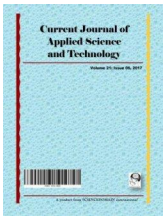

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In vitro Callussing of Carnation (*Dianthus caryophyllus* L.) cv. Scania and Indios

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ZAQ and Neelofar designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AD, NHM and MAW managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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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

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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'.

Keywords: Tissue culture; carnation; in vitro callusing.

ABBREVIATIONS

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.

1. INTRODUCTION

Since carnation is one of the top selling ornamental crops worldwide [1,2]. 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 [3] 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. 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 [4]. 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 programmes [5].

Plant callus is a growing mass of unorganized plant parenchyma cells. In living plants, callus cells are those cells that cover a plant wound. Callus formation is induced from plant tissue explants after surface sterilization and plating onto tissue culture medium in vitro. The culture medium is supplemented with plant growth regulators, such as auxins, cytokinins, and gibberellins, to initiate callus formation or somatic embryogenesis. Specific auxin to cytokinin ratios in plant tissue culture medium give rise to an unorganized growing and dividing mass of callus cells. Callus cultures are often broadly classified as being either compact or friable. Friable calluses fall apart easily, and can be used to generate cell suspension cultures. Callus can directly undergo direct organogenesis and/or embryogenesis where the cells will form an entirely new plant and the process is known as callus culture. Thakur et al. [6] obtained friable and pale yellow callus from internodal segments of two cultivars of perpetual carnation 'Cabaret' and Feyenoord' on MS medium containing 0.5 ppm each of 2, 4-D and NAA. The callus induced on medium supplemented with 2, 4-D alone or in combination with NAA was unable to regenerate shoots when transferred directly to the regeneration medium. Shoot initiation only occurred after culturing the calli on MS basal medium containing 0.3% activated charcoal. The callus turned green on this medium and several shoot primordia appeared after one month of culture period.

Carnation is well adapted to the climatic conditions of Jammu and Kashmir. It grows successfully in Kashmir valley with 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 carnations are imported from European countries, particularly the Netherlands on very high cost prices. To overcome this problem, a quick micro-propagation system is exigent needed, on which no work has been done in our state so for. Therefore, it is important to devise an efficient protocol of callus proliferation to start in vitro manipulation of carnation for production as well as for introducing novel varieties with the objectives.

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

2. MATERIALS AND METHODS

Salient features of the experiments carried out in the present investigation involving the study of different types of explants, media, hormonal regimes for *in vitro* callusing studies of two cultivars of carnation 'Scania' and 'Indios' (Figs. 1 and 2).

2.1 Culture Media

Murashige and Skoog [7] medium was employed during the course of present investigation (Table 1). In addition to above, some modifications in Murashige and Skoog [7] medium were also employed details of which have been provided in the appropriate sections.

2.2 Preparation of Stock Solutions

Different types of stock solutions of macro elements, micro elements and organics were prepared for each medium and stored in reagent bottles for use. Usually four types of stock solutions (A-D) are made for Murashige and Skoog [7] basal medium. However, for longer storage seven types of stock solutions (A, B, C, D, E, F, and G) of Murashige and Skoog [7] are generally prepared in the Biotechnology Laboratory of the Division of Fruit Science and same was followed in the current investigation (Tables 3, 4). During the entire investigation period, 200 ml of each stock solution was prepared each time which was sufficient for making 20 litres of medium. For making one litre of medium, 10 ml of each stock solution was added to 600 ml of double distilled water in a beaker and final volume made to one litre by adding double distilled water.

Seven stock solutions were used in the preparation of MS medium. Stock solution (A/B/D) containing only one chemical was prepared by dissolving required quantity of each chemical (quantity of chemical required for making 20 litres of medium) in 150 ml of double distilled water and final volume made to 200 ml with double distilled water. Thus stock solution with strength of 100 X was obtained. Stock solution containing more than one chemical (C/E/F/G) was prepared by weighing the required quantity of each chemical (quantity of chemical required for making 20 litres of medium) separately and dissolved to the last particle in double distilled water followed by mixing them together slowly with continuous stirring. Final volume of 200 ml of the mixture was made by addition of double distilled water. Stock solutions were stored in corning reagent bottles at 4°C. The required quantity of cobalt chloride was too small (0.5 mg) to be weighed properly on the available digital balance. This quantity was multiplied by 100 and the quantity obtained (50 mg) was dissolved in 100 millilitres of water. Then one millilitre of this solution was added to stock solution C and final volume of 200 ml was made by adding double distilled water**.** Similar method was used for adding required quantity of cupric sulphate and thiamine to their respective stock solutions.

Fig. 1. *Dianthus caryophyllus* L. cv. Scania

Fig. 2. *Dianthus caryophyllus* L. cv. Indios

Table 1. Composition and preparation of stock solutions for Murashige and Skoog (1962) medium

Stock	Components	Chemical formula	Qty. mgl^{-1}	Qty. for 20 litres	Qty. of stock solution	Conc. of stock solution
A	Ammonium nitrate	NH_4NO_3	1650	33.00 g	200 ml	100 X
B	Potassium nitrate	KNO_3	1900	38.00 g	200 ml	100 X
C	Potassium iodide	KI	0.83	16.6 mg	200 ml	100 X
	Boric acid	H_3BO_3	6.2	124 mg		
	Potassium phosphate, monobasic	KH_2PO_4	170	3.4 g		
	Molybdic Acid (Sodium Salt)•$2H_2O$	$Na_2MoO_4.2H_2O$	0.25	5 mg		
	Cobaltous chloride hexahydrate	$CoCl_2.6H_2O$	0.025	0.5 mg		
D	Calcium chloride, dihydrate	$CaCl_2.2H_2O$	440	8.8 g	200 ml	100 X
E	Magnesium sulphate, heptahydrate	$MgSO_4.7H_2O$	370	7.4 g	200 ml	100 X
	Zinc sulphate, heptahydrate	$ZnSO_4.7H_2O$	8.6	172 mg		
	Manganese Sulfate, tetrahydrate	$MnSO_4.4H_2O$	22.3	446 mg		
	Cupric Sulfate pentahydrate	$CuSO_4.5H_2O$	0.025	0.5 mg		
F	Ethylenediaminetetra acetic acid disodium salt	$Na_2EDTA•2H_2O$	37.3	746	200 ml	100 X
	Ferrous Sulfate heptahydrate	$FeSO4.7H_2O$	27.8	556 mg		
G	Glycine	-----	2.00	40 mg	200 ml	100 X
	Nicotinic acid	-----	0.5	10		
	Pyridoxine HCl	-----	0.5	10		
	Thiamine HCl	-----	0.1	2		

For the preparation of the iron stock solution (stock solution F), required quantities of $FeSO_4.7H_2O$ and Na_2EDTA were dissolved separately in 75 ml double distilled water. Na_2EDTA solution was heated over hot plate and stirred to dissolve it completely. It was then added to $FeSO_4.7H_2O$ solution gently and stirred well to get a homogeneous light yellow solution. Final volume (200 ml) of the mixture was made by adding double distilled water and stored in an amber-coloured reagent bottle.

2.3 Preparation of Stock Solutions of Growth Regulators

Basal medium was supplemented with different types of growth regulators. Small quantity of Stock solution (50 ml) of each growth regulator was prepared on weekly/monthly basis and stored in refrigerator at 4°C. Stock solutions of benzyl-aminopurine (BAP), kinetin, naphthalene-acetic-acid (NAA), indole-3-aceticacid (IAA) and indole-3-butyric-acid (IBA) were prepared by dissolving 50 mg of each growth regulator in 25 ml of 0.50 N NaOH and then volume was made to 50 ml with double distilled water. However, 2, 4-D was dissolved directly in sterile double distilled water (50 $mg^{-50\ ml}$).

2.4 Preparation of Culture Media

The required quantity of sucrose (usually 30 g l^{-1}) and myo-inositol was dissolved in double distilled water in a beaker. Required quantity of stock solutions containing macro elements, microelements, vitamins and growth regulators were added as per the treatment requirement. Final volume of the medium was made with the

addition of double distilled water. The pH of medium solution was adjusted at 5.7 by adding 1N NaOH or 1 N HCl drop wise with a micropipette. Care was taken that electrode does not come in direct contact with the walls of beaker and bulb of electrode remains completely immersed in the solution. The medium was slightly heated in microwave oven and then required quantity of agar was added, stirred well with scalpel or stirrer and again heated in microwave oven to boil so as to dissolve the agar completely. The medium was allowed to cool for few minutes and subsequently dispensed into culture vessels. Non absorbent cotton plugs covered with news paper were used to plug the culture vessels.

2.5 Explant and Hormonal Regimes for Callussing

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 2). Each treatment combination was assigned 10 explants with one explant per test tube and replicated thrice. Data for callusing was recorded under following parameters.

Table 2. 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

2.5.1 Days to callus initiation

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

2.5.2 Callus induction (%)

Callus induction means callus has developed after visible to naked eye and can be subcultured to proliferate / multiply. Data was recorded after 4 weeks of culture.

2.5.3 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.

2.5.4 Callus type

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

2.6 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 (CRD) with three replications [8]. To satisfy model assumptions for analysis of variance, percentage data were subjected to angular transformation as suggested by Steel and Torrie [9].

3. 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 (Tables 3 and 4) 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 (Figs. 3 and 5). 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. [10] 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 [11], 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 [12] 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 in 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 (Tables 4 and 5). Nodal sections incubated in 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

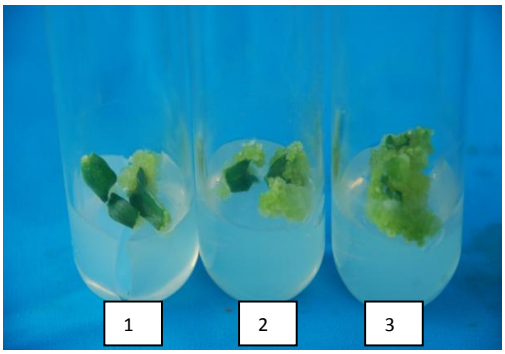

Fig. 3. Callusing in leaf explant cv. 'Scania'
1) BAP + 2,4-D = 1.00 + 1.00 mg l^{-1}
2) BAP + 2,4-D = 1.00 + 1.50 mg l^{-1}
3) BAP + 2,4-D = 1.00 + 2.00 mg l^{-1}

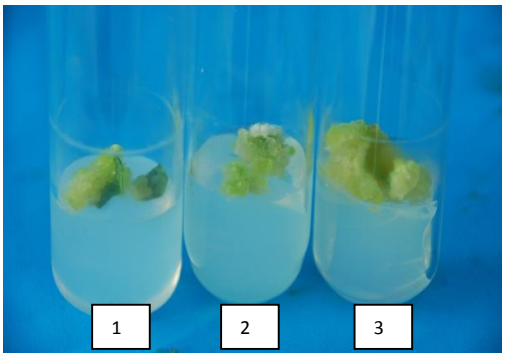

Fig. 4. Callusing in nodal explant cv. 'Scania'
1) BAP + 2,4-D = 1.00 + 1.00 mg l^{-1}
2) BAP + 2,4-D = 1.00 + 1.50 mg l^{-1}
3) BAP + 2,4-D = 1.00 + 2.00 mg l^{-1}

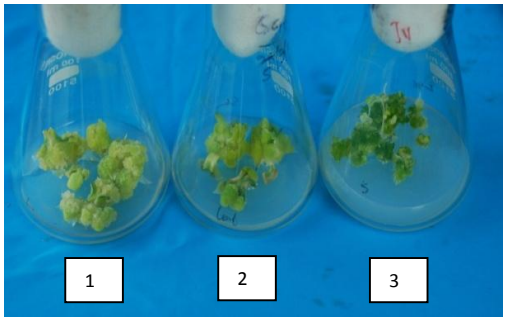

Fig. 5. Callusing in leaf explant cv. 'Indios'
1) BAP + NAA = 1.00 + 1.00 mg l^{-1}
2) BAP + NAA = 1.00 + 1.50 mg l^{-1}
3) BAP + NAA = 1.00 + 2.00 mg l^{-1}

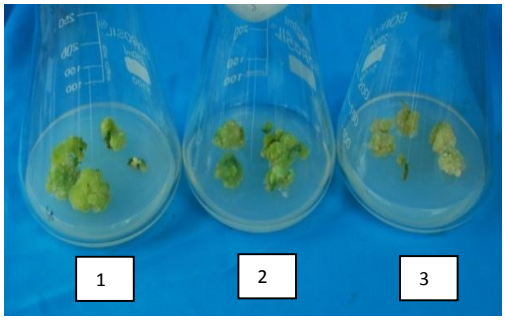

Fig. 6. Callusing in nodal explant cv. 'Indios'
1) BAP + 2,4-D = 1.00 + 1.00 mg l^{-1}
2) BAP + 2,4-D = 1.00 + 1.50 mg l^{-1}
3) BAP + 2,4-D = 1.00 + 2.00 mg l^{-1}

Table 3. Influence of growth regulators on callusing in leaf explants of carnation (*Dianthus caryophyllus* L.) cv. Scania

PGR's	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 (55.96)	1.38	Soft yellowish green
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.

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 (Figs. 4 and 6). Kanwar and Kumar [11] 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 [13] 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 4. Influence of growth regulators on callusing in leaf explants of carnation (*Dianthus caryophyllus* L.) cv. Indios

PGR's	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

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

PGR's	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

PGR's	Concentration (mg l^{-1})	Days to callus initiation	* Callus induction (%)	** Callus weight (g $explant^{-1}$)	Callus type
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**	

**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 6. Influence of growth regulators on callusing in nodal segment explants of carnation (*Dianthus caryophyllus* L.) cv. Indios

PGR's	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

4. 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'.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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