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Full Length Research Paper

Can a general shoot proliferation and rooting medium be used for a number of carnation cultivars?

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This investigation was conducted to find the best shoot proliferation and rooting media for 13 virus-free cultivars of carnation (*Dianthus caryophyllus* L.) recently imported from The Netherlands to Iran. The best shoot proliferation media were Murashige and Skoog (MS) containing 3 mgL^{-1} (13.95 μM) kinetin and 0.5 mgL^{-1} (2.69 μM) NAA or 1 mgL^{-1} (4.44 μM) BA and 1 mgL^{-1} (5.37 μM) NAA. Average of shoot numbers produced in the establishment media was 2-3, which increased to 30 or higher with some cultivars in subsequent subcultures. For rooting, the in vitro shoots were cultured on the best MS rooting media which is discussed. Transferring to a mixture of sand, leaf-mold and vermiculite (1:1:1, v/v/v) successfully acclimatized the plantlets. It can be concluded that using a general medium for shoot proliferation of used carnation cultivars is possible, but for rooting it is impossible.

Key words: culture medium, *Dianthus caryophyllus* L., in vitro culture, micropropagation.

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is a member of Caryophyllaceae and a native of the Mediterranean area (Dole and Wilkins, 1999). This plant is one of the world's most popular, economic and important cut flowers due to perpetual flowering (Mii et al., 1990) and presence of new single- and multi-color cultivars (Dole and Wilkins, 1999). Carnations are divided to two groups, standard and miniature (spray), that produce in each stem, one large terminal flower and several smaller lateral flowers, respectively (Dole and Wilkins, 1999).

Carnation cultivars have high heterozygosity and must be vegetatively propagated (Mii et al., 1990). Generally, one or more viruses (Bierly, 1964; Bierly and Smith, 1957; Kassanin, 1955; Ram and Zaidi, 1999) or bacteria and fungi (Forsberg, 1963; Holley and Baker, 1963; Thakur et al., 2002) infect stem cuttings of this plant. Tissue culture and micropropagation techniques can aid us in producing uniform and pathogen-free plants suitable for export to other countries around the world. Micropropagation methods of this plant have been reviewed (Mii et al., 1990) and some other reports are also available (Choudhury and Garg, 1999; Cuzzuol et al., 1996; Ilahi et al., 1995; Jagannatha et al., 2001; Kallak et al., 1996; Watad et al., 1996). In these studies, various cultivars are used, and although most of them are established on Murashige and Skoog (1962) medium (MS), but with different growth regulators or different concentrations.

The main purpose of this study was to investigate the possibility of using a general medium for shoot proliferation and rooting of a number of carnation cultivars.

MATERIALS AND METHODS

Plant materials

Ident virus-free plants of 13 carnation cultivars recently imported from The Netherlands included a) Standards: 1. 'Tempo', 2. 'Alvin', 3. 'Leila', 4. 'Miledy', 5. 'Venezia', 6. 'Flipper Light', 7. 'Pink Castellaro' and 8. 'Voltaire'; and b) Miniatures: 9. 'Lunetta', 10. 'Rotem', 11. 'Yellow Rotem', 12. 'Graziella' and 13. 'Coral Graziella'.

Abbreviations: BA, N^6–benzyladenine; IBA, indole-3-butyric acid; kinetin, 6-furfurylaminopurine; MS, Murashige and Skoog medium; NAA, α-naphthaleneacetic acid.

Forty plants per cultivar were used in this study. These were purchased from a commercial greenhouse in Tehran province and transported to the greenhouse of Department of Horticultural Science, Shiraz University, Shiraz, Iran.

Based on preliminary experiments, single-node and shoot-tip explants were used for further investigations.

Surface sterilization

Shoot explants of about 10 cm length, after defoliation were prewashed in water supplemented with 0.2% of a weak household detergent solution ('Rika') for 10 min and then placed under running tap water for 1 h. Disinfestation was done using 70% ethanol for 0.5 or 1 min and 10% chlorox (containing 5.25% sodium hypochlorite) for 5, 10, 15, 20, 25 or 30 min. The explants were then rinsed three times with sterilized distilled water.

Culture

Single-node or shoot-tip explants with about 0.5 cm length were cultured on the basic MS medium supplemented with 30 gL^{-1} sucrose and 8 gL^{-1} agar and various combinations of plant growth regulators (see below). The pH of media was adjusted to 5.7-5.8 with 0.1 N NaOH before autoclaving for 15 min at 121°C and 1.5 kg cm^{-2} pressure. 20 ml of each medium was poured into 50 ml flasks. One explant was cultured in each flask. Sometimes, several explants have been cultured in one flask for high multiplication rate.

In preliminary experiments, a combination of 0, 1, 2 or 3 mgL^{-1} (0.00, 4.65, 9.30 or 13.95 μM) kinetin and 0.0, 0.5 or 1.0 mgL^{-1} (0. 00, 2.69 or 5.37 μM) NAA or 0, 1, 2 or 3 mgL^{-1} (0.00, 4.44, 8.88 or 13.32 μ M) BA and 0, 1, 2 or 3 mgL^{-1} (0.00, 5.37, 10.74 or 16.11 μM) NAA were used for establishment (medium that used for selecting the healthy and vigorous explants for further subcultures) and shoot proliferation media. Further investigations were conducted on a medium with 1 mgL^{-1} BA (4.44 μM) and NAA (5.37 μM), through fifth subculture, with 4 weeks intervals.

In rooting experiments, in vitro shoots were cultured on MS media supplemented with 0.0, 0.5, 1.0, 1.5 or 2.0 mgL^{-1} of NAA (0.00, 2.69, 5.37, 8.05 or 10.74 μM) or IBA (0.0, 2.47, 4.93, 7.40 or 9.86 μ M). All the plant growth regulators were purchased from Sigma Co.Cultures were kept under a 16 h photoperiod, 30 mmolm-2s-1, provided by cool-white fluorescent lamps at 25 ± 2°C.

Acclimatization

Rooted plantlets were transferred to a pasteurized soil mixture consisted of sand, leaf-mold and vermiculite (1:1:1, v/v/v) for acclimatization. Acclimatized plants were transferred to greenhouse after 3 weeks.

Data recording and analysis

Number of shoots produced on establishment and shoot proliferation media (with 4 weeks intervals) and rooting percentage, mean root number and length in each plantlet were recorded 4 weeks after culture. All the experiments were conducted in a completely randomized design (in the case of subcultures, with factorial arrangement) with about 30 replicates in each treatment, and repeated at least twice. Data represented the percentage were analyzed after appropriate transformation [ArcSin Sqrt(X/100)]. Data were statistically analyzed by one-way ANOVA (in the case of subcultures, by FACTOR analysis) in MSTATC program and the means were compared using Duncan's multiple range test (DMRT) at 1% level.

RESULTS AND DISCUSSION

Surface sterilization

The best disinfestation treatment was using the 70% ethanol for 1 min and then 10% chlorox for 25 min (data are not shown).

Establishment

Based on preliminary experiments the best establishment media for all cultivars were as follows:

a. 3 mgL^{-1} (13.95 μM) kinetin with 0.5 mgL^{-1} (2.69 μM) NAA.
b. 1 mgL^{-1} BA (4.44 μM) with 1 mgL^{-1} NAA (5.37 μM).

The mean shoot numbers were 1.75 and 2.67 for a and b, respectively (data are not shown). Thus, the second medium was used for further investigations and subcultures. These results are similar to some reports (Mii et al., 1990) but in contrast to others (Hemple, 1979; Kozak and Hemple, 1979; Petru and Landa, 1974) in which the high concentration of cytokinins with low concentration of auxins were the best treatments for shoot proliferation. It may be due to different cultural conditions or growth regulator types.

Cultivars

Comparison between different cultivars due to mean shoot proliferation in five subcultures is shown in Table 1. 'Rotem' and 'Lunetta' cultivars had the highest and lowest shoot proliferation, respectively. This is in accordance with Kallak et al. (1996) results. Because both the highest and lowest shoot proliferation rates are observed in miniature cultivars (Table 1), it can not be concluded that a relation is existing between two types of cultivars and shoot proliferation rates.

Subcultures

Comparison between mean shoot proliferation rate of all the cultured cultivars in different subcultures is shown in Table 1. The lowest and highest shoot proliferation rates were observed in first and second (Figure 1) subcultures, respectively, and gradually decreased in subsequent subcultures (Table 1). There is no available report on comparison between different subcultures in carnation but in accordance with results of a similar research in miniature roses (Salehi and Khosh-Khui, 1996), the gradual reduction in shoot proliferation in subsequent

Table 1. Interaction between different cultivars of carnation and subcultures due to shoot proliferation rates [on the medium with 1 mgL^{-1} of BA (4.44 μm) and NAA (5.37 μm)] and comparison between total means of cultivars and subculture stages.

Cultivars	Shoot proliferation rates					
	Subcultures					
	First	Second	Third	Fourth	Fifth	Total mean
'Tempo'	2.2ijk	8.0c	5.0fgh	5.2efg	4.5gh	5.0C
'Alvin'	1.5jkl	5.0fgh	3.0ia	3.0ia	2.0ijkl	2.9E
'Leila'	2.5ij	7.5c	6.0de	5.0fgh	3.0ia	4.8C
'Miledy'	1.0l	4.5gh	3.0ia	3.0ia	3.0ia	2.9E
'Venezia'	1.2kl	5.0fgh	4.0h	2.0ijkl	2.0ijkl	2.8E
'Flipper Light'	1.2kl	3.0l	2.0ijkl	1.0l	1.0l	1.6F
'Pink Castellaro'	1.5jkl	5.0fgh	5.0fgh	4.0h	3.0ia	3.7D
'Voltaire'	1.5jkl	9.5b	8.0c	8.5c	6.0de	6.7B
'Lunetta'	1.0l	1.0l	1.0l	1.0l	1.0l	1.0G
'Rotem'	1.2kl	15.0a	10.0b	8.0c	6.5d	8.1A
'Yellow Rotem'	2.0ijkl	8.0c	6.0def	5.0fgh	3.0ia	4.8C
'Graziella'	3.0ia	5.0fgh	3.0ia	3.0ia	2.0ijkl	3.2E
'Coral Graziella'	2.0ijkl	10.0b	5.0fgh	4.0h	3.0ia	4.8C
Total mean	1.7E	6.6A	4.7B	4.1C	3.1D	

Means followed by the same letters (small letters for means and capital letters for total means) are not significantly different according to DMRT at 1% level.

Figure 1. High shoot proliferation in 'Rotem' at second subculture. Left: bottom of flask view, Right: front view.

gradual reduction in shoot proliferation in subsequent subcultures may be due to using the more terminally buds with too matured tissues. Further investigation may be necessary for determining the best media for each subculture stage.

Table 2. The best MS rooting media selected for each cultivar of carnation.

Cultivars	Rooting media
'Tempo'	2.0 mgL^{-1} (9.86 μM) IBA
'Alvin'	2.0 mgL^{-1} (10.74 μM) NAA
'Leila'	1.0 mgL^{-1} (5.37 μM) NAA
'Miledy'	growth regulator-free
'Venezia'	1.5 mgL^{-1} (7.40 μM) IBA
'Flipper Light'	1.5 mgL^{-1} (7.40 μM) IBA
'Pink Castellaro'	1.5 mgL^{-1} (7.40 μM) IBA
'Voltaire'	2.0 mgL^{-1} (9.86 μM) IBA
'Lunetta'	2.0 mgL^{-1} (10.74 μM) NAA
'Rotem'	1.0 mgL^{-1} (5.37 μM) NAA
'Yellow Rotem'	1.0 mgL^{-1} (5.37 μM) NAA
'Graziella'	without rooting
'Coral Graziella'	1.5 mgL^{-1} (7.40 μM) IBA

Table 3. Comparison between different cultivars cultured on suitable rooting media (see Table 2), due to rooting factors.

Cultivars	Rooting percentage (rounded)	Mean root number per plantlet	Mean root length per plantlet (cm)
'Tempo'	100a	7.0c	2.25abc
'Alvin'	50b	4.0e	2.00abcd
'Leila'	100a	4.0e	2.50ab
'Miledy'	100a	4.0e	1.25cd
'Venezia'	50b	8.0b	1.50bcd
'Flipper Light'	50b	7.0c	2.00abcd
'Pink Castellaro'	50b	9.0a	1.00d
'Voltaire'	100a	4.0e	2.75a
'Lunetta'	50b	3.0f	3.00a
'Rotem'	100a	5.5d	2.50ab
'Yellow Rotem'	100a	3.0f	2.75a
'Graziella'	0c	-	-
'Coral Graziella'	100a	6.5c	2.50ab

In each column, means followed by the same letters are not significantly different according to DMRT at 1% level.

Interaction of different cultivars and subcultures

Interaction between different cultivars and subcultures due to shoot proliferation rate are presented in Table 1. 'Rotem' at second subculture and 'Lunetta' at all the subcultures had the highest and lowest shoot proliferation rates, respectively (Table 1). These findings confirmed the different reactions of various cultivars of carnation to culture medium at different subcultures. The total means shoot proliferation at different subcultures with standard cultivars was 3.81 and with miniature cultivars, was 4.43. This may be due to interaction effect of cultivar × subculture stage, because in cultivar effect, singly, there was no relation between two types of cultivars and their shoot proliferation rates.

Rooting and acclimatization

Due to high variability observed between different cultivars for rooting medium and rooting percentage, the best media were selected for each cultivar (Table 2).

There are several reports (e.g., Ilahi et al., 1995; Mii et al., 1990) on suitability of growth regulator-free medium for rooting of carnation cultivars. But in this research, similar to results of Cuzzuol et al. (1996) and Jagannatha et al. (2001), except for 'Miledy', growth regulator-free medium did not result in good rooting percentage, may be due to genotype differences.

'Graziella' had no rooting in all the media used, which need further investigations. With other cultivars, rooting percentage ranged from 50 to 100 (Table 3). The highest

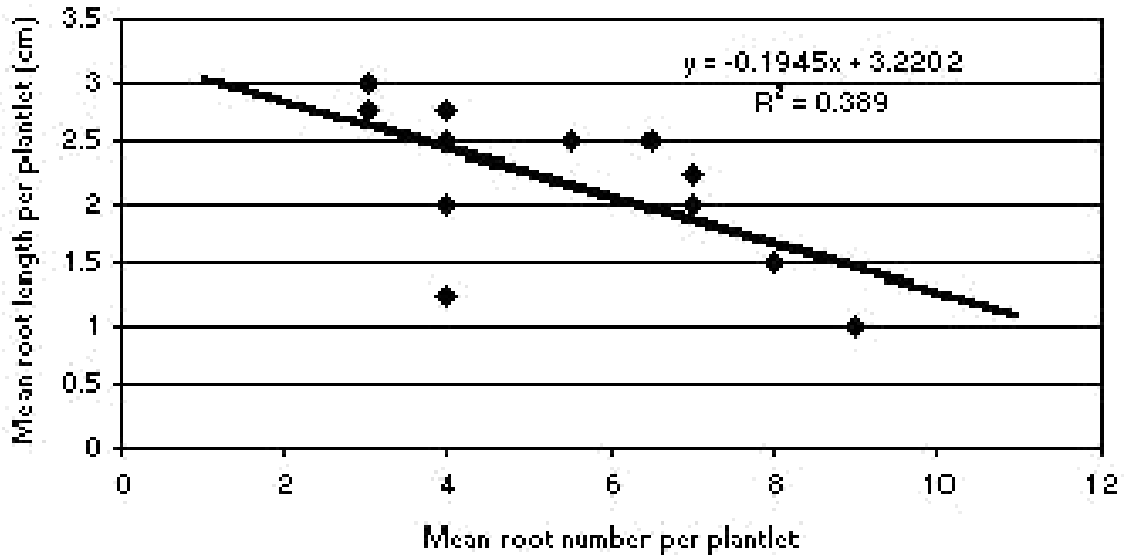

Figure 2. Mean root length as a function of mean root number per plantlet (averaged over the cultivars that respond to the rooting media).

and lowest mean root number per plantlet were produced by 'Pink Castellaro' and both 'Lunetta' and 'Yellow Rotem', respectively (Table 3). It is shown that plantlets with the higher root numbers have the lower root lengths (Figure 2). In this regard, there is no available report on carnation but is similar to results obtained with miniature roses (Salehi and Khosh-Khui, 1996). Plantlets were successfully acclimatized and transferred to greenhouse, with a 90% success.

Conclusion

It can be concluded that using the MS medium supplemented with 1 mgL^{-1} BA (4.44 μM) and NAA (5.37 μM) for shoot proliferation of used carnation cultivars is possible. In this medium, the used cultivars produced about 1 to 8 shoots in their subcultures, with a mean of about 4 shoots. This general medium can be used easily for commercial multiplication of virus-free carnation plants. According to the results, rooting medium in used carnation cultivars is highly genotype dependent and can not recommend a general medium for rooting all of them.

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REFERENCES

Bierly P (1964). Effects of four viruses on yield and quality of 'King Cardinal' carnations. Plant Dis. Rep. 48: 5-7.
Bierly P, Smith FF (1957). Carnation viruses in the United States. Phytopathol. 47: 714-721.
Choudhury PR, Garg GK (1999). In vitro shoot proliferation and elongation in carnation. J. Ornament. Hort. (New Series) 2: 71-73.
Cuzzuol GRF, Gallo LA, Crocomo OJ (1996). Rooting of carnation (*Dianthus caryophyllus* L.) in vitro and ex vitro. Sci. Agric. 53: 60-66.
Dole JM, Wilkins HF (1999). Floriculture, Principles and Species. Prentice Hall Inc., New Jersey, USA.
Forsberg JL (1963). Diseases of Ornamental Plants. Special Pub. No. 3, Univ. Illinois, USA.
Hemple M (1979). Studies on in vitro multiplication of carnations. I. The influence of some cytokinins on the differentiation of shoot apices. Acta Hort. 91: 317-321.
Holley WD, Baker R (1963). Carnation Production. WC Brown, Dobuque, Iowa, USA.
Ilahi I, Aziz F, Jabeen M (1995). Tissue culture studies for micropropagation of carnation (*Dianthus caryophyllus* L.). Pakistan J. Bot. 27: 411-415.
Jagannatha J, Ashok TH, Sathyanarayana BN (2001). In vitro propagation in carnation cultivars (*Dianthus caryophyllus* L.). J. Plant Biol. 28: 99-103.
Kallak H, Hilpus I, Virumae K (1996). Influence of genotype and growth regulators on morphogenetic processes in carnation shoot apex culture. Sci. Hort. 65: 181-189.
Kassanin B (1955). Some properties of four viruses isolated from carnation plants. Annu. Appl. Biol. 43: 103-113.

Kozak D, Hempel M (1979). Studies on in vitro multiplication of carnations. III. The optimalization of multiplantlets formation. Acta Hort. 91: 333-337.
Mii M, Buiatti M, Gimelli F (1990). Carnation. In: Ammirato PV, Evans DA, Sharp WR, Bajaj YPS (Eds.), Handbook of Plant Cell Culture. McGraw-Hill Pub. Co., New York, USA pp. 284-318.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant 15: 473-497.
Petru E, Landa Z (1974). Organogenesis in isolated carnation plant callus tissue cultivated in vitro. Biol. Plant. 16: 450-453.
Ram R, Zaidi AA (1999). Meristem tip culture and carnation vein mottle virus tested perpetual flowering carnations. Indian J. Virol. 15: 43-46.
Salehi H, Khosh-Khui M (1996). Micropropagation of miniature rose cultivars. Iran Agric. Res. 15: 51-67.
Thakur M, Sharma DR, Sharma SK (2002). In vitro selection and regeneration of carnation (*Dianthus caryophyllus* L.) plants resistant to culture filtrate of *Fusarium oxysporum f.sp.* dianthi. Plant Cell Rep. 20: 825-828.
Watad AA, Zuker A, Shejtman H, Nissim A, Vainstein A (1996). Adventitious shoot formation from carnation stem segments: a comparison of different culture procedures. Sci. Hort. 65: 313-320.