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Effect of plant growth regulators and genotypes on callus induction in carnation (*Dianthus caryophyllus* L.)

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Abstract
The effect of plant growth regulators, genotypes and their interaction on callus induction was studied in *Dianthus caryophyllus*. The nodal explants of carnation cultivars 'Tempo' and 'Raggio -de- Sole' were cultured on Murashige and Skoog medium having different concentrations and combinations of NAA, 2,4-D and Kinetin. Highest per cent callus induction (96.40 %), ealiest callus intiation (9.82 days), and minimum number of days taken to optimal callus growth (23.05 days) was obtained in MS medium containing 10 µM NAA and 10 µM Kinetin. Both cultivars formed friable and creamish calli in MS medium with combinations of NAA and 2,4-D. Among Cultivars, 'Raggio-de-Sole' proved better over 'Tempo' for all the callus induction parameters.

Keywords: growth regulators, genotypes, carnation, tissue culture

Introduction
Carnation (*Dianthus caryophyllus* L.) is a member of family Caryophyllaceae and a native of Mediterranean area (Dole and Wilkins, 1999) [3]. This flower is one of the world's most popular and economic cut flowers due to its keeping quality, wide range of forms, colors and ability to withstand long distance transportation. It is preferred by growers to roses and chrysanthemum in several flower exporting countries. Carnation is a vegetatively propagated flower crop and commercially multiplied through stem cuttings.

In vegetatively propagated crops, tissue culture techniques can be used for crop improvement and for quick multiplication of disease free planting material. These cell and tissue culture techniques aid in developing the genotypes with altered plant morphology, with a series of flower colour variations and resistance to biotic and abiotic stress as desired by the breeders. A prime requirement for crop improvement through tissue culture is to establish an efficient culture system consisting of suitable genotype, explants source and plant growth regulators. Thus, the present study was aimed to understand the effect of different plant growth regulators, genotypes and their interaction on callus induction in carnation and to standardize a protocol which could be the basis for crop improvement and in multiplication and conservation of carnation resources.

Material and methods
The present experiment was conducted at the tissue culture laboratory of Department of Floriculture and Landscaping, College of Horticulture, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan. Stem cuttings were taken from the healthy mother plants maintained at the experimental field of the Department. The leaves covering the stem were removed gently. Nodes were excised from the stem cuttings and treated with Dithane M-45 (0.2%) and Bavistin (0.1%) for 10 minutes followed by washing under running tap water for 15-20 minutes. The explants were then treated in the 0.1% teepol solution for 10 minutes and washed with distilled water. The explants were cut to a size of 5-7 mm and under laminar flow cabinet, treated with Mercuric Chloride (0.1%) for 4-5 minutes. After surface sterilization, explants were washed thrice with the distilled water before inoculation.

The sterilized explants were inoculated on Murashige and Skoog nutrient medium containing 3.0% sucrose, 0.8 % agar and different concentrations of NAA, 2,4-D and Kinetin alone or in combinations. The pH of the medium was adjusted to 5.8 before autoclaving at 121° C at the pressure of 1.1 kg/cm^2 for 15 min.

Three replications with 10 explants each replication were maintained for each treatment and the data was analyzed statistically using factorial CRD (Gomez and Gomez, 1975) [5].

Result and discussion

Days taken for callus initiation

Data presented in Table 1. Reveals that treatments differed significantly with regard to days taken to initiation of callus. Earliest callus initiation (9.82 days) took place on MS medium containing 10 µM each of NAA and kinetin. In contrast, maximum time for callus initiation taken in the medium supplemented only with 2.5µM 2,4-D (25.77 days) was found to be at par with 5 µM 2,4-D (24.74 days). In general, earlier callus initiation was observed in cultivar 'Raggio-de-Sole' (17.59 days) than 'Tempo' (20.23 days). The interaction between treatments and cultivars revealed earliest callus initiation in the nodal section explants of 'Raggio-de-Sole' (8.00 days) when cultured onto MS medium containing 10 µM each of NAA and kinetin. In contrast, maximum time for callus initiation taken by the explant in case of 'Tempo' (26.36 days) when cultured onto MS medium containing 2.5 µM 2, 4-D, was found to be at par with 'Raggio-de-Sole' (25.18 days) in the similar medium and also when medium was supplemented with 5 µM 2, 4-D in case of 'Tempo' (25.80 days). Further, like 'Raggio-de-Sole', cultivar 'Tempo' (11.64 days) also showed earliest callus initiation with a treatment combination of NAA and kinetin (10 µM each).

NAA in all combinations with 2,4-D or kinetin induced callus formation. Earliest callus initiation was obtained on MS medium containing 10µM each of NAA and kin. These results are similar to some reports (Garg, 1995; Mehta, Rai *et al*. (2015 2004) [4, 8] [14] in which equal concentrations of NAA and kinetin in MS medium was the best treatment for callus initiation. In contrast, Sharma (2005) [11] observed inhibitory effects of NAA and kinetin on callus formation in leaf explant of carnation. The response of different explants for *in vitro* callus induction varied with the genetic makeup. Particular combinations of growth regulator suitable for one cultivar may not hold true for the other (Thakur, 1999)

Days taken for optimal callus growth

Days taken for optimal callus growth were counted from the day of inoculation to the stage till calli continued further growth which was noted by weighing the regenerated calli along with explant. Data presented in Table 1. Revealed that optimal callus growth was observed earliest (23.05 days) when basal MS medium was supplemented with 10 µM each of NAA and kinetin (Plate 6c & 7c). In contrast, maximum time (40.59 days) taken by the cultures for optimal callus growth in 2.5 µM 2,4-D, was found to be at par when medium was supplemented either with 10 µM NAA (39.45 days) or 5.0 µM 2,4-D (39.52 days) or NAA 5.0 µM + 2, 4-D 2.5 µM (39.12 days). In general, optimal callus growth was observed earlier in case of 'Raggio-de-Sole' (32.18 days) than 'Tempo' (33.67 days). Like the average effect of treatments, interaction data also revealed earliest optimal callus growth in both cultivars when MS medium was supplemented with 10 µM each of NAA and kinetin. Earliest optimal callus growth observed in case of 'Raggio-de-Sole' (21.95 days) with 10µM each of NAA and kinetin, was found to be at par with 'Tempo' (24.16 days) in the same medium and 'Raggio-de-Sole' when medium was supplemented with 10 µM NAA and 5 to 7.5 µM kinetin (23.73 and 24.11 days). In contrast, maximum time for optimal callus growth noted in case of 'Tempo' (40.70 days) with 2.5 µM 2, 4-D, was found to be at par with T_1 (38.68, 40.22 days), T_3 (39.47, 39.57 days), T_4 (38.00, 40.25 days) and T_5 (37.80, 38.70 days) treatments in both cultivars 'Tempo' and 'Raggio-de-Sole', respectively and T_6 in case of cultivar 'Raggio-de-Sole' (37.75 days). Similar results were obtained by Garg (1995) [4], Palai *et al.* (1996) [10] and Mehta (2004) [8]

Per cent callus induction

Data presented in Table1. On per cent callus induction reveals that significant differences were observed among different treatments for callus induction. Maximum explants (96.40%) showed callus induction when cultured onto MS medium containing 10 µM each of NAA and kinetin. In contrast, minimum per cent callus induction was observed in the medium supplemented with 10 µM NAA (30.28%). In general, per cent callus induction was observed more in 'Raggio-de-Sole' (70.21%) than 'Tempo' (63.46%). The interaction between treatments and cultivar revealed that maximum per cent callus induction (97.33) was obtained onto MS medium containing 10 µM each of NAA and kinetin while minimum per cent callus induction (20.16) was observed in the medium supplemented with 10 µM NAA in cultivar 'Tempo' Further like 'Tempo', cultivar 'Raggio-de-Sole' showed maximum per cent callus induction (95.51) with a treatment combination of NAA and kinetin (10 µM each) which was found to be at par with 10 µM NAA + 7.5 µM kinetin, (95.41%).

Maximum explants (96.40%) showed callus induction when cultured onto MS medium containing 10 µM each of NAA and kinetin. Maximum number of explants showing callus formation with 1mg/l each of NAA and mg/l kinetin in MS medium was also reported by Radojevic *et al.* (1990). Garg (1995) [4] found 100 per cent callus induction when leaf and stem segments of carnation were cultured on MS medium containing 10 µM NAA + 10 µM kinetin. Maximum per cent callus induction with equal concentration of NAA and kinetin was also reported by Mehta (2004) [8]. Ruffoni *et al.* (1990) reported callus induction from fragments of *in vitro* multiplied carnation plantlets on MS medium supplemented with 1 mg^{-1} each of NAA and BAP.

Higher concentration of auxins are required for dedifferentiation of explants into callus (Ziv, 1970; Bajaj *et al.*, 1983) [13, 1] but cytokinins also stimulates callus induction by stimulating cell division that lead to the formation of undifferentiated cell mass (Kiss and Mandy, 2003) [6].

Callus type

Type of callus noted by seeing its texture and colour has been given in Table 2. The best quality callus i.e. friable and light green in colour have been obtained with T_4, T_6, T_7 and T_{16} in case of 'Tempo' and T_3 and T_4 in case of 'Raggio-de-Sole'. The best quality callus i.e. friable and light green was obtained with NAA + 2,4-D and NAA + kinetin. Similar observations have been reported by others (Palai *et al.*, 1998; Thakur, 1999; Mehta, 2004), and Rai *et al.* (2014) [14]. [8].

Callus intensity

Callus intensity was considered as growth rate of calli and was found excellent in the MS medium supplemented with 10 µM each of NAA and kinetin in cultivar 'Tempo' and with 10 µM NAA and 5 µM kinetin in cultivar 'Raggio-de-Sole' (Table 4.2).

Growth rate of calli was excellent in MS medium supplemented with 10 µM NAA + 5 µM kinetin in 'Raggio-de-Sole' and with 10µM each of NAA and kinetin in 'Tempo'. Most intensive growth rate of callus was also observed by Kiss *et al.* (2001) [7] on MS medium supplemented with 0.5 mg/l each of NAA and kinetin in carnation. Similar report was given by Mehta (2004) [8].

In present studies, callus formation was mostly stimulated by NAA and kinetin and it was less enhanced by 2, 4-D alone or in combination with NAA. It is very difficult to comment these results because the concentrations of endogenous auxin and cytokinin are not known, but under the described conditions it seems that slightly increased ratios between the externally added NAA and kinetin exhibited the most favourable influence on callus formation of the nodal segment. Dietert *et al.* (1982) [2] and Mehta (2004) [8] have also observed inhibitory effects of 2, 4-D on callus growth.

In these results, cultivar 'Raggio-de-Sole' proved better over 'Tempo' for earliest callus induction, optimal callus growth and maximum per cent callus induction. According to Naryanswamy (1994) [9] callus induction and its rate of proliferation depends upon the source and ingredients in the nutrient medium. In our results, variation between cultivars with same growth regulators treatment in MS medium might be due to genetic differences.

Table 1: Standardization of medium for *in vitro* callus induction in carnation cultivars 'Tempo' and 'Raggio-de-Sole'

Treatment (Conc. µM)		Days taken for callus initiation			Days taken for optimal callus growth			Per cent callus induction		
		Tempo	Raggio-de-Sole	Mean	Tempo	Raggio-de-Sole	Mean	Tempo	Raggio-de-Sole	Mean
	NAA, 2,4-D									
T_1	10.0,0.0	23.79	22.31	23.05	38.68	40.22	39.45	20.16 (26.67)	40.41(39.47)	30.28 (33.07)
T_2	0.0, 2.5	26.36	25.18	25.77	40.70	40.48	40.59	30.58 (33.57)	50.16 (45.09)	40.37 (39.33)
T_3	0.0, 5.0	25.80	23.68	24.74	39.47	39.57	39.52	30.02 (33.22)	55.47 (48.16)	42.75 (40.69)
T_4	5.0, 2.5	22.45	23.25	22.85	38.00	40.25	39.12	36.22 (37.00)	50.16 (45.09)	43.19 (41.05)
T_5	5.0, 5.0	23.87	21.13	22.50	37.80	38.70	38.25	50.63 (45.36)	50.57 (45.33)	50.60 (45.35)
T_6	7.5, 2.5	20.84	20.58	20.71	34.22	37.75	35.99	52.75 (46.58)	50.61 (45.33)	51.68 (45.97)
T_7	7.5, 5.0	19.39	20.10	19.74	34.52	35.27	34.89	45.32 (42.31)	70.06 (56.84)	57.60 (49.57)
	NAA, Kin									
T_8	5.0, 5.0	23.96	18.49	21.22	34.68	34.75	34.71	50.60 (45.35)	70.81 (57.31)	60.70 (51.33)
T_9	5.0, 7.5	21.35	17.80	19.57	33.21	30.94	32.07	80.73 (64.02)	75.50 (60.36)	78.10 (62.19)
T_{10}	5.0, 10.0	19.45	16.70	18.07	34.25	28.08	31.16	85.59 (67.77)	70.78 (57.28)	78.18 (62.52)
T_{11}	7.5, 5.0	20.11	15.74	17.92	33.67	27.20	30.43	85.54 (67.75)	80.69 (63.95)	83.10 (65.85)
T_{12}	7.5, 7.5	19.27	13.52	16.26	31.64	26.50	29.07	85.43 (67.57)	83.75 (66.25)	84.59 (66.91)
T_{13}	7.5, 10.0	15.32	12.00	13.66	28.74	25.34	27.04	80.74 (63.97)	90.78 (72.34)	85.70 (68.15)
T_{14}	10.0, 5.0	16.27	12.73	14.50	27.92	23.73	25.82	92.93 (74.68)	92.65 (74.33)	92.70 (74.51)
T_{15}	10.0, 7.5	14.05	10.27	12.16	27.08	24.11	25.59	90.82 (72.38)	95.41 (77.68)	93.11 (75.03)
T_{16}	10.0, 10.0	11.64	8.00	9.82	24.16	21.95	23.05	97.33 (81.10)	95.51 (77.82)	96.40 (79.46)
Mean		20.23	17.59	-	33.67	32.18	-	63.46 (54.33)	70.21 (58.29)	-
Figures in the parenthesis are arc sine transformed values										
$CD_{0.05}$:										
Treatments				1.05			2.31			(1.98)
Cultivars				0.37			0.82			(0.70)
Treatments x Cultivars				1.48			3.28			(2.80)

Table 2: Effect of different growth regulator treatments on callus type and intensity in carnation cultivars 'Tempo' and 'Raggio-de-Sole'

Treatment (Conc. µM)		Tempo			Raggio-de-Sole		
		Callus Type		Intensity	Callus Type		Intensity
		Texture	Colour		Texture	Colour	
	NAA, 2,4-D						
T_1	10.0,0.0	Compact	Green	+	Compact	Dark Green	+
T_2	0.0, 2.5	Compact	Light Green	+	Compact	Light Green	+
T_3	0.0, 5.0	Friable	Creamish	++	Friable	Light Green	+
T_4	5.0, 2.5	Friable	Light Green	++	Friable	Light Green	++
T_5	5.0, 5.0	Friable	Creamish	++	Friable	Creamish	+++
T_6	7.5, 2.5	Friable	Light Green	++	Friable & compact	Light Green	++
T_7	7.5, 5.0	Friable	Light Green	+++	Friable & Compact	Light Green	+++
	NAA, Kin						
T_8	5.0, 5.0	Compact	Dark Green	+	Compact	Dark Green	+
T_9	5.0, 7.5	Compact	Dark Green	+	Compact	Dark Green	++
T_{10}	5.0, 10.0	Compact	Dark Green	+	Compact	Green	+++
T_{11}	7.5, 5.0	Compact	Light Green	++	Compact	Green	++
T_{12}	7.5, 7.5	Compact	Light Green	++	Compact	Light Green	+++
T_{13}	7.5, 10.0	Compact	Light Green	+++	Compact	Light Green	+++
T_{14}	10.0, 5.0	Friable & Compact	Light Green	+++	Friable & Compact	Light Green	++++
T_{15}	10.0, 7.5	Friable &Compact	Light Green	+++	Friable & Compact	Light Green	+++
T_{16}	10.0, 10.0	Friable	Light Green	++++	Compact	Green	+++

* Intensity of callus in terms of growth rate of calli

Little +
Fair ++
Good +++
Excellent ++++

References

1. Bajaj YPS, Sindhu MMS, Gill APS. Some factors affecting the *in vitro* propagation of gladiolus. Scientia Horticulturae. 1983; 18:269-275.
2. Dietert MF, Barron SA, Yoder OC. Effects of genotype on *in vitro* culture in the genus Brassica. Plant Science Letters. 1982; 26:233-240.
3. Dole JM, Wilkins HF. Floriculture, Principles and Species. Prentice Hall Inc., New Jersy, USA, 1999.
4. Garg M. *In vitro* multiplication of virus tested carnations. M.Sc. Thesis, submitted to Dr. YS, 1995.
5. Gomez KA, Gomez A. Statistical procedure for agricultural research. 2nd edition. John Wiley and Sons, Inc., Singapore, 1975, 680.
6. Kiss ZO, Mandy A. Influence of different growth regulators on the *in vitro* morphogenesis of an ornamental variety of carnation. International Journal of Horticultural Science. 2003; 9(3/4):55-57.
7. Kiss ZO, Balogh A, Fodorpataki L. Investigation of the *in vitro* regeneration of mericlones in the Caribe variety of carnation. International Journal of Horticultural Science. 2001; 7(3-4):87-89.
8. Mehta R. *In vitro* selection and biochemical characterization of carnation (*Dianthus caryophyllus* L.) callus culture tolerant of *Alternaria dianthi*. M.Sc. Thesis, submitted to Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), 2004.
9. Narayanswamy S. Plant Cell and Tissue Culture, Tata MacGraw Hill Publishing Company Ltd., New Delhi, 1994, 700.
10. Palai SK, Rout GR, Das P. *In vitro* plant regeneration *Dianthus caryophyllus* cv. Alas Red through callus culture. Indian Journal of Horticulture. 1996; 53(1):1-7.
11. Sharma C. Studies on cell selection in carnation (*Dianthus caryophyllus* L.) cv. Raggio-de-Sole against culture filtrate of *Rhizoctonia solani* Kuhn. M.Sc. Thesis, submitted to Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), 2005.
12. Thakur M, Sharma DR, Pathania NS. *In vitro* callus initiation and organogenesis from internodal segments in perpetual carnation. In: Floriculture Research Trend in India, RL Mishra and Sanyat Mishra (eds). Indian Society of Ornamental Horticulture, Division of Floriculture and Landscaping, IARI, New Delhi, 2002, 164-168.
13. Ziv M. The effect of growth retardants on shoot proliferation and morphogenesis in liquid cultured gladiolus plants. Acta Horticulturae. 1970; 280:207-214.