Cost Effective Culture Medium for Micropropagation of Paulownia (Paulownia tomentosa Steud.) and Catalpa (Catalpa bignonioides Walt.)

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المستخلص :

أجريت محاولة للحصول على وسط غذائي متوازن ذو تكاليف اقتصادية قليلة للاكثار الدقيق لنباتي الباولونيا والكتالبا من خلال اختبار عدة أوساط غذائية وهي MS وWPM وGD مع اضافة تراكيز متباينة من BA وNAA في المراحل الاكثار المتوالية. أظهرت النتائج على الحصول على ١٠٠% من المزارع المعقمة من خلال تعقيم البذور لكلا النباتين في ٢.٥% من هايبوكلورات الصوديوم لمدة ١٠ دقائق. وصلت نسب انبات البذور خارج الجسم الحي للباولونيا الى ٩٨% وللكتالبا الى ٩٥% على التوالي. عند زراعة العقد المتبادلة كأجزاء نباتية في وسط MS المزود بـ٢.٠ ملغم/ لتر من BA تم الحصول على أكبر عدد من الفروع للجزء النباتي (٢.٤٦ فرع/ جزء نباتي). بينما أقل عدد من الفروع (١.٣٧ فرع/ جزء نباتي) تم الحصول عليه من خلال الزراعة في وسط GD الخالي من BA. أطول الفروع تم الحصول عليها من خلال الزراعة في وسط WPM المزود بـ ٢.٠ ملغم/ لتر BA (١.٣٩ سم). اضافة BA قد أدى الى زيادة عدد الفروع ومتوسط الطول للكتالبا (١.٨٦ فرع/ جزء نباتي). أكبر عدد من الفروع (١.٩٢) تم الحصول عليه عند الزراعة في وسط MS المزود بـ ٢.٠٠ ملغم/ لتر BA. إضافة ١.٠ ملغم لتر من NAA ادت الى زيادة عدد الجذور الى ٦.٨٤ جذرواعلى نسبة تجذير وصلت الى ١٠٠% للباولونيا النامية في وسط WPM. أعلى نسبة تجذير (١٠٠%) وأطول الجذور (٤.٨٨ سم) تم تسجيلها من خلال الزراعة في وسط WPM الخالي من NAA. اضافة ١.٠ ملغم/ لتر من NAA الى WPM كان كفيلاً قي زيادة عدد الجذور الى ١٤.٢٦ جذر/ جزء نباتي ونسبة التجذير الى ٩٨% للكتالبا. تم أقلمة ونقل

النبيتات الناتجة بنجاح الى خارج المختبر بنسبة نجاح وصلت الى ١٠٠% لكلا النباتين.

Abstract:

An attempt was done to achieve a cost effective culture medium for paulownia and catalpa micropropagation. Different types of culture media including MS, WPM and GD were tested with the addition of BA and NAA at various concentration in the successive micropropagation stages. The results showed that 100% aseptic cultures were obtained through disinfesting the seeds of both plants in 2.5% sodium hypochlorite for 10 minutes. *In vitro* seed germination rates of paulownia and catalpa reached to 98% and 95% respectively. Paulownia alternative node explants grown on MS medium enriched with 2.0 $mg.l^{-1}$ BA gave the highest number of shoots per explant (2.46 shoots/ explant). Whereas, the least number of shoots (1.37 shoots/ explant) were recorded for explants grown on GD medium with 00.0 $mg.l^{-1}$ BA. The longest shoots were found for explants grown on WPM medium enriched with 2.0 $mg.l^{-1}$ BA (1.39 cm). The addition of BA was increased the number of shoots per explants and the mean length of shoots of catalpa by giving 1.86 shoots/ explant and 1.29 cm respectively. The highest number of shoots per explant 1.92 shoots/ explant was recorded for the explants grown on MS medium with 2.0 mg/l^{-1} BA. The addition of 1.0 $mg.l^{-1}$ NAA raised the number of roots per explant by producing 6.84 roots/ explant and the highest rooting percentage (100%) on paulownia micro-shoots grown on WPM. The highest rooting percentage (100%) and longest roots (4.88 cm) were found on micro-shoots grown on NAA-free WPM. The addition of 1.0 $mg.l^{-1}$ NAA to WPM was significantly effective in raising the number of roots per explant to reach 14.26 roots/ explant and the rooting percentage to 98% for catalpa micro-shoots. NAA-free WPM produced the longest roots (4.40 cm) with the 98% of

rooting. A 100% successful acclimatization process was achieved for both tree plantlets.

Keywords: *In vitro*, MS, WPM, GD, Growth Regulators

INTRODUCTION

Plant tissue culture techniques became the prior option for plant propagation worldwide. This is because the advantageous perspectives drawn from like; mass production, year round production, production of disease-free plants in a short period of time and the cost effective propagation in long-term projects to produce uniform plats and establishment of commercial plantations (Toma *et al*., 2012). The propagation of woody plants is usually more difficult than the propagation of herbaceous plants in terms of poor multiplication and rooting (George, 2018). Paulownia is a deciduous tree originated in China characterized by a very fast growing habit. Catalpa or catawa are the common names of *Catalpa* which represents a genus of flowering plants belonging to the family *Bignoniaceae* native to warm temperate and subtropical regions of East Asia, the Caribbean and North America (Maroni *et al*., 2013).

In term of high micropropagation costs, there is a need to develop a cost effective system for large scale production of these two important forestry trees wherein the local farmer could purchase the transplants at minimal rates hence guarantee monetary gain to the growers (Pant *et al*., 2015).

Paulownia trees are fast growing and the harvesting starts within eight to ten years and can proceed every year for a long time as desired. They are extremely hardy and their plantation requires only less inputs from the grower than other trees. Their value as being a short-rotation woody plants (Bergmann and Moon, 1997), afforestation (Zhu et al., 1986) and mine site reclamation (Carpenter, 1997) has been reported. Paulownia trees have remarkable combination of qualities that resulted in

the use for a wide range of purposes. Their wood is characterized by being soft, light-weight with excellent machining and finishing properties (Akyildiz and Sahin, 2010). Its stem bark has been used in Chinese herbal medicine as a component remedies for some infection diseases. Aside from their timber products, some Paulownia species have also ornamental use (Shtereva *et al.*, 2014).

On the other hand, catalpa trees are woody plants native to North America. They are 40- to 70 feet tall trees with arching canopies. Their leaves are arrow-shaped with glossy bright green. In fall they turn a bright yellow-green before dropping as cold temperatures and chilly winds arrive. Flowers appear in spring and last into early summer. The fruit is a long bean-shaped pod, 20 to 48 cm long. The tree is useful as a shade tree, along streets and in dry, hard-to-plant sites. However, the pods can become a litter problem (Grant, 2018).

To overcome the obstacles of conventional propagation of these two important trees, this investigation was carried out to improve a reliable and cost-effective micropropagation protocol through testing different available tissue culture media enriched with various types and concentrations of plant growth regulators.

MATERIALS AND METHODS

The current investigation was carried out at plant tissue culture laboratory of Horticulture Department, College of Agriculture at the University of Duhok. The seeds of *Catalpa bignonioides* Walt. and *Paoulownia tomentosa* Steud. purchased from Sheffield's Seed Company (269 Route 34 Locke, NY 13092 USA) were cultured on growth regulators-free MS medium. Seeds disinfestations were done by immersing in 2.5% sodium hypochlorite for 10 minutes. At initiation stage, alternative nodes were taken from germinated seedlings of catalpa and paulownia after three weeks in culture media.

For shoot multiplication stage, *in vitro* grown explants were cultured on MS, WPM and GD media enriched with 0 and 2 mg.l^{-1} BA. After six weeks in culture, the number of formed shoots and their mean lengths were recorded. At root formation stage, NAA at 0, 0.5 and 1.0 mg.l^{-1} was tested on MS, WPM and GD media for both plants. After six weeks in culture, the number of producing roots, their mean lengths and rooting percentage were recorded as rooting parameters. The cultures were kept at 25± 2° C and 1000 lux light intensity and 16 hours light+ 8 hours darkness. The experiments were designed according to Completely Randomized Design (CRD) and the comparisons between means were done according to Duncan's multiple range test ($P \leq 0.05$) by using a computerized program of SAS (SAS, 2000). At the end of the experiment, the produced plantlets from both trees, were acclimatized and gradually moved to greenhouse conditions.

RESULTS

The results of seed disinfestations were very high by achieving 100% of healthy and aseptic cultures at seed culture stage of both paulownia and catalpa plants. Paulownia seed germination rate was 98% and was accomplished in one week. Whereas catalpa seed germination took about two weeks to reach 95% germination rate. At initiation stage, the response of alternative node explants was very high in both plants by giving a good number of stocks to be used as sources for next developmental stages. Table (1) reveals the responses of paulownia alternative node explants to the culture on MS, WPM and GD media enriched with 0 and 2.0 mg.l^{-1} BA after six weeks in culture. The addition of BA had no significant effects neither on the number of shoots per explants nor on mean length of shoots. Explants grown on MS medium enriched with 2.0 mg.l^{-1} BA gave the highest number of shoots per explant (2.46 shoots/

explant). Whereas, the least number of shoots (1.37 shoots/ explant) were recorded for explants grown on GD medium with 00.0 mg.l^{-1} BA. On the other hand, the longest shoots were found for explants grown on WPM medium enriched with 2.0 mg.l^{-1} BA by reaching 1.39 cm. The shortest shoots (0.48 cm) were found on explants grown on BA-free GD medium.

Table (1): Response of shoot multiplication stage of Paulownia explants grown on different culture media to Benzyl Adenine after six weeks in culture

BA (Mgl^{-1})	Culture Media	Number of shoots/ explant	Mean length of shoots (cm)
0.0	MS	2.11 b	0.44 c
	WPM	1.60 c	1.33 a
	GD	1.37 d	0.48 c
2.0	MS	2.46 a	0.92 b
	WPM	1.89 c	1.39 a
	GD	1.63 c	0.55 c
Means of BA (Mgl^{-1})	Number of shoots/ explant	Mean length of shoots (cm)	
0.0	1.69 b	0.75 a	
2.0	1.99 a	0.95 a	
Means of culture media	Number of shoots/ explant		Mean length of shoots (cm)
MS	2.28 a		0.68 b
WPM	1.74 b		1.36 a
GD	1.5 b		0.51 b

Table (2) shows the responses of catalpa alternative node explants to the culture on MS, WPM and GD media enriched with 0 and 2.0 mg.l^{-1} BA after six weeks in culture. The addition of BA was very effective on the number of shoots per explants and on mean length of shoots by giving 1.86 shoots/ explant and 1.29 cm respectively. The highest number of shoots per explant 1.92 shoots/ explant was recorded for the explants grown on MS medium with 2.0 mg/l^{-1} BA. But this increase did not reach the

significance rates as compared with the rest of treatments except BA-free WPM and GD media. The longest shoots (1.86 cm) were found for WPM enriched with 2.0 mg/l^{-1} which showed significant differences when compared to the three media with 0.0 mg/l^{-1} BA.

Table (2): Response of shoot multiplication stage of Catalpa explants grown on different culture media to Benzyl Adenine after six weeks in culture

BA (Mgl^{-1})	Culture Media	Number of shoots/ explant	Mean length of shoots (cm)
0.0	MS	1.87 a	0.65 b
	WPM	1.13 b	1.42 b
	GD	1.00 b	0.49 b
2.0	MS	1.92 a	1.34 a
	WPM	1.83 a	1.86 a
	GD	1.84 a	0.69 b
Means of BA (Mgl^{-1})	Number of shoots/ explant	Mean length of shoots (cm)	
0.0	1.33 b	0.85 b	
2.0	1.86 a	1.29 a	
Means of culture media	Number of shoots/ explant		Mean length of shoots (cm)
MS	1.89 a		0.99 b
WPM	1.48 b		1.64 a
GD	1.42 b		0.59 c

Table (3) shows the effect of NAA on rooting parameters of paulownia shoots grown on different culture media. The addition of 1.0 mg.l^{-1} NAA was very effective in increasing the number of roots per explant by producing 6.84 roots/ explant and the highest rooting percentage (100%) on paulownia micro-shoots grown on WPM as compared to only 2.00 roots/explant and 87% rooting percentage produced by those grown on NAA-free GD medium. The highest rooting percentage (100%) and

longest roots (4.88 cm) were found on micro-shoots grown on NAA-free WPM. The various stages of paulownia micropropagation are shown on Figure (1).

Table (3): Effect of NAA concentrations on rooting response of Paulownia shoots grown on different culture media after six weeks in culture

NAA (Mgl^{-1})	Culture Media	Number of roots/ explant	Mean length of roots (cm)	Rooting Percentage (%)
0.0	MS	0.50 f	1.77 c	88
	WPM	3.80 d	4.88 a	100
	GD	2.00 e	4.55 a	87
0.5	MS	2.33 e	1.68 c	90
	WPM	5.73 b	2.45 b	99
	GD	3.33 d	1.80 c	90
1.0	MS	4.93 c	1.98 c	89
	WPM	6.84 a	2.35 b	100
	GD	3.06 d	1.76 c	87
Means of NAA (Mgl^{-1})	Number of roots/ explant	Mean length of roots (cm)		Rooting Percentage (%)
0.0	2.10 a	3.73 a		91.67
0.5	1.96 a	1.97 b		93.00
1.0	2.04 a	2.03 b		92.00
Means of culture media	Number of roots/ explant		Mean length of roots (cm)	Rooting Percentage (%)
MS	2.58 b		1.81 b	89.00
WPM	5.45 a		3.22 a	99.67
GD	2.79 b		1.74 b	88.00

On the other hand, the addition of 1.0 $mg.l^{-1}$ NAA to WPM was significantly effective in raising the number of roots per explant to reach 14.26 roots/ explant and the rooting percentage to 98% for catalpa micro-shoots as shown on Table (4). Whereas, NAA-free WPM produced the longest roots (4.40

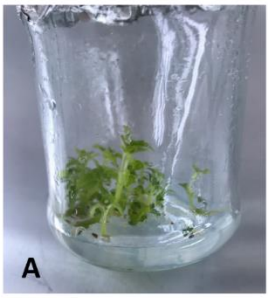
cm) with the 98% of rooting. The various stages of catalpa micropropagation are shown on Figure (2). The produced paulownia and catalpa plantlets were successfully acclimatized and gradually moved to the open-air condition with a rate of 100%.

Table (4): Effect of NAA concentrations on rooting response of Catalpa shoots grown on different culture media after six weeks in culture

NAA (Mgl^{-1})	Culture Media	Number of roots/ explant	Mean length of roots (cm)	Rooting Percentage (%)
0.0	MS	1.06 d	1.30 c	86
	WPM	2.40 d	4.40 a	98
	GD	2.06 d	0.77 d	84
0.5	MS	5.66 c	1.28 c	89
	WPM	8.18 b	2.77 b	97
	GD	10.46 b	1.22 c	87
1.0	MS	9.06 b	1.33 c	86
	WPM	14.26 a	1.86 c	98
	GD	9.06 b	0.78 d	80

Means of NAA (Mgl^{-1})	Number of roots/ explant	Mean length of roots (cm)	Rooting Percentage (%)
0.0	1.84 c	2.15 a	89.33
0.5	8.10 b	1.75 b	91.00
1.0	10.79 a	1.33 b	88.00

Means of culture media	Number of roots/ explant	Mean length of roots (cm)	Rooting Percentage (%)
MS	5.26 b	1.30 b	87.00
WPM	8.28 a	3.01 a	97.67
GD	7.19 a	0.92 b	83.67



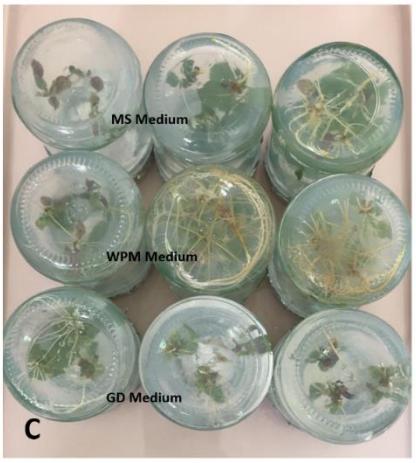
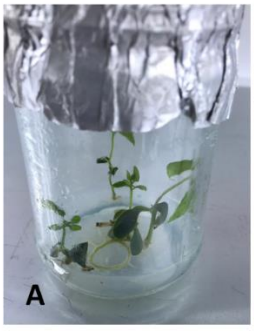
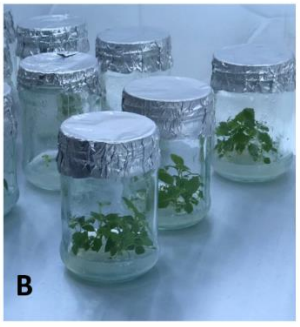


Figure (1):

Developmental Stages of Paulownia Micropropagation

A. Initiation Stage
B. Shoot Multiplication Stage
C. Root Formation Stage





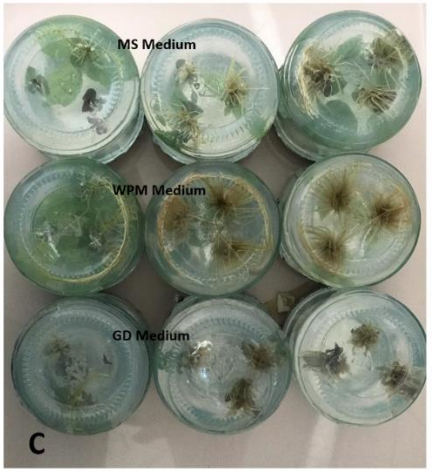


Figure (2):

Developmental Stages of Catalpa Micropropagation

A. Initiation Stage
B. Shoot Multiplication Stage
C. Root Formation Stage

DISCUSSION

The promoted role of BA on shoot multiplication is usually due to its great effect in releasing lateral buds from the dominance of terminal buds without the need to remove the apical bud by enhancing the formation of vascular tissues of buds which ease the transformation of water and nutrients leading to lateral bud growth (Mohammed and Al-Younis, 1991). Further than, it's prominent role in the increasing the enhancing of RNA, protein and enzymes inside the cell which promote bud growth too (Al-Rifae´e and Al-Shobaki, 2002). As well as, the genetic structure has an influence on the response of cultured explant because of its effect on endogenous hormone content (Singh *et al.*, 1994*)*. The different performance of explants on different culture media is highly related to the different salts content especially those related to C/N ratios (Travers *et al*., 1989). The results of the current investigation proved that NAA has a positive role in rooting process since it promotes adventitious roots initiation on the bases of cultured shoots (Toma and Tamer, 2015). These results are in agreement with what has been found by Shtereva *et al*. (2014); Bergmann and Moon (1997) and Wysokinska and Swiatek (1989). In general, *in vitro* propagation technology is more expensive than the conventional plant propagation methods. The prices of pre-mixed culture media are varied according to the type of the type and other ingredients. Usually, WPM is cheaper than MS medium in the market. In the current study, the use of WPM was superior upon MS medium almost in all shoot multiplication and root formation stages. Achieving such cost effective propagation protocols will defiantly reduce the whole propagation costs. In conclusion, it can be said that through applying this micropropagation protocol for both paulownia and catalpa trees is highly recommended for local nurseries toward mass

production of these two economically important trees with low cost projects.

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