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Employment of *Tissue Culture* Techniques in Improvement Propagation of *Paulownia Tomentosa* Plant

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ABSTRACT

Different media and explant types, anti-oxidant pre-treatments, additives, cytokinin types &concentrations as well as medium strength, GA3 concentrations, auxin types &concentrations were studied to find out the best protocol of *in vitro* propagation of *Paulownia tomentosa*. The results indicated that culturing of pre-treated shoot tips with anti-oxidant solution on modified Murashige&Skoog medium supplemented with PVP and activated charcoal as anti-oxidants, as well as adenine sulphate and coconut milk as additives maximized survival percentage and improved explant development. Also, using of 2mg/ L BAP increased proliferation. Addition of 1.0 mg/ L GA3 to half strength medium maximized shoot length. Moreover, addition of 1.0 IBA to the culture medium encouraged the highest shoot length and number of roots.

Keywords: *In vitro* culture, *Paulownia tomentosa*, woody plants.

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INTRODUCTION

Paulownia tomentosa (Thunb.) Steud. is a very fast growing hard wood tree known as Empress tree which belonging to the family Paulowniaceae. It is planted as 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. Traditionl methods of vegetative propagation are slow and can not cover the increasing demand every year. Tissue culture is the only tool which helps in producing high quality planting material in large quantity. Also, is a sophisticated technique which involves different stages which have to be performed carefully to successfully produce the planting material (*Chesha et al.* 2015). The propagation of *Paulownia tomentosa* was achieved mainly by using either seeds (Ozaslan *et.al*.2005) or nodal explants (Rout *et.al*. 2001).The conventional methods are not recommended as result of low number produced , susceptibility to pests & diseases as well as poor germination and slow growth (Bergmann and Moon,1997).However, *in vitro* propagation encouraged production of huge numbers of healthy, homogenous, free from bacterial & fungal diseases, and with great resemblance to the mother trees. Thus, application of tissue culture is greatly recommended for enhancing the scope and potentiality of mass propagation by exploiting regeneration behavior in a wide rang of selected horticultural plants (Bajaj.1986,Bonga and Durzan. 1987). The ultimate goals of this investigation are to find out the possibilities of the alternatives of conventional procedures for propagation of *Paulownia tomentosa* to cover the progressive demand of this plant .Also, production of higher numbers in short time with fewer expenses.

MATERIALS AND METHODS

This study was carried out at the Tissue Culture Unit ,Horticulture Department , Faculty of Agriculture, Benha University during the period from 2013 to 2014. New growing branches from good growing, healthy *Paulownia tomentosa* trees were taken from the Nursery of Tissue Culture Unit and subjected to running water for 5 minutes and divided into small parts. Then sterilized by using 10 % Clorox with 2 drops of Tween-20 for 15 minutes and immersed in distilled water. Then all the following steps were done under asceptic conditions. Shoot tips were excised from terminal parts with 0.5-1.0 mm length. The remaining parts were divided into one node cuttings as explants. The prepared explants were cultured on different nutrient media supplemented with 30 gm/L sucrose,0.1mg/L IBA (Indole -3- butyric acid),1.0/L BAP (6-benzylaminopurine), and 7.0 g/L Difico Bacto agar. The PH was adjusted to 5.7 and autoclaved at 121ºC and 15 Ib/in for 15 minutes.The cultured explants were incubated under16 hours of artificial light (Fluorescent light) and 8 hours of darkness at average temperature of 27-28ºC.

The following experiments were carried out:

I-Establishment stage:

A-Effect of the culture medium and explant type:

Different explants i.e. shoot tips and one node cuttings were cultured on different nutrient media i.e. Murashige & Skoog, modified Murashige & Skoog, Woody Plant Medium (W.P.M) to find out the most effective explants and medium type.

B- Effect of anti-oxidant treatment:

Variable anti-oxidant compounds were evaluated to determine the best anti-oxidant treatment valuable in reducing the accumulation of free phenolic compounds which is toxic and causing necrosis for the explants.

The anti-oxidant compounds used in this study were as follow:

1-Control: the explants were immersed in sterilized distilled water as pre-treatment for2 hours.

2-Anti-oxidant solution (A.O.S.): the explants were immersed in anti-oxidant solution (consists of a mixture of 100 mg/L ascorbic acid and150 mg/Lcitric acid) for2 hours as recommended by Wang *et.al.(*1994).

3-Activated charcoal (A.C.): 3000 mg/L of plant activated charcoal was supplemented to the culture medium.

4-Polyvinylpyrrolidone (P.V.P.): 100 mg/L of P.V.P. was added to the culture medium as recommended by Siqueira *et.al.*(1991*)*

5-Combination of anti-oxidant solution and activated charcoal.

6-Combination of anti-oxidant solution and Polyvinylpyrrolidone.

7- Combination of A.O.S. and A.C. and P.V.P.

C-Effect of additives:

Different additives were supplemented to the culture medium to study the effect of these additives and select the most suitable one.

The following additives were evaluated in this study:

1- Control (no additives were used in the culture medium)

2-80 mg/L of adenine sulphate was added to the culture medium

3-10% of coconut milk was supplemented to the culture medium

4- Combination of adenine and coconut milk were added to the culture medium

II-Effect of cytokinin types and concentrations:

Different cytokinin types i.e. control (cont.), kinetin (Kin.)**,** 6-benzylaminopurin (BAP), 2-isobentenyl-adenin (2-ip) with **d**ifferent concentrations i.e. 0.0, 1.00, 2.00, and4.00 mg /L were studied to select the best cytokinin type with suitable concentration that enhanced the highest proliferation.

III- Rooting :

A- Effect of medium strength:

Different medium strengths i.e. full, one half, one-fourth, one eighth were treated to detect the more suitable medium strength induced the best shoot length and number of roots.

B-Effect of gibberellic acid (GA3) concentrations:

GA3 was supplemented to the culture medium with different concentrations i.e, 0.0, 0.5, 1.0, 2.0 mg/L to verify the most suitable concentration of GA3 that encouraged the highest shoot length and root numbers.

C- Effect of auxin type :

Addition of different auxin types i.e. indole acetic acid (IAA), indole-3-butyric acid
(IBA) and Naphthalene acetic acid (NAA) at 1.0 mg/L level to point out the best auxin type maximized shoot length and number of roots.

D-Effect of indole-3-butyric acid (IBA) concentrations:

Different IBA concentrations i.e. 0.0, 1.0, 2.0, 3.0 mg/L were tested to determine the most effective concentration in inducing the best shoot length and number of roots..

Data and Calculations:

Scores were given for growth, greening, and explant development. These scores were estimated as follow: negative results = 1, below average = 2, average = 3, above average = 4 and excellent results = 5 .However, the reverse is true for browning,greening and necrosis according to (pottino, 1981). On the other hand, proliferation and number of roots parameters are estimated by counting their numbers. Shoot length determined by measuring the shoot length (cm).

Survival percentage was calculated as:

Survival % = No. of survived plants / total No. of starting plants×100

Statistical analysis:

All treatments used in this study were arranged in a complete randomized block design and replicated 4 times with 3 jars for each replicate. The obtained date were subjected to analysis of variance and statistically analyzed according to Duncan's multiple range test (Duncan, 1955) at 1%level.

RESULTS AND DISCUSSION

I-Establishment stage:

A-Effect of the culture medium and explants type:

Table (1) reflects the effect of different explants and medium types on survival percentage and explant development parameters of *Paulownia tomentosa* .It is clear from Table (1-A) and Photo (1) that survival % was significantly increased when shoot tips were used as compared with one node cuttings .However , the revese is true when necrosis parameter is considered. On the other hand, statistical differences were lacked between shoot tips and one node cuttings when explant development, greening, and browning parameters were concerned.

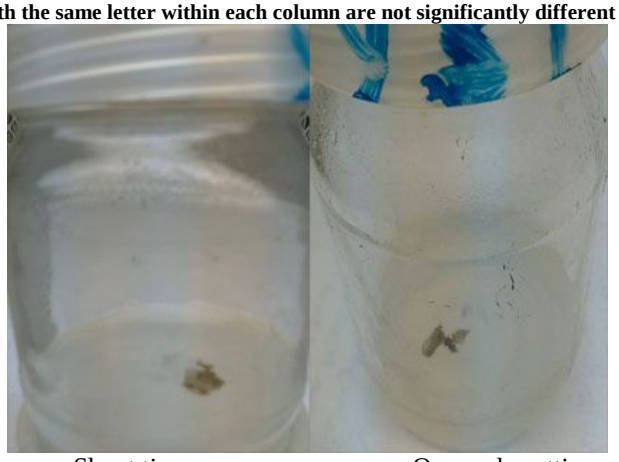
Moreover, Table (1-B) showed that modified Murashige & Skoog was superior in increasing of Survival %, explant development, and greening parameters as compared with the other media types. Otherwise, necrosis and browning behaved differently in relation to Woody Plant Medium. Referring to the interaction between explant and medium types ,its appear from Table (1-C) that combination of either shoot tips or one node cuttings and modified Murashige & Skoog medium was significantly enhanced explants development parameter in relation to others. Meanwhile, combination of shoot tips and modified Murashige & Skoog medium induced the best statistical survival % and greening parameters in comparison with the other combinations. On contrast, the lowest significant decrease of necrosis appeared when combinations of shoot tips and either Murasige & Skoog or Modified Murasige & Skoog medium was used.

The above mentioned results reveals that culturing of shoot tips on modified Murashige & Skoog medium maximized survival percentage, explant development, while reduced both necrosis and browning parameters. These results somewhat go in line with the findings of Emam (2006) on *Pyrus communis* stated that shoot tips surpassed one nodal cutting in improving explant development parameter.

Table (1-A): Effect of explant type on survival % and explant development parameters of *Paulownia tomentosa*.

Parameters / Explant type	Necrosis (Scores)	Survival %	Explant development (Scores)	Greening (Scores)	Browning (Scores)
Shoot tips	1.98b	17.00a	2.22a	1.98a	2.58a
One node cuttings	3.12a	11.00b	2.67a	1.90a	2.73a

Means of explant type followed with the same letter within each column are not significantly different from each other at 1% level.



Shoot tip One node cutting

Photo (1): Effect of explant type on explant development of *Paulownia tomentosa.*

Table (1-B):Effect of medium type on survival % and explant development parameters of *Paulownia tomentosa*.

Parameters / Medium type	Necrosis (Scores)	Survival %	Explant development (Scores)	Greening (Scores)	Browning (Scores)
MS.	2.62b	8.00b	2.04b	1.81b	2.60b
Modified MS.	1.98c	31.00a	2.99a	2.64a	2.25b
W.P.M	3.05a	3.00b	1.39c	1.38c	3.12a

Means of medium type followed with the same letter within each column are not significantly different from each other at 1% level.

Table (1-c): Effect of combinations of medium and explant types on survival % and explant development parameters of *Paulownia tomentosa*.

Explant type	Medium type	Necrosis (Scores)	Survival %	Explant development (Scores)	Greening (Scores)	Browning (Scores)
Shoot tips	MS.	1.85d	13.00c	2.32b	1.80bc	2.30bc
	Modified MS.	1.86d	36.00a	3.08a	2.82a	2.10c
	W.P.M	2.23c	3.00d	1.26d	1.26c	3.55a
One node cuttings	MS.	3.40b	3.00d	1.76c	1.76c	2.90ab
	Modified MS.	2.11c	26.00b	2.91a	2.46ab	2.40bc
	W.P.M	3.86a	3.00d	1.52cd	1.49c	2.90ab

Means of combinations of medium and explants types followed with the same letter within each column are not significantly different from each other at 1% level.

B- Effect of anti-oxidant treatments:

Table (2) clarifies the effect of different anti-oxidant treatments on explant development parameters. It is clear that combination treatment of anti-oxidant solution as pre-treatment and addition of both activated charcoal and Polyvinylpyrrolidone encouraged the most suitable conditions for statistical increase of explant development and greening parameters while significantly reducing both necrosis and browning in relation to other studied treatments.

Table (2): Effect of different antioxidant treatments on explant developme parameters of *Paulownia tomentosa*

Parameters / Antioxidant treatments	Necrosis (Scores)	Browning (Scores)	Explant development (Scores)	Greening (Scores)
Control	3.60a	3.72a	2.20cd	1.63e
Antioxidant soulution	2.32d	2.80c	2.25cd	2.49b
Poly vinyl pyrrolidone (P.V.P)	2.80c	2.76c	2.36bc	2.26c
Activated charcoal	3.20b	3.20b	1.77d	1.95d
Antioxidant solution+ Poly vinyl pyrrolidone	1.69e	1.88e	2.85b	2.68b
Poly vinyl pyrrolidone + Activated charcoal	2.20d	2.56d	2.41bc	2.51b
Antioxidant solution+ Poly vinyl pyrrolidone+ Activated charcoal	1.50e	1.63f	3.56a	3.58a

Means of antioxidant treatments followed with the same letter within each column are not significantly different from each other at 1% level.

The aforementioned results indicated the importance of combination treatment of anti-oxidant solution, activated charcoal and Polyvinylpyrrolidone in reducing free phenolic compounds which induced browning and toxic for explants causing necrosis. Thus, reducing of free phenols is recommended for inducing the best conditions for improving the results. Anti-oxidant solution and Polyvinylpyrrolidone encouraged changing free phenols to conjugated phenols while activated charcoal adsorbing free phenols(Pierik,1987).These results are ingeneral agreement with the findings of Abd El-Kader (2004*)* who found that combination of anti-oxidant solution plus P.V.P. succeeded in reducing browning of taxodium plant.

C-Effect of additives:

Data in Table(3) showed that combination treatment of addition adenine sulphate and coconut milk enhanced significant increase of survival percentage ,explant development, and greening parameters while the revese is true for both necrosis and browning parameters as compared with the other additives.

Generally, supplementation of the culture medium with adenine sulphate and coconut milk improved all parameters under study as they contained some promotive effect.

Table (3):Effect of different additives treatments on explant development parameters of *Paulownia tomentosa*.

Parameters / Additives	Necrosis (Scores)	Browning (Scores)	Survival %	Explant development (Scores)	Greening (Scores)
Control	2.80a	2.69a	23.00c	1.46d	1.29d
Adenine sulphate	1.56c	1.48c	63.00b	2.92c	2.88c
Coconut milk	1.93b	2.10b	66.00b	3.82b	3.60b
Adenine sulphate+ Coconut milk	1.46d	1.20d	83.00a	4.71a	4.06a

Means of additives followed with the same letter within each column are not significantly different from each other at 1% level.

II-Proliferation:

A-Effect of cytokinin types and concentrations:

It is quit evident from Table (4) that using kinetin statistically improved growth and reduced necrosis parameters in relation to other cytokinins. However, using of BAP was significantly maximized proliferation while 2-ip took the second rank in this concern.

Furthermore, Table (4) showed that growth was significantly improved by adding 1.0 mg /L of kinetin as compared with the other concentrations. However, proliferation was significantly increased through increasing concentration up to 2.0 mg /L then decreased again as 4mg /L was used. Moreover, increasing concentration up to 4.0 mg /L was statistically maximized necrosis parameter in relation to other treatments.

The above mentioned study conclude that using 2.0 mg/L BAP is recommended for maximizing proliferation of *Paulownia tomentosa*. These results are confirmed by the findings of Abd El-Kader (2004) who told that using 2.0 mg/L BAP increased proliferation of *Taxodium distichum* plants.

Table (4): Effect of different of cytokinin types with different cocentrations on proliferation and growth parameters of *Paulownia tomentosa*.

Cytokinin type	Concentrations(mg/L.)	Necrosis	Growth	Proliferation
Control	0.0	2.90bc	2.12c	2.13e
Ki	1.0	1.72fg	3.80a	1.64f
	2.0	1.87f	2.68b	2.76d
	4.0	2.56d	1.33f	3.07c
BAP	1.0	1.70fg	2.87b	3.80b
	2.0	2.80c	2.00cd	4.73a
	4.0	3.77a	1.60e	2.80d
2-IP	1.0	1.63g	2.76b	2.36e
	2.0	2.17e	2.08c	3.70b
	4.0	3.03b	1.76de	3.60b

Means of cytokinin type with concentrations mg/L. followed with the same letter within each column are not significantly different from each other at 1% level.

III- Rooting :

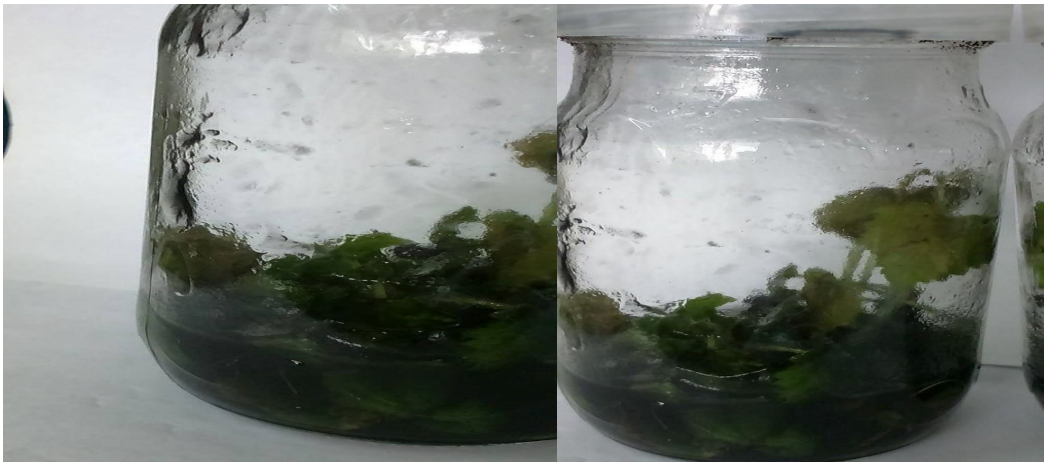
A-Effect of medium strength:

Table (5) and Photo(2) explain the effect of medium strength on shoot length and number of roots parameters. It appears that half strength medium was significantly increased shoot length, greening and number of roots parameters in relation to other medium strengths. However, full strength medium maximized necrosis in comparison with the other treatments.

Table (5):Effect of medium strength on shoot length and number of roots parameters of *Paulownia tomentosa.*

Parameters / Medium strength	Necrosis	Greening	Shoot length (cm)	No. of Roots
Full	1.96a	3.12b	3.10b	2.87c
One half strength	1.84ab	3.93a	4.80a	4.03a
One fourth strength	1.70b	2.93c	3.50c	3.03b
One eigth strength	1.66b	1.80d	2.50d	2.20d

Means of medium strength followed with the same letter within each column are not significantly different from each other at 1% level



Full medium strength **Half medium strength**

Photo (2): Effect of medium strength on shoot length of *Paulownia tomentosa*.

The above results summarized that reducing medium strength to one half was valuable in increasing free water and decreased osmotic pressure of the cultured medium which reflected in an increase of absorption ability and in turn improved most of the parameters under study.These results are in harmony with the studies of Aydieh *et.al.* (1999).They recommended half strength M.S. medium induced the best rooting of pineapple plants.

B-Effect of gibberellic acid (GA3) concentrations:

Table (6) and Photo (3) reveal the effect of different GA3 concentrations on shoot length and number of roots parameters .It is clear that either shoot length or greening parameter was significantly superior as 1.0 mg/L GA3 was supplemented to the culture medium in relation to other GA3 concentrations under study.Meanwhile,supplementation of the culture medium with 0.5 mg/L encouraged the highest significant number of roots in comparison with the other GA3 concentrations.

In general the results declared that 1.0 mg/L GA3 maximized shoot length and most other parameters under study. These results confirmed the results of Kiran *et.al . (2004).*They found that dwarf shoots of *Mentha piperite L.* were elongated on MS medium supplemented with 1.0 mg/L GA3.

Table (6): Effect of different GA3 concentrations on shoot length and No.of roots of *Paulownia tomentosa*.

Parameters / GA3 conc.(mg/L.)	Necrosis	Greening	Shoot length (cm)	No. of Roots
0.0	1.91b	2.71c	2.03	3.10b
0.5	1.90b	3.86a	2.52c	4.00a
1.0	2.16ab	3.23b	6.10a	2.00c
2.0	2.54a	3.10b	4.55b	2.00c

Means of different GA3 concentrations followed with the same letter within each column are not significantly different from each other at 1% level.

0.5 mg/L GA3 **1.0mg/L GA3**

Photo (3): Effect of different concentrations of GA3 on shoot length of *Paulowniatomentosa*

C- Effect of auxin types:

Table (7) refer to the effect of different auxin types on shoot length & number of roots parameters .It is appear that shoot length and necrosis were significantly increased when naphthalene acetic acid (NAA) was used as compared with using of indole-3-butyric acid. However, number of root parameter was statistically maximized as indole-3-butyric acid (IBA) was used.

The aforementioned results summarized that indole-3-butyric surpassed either IAA or NAA in increasing number of roots. These results are in harmony with the findings of Arikat *et. al.* (2004).They found that IBA was more active than IAA or NAA in promoting root development of *Salvia fruticosa* Mill.

Moreover, Table (8) &Photos (4,5) verified that lower IBA concentration (1.0 mg/L) induced the highest shoot length and number of roots in relation to both other concentrations under study.

Table (7): Effect of different auxin types on shoot length and number of roots parameters of *Paulownia tomentosa*.

Parameters / Auxin type (1.0 mg/L)	Necrosis	Shoot length (cm)	No.of Roots
IBA	3.03a	6.80c	3.30a
NAA	2.60b	8.45a	2.20b
IAA	2.06c	7.20b	1.60c

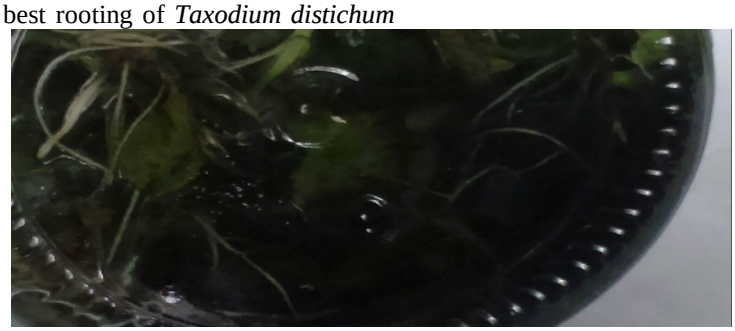
Means of auxin type followed with the same letter within each column are not significantly different from each other at 1% level.

Table (8): Effect of different IBA concentrations on shoot length and number of roots parameters of *Paulownia tomentosa*.

Parameters / IBA conc. (mg/L.)	Necrosis	Shoot length (cm)	No.of Roots
0.0	1.28c	8.77a	1.56d
1.0	1.32c	8.83a	3.45a
2.0	2.93b	6.33b	2.96b
3.0	3.30a	5.62b	2.50c

Means of IBA concentrations followed with the same letter within each column are not significantly different from each other at 1% level.

The above study conclude that adding of 1.0mg/L IBA to the culture medium encouraged the highest growth and number of roots. These results are in harmony with findings of Abd El-Kader (2004) who mentioned that the best rooting of *Taxodium distichum* was obtained when 1.0 mg/L IBA was used., Asghar *et al*. (2016) stated that rooting occurred with the highest frequency in *Rosa damascene* cultured on a medium containing 1.0 mg L IBA .



1.0 mg/L IBA (No.of Roots)

Photo(4): Effect of 1.0 mg /L IBA on No.of Roots of *Paulownia tomentosa*.

0.0 mg/L IBA 3.0 mg/L IBA 2.0 mg/L IBA 1.0 mg/L IBA

Photo(5): Effect of different IBA concentrations on shoot length and number of roots of *Paulownia tomentosa*.

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توظيف تقنية زراعة الأنسجه فى تحسين اكثار نبات البولونيا

ياسر عبد الفتاح عبد العاطي غطاس

قسم البساتين – كليه الزراعه– جامعه بنها- مصر

اشتملت الدراسه على العديد من التجارب مثل دراسة تحديد البيئه والجزء النباتى المناسبين وتاثير مضادات الأكسده واضافات البيئه للحصول على أفضل نسبه مئويه للبقاء وتطور النسيج كما تمت دراسة أنواع مختلفه من السيتوكينينات واختبار التركيز المناسب من ال ٦-بنزايل أمينوبيورين لاحداث أكبر زياده عدديه ممكنه وكذادراسة قوى مختلفه من البيئه وتركيزات من الجبريللين لتحقيق أفضل طول للنمو وعدد جذور كما تمت دراسه أنواع مختلفه من الاكسينات وكذا تركيزات مختلفه من اندول حمض البيوترك للحصول على أفضل طول نمو و تجذير. و أظهرت النتائج الحصول على بروتكول متكامل لاكثار البولونيا داخل الأنابيب وذلك من خلال زراعة القمه الناميه المعامله بمحلول مضادات الأكسده على بيئة موراشيج وسكوج المعدله مع اضافة البولى فينيل بيرلدون والفحم النباتى النشط كمضادات أكسده واضافة الادينين ولبن جوز الهند كإضافات للبيئه وذلك للحصول على أعلى نسبه مئويه للبقاء وتطور النسيج وكذلك اضافة ٢ملجم/لتر من ٦-بنزايل أمينوبيورين لاحداث أكبر زياده عدديه ممكنه وأثبتت الدراسه كذلك ان اضافة ٠.٥ملجم من الجبريللين الى بيئه نصف قوه ساعد على أفضل زياده فى طول النموات كما ان اضافة ٠.١ملجم/لتر من اندول حمض البيوترك شجعت على تكوين أفضل عدد من الجذور.