7.99 shoots, a significant difference from the treatment with concentration (1 mg). L^{-1}) of the NAA and amounted to 5.21 shoots, Where, the lowest number was recorded in the control treatment, which amounted to 2.66 shoots . The addition of the cytokinin growth regulator TDZ to the MS medium led to an increase in the number of adventitious shoots formed from callus, where the highest percentage was achieved when treated with concentration (2 mg.L^{-1}) of the growth regulator TDZ, which amounted to 8.16 adventitious shoots consisting of callus. which differed significantly with the treatment with the concentration (3 mg.L^{-1}) of the growth regulator TDZ, which amounted to 5.21 shoots, and the formation of adventitious shoots was not observed when the comparison treatment. As for the values of the interaction treatments of the auxin growth regulator NAA and the treatments of the cytokinin growth regulator TDZ, which led to an increase in the number of adventitious shoots resulting from the callus tissue. NAA with concentration (2 mg.L^{-1}) of TDZ and it reached 8.66 shoots. Followed by the values of the interaction treatment with concentrations (1 mg.L^{-1}) of NAA with (2 mg.L^{-1}) of TDZ, which amounted to 7.66 cross-sections, which were significantly similar to the treatment nested with concentration (0.5 mg.L-1) of NAA with the treatment. The concentration (3 mg.L^{-1}) of TDZ, which amounted to 7.33 adventitious shoots, differed significantly from the rest of the concentrations of the other interaction treatments. The formation of adventitious shoots was not observed when the control treatment of the interactions except for the treatment with concentration (0 mg.L^{-1}) of NAA with (3 mg.L^{-1}) of TDZ, which amounted to 2.66 days.

Table (6) The effect of the growth regulator auxin NAA and cytokinin TDZ on the formation of the number of adventitious shoots of the callus tissue (shoots)

NAA concentration mg.L^{-1}	TDZ concentration mg.L-1				NAA average
	control	1	2	3	
control	--	--	--	e 35.30	B 35.3
0.5	--	--	a 20.00	c 28.00	A 24.0
1	--	d 33.00	a 20.33	b 26.00	A 26.44
TDZ average	--	33.00C	20.16A	B29.70	
R.L.S.D	NAA*BAP=1.81		TDZ=2.56	NAA=2.96	

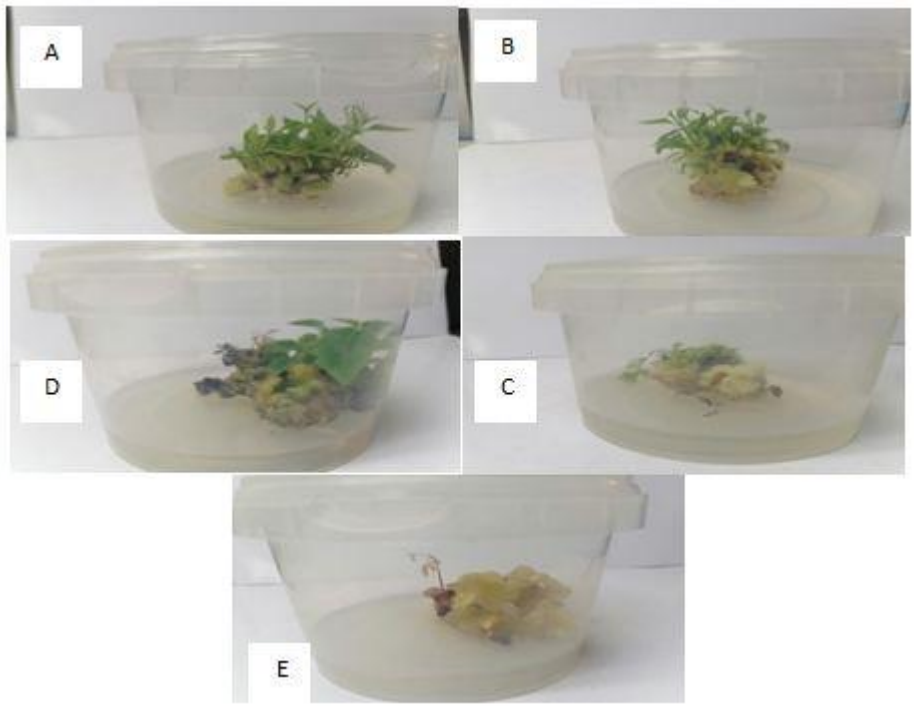
The length of adventitious shoots

The results in Table (7) show the effect of adding the growth regulator auxin NAA and cytokinin TDZ on the length of the adventitious shoots formed from the callus tissue. The addition of auxin NAA to the MS medium with full strength led to an increase in the length of adventitious shoots. The treatment with the concentration (0.5 mg.L^{-1}) of the growth regulator NAA gave the highest longitudinal increase of the adventitious shoots and it was 5.16 cm, and a significant difference from the treatment with the concentration (1 mg.L^{-1}) of NAA which was 4.71 cm,

and the lowest length was recorded when the control treatment was 2.20 cm. The addition of the cytokinin growth regulator TDZ to the MS medium with full strength also led to an increase in the length of the adventitious shoots arising from the callus, where the treatment with concentration (2 mg.L^{-1}) gave the highest longitudinal increase of adventitious shoots and reached 5.36 cm, which differed significantly with The treatment in concentration (3 mg.L^{-1}) was 4.13 cm. The formation of adventitious shoots was not observed when the control treatment of TDZ concentrations. As for the interaction treatments between the auxin growth regulator NAA and the cytokinin TDZ, the interaction treatment with concentration (1 mg.L^{-1}) of NAA with the treatment with concentration (2 mg.L^{-1}) of TDZ gave the highest increase in the length of adventitious shoots and reached 5.80 cm. It was followed by the interaction treatment that was significantly similar with it and the concentration (0.5 mg.L^{-1}) of NAA with (3 mg.L^{-1}) of TDZ and it amounted to 5.40 cm, which differed significantly with the other interaction treatments, and they were followed by the two interaction treatments that were significantly similar to each other. The first was treated with concentration (0.5 mg.L^{-1}) of NAA with a concentration (2 mg.L^{-1}) of TDZ and it reached 4.93 cm, and the second was treated with concentration (1 mg.L^{-1}) of NAA with concentration (3) mg.L^{-1}) of TDZ and it was 4.80 cm, which differed significantly from the rest of the other interaction treatments.

Table (7) The effect of the growth regulator auxin NAA and cytokinin TDZ on the length of the adventitious shoots formed from the callus tissue (cm)

NAA concentration mg.L^{-1}	TDZ concentration mg.L-1				NAA average
	control	1	2	3	
control	--	--	--	d 2.20	C2.66
0.5	--		b 4.93	a 5.40	A 7.99
1	--	c3.53	a 5.80	b 4.80	B 5.21
TDZ average	--	3.53 C	A 5.36	B 4.13	
R.L.S.D	NAA*BAP=0.59		TDZ=1.13	NAA=0.98	



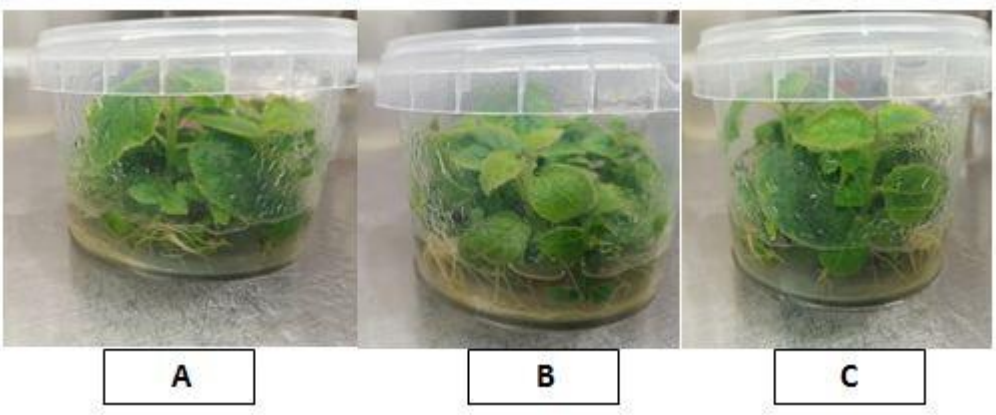
Picture (3) The adventitious shoots formed on the callus tissue by the influence of growth regulators A(0.5 NAA,2 TDZ) B(0.5 NAA,3TDZ) C(1NAA,2TDZ),D(1 NAA,3 TDZ) E(0 NAA, 0TDZ)

Table (8) The effect of the growth regulator TDZ on the doubling of vegetative shoots

TDZ concentration $mg.L^{-1}$	number of leaves	shoots length (cm)	number of shoots
0	3.6 C	4.6 C	3.6 C
2.5	5.3 B	5.6 BC	14.0 B
5	6.6 A	7.3 A	22.3 A
7.5	5.0 B	6.6 AB	20.6 A
RLSD	LSD = 1.2	LSD = 1.5	LSD=3.3

The effect of the growth regulator TDZ on the multiplying of vegetative shoots

A = TDZ (5mg.L-1) B= TDZ (7.5mg.L-1) C= TDZ (2.5mg.L-1)



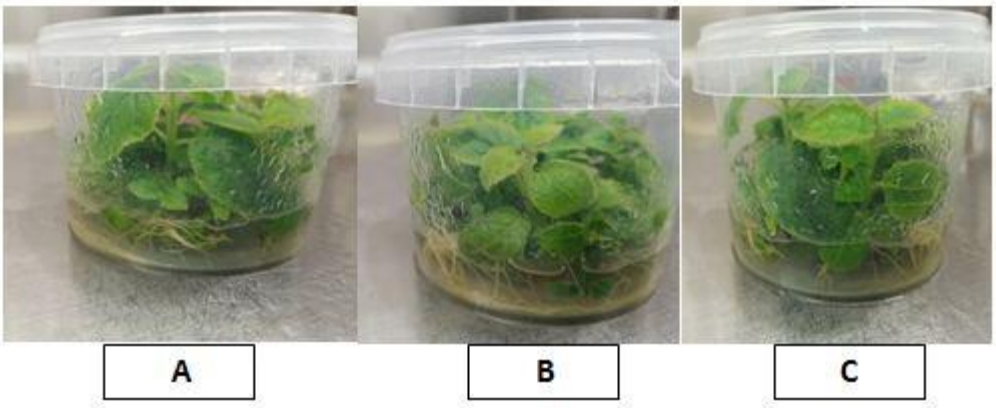
vegetative multiplication

The analysis table (9) shows the effect of the growth regulator IBA on the formation and number of roots emerging from the vegetative shoots , where the treatment with concentration (1 mm. L^{-1}) of IBA achieved the highest value in the formation of the number of roots from the adventitious shoots and reached (12 roots), which did not differ Significantly with the treatment that followed it and at the concentration (2 mg.L^{-1}) IBA and it reached (10 roots) Which differed significantly with the treatment that followed it with concentration (3 mg.L^{-1}) IBA and reached (7.66 root), followed by the treatment that was significantly similar with it with concentration (0 mg.L^{-1}) IBA and reached (6.66 root) Picture (4).As for the traits of the length of the roots, it is shown through the same table, the effect of adding the growth regulator auxin IBA on the length of the roots formed from the adventitious shoots , where the best percentage of the length of the roots formed when treated with the concentration (1 mg.L^{-1}) of IBA reached (6.66 cm) ,It was followed by the treatment that significantly differed with it in concentration (3 mg.L^{-1}) of IBA and reached (5 cm), then followed by the treatment that was significantly similar with it and in concentration (2 mg.L^{-1}) of IBA and reached (4.66 cm).Which was not significantly similar with the treatment with concentration (0 mg.L^{-1}) IBA, which amounted to (2 cm). As it is clear from the above table, the effect of the auxin growth regulator IBA on the number of leaves, where the best treatment was achieved in the emergence and the number of leaves at a concentration (1 mg.L^{-1}) of IBA reached (6.66 leaves),It was followed by the treatment that was significantly similar with it in concentration (2 mg.L^{-1}) and it reached (6.66 leaves), while the treatment with concentration (3 mg.L^{-1}) of IBA was significantly different and it reached (5.66 leaves), as well as the last treatment that in concentration differed significantly from them. (0 mg.L^{-1}) of IBA and it reached (3.33 leaves).

Table (9): The effect of auxin growth regulator IBA on rooting of vegetative shoots

NAA concentration mg.L^{-1}	number of roots	root length (cm)	number of Leaves
0	3.6 C	2.0 D	3.3 C
1	12.0 A	6.6 A	5.6 A
2	10.0 A	4.6 C	6.3 A
3	7.6 B	5.0 B	4.6 B
RLSD	LSD= 2.2	LSD = 1.3	LSD = 0.9

Picture (4) The effect of auxin growth regulator IBA on rooting of vegetative shoots



Acclimatization

Effect of irrigation with MS salts on acclimatization of the resulting plants

Table (10) Effect of MS salts on acclimatization of plants

NAA concentration $mg.L^{-1}$	The number of acclimatized plants %	plant length (cm)	number of Leaves
control	**66.6**	**11.66**	**5.66**
quarter strength	**100**	**14.00**	**7.66**
half strength	**90**	**16.66**	**12.33**
full strength	**60**	**11.00**	**7.00**
RLSD	**LSD= 22.3**	**LSD =3.75**	**LSD =1.93**

The results in Table (10) indicated the effect of irrigation with MS salts on the acclimatization of the plants produced from the tissue culture, where the highest percentage of the number of acclimatized plants at the concentration was one-fourth of the strength and amounted to (100%).Which did not differ significantly from the treatment that followed it with a half-strength concentration and amounted to (90%), which differed significantly from the control treatment, which amounted to (66.6%), and there were no significant differences with the treatment with full-strength concentration, which amounted to (60%).The results in the table also showed the effect of irrigation with MS salts on plant height, where irrigation with MS salts and the mentioned concentrations led to a longitudinal increase for the plant, where the highest longitudinal increase was at a concentration of half the strength and reached (16.6 cm)Which did not differ significantly from the treatment that followed it with a quarter-strength concentration and amounted to (14 cm), while there were significant differences with the control treatment and amounted to (11.6 cm) and the treatment that was significantly similar with it at a full-strength concentration amounted to (11 cm).The table also showed the effect of irrigation with MS salts on the formation and number of leaves in the resulting plants, where irrigation with MS salts led to an increase in the number of leaves in acclimatized plants and achieved the highest number at the half-power concentration and reached (12.33),Which differed significantly from the treatment that followed it with a

quarter-strength concentration and amounted to (7.66), which did not differ significantly from the treatment with full-strength concentration, which amounted to (7), while it differed significantly from the rest of the treatments treated with a control concentration, which amounted to (5.66) Picture (5).

Picture (5) acclimatized paulownia plants under the influence of irrigation with MS salts

Table (11) Effect of MS salts on the biochemical traits of plants

MS . salt concentration	Chlorophyll content %	Carbohydrates mg.g^{-1}	Proline µg.g^{-1}
control	**33.56**	**22.40**	**1.60**
quarter strength	**35.23**	**28.26**	**1.96**
half strength	**38.96**	**35.50**	**2.35**
full strength	**31.43**	**26.33**	**3.31**
RLSD	**LSD= 4.31**	**LSD = 7.01**	**LSD =0.93**

The results of the above statistical table (11) indicated the effect of irrigation with MS salts on the chemical traits of the resulting plants, where the highest percentage of chlorophyll content was recorded in the plants produced when treated with the half-strength concentration, which amounted to (38.96%), It did not differ significantly from the treatment that followed it with a quarter-strength concentration (35.23%), while there were significant differences in the control treatment, which amounted to (33.56%), which did not differ significantly with the full-strength treatment (31.43%).The above table also indicated the effect of irrigating with MS salts on the carbohydrate content of the resulting plants, measured in mg.g^{-1}. Irrigation with MS salts led to an increase in the percentage of carbohydrates formed in the plants, reaching the highest value when treated with a concentration of half the strength and reached (35.50). mg.g^{-1}),The treatment with a quarter-strength

concentration was significantly different from it (28.26 mg.g^{-1}), which was significantly similar to the treatment with a full-strength concentration of (26.33 mg.g^{-1}), while the last treatment did not differ significantly from it by the control concentration, which amounted to (22.40 mg.g^{-1}).The same table also shows the vegetative content of proline, measured in µg.g-1. Where the lowest value of the proline content when the control concentration was (1.60 µg.g-1),It was followed by the treatment that did not differ significantly from it with a quarter-force concentration (1.96 µg.g-1), then followed by the treatment that did not differ significantly from them with a half-force concentration (2.35 µg.g-1), while it differed significantly from the rest of the treatments in the last treatment And at a full strength concentration of (3.31 mcg.g^{-1}).Plant tissue culture is concerned with the propagation of many plant species under laboratory conditions and in large numbers, as well as the use of technology in the production of new species or strains or the propagation of average strains or the study of scientific research and dissemination of the results to give initial perceptions and develop strategies for the propagation of multiple plants . Referring to the sterile growth of cells, tissues and organs in artificial medium, although the culture of plant cells and tissues has long been a tool for plant physiologists, this technology is now increasingly used as a means of rapid reproduction of plants (Bhatia, 2015).

The method of propagation using tissue culture technology is also one of the modern and good methods for producing large numbers of plants that are free of diseases and compatible with the cultivar (Bhatia & Sharma, 2015).

Some studies have been conducted on the propagation of the paulownia plant using the technique of plant tissues culture and of different cultivars. The research indicated the possibility of propagation of the paulownia by the plant tissues culture and it gave good results in the production of seedlings that match the cultivar as well as the resulting numbers compared to using the traditional method of propagation by seeds (Bergmann & Moon, 1997; Kumar et al., 1998; Marcotrigiano & Stimart, 1983)(Bergmann & Moon, 1997) indicated that the multiplication of Paulownia from the lateral buds of the soft green parts and the formation of callus and vegetative embryos on MS medium prepared with the growth regulators NAA and benzyl adenine.The nutritional medium containing MS salts is the most widely used medium in tissue culture due to its excelled on other ancient medium and its ability to push the process of formation of callus tissues and organs, especially the formation of buds and vegetative shoots(Suman et al., 2013)Plant growth regulators such as auxins and cytokinins are one of the most important components of the nutrient medium affecting the success of tissue culture (Schaller et al., 2015), and auxins play a key role in the formation of callus and its development into vegetative embryos and their germination, the most important of which is (2,4-D)2,4-dichlorophenoxy acetic acid and NAA 1-naphthalene acetic acid, while cytokinins are important factors in the formation of vegetative shoots and induction of somatic embryos, including (BA) benzyladenine, (KN) kinetine and (2iP) (Davies, 2020).A study (Rout et al., 2001) on the propagation of Paulownia by plant tissue culture showed that the apical buds were used on the MS food medium and a combination of plant growth regulators (NAA, IBA and BAP) during the different stages of growth. The study indicated that the use of NAA with BAP led to the stimulation of lateral shoots formation, while IBA was used in the rooting stage, and this was confirmed by (Bahri & Bettaieb, 2013; Tang et al., 2010).Whereas (Rahman et al., 2013) indicated that stimulating the lateral shoots of Paulownia plant propagated by plant tissue culture is affected by the levels of plant growth regulators, where it was shown that the use of 2.5 mg.l-1 of BAP with 0.5 mg.L^{-1} of NAA led to an increase an Average number of vegetative shoots formed on the apical buds planted.

References

Bahri, N. Ben, & Bettaieb, T. (2013). In vitro propagation of a forest tree Paulownia tomentosa (Thunb.) Steud. - A valuable medicinal tree species - ProQuest. Albainian Journal of Agricultural Sciences, 12(1), 37–42. http://search.proquest.com.ezproxy.lib.rmit.edu.au/docview/1315137090/C83BFB910C4946FDPQ/20?accountid=13552

Bergmann, B. A., & Moon, H.-K. (1997). In vitro adventitious shoot production inPaulownia. Plant Cell Reports, 16(5), 315–319. https://doi.org/10.1007/bf01088288

Bhatia, S. (2015). Application of Plant Biotechnology. In Modern Applications of Plant Biotechnology in Pharmaceutical Sciences. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802221-4.00005-4

Bhatia, S., & Sharma, K. (2015). Plant Tissue Culture-Based Industries. In Modern Applications of Plant Biotechnology in Pharmaceutical Sciences. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802221-4.00014-5

Burger, D. W. (1989). Empress Tree (Paulownia tomentosa Steud.). 5, 359–369. https://doi.org/10.1007/978-3-642-61535-1_18

Çelik, Ö., Atak, Ç., & Rzakulieva, A. (2008). Stimulation of rapid regeneration by a magnetic field in Paulownia node cultures. Journal of Central European Agriculture, 9(2), 297–303. https://doi.org/10.5513/jcea.v9i2.670

Chunchukov, A., & Yancheva, S. (2015). Micropropagation of Paulownia Species and Hybrids. Peoceedings of First National Conference of Biotechnology, 100, 223–230.

Davies, P. J. (2020). Plant Hormones Biosynthesis. https://doi.org/10.1007/978-1-4020-2686-7

Icka, P., Damo, R., & Icka, E. (2016). Paulownia Tomentosa, a Fast Growing Timber. Annals ”Valahia” University of Targoviste - Agriculture, 10(1), 14–19. https://doi.org/10.1515/agr-2016-0003

Kumar, P. P., Dimps Rao, C., & Goh, C. J. (1998). Influence of petiole and lamina on adventitious shoot initiation from leaf explants of Paulownia fortunei. Plant Cell Reports, 17(11), 886–890. https://doi.org/10.1007/s002990050503

Marcotrigiano, M., & Stimart, D. P. (1983). In vitro organogenesis and shoot proliferation of Paulownia tomentosa steud. (empress tree). Plant Science Letters, 31(2–3), 303–310. https://doi.org/10.1016/0304-4211(83)90069-X

Matkowski, A. (2008). Plant in vitro culture for the production of antioxidants - A review. Biotechnology Advances, 26(6), 548–560. https://doi.org/10.1016/j.biotechadv.2008.07.001

Millam, S. (2013). Plant Tissue Culture. Techniques and Experiments. Third edition. By R. H. Smith. Amsterdam: Academic Press (2013), pp. 188, £54.99. ISBN 978-012-415920-4. Experimental Agriculture, 49(2), 317–317. https://doi.org/10.1017/s0014479712001433

Petrus-vancea, A. (2018). Paulownia Tomentosa L . in Vitro Propagation. June 2016, 30–37.

Pospíšilov, J., Synková, H., Haisel, D., & Semorádová, Š. (2007). Acclimation of plantlets to Ex vitro conditions:

Effects of air humidity, irradiance, CO2 concentration and abscisic acid (a Review). Acta Horticulturae, 748, 29–38. https://doi.org/10.17660/actahortic.2007.748.2

Rahman, M. A., Rahman, F., & Rahmatullah, M. (2013). In vitro regeneration of Paulownia tomentosa Steud. plants through the induction of adventitious shoots in explants derived from selected mature trees, by studying the effect of different plant growth regulators. American-Eurasian Journal of Sustainable Agriculture, 7(4), 280–289.

Rout, G. R., Reddy, G. M., & Das, P. (2001). Studies on in vitro clonal propagation of Paulownia tomentosa Steud. and Evaluation of genetic fidelity through RAPD marker. Silvae Genetica, 50(5–6), 208–212.

Salkić, B., Salkić, A., Keran, H., Noćajević, S., Salkić, E., & Imširović, E. (2018). Production of Seedlings of Fast - Growth Tree of Paulownia elongata S. Y. Hu. International Journal of Plant & Soil Science, 25(1), 1–8. https://doi.org/10.9734/ijpss/2018/43348

Schaller, G. E., Bishopp, A., & Kieber, J. J. (2015). The yin-yang of hormones: Cytokinin and auxin interactions in plant development. Plant Cell, 27(1), 44–63. https://doi.org/10.1105/tpc.114.133595

Suman, S., Rajak, K. K., Kishore, C., & Kumar, H. (2013). Micropropagation of banana cv. Champa. Biochemical and Cellular Archives, 13(2), 291–295.

Tang, Z. Q., Chen, D. L., Song, Z. J., He, Y. C., & Cai, D. T. (2010). In vitro induction and identification of tetraploid plants of Paulownia tomentosa. Plant Cell, Tissue and Organ Culture, 102(2), 213–220. https://doi.org/10.1007/s11240-010-9724-6

Toma, R. S. (2019). Cost Effective Culture Medium for Micropropagation of Paulownia (Paulownia tomentosa Steud.) and Catalpa (Catalpa bignonioides Walt.). 46–33 ,(4)2 ,المجلة العربية للعلوم الزراعية. https://doi.org/10.21608/asajs.2019.52886

Venkateswarlu, B., Mukhopadhyay, J., Sreenivasan, E., & Moses Kumar, V. (2001). Micropropagation of Paulownia fortuneii through in vitro axillary shoot proliferation. Indian Journal of Experimental Biology, 39(6), 594–599.

Yadav, N. K., Vaidya, B. N., Henderson, K., Lee, J. F., Stewart, W. M., Dhekney, S. A., & Joshee, N. (2013). A Review of <i>Paulownia</i> Biotechnology: A Short Rotation, Fast Growing Multipurpose Bioenergy Tree. American Journal of Plant Sciences, 04(11), 2070–2082. https://doi.org/10.4236/ajps.2013.411259

Ziv, M. (1995). 20. In vitro. 493–516.