Original Research Article

In vitro callussing of Carnation (*Dianthus caryophyllus* L.) cv. Scania and Indios

ABSTRACT

An experiment was conducted to standardize the protocol for *in vitro* callusing of two carnation (*Dianthus caryophyllus* L.) cultivars viz., 'Scania' and 'Indios'. Different explants tried were leaf segments, nodal segments, shoot tips and apical shoots. Apical shoots were also used with or without leaves, besides nodal segments from two environments i.e., open field and polyhouse. Among all explants leaf segments and nodal segments proved best for callusing in both the cultivars. MS medium supplemented with BAP 1.00 + 2, 4-D 2.00 mg l^{-1} resulted in best callusing of leaf and nodal segment explants in terms of minimum days to callus initiation, maximum callus induction and callus weight per explant in both the cultivars. Minimum time (10.50 and 8.33 days) to callus initiation , highest callus induction of 91.66 and 91.00 per cent and significantly maximum mean callus weight 2.15 and 2.52 g explant^{-1} was recorded in media fortified with BAP 1.00 + 2, 4-D 2.00 mg l^{-1} in case of leaf and nodal segment explant of scania while as BAP 1.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1} took lowest (12.00 and 8.00) days for initiation of callusing , 85.00 and 89.00 per cent for callus induction , and significantly maximum mean callus weight 1.93 and 2.14 g explant^{-1}in leaf and nodal segment explants of cv. 'Indios'.

Key words: Tissue culture, Carnation, *In vitro* Callusing.

Abbreviations used—BAP; 6-Benzyl amino purine, BA; 6-Benzyladenine, 2, 4-D; 2,4dichloro-phenoxyacetic acid, GA3; Gibberelic acid, IAA;Indole-3-acetic acid, IBA; Indole-3-butyric acid, MS; Murashige and Skoog's medium, NAA; Naphthalene acetic acid and µm; Micro molar.

INTRODUCTION

Since carnation is one of the top selling ornamental crops worldwide (Casas *et al*., 2010 and Gutierrez-Miceli *et al*., 2010). Himachal Pradesh, Punjab, West Bengal, Jammu and Kashmir and Karnataka are the important states producing carnation. Ornamental and flower crops have been benefited most by plant tissue culture techniques. Murashige (1990) reports that out of about 1000 plant species demonstrated to be micropropagatable, more than three fourth of them are ornamentals. About 300 commercial companies are engaged in tissue culture, producing approximately 400 million plants annually in USA, England, The Netherlands, France, Germany and Australia. In India about 40 companies are now engaged in tissue culture (Chaturvedi *et al*., 1995). The number of commercial tissue culture laboratories in India has gone upto 76 with an installed capacity of 300 million plants per year. These laboratories produced a total of 50 million plants in 2003 and this number is likely to increase in coming years (Prakash, 2006). Indian industry produces hardened plants

for domestic use and *in vitro* rooted plantlets for the export market. Micro-propagation by axillary branching for sufficient cycles can potentially allow isolation of solid mutants with higher efficiency and thus save on space and time in any breeding programme (Donini and Micke, 1984).

Carnation is well adapted to the climatic conditions of Jammu and Kashmir. It grows successfully in Kashmir valley having temperate cum Mediterranean climate. Non-availability of the quality planting material is a major problem faced by the valley growers. Presently quality planting material of carnation is imported from European countries, particularly the Netherlands on very high cost prices. To overcome this problem, a quick micro-propagation system is exigently needed, on which no work has been done in our state so for. Therefore, keeping in view the importance of carnation as a cut flower and the potential scope of its tissue culture production as well as the need for introducing novel varieties, present studies have been undertaken with the following objectives.

1. Identification of suitable explant for *in vitro* callusing of carnation.
2. Standardization of media and growth regulator regimes for *in vitro* callusing.

MATERIALS AND METHODS

Salient features of the experiments carried out in present investigation involving the study of different types of explants, media, hormonal regimes for *in vitro* callusing and regeneration studies of two cultivars of carnation.

3.1 Callusing

For callus induction leaf and nodal segment explants of two cultivars ‘Scania’ and ‘Indios’ were used in standardization experiments. Explants after sterilization were placed on media containing different auxin and cytokinin combinations (Hi-Media Laboratories Pvt. Ltd) (Table 1). Each treatment combination was assigned 10 explants with one explant per test tube and replicated thrice. Data for callusing was recorded under following parameters.

3.2 Days to callus initiation

Measured from the date of inoculation to the date when callus became visible to naked eye.

3.3 Callus induction (%)

Data was recorded after 4 weeks of culture

3.4 Callus weight (g explant^{-1})

Callus weight was recorded at the end of six weeks of culture**.** Test tubes/flasks with fresh media were weighed individually. Callus from growing cultures was separated from the explants and transferred to pre weighed test tubes/flasks with fresh media. The test tubes/flasks were weighed again. The difference between the two measurements was recorded as the callus weight per explant.

3.5 Callus type

Callus type in each case was visually assessed and observations recorded accordingly.

Table 1: PGR supplements used for standardization of callus induction and growth

S. No.	Growth regulators	Conc. (mg l^{-1})
1	BAP + 2, 4-D	0.50 + 1.00
2	BAP + 2, 4-D	0.50 + 1.50
3	BAP + 2, 4-D	0.50 + 2.00
4	BAP + NAA	0.50 + 1.00
5	BAP + NAA	0.50 + 1.50
6	BAP + NAA	0.50 + 2.00
7	BAP + 2, 4-D	1.00 + 1.00
8	BAP + 2, 4-D	1.00 + 1.50
9	BAP + 2, 4-D	1.00 + 2.00
10	BAP + NAA	1.00 + 1.00
11	BAP + NAA	1.00 +1.50
12	BAP + NAA	1.00 +2.00

3.6 Statistical analysis

Statistical analysis of the data collected for different parameters during the present investigation was subjected to analysis of variance for completely randomized design with three replications (Gomez and Gomez, 1983). To satisfy model assumptions for analysis of variance, percentage data was subjected to angular transformation as suggested by Steel and Torrie (1980).

RESULTS AND DISCUSSION

In present investigation twelve growth regulator combinations consisting auxins (2, 4-D and NAA at 1.00, 1.50 and 2.00 mg l^{-1}) in combination with cytokinin (BAP at 0.50 or 1.00 mg l^{-1}) were used in standardization of various callusing parameters of leaf and nodal segment explants of both the cvs. 'Scania' and 'Indios'.

In case of leaf explant, all the growth regulator combinations induced callus in both the cultivars. Perusal of data (Table 2 and 3) reveals that there were significant differences in means of callusing parameters viz., days to callus initiation, callus induction per cent ,callus weight explant^{-1}, and callus type under the influence of various growth regulator combinations (Fig.a and c). Minimum days taken to callus induction were recorded in leaf explant incubated on higher concentrations of 2, 4-D (1.50 and 2.00 mg l^{-1}) in combination with BAP (0.50 or 1.00 mg l^{-1}). In comparison, callus induction was significantly delayed in explants on media supplemented with NAA (1.50 and 2.00 mg l^{-1}) + BAP (0.50 or 1.00 mg l^{-1}). Mubarack *et al*. (1991) also reported that MS media supplemented with 2, 4-D + NAA (0.50 mg l^{-1} each) resulted in early callus formation, whereas NAA + BA (0.50 mg l^{-1} each) delayed it in leaf explants of carnation cultivars 'Shocking Pink', 'Arthur Sim' and 'William Sim'.

Perusal of data regarding per cent callus induction followed a similar trend i.e., significantly highest on MS media supplemented with 2, 4-D at higher concentration in combination with BAP at 1.00 mg l^{-1}. Whereas, significantly lowest per cent callus induction was observed in NAA treatments at higher concentrations in combination with BAP at 0.50 or 1.00 mg l^{-1}. The results are in conformity with Kanwar and Kumar (2009), who reported highest callus induction in leaf explant of carnation cv. 'Indios' achieved on MS media supplemented with 2.00 mg l^{-1} 2, 4-D in combination with 1.00 mg l^{-1} BA and lowest on media containing NAA or kinetin. Devi and Gupta (2004b) reported 100.00 per cent callus induction from leaf and internodal stem segments of carnation with the increase in concentration of 2, 4-D from 0.50 to 1.00 mg l^{-1} alone in the medium. Perusal of data regarding callus weight explant $^{-1}$ revealed maximum callus development on media containing 2, 4-D 2.00 mg l^{-1} in combination with BAP 1.00 mg l^{-1}. Other 2, 4-D treatments also resulted in higher callus weight in comparison to NAA based treatments. Auxins which

help in cell division are also known to increase cell volume by a process called acid growth where in cell wall loosens up through acidification, thus allowing increase in volume through uptake of more water. The increased cell volume means increase in individual cell weight and hence increased weight of the callus.

Callusing of nodal segment explants of two cultivars recorded a trend similar to the callusing behaviour of leaf explants (Table 4 and 5). Nodal sections incubated on MS media fortified with 2, 4-D (1.00, 1.50 or 2.00 mg l^{-1}) in combination with BAP 1.00 mg l^{-1} callused significantly earlier. Whereas, it took more days to callusing on media supplemented with higher concentration of NAA in combination with BAP 0.50 or 1.00 mg l^{-1}. Similarly, per cent callus induction was significantly high in the above mentioned 2, 4-D formulation, while as NAA based treatments at 1.00, 1.50 and 2.00 mg l^{-1} in combination with BAP 0.50 mg l^{-1} recorded lowest percentage of callusing (Fig b and d). Kanwar and Kumar (2009) reported highest callus induction in internodal sections of carnation cv. 'Indios' on MS media supplemented with 2, 4-D 2.00 mg l^{-1} in combination with BA 1.00 mg l^{-1} and lowest on media containing NAA and kinetin. Kiss and Mandy (2003) also reported callus induction on MS media supplemented with 2, 4-D and BA at 0.50 + 2.00, 1.00 + 1.50, 1.50 + 1.00 and 2.00 + 0.50 mg l^{-1} from explants of carnation cv. 'Rimini'. They found callus formation was more stimulated in the medium containing 1.50 mg 2, 4-D + 1.00 mg BA per litre than in the medium containing 2, 4-D 0.50 + BA 2.00 mg l^{-1}. On further perusal of data regarding callus weight explant^{-1}, it was observed that higher concentrations of 2, 4-D in combination with BAP at 1.00 mg l^{-1} produced significantly highest callus weight than other treatments including NAA based growth regulator combinations in both the cultivars.

Table 2: Influence of growth regulators on callusing in leaf explant of carnation (*Dianthus caryophyllus* L.) cv. Scania

MS + PGRs	Concentration (mg l^{-1})	Days to callus initiation	* Callus induction (%)	** Callus weight (g explant^{-1})	Callus type
BAP + 2,4-D	0.50 + 1.00	13.83	74.00 (59.35)	1.58	Compact, creamy white
BAP + 2,4-D	0.50 + 1.50	13.50	74.33 (59.58)	1.61	Compact, creamy white
BAP + 2,4-D	0.50 + 2.00	12.16	78.33 (62.27)	1.69	Nodular, creamy white
BAP + NAA	0.50 + 1.00	14.66	68.66	1.38	Soft yellowish green

			(55.96)		
BAP + NAA	0.50 + 1.50	15.75	67.33 (55.15)	1.30	Soft yellowish green
BAP + NAA	0.50 + 2.00	17.00	62.33 (52.14)	1.05	Soft yellowish green
BAP + 2,4-D	1.00 + 1.00	12.00	79.33 (62.97)	1.86	Nodular creamy white
BAP + 2,4-D	1.00 + 1.50	11.00	80.00 (63.44)	1.99	Nodular creamy white
BAP + 2,4-D	1.00 + 2.00	10.50	91.66 (73.41)	2.15	Nodular creamy white
BAP + NAA	1.00 + 1.00	14.83	74.00 (59.34)	1.45	Soft yellowish green
BAP + NAA	1.00 + 1.50	16.50	70.00 (56.79)	1.39	Soft yellowish green
BAP + NAA	1.00 + 2.00	17.50	70.00 (56.79)	1.18	Nodular yellowish green
SE $_{diff.}$		**0.41**	**1.65**	**0.05**	
LSD$_{(P=0.05)}$		**0.85**	**3.41**	**0.11**	

*Data recorded after 4 weeks of culture

** Callus weight recorded after 6 weeks of culture

Figures in the parenthesis are arcsine transformed value of the percentage data.

Table 3: Influence of growth regulators on callusing in leaf explant of carnation (*Dianthus caryophyllus* L.) cv. Indios

MS + PGRS	Concentration (mg l^{-1})	Days to callus initiation	* Callus induction (%)	** Callus weight (g $explant^{-1}$)	Callus type
BAP + 2,4-D	0.50 + 1.00	15.33	67.66 (55.35)	1.30	Compact creamy white
BAP + 2,4-D	0.50 + 1.50	14.00	69.66 (56.58)	1.41	Compact creamy white
BAP + 2,4-D	0.50 + 2.00	14.00	70.00 (56.79)	1.40	Compact creamy white
BAP + NAA	0.50 + 1.00	15.66	62.00 (51.94)	1.06	Soft yellowish green
BAP + NAA	0.50 + 1.50	16.00	58.33 (49.79)	1.04	Nodular yellowish green
BAP + NAA	0.50 + 2.00	18.00	58.00 (49.60)	0.87	Nodular yellowish green
BAP + 2,4-D	1.00 + 1.00	12.66	71.66 (57.86)	1.54	Nodular creamy white
BAP + 2,4-D	1.00 + 1.50	12.16	74.66 (59.79)	1.72	Nodular creamy white
BAP + 2,4-D	1.00 + 2.00	12.00	85.00 (67.24)	1.93	Nodular creamy white
BAP + NAA	1.00 + 1.00	15.50	65.33 (53.93)	1.24	Soft yellowish green
BAP + NAA	1.00 + 150	17.66	64.33 (53.33)	1.09	Soft yellowish green
BAP + NAA	1.00 + 2.00	19.00	64.00 (53.13)	1.04	Soft yellowish green
$SE_{diff.}$		**0.67**	**1.55**	**0.09**	
$LSD_{(P=0.05)}$		**1.39**	**3.20**	**0.19**	

*Data recorded after 4 weeks of culture

** Callus weight recorded after 6 weeks of culture

Figures in the parenthesis are arcsine transformed value of the percentage data

CONCLUSION

Among all explants leaf segments and nodal segments proved best for callusing in both the cultivars. MS medium supplemented with BAP 1.00 + 2, 4-D 2.00 mg l^{-1} resulted in best callusing of leaf and nodal segment explants in terms of minimum days to callus initiation, maximum callus induction and callus weight per explant in both the cultivars. Minimum time (10.50 and 8.33 days) to callus initiation , highest callus induction of 91.66 and 91.00 per cent and significantly maximum mean callus weight 2.15 and 2.52 g $explant^{-1}$ was recorded in

media fortified with BAP 1.00 + 2, 4-D 2.00 mg l^{-1} in case of leaf and nodal segment explant of scania while as BAP 1.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1} took lowest (12.00 and 8.00) days for initiation of callusing , 85.00 and 89.00 per cent for callus induction , and significantly maximum mean callus weight 1.93 and 2.14 g explant^{-1}in leaf and nodal segment explants of cv. 'Indios'.

Table 4**: Influence of growth regulators on callusing in nodal segment explant of carnation (*Dianthus caryophyllus* L.) cv. Scania**

MS + PGRs	Concentration (mg l^{-1})	Days to callus initiation	* Callus induction (%)	** Callus weight (g explant^{-1})	Callus type
BAP + 2,4-D	0.50 + 1.00	13.00	75.33 (60.22)	1.60	Compact creamy white
BAP + 2,4-D	0.50 + 1.50	12.33	75.66 (60.45)	1.65	Compact creamy white
BAP + 2,4-D	0.50 + 2.00	12.00	77.33 (61.58)	1.78	Compact creamy white
BAP + NAA	0.50 + 1.00	13.33	70.00 (56.79)	1.32	Soft yellowish green
BAP + NAA	0.50 + 1.50	15.08	67.33 (55.14)	1.28	Soft yellowish green
BAP + NAA	0.50 + 2.00	16.16	65.00 (53.73)	1.16	Soft yellowish green
BAP + 2,4-D	1.00 + 1.00	11.16	77.33 (61.58)	2.15	Compact creamy white
BAP + 2,4-D	1.00 + 1.50	9.66	82.66 (65.43)	2.30	Compact creamy white
BAP + 2,4-D	1.00 + 2.00	8.33	91.00 (72.55)	2.52	Compact creamy white
BAP + NAA	1.00 + 1.00	13.50	73.00 (58.70)	1.58	Compact dark green
BAP + NAA	1.00 + 150	15.83	72.33 (58.26)	1.51	Compact dark green
BAP + NAA	1.00 + 2.00	16.50	71.66 (57.86)	1.45	Compact dark green
SE $_{diff.}$		**0.55**	**1.59**	**0.04**	
LSD$_{(P=0.05)}$		**1.14**	**3.28**	**0.09**	

173 *Data recorded after 4 weeks of culture

174 ** Callus weight recorded after 6 weeks of culture

175 Figures in the parenthesis are arcsine transformed value of the percentage data.

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Table 5: Influence of growth regulators on callusing in nodal segment explant of carnation (*Dianthus caryophyllus* L.) cv.Indios

MS + PGRS	Concentration (mg l^{-1})	Days to callus initiation	* Callus induction (%)	** Callus weight (g $explant^{-1}$)	Callus type
BAP + 2,4-D	0.50 + 1.00	12.83	68.66 (55.97)	1.40	Compact creamy white
BAP + 2,4-D	0.50 + 1.50	12.00	73.00 (58.70)	1.49	Compact creamy white
BAP + 2,4-D	0.50 + 2.00	11.66	77.66 (61.83)	1.66	Compact creamy white
BAP + NAA	0.50 + 1.00	13.00	61.00 (51.35)	1.20	Soft yellowish green
BAP + NAA	0.50 + 1.50	13.66	59.66 (50.59)	1.18	Soft yellowish green
BAP + NAA	0.50 + 2.00	15.16	59.33 (50.38)	1.13	Nodular yellowish green
BAP + 2,4-D	1.00 + 1.00	10.66	77.33 (61.58)	1.71	Compact creamy white
BAP + 2,4-D	1.00 + 1.50	9.66	80.33 (63.68)	1.84	Compact creamy white
BAP + 2,4-D	1.00 + 2.00	8.00	89.00 (70.83)	2.14	Compact creamy white
BAP + NAA	1.00 + 1.00	13.08	68.33 (55.77)	1.41	Compact dark green
BAP + NAA	1.00 + 150	14.83	66.66 (54.73)	1.30	Compact dark green
BAP + NAA	1.00 + 2.00	15.83	63.33 (52.73)	1.29	Compact dark green
$SE_{diff.}$		**0.46**	**2.26**	**0.04**	
$LSD_{(P=0.05)}$		**0.95**	**4.66**	**0.09**	

*Data recorded after 4 weeks of culture

** Callus weight recorded after 6 weeks of culture

Figures in the parenthesis are arcsine transformed value of the percentage data

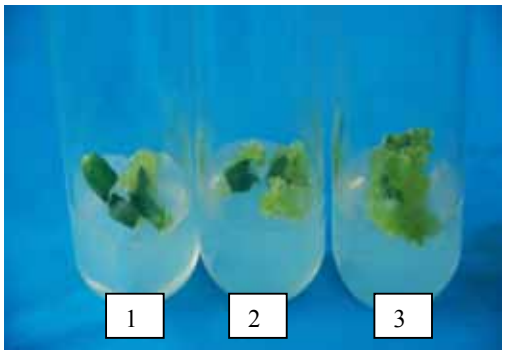

Fig. a) Callusing in leaf explant cv. 'Scania'

1) BAP + 2,4-D = 1.00 + 1.00 mg l^{-1}
2) BAP + 2,4-D = 1.00 + 1.50 mg l^{-1}
3) BAP + 2,4-D = 1.00 + 2.00 mg l^{-1}

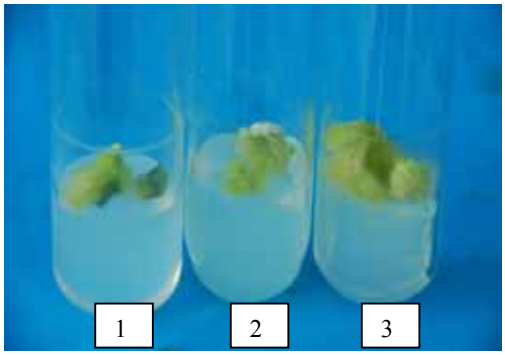

Fig.b) Callusing in nodal explant cv. 'Scania'

1) BAP + 2,4-D = 1.00 + 1.00 mg l^{-1}
2) BAP + 2,4-D = 1.00 + 1.50 mg l^{-1}
3) BAP + 2,4-D = 1.00 + 2.00 mg l^{-1}

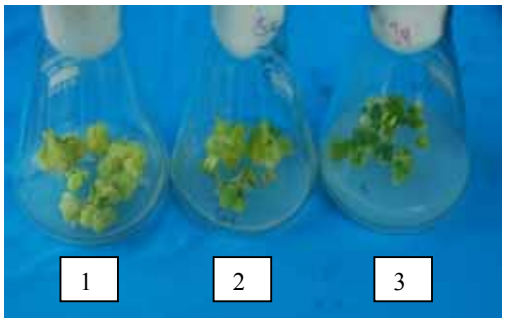

Fig.c) Callusing in leaf explant cv. 'Indios'

1) BAP + NAA = 1.00 + 1.00 mg l^{-1}
2) BAP + NAA = 1.00 + 1.50 mg l^{-1}
3) BAP + NAA = 1.00 + 2.00 mg l^{-1}

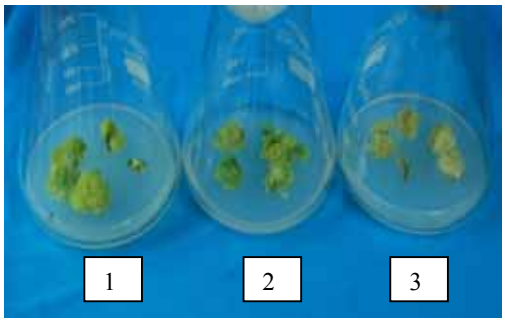

Fig.d) Callusing in nodal explant cv. 'Indios

1) BAP + 2,4-D = 1.00 + 1.00 mg l^{-1}
2) BAP + 2,4-D = 1.00 + 1.50 mg l^{-1}
3) BAP + 2,4-D = 1.00 + 2.00 mg l^{-1}

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