

Original Research Article

In vitro callusing of CARNATION (*Dianthus caryophyllus* L.) cv. Scania and Indios

ABSTRACT

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. 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.

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

INTRODUCTION

Carnation is indigenous to the Mediterranean area. It is believed that carnation was cultivated by the Muslims of Africa and introduced to Europe from Tunis in the thirteenth century. This may be true to one of the garden forms, but the plant was certainly known, even in England, before that date (Coats, 1956). The areas of natural climates for carnation occur near 30° North or South latitudes and on the western edges of the continents. Southern California, the Mediterranean area, Australia, Valparaiso, Chile and the Republic of South Africa are the places where large quantities of carnation are produced. Altitude can modify latitude for other suitable carnation areas: Bogota (Colombia), parts of Kenya in Africa are such examples. Bogota is only 4° North of the equator but at an altitude of 2800 metres, the climate for carnation production is as near ideal as any congenial place in the world. That is why the best carnations in the world are known to be produced in this part of South America and the supply of these Colombian carnations dominates the world flower markets during the northern winter. Carnations are cultivated on a large scale in France, Holland, Italy, Israel and Germany for their flowers.

Since carnation is one of the top selling ornamental crops worldwide (Casas *et al.*, 2010 and Gutierrez-Miceli *et al.*, 2010). The development of ‘Sim’ strains grown under heated glasshouses and polythene cover in most of the western countries and the U.S.A, has revolutionized the world carnation trade. In India, only very recently the cultivation of this

group received an attention and several cultivars have been introduced and are being tested. 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 far. 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*
 - Callus induction
 - Callus proliferation
 - Caulogenesis

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.

3.1.3.2 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 (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.

3.1.3.2.1 Days to callus initiation

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

3.1.3.2.2 Callus induction (%)

Data was recorded after 4 weeks of culture

3.1.3.2.3 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.

3.1.3.2.4 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

92 RESULTS AND DISCUSSION

93 4.1.3.1 Callusing from leaf explant of cv. 'Scania'

94 Data related to the days taken to callus initiation, callus induction (%), callus weight and
 95 callus type under the influence of different growth regulator concentrations in leaf explant of
 96 carnation (*D. caryophyllus* L.) cv. 'Scania' are presented in (Table 2). Leaf explants in MS
 97 medium fortified with BAP at 0.50 or 1.00 mg l⁻¹ in combination with 2, 4-D or NAA at 1.00,
 98 1.50 and 2.00 mg l⁻¹ recorded significant differences in means for days taken to callus
 99 initiation, callus induction (%) and callus fresh weight (g explant⁻¹) in cv. 'Scania'. Perusal of
 100 data revealed that days to callus initiation reduced with the increase in concentration of 2, 4-
 101 D from 1.00 to 2.00 mg l⁻¹. Minimum time (10.50 days) to callus initiation was recorded in
 102 media fortified with BAP 1.00 + 2, 4-D 2.00 mg l⁻¹, followed by BAP. 100 + 2, 4-D 1.50 mg
 103 l⁻¹ (11.00 days). Both the treatment combinations were at par with each other. However, a
 104 reverse trend was observed when NAA was used. Increase in concentration of NAA from
 105 1.00 to 2.00 mg l⁻¹ days to callusing were significantly delayed highest up to 17.50 recorded
 106 with BAP 1.00 + NAA 2.00 mg l⁻¹. Among NAA treatments, minimum duration of 14.66
 107 days to callus induction was recorded with BAP 0.50 + NAA 1.00 mg l⁻¹.

108 Both the auxins when used in combination with BAP had a significant effect on the callus
 109 induction. Auxin 2, 4-D proved superior to NAA in improving callus induction percentage in
 110 leaf explants. It increased with the increase in concentration of 2, 4-D but decreased at higher
 111 concentration of NAA. Highest callus induction of 91.66 per cent was recorded with growth
 112 regulator combination BAP 1.00 + 2, 4-D 2.00 mg l⁻¹ (Fig a). This was a significant
 113 improvement over the means of 80.00, 79.33, 78.33 and 74.33 per cent respectively recorded
 114 with BAP 1.00 + 2,4-D 1.50 mg l⁻¹, BAP 1.00 + 2, 4-D 1.00 mg l⁻¹, BAP 0.50 + 2, 4-D 2.00
 115 mg l⁻¹, BAP 0.50 + 2,4-D 1.50 mg l⁻¹. The other treatment combinations which recorded

70.00 or more than 70.00 per cent callus induction in leaf explants of cv. 'Scania' were BAP 0.50 + 2, 4-D 1.00 mg l⁻¹ and BAP 1.00 + NAA 1.00 mg l⁻¹ (74.00 % each) and BAP 1.00 mg l⁻¹ + NAA 1.50 or 2.00 mg l⁻¹ (70.00 % each). Significantly lowest 62.33 per cent callus induction was recorded by growth regulator combination BAP 0.50 + NAA 2.00 mg l⁻¹, followed by BAP 0.50 + NAA 1.50 mg l⁻¹ (67.33%) and BAP 0.50 + NAA 1.00 mg l⁻¹ (68.66 %). Higher concentration of BAP (1.00 mg l⁻¹) yielded significantly better callus induction percentage compared to lower concentration (0.50 mg l⁻¹).

Callus fresh weight recorded after 6 weeks of culture was significantly more in leaf explants incubated on 2, 4-D based growth regulator combinations than in NAA based ones. Significantly maximum mean callus weight 2.15 g explant⁻¹ was measured under BAP 1.00 + 2, 4-D 2.00 mg l⁻¹, followed by 1.99 g explant⁻¹ under BAP 1.00 + 2,4-D 1.50 mg l⁻¹ and 1.86 g explant⁻¹ under BAP 1.00 + 2, 4-D 1.00 mg l⁻¹. These treatments also differed significantly from each other. The other treatments involving 2, 4-D at 1.00, 1.50 and 2.00 mg l⁻¹ in combination with BAP 0.50 mg l⁻¹ measured statistically at par callus weight of 1.58, 1.61 and 1.69 g explant⁻¹, respectively. Lowest callus fresh weight of 1.05 g explant⁻¹ was produced with BAP 0.50 + NAA 2.00 mg l⁻¹, followed by BAP 1.00 + NAA 2.00 mg l⁻¹ (1.18 g explant⁻¹). The other NAA treatment combinations varied between 1.30-1.45 g explant⁻¹ callus fresh weight. BAP significantly improved the callus fresh weight when used in combination with auxins. There was a significant increase in the callus fresh weight when the concentration of BAP was increased from 0.50 to 1.00 mg l⁻¹.

Callus type in cv. 'Scania' was compact or nodular and creamy white in colour when 2, 4-D was used in combination with BAP (Fig.e) whereas it was either soft or nodular and yellowish green in colour when NAA was used in treatment combinations (Fig.f).

4.1.3.2 Callusing from leaf explant of cv. 'Indios'

Data regarding the days to callusing, callus induction (%), callus fresh weight and callus type under different growth regulator concentrations in leaf explant of carnation (*D. caryophyllus* L.) cv. 'Indios' are presented in Table 3.

Perusal of data reveal that there were significant differences in means recorded for days to callusing, callus induction per cent and callus fresh weight under the influence of various growth regulator combinations. Treatments comprised of BAP 1.00 mg l⁻¹ in combination with 2,4-D at 2.00, 1.50 and 1.00 mg l⁻¹ took lowest 12.00, 12.16 and 12.66 days, respectively for initiation of callusing in leaf explants of cv. 'Indios'. All the three treatments were at par with one another. Whereas, highest 19.00 days to callusing were

recorded with BAP 1.00 + NAA 2.00 mg l⁻¹, followed by BAP 0.50 + NAA 2.00 mg l⁻¹ (18.00 days) and BAP 1.00 + NAA 1.50 mg l⁻¹ (17.66 days). These treatment combinations were statistically at par with each other, but significantly different from rest of the treatments, which varied between 14.00 to 16.00 days taken to callusing.

Callus induction 85.00 per cent was noticed significantly highest with BAP 1.00 + 2, 4-D 2.00 mg l⁻¹. Next to this combination were also 2, 4-D based treatments i.e., BAP 1.00 + 2, 4-D 1.50 or 1.00 mg l⁻¹ (74.66 and 71.66 %) and BAP 0.50 + 2, 4-D 2.00, 1.50 or 1.00 mg l⁻¹ recording 70.00, 69.66 and 67.66 per cent callusing, respectively. The remaining six treatment combinations based on NAA concentrations recorded less percentage of callus induction as compared to 2, 4-D based treatments. Significantly lowest callusing 58.00 and 58.33 per cent was observed with BAP 0.50 + NAA 2.00 or 1.50 mg l⁻¹, respectively. Both the treatments were statistically at par with each other. Among NAA based treatments, maximum callus induction 65.33 per cent took place in BAP 1.00 + NAA 1.00 mg l⁻¹ (Fig. c). Remaining three combinations ranged between 62.00 to 64.33 per cent callus induction. Treatments containing BAP 1.00 mg l⁻¹ recorded significantly more callus induction percentage than with BAP 0.50 mg l⁻¹ in combination.

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 and callus weight explant⁻¹ under the influence of various growth regulator combinations. 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'.

180 **Table 2: Influence of growth regulators on callusing in leaf**
 181 **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 (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	

182 *Data recorded after 4 weeks of culture

183 ** Callus weight recorded after 6 weeks of culture

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

185

186 **Table 3: Influence of growth regulators on callusing in leaf**
 187 **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 + 1.50	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	

188 *Data recorded after 4 weeks of culture

189 ** Callus weight recorded after 6 weeks of culture

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

191
 192 Perusal of data regarding per cent callus induction followed a similar trend i.e.,
 193 significantly highest on MS media supplemented with 2, 4-D at higher concentration in
 194 combination with BAP at 1.00 mg l⁻¹. Whereas, significantly lowest per cent callus induction
 195 was observed in NAA treatments at higher concentrations in combination with BAP at 0.50
 196 or 1.00 mg l⁻¹. The results are in conformity with Kanwar and Kumar (2009), who reported
 197 highest callus induction in leaf explant of carnation cv. 'Indios' achieved on MS media
 198 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. 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.

230 Table 4: Influence of growth regulators on callusing in nodal segment
 231 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 + 1.50	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	

232 *Data recorded after 4 weeks of culture

233 ** Callus weight recorded after 6 weeks of culture

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

235

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 + 1.50	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

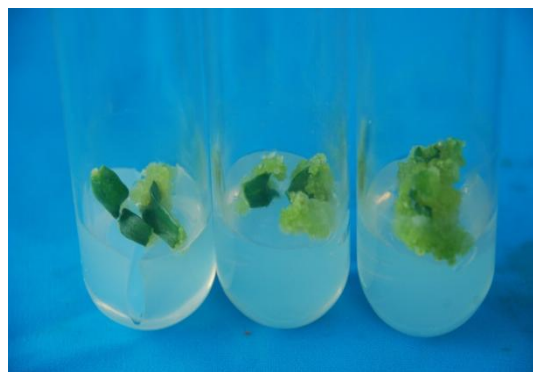


Fig.a) Callusing in leaf explant cv. 'Scania'

- 1) BAP + 2,4-D = 1.00 + 1.00 mg l⁻¹
- 2) BAP + 2,4-D = 1.00 + 1.50 mg l⁻¹

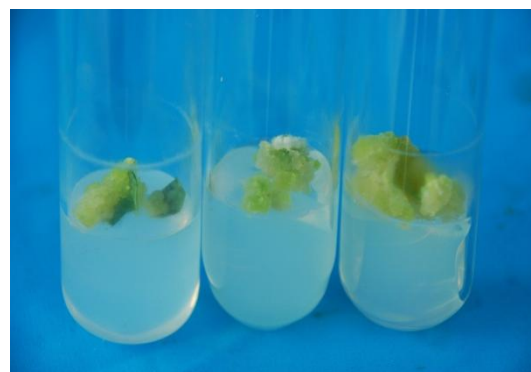


Fig.b) Callusing in nodal explant cv. 'Scania'

- 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

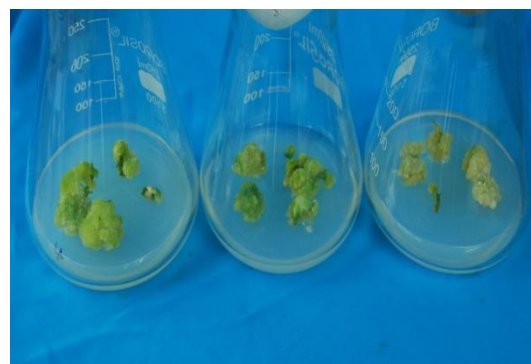


Fig.d) Callusing in nodal explant cv. 'Indios'

- 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

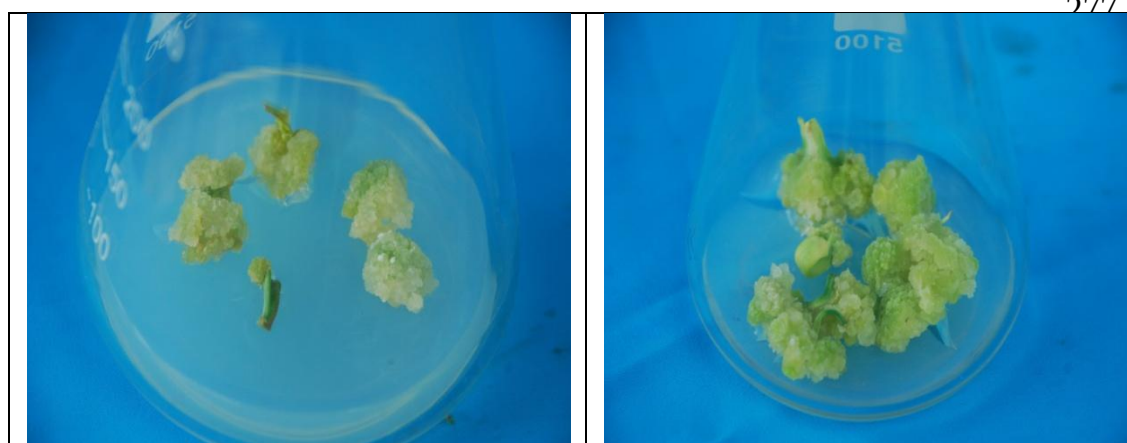


Fig.e) Callus type-I (compact, creamy white)

Fig.f) Callus type-II (soft, yellowish green)

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