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In vitro CULTURE AND ESTABLISHMENT OF *Dianthus caryophyllus* L.

Maghazy,Eman,I.; H.K. Atta-Alla; A. K.Waly and I. H. Abo El Soad
Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt.

ABSTRACT

Shoot tips of *Dianthus caryophyllus* L. cvs., Lelia, Maragia, Sorriso and Aicardi were excised from the plants grown in the greenhouse and cultured on MS medium containing BA at 0.0, 0.5, 1.0 and 2.0 mg/l. The medium was solidified with 8.0, 10.0, 12.0 and 14.0 g/l agar. After 8 weeks, the results showed that the treatment with BA at 2.0 mg/l and agar at 8.0 g/l produced the highest number of shoots as 8.3 and 10.1 shoots /explant with Maragia and Aicardi cultivars. Increasing BA levels in the culture medium increased vitrification percentage. On the other side, increasing agar concentration decreased both the multiplication rate and the percentage of vitrified shoots. The developed shoots were transferred to a rooting medium (MS medium supplemented with NAA at 0.0 and 1.0 mg/l).The medium was solidified with agar at 7.0 or 8.0 g/l. The treatment with MS medium containing NAA at 1.0 mg/l produced the largest number of roots with Maragia and Sorriso cultivars. The plantlets were then transferred from the rooting medium and cultured on small plastic pots containing peatmoss: vermiculite and perlite. (1:1:1, vv:v). The pots were kept under mist system for 2 weeks. The plants grew normally and the survival percentage reached 80% with all cultivars.

Abbreviations: BA- Benzyladenine; MS- Murashige and Skoog (1962) medium; NAA- Naphathaleneacetic acid

INTRODUCTION

Carnation (*Dianthus caryophyllus* L) is a member of the Caryophyllaceae family, it is one of the most important three cut flowers in world trade (Armitage, 1993). Carnation grows commercially in many countries of the world. The plants are propagated by cuttings. The greenhouse carnation flowers at year round. The carnation is used for cultivation in the borders and flowers beds as well as pot plant, but mainly is used as a popular cut flower. El Saied and Hossnei (1999) reported that about 2.3 million tons of carnation representing a value of US$ 267 million were imported by the European countries from different parts of the world.

Propagation of carnation by *in vitro* culture has been reported by many workers. Effect of BA on the shoot multiplication of carnation was studied by Choudhary *et al.*, (1990) who produced the best shoot proliferation by culturing the axillary buds of carnation cv. Scania Red on Gamborg s medium supplemented with BA at 1.0 mg/l and 8.0 g/l agar. Each proliferated shoot produced 10 –12 shoots. Kovac (1995) reported that the highest multiplication rate from the nodal segments of carnation was achieved on modified MS medium containing 1.0 mg/l BA. Ihsan *et al.*, (1995) found that the greatest number of carnation plantlets per flask was produced on MS medium containing BA at 5.0 mg/l.

Concerning the effect of the interaction between BA and NAA, Ghosh (1986) and Kim and Kang (1986) concluded that the combination between BA and NAA at different concentrations yielded new shoots / explants from shoot tips and nodes of carnation cultivars. On the other side, Mujib and Pal (1995)

produced the highest number of shoots of carnation cv. Candy Sim in the presence of low concentrations of BA (0.5 mg/l). They added that the addition of NAA did not promote shoot proliferation but increased the shoot length.

Some micropropagated plants produced abnormal vitrified plants (translucent plantlets with short stems and fragile leaves). Vitrification is a physiological disorder ferquently affecting plants propagated *in vitro*. Debergh *et al.*, (1981) reported that vitreous leaves did not contain palisade tissue. Kevers *et al.*, (1988) added that the cellulose and lignin levels were lower in vitrified stems and leaves. Leshem (1986) stated that about one half of explants of carnation cv. Cerise Royallette produced vitrified plants on MS medium supplemented with BA and NAA. Ku and Tsay (1994) reported that addition of BA or kinetin to the culture medium increased the vitrification on the shoot tip cultures of carnation cvs. Opal and Pinky. The increase was greater at high BA or kinetin concentration.

Han *et al.* (1991) found that BA at 0.5–2.0 mg/l was the most effective cytokinin for shoot proliferation of *Gypsophila paniculata*, but released in a higher percentage of vitrified plantlets. Combination of BA and IAA increased the vitrification. Pedroza *et al.*, (1997) reported that the highest number of non vitrified shoots was observed in the medium supplemented with 0.5 mg/l BA , 2.0 mg/l calcium patothenate, 4% sucrose and 0.7% agar.

Concerning the effect of agar on shoot multiplication and vitrification Ziv *et al.*, (1983) reported that the rate of shoot multiplication of carnation was decreased by adding more agar to the culture medium. This may be due to the effect of agar on lowering the total water potential of medium, they added that the percentages of abnormal (vitrescent) leaves and shoots in carnation shoot tips were reduced by raising the agar concentration from 0.8 to 1.8%. Leshem (1983) stated that increasing the agar concentration in the culture medium increased the proportion of normal shoot developing of carnation from 46 to 77% . Kim *et al.*, (1988) found that using higher concentration of agar more than 15 g/l in the medium produced normal plantlets of carnation. Choudhary *et al.*, (1993) reported that the percentage of vitrified plantlets of carnation was decreased with increasing agar concentration, but with concentrations above 1.2 % the apices developed into small shoots without proliferation.

As for *in vitro* rooting, there are many factors affecting the *in vitro* rooting such as auxins, medium composition, sugars and other cultural environmental like light, aeration and temperature.

Can and Koc (1992) demonstrated that the shoots of carnation cultured on MS medium containing 2.0 mg/l NAA + 0.1 mg/l IAA showed better root development than those cultured on media containing combinations between auxins and cytokinins. Cuzzuol *et al.*, (1996) found that adventitious shoots from carnation cultures were rooted on MS medium supplemented with NAA, IAA or IBA each at 0.0, 0.25. 0.5 or 1.0 mg/l. Root development was similar in all media including the control medium (without plant growth regulators).

Effect of NAA on rooting was studied by Harazy *et al.*, (1985). They obtained the rooting of *Limonium sinuatum* by culturing the shoots on ½ MS medium with 0.5 mg/l NAA. Bach and Pawlowska (1992) reported that shoots

of *Limonium tataricum* were rooted in the presence of NAA at 0.05-0.8 mg/l on the MS rooting medium. Lledo *et al.*, (1996) produced the rooting formation on the proliferated shoots of *Limonium thiniense* on MS medium supplemented with NAA, IAA or IBA.

Regarding the effect of salt concentrations of medium on the *in vitro* rooting, Matsumoto *et al.*, (1997) stated that the most plants of hybrid statice (*Limonium altaica* x *L. capsicum*) were rooted after 30 days on solidified hormone free ½ medium. Zamorano and Meigia (1994) reported that the root formation was occurred on regenerated shoots of *Gypsophila paniculata* cv. Perfecta on MS medium without growth regulators. Choudhary (1992) induced rooting of carnation cultures cv. William Sim by placing the developed shoots on both solid and liquid MS medium. Ihsan *et al.*, (1995) found that rooting was stimulated by subculturing the proliferated shoots of carnation on ½ MS medium with 3% sucrose and 1% agar. Barbosa *et al.*, (1994) and Pierik *et al.*, (1995) found that the rooting of Gerbera and Rose was obtained on MS medium at full strength salts. Arnold *et al.*, (1995) reported that the salt concentrations of MS medium had a minimal effect on the rooting percentage and the root length of Rose plantlets.

The objective of this work was to use the *in vitro* culture as a recent fast technique for multiplication, rooting, hardening off the plantlets as well as overcoming the vitrification phenomenon on the carnation (*Dianthus caryophyllus* L) cultivars Lelia, Maragia, Sorriso and Aicardi.

MATERIALS AND METHODS

This work was carried out at the Plant Tissue Culture Laboratory in the Horticulture Department, Faculty of Agriculture, Suez Canal University during the years 2000–2001.

Shoot tips of carnation (*Dianthus caryophyllus* L) cultivars, Lelia, Maragia , Sorriso and Aicardi were collected from the farm and transferred to the Tissue Culture Laboratory. The shoot tips were washed with running tap water followed by immersion in 70% ethanol for few seconds. Then they were soaked in sodium hypochlorite (commercial Clorox) at 10% for 10 min. followed by washing with 3 changes of sterile distilled water to remove all traces of disinfectants. The shoot tips were trimmed into about 1.0 cm and placed into the culture vessels (200 ml glass jars) containing 35 ml of MS medium (Murashige and Skoog, 1962) as initiation medium. The medium was supplemented with 100 mg/l myo-inositol, 30 g/l sucrose and solidified with 8.0 g/l agar. No growth regulators were added to the initiation medium. The pH of medium was adjusted to 5.7 before autoclaving. The aim of this stage was to establish the rapid developing and to obtain non contaminated explants.

After 4 weeks, the shoot tips about 0.5 cm long were excised from the developing plantlets and cultured on multiplication medium containg MS macro and micro salts. The medium was supplemented with 100 mg/l myo-inositol and 40 g/l sucrose. Factorial combinations of BA at 0.0 , 0.5 , 1.0 and 2.0 mg/l and agar at rates of 8.0, 10.0 , 12.0 and 14.0 g/l were added to the media. The aim of this experiment was to study the effect of BA and agar on

the shoot multiplication as well as on the vitrification percentage of the four cultivars of carnation

Two shoot tips from each cultivar were cultured in each glass jar (200 ml) containing 35 ml of the medium. Each treatment included 20 replicates (jars).

The culture vessels were maintained in growth room at 26^{o}C under 16 hours light and 8 hours dark cycle at light intensity of approximately 1500 Lux.

After 4 weeks, the cultures were transferred to the same fresh media (recultured) and after 8 weeks from beginning the experiment, the following data were recorded: number of shoots/explant, shoot length (cm), number of leaves/explant and the percentage of vitrification .

To study the *in vitro* rooting, an experiment was designed to investigate the effect of NAA and agar on root formation of the carnation cultivars under study .

The regenerated shoots were individually separated from the multiplication stage and cultured on a rooting medium. The medium was supplemented with myo–inositol at 100 mg/l , sucrose at 30 g/l., NAA at 0.0 and 1.0 mg/l and agar at rates of 7.0 and 8.0 g/l

One shoot was cultured per culture tube and each treatment included 20 replicates (culture tubes). The culture tubes were kept under the same environmental conditions as described with the multiplication experiment.

After 4 weeks, the following data were recorded : number of roots/ plantlet and the root length in cm .

The rooted shoots which produced from the rooting stage were carefully removed from the culture tubes, washed with fungicide 1% to reduce the fungal contamination. The plantlets were cultured in small plastic pots. The mixture medium contained 1 :1 : 1 peat moss : vermiculite : perlite by volume. The pots were placed under mist for 2 weeks, then they were transferred to grow under greenhouse conditions. The survival percentage of the plants was recorded after 3 weeks of transplanting.

The two experiments were set up in a factorial design. Data with percentage of vitrification were transformed using square root transformation ($\sqrt{x + 1}$) according to Snedecor and Cochran (1967) before statistical analysis. The data were computed using SAS program and analyzed by analysis of variance with means separated by Duncan's Multiple Rang Test according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1- Effect of BA and agar on shoot multiplication :

As shown in Table (1), addition of BA to the culture media significantly increased the number of proliferated shoots on all cultivars of carnation under study. The number of obtained shoots was increased with increasing BA concentrations in most cases. The treatment with BA at 2.0 mg/l produced the highest number of shoots (5.8 and 4.1 shoots/explant) with Maragia and Sorriso cultivars. While BA at 1.0 mg /L gave the highest number of shoots as 6.0 shoots/explant with Aicardi cultivar. Increasing the number of proliferated shoots on carnation by adding BA to the culture medium has been reported by

many workers. Choudhary *et al.*, (1990) produced the best shoot proliferation (10–12 shoots) by culturing the axillary buds of carnation cultivar Scania Red on medium supplemented with BA at 1.0 mg/l Mujib and Pal (1995) reported that the highest number of shoots of carnation cv. Candy Sim was observed by culturing the shoot tips on MS medium supplemented with BA at 0.5 mg/l.

As for shoot length, data in Table (1) indicated that BA a had reducing effect on the length of developed shoots of carnation .This observation was true with the all cultivars. The treatment with MS medium containing BA at 2.0 mg/l produced the shortest shoots, while MS medium without BA gave the tallest shoots with all cultivars.

Concerning the effect of BA on the number of developed leaves of carnation cultivars, data presented in Table (1) showed that increasing BA rates significantly increased the number of leaves/explant. BA at 1.0 mg/l produced the highest number of leaves (25.5 and 24.1) with Lelia and Aicardi cultivars, while BA at 2.0 mg/l resulted the highest number of leaves (23.8) with Maragia cultivar.

Increasing the leaf number/explant may be attributed to increasing the number of proliferated shoots which has been occurred as a result to BA treatment.

According to effect of agar, data presented in Table (2) indicated that increasing the rate of agar on the culture medium, generally decreased the number of proliferated shoots on all cultivars of carnation under study. With Maragia and Aicardi cultivars, the best results (4.5 and 5.3 shoots/explant) were obtained on MS medium containing 8.0 g / L agar (least rate).

On the other side, the highest rate of agar as 14.0 g/l produced the lowest values of shoot number with all cultivars. Agar is one of the most common organic complexes used in tissue culture, the amount of agar added to the medium varies considerably. Increasing the agar concentrations minimize water loss via evaporation but the medium will not be soft enough to allow good nutrient diffusion. This phenomenon may be leading to inhibit the growth of explants and decrease number of the obtained shoots.

A similar trend of results was obtained by Ziv *et al.*, (1983) who found that adding agar to the medium decreased the rate of shoot multiplication.

It was obvious from data in Table (2) that raising the rate of agar reduced shoot length of carnation cultivars. The shortest shoots as 2.80, 3.0 , 3.3 and 3.4 cm were obtained from the medium containing 14.0 g/l agar for Lelia, Maragia, Sorriso and Aicardia cultivars, respectively.

Concerning the effect of agar on the number of developed leaves , it is clear from data in Table (2) that the number of leaves was decreased by increasing the concentration of agar. This was true with all cultivars of carnation.

Effect of interaction between BA and agar on the shoot multiplication was presented in Table (3). With Maragia and Aicardi cultivars, treatment with BA at 2.0 mg/l + agar at 8.0 g/l produced the highest number of shoots per explant (8.3 and 10.1 shoots) respectively (Fig.1.a). BA at 2.0 mg/l combined with agar at 10.0 g/l gave the highest number of shoots as (7.0 shoots/explant) with Sorriso cultivar.

Table (1): Effect of BA on shoot multiplication of *Dianthus caryophyllus* cvs. Lelia, Maragia, Sorriso and Aicardi.

Cvs.	Lelia			Maragia			Sorriso			Aicardi		
BA (mg/L)	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant
0.0	1.3	4.6	10.7	1.2	3.5	7.5	1.0	4.0	6.7	1.1	4.6	7.7
0.5	3.7	3.1	20.4	1.7	3.9	9.5	2.4	3.7	13.1	3.7	3.6	20.2
1.0	5.7	2.8	25.5	5.1	3.0	21.8	3.8	3.8	17.5	6.0	3.1	24.1
2.0	4.8	3.1	24.2	5.8	3.0	23.8	4.1	3.1	16.1	5.3	3.0	21.4
LSD 5%	0.55	0.27	2.73	0.67	0.27	2.57	0.43	0.45	1.83	0.62	0.37	2.19

Table (2): Effect of Agar on shoot multiplication of *Dianthus caryophyllus* cvs. Lelia, Maragia, Sorriso and Aicardi.

Cvs.	Lelia			Maragia			Sorriso			Aicardi		
Agar (g/L)	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ Explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant
8.0	3.9	3.8	22.9	4.5	3.5	17.9	2.9	3.9	14.9	5.3	3.8	22.1
10.0	4.3	3.5	21.7	4.1	3.5	18.4	3.5	3.9	14.7	4.2	3.5	18.9
12.0	4.5	3.5	20.4	3.3	3.5	15.3	3.1	3.5	13.7	4.3	3.5	18.9
14.0	2.7	2.8	15.7	1.9	3.1	10.6	1.8	3.3	10.3	2.3	3.4	15.3
LSD 5%	0.55	0.27	2.73	0.67	0.27	2.57	0.43	0.45	1.83	0.62	0.37	2.19

Table (3): Effect of BA x agar on shoot multiplication of *Dianthus caryophyllus* cvs. Lelia, Maragia, Sorriso and Aicardi.

BA (mg/L)	Cvs.	Lelia			Maragia			Sorriso			Aicardi		
	Agar (g/L)	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ Explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant	No. of shoots/ Explant	Shoot length (cm)	No. of leaves/ explant	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ explant
0.0	8.0	1.3	4.7	11.4	1.0	4.0	5.9	1.0	3.9	6.6	1.1	4.9	7.8
	10.0	1.3	4.8	9.4	1.4	3.8	7.9	1.0	3.9	7.0	1.1	4.1	7.8
	12.0	1.4	4.8	12.0	1.4	3.5	9.6	1.1	4.1	6.8	1.0	4.9	7.5
	14.0	1.4	3.9	10.0	1.0	2.6	6.8	1.0	4.3	6.5	1.0	4.4	7.8
0.5	8.0	3.8	3.9	20.3	2.0	4.0	10.3	1.9	4.6	12.0	3.4	4.5	20.3
	10.0	4.1	3.1	25.4	1.9	3.9	9.8	2.4	4.6	12.8	4.4	3.6	23.0
	12.0	4.4	3.0	23.1	1.6	4.0	9.8	3.3	2.8	15.4	4.9	3.0	23.6
	14.0	2.5	2.5	12.8	1.4	3.8	8.3	2.0	2.8	12.1	2.0	3.4	14.0
1.0	8.0	5.9	2.9	29.1	6.6	3.4	26.4	5.9	3.4	26.4	6.6	3.0	27.4
	10.0	5.8	2.9	28.3	6.9	3.1	28.4	3.6	4.5	15.3	7.3	2.8	27.3
	12.0	8.0	2.8	27.6	4.5	3.3	20.3	3.6	3.3	16.3	7.6	3.3	26.6
	14.0	2.6	2.4	16.9	4.5	2.6	12.0	2.3	3.9	12.0	2.6	3.3	15.0
2.0	8.0	4.6	3.6	30.9	8.3	2.7	29.0	3.0	3.8	14.4	10.1	2.9	33.1
	10.0	6.0	3.1	23.9	6.4	3.2	27.5	7.0	2.6	23.8	4.0	3.3	17.4
	12.0	4.1	3.4	18.9	5.6	3.0	21.4	4.3	3.8	15.9	3.5	3.0	17.8
	14.0	4.3	2.5	23.0	2.8	3.1	15.5	2.0	2.3	10.5	3.4	2.6	17.3
Mean		3.8	3.4	20.1	3.5	3.4	15.5	2.8	3.6	13.3	4.0	3.6	18.3
LSD at 5%		1.79	0.90	8.90	2.20	0.89	8.40	1.41	1.47	5.96	2.04	1.19	7.16

The interaction between BA and agar had different effects on length of the developed shoots of the cultivars of carnation as shown in Table (3).

The treatment with zero BA + agar at 8.0 g/l produced the longest shoots (4.0 and 4.9 cm) with Maragia and Aicardi cultivars , while the treatment with BA at 0.5 mg/l + agar at 8.0 or 10.0 g/l resulted the longest shoots with Sorriso cultivar.

Concerning the number of leaves, data in Table (3) show that MS medium supplemented with BA at 2.0 mg/l and agar at 8.0 g /L significantly increased the number of leaves to 29.0 and 33.1 for Maragia and Aicardi cultivars, respectively. On the other side, BA at 1.0 mg/l with agar at 10.0 g/l produced the highest number of leaves (26.4 leaves/explant) with Sorriso cultivar.

In general, the cultivars of carnation showed different responses to treatments with BA and agar as shown in Table (3). Aicardi cultivar produced the highest number of shoots (4.0 shoots/explant) followed by Lelia (3.4 shoots). The longest shoots (3.6 cm) were obtained by Aicardi and Sorriso cultivars, while, the highest number of leaves/explant as 20.1 and 18.3 was observed on Lelia and Aicardi cultivars, respectively.

The obtained results indicated that the vegetative growth of plants depended on the genotypes. Similar observation was reported by Mirghis and Mirghis (1995) who found that the number of shoots developed per explant of Rose depended on genotype.

Concerning the effect of BA on the vitrification, data in Table (4) cleary showed that increasing the levels of BA on the culture medium increased the occurrence of vitrification phenomenon (Fig. 1 b). It was clear that the conditions which promote extensive shoot proliferation often result in vitreous plants (abnormal leaves and shoots) The vitreous leaves of several plants propagated *in vitro* have abnormal mesophyll (lack cuticular waxes and their stomatal functioning is impaired . (Leshem, 1983).

As for the effect of agar on vitrification percentage, data in Table (4) showed that increasing agar concentration decreased the percentage of vitrification on all cultivars. However, the vitreous leaves disappeared with the highest rate of agar (14.0 g/l) and the plantlets grew normally.

Inhibition of vitrification by raising the agar concentration in the culture medium may be due to effect of agar on water potential and its effect on decreasing the water loss through evaporation.

From the results, increasing agar rates had positive effect on reducing, preventing the vitrification and producing normal plantlets. However, increasing the agar had a negative effect on growth of plantlets where it decreased the number of developed shoots.

Similar results on carnation cv. Cerise were reported by Leshem (1983) who found that increasing the agar from 0.8 to 1.2 % increased the proportion of normal shoots developing from 46 to 77 % Choudhary *et al.*, (1993) reported that the percentage of vitrified shoots of carnation cv. Scania was decreased with increasing agar from 0.6 to 1.4 % but the apices developed into small shoots.

Table (4): Effect of BA and agar on the vitrification percentage of Dianthus caryophyllus cvs. Lelia, Maragia, Sorriso and Aicardi.

BA (mg/L)	Agar (g/L)	Vitrification % Original data				Vitrification Transformed data*			
		Lelia	Maragia	Sorriso	Aicardi	Lelia	Maragia	Sorriso	Aicardi
0.0	8	50	50	50	50	7.1	7.1	7.1	7.1
	10	25	25	25	25	5.1	5.1	5.1	5.1
	12	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
	14	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
0.5	8	50	50	50	50	7.1	7.1	7.1	7.1
	10	25	25	25	25	5.1	5.1	5.1	5.1
	12	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
	14	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
1.0	8	50	50	50	50	7.1	7.1	7.1	7.1
	10	50	50	50	50	7.1	7.1	7.1	7.1
	12	25	25	25	0.0	5.1	5.1	5.1	5.1
	14	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
2.0	8	75	75	75	50	8.7	8.7	8.7	7.1
	10	50	50	50	50	7.1	7.1	7.1	7.1
	12	25	25	25	25	5.1	5.1	5.1	5.1
	14	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
LSD at 5%						0.73	0.91	0.82	0.76
BA (mg/l)	0.0	18.8	18.8	18.8	18.8	3.6	3.6	3.6	3.6
	0.5	18.8	18.8	18.8	18.8	3.6	3.6	3.6	3.6
	1.0	31.3	31.3	31.3	25.0	5.1	5.1	5.1	4.1
	2.0	37.5	37.5	37.5	31.3	5.5	5.5	5.5	5.1
LSD at 5%						0.37	0.46	0.41	0.38
Agar (g/l)	8	56.3	56.3	56.3	50.0	7.5	7.5	56.3	7.1
	10	37.5	37.5	37.5	37.5	6.1	6.1	37.5	6.1
	12	12.5	12.5	12.5	6.3	3.1	3.1	12.0	2.0
	14	0.0	0.0	0.0	0.0	1.0	1.0	0.0	1.0
LSD at5%						0.37	0.46	0.41	0.38

* Data were transformed using square root transformation.

It was detected from the data in Table (4) that the combination between the highest level of BA (2.0 mg/l) and the lowest level of agar (8.0 g/l) obtained the highest percentage of vitrification (75%) with Maragia and Sorriso cultivars. However, the vitrification percentage reached zero and the vitreous leaves disappeared with agar at the highest rate (14.0 g/l) with each level of BA.

2- Effect of NAA and Agar on the *in vitro* rooting

Data in Table (5) showed the effect of NAA, agar and their combinations on the *in vitro* rooting of carnation cvs. Lelia, Maragia, Sorriso and Aicardi. The number of developed roots varied between the cultivars. The Aicardi cultivar produced the highest number of roots as 13.3 followed by 12.1, 11.0 and 9.5 for Sorriso, Lelia and Maragia cultivars respectively.

Application of NAA at 1.0 mg/l to the culture medium increased the number of roots/plantlet with all cultivars of carnation comparing with MS medium without NAA.

Table (5): Effect of NAA, agar and their combinations on the *in vitro* rooting of *Dianthus caryophyllus* cvs. Lelia, Maragia, Sorriso and Aicardi.

Treatments	Lelia		Maragia		Sorriso		Aicardi	
	No. of roots/ plantlet	Root length (cm)	No. of roots/ plantlet	Root length (cm)	No. of roots/ plantlet	Root length (cm)	No. of roots/ plantlet	Root length (cm)
NAA at (0.0 mg/L)	8.6	2.2	6.1	1.5	7.3	2.0	9.9	1.4
NAA at (1.0 mg/L)	13.8	2.9	13.6	1.9	15.1	2.3	16.4	3.3
Agar at (7.0 g/L)	9.4	1.9	8.1	1.5	7.6	1.2	8.2	1.6
Agar at (8.0 g/L)	12.9	3.2	11.5	1.8	14.8	3.0	18.1	3.2
NAA (0.0 mg/L) x (agar (7g/L)	6.2	1.1	6.4	1.1	1.0	0.1	1.0	0.1
NAA (0.0 mg/L) x agar (8g/L)	10.9	3.3	5.8	1.9	13.5	3.9	18.9	2.8
NAA (1.0 mg/L) x agar (7g/L)	12.5	2.6	9.9	2.0	14.3	2.3	15.4	3.1
NAA (1.0 mg/L) x agar (8g/L)	15.0	3.1	17.3	1.7	16.0	2.2	17.4	3.6
LSD for NAA x Agar at 5%	2.52	1.48	7.69	1.81	5.17	0.71	7.57	1.19

On the other side, increasing the agar concentration from 7.0 to 8.0 g/l increased the root number /plantlet with all cultivars. The best results were reported with Aicardi cultivar, where MS medium containing 8.0 g/l agar produced the highest number of roots (17.4 and 17.3) for Aicardi and Maragia cultivars. The lowest number of roots was obtained from the treatment with MS medium without NAA and supplemented with agar at 7.0 g/l.

A similar trend of results was found by Can and Koc (1992) who found that the shoots of carnation produced roots on MS medium containing NAA and IAA. Also, Cuzzuol *et al.*, (1996) produced the roots on carnation by culturing the shoots on MS medium with NAA, IAA or IBA.

As for the length of developed roots, data in Table (5) indicated that the length of roots varied between the cultivars of carnation under study. Aicardi cultivar produced roots longer than the other cultivars. Generally, MS medium with NAA at 1.0 mg/l increased the root length comparing to MS medium without NAA (Fig. 1 c)

Regarding effect of agar, data indicated that increasing the rates of agar to 8.0 g/l increased the length of roots for all cultivars in comparison with MS medium with 7.0 g/l. However, the root length reached 3.2 and 3.1 cm for Aicardi and Lelia cultivars respectively.

Meantime, the treatment with MS medium containing 1.0 mg/l and 8.0 g/l agar significantly produced the tallest roots as 3.6 cm with Aicardi when compared to the other treatments and the other cultivars.

The obtained plantlets of carnation were carefully removed from the rooting medium, washed with fungicide and transplanted into a medium consisting of peatmoss: vermiculite: perlite (1:1:1). The pots were placed on benches in the greenhouse under intermittent mist. Plants were growing well under these conditions (Fig. 1 d). The survival percentage reached 80% with all cultivars.

Finally in conclusion, propagation of carnation using tissue culture techniques is recommended as a method for mass production of plants. It is necessary to make balance between growth regulators and agar to overcome the vitrification and to obtain sufficient multiplication rate during the multiplication stage.

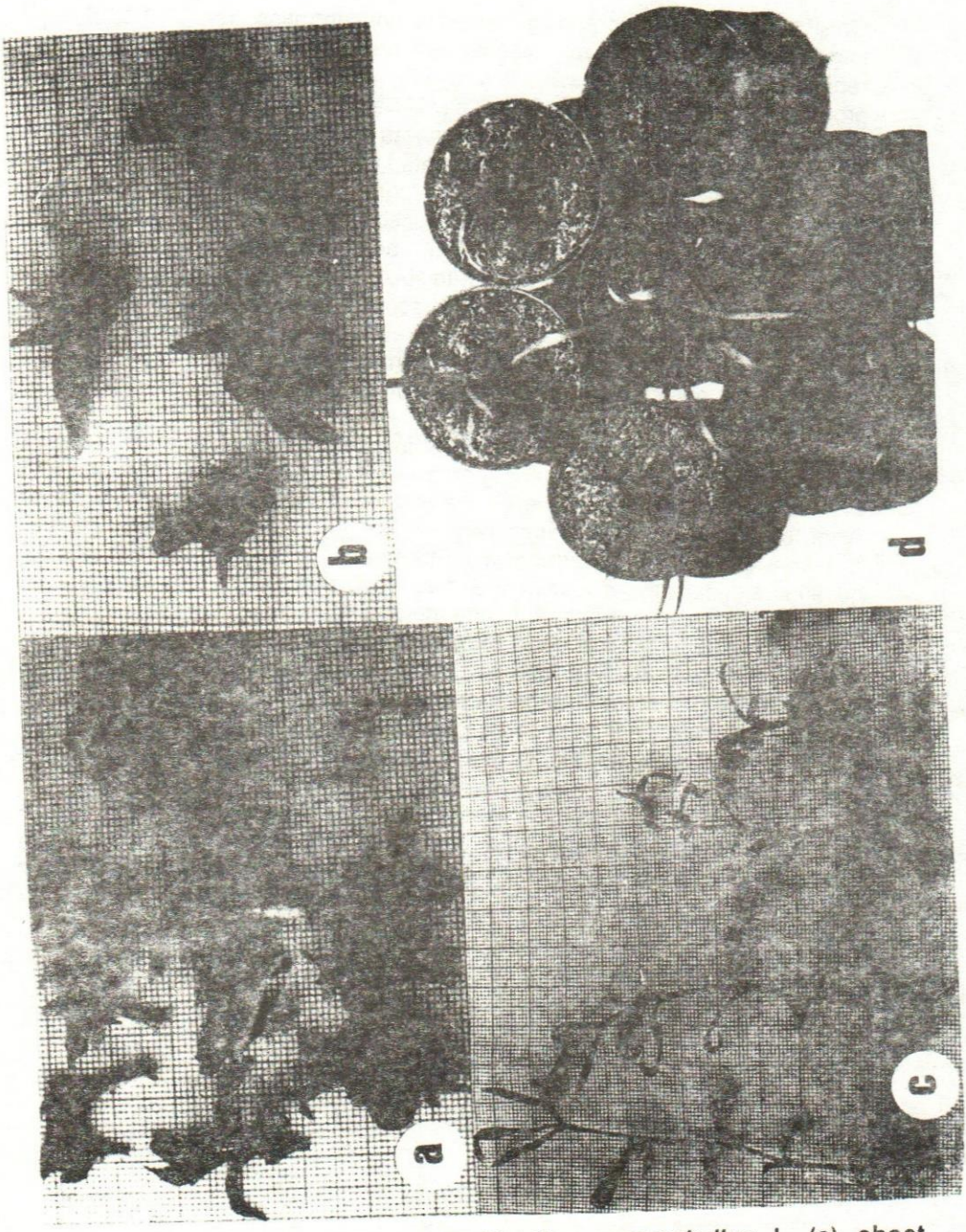

Fig (1): *In vitro* culture of *Dianthus caryophyllus* L (a) shoot multiplication from shoot tips after 8 weeks from culture. (b) vitrified plantlets of carnation with high rates of BA. (c) *in vitro* rooting after 4 weeks. (d) The carnation plants establishement.

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الإكثار الدقيق والأقلمة لنباتات القرنفل

إيمان إسماعيل مغازى ، حمدى كمال عطا الله ، عبدالقوى والى و إسلام أبوالسعود
قسم البساتين ، كلية الزراعة ، جامعة قناة السويس الإسماعيلية ٤١٥٢٢٢ مصر

أجرى هذا البحث فى معمل زراعة الأنسجة قسم البساتين ، كلية الزراعة جامعة قناة الويس خلال الفترة من ٢٠٠٠ إلى ٢٠٠١ ، تم فصل القمم النامية لنباتات القرنفل من أصناف (Lelia, Maragia, Sorriso and Aicardia) وزرعت على بيئة مورشاج وسكوج والتى تحتوى على البنزيل أدنين بمعدلات صفر ، ٠,٥ ، ١ ، ٢ ملجم/لتر وتم إضافة الأجار إلى البيئة بمعدلات ٨ ، ١٠ ، ١٢ جم/لتر .

بعد ٨ أسابيع من الزراعة أوضحت النتائج أن المعاملة بالبنزيل أدنين بمعدل ٢ ملجم/لتر والأجار بمعدل ٨ جم/لتر أعطت أكبر عدد من الأفرع المتكونة (٨,٣ و ١٠ أفرع/قمة نامية منزرعة) فى كل من صنفى Aicardi, Maragia على التوالى. ولكن زيادة تركيز البنزيل أدنين فى البيئة أدى إلى زيادة تكوين الأوراق الزجاجية (Vitrification) ومن ناحية أخرى أوضحت النتائج أن زيادة معدل الأجار فى البيئة ادى إلى حدوث نقص فى عدد الأفرع المتكونة وكذلك التقليل من تكوين الأوراق الزجاجية. بعد ذلك تم نقل الأفرع الناتجة وزراعتها على بيئة تجذير .

تبين من النتائج أن بيئة مورشاج وسكوج والمحتوية على ١ ملجم/لتر نفثالين أسيتيك أسيد مع الأجار بمعدل ٧ أو ٨ جم/لتر أعطت أكبر عدد من الجذور مع الأصناف Sorriso, Maragia.

نقلت النباتات من بيئة التجذير وزرعت فى أصص صغيرة تحتوى على بيئة مكونة من البيت موس والفرمكوليت والبيرليت (١:١:١) ووضعت الأصص فى الصوبة البلاستيكية مع الرى بالرزاز لمدة أسبوعين وكانت نسبة النباتات الناجحة ٨٠% فى كل أصناف القرنفل المستخدمة.