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Impact of Explants, Plant Growth Regulators and their Interaction on Micropropagation of *Impatiens balsamina*, L.

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ABSTRACT

The aim of this study is to improve the propagation of *Impatiens balsamina*, L by an efficient protocol for *in vitro* propagation was successfully established through tissue culture technique. Cotyledonary node with sections of cotyledon, section of cotyledons and hypocotyls were derived from 21 days *in vitro* germinating seeds after surface sterilization. They were cultured for shoot multiplication and callus induction on MS medium fortified with various concentrations of BAP, TDZ or combination between BAP and NAA. It was determined that cotyledonary node with sections of cotyledon on MS medium supplemented with 4.5 mg/L BAP for maximum shoots number (4.33shoots) and callus induction 3.78. The best shoot length (3.08 cm) within 8 weeks from regeneration was achieved on MS medium fortified with 1.0 mg/L BAP + 0.5 mg/L NAA. The number of shoots formed per explants increased by re-culturing for six weeks on the same medium (4.5 mg/L BAP alone), since it produced (9 shoots). While the best shoot length (4cm) was obtained by sub culturing the explants on MS medium received 1.0 mg/L BAP + 0.5 mg/L NAA to MS without growth regulators after six weeks. The best callus fresh weight (9.32 g) and callus volume (7.76 cm^3) were recorded with using TDZ at 3.0 mg/L that sub cultured on medium fortified with 4.5 mg/L BAP. In addition the highest dry weight (0.81 g) was obtained by re - culturing on the same medium that contained with 4.5 mg/L BAP.

Keywords: Tissue culture, balsam, auxin, cytokinin, gibberellins, BAP, TDZ, IBA, NAA, callus.

INTRODUCTION

Balsam (*Impatiens balsamina*, L.) belonging to Family Balsaminaceae. It is an annual herbaceous plant, growing in tropical regions of Asia and Africa (Fleming, 2000). The flowers produced different color (purple, pink, white and red). Also, it considered as important medicinal plants. All the balsam parts have many active constituents (phenolics, naphthoquinone derivatives, triterpenoids, glycosides, fatty acids, alkaloids and flavonoids (Sakunphueak and Panichayupakaranant, 2012). The whole plant is used for the medicinal, cosmetic and traditional purpose. (Farnsworth and Nanthawan, 1992). Red pigments can be extracted from flowers and used as a substitute for Henna (*Lawsonia inermis*) in cosmetics. The seeds, leaves, flowers of garden balsam are edible (Lim, 2016). Balsam is propagated from seeds, but the seeds are not available in Egypt except through import from abroad and it is very difficult to fulfill the demand from it for its high cost, which is increasing every year and this contributes to the high production cost of the seedling. So, there was a need to develop its propagation technology through tissue culture technique for rapid and large-scale propagation to produce large quantities of it at a lower cost and expand its cultivation in Egypt. The practice of plant tissue culture has contributed towards the propagation of a large number of plants from small pieces of stock plants in a relatively short period of time (Daniel, 1998). Callus is an important source for indirect plant organogenesis and embryogenesis. Over one thousand plant species have been regenerated *in vitro* via organogenesis and embryogenesis. These two methods are among the most striking processes in plant micro propagation.

The plant growth regulators are the critical in the *in vitro* media which affect the cell, tissue, organ development and differentiation. Since, it includes the auxins, cytokinins and the gibberellins. These plant growth regulators are defined as organic compounds which accelerate the control growth and other functions development of the plants through affecting the physiological processes (Rout and Jain, 2004). A relation between high ratio of cytokinin and low ratio from auxin could be induced the shoot formation, whereas reverse favors root formation. The most frequently used auxins 2, 4-dichlorophenoxy acetic acid (2, 4-D), Indole-3-acetic acid (IAA), Naphthalene acetic acid (NAA) and Indol butyric acid (IBA). N-6-furfurylaminopurine (Kin) and 6-benzylaminopurine (BAP) are commonly used as cytokinins. Thidiazuron (TDZ) a substituted phenylurea (N-phenyl-N-1, 2, 3-thiadiazol-5-ylurea) is used as a synthetic herbicide and a plant growth regulator to stimulate the high rate of axillary shoot proliferation in many woody plant species and stimulates shoot formation in a wide variety of plant species. TDZ have ability to remove an apical dominance (Durkovic and Mišalová, 2008). It has been considered to be more potent than most of the commonly used cytokinins. TDZ directly promotes growth due to its own biological activities in a fashion similar to that of an N- substituted cytokinin or it may induce the synthesis and accumulation of an endogenous cytokinin. In woody plant species, low levels of TDZ induce the axillary shoot proliferation, whereas BAP and other cytokinins are not effective Guo *et al*., (2011), but higher levels may inhibit it. Moreover, TDZ used for stimulating somatic embryo formation (Rugini and Silvestri, 2016). TDZ also promotes callus formation in many state more than other regulators Traore *et al*., (2003). The success of *in vitro* micro propagation is also affected by explants type such as cotyledon, hypocotyle, epicotyle , root explants (Shah and George, 2019),and cotyledonary node Kumar *et al*., (2016) which have huge effect on tissue culture plants.

In this context, the aim of this research was to study the effects of many factors such as explants types, auxin and cytokinin types and concentrations plus their combinations via *in vitro* culture and find out a commercial method for micro propagation of *Impatiens balsamina*, L.

MATERIALS AND METHODS

This study was achieved during the seasons of (2017-2018) at the Tissue Culture Laboratory of Vegetable and Floriculture Department, Faculty of Agriculture,

Mansoura University, Egypt, to improve the *in vitro* propagation of garden balsam.

Surface sterilization and seeds germination:

Seeds of balsam plant were obtained from the Flower Goddess Company (China). The seeds were washed under running tap water containing a few amount of household detergent for one hour, then it surface sterilized by immersing in ethanol alchohol (70%) at different durations (30 or 60 sec) followed by (30%) commercial colorex solution for (5, 10 or 15 min). After that it was rinsed three times in sterilized distilled water in laminar air flow hood to remove the residuals. Finally, sterilized seeds were cultivated on autoclaved water with medical cotton in 250 ml jars under aseptic conditions and the culture kept in growth chamber at 25± 2°C with 16 h. light/ followed by 8h dark cycle and left for germination for two weeks, then transferred aseptically to sterilized culture jars containing MS according to (Murashige and Skoog ,1962) free hormone solid medium.

Media preparation and culture conditions:

Two weeks old seedlings that produced from the previous stage were cultured on full strength MS media fortified with 8 g/L agar and sucrose at 30 g/L .The pH of the medium was adjusted to 5.7 prior to addition of agar and before autoclaving for 20 min at 121 °C under 1.1 kg/cm2 pressures, then left to cool. All the cultured jars (250 ml) contained 30 ml of medium each of which were incubated in plant growth room at 25± 2°C under constant fluorescent light of 2500 Lux for 16 h. light/ followed by 8h. dark photoperiod, and left for one week on this medium.

Exp. I: Shoot multiplication and callus production:

Three types of explants (cotyledonary node with sections of cotyledon, section of cotyledon and hypocotyl explants) of balsam were excised in a laminar flow hood from 21 days old grown seedlings and cultured on MS free hormone medium as a control or fortified with different combinations and concentrations of cytokinins named 6-benzylaminopurine (BAP) at 1.5, 3.0, 4.5 or 5.5 mg/L , Thidiazuron (TDZ) at 0.5, 1.5 or 3.0 mg/L or 6-benzylaminopurine (BAP) at 1.0, 3.0 and 5.0 mg/L in combination with Naphthalene acetic acid (NAA) at 0.5 or 1.0 mg/L) to evaluate the callus size by three persons as different degrees for the three examined explants types and shoots (number and length) number of (leaves and root) of cotyledonary node. Cultures were incubated for eight weeks under the same conditions as mentioned above.

Exp. II: Re - culture or Sub culture of regenerated explants:

In this stage shoots from multiplications stage were transferred aseptically on fresh MS medium supplemented with 6- benzylaminopurine (BAP) at 3.0 or 4.5 mg/L or Thidiazuron (TDZ) at 3.0 mg/L or 6- benzylaminopurine (BAP) at 1.0, 3.0 and 5.0 mg/L in combination with Naphthalene acetic acid (NAA) at 0.5 and 1.0 mg/L and medium without growth regulators as illustrated in Table (1) to study the influence of different growth regulators and re or subcultures on shoot multiplication, callus weight fresh and dry (g) and volume (cm^3) of cotyledonary node with sections of cotyledon. Cultures were incubated for six weeks under the same conditions as mentioned above.

Table 1. The composition of the growth regulators in the tested re-culture or subculture medium on the multiplication and callus production stage.

Explant type: Cotyledonary node with section of cotyledon explants		
Multiplication and callus production media		Re or sub culture media (mg/L)
Plant growth regulators	Concentration (mg/L)	
BAP	1.5	
TDZ	0.5	Without growth regulators
TDZ	1.5	
BAP: NAA	1.0:0.5	
BAP	3.0	
BAP	4.5	BAP 4.5
TDZ	3.0	
BAP: NAA	5.0:0.1	TDZ 3.0
BAP: NAA	3.0:1.0	BAP: NAA
BAP: NAA	5.0:0.1	3.0:1.0

Statistical analysis:

Exp. I (Multiplication and callus production media) contained fourteen treatment and exp. II contained six treatment of three replicates, each of which contained four jars. Treatments were arranged in a Completely Randomized Designs (CRD), COSTAT v.63 statistical software was used for analysis of variance (ANOVA) and subsequently Least Significant Differences (LSD) method according to (Steel and Torrie, 1980) was done for means comparison at $P \leq 0.05$.

RESULTS AND DISCUSSION

Exp. I: Shoot multiplication and callus production:

Effect of explant types, plant growth regulator types, concentration and their interactions on callus size and callus morphology after 8 weeks

The obtained results in Table (2) and photo (1) revealed that the best result for callus induction were observed from cotyledonary node with sections of cotyledon when compared to the other explants, where the callus induction was varied depending on the explants types and concentration of growth regulators that used, In addition this result agreed with the study of Das *et al*., (2013) who believed that the different plant tissues have different levels of endogenous PGRs, so explants types could have an effect on the callus induction process. Moreover, section of cotyledon explants produced callus than hypocotyl explants in this study and a similar result was observed by Vaezi *et al*., (2015) on *Trigonella foenumgraecum* L. Also 4.5 mg/L BAP was more effective for induction of a big callus compared with the other treatments. Since, the big callus (3.78) was obtained from a cotyledonary node with sections of cotyledon, followed by (3.37) for section of cotyledon explants. On the other side, the hypocotyl explants didn't produce any callus. Followed by 3.00 mg/L BAP that produced (3.60) for cotyledonary node of sections of cotyledon and (3.00) for section of cotyledon, respectively. But, it was clear that BAP at low concentration produced the lowest callusing, besides to the higher concentrations of BAP 5.5 mg/L, since it inhibited the callus formation except with hypocotyls (3.49). BAP is required at concentrations ranging from 3.0 to 4.5 mg/L, but higher and lower concentrations of BAP had an adverse effect on callusing rate formation.

Also from Table (2) we found that the largest callus size of (3.65) obtained from hypocotyl explants that cultured on medium contained 3.0 mg/L TDZ only.

Table 2. Effect of explants type, plant growth regulator type, concentration and their interaction on callus size and callus morphology of balsam plant after 8 weeks:

Plant growth regulators types	Conc. (mg/L)	Explant types			Callus morphology	
		Callus size				
		Cotyledonary node with sections of cotyledon	Section of cotyledon	Hypocotyls	Texture	Color
Control	Without growth regulators	1.00	1.00	1.00	-	-
BAP	1.5	2.00	1.00	1.00	C&F	P to G
	3.0	3.60	3.00	1.00	C&F	P,G,B,R to W
	4.5	3.78	3.37	1.00	C&F	P,G,B to W
	5.5	1.00	2.17	3.49	C&F	P to G
TDZ	0.5	2.00	2.33	1.00	C&F	G,B to W
	1.5	2.11	2.22	2.00	C&F	G to B
	3.0	2.85	2.71	3.65	C	G,P to B
BAP: NAA	1.0:0.5	2.55	1.28	1.00	C&F	G to B
	1.0:1.0	1.00	1.00	1.00	-	-
	3.0:0.5	1.00	1.00	3.00	F	B,W
	3.0:1.0	2.00	1.00	1.00	F	B ,Y to W
	5.0:0.5	1.00	2.41	1.00	F	B
	5.0:0.1	3.00	1.00	1.00	F	B,W to G

Size of callus at different degrees: 1: No callus, 2: Small callus, 3: Medium callus, 4: Big callus, G: green, B: brown, Y: yellow, W: white, P: pink, R: red, C: compact, F: friable.

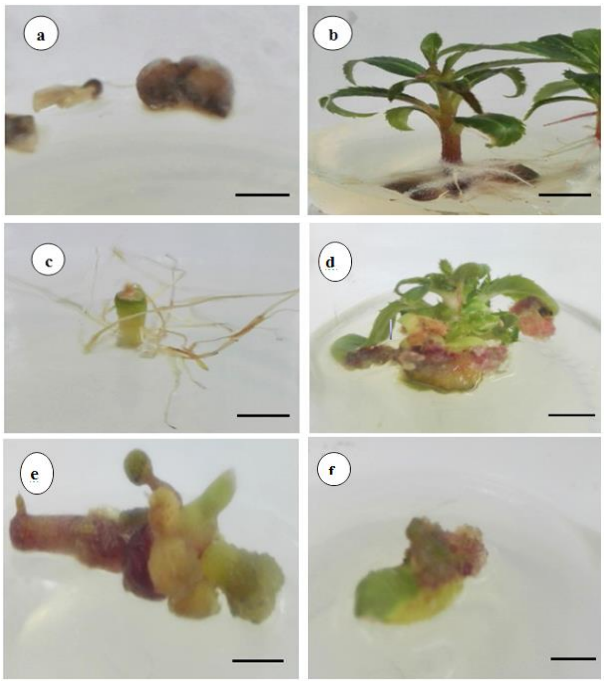

Photo. 1. Effect of explants type, plant growth regulator type concentration and their interaction on means of callus size of balsam plant after 8 weeks.

a. section of cotyledon cultured on free hormone medium.
b. cotyledonary node with section of cotyledon cultured on free hormone medium.
c. hypocotyl cultured on free hormone medium.
d. cotyledonary node with section of cotyledon cultured on 4.5 mg/LBAP.
e. section of cotyledon cultured on 4.5 mg/LBAP.
and f. hypocotyl cultured on medium fortified with 3.0 mg/L TDZ.
***Scale bars = 1 cm.**

But the other concentrations of TDZ gave the lowest callus size for the other both explants types. The interaction between BAP and NAA gave a weak response for most type of explants except cotyledonary node with sections of cotyledon that cultured on medium fortified with 5.0 mg/L BAP + 0.1 mg/L NAA produced a medium size of callus (3.00). A conflict results were observed by Modarres *et al*., (2018) who found that the combination of 5 mg/L BAP + 5 mg/L NAA on MS medium gave the highest percentage of callus induction rate (100%) and callus dry weight (0.38 g) in *Salvia leriifolia benth* and these conflicting results could be attributed to the use of different species as well as the possible effects of different genotypes.

The obtained data show that no callus was observed with any explants cultured on medium free of growth regulators. This result agreement with that of (Prakasha and Umesha, 2018) who found that the MS medium, without any growth hormones, was unable to induce callus in *Nerium odorum*. Also section of cotyledon turned to black color and died as shown in photo (1, a). Different colors (pink, Greenish Yellow, green and white) of callus and textures (Compact and friable) were observed.

Section of cotyledon and hypocotyl explants didn't induces any shoots and this similar to previous research in balsam plant Taha *et al*., (2009) with any type and concentrations of plant growth regulators. Also no shoots were induced from callus that produced from section of cotyledon and hypocotyls on the shoot induction media on the present study of balsam plant.

Effect of plant growth regulator types, concentrations and their interaction on shoots number, shoots length, leaves number and roots number per shoots of cotyledonary node with sections of cotyledon of balsam plant after 8 weeks:

Data in Table (3) and photo (2) indicated that among the various cytokinins, BAP was found to be more efficient than the others treatments for shoots regeneration. As using BAP showed appositive results than the other cytokinins. A similar result was obtained on *Alpinia galangal* Shamsudheen *et al*., (2018) and *Thymus lotocephalus* Coelho *et al*., (2012).

In addition, cotyledonary node explants was more responsive for shoot regeneration when compared to the other explants and this has been reported previously in *Trigonella foenum-graecum* L., since a cotyledon node explants has been used for successful shoot regeneration Aasim *et al*., (2010). The maximum number of shoots was observed on medium fortified with BAP at 4.5 mg/L since it produced (4.33 shoots photo (2a) within 8 weeks followed by (3.16) for medium supplemented with BAP at 3.0 mg/L which showing

a gradual increase with higher applications and reaching a maximum at 4.5 mg/l before decreasing at higher concentrations and this were agreement with Bekircan *et al.*, (2018) in *Thymus lotocephalus*. Also, BAP inhibited shoots elongation at high concentrations and this were agreement with Ashraf *et al.*, (2014) in *Chlorophytum borivilianum Sant.* & *Fernandez.* But, for shoot length medium that supplemented with combination of 1.0 mg/L BAP + 0.5 mg/L NAA showed a lower percentage of shooting (1 shoot) with the tallest shoot (3.08 cm) and this were agreement with Sjahril *et al*., (2016) who observed that media supplemented with combination of BAP and NAA 0.5:0.5 mg/L in the MS medium promoted shoot length for *Chrysanthemum morifolium*. Also, it produced the maximum leaves number (16.17 leaves) and this was similar for report of Sen *et al.*, (2014) who observed that the highest leaf number of *Achyranthes aspera* L was obtained from combination of BAP 2.0 mg/L and NAA 0.5 mg/L, also the number of roots were comparatively more in combination of BAP and NAA and roots number (16.33 roots) within 8 weeks. But, all the other combination of BAP and NAA in this experiment inhibited shoots elongation .These results are conformed by Taha *et al.*, (2009) on balsam plant. Shoots formation was also obtained in some concentrations of TDZ, but the percentage was lower compared to BAP treatments, since BAP was more effective in uptake and led to the high shoot induction compared to TDZ (Sakikabara, 2004).

Table 3. Effect of plant growth regulators types, concentrations and their interaction on shoots number, shoots length, leaves number per shoots and roots number of cotyledonary node with section of cotyledon explants of balsam plant after 8 weeks.

Plant Growth Regulators Types	Conc. (mg/L)	Cotyledonary node with section of cotyledon explants			
		Shoots number	Shoots length(cm)	Leaves number	Roots number
Control	Without growth regulators	0.50	1.83	5.17	9.00
BAP	1.5	1.00	0.38	2.62	0.01
	3.0	3.16	1.12	6.39	4.50
	4.5	4.33	0.89	6.36	3.60
	5.5	0.01	0.01	0.01	0.01
TDZ	0.5	1.16	0.93	5.25	0.01
	1.5	1.00	0.96	6.50	0.01
	3.0	1.16	0.73	8.27	0.01
BAP: NAA	1.0:0.5	1.00	3.08	16.17	16.33
	1.0:1.0	0.01	0.01	0.01	0.01
	3.0:0.5	0.01	0.01	0.01	0.01
	3.0:1.0	0.33	0.33	3.00	0.01
	5.0:0.5	0.01	0.01	0.01	0.01
	5.0:0.1	0.50	0.26	1.50	0.01
LSD (5%)		0.97	0.79	3.80	4.46

Also, the shoots length decreased and become fascinated, distorted and hydricited, and caused uncontrolled callusing, vitrification, abnormal shoot growth, and difficulty in rooting whereas, the duration of exposure to TDZ was increased and this was agreement with Aasim *et al.*, (2009). The control medium without growth regulators failed to give any multiple shoots, but gave a good length for shoots (1.83 cm).

Finally, the cytokinins have an important role to stimulate the cell division, shoot multiplication and auxiliary bud formation, initiation and activity of axillary meristems George *et al*., (2008) , which result in shoot induction and formation since it activating the RNA synthesis and stimulate protein synthesis as well as enzyme activity Hrtyan *et al*., (2015). In addition a balance between auxin and cytokinin growth regulators is most often required for the formation of adventitious shoot and root meristems. Which both of auxin and cytokinin are usually required for growth or morphogenesis. Auxin blocks cytokinin signaling (Müller *et al*., 2017) while cytokinins can inhibit at least some of the action of auxin.

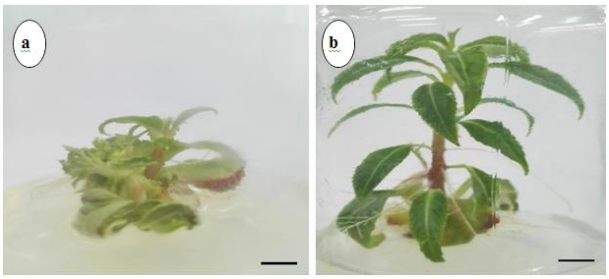

Photo 2. Effect of plant growth regulators types, concentrations and their interaction on shoots number, shoots length, leaves number per shoots, roots number and length of cotyledonary node with section of cotyledon explants of balsam plant pink after 8 weeks.

a: cotyledonary node with sections of cotyledon cultured on on 4.5 mg/LBAP

b: cotyledonary node with section of cotyledon cultured on 1.0 mg/l BAP+ 0.5 mg/l NAA.

Scale bars = 1 cm.

Exp. II: Re-culture or Sub culture of regenerated explants: Effect of plant growth regulator types, concentrations and their interaction on fresh weight, dry weight, volume and callus morphology of cotyledonary node with section of cotyledon explants of balsam plant after 6 weeks.

Cotyledonary node with section of cotyledon was subcultured or re-cultured in MS medium containing PGR according to treatments. Results showed the rate of callus weight, dry weight and volume gained after 6 weeks, the maximum fresh weight and volume of callus 9.32 gm and 7.76 cm3 respectively was observed when shoots culture on MS medium fortified with TDZ 3.0 (mg/L) were subculture on medium contained BAP 4.5 (mg/L) as shown in Table (4), photo (3,b) and Fig (1). Moreover, the induced callus was friable and brownish color and this was in agreement with Sitorus *et al*., (2012) since, the fresh weight of callus is caused by the high content of water.

Also, from the previous stage it was observed that the long duration of exposure to TDZ had an inhibitory effect on callus, shoot number and length, with producing fascinated and distorted shoots Faisal *et al*., (2005). So that, it was necessary to transfer shoots on medium containing a lower level of TDZ and/or another cytokinin BAP and that may be explained by the fact of elimination of accumulated TDZ in tissues and undesirable side effect of TDZ. In addition, an increase in the callus fresh weight is due to an increasing of cell division and the increase in cell enlargement. Also, the callus fresh weight physiologically affected by the water and the carbohydrates in the culture medium (Indah and Ermavitalini 2013), and the amount of fresh weight produced highly depends on how fast these cells divide, multiply, and then producing a callus (Andaryani, 2010). Also from the same table, the best dry weight value of 0.81 gm was observed at the shoots that re-culture on the same medium of BAP at 4.5 mg/L as shown in Fig (1).

Table 4. Effect of plant growth regulators types, concentrations and their interaction on fresh weight, dry weight, volume and callus morphology of cotyledonary node with sections of cotyledon explants of balsam plant after 6 weeks.

Treat	Multiplication and callus production media		Re or sub culture media (mg/L)	Explants type:Cotyledonary node with section of cotyledon explants				
	Plant growth regulators	Concentration (mg/L)		Fresh weight \ gm	Dry weight \ gm	Volume \ cm^3	Callus morphology Texture	Color
T1	BAP	3.0	BAP 4.5	5.21	0.25	2.50	Friable& Compact	P,W,Gt B
T2	BAP	4.5		1.39	0.81	0.84	Friable& Compact	B to G
T3	TDZ	3.0		9.32	0.28	7.76	Friable	B to G
T4	BAP: NAA	5.0:0.1	TDZ 3.0	2.77	0.25	2.71	Friable	B to G
T5	BAP: NAA	3.0:1.0	BAP: NAA	2.43	0.21	1.95	Friable	W,G to B
T6	BAP: NAA	5.0:0.1	3.0:1.0	1.64	0.11	2.01	Friable	B to G
L.S.D. at 5%				2.03	0.26	2.17		

Means with the same letter are not significantly different at P < 0.05. **Callus morphology: G: green, B: brown, W: white, P: pink.**

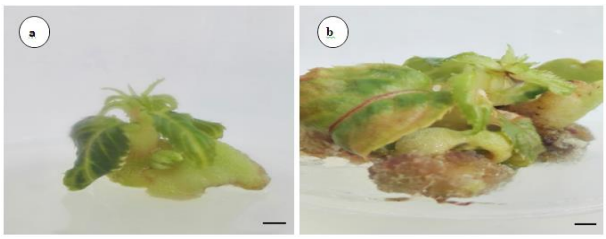

Photo 3. Effect of plant growth regulators types, concentrations and their interaction on callus volume of cotyledonary node with sections of cotyledon explants of balsam plant after 6 weeks from subculture

a:cotyledonary node with sections of cotyledon that subculture on 3.0 mg/LTDZ from medium fortified with 4.5 mg/L BAP after one week
b:cotyledonary node with sections of cotyledon that subculture on 3.0 mg/l TDZ from medium fortified with 4.5 mg/L BAP after 6 week.
Scale bars = 1 cm.

Effect of plant growth regulator types, concentrations and their interaction on shoots number, shoots length, leaves number and roots number per shoots of cotyledonary node with sections of cotyledon explants of balsam plant after 6 weeks:

Data in Table (5) and photo (4) indicated that, the significantly highest shoot numbers 9.00 shoots was derived from shoots that re-culture from medium containing BAP 4.5mg/L to same medium as shown in photo (4b) and this in agreement with Srivastava *et al.* (2015) which found that re-culturing the shoots from old medium to fresh medium gave increments in number of shoots and become very suitable for rooting. Shoots that grown on medium fortified with 5.0 mg/L BAP + 0.1 mg/L NAA then transferred to TDZ 3.0 mg/L and shoots that grown also on medium supplemented with 3.0 mg/L BAP + 1.0 mg/L NAA then transferred to the same medium recorded the lowest number of shoots (0.50 shoots). Besides to, shoots that cultured on medium fortified with TDZ 1.5 mg/L then subcultured on medium without growth regulators.

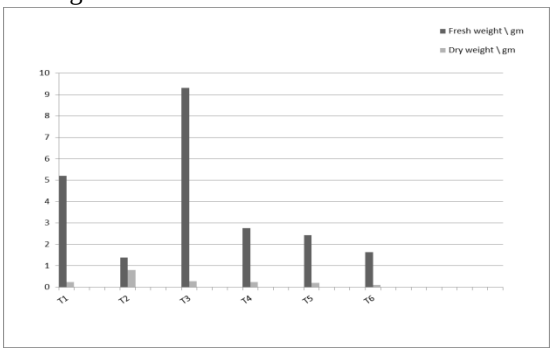

Fig. 1. Effect of plant growth regulators types, concentrations and their interaction on fresh weight and dry weight of cotyledonary node with sections of cotyledon explants of balsam plant after 6 weeks

T1= BAP 3.0(mg/L) subculture on medium containing BAP 4.5 (mg/L).
T2= BAP 4.5 (mg/L) re-culture on medium containing BAP 4.5 (mg/L).
T3= TDZ 3.0 (mg/L) subculture on medium containing BAP 4.5 (mg/L).
T4= BAP: NAA 5.0:0.1(mg/L) subculture on medium containing TDZ 3.0 (mg/L).
T5= BAP: NAA 3.0:1.0(mg/L) re-culture on medium containing BAP: NAA 3.0:1.0(mg/L).
T6=BAP: NAA 5.0:0.1(mg/L) subculture on medium containing BAP: NAA 3.0:1.0(mg/L).

Table 5. Effect of plant growth regulator types, concentrations and their interaction on shoots number, shoots length, leaves number per shoots, roots number and length of cotyledonary node with section of cotyledon explants of balsam plant after 6 weeks:

Multiplication and callus production media		Re or sub culture media (mg/L)	Explants type: Cotyledonary node with section of cotyledon explants			
Plant Growth Regulators	Concentration. (mg/L)		Shoots number	Shoots length(cm)	Leaves number	Roots number
BAP	1.5	Without Growth Regulators	0.83	0.50	1.63	2.50
TDZ	0.5		0.01	0.01	0.01	0.01
TDZ	1.5)		0.50	0.62	2.86	0.01
BAP: NAA	1.0:0.5		0.83	4.00	17.00	20.00
BAP	3.0)	BAP 4.5	5.83	1.59	7.04	11.00
BAP	4.5		9.00	1.55	6.86	1.33
TDZ	3.0		2.33	1.08	5.14	0.01
BAP: NAA	5.0:0.1	TDZ 3.0	0.50	1.35	5.66	0.01
BAP: NAA	3.0:1.0	BAP: NAA	0.50	0.71	2.33	3.00
BAP: NAA	5.0:0.1	3.0:1.0	0.66	0.66	3.00	0.01
LSD (5%)			1.680	1.25	5.32	8.49

While, treatment that transferred from medium containing TDZ 0.5 mg/L for medium without growth regulators turned to a black color and died. From the same (Table 5 and photo 4) data also cleared that, explants that sub cultured from medium containing 1.0 mg/L BAP + 0.5 mg/L NAA to medium without growth regulators resulted in the significant tallest shoots (4.00cm) as shown in photo (4d), leaves number (17 leaves) and maximum roots number (20 roots) than all treatments and this similar to Faisal and Anis (2006) on *Psoralea corylifolia* which found that sub culturing to free hormone increase the length of shoots , also rejuvenation of explants tissues due to repeated transfer of explants on media which promoted activation and conditioning of meristems (Moharana *et al*., 2017).

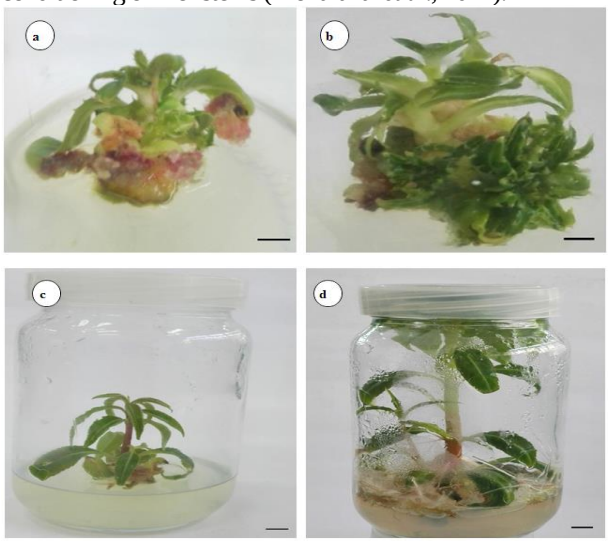

Photo 4. Effect of plant growth regulator types, concentrations and their interaction on shoots number, shoots length, leaves number per shoots, roots number and length of cotyledonary node with section of cotyledon explants of balsam plant after 6 weeks.

a: cotyledonary node with sections of cotyledon that that re-culture from medium containing BAP 4.5mg/L to same medium after one week.

b: cotyledonary node with sections of cotyledon that re-culture from medium containing BAP 4.5mg/L to same medium after six week .

c: cotyledonary node with sections of cotyledon that sub cultured from medium containing 1.0 mg/L BAP + 0.5 mg/L NAA to medium without growth regulators after one week.

d: cotyledonary node with sections of cotyledon that sub cultured from medium containing 1.0 mg/L BAP + 0.5 mg/L NAA to medium without growth regulators after six week .

Scale bars = 1 cm.

CONCLUSION

In this research we have been successfully developed a reliable and efficient method for an efficient *in vitro* regeneration protocol of balsam plant using different types of plant growth regulator and explants. Most of the shoot multiplication and callus induction was achieved from the cotyledonary node with sections of cotyledon derived from 21days old seedlings cultured on MS medium supplemented with 4.5 mg/l BAP and increased by re-culture on the same medium. Full regeneration of balsam plant has been achieved within 8 weeks in culture and increased by re-culture for six weeks. The protocol could be useful for the production of large scale and obtaining high numbers of plants and overcome the difficult conditions of reproduction and the price of seeds.

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تأثير الأجزاء النباتية ، منظمات النمو وتفاعلها على التكاثر الدقيق لنبات البلسم

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يعتبرنبات البلسم (*impatiens balsamina* , L.) كنبات زينه وأيضا كنبات طبى هام ينتمى لعائلة *Balsaminaceae* الهدف من هذه الدراسة وضع بروتوكول فعال من أجل الاكثارالدقيق لنبات البلسم في المختبر من خلال تقنيات زراعة الأنسجة, ودراسة تأثير الأجزاء النباتيه المختلفه (العقدة الفلقية مع جزء من الفلقة، جزء من الفلقة و سويقة تحت فلقية) من الشتلات المزروعه من البذور التى تم زراعتها معمليا بعمر ٢١ يومًا من الزراعه ، وتم زراعتها للتضاعف وحث انتاج الكالس على بيئة موراشيج وسكوج المدعمة بتركيزات مختلفة من الثيديزورون (TDZ) و البنزيل امينو بيورين (BAP) بمفردها أو مع نفثالين حمض الخليك (NAA) . وقد لوحظ ان العقدة الفلقية مع جزء من الفلقة كان الأكثرفعالية لحث الكالس و لوحظ ايضا ان أعلى معدل لحجم الكالس في العقدة الفلقية فى بيئة مدعمه ب ٤.٥ ملغم / لتربنزيل أمينوبيورين كان الأفضل من حيث التضاعف حيث اعطي أكبرعدد من الأفرع ٤.٣٣وحجم كالس٣.٧٨ وأفضل طول ٣.٠٨ سم, وقد تحقق فى غضون ٨ اسابيع من الزراعه على بيئة موراشيج سكوج محتوية على ٠.٥ ملغم/ لتر نفثالين حمض الخليك + ١.٠ملغم / لتر ملغم البنزيل أمينو بيورين. ارتفع عدد النباتات من خلال إعادة الزراعة للعقدة الفلقيه مع جزء من الفلقه لمدة ستة أسابيع على نفس الوسائط المكملة التى تحتوي على ٤.٥ ملغم / لتر بنزيل أمينو بيورين (BAP) على حده حتى ٩نباتات بينما تم الحصول على أفضل طول ٤سم من خلال إعادة الزراعه للعقدة الفلقيه مع جزء من الفلقه من بيئه موراشيج وسكوج مزودة ب٠.٥ ملغم / لتر نفثالين حمض الخليك + ١.٠ملغم / لتر البنزيل امينو بيورين الى بيئه موراشيج وسكوج خالية من منظمات النمو لمده ستة اسابيع كما تم تسجيل أفضل وزن طازج للكالس ٩.٣٢ جم وحجم الكالس ٧.٧٦ سم تم عند الزراعه على بيئه تحتوى على ٣.٠ ملغم الثيديزورون ثم اعادة الزراعه على بيئة تحتوى على ٤.٥ ملغم / لتر بنزيل امينو بيورين ، ولكن أعلى وزن جاف ٠.٨١جرام تم الحصول عليه عن طريق اعادة الزراعه على نفس البيئة التى تحتوي على ٤.٥ ملغم / لتر بنزيل امينو بيورين.