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TISSUE CULTURE AND POST HARVEST STUDIES ON SOME CUT FLOWERS

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ABSTRACT

This study was carried out for two parts of experimental work: tissue culture and post harvest. The present research is devoted for tissue culture study. The present research was carried out at Horticulture Department, Faculty of Agriculture, Mansoura University to study the effect of explant types, shoot tip and nodal cuttings with 1,2 and 3 axillary buds and different growth regulators (BA, Kin, AS, BG) on micropropagation of *Aster novi-belgii* (F. Asteraceae). Murashige and Skoog (1962) medium was used and supplemented with sucrose (30g/l) and 7g/l Agar agar.

The obtained results showed that benzyleadenine (BA) at 1 ppm was significantly effective in enhancing the number of shoots per explant and induced more callus formation on the base of shoot tip explant than the other used growth regulators, i.e, kinetin (Kin), adenine sulphate (AS) and phloroglucinol (PG). Concerning the response of nodal explant type to growth regulators, the data indicated that the explants have 2 or 3 nodes were significantly effective in enhancing the number of shoots / explant, shoot length, leaves number per shoot and callus formation. Also, the highest response of shoot number / explant was recorded by benzyleadenine (BA).

The effect of interaction between nodes number / explant and growth regulators showed that the highest number of shoots / explant was obtained with the combination of three nodes / explant and benzyleadenine(BA) at 1 ppm, while kinetin (Kin) at 1 ppm combined with three nodes / explant was the most effective in enhancing roots number / shoot. Well rooted shoots were acclimatized successfully on a media of (1:1 , v/v) Peat moss and sand to obtain good seedling of *Aster novi-belgii*.

INTRODUTION

Aster is a new crop that is among the top 25 most popular cut flowers around the world. This new crop could be adopted to be produced under the natural Egyptian conditions. The genus aster belong to the family Asteraceae includes several species ranked as a very important ornamental plant. *Aster novi-belgii* is one of these species. Application of tissue culture technique is recommended as a very effective method that used very small pieces and small space are required to multiply large number of plants in a given short time. The effect of explant type on micropropagation of members of family Asteraceae was reported in many studies (Ihsanul-Haq *et al.*, 1998; LE,1998; Iqbal and Srivastava,2000; Aswath and Nazneen,2004). The promotive effect of BA on proliferation has been identified in several plants of family Asteraceae: *Coreopsis lanceolata* (Chiwon *et al*.,1994), *Achillea asplenifolia* (Wawrosch *et al.,*1994), *Chrysanthemum morifolium* (Ihsanul-Haq *et al.,* 1998)and *Gerbera jamesonii* (Nagaraju *et al.*,1998; Le *et al.*, 1999; Thaker *et al.*,2004).

Tissue culture experiments aimed to find the optimum explant (shoot tips or nodal explant),and the optimum dose of different growth regulators (Kin, BA, AS and PG) required to obtain the best growth and number of shoots during proliferation stage *Aster novi-belgii*.

MATERIALS AND METHODS

This investigation was carried out during the period from 2006 – 2007 at the laboratory of plant tissue culture, faculty of Agriculture, Mansoura University. The shoot tips (1.5 cm) and nodal cuttings with 1, 2 and 3 axillary buds (1, 1.5, 2 cm, respectively) were excised from in vivo seedlings and used as explants The excised explants were thoroughly washed with running tap water for one hour (outside the culture cabinet). Thereafter, they transferred to inside the culture cabinet since, they were surface sterilized by immersion in sodium hypochlorite at 2% solution containing 5 drops of Tween 20 wetting agent for 8 minutes and during this step of surface sterilization, container which contained the explants was shaken as much as possible. Then, the explants were thoroughly rinsed 4 times in sterile distilled water changed for 3 minutes each.

Uniform size shoot tip explants were subjected to the first experiment This experiment was conducted aiming to investigate the response of shoot tip to different growth regulators. The treatments of growth regulators were control, 1 ppm benzyladenine (BA), 1 ppm Kinetin (Kin), 40 and 80 ppm adenine sulphate (As) and 2 or 4 ppm phloroglucinol (PG). The experiment continued for six weeks. This experiment was designed as simple experiment in complete randomized block design with three replicates, each replicate contained four jars.

The second experiment was conducted aiming to investigate the response of nodal explant type to growth regulators. The types of nodal explant were: one node / explant (1 cm length) two nodes / explant (1.5 cm length) or three nodes / explant (2 cm length). The tested growth regulators were 1 ppm BA (benzyle adenine), 1 ppm kinetin, 40 and 80 ppm adenine sulphate and 2 or 4 ppm phloroglucinol. The experiment continued for six weeks. This experiment was designed as factorial experiment between explant type and growth regulators in three replicates, each replicate contained four jars.

In all above treatments, 30 ml of Murashige and Skoog (1962) supplemented with sucrose (30 gm/l), 7 gm/l Agar agar and different growth regulators were dispensed into 200 ml jars for explant culture, one explant was cultured per jar. They were incubated at 25 ± 2 ºC under 16 h/day photoperiod of white fluorescent light lamp with light intensity (2500 lux) during multiplication stages. After six weeks the following characters were recorded: number of shoots / explant, shoot length (cm), number of leaves / shoot, number of roots / shoots, callus formation rate. Well rooted shoots were acclimatized successfully on a media of (1:1, v/v) Peat moss and sand to obtain good seedling of *Aster novi-belgii*.

All obtained data were statistically analyzed, and the means were compared using Duncan multiple range test (Little and Hills, 1978).

RESULT

1. Effect of growth regulators on shoot tip organogenesis of *Aster novi-belgii* after six weeks:

Data presented in Table (1) show that benzyladenine at 1 ppm was significantly effective in enhancing the number of shoots / explant than the other used growth regulators.

On the other side shoot length was enhanced by benzyladenine or kinetin at 1 ppm for each without significant differences between them. The number of leaves / shoot recorded the highest values with benzyladenine or kinetin.

Table (1): Effect of growth regulators on shoot tip organogenesis of *Aster novi-belgii* after in six weeks.

Treatment		Measurement				
Growth regulator	Conc. (mg/l)	Shoots number/ explant	Shoot Length (cm)	Leaves number/ shoot	Roots number/ shoot	Callus size
Control	0	1.00 a	3.30 a	7.99 a	2.77 b	• ----
BA	1	1.44 b	4.66 b	11.16 b	0.00 a	+ + +
Kin	1	1.10 a	5.83 b	11.10 b	6.77 c	+
AS	40	1.00 a	3.99 ab	9.99 ab	3.55 b	----
	80	1.00 a	3.60 ab	8.55 a	2.77 b	----
PG	2	1.00 a	2.71 a	8.66 ab	2.99 b	----
	4	1.00 a	3.27 a	7.66 a	3.55 b	----

- **---- No callus formation at the base of the explant.**
- **+ Less callus formation at the base of the explant.**
- **+ + + more callus formation at the base of the explant.**

Values within each column followed by the same letter are not significantly different by Duncan's new multiple range test (p= 0.05).

As for the number of roots / shoot, the data clear that benzyladenine failed to induce rooting where kinetin was effective in this regard, since it resulted in the highest roots number/ shoot (6.77). The other growth regulators, i.e, adenine sulphate and phloroglucinol were similar to control in this regard and produced nearly similar magnitudes of rooting ranging 2.77 - 3.55 roots / shoot without significant differences between them.

The data also clear that benzyladenine induced more callus formation on the base of explant than kinetin while the other growth regulators, i.e, adenine sulphate and phloroglucinol or control did not show any callus formation on explant base.

2. Response of nodal explant type to growth regulators:

Data in Table (2) show the main effect of explant which indicate that the explants have two or three nodes were significantly effective in enhancing shoots number / explant than one node explant.

Shoot length was significantly higher for two and three nodes explant than the one node explant, while leaves number / shoot was not significantly affected by the number of nodes per explant. The data also clear that roots number / shoot was significantly higher for three nodes explant and This was concomitant by less callus formation on shoot base.

Table (2): Effect of nodes number / explant on organogenesis of *Aster novi-belgii* plant after six weeks.

Nodes number/ explant	Shoots number/ explant	Shoot length/ (cm)	Leaves number/ shoot	Roots number/ shoot	Callus Size
One node	1.00 a	3.27 a	6.98 a	1.58 ab	+ +
Two nodes	1.20 b	4.75 b	7.53 a	1.33 a	+ + + +
Three nodes	1.22 b	4.50 b	7.29 a	2.01 b	+ +

Values within each column followed by the same letter are not significantly different by Duncan's new multiple range test (p= 0.05)

Data in Table (3) declare the main effect of growth regulators on organogenesis of *Aster novi-belgii*. The highest response of shoots number / explant was recorded by benzyladenine followed descendenigly by the other growth regulators with same magnitude for them, where the least shoot number was obtained with control.

Table (3): Effect of growth regulators on organogenesis of *Aster novi-belgii* plant after six weeks.

Treatment		Measurements				
Growth Regulator	**Con. (mg/l)**	**Shoots Number/ explant**	**Shoot Length (cm)**	**Leaves Number/ shoot**	**Roots Number/ shoot**	**Callus size**
Control	0	1.00 a	3.99 ab	8.34 c	2.02 b	----
BA	1	1.39 c	4.51 b	4.58 a	0.00 a	+ + +
Kin	1	1.09 b	3.54 a	6.87 b	3.31 c	+
AS	40	1.15 b	4.14 b	7.84 bc	1.79 b	----
	80	1.09 b	3.79 ab	7.46 bc	1.44 b	----
PG	2	1.11 b	4.10 b	7.93 bc	1.46 b	----
	4	1.14 b	5.16 c	7.86 bc	1.45 b	----

Values within each column followed by the same letter are not significantly different by Duncan's new multiple range test (p= 0.05).

The highest response for shoot length was recorded by phloroglucinol at 4 ppm (5.16 cm) followed descendenigly by benzyladenine at 1 ppm, adenine sulphate at 40 ppm and phloroglucinol at 2 ppm (4.51, 4.14 and, 4.10cm, respectively) without significant differences among them. On the contrary the least shoot length was recorded with kinetin at 1 ppm, adenine sulphate at 80 ppm and control (3.54, 3.79 and 3.99 cm, respectively).

Data also clear that although benzyladenine resulted in the highest shoot number / explant and moderate shoot length, it resulted in the least leaves number / shoot while the other growth regulations the kinetin, adenine sulphate, phloroglucinol or control were nearly of similar magnitude and

resulted in high leaves number than benzyladenine. On the other side, the highest roots number / shoot was recorded by kinetin at 1 ppm (3.31 roots / shoot) followed by control or the other growth regulators (1.44 – 2.02 roots /shoot) without significant differences among them. On the other side, benzyladenine at 1 ppm failed to induce rooting, while the highest callus formation was obtained by benzyladenine followed by kinetin, where the other growth regulator did not induce any callus formation.

Data in Table (4) show the effect of interaction between nodes number / explant and growth regulators. The data clear that the highest number of shoots / explant was obtained with the combination of three nodes / explant and benzyladenine at 1 ppm (3.10 shoots / explant) followed by the combination of two nodes explant and benzyladenine at 1 ppm (2.33 shoots / explant), while the least shoot number was obtained by control with the three types of explant (one or two or three nodes) and also with the one node explant combined with all growth regulators.

Table (4): Effect of interaction between nodes number / explant and growth regulators on shoot of *Aster novi-belgii* after six weeks.

Treatment			Measurements				
Explant type	Growth regulators (mg/l)		Shoots number / explant	Shoot length (cm)	Leaves number / shoot	Roots number / shoot	Callus size
One node / explant	Control	0	1.00 a	3.92 cd	7.77 a	1.88 bc	----
	BA	1	1.00 a	0.37 a	3.10 a	0.00 a	+ +
	Kin	1	1.00 a	2.39 b	6.32 a	2.97 c	+ +
	AS	40	1.00 a	3.22 bc	7.77 a	1.61 bc	----
		80	1.00 a	3.61 c	7.10 a	1.22 b	----
	PG	2	1.00 a	4.38 cd	8.66 a	1.72 b	----
		4	1.00 a	5.05 d	8.11 a	1.66 bc	----
Two nodes / explant	Control	0	1.00 a	4.06 cd	8.25 a	1.55 bc	----
	BA	1	2.33 d	6.60 e	7.02 a	0.00 a	+ + + +
	Kin	1	1.33 b	3.13 bc	6.66 a	1.85 bc	+
	AS	40	1.66 c	5.49 d	7.50 a	1.73 bc	----
		80	1.00 a	3.83 c	7.99 a	1.77 bc	----
	PG	2	1.55 bc	4.72 d	7.52 a	1.21 bc	----
		4	1.55 bc	5.44 d	7.81 a	1.24 b	----
Three nodes / explant	Control	0	1.00 a	3.99 cd	8.34 a	2.66 bc	----
	BA	1	3.10 e	6.55 e	3.62 a	0.00 a	+ +
	Kin	1	1.15 b	5.10 d	7.63 a	5.10 d	+
	AS	40	1.44 bc	3.72 c	8.26 a	2.02 bc	----
		80	1.66 c	3.94 cd	7.28 a	1.33 b	----
	PG	2	1.22 ab	3.22 bc	7.61 a	1.47 bc	----
		4	1.44 bc	4.99 d	7.66 a	1.47 bc	----

Values within each column followed by the same letter are not significantly different by Duncan's new multiple range test (p= 0.05

As for shoot length, The highest shoot length was obtained by the combination of two or three nodes / explant with benzyladenine (6.604 6.55 cm) without significant differences between them, while the least shoot length (0.37 cm) was recorded by the combination of one node explant with benzyladenine at 1 ppm. Also phloroglucinol was effective in enhancing shoot length regardless explant type and come in the second order (4.99 - 5.44 cm).

The interaction between explant type and growth regulators did not show significant effect on leaves number / shoot, however the least leaves number / shoot was belong to one node explant combined with benzyladenine at 1 ppm.

Concerning the number of roots / shoot, the interaction between explant type and growth regulators proved that kinetin at 1 ppm combined with three nodes / explant was the most effective treatment in enhancing roots number / shoot (5.10 roots / shoot) while benzyladenine when combined with all explant types (one, two or three nodes) failed to induce rooting. The other growth regulators, i.e., adenine sulphate and phloroglucinol or control resulted in rooting with less magnitude regardless explants type.

The callus formation on explant base was the highest under two nodes / explant combined with benzyladenine then followed by one or three nodes / explant. Kinetin resulted in less callus formation than benzyladenine with the three types of explant (one, two or three nodes). The other growth regulators, i.e., adenine sulphate and phloroglucinol or control did not induce callus formation with all explant types.

DISCUSSION

The previous results proved the efficiency of benzyladenine on enhancing shoots proliferation for *Aster novi-belgii* plant than kinetin, adenine sulphate or phloroglucinol. On the other side, both of benzyladenine and kinetin showed enhancing effect on shoot length than the other used growth regulators, this was concomitant with high leaves number / shoot. In this regard, Evans *et al.* (1983) mentioned that although a small quantity of cytokinins may be synthesized by shoot growth in vitro, roots were the principle site of cytokinins biosynthesis. It is unlikely that the meristem, shoot tip and bud explants have sufficient endogenous cytokinins to support growth and development. Thus, 85 % of the initiation stage needs supplement with cytokinins, hence the added BA in the herein work supported the shoot differentiation and shoot length. According to Evans *et al.* (1983) there are three cytokinins frequently used, kinetin (Kin), benzyladenine (BA) and isopentenyl-adenine (2-ip). BA was the most effective for meristem, shoot tip and bud cultures, followed by Kin and 2-ip has been used less frequently, one should be aware of the fact that, although a given cytokinins may not work well in certain species; it may be quite effective in others. In the herein work, BA proved to be more effective than Kin for increasing shoots number for either shoot tip or nodal explants which have one, two or three nodes / explant. The efficiency of benzyladenine than kinetin in enhancing shoots proliferation was also found by Sujatha (1997) who reported that the number of shoots / explant of *Guizotia abyssinica* was the highest on BA supplemented media (6.3 – 85.2) comparing with kinetin supplemented media (0.3 – 18.9). Similar results were obtained with *Gerbera jamesonii* by Olivera *et al.* (2000). Also, Chandramu *et al* (2003) on *Vitex negundo* and Arikat *et al* (2004) on *Salvia fruticosa,* who reported that BA was found to be more effective than kinetin in the induction of multiple shoot from the nodal

explants. The promotive effect of BA on proliferation has been identified in several plants of family Asteraceae: *Coreopsis lanceolata* (Chiwon *et al*.,1994), *Achillea asplenifolia* (Wawrosch *et al.,*1994), *Chrysanthemum morifolium* (Ihsanul-Haq *et al.,* 1998)and *Gerbera jamesonii* (Nagaraju *et al.*,1998; Le *et al.*, 1999; Thakur *et al.*,2004).

Moreover using three nodes / explant combined with BA treatment was more effective in enhancing shoots number (3.10 shoots / explant) than single node or shoot tip culture combined with BA treatment. Concerning the effect of explant type, it was found that using three nodes / explant culture resulted in the highest values of the shoot length and this was concomitant with high leaves number / shoot. The obtained results go in line with the findings of Iqbal and Srivastava (2000) on *Sliybum marianum* and Begum *et al.* (2000) on *Ocimum basilicum*, who indicated that the proliferation of nodal explants from mature plants was significantly higher than that of shoot tip explants.

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دراسات على زراعة الأنسجة وما بعد الحصاد على بعض نباتات زهور القطف

محمد نزيه شرف الدين ، حسين على أحمد ، هشام هاشم عبد القادر و هبه يوسف البنا
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أجري هذا البحث في قسم البساتين كلية الزراعة جامعة المنصورة لدراسة تأثير نوع الجزء النباتي (القمة النامية والعقلة الساقية ذات البرعم، والبرعمين، والثلاثة براعم) ومنظمات النمو المختلفة (بنزيل أدينين، كينيتين، سلفات الأدينين، الفلوروجليسينول) على الإكثار الدقيق على نبات *Aster novi- belgii*. استعملت بيئة موراشيج وسكوج (١٩٦٢) بعد إمدادها بالسكر (٣٠ جم/لتر) و(٧ جم/لتر) أجار أجار. وقد أظهرت أن البنزيل أدينين بتركيز ١ جزء في المليون أدى إلى زيادة معنوية في عدد الأفرع / جزء نباتي وشجع تكوين الكالوس أكثر من منظمات النمو الأخرى مثل الكينيتين وسلفات الأدينين الفلوروجليسينول.

وفيما يتعلق باستجابة نوع العقلة النباتية لمنظمات النمو بينت النتائج أن الجزء النباتي الذي يحتوي على عقدتين أو ثلاثة كان أكثر تأثيراً في زيادة عدد الأفرع وطولها وكذلك عدد الأوراق على كل فرع وتكوين الكالوس وقد سجل استعمال البنزيل أدينين أعلى عدد أفرع / جزء نباتي.

وبالنسبة لتأثير التفاعل بين نوع العقلة النباتية ومنظمات النمو أظهرت النتائج أن استعمال البنزيل أدينين بتركيز واحد جزء في المليون مع الجزء النباتي ذات الثلاث عقد أدى إلى الحصول على أكبر عدد من الأفرع بينما أدى استعمال الكينيتين بتركيز ١ جزء في المليون مع الجزء النباتي ذات الثلاث عقد إلى زيادة عدد الجذور بالنسبة لكل فرع وقد أمكن أقلمت هذه النباتات بنجاح عند زراعتها في مخلوط من البيت موس والرمل (١:١ حجماً).