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PROPAGATION OF ASTER ERICOIDES CV. "MONTE CASINO" BY IN VITRO CULTURE TECHNIQUE

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ABSTRACT

In vitro culture trials were carried out to study the micropropagation procedure of *Aster ericaides* cv. Monte casino for both apical and lateral buds the best survival recanting (highest values) and contamination percentage (lowest values) were all obtained with 30% Clorox and 20 min. for apical buds and 20% Clorox and 20 min for lateral buds time of explants exposure. To obtain the highest values of number of shots, length of shoots and number of leave MS supplemented with 1 ppm BA was used during the multiplication stage. The best treatment for either root number or root length was obtained when 0.4 ppm NAA was used in MS medium to obtain the highest survival percentage after acclimatization when small pots with sterilized and or sand plus peatmoss (1:1 v/v) was used.

Key words: Propagation – *in vitro* culture technique – *Aster ericoides*

INTRODUCTION

Establishment stage:

In general, the primary objectives of this stage is not only to establish the explant in culture but also to stabilize the culture for multiple shoot development. This may involve the elongation of the apical meristems, the stimulation of axillary shoots and the imitation of adventitious shoots on excised shoots. leaves, buds, scales, flowers. scarps, cotyledon or other organs (Wcthcrdll, 1982; Hartmann *et al*., 1990).

Factors that influence the success of this stage include the choice of the explant (source and type), elimination of contamination and

culture conditions which include culture media composition, light, temperature arid type of explant support (WctherclJ, 1982; Dodds & Roberts 1985; Hartmann *et al*., 1990).

Multiplication stage:

The use of shoot tip and axillary buds reduce some colonal variation and therefore are used to propagate plant clonally in a pure

The culture media and growth conditions used in this stage are fine turned and optimized for maximum rate of multiplication. In further detail, the propagation in this stage is conditioned in a high cytokinin medium to favor shoot proliferation (Hartmann *et al*., 1990).

Pence *et al*.,(1997) indicated that in vitro germinated seedling tips of *Aster vialis* were multiplied on MS medium with 0.5 mg/I Benzyl adenine and 0.05 mg/l Naphthalene acetic aced, giving an average 5-fold increase over 6 weeks. An average of 2- G4 shoots from buds culture were obtained in *Aster ericoides* cv. *Monte casino*, Salazav *et al.,* (2005).

Chitra *et al*. (2006) suggested that the new multiple shoot regeneration response was 95% and 80% for nodal segment Patil *et al.,* (2005) found that the shoot tip explants of *Chrysanthemum* cv. pkv shubra gave better results when cultured on MS medium supplemented with 2 mg/l BAP and 0.5 mg/l NAA.

Quoirin *et al*., (2008) found that the multiplication of nodal segments of *Gypsophila paniculata* was most pronounced in the MS medium containing 0.5 m M kinetin and 0.3 m M GA_3.

Drefahl *et al*., (2007) clarified that the best results for micro propagation of *Rose hybrida* cv. *Vegas* were obtained with buds containing 5 to 7 primordia developed in MS culture media supplemented with 0.5 mg/l BA + 0.25 mg /l GA_3 + 0.01 mg/l IAA. Also, Nikbakht *et al.,* (2005) found that the highest multiplpcation rate for *Rosa damascene* single node segments with buds were obtained with 1-2 mg/l BA, 0.1 mg/l GA_3 and 0-0.1 mg/l NAA for cv. Azaran and with BA and GA_3 at the same concentrations, but no NAA, for cv. Ghamsar. A short exposure of explants to TDZ or CPPU before subculture onto normal shoot proliferation medium during stage- ll, can significantly enhance axillary shoot proliferation in hybrid tea rose cvs. for commercial multiplication Singh and Jyamal (2001).

Recentaly, a high multiplication rate of *in vitro* micropropagation for *Limonium cordatum* L. was obtained when MS

medium supplemented with 0.2 mg/l 6- BA and 0.5 mg/l IAA Savona *et al* (2009).

Rooting stage:

Pence *et al*. (1997) found that hormone-free woody plant medium gave up to 70% rooting, but this was increased to over 95% on MS medium with 0.5 mg/l IBA. Also, Joshi and Dhav (2003) mentioned that 100% *in vitro* rooting of *saussured obvallata* shoots was obtained in half-strength MS supplemented with 0.5mg/1IBA.

Patil *et al.* (2005) recorded that the best, rooting % for chrysanthum shoots were obtained in MS + 1 mg/l NAA. Lakhshmi *et al.* (2006) indicated hat the shoots ،propagated were root well on 0.5 MS + 1.0 NAA mg/l and 0.5 MS + 1.0 mg/l IAA for chrysanthenum cv. Pkvshabler.

Zaki zadea *et al.,* (2008) indicated that shoots of *in vitro* grown callus of *Rosa hybrida* were rooted on $^1/_2$ MS medium supplemented with 5.7 micro M NAA and 9.84 micro M IBA and transferred to the greenhouse.

Drefahl et al., (2007) found that rooting of multiplied shoots Rosa hybrida were produced in $^1/_2$ MS without hormones Nikbakht (2005) found that for rooting of the proliferated micro shoots of Rosa damascene Mill, were enhanced more effectively when treated with 200 ppm IBA for 10 second then cultured in $^1/_2$ MS medium.

c. Acclimatizing stage:

Plantlets developed *in vitro* are very susceptible to the environmental conditions *ex. vitro.* The stage of acclimation thus is a very important procedure in order for the tissue culture. process to be profitable one components of the potting, Pence *et al* (1997) found that rooted plants were acclimated in soil under a humidor for 2 month before transfer to open pots. One the other hand, Motooka *et al.,* (1992) were concluded that there were no differences in survival rate between aster plants into perlite/ vermiculate/ peat moss 1:1:1 and aster plants into perlite/ vermiculite mixture. Salazar *et al*., (2005) found that the plant regenerate by organogenesis were transferred to pots and 8% of plant acclimatization was obtained in *Aster ericoides* cv. Monte casino.

MATERIALS AND METHODS

1. Location and duration:

This study was carried out at El-Zohria tissue culture lab. El-doky – Cairo – Egypt in 2006-2008.

2. Plant material and explant types:

Vegetative seedlings were bought from Pico company in one cultivar of Aster ericoides (cv. Monte casino) was used as experimental plant material which gave white flowers. This cultivar is allowed to propagated by tissue culture (vegetative propagation.

Seedling about 10 cm in length, were directly transferred to the tissue culture lab where brown leaf and the basal parts were all discarded. Apical bud 1 cm in length with leaves. The explants were trimmed to their final size as will be described later.

3. Culture media:

A basic Murashige and Skoog (1962) medium (MS) was used for the micropropagation of aster which consisted of salts and vitamins at full strength the hormonal additions differed according to the particular aim and individual situation, as will be mentioned later on for each stage.

All types of media were solidified with 6g/l purified agar. The pH was adjusted to 5.6 using NaOH and HC1. For sterilization, all media were autoclaved at 100 kpa and 12 1°C for 20 mm then left to cool and stored at 27±2°C for one week before being used

4. Glassware equipment:

Test tubes 150 x 15 mm were used during establishment and rooting stage. Glass jars 250 ml were used during multiplication stage. All glassware were washed by tap water with a commercial cleaning powder and later autoclaved at 100 Kpa and 12 1°C for 30 mm.

5. Culture room conditions:

All culture procedures were carried out under a laminar air-flow Cabinat. Cultures were incubated in a culture room under controlled conditions, where temperature was maintained at 27±2°C and light intensity Kept explants level at 2500 lux. The photo-periods inside the culture room was adjusted to a 16 h light and 8 h dark cycle

6. Surface sterilization of explants:

The target of this experiment was to specify the most suitable sterilization procedure needed for the effect killing of all microorganisms connected to the explant tissues and thus providing effective prevention from contamination.

The explants of aster (lateral buds and apical bads) were placed in an erlenmeyer flask (0.5 litre) and washed with a few drops of a commercial liquid detergent. Then, they were rinsed thoroughly for 1 h with running tap water. The explants were then surface sterilized using a sodium hypochlorite (NaOCI) solution as commercial blench Clorox" (5.25% available chlorine at 10%, 20%, 30% and 40% (v/v of commercial blech) with a few added drops of commercial liquid detergent 5, 10, 15, 20 and 25 mm. The explants were rinsed three times with distilled sterile water before being dissected on sterile paper with the use of a sterile scalpel (No. 11 blade) and forceps.

At this stage, one or two of outermost leaves of the apical bud were removed. Shoot tips were trimmed to 1 cm in length. The dissected explants were transferred immediately to culture tubes filled with MS free hormone medium. For lateral buds, the stems under shoot tips were cut into small segments about 1 cm in length.

7. Shoot proliferation from buds

a. Establishment stage

The main target of this stage was to produce single shoots free from microorganisms. In this stage, explants of apical and lateral buds were cultured in test tubes filled with 10 ml MS fresh hormone medium and capped each with apiece of aluminum foil.

b. Multiplication stage

The main target of this stage was to maximize the number of shoots per explant using different BAP concentrations single shoot- which have been produced from both apical and lateral buds during establishment of cultures stage were transferred to jars with transparent ploypropylene film. The jars were filled before hand with 25 ml MS medium supplement with BAP and NAA at different concentration as follow: 1- MS, 2- MS + 0.5 mg/l BA 3-MS + 1 mg/l BA, 4-MS + 0.1 mg/l NAA, 5- MS + 0.1 mg/l NAA + 0.5 mg/l BA and 6- MS + 0.1 mg/l NAA + 1 mg/l BA. Routine subculture was carried out every four weeks up to three times.

In each subculture, shoot clusters were trimed to 0.5 cm in length and transferred to the medium used before subculture.

c. Rooting stage:

Single shoots were transferred to half MS medium supplemented with NAA at 0.0, 0.2, 0.4 and 0.6 mg/l.

The culture were left till rooting (about 1 month) then transferred to acclimatization stage.

d. Acclimatization stage:

Plantlets from the rooting media were subcultured in the following sterilized media in plastic small pots:

1- Sand medium
2- Sand medium + peatmoss medium (1 :1 v/v)
3- Sand + loam medium (1 : 1 v/v)

9. Experimental design and statistical analysis:

All experiments were arranged in a complete randomized design. Each treatment consisted 10 explants in the surface sterilization experiment and rooting stage. Meanwhile, each treatment in the shoot proliferation from buds consisted of three replication with three explant per replicate.

Each treatment in Acclimatization stage consisted of three replication with two plantlets per replicate.

To statistically test the results, one or two way analysis variance as required was carried out as described by Snedecor and Cochran (1989).

The means were compared by determining the LSD (least Significant difference) at a probability level of 5% (mentioned only whenever proven significant.

RESULTS AND DISCUSSION

1- Establishment stage :

1-1- Apical buds:

Data in Table (1) showed that the highest survival percentage was produced when apical buds treated with 30% Clorox and 40% compared to the treatments. For the time of expos or the best survival result obtained when 20 min or 25 min were used compared to the other treatments.

The interaction between time and Clorox % showed that the highest survival value obtained with 30% Clorox for 20 min. and 30, 40% Clorox for 25 min. compared to the other interaction.

Also data in Table (1) showed that the lowest contamination value obtained with 40% Clorox compared to the other treatments.

Table (1): Effect of time of clorox exposor and clorox concentration treatments on either survived percentage or contamination percentage of *Aster ericoides* cv. "Monte casino" during establishment stage in apical bud.

	Survival percentage						Contamination percentage					
Time Clorox%	5min	10 min	15 min	20 min	25 min	Mean	5min	10 min	15 min	20 min	25 min	Mean
10%	0.0	0.0	0.0	0.0	0.0	0.00	1.0	1.0	1.0	1.0	1.0	1.00
20%	0.0	0.0	0.2	0.4	0.5	0.22	1.0	1.0	0.8	0.5	0.2	0.70
30%	0.2	0.4	0.5	0.9	0.7	0.54	0.0	0.6	0.3	0.0	0.2	0.42
40%	0.1	0.3	0.4	0.6	0.7	0.42	0.2	0.3	0.3	0.2	0.1	0.22
Mean	0.075	0.175	0.275	0.475	0.500	Mean	0.750	0.725	0.650	0.425	0.375	

L.S.D. Time : 0.1333 Time : 0.113
Clorox% 0.149 Clorox% 0.1264
Time X Clorox % 0.2981 Time X Clorox % 0.258

For the time of Clorox exposor, the best results (lowest contamination percentage) obtained with 20 or 25 min. when compared to the other treatments.

The interaction showed that the lowest contamination values obtained with 30% Clorox for 5, 20 and 25 min. and 40% Clorox for 20, 25 min.

For both highest survival percentage and lowest contamination percentage Clorox at 30% for 20 and 25 min of explant exposor was used.

Data in Table (2) showed that the highest survival percentage was obtained when lateral buds treated with 20% and 30% Clorox when compared the other treatments for time of exposr the high value obtained with 20 and 25 min. compared to the other treatments.

For the interaction, the best result obtained with 20% Clorox for 20 and 25 min. compared to the other interactions.

Also, data in Table (2) showed that the lowest contamination percentage obtained with 40% Clorox compared to the other treatments.

For time of explant exposor the best value obtained with 20 or 25 min when compared to the others.

For the interaction, the lowest contamination value obtained with 20% Clorox for 20, 25 min. or 30% Clorox with either 20 or 25 min time of exposor, at 40% Clorox for 10, 15, 20 or 25 min.

Nevertheles, the best results for the surface sterilization of lateral buds obtained with 20% Clorox for 20 min.

Table (2): Effect of time of clorox exposor and clorox concentration treatments on either survived percentage or contamination percentage of *Aster ericoides* cv. "Monte casino" during establishment stage in lateral buds.

	Survival percentage						Contamination percentage					
Time Clorox%	5min	10 min	15 min	20 min	25 min	Mean	5min	10 min	15 min	20 min	25 min	Mean
10%	0.0	0.0	0.2	0.4	0.5	0.22	1.0	1.0	0.8	0.5	0.4	0.74
20%	0.1	0.3	0.6	0.9	0.7	0.32	0.9	0.6	0.3	0.0	0.1	0.38
30%	0.0	0.1	0.3	0.4	0.4	0.24	0.6	0.5	0.1	0.0	0.0	0.24
40%	0.0	0.1	0.2	0.3	0.2	0.16	0.3	0.2	0.0	0.0	0.0	0.10
Mean	0.025	0.125	0.325	0.500	0.450	Mean	0.70	0.575	0.300	0.125	0.125	

L.S.D. Time : 0.1164
Clorox% 0.1302
Time X Clorox % 0.2604

Time : 0.1151
Clorox% 0.1287
Time X Clorox % 0.2574

For the interaction, the best result obtained with 20% Clorox for 20 and 25 min. compared to the other interactions.

Also, data in Table (2) showed that the lowest contamination percentage obtained with 40% Clorox compared to the other treatments.

For time of explant exposor the best value obtained with 20 or 25 min when compared to the others.

For the interaction, the lowest contamination value obtained with 20% Clorox for 20, 25 min. or 30% Clorox with either 20 or 25 min time of exposor, at 40% Clorox for 10, 15, 20 or 25 min.

Nevertheles, the best results for the surface sterilization of lateral buds obtained with 20% Clorox for 20 min.

It is well knowen that the most commonly used material for surface sterilization of explants either the hypochlorite (on which is released from sodium hypochlorite or calcium hypochlorite) or simple alcohols (mainly ethanol). According to the type plant natural and to the time of exposor, the final concentration required for such compounds is determined. This may very from 0.25-2% w/v for sodium hypochlorite with or without dipping in ethanol (George, 1993). The germicidal effectivaess of sodium hypochlorite may be due to its oxidizing capacity of chlorine (Smith & Thrope, 1975; Dodds & Roberts, 1985).

2- Multiplication stage:

Data in Table (3) showed that the best results of shoot numbers, shoot length and leave number were all obtained in the third subculture compared to the others.

For Media composition the highest shoot number obtained with MS with 1 ppm BA; the highest shoot length and leave number obtained with media number 2, 3, 5, 6 compared to the others.

For the interaction between media composition and subculture, the highest values obtained with MS + 1 ppm BA and the third subculture for shoot number, obtained with media No. 2, 5 or 6 at the third subculture and No. 6 at second subculture.

For both shoot length and number of leave in conclusion, the best shoot number, shoot length and leave number obtained in the subculture No. 3 and with MS + 1 ppm BA. These results are not similar to other results obtained on Aster plants by Pence *et al.,* (1997) and Salazer *et al.,* (2005).

Although, El-Gendy *et al.,* (2001) were propagate gladiolus in vitro using BAP at 0.5 or 1 mg/l. Cytokinins appear to be necessary for plant cell division in tissue culture trials. Subculture on to a medium containing cytokinines can cause the cells of tissue to divide synchronously (George, 1993). Also to an courage the growth of exillerey buds and reduce apical dominance in shoot cultures one or mor cytokinines are usually recommended (Bhojwani & Razdan, 1983;Hussey,1986).

3- Roots stage:

Data in Table (4) showed that best number of roots were obtained with either 0.4 or 0.6 ppm of NAA levels compared to the other treatments. Also, the best results for root length obtained with 0.4 ppm NAA.

For both root number and length the highest results obtained with 0.4 ppm NAA. Auxin are weel-known rooting promoters. Also, the best rooting % obtained in chrysanthemum when NAA was used in the medium (Lakhshmi *et al.*, 2006).

Table (3): Effect of number of subculture and Media treatments on number of shoots, length of shoots and number of leaves of Aster ericoides cv. "Monte casino" during in vitro multiplication stage.

Subculture / Media*	Shoot number				Shoot length				Leave number			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
1	0.00	0.11	0.44	0.19	0.00	1.00	2.67	1.22	0.00	0.67	1.89	0.89
2	0.33	1.22	2.33	1.30	0.89	3.44	5.33	3.22	0.89	3.44	4.67	3.00
3	0.67	2.56	8.22	3.82	1.78	3.56	4.67	3.33	1.67	3.56	3.56	2.93
4	0.00	0.11	0.67	0.26	0.00	0.56	3.67	1.41	0.00	0.33	2.44	0.93
5	0.44	0.89	2.11	1.15	1.78	3.56	5.33	3.56	1.22	2.56	4.78	2.85
6	0.56	2.00	2.22	1.59	1.44	4.00	4.56	3.33	1.44	3.67	3.78	2.96
Mean	0.33	1.15	2.67	Mean	0.98	2.69	4.37	Mean	0.87	2.37	3.52	

LSD : Subculture = 0.268 Subculture = 0.8556 Subculture = 0.6429
Media = 0.1895 Media = 0.6050 Media = 0.4546
Subculture X Media= 0.4642 Subculture X Media = 1.482
Subculture X Media = 1.114

*1- MS free hormone
2- MS + 0.5 ppm BA
3- MS + 1 ppm BA
4- MS + 0.1 ppm NAA
5- MS + 0.5 ppm BA + 0.1 ppm NAA
6- MS + 1 ppm BA + 0.1 ppm NAA

Table (4): Effect of NAA concentration levels on either root number or root length of *Aster ericoides* cv. "Monte casino" during in vitro roting stage.

NAA levels (ppm)	Roots number	Root length
0.0	3.633	1.997
0.2	3.330	2.487
0.4	4.660	4.277
0.6	5.997	2.863
LSD	1.624	0.722

Table (5): Effect of media composition on survival percentage after acclimatization stage for in vitro plantlests of *Aster ericoides* cv. "Monte casino".

Media composition	Survival percenyage
Sand	100
Sand : petmoss (1:1)	100
Sand : loam (1:1)	16.67

LSD 33.30

4- Acclimatization stage:

4-1- Data in Table (5) showed that best survival percentage (100%) was obtained when sand or sand + peat (1:1 v/v) was used compared to sand + loaum (1:1 v/v).

This result is more or less similar to the resultes obtained by Salazer *et al.* (2005). Also, may be this result regards to the fact that sand or sand with peatmoss are less contaminated by microorganisms than sand and loam medium plate (1).

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إكثار نبات الاستر مونت كازينو بواسطة زراعة الأنسجة

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اجرت هذه الدراسة فى خلال الفترة من 2006-2008 فى معمل زراعة الانسجة بالزهرية التابع لمعهد بحوث البساتين وزارة الزراعة – الدقى – القاهرة – جمهورية مصر العربية. واجريت تجارب تعقيم منفصلات البراعم الطرفية والجانبية باستخدام الكلوراكس بتركيزات مختلفة وهى 10%، 20%، 30%، 40% لفترات مختلفة تشمل 5، 10، 15، 20، 25 دقيقة. ومن الدراسة اتضح ان أفضل معاملة من حيث تعقيم لكلا المنفصلين فى نبات الاستر مونت كازينو هى 30% كلوركس لمدة 20 دقيقة. وكذلك اجريت دراسة تأثير منظمات النمو على عدد الافرع وطولها وعدد الاوراق حيث استخدمت بيئة مواشيج وسكوج مضافا لها بنزيل ادنين ونفثالين استيك اسيد بتركيزات مختلفة وكانت المعاملات كما يلى:

بيئة مواشيج وسكوج بدون هرمون (كونترول) وبيئة مواشيج وسكوج مضافا لها بنزيل ادنين بتركيزات 0.5، 1ملجم/لتر كذلك بيئة مواشيج وسكوج مضافا لها نفثالين استيك اسيد بتركيز 0، 0.1 ملجم/لتر منفردا او مع بيئة بنزيل ادنين. وكان أهم النتائج أن بيئة مواشيج وسكوج بالإضافة لـ 1 جزء فى المليون بنزيل ادنين فى النقلة الثالثة أعطت أعلى قيمة لعدد الأفرع وبيئة مواشيج وسوج بالإضافة إلى بنزيل ادنين بتركيز 0.5 جزء فى المليون مع أو بدون نفتالين استيك اسيد وكذلك بيئة موارشيج وسكوج مع بنزيل ادنين 1 جزء فى المليون مع نفتالين استيك اسيد أعطت أعلى نتائج فى النقلة الثالثة أيضاً بالنسبة لطول الأفرع وعدد الأوراق. كما وجد أنه لتفس الصفة كانت القيمة مرتفعة مع النقلة الثانية فى حا له استخدام مواشيج وسكوج مع بنزيل ادنين 1 جزء فى المليون ونفثالين استيك اسيد 0.1 جزء فى المليون. كما اجريت تجربة اخرى لمعرفة اثر نفثالين استيك اسيد على التجذير فى مرحلة التجذير حيث استخدمت مستويات مختلفة من نفثالين استيك اسيد وهى 0، 0.2، 0.4، 0.6 ملجم/لتر واتضح من الدراسة ان افضل المعاملات من حيث طول وعدد الجذور عند استخدام 0.4ملجم/لتر نفثالين استيك اسيد. اما التجربة الاخيرة فكانت على دراسة تأثير مكونات بيئة الاقلمة على نسبة البقاء حيث استخدمت بيئة الرمل منفردا وبيئة الرمل + البتموس بنسبة 1:1 وبيئة الرمل + الطمى بنسبة 1:1 حجم/حجم وكانت اعلى نسبة بقاء 100% فى حالة استخدام بيئة الرمل او بيئة الرمل والبيتموس مقارنة ببيئة الرمل والطمى.