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- Short communication

Indirect regeneration of carnation (*Dianthus caryophyllus* L.) through *in vitro* culture

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Carnation (*Dianthus caryophyllus* L.) is most famous for its use as a cut flower in the florist trade, but also performs well in the garden as a bedding plant (Burich *et al.*, 1996). The importance of this ornamental flower is due to its beauty, diversity of colors, excellent keeping quality and wide range of different forms (Ali *et al.*, 2008., Kanwar & Kumar, 2009). Carnation a member of the family Caryophyllous has 88 genera and 1750 species (Ali *et al.*, 2008). The consumption of carnation in world floriculture market has been increased day by day. Considering the benefits of this crop and to fulfill the world's demand for carnation, Japan, India, Pakistan and Bangladesh have started to propagate it vegetatively, thus varieties are maintained year after year by cutting or by other vegetative propagules (Karami, 2008). In this way the plants remain same phenotypically and genotypically. They may become internally infected by pathogen like fungi, bacteria and viruses which decrease their yield significantly. Genetic transformation based on tissue culture technology provides an alternative way to overcome these problems. Indirect regeneration technique is more suitable for gene transfer (Carman, 1990).

Though many authors previously reported direct *in vitro* regeneration of carnation (Altvorst *et al.*, 1992.; Miller *et al.*, 1991; Leshem, 1983), but indirect regeneration of carnation is not well documented. So far my knowledge there is no report on indirect regeneration of carnation in our country. In this regards, attempt was made to develop an efficient protocol of indirect plant regeneration in carnation.

Young leaves and internodes were collected from garden grown plants as plant materials. After sterilization, leaves and internodes were cut into small pieces (0.5-1.0 cm) for explants. Leaf segments and internodal segments were cultured on medium supplemented with different concentrations of 2,4-D alone or in combination with NAA for callus induction. Induced calli were transferred to MS with different concentrations of BAP and

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Kin alone or in combination with IAA for shoot initiation. Coconut water (CW) (5-20%) was added to the medium for further development of shoot multiplication. In order to induce root system, shoots were excised individually and cultured on half-strength of MS supplemented with different concentrations and combinations of IBA, IAA and NAA. The cultures were incubated at 25±2°C with a 16h photoperiod.

Between two types of explants of carnation, the best callus induction rate was observed in internodal explants and it was 90% at the concentration of 2.0 mg/l 2,4-D + 0.5 mg/l NAA with MS medium (Table 1). The color of the calli was greenish and loose in nature in this combination (Plate 1). The second best callusing rate was 80% in the same medium for leaf segment explants (Table 1). The calli were white friable in nature. Callus was optimally induced from both explant types within 18-20 days of inoculation when placed on above mentioned medium. Callusing was initiated at the cut ends of the explants.

Table 1. Effects of different concentrations of 2,4-D and 2,4-D with NAA employed in MS on callus induction of carnation, *Dianthus caryophyllus* L.

Growth regulators (mg/l)	Leaf segment explants		Internodal explants	
	% of explant responded	Nature of callus	% of explant responded	Nature of callus
2,4-D				
0.5	20	WC	40	WF
1.0	32	WC	48	WC
1.5	48	WC	50	WC
2.0	60	WF	70	WF
2.5	50	WF	62	WF
3.0	42	WC	50	WF
3.5	36	WC	42	WF
2,4-D + NAA				
0.5+0.5	58	WC	60	WF
1.0+0.5	60	GL	72	WF
1.5+0.5	64	WF	78	GL
2.0+0.5	**80**	**WF**	**90**	**GL**
2.5+0.5	72	WF	76	GL
3.0+0.5	50	WF	68	GL
1.5+1.0	48	WC	50	WC
2.0+1.0	60	WC	70	WC
2.5+1.0	42	WC	50	WC
3.0+1.0	40	WC	48	WC

WC= White compact, WF= White friable, GL= Greenish loose

Lumbomski & Jerzy (1989) used 2.0 mg/l 2,4-D and 0.25 mg/l NAA with MS medium for better callus induction and they got 80% greenish callus induction frequency. It is concluded that combined action of 2,4-D and NAA was more effective on greenish callus induction. Greenish calli were used for multiple shoots formation. Multiple shoots

induction occurred when the calli were transferred on MS supplimented with BAP and Kin alone or in combination with IAA. In combinations of BAP and IAA was found to be the most responsive for shoot formation from the greenish calli and 2.0 mg/l BAP + 0.5 mg/l IAA was found optimum, in which 70% of the cultured calli produced shoots within 20 days of culture. The highest mean number of shoots per culture was 10±1.24 (Table 2, Plate 2). Kim & Williams (1985) obtained highest number of shoot regeneration from carnation calli on MS+ 2.5mg/l BAP + 0.5 mg/l IAA.

For further development of the medium and enhanced shoot proliferation, coconut water (CW)(5-20% v/v) was added to the medium. Addition of 10% CW to the medium increased the number of shoots up to 15 per culture (Plate 3).

Table 2. Effects of different concentrations of cytokinins (BAP and Kin) alone or in combination with auxin (IAA) in MS on shoot proliferation from greenish calli of carnation

Growth regulators (mg/l)	% of calli regenerated shoots	Average No. of shoots per callus
BAP		
0.5	-	-
1.0	-	-
1.5	10	4.0±0.25
2.0	40	5.0±0.22
2.5	-	-
BAP+IAA		
1.0+0.5	30	5.0±0.20
1.5+0.5	48	7.0±1.12
2.0+0.5	**70**	**10.0±1.24**
2.5+0.5	20	4.0±0.25
1.5+1.0	-	-
2.0+1.0	10	3.0±0.50
2.5+1.0	-	-
3.0+1.0	-	-
Kin		
0.5	-	-
1.0	-	-
1.5	20	5.0±0.25
2.0	-	-
Kin+IAA		
1.0+0.5	-	-
1.5+0.5	30	5.0±0.40
2.0+0.5	-	-
2.5+0.5	-	-

Roy (2008) reported that addition of 10% CW in the medium increased the number of shoots in *Boerhaavia diffusa* L. culture. Regenerated shoots need to root formation for their healthy growth. So, an experiment was conducted with half-strength MS supplemented with different types of auxins (IBA, IAA and NAA). Auxin plays a major role in root induction through its effect on the first cell division which forms root initials (Farooq *et al.*, 2008). The best result was obtained in half-strength MS supplemented with 1.0 mg/l NAA (Table 2). In this combination, it was observed that 90% shoots rooted well within 15-17 days of culture and each microcutting produced 10-12 roots (Plate 4). Kharrazi *et al.* (2011) reported that NAA was more effective for *in vitro* rooting of carnation. They observed that MS with 0.5 mg/l NAA was suitable combination for best rooting. After sufficient roots development, plantlets were transplanted in small earthen pots containing a mixture of soil, sand and compost (2:1:1) (Plate 5). Sixteen plantlets were survived out of twenty in potted soil condition. The results of the study showed an efficient callus induction and plant regeneration protocol of carnation through *in vitro* culture.

Table 3. Effects of different concentration of IBA, IAA and NAA singly or in combination in half-strength MS on root induction from regenerated shoots of carnation

Growth regulators (mg/l)	% of shoots rooted	Days required for rooting	No. of roots per shoot	Average root length (cm)
IBA				
0.5	-	-	-	-
1.0	20	18-20	4-6	3±0.3
1.5	40	18-20	5-7	4±0.2
2.0	30	20-22	4-5	4±0.2
2.5	10	20-22	4-5	3±0.2
IAA				
0.5	-	-	-	-
1.0	-	-	-	-
1.5	30	20-22	3-5	4±0.3
2.0	-	-	-	-
2.5	-	-	-	-
NAA				
0.5	60	18-20	5-6	3±0.3
1.0	**90**	**15-17**	**10-12**	**5±0.2**
1.5	70	18-20	6-8	4±0.2
2.0	40	16-18	5-7	4±0.3
IBA+IAA				
0.5+0.5	-	-	-	-
1.0+0.5	30	20-22	4-6	4±0.2
1.5+0.5	40	18-20	5-6	4±0.2
IBA+IAA+NAA				
1.0+0.5+0.5	20	20-22	4-6	3±0.2
1.0+1.0+1.0	50	18-20	5-7	4±0.3
1.5+1.5+1.0	40	18-20	4-6	3±0.2

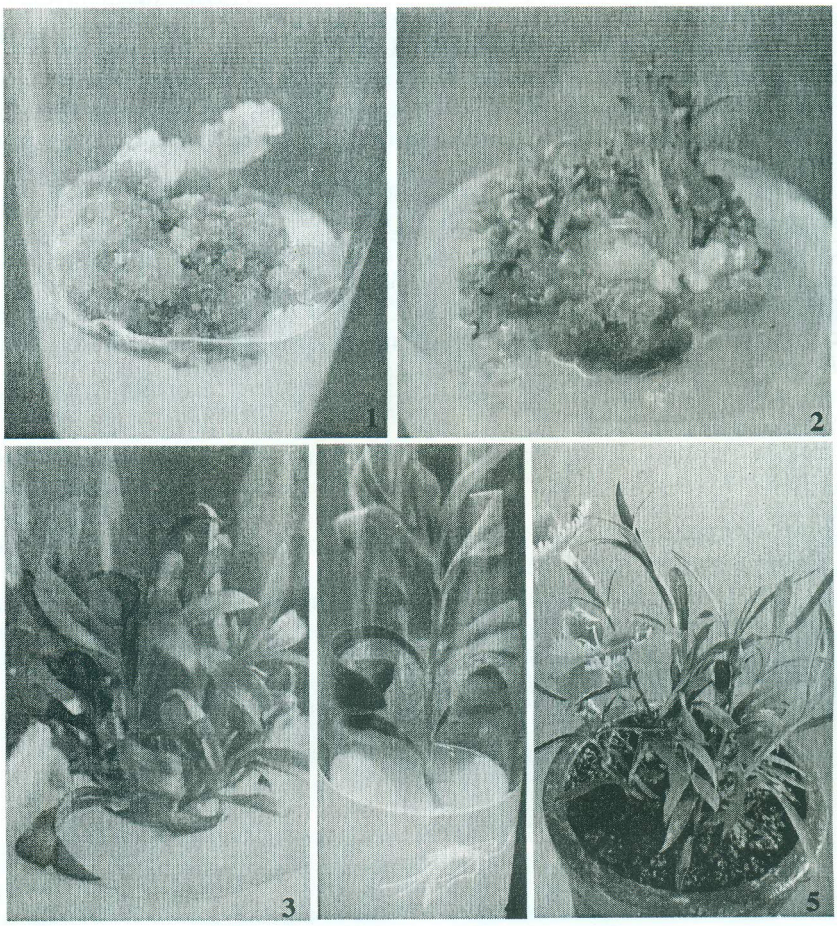

Plate 1-5. Indirect regeneration of carnation, *Dianthus caryophyllus* L. 1. Greenish callus induced from intermodal explant on MS supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l NAA. 2. Shoot regeneration from greenish callus on MS with 2.0 mg/l BAP and 0.5 mg/l IAA. 3. Positive effect of coconut water (10%) on shoot multiplication. 4. Root induction on half-strength MS supplemented with 1.0 mg/l NAA. 5. Regenerated plantlet in potted soil

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