Journal of the Arkansas Academy of Science

Volume 49 Article 7

1995

Effect of Thidiazuron and Benzylaminopurine on In Vitro Shoot Proliferation of Carnation (Dianthus caryophyllus L.)

Mohanjeet S. Brar
University of Arkansas, Fayetteville

Jameel M. Al-Khayri
University of Arkansas, Fayetteville

Gerald L. Klingaman
University of Arkansas, Fayetteville

Effect of Thidiazuron and Benzylaminopurine on In Vitro Shoot Proliferation of Carnation (*Dianthus caryophyllus* L.)

Mohanjeet S. Brar, Jameel M. Al-Khayri and Gerald L. Klingaman
Department of Horticulture
University of Arkansas
Fayetteville, AR 72701

Abstract

Carnations (*Dianthus caryophyllus* L.) are among the most widely used cut flowers in the world. Tissue culture techniques offer an efficient method for the micropropagation of carnations. This study was conducted to test the effect of thidiazuron (TDZ) and benzylaminopurine (BAP), artificial cytokinins, on shoot multiplication of two carnation cultivars, Barlo II Nora and Raggio di Sole. Isolated axillary buds were cultured on Gamborg's (B-5) basal medium supplemented with 30 g/L sucrose and 8 g/L agar. The cultures were maintained at a 10-h photoperiod (40 $\mu Em^{-2}s^{-1}$) and 23°C±2C°. Number of multiple shoots produced was dependent upon the genotype and was also influenced by the cytokinin type and concentration. Barlo II Nora produced the highest shoot number with 14 shoots per explant on a medium containing 20 mg/L BAP. The cultivar Raggio di Sole cultured on BAP-containing media produced a maximum of 4 shoots per explant. Barlo II Nora cultured on TDZ-containing media produced a maximum of 8 shoots per explant, however, large amounts of calli were associated with these shoots. Increasing the concentration of cytokinin was associated with an increase in shoot number and a decrease in shoot height. Shoots were rooted on Gamborg's medium containing 2 mg/L of 3-indole-butyric acid (IBA) and then transferred to pots. Once acclimatized the carnations were transferred to a greenhouse where they exhibited normal growth. This method could be useful for the rapid propagation of carnations in commercial production.

Introduction

Carnations (*Dianthus caryophyllus* L.) are among the three most important cut flowers in the world. Tissue culture of carnation has progressed rapidly from the first application as a means of virus elimination to its current extensive use in micropropagation. Adventitious shoot regeneration of carnation has been achieved with many different explants varying from hypocotyls (Petru and Landa, 1974), petals (Gimelli et al., 1984; and Frey and Janick, 1991), ovules (Demmink et al., 1987), anthers (Villalobos, 1981), leaf (Altvorst et al., 1992), nodal stems (Roest and Bokelmann, 1981), axillary buds (Choudhary, 1991; Miller et al., 1991), to shoot tips (Johnson, 1980).

Axillary bud culture can be used for the clonal multiplication of carnations since there is no callus phase, and therefore, the shoots that develop are genetically identical to the parent (Broertjes and Keen, 1980). This technique can also be used for crop improvement through *Agrobacterium*-mediated transformations since the lack of a callus phase reduces the chance of somaclonal variation (Altvorst et al., 1992). Explant, culture environment, plant genotype, and hormonal type and concentration affect the regeneration capacity of a plant, with the parental genotype potentially exerting the greatest influence (Gimelli et al., 1984). The intent of this study was to evaluate the effectiveness of axillary bud explants in the micropropagation of two carnation cultivars which have not been previously reported.

The objectives of this experiment were to: 1) test the effect of thidiazuron (TDZ) and benzylaminopurine (BAP) on shoot multiplication, 2) examine the genotypic response of cultivars Raggio Di Sole and Barlo II Nora to shoot multiplication treatments, and 3) induce the rooting of regenerated shoots and the establishment of plants in soil.

Materials and Methods

Disinfection of plant material.--This study was conducted with rooted cuttings obtained from California Florida Plant Co. (P.O. Box 5310 Salinas, CA 93915). Roots and leaves were removed and the stems were washed thoroughly with tap water. The plant material was then washed in a diluted soap solution (5 drops liquid soap/L of water) and surface-sterilized for 30 s in 70% ethanol followed by immersion for 20 minutes in 20% vol/vol Clorox (commercial bleach) containing 3 drops of Tween 20, a detergent, (Sigma Chemical Company, St. Louis, MO) per 100 ml Clorox solution. The plants were then rinsed 4 times with sterile distilled water. The axillary buds were aseptically removed (Fig. 1) and cultured onto 16 x 100-mm culture tubes (5ml/tube) with the basal

end inserted into the medium.

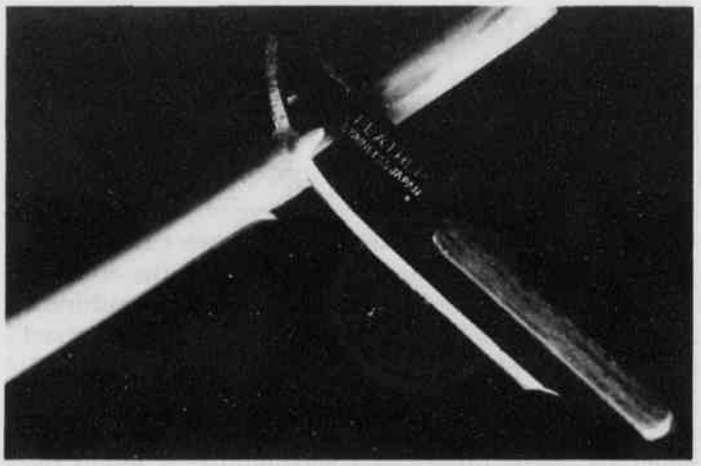

Fig. 1. Carnation axillary bud being removed in preparation for culturing.

Culture medium and conditions.--The culture medium contained Gamborg's salt (Gamborg et al., 1968) augmented with 70 mg/L myo-inositol, 50 mg/L casein hydrolysate, 0.1 mg/L d-pantothenic acid, 1 mg/L nicotinic acid, 1 mg/L pyridoxine-HCI, 1 mg/L thiamine-HCI, 2 mg/L gycine, 30 g/L sucrose, and 8 g/L tissue culture grade agar [Agar-agar/Gum agar](Sigma). The pH of the medium was adjusted to 5.7 with 1 N KOH and 1 N HCI. To test the in vitro response of the cultivar Raggio di Sole, the medium was supplemented with one cytokinin (BAP) at 2.5, 5, 10, 15, and 20 mg/L. To test the in vitro response of the cultivar Barlo II Nora, the medium was supplemented with the same concentrations of BAP or TDZ at 0.01, 0.05, 0.5, and 1 mg/L. The media was autoclaved at 121°C and 1 x 10^5 Pa (10.8 N/cm^2) for 15 min.

Cultures were maintained at 23°C±2C° under a 10-h photoperiod of cool-white fluorescent light (40 $\mu Em^{-2}s^{-1}$). Six weeks after culture initation, data were taken on number of explants that exhibited multiplication, number of shoots per explant and shoot length. Observations on callus formation and shoot vitrification were also made.

Plant establishment.--Regenerated shoots were rooted in GA-7 Magenta vessels (Magenta Corp., Chicago, IL) containing 50 ml of rooting medium which consisted of the same medium used in multiplication, except the cytokinins were replaced with the addition of 2 mg/L of IBA. After 3 weeks in culture, the plantlets were removed from the culture vessels and the roots washed to remove the agar. Plantlets were transplanted into pots containing a potting mix (Redi-Earth Peat-Lite Mix, Grace-Sierra Hort. Products Co., Milpitas, CA), then misted and covered with clear plastic containers to maintain high humidity. The misting and cover was gradually reduced to acclimatize the plants to the ambient atmosphere. The plantlets were maintained under cool-white fluorescent light for 3 weeks after which they were transferred to a greenhouse.

Results and Discussion

The explants began to enlarge within 24 h of culturing. After 4 to 5 days shoot growth was observed from the buds and, in six weeks, multiple shoots developed (Fig. 2). The percent shoot multiplication for Barlo II Nora was 100 percent at all BAP levels. This cultivar showed increases in average shoot number with increased concentrations of BAP (Table 1). Levels of BAP higher than 20 mg/L may further promote shoot multiplication and merits further study. There seems to be a negative correlation between BAP concentration and shoot length (Table 1). This is in accordance with the findings of Sankhla et al., (1994) with silktree shoots.

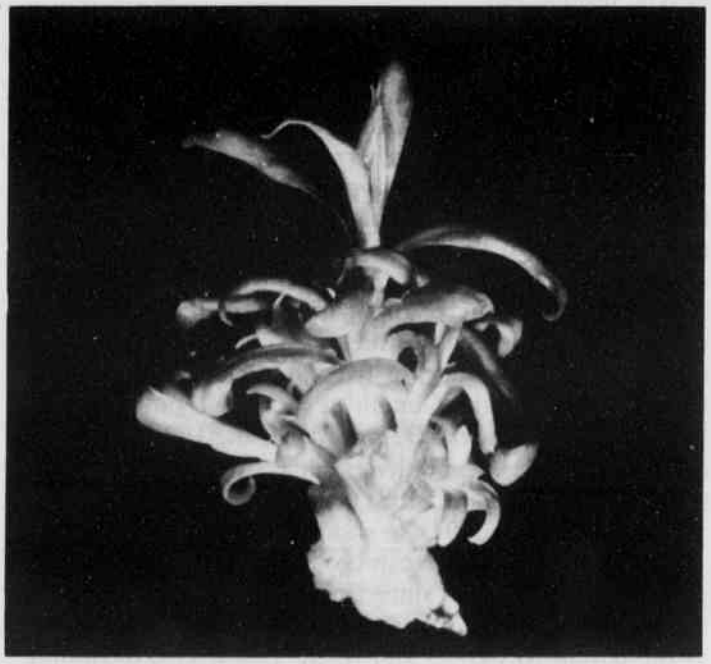

Fig. 2. Shoot multiplication from axillary bud of carnation.

Callus formation was observed at the base of the explants. The percent of explants that produced calli varied from 70 to 90 percent. The presence of a callus is not desirable in shoot multiplication and, therefore, treatments that produce minimal or no callus are preferred. The calli produced on BAP-containing media (2.5 to 1.5

mg/L) were approximately 3 mm or less in size and increased to 3 to 6 mm as the concentration increased to 20 mg/L (Table 1). Callus production, however, did not interfere with the shoot multiplication.

Table 1. Effects of BAP on shoot multiplication and callus formation in the carnation cultivar Barlo II Nora.

	Shoot Multiplication			Callus Formation	
BAP (mg/L)	%[1] Mult.	Shoot[2] No.±SE	Shoot Length (cm)±SE	%[3] Callus	Callus[4] Size (mm)
2.5	100	4.9±1.1	2.5±1.0	70	small
5	100	6.2±1.9	3.8±0.6	80	small
10	100	7.7±1.1	2.6±1.0	60	small
15	100	13.0±2.0	1.4±0.4	70	small
20	100	14.0±1.5	1.2±0.3	90	medium

[1]Percent of explants which produced multiple shoots.
[2]Mean shoot number ±SE (n=10).
[3] Percent of explants which produced callus (n=10).
4Relative callus size (diameter): small <3mm, medium 3-6mm, large >6mm.

The percentage of explants that produced multiple shoots from Raggio di Sole at 2.5 mg/L was 70 percent. The mean shoot number per explant in Raggio di Sole increased with the increase of BAP up to 15 mg/L where it appeared to level off (Table 2). The maximum mean number of shoots produced is significantly less than that of Barlo II Nora. This difference may be attributable to the genotype, since the plant genotype has been shown to be an important factor in plant regenerability (Gimelli et al., 1984). Shoot length decreased with the increase of BAP concentration.

Table 2. Effects of BAP on shoot multiplication and callus formation in the carnation cultivar Raggio di Sole.

	Shoot Multiplication			Callus Formation	
BAP (mg/L)	%[1] Mult.	Shoot[2] No.±SE	Shoot Length (cm)±SE	%[3] Callus	Callus[4] Size (mm)
2.5	70	1.3±0.3	6.4±1.4	70	small
5	90	2.5±0.4	5.5±1.5	90	small
10	100	3.7±0.5	4.5±1.5	80	small
15	100	4.1±0.5	4.0±1.0	90	medium
20	100	4.0±0.6	2.0±0.5	80	medium

[1]Percent of explants which produced multiple shoots.
[2]Mean shoot number ±SE (n=10).
[3] Percent of explants which produced callus (n=10).
4Relative callus size (diameter): small <3mm, medium 3-6mm, large >6mm.

Calli developed in all treatments at a rate of 70% to 90%. On media containing BAP at 15 mg/L and 20 mg/L, calli were larger than that obtained on 2.5 to 10 mg/L (Table 3). As in Barlo II Nora, the formation of callus did not seem to adversely affect shoot multiplication (Table 1).

Table 3. Effects of TDZ on shoot multiplication and callus formation in the carnation cultivar Barlo II Nora.

	Shoot Multiplication			Callus Formation	
TDZ (mg/L)	%[1] Mult.	Shoot[2] No.±SE	Shoot Length (cm)±SE	%[3] Callus	Callus[4] Size (mm)
0.01	40	1.6±0.4	3.1±0.7	100	medium
0.05	100	2.1±0.5	2.4±0.3	90	large
0.5	80	2.0±0.4	1.9±0.3	100	large
1	80	8.3±1.9	1.8±0.4	100	large

[1]Percent of explants which produced multiple shoots.
[2]Mean shoot number ±SE (n=10).
[3] Percent of explants which produced callus (n=10).
4Relative callus size (diameter): small <3mm, medium 3-6mm, large >6mm.

Shoot multiplication of 40% was obtained from explants of Barlo II Nora cultured on a medium containing 0.1 mg/L TDZ. The addition of 0.01 to 0.5 mg/L TDZ to Barlo II Nora did not significantly affect the shoot number (Table 3). The low mean shoot numbers obtained in these two treatment could be attributed to the large amounts of callus that formed. Treatments containing BAP, produced less callus than TDZ-containing treatments (Table 1 and 3). The addition of 1 mg/L TDZ caused a significant increase in shoot number, Concentrations higher than 1 mg/L TDZ may further promote shoot multiplication for this cultivar. As was observed with BAP, the shoot length decreased with increasing TDZ concentrations.

The callus size obtained on 0.01 mg/L of TDZ ranged from 3-6 mm. All other treatments produced calli larger than 6 mm (Table 3). The large quantity of callus tissue produced in these treatments appeared to inhibit shoot multiplication. However, at a 1 mg/L TDZ the increase in shoot number is attributed to the high level of cytokinin that may have had overriding activity on the callus affect (Table 3). Vitrification is a common problem in carnation micropropagation (Lesham, 1985). The amount of vitrification observed in this study, 7% of regenerants, was negligible. The rooted plantlets were transferred to a green house where they displayed normal growth.

Conclusions

There was a significant difference in the average number of shoots produced between the cultivars; Barlo II Nora produced higher average shoot numbers than Raggio di Sole. Within the BAP concentrations in this experiment, the optimum range was between 15 and 20 mg/L. Levels of BAP higher than 20 mg/L may further promote shoot multiplication for Barlo II Nora. The amount and percent of callus produced with BAP did not seem to be a limiting factor for shoot multiplication in either cultivar.

The optimal TDZ concentration within the range tested was 1 mg/L, which resulted in a maximum average of 8.3 shoots per explant in Barlo II Nora. The shoot length tended to decrease with increasing cytokinin concentrations. Large amounts of callus were produced on TDZ-containing media. The addition of 1 mg/L of TDZ to the multiplication media promoted shoot multiplication. Higher levels of TDZ may further increase shoot multiplication for this cultivar.

The maximum shoot numbers obtained in our study were higher than those previously reported for carnation axillary bud explants (Miller et al., 1991; Choudhary, 1991). This study resulted in the development of a micropropagation system for these two genotypes. Furthermore, it provided an improvement in shoot multiplication of axillary buds that could be applied to other cultivars. The fact that the highest multiplication was achieved at the highest concentration of cytokinin tested indicates the possibility of further increase of the multiplication rate with the increase in concentration of these cytokinins. This is an area that merits further investigation.

Literature Cited

Altvorst, A.C., H.J.J. Koehorst, T. Bruinsma, J. Jansen, J.B.M. Custers, J. Jong and **J.J.M. Dons.** 1992. Adventitious shoot formation from in vitro leaf explants of carnation (*Dianthus caryophyllus* L.). Scientia Hort. 51:223-235.

Broertjes, C. and **A. Keen.** 1980. Adventitious shoots: do they develop from one cell? Euphytica 29:73-87.

Choudhary, M.L. 1991. Vegetative propagation of carnation in vitro through multiple shoot development. Indian J. Hort. 48:177-181.

Demmink, J.F., J.B.M. Custers and **J.H.W. Bergervoet.** 1987. Gynogenesis to bypass crossing barriers between diploid and tetraploid *Dianthus* species. Acta Hort. 216:343-344.

Frey, L. and **J. Janick.** 1991. Organogenesis in carnation. J. Am. Soc. Hort. Sci. 116:1108-1112.

Gamborg, O.L., R.A. Miller and **K. Ojima.** 1968. Nutrient requirements of suspension culture of soy bean root cells. Exp. Cell. Res. 50:151-158.

Gimelli, F., G. Ginatta, R. Venturo, S. Positano and **M. Buiatti.** 1984. Plantlet regeneration from petals and floral induction in vitro in the mediterranean carnation (*Dianthus caryophyllus* L.) Riv. Ortoflorofrutt. It. 68:107-121.

Johnson, R.T. 1980. Gamma irradiation and in vitro induced separation of chimeral genotypes in carnation. HortScience 15:605-606.

Lesham, B. and **T. Sachs.** 1985. Vitrified *Dianthus*-teratomata in vitro due to growth factor imbalance. Ann. Bot. 56:613-617.

Miller, R.M., V. Kaul, J.F. Hutchinson and **D. Richards.** 1991. Adventitious shoot regeneration in carnation (*Dianthus caryophyllus*) from axillary bud explants. Ann. of Bot. 67:35-42.

Roest, S. and **G.S. Bokelmann.** 1981. Vegetative propagation of carnation in vitro through multiple shoot development. Scientia Hort. 14:357-366.

Petru, E. and **Z. Landa.** 1974. Organogenesis in isolated carnation plant callus tissue cultivated in vitro. Biol. Plant. 16:450-453.

Sankhla, D., T.D. Davis and **N. Sankhla.** 1994. Thidiazuron-induced in vitro shoot formation from roots of intact seedlings of *Albizzia julibrissin*. Plant Growth Reg. 14:267-272.

Villalobos, V. 1981. Floral differentiation in carnation (*Dianthus caryophyllus* L.) from anthers cultured in vitro. Phyton 41:71-75.