Journal on plant Sciences Researches, Vol.1, No.3, Autumn 2006

In vitro culture of carnation and acclimatization of the plantlet to *ex vitro* condition

[*]Ghorbanli, M.[1], Nouri-Cootanai, F.[1], Allahverdi Mamaghani, B.[2]

1. Biology Department, Islamic Azad University, Tehran North Branch, Iran
2. Research Institute of Forest and Rangland, Tehran, Iran

Abstract

Dianthus caryophyllus is one of the most important species in Dianthus genus. In order to propagation virus-free plant material, *in vitro* culture of this species var cerise royallette were studied. Explants were cultured in Anderson medium containing 1 mgl^{-1} 6-benzylaminopurine. 6-benzylaminopurine alone induced callus in nodes and internodes explants. To achieve organogenesis, calli subcultured onto Anderson medium with or without plant growth regulator. The highest shoot length was obtained in medium without any hormone. The most suitable adventitious shoot regeneration medium was Anderson medium supplement with 4 mgl^{-1} Kin and 0.1 mgl^{-1} NAA. Root regeneration was observ ed in medium with 0.5 and 1 mgl^{-1} naphtalenacetic acid. Rooted plantlet transferred into pot with perlite and irrigated by different nutrient solution. The effect of different nutrient solution (An, OM, MS, MC) on growth and development of plantlet were compared. The results showed Anderson medium was more effective than the others. Acclimatized plants were transferred to soil. All plants were survived in field condition and produced flower after 4 months.

Key words: *Dianthus caryophyllus* var cerise royallette, Micropropoagation, Nutrient solution, Plant growth regulator

Abbreviation: BAP: 6-benzylaminopurine, 2iP: 2-Amino purine, IBA: indol-3-butyric acid, IAA: indol acetic acid, Kin**:** kinetin**,** OM: Olive Medium, NAA: naphtalenacetic acid, An: Anderson (1975), MS: Murashige & Skoog (1962), MC: Misra & Chaturvedi (1984), min: minute, PGR: plant growth regulator.

Introduction

Carnation is one of the three important cut flower in the world. Conventional propagation of this plant is cutting of auxillary shoot and seed. These methods are not only practical in all carnation cultivar but also plant material have viral and fungal contamination. Micropropagation provides much more rapid cloning from selected cultivar than would be expected from seasonally limited conventional propagation (Hammatt et al., 1997). However its more widespread use is restricted by the often high percentage of plants lost or damaged when transferred to *ex vitro* condition (Pospislova, 1999). Nugent et al. (1991) reported plant regeneration of four cultivar in carnation from petal explant. Miller et al. (1991) used MS basal medium supplement with 4-8 µM naphtalenacetic acid and 6-benzylaminopurine for adventitious shoot regeneration of carnation. Miller et al. (1991) showed adventitious shoot regeneration from auxillary buds explants of 15 carnation cultivar. They indicated that auxillary bud size and the type between subcultures of source material influenced the production of adventitious shoots. Jethwani et al (1996) demonstrated that phenylacetic acid in combination with 6-benzylaminopurine was essential to trigger shoot regeneration from

[*]*e.mail: ghorbanli@yahoo.com*

cultured leaf callus of *Dianthus chinensis*. Fal et al., (1999) showed ethylene improved the quality of cv. BarbaretTanga explants, decreasing hyperhydricity and increasing the number of shoots, the length of the main shoot and the multiplication coefficient. Majada et al. (2000) concluded vessel ventilation promoted *in vitro* hardening of micropropagated carnation shoots and pushed the culture-induced phenotype closer to that of *ex vitro* acclimatized plants. Nontaswatsri et al. (2002) Reported shoot regeneration of leaf and nod explant of 38 cultivar in carnation. They indicated correlation coefficient between shoot regeneration percentage of nod and leaf explant was significantly positive.

There is no published protocol on in vitro culture of *Dianthus caryophyllus* var cerise royallette. One aim of these experiments was to invstigated the effect of PGR on organogenesis of carnation var cerise royallette. Another aim was to assess wether the effect of nutrient solution on growth and acclimatization of plantlet in green house condition.

Materials and methods

Explants were taken from plants without bud dormancy, bacterial symptoms and water stress. Leaves, nodes and internodes segment, 5-10 cm long, rinsed in tap water for 30 min and washed with water and detergent. Then, explants were surface sterilized by dipping them in benomile solution (0.5%) for 20-30min, ethanol (70%) for 20-30 S followed with immersion them in hypocholorite solution (5%) for 20-30 min. Then explants transferred to laminar air flow cabinet and rinsed three times with sterile distilled water. The resulting explants were cultured on An Medium (Anderson, 1978-1980) containing 1mgl^{-1} BAP, 30gl^{-1} sucrose at PH 5.7±0.1 and solidified with 8gl^{-1} Agar. After callus induction, Calli transferred on An Medium supplement with different concentration of BAP, 2iP and kin (0, 1, 2, 3, 4, 5mgl^{-1}) with combination to NAA (0, 0.1, 0.5, 1mgl^{-1}). After six weeks shoot length, shoot number, internodes number, Root number and root length were recorded. Cultures were subcultured at six weeks interval. They maintained at 25±2 with 16 photoperiod provided by Double cool fluorescent tube with 1000 lux intensity.

Plantlet with normal and healthy roots was carefully washed by water. Then, plantlet immersed in 0.5% benomile solution for 20 min to avoid fungal infection. Rooted plantlet inserted in pot containing sterile Perlit. Pots were kept in the shad and high relative humidity. Plantlet were irrigated by nutrient solution containing macro and micro nutrient of An, OM (Olive Medium), MS (Murashig and Skoog, 1962), MC (Misra and chatuvedi, 1984) and water (control). Irrigation was done 3 days interval during three weeks. After this period growth and development of shoots and roots were evaluated. Acclimatized plantlet transferred to soil and exposed to long day and 20- 25c̊ temperature.

All treatments were conducted in complete randomized design. For each trait means and standard error of means were calculated. Analysis of variance was done at $P \leq 0.05$. Means comparison was made by least significant difference (LSD).

Results

Effect of BAP and NAA

The highest shoot length was observed in hormone –free medium (table 1 and figure 1). In this medium shoots had dark green leaves and internodes length were 1cm. The average of adventitious shoots and shoot length decreased as the BAP concentration increased (table 1 and figure 1). The highest shoot proliferation (4.62) was observed in A_9. In medium containing BAP alone,. In culture with 0.1mgl^{-1} NAA and different concentration of BAP, there was no significant difference among A_9 to A_{11} for shoot length and A_8 to A_{10}, A_{12} to A_{13} for shoot number. In medium with 1 mgl^{-1} NAA,. Shoots had thin leaves with light green color. In medium with 0.5 mgl^{-1} NAA There was no significant difference among A_{17} to A_{20} for shoot length and A_{15} to A_{16} for shoots number and $A_{18}$to A_{20} for internodes number. In medium with 1 mgl^{-1} NAA there was no significant difference among A_{23} to A_{25} and A_{26} to A_{28} (Table 2). NAA alone did not promote multiple shoots (figure 1). Shoot length was higher in medium with BAP and NAA together.

Optimum root length and root number were obtained in 0.5 and 1 mgl^{-1} NAA. In hormone –free medium root formation and growth was observed. The highest root number was in A_{22} and the optimum root length was in A_{15}. Increased of BAP to 1 mgl^{-1} tend to decrease of root regeneration. High content of BAP inhibited root formation (Table 1 and figure 2).

Effect of 2iP and NAA

Highest shoot length (4.21) was observed in medium without PGR. The best shoot proliferation (4.63) was obtained in medium supplement with 1 mgl^{-1} 2iP. Shoot length decreased slowly with increased 2iP concentration (table2 and figure2). Increasing of 2iP into 1mgl^{-1} promoted shoot regeneration. But high level of 2iP had an inhibitory effect on shoot formation. In culture with 0.1mgl^{-1} NAA and different concentration of 2iP, There was no significant difference among B_8 to B_9, B_{10} to B_{11} and B_{12} to B_{14} for shoot number and B_9 to B_{11} for internodes number.. In medium with 0.5 mgl^{-1} NAA There was no significant difference among B_{17} to B_{21} for shoot length, B_{15} to B_{17} and B_{19} to B_{21} for internodes number, B_{15} to B_{18} and B_{19} to B_{21} for shoots number. In medium with 1 mgl^{-1} NAA There was no significant difference among B_{22} to B_{24} and B_{26} to B_{27} for internodes number, B_{22} to B_{25} and B_{26} to B_{28} for shoots number (table2).

Root formation induced in all concentration of NAAwith no 2iP. High concentration of NAA increased root regeneration. Increasing of 2iP up to 0.5 mgl^{-1} lead to root regeneration. Although, high level of 2iP prevented root formation (figure 2). Optimum root length and root number was in B_{15} and B_{22}.

Effect of Kin and NAA

The highest shoot length was observed in hormone- free medium. In treatment containing Kin alone. the best shoot proliferation were obtained in 0.5mgl^{-1} Kin. Increased of Kin content decreased shoot number (figure 3). However in treatment involved 0.1 mgl^{-1} NAA and different concentration of Kin, shoot induction was slowly decreased and the highest shoot number (10.2) was observed in C_{13} (table 3). In culture with 0.1mgl^{-1} NAA and different concentration of Kin, There was no significant difference among C_9, C_{10} C_{12} and C_8, C_{11}, C_{13} for shoot length and C_8 , C_{11} and also C_9, C_{12} for shoots number In medium with 0.5 mgl^{-1} NAA there was no significant difference among C_{17} to C_{21}, C_{15} to C_{16} for shoot length, C_{16} to B_{18} and C_{19} to C_{21} for shoots number, C_{17} to C_{21} and C_{16} to C_{20} for internodes number. In medium with 1 mgl^{-1} NAA There was no significant difference among C_{23} to C_{25} For shoot length and C_{22}, C_{23} and C_{26} and C_{24} to C_{25} for shoots number, C_{22} to C_{23} and $C_{24}$to C_{25} for internodes number (figure 3).

Growth and development of root in medium with Kin was better than medium containing 2iP and BAP. Optimum root length and root number were observed on medium with 0.5 and 1 mgl^{-1} NAA respectively. In medium with Kin alone the highest root number and root length were in C_3 and C_2. Kinetin up to 1mgl^{-1} promoted root formation. Although, high concentration of Kin had an inhibitory effect on rooting process (figure 3).

Acclimatization of the plantlet to *ex vitro* condition

The highest shoot length was in An medium and shoot length was minimum in control. There is no significant difference between MS and OM medium in relation to root length. The best shoots, internodes and roots number were obtained in An culture medium. In contrast shoot and root number were decreased in MC and MS medium. Plantlet irrigated with An solution have an expanded aerial and root system with normal shoots. Leaves of shoots were long with dark green color. Roots were white, branched and have root hair. In contrast with OM medium, shoot proliferation and internodes length were decreased. Root system was inhibited. Roots were short, branched and dark brown color. With MS solution shoot number inhibited. But shoots have normal leaves. Root system was similar to OM medium. Using of MC medium decreased shoot number. Leave size was inhibited in comparison to An medium. Roots were long and branched with root Hair. In control treatment shoot number and length was reduced. Leaves were normal with light green color. Roots were thin and branched without suitable root hair.

Discussion

There was no adventitious shoot regeneration in leaves explants. Miller et al. (1991) concluded that use of leaf and stem explant was not successful largely due to explant senescence. Jethwani and Kothari (1996) reported Shoot regeneration from leaf derived callus of *Dianthus chinensis* using Phenylacetic acid. The optimum shoot regeneration was found in medium containing Kin and NAA. Casanova et al. (2004) reported that thidiazuron alone as well as synergistically with NAA promoted shoot organogenesis in petal and was more active than BAP. It is hypothesized shoot differentiation in carnation depend on explant source, cultivar and

balance of plant growth regulator. Produced shoots in medium containing Kin showed light green toward yellow color due to leaves senescence in presence of cytokinin (Miller et al., 1991). Genkov et al. (1997) showed that BAP, N-phenyl-N´-(2-chloro-4-pyridyl) urea and TDZ increased chlorophyll content and change chlorophyllase activity but TDZ decreased photosynthetic membrane stability. In all treatment highest shoot length was in hormone-free medium. It is suggested that PGR promoted cell division and inhibited cell elongation. Vitrification of organ was observed in treatment with NAA and 2iP as well as NAA and Kin. High level of BAP induced hyperhydric organ. Vitrification of shoots depend on culture medium composition and growth regulator. Yadav et al. (2003) reported that increased concentration of iron and magnesium reduced hyperhydricity in three commercial varieties of carnation. It seemes that hyperhydricity of shoots caused by oxidative stress. Saher et al (2005) showed that ethylene content, H_2O_2 production and antioxidative enzymes were higher in hyperhydric tissues of carnation. The percentage of hyperhydric shoots showed a significant decrease under bottom cooling condition (Saher et al., 2005).

Rooting of cutting is an essential process in horticultural mass production. Rhizogenesis controlled by external and internal factor. External factor are such as temperature, light and medium composition. Internal factor are such as endogenous production of PGR, carbohydrates as well as phenolic compounds (Podwyszynska, 2003). Root induction in all treatment was similar. However root regeneration in medium containing Kin and NAA was expanded. Interaction between Auxin and cytokinin induced root regeneration in carnation. Root formation in hormone-free medium was paralleled with Leshem et al. (1986).

High level of nitrogen and potassium as well as low content of them decreased shoot and root growth. A medium had low level of nitrogen, amonium and potassium. This medium was suitable for shoot proliferation and rooting. Shoots Growth depend on growth and development of root system. OM and MS medium had high content of nitrate, ammonium and potassium and inhibited root regeneration and subsequent shoot growth. In control treatment internodes number and shoot length were decreased because of mineral nutrition deficiency.

All plantlet were survived after transferring. Flower formation was achieved after 4 months. Nugent et al. (1991) reported flower formation was induced after 8 months. They indicated that flower was formed on short shoots and flower color was similar to mother plant such as our result. However Leshem (1986) indicated flower color of *in vitro* plantlet was different.

Table1. Shoot proliferation and rooting in different concentration of BAP and NAA

mgl^{-1}		code	Shoot proliferation			Root regeneration	
NAA	BAP		Shoot length	Shoot number	internodes number	Root length	Root number
0	0	A_1	4.26±0.6	3.81±0.74	6.01±0.89	2.5±0.54	3.01±0.63
0	0.5	A_2	1.42±0.37	1.44±0.48	1.85±0.74	-	-
0	1	A_3	2.10±0.37	4.21±0.74	3.41±0.48	-	-
0	2	A_4	1.82±0.24	2.60±0.8	2.60±0.48	-	-
0	3	A_5	1.74±0.24	3.60±0.48	2.86±0.47	-	-
0	4	A_6	1.93±0.2	2.87±0.74	2.47±0.8	-	-
0	5	A_7	0.52±0.31	1.21±0.74	1.08±0.63	-	-
0.1	0	A_8	2.60±0.2	2.8±0.4	4.23±0.74	2.8±0.6	3.27±0.74
0.1	0.5	A_9	1.5±0.31	4.62±0.48	3.09±0.63	2.7±0.6	2.6±1
0.1	1	A_{10}	1.08±0.31	2.26±1.1	1.89±0.74	2.25±0.24	1.24±0.4
0.1	2	A_{11}	1.24±0.24	2.61±1	2.61±0.8	-	-
0.1	3	A_{12}	0.21±0.24	0.41±0.48	0.44±0.48	-	-
0.1	4	A_{13}	0.14±0.2	0.27±0.4	0.21±0.4	-	-
0.1	5	A_{14}	-	-	-	-	-
0.5	0	A_{15}	2.73±0.24	2.69±0.8	3.22±0.74	6.47±0.73	9.27±0.74
0.5	0.5	A_{16}	1.71±0.24	2.81±0.47	2.85±0.47	0.89±0.24	1.49±0.48
0.5	1	A_{17}	0.74±0.24	1.23±0.4	1.29±0.4	-	-
0.5	2	A_{18}	1±0.31	2.65±0.48	1.81±0.74	-	-
0.5	3	A_{19}	0.83±0.24	2.42±1	1.02±0	-	-
0.5	4	A_{20}	0.72±0.24	2.4±0.8	1.22±0.4	-	-
0.5	5	A_{21}	0.3±0.24	0.61±0.48	0.62±0.48	-	-
1	0	A_{22}	2.62±0.21	2.85±0.74	3.2±0.9	6.31±0.6	9.24±0.48
1	0.5	A_{23}	1.8±0.24	3.82±0.4	2.63±0.8	0.72±0.24	2.12±0.89
1	1	A_{24}	1.92±0.37	4.09±0.63	2.61±0.48	-	-
1	2	A_{25}	1.7±0.24	3.8±0.74	2.29±0.4	-	-
1	3	A_{26}	0.54±0.31	0.87±0.4	1.05±0.63	-	-
1	4	A_{27}	0.44±0.37	0.65±0.48	0.82±0.74	-	-
1	5	A_{28}	0.25±0.24	0.6±0.8	0.42±0.48	-	-

Table 2. Shoot proliferation and rooting in different concentration of 2iP and NAA

mgl^{-1}		code	Shoot proliferation			Root regeneration	
NAA	2iP		Shoot length	Shoot number	internodes number	Root length	Root number
0	0	B_1	4.26±0.6	3.81±0.7	6.01±0.89	2.5±0.54	3.01±0.63
0	0.5	B_2	2.4±0.48	2.87±0.4	3.42±0.48	2.09±0.54	1.89±0.74
0	1	B_3	2.6±0.39	4.63±0.48	3.87±0.74	-	-
0	2	B_4	2.17±0.12	3.21±0.74	3.65±0.8	-	-
0	3	B_5	0.46±0.25	3.45±0.8	1±0.63	-	-
0	4	B_6	0.4±0.2	1.09±0.63	0.81±0.4	-	-
0	5	B_7	0.51±0.31	1.6±1.2	1.2±0.74	-	-
0.1	0	B_8	2.6±0.2	2.8±0.4	4.23±0.74	2.8±0.6	3.25±0.7
0.1	0.5	B_9	1.11±0.2	3.2±0.4	1.84±0.7	1.14±0.19	1.63±0.8
0.1	1	B_{10}	1.55±1	2.22±0.4	2.27±0.4	0.94±0.23	1.2±0.4
0.1	2	B_{11}	1.84±0.5	1.8±0.74	2.05±0.4	-	-
0.1	3	B_{12}	0.31±0.24	1.01±0.8	0.86±0.7	-	-
0.1	4	B_{13}	0.6±0.48	1.28±0.9	1.21±0.97	-	-
0.1	5	B_{14}	0.37±0.24	0.81±0.74	0.6±0.48	-	-
0.5	0	B_{15}	2.73±0.24	2.69±0.8	3.22±0.74	6.47±0.73	9.27±0.74
0.5	0.5	B_{16}	1.8±0.6	2.81±0.7	2.41±1.3	1.02±0.32	2.43±0.48
0.5	1	B_{17}	0.73±0.24	2.6±0.8	1.18±0.8	-	-
0.5	2	B_{18}	0.44±0.12	2.4±1	1.2±0.4	-	-
0.5	3	B_{19}	0.4±0.37	1.47±1.2	1±0.89	-	-
0.5	4	B_{20}	0.57±0.31	1.61±1.2	1.22±0.74	-	-
0.5	5	B_{21}	0.6±0.37	2.08±0.7	1.42±0.8	-	-
1	0	B_{22}	2.62±0.21	28.5±0.74	3.2±0.9	6.31±0.6	9.42±0.48
1	0.5	B_{23}	1.63±0.37	3.41±0.8	3±0.63	2.44±0.33	4.21±0.7
1	1	B_{24}	1.7±0.4	2.8±0.4	2.81±0.4	-	-
1	2	B_{25}	1±0.63	2.62±0.48	1.8±0.74	-	-
1	3	B_{26}	0.18±0.18	1.22±0.9	0.8±0.74	-	-
1	4	B_{27}	0.12±0.09	0.8±0.7	0.69±0.4	-	-
1	5	B_{28}	0.8±0.09	0.42±0.48	4.02±0.4	-	-

Table 3. Shoot proliferation and rooting in different concentration of Kin and NAA

mgl^{-1}		code	Shoot proliferation			Root regeneration	
NAA	Kin		Shoot length	Shoot number	internodes number	Root length	Root number
0	0	C_1	4.26±0.6	3.81±0.74	6.01±0.89	2.5±0.54	3.01±0.63
0	0.5	C_2	2.7±0.24	7.1±1.2	3.48±0.4	3.2±0.67	2.8±0.7
0	1	C_3	2.74±0.22	5.8±0.74	3.6±0.48	2.62±0.37	4.2±0.74
0	2	C_4	1.72±0.19	5.43±0.8	3.9±0.5	1.11±0.37	2.45±1.2
0	3	C_5	1.76±0.24	5.6±0.8	4.41±0.4	2.21±0.5	1.8±0.74
0	4	C_6	-	-	-	-	-
0	5	C_7	-	-	-	-	-
0.1	0	C_8	2.6±0.2	2.8±0.4	4.23±0.74	2.8±0.6	3.27±0.74
0.1	0.5	C_9	2.11±0.3	4.43±1	5.2±0.7	1±0.31	1.81±0.4
0.1	1	C_{10}	2.14±0.31	5.61±0.48	3.4±0.4	4.6±0.96	5.8±1.1
0.1	2	C_{11}	2.5±0.44	2.81±1.3	3.29±0.7	-	-
0.1	3	C_{12}	2.25±0.6	4.4±0.48	3±1.2	-	-
0.1	4	C_{13}	2.9±0.37	10.02±1	5±0.89	-	-
0.1	5	C_{14}	-	-	-	-	-
0.5	0	C_{15}	2.73±0.24	2.69±0.8	3.22±0.74	6.47±0.73	9.27±0.74
0.5	0.5	C_{16}	2.5±0.31	4.81±0.4	5.8±0.4	3.5±0.44	2.8±0.74
0.5	1	C_{17}	2.04±0.08	5±0.63	5.22±0.4	-	-
0.5	2	C_{18}	1.84±0.2	5.2±0.7	4.6±0.66	-	-
0.5	3	C_{19}	2±0.31	3.8±1.4	5.01±0.63	-	-
0.5	4	C_{20}	2.1±0.37	4.21±1.4	5.2±0.74	-	-
0.5	5	C_{21}	1.8±0.4	4.4±0.8	4.6±0.8	-	-
1	0	C_{22}	2.62±0.21	2.85±0.74	3.2±0.9	6.31±0.6	9.42±0.48
1	0.5	C_{23}	1.4±0.37	3±1	3.82±0.74	1.7±0.4	1.6±0.8
1	1	C_{24}	1.84±0.28	4.6±0.48	4.6±0.6	1.2±0.24	1.2±04
1	2	C_{25}	1.61±0.37	4.22±0.74	4.2±0.7	-	-
1	3	C_{26}	0.93±0.3	2.4±1.2	2.63±0.48	-	-
1	4	C_{27}	-	-	-	-	-
1	5	C_{28}	-	-	-	-	-

Table 4. Comparison of growth and development of shoot and root in different nutrient media

Nutrient solution	Shoot proliferation			Rooting	
	Shoot length (cm)	Shoot number	internodes number	Root length (cm)	Root number
An	10.11±0.9	9.6±0.48	24.8±1.6	4.7±0.7	8.2±0.74
OM	8.9±0.96	4.8±0.74	19.62±1.4	1.72±0.24	6.21±0.74
MS	8.82±0.87	5.23±0.74	18.81±2	1.52±0.3	5.4±0.48
MC	9.26±0.9	7.41±0.48	21.22±1.6	5.31±0.67	8.11±0.53
C	5.94±0.91	6.81±0.74	12.8±0.97	2.5±0.44	5.6±0.8

An: Anderson (1975) OM: Olive Medium MS: Murashige & Skoog (1962) MC: Misra & Chaturvedi (1984) C: Control

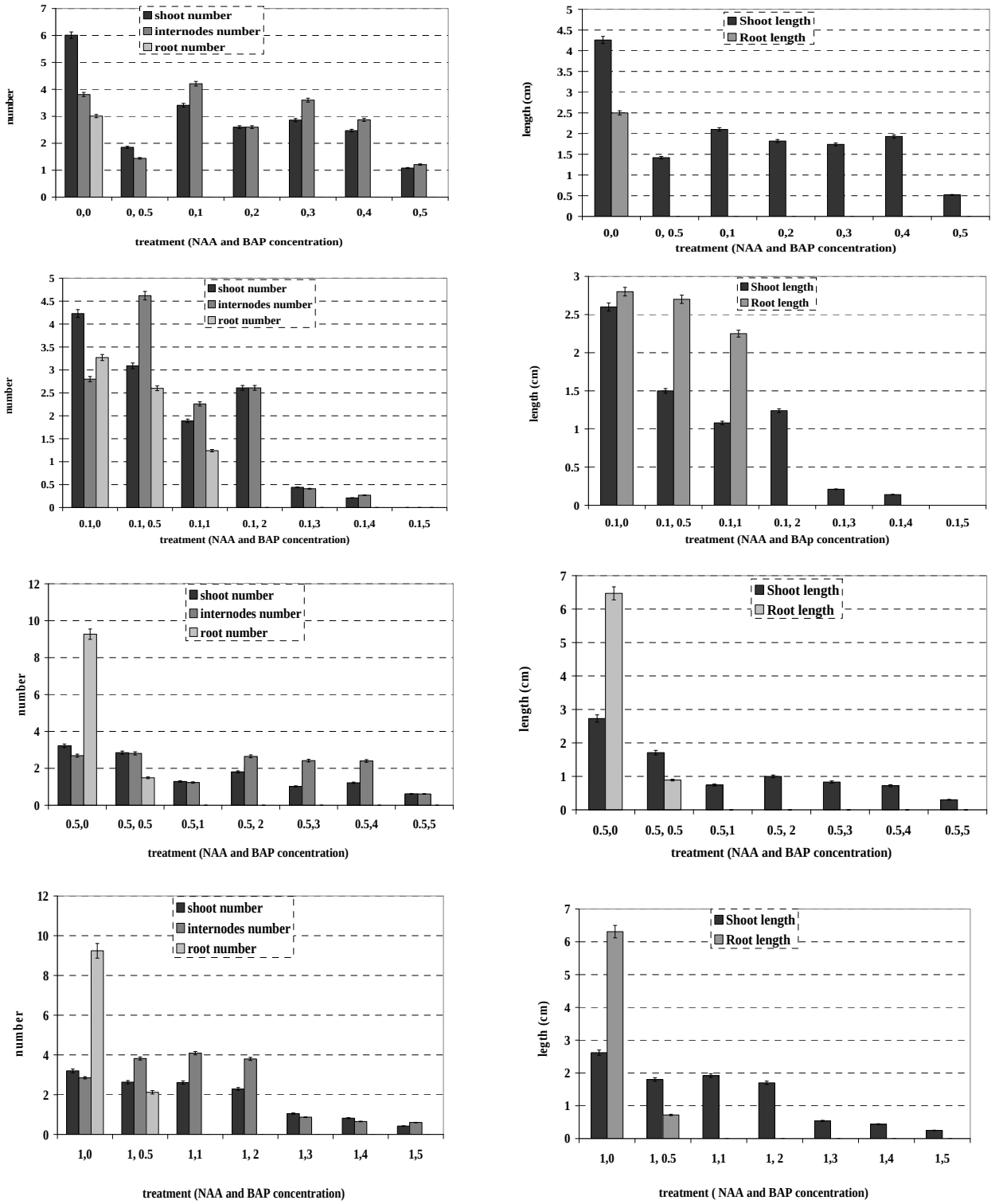

Figure1. Shoot proliferation and rooting in different concentration of BAP and NAA

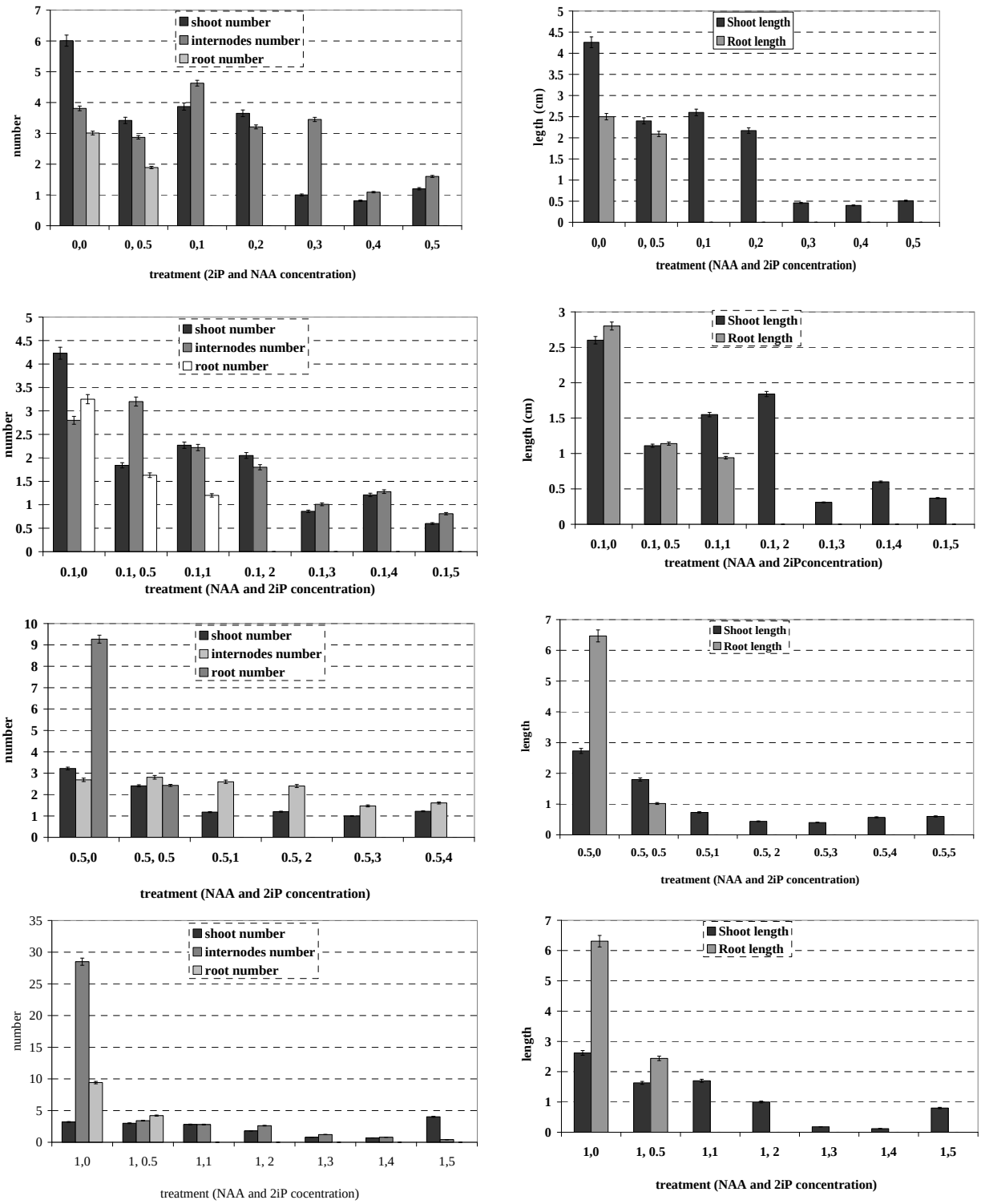

Figure 2. Shoot proliferation and rooting in different concentration of 2iP and NAA.

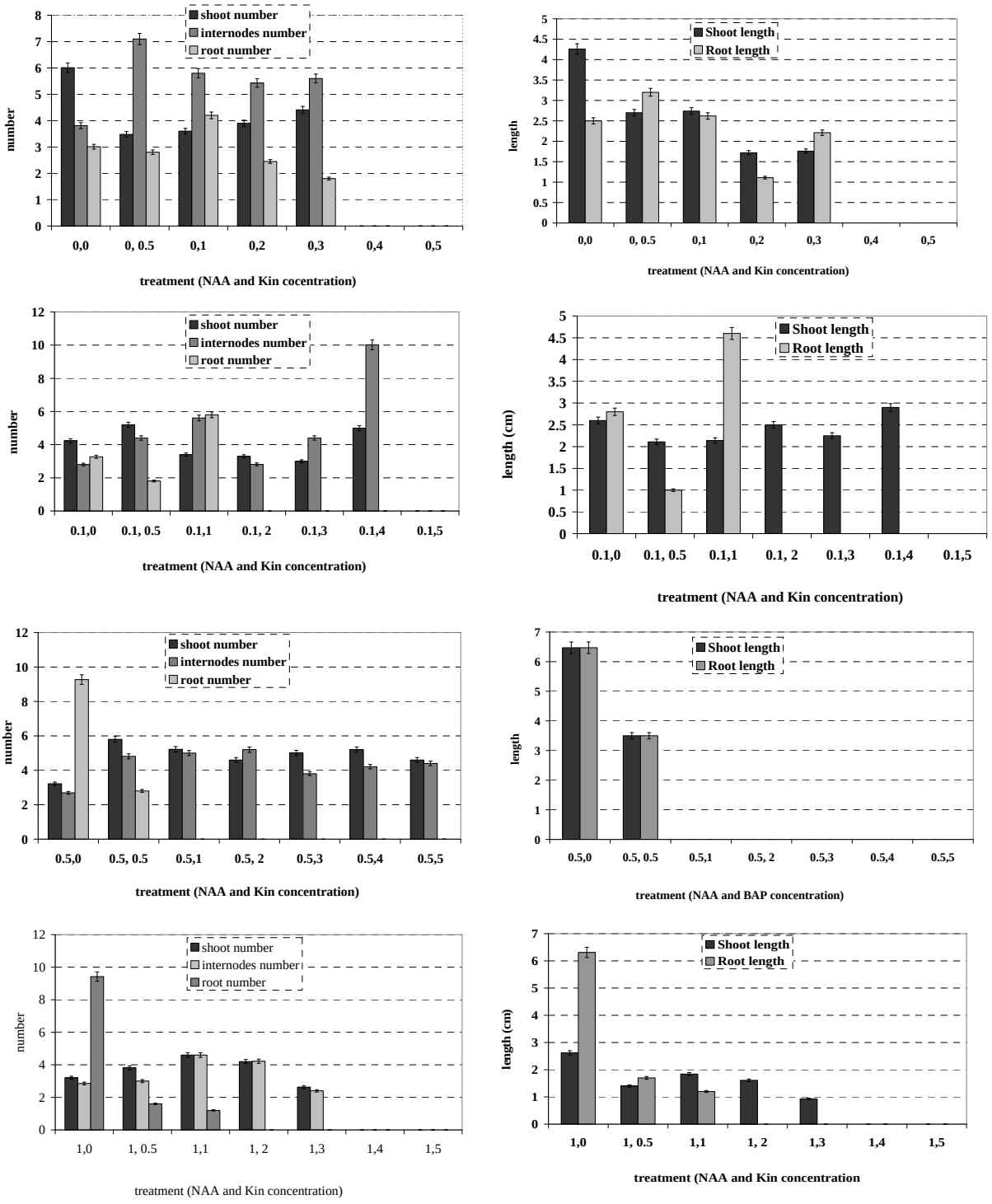

Figure 3. Shoot proliferation and rooting in different concentration of Kin and NAA.

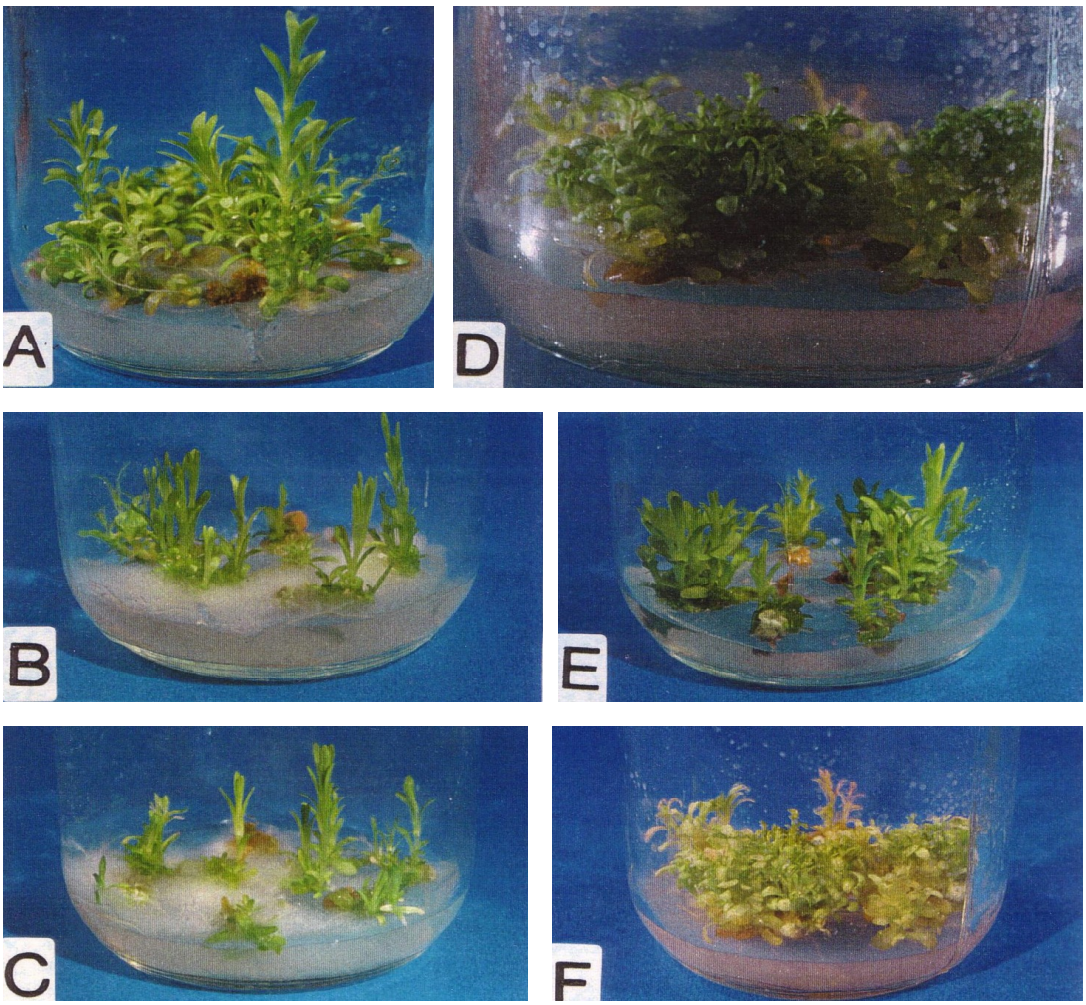

Figure 4. Shoot proliferation and rooting on media with different concentration of BAP, 2iP and kin combine with NAA. A: Shoot elongation in medium without PGR.B and C: Rooting of produced shoots in media of supplement with 0.5 and 1mgl^{-1} NAA respectively .D and E: Shoot proliferation media .F: Symptome of vitrification.

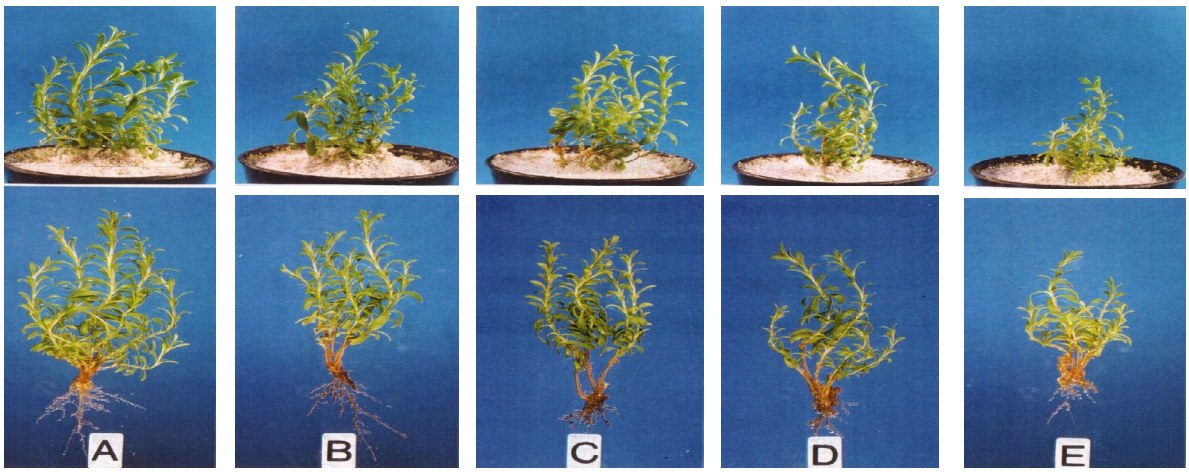

Figure 5. Comparison of growth and development of plantlet irrigated with different nutrient solution. A: Anderson medium, B: MC medium, C: OM medium, D: MS medium, E: Control.

Refrences

Anderson W C, (1975) Tissue culture propagation of Rhododendrons *in vitro*. 14: 334 (abs).

Casanova E, valdes AE, Fernandez B, Moysset L, Trillas, M I (2004) Levels and immunolocalization of endogenous cytokinins in thidiazuron–induced shoot organogenesis in carnation, Journal of Plant Physiology, 161 (1): 95-104.

Genkov T, Tsoneva P, Ivanova I, (1997) Effect of cytokinins on photosynthetic pigments and chlorophyllase activity in *in vitro* cultures of auxillary buds of *Dianthus caryophyllus* L. Journal of Plant growth regulation, 16 (3): 169-172.

Hammatt N, Grant NJ, (1997) Micropropagation of mature british wild cherry, Plant cell, Tissue and Organ Culture, 47: 103-110.

Fal MA, Majada JP, Gonzalez A, Sanches Tames R, (1999). Difference between *Dianthus caryophyllus* L. cultivar in in vitro growth and morphogenesis are related to their ethylene production. Plant growth regulation, 27(2): 131-136.

Jethwani V, Kothari S L, (1996) phenylacetic acid induced organogenesis in cultured leaf segments of *Dianthus chinensis*. Plant Cell Report, 15 (11): 869-872.

Leshem B, (1986) Carnation Plantlet from vitrified plants as a source of somaclonal variation. Hort Science, 21: 320-321.

Nontaswatsri C, Fukai S, Touma T, Goi M, (2002). Compartion adventitious shoot formation from node and leaf explants of various carnation *Dianthus caryophyllus* L. cultivars, The journal of horticultural science and biotechnology. 77(5): 520-525.

Nugent, G., Wardley-Richardson, T. and Lu, C. (1991) Plant regeneration from stem and petal of carnation (*Dianthus caryophyllus* L.), Plant Cell Report, 10(9):477-480.

Majada JP, Tadeo F, Fal M.A, Sanchez-Tames R, (2000) Impact of culture vessel ventilation on the anatomy and morphology of micropropagated carnation. Plant cell, Tissue and Organ Culture, 63 (3), 207-214.

Miller RM, Kaul V, Hutchinson JF, Richards D, (1991a) Adventitious shoot regeneration in carnation (*Dianthus caryophyllus* L.) from auxillary bud explants. Annals of Botany, 67(1): 33-42.

Miller RM, Kaul V, Hutchinson JF, Mahesvaran G, Richards D, (1991b) Shoot regeneration from fragmented flower buds of carnation (*Dianthus caryophyllus*). Annals of Botany, 68(6): 563-568.

Misra P, and Chaturvedi H C, (1984) Micropropagation of *Rosmarinus officinalis* L. Plant cell, Tissue and Organ Culture, 3: 163-168

Murashige T, and Skoog F, (1962) Revised medium for rapid growth and bioassay with tobacco tissue culture, Physiologia Plantarum,15: 473-479

Podwyszynska M, (2003) Cell Tissue and Organ culture (Rooting of micropropagated shoot). In: Roberts AV, Debener T, and Gudin S, Encyclopedia of Rose science. Elsevier Press.

Pospislova J, Ticha I, Kadlecek P, Haisel D, (1999) Acclimatization of micropropagated plants to *ex vitro* condition. Biologia Plantarum, 42(4): 481-497.

Saher S, Piqeras A, Hellin E, Olmos E, (2004) Hyperhhydricity in micropropagated carnation shoots: the role of oxidative stress. Physiologia Plantarum, 120 (1); 152-161.

Saher S, Piqeras A, Hellin E, Olmos E, (2005) Prevention of hyperhydricity in micropropagated carnation shoots by bottom cooling : implications of oxidative stress, Plant cell, Tissue and Organ Culture, 81(2): 149-158.

Yadav MK, Gaur AK, Garg GK, (2003) Development of suitable protocol to overcome hyperhydricityin carnation during micropropagation, Plant cell, Tissue and Organ Culture, 72(2): 153-156.

کشت در شیشه میخک و خوگیری گیاهچه در شرایط خارج از شیشه

قربانلی، م.[۱]، نوری کوتنانی، ف.[۱]، الله‌وردی ممقانی، ب.[۲]

۱. گروه زیست‌شناسی دانشگاه آزاد اسلامی واحد تهران شمال، ایران
۲. مرکز تحقیقات جنگل‌ها و مراتع، تهران، ایران

چکیده

Dianthus caryophyllus یکی از مهمترین گونه‌های جنس میخک است. برای تکثیر گیاهان بدون ویروس کشت در شیشه این گیاه، واریته سوریز روآیالت مورد مطالعه قرار گرفته است. جداکشت‌ها در محیط کشتی که محتوی ۱ میلی‌گرم در لیتر ٦-بنزیل آمینو پورین بود، کشت داده شوند. ٦-بنزیل آمینو بورین به تنهایی در جداکشت‌های گره و بین گره کالوس القا کرد. برای بدست آوردن اندام‌زایی، کالوس‌ها در محیط اندرسون با، یا بدون تنظیم کننده رشد، واکشت گردیدند. بیشترین طول اندام هوایی در محیط بدون هرگونه تنظیم کننده رشد بدست آمد. بیشترین تولید شاخه‌ها در محیط اندرسون با مکمل ٤ میلی‌گرم در لیتر کینتین و ۰/۱ میلی‌گرم در لیتر نفتالن استیک اسید بدست آمد. پیدایش ریشه در محیط با ۰/۵ و ۱ میلی‌گرم در لیتر نفتالن استیک اسید مشاهده شد. گیاهچه‌های ریشه‌دار شده به داخل گلدان دارای پرلیت منتقل و بوسیله محلول‌های غذایی مختلف آبیاری شدند. اثر محلول‌های غذایی (MC, MS, OM, An) مختلف بر رشد و نمو گیاهچه‌ها مقایسه شد. نتایج نشان داد که محیط اندرسون نسبت به سایر محیط‌ها موثرتر بود. گیاهان سازگار شده به خاک منتقل شدند. تمام آنها در شرایط مزرعه به زندگی ادامه داده و پس از ٤ ماه گل دادند.

واژه‌های کلیدی: تنظیم کننده‌های رشد گیاه، ریز ازدیادی، محلول غذایی، *Dianthus caryophyllus* واریته سوریز روآیالت