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In vitro Regeneraton and Field Evaluation of Carnation (*Dianthus caryophyllus* L.) Through Shoot Tip and Node Culture

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ABSTRACT

An efficient and reproducible *in vitro* protocol for the large scale propagation of Carnation (*Dianthus caryophyllus* L.) has been described. Shoot tip and nodal segments were used as explants in the present experiment. Nodal explants showed more effective for shoot proliferation than shoot tip explant. Among two types of explants, nodal segment explants produced the highest number of shoots (25±0.4) when they were cultured on MS+1.0mg/lBAP. Shoot tip explants also produced multiple shoots, but their performance was not as good as nodal explants. With repeated sub culture and addition of 10% coconut water (CW) to the above mentioned medium enhanced the number of shoots per culture and incorporation of 100mg/l urea to the medium increased the length of shoots. For best rooting, well developed shoots were excised and implanted individually onto the rooting medium containing half-strength MS fortified with 1.0mg/l NAA. Within 15 days of transfer to the rooting medium, 80% microcuttings produced 10-12 roots. The complete plantlets were successfully acclimatized in potted soil. Finally, the plantlets were transferred in the experimental field. The survival rate was 80% of the planted tissue cultured plantlets.

Key words: *In vitro* regeneration, Explants, *Dianthus caryophyllus* L.

Abbreviations: MS: Murashige and Skoog (1962) basal medium, BAP: 6-benzyl amino purine, NAA: Napthalene acetic acid, CW: Coconut Water, IAA: Indole-3-acetic acid, IBA: Indole-3-butyric acetic, Kn: Kinetin.

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is one of the major floriculture crops in many countries all over the world with high ornamental and commercial interest [1]. The importance of this ornamental flower is due to its beauty, diversity of colors, excellent keeping quality and wide range of different forms [2, 3]. The name Carnation is derived from the Latin term ‘carnatio’ meaning fleshness. Caryophyllus means pink refers to the color of blooms of the original species.

For commercial cut flower production, carnations are grown in green houses by maintaining optimum growing environment. The optimum night temperature for carnation is 11-12°C during winter and 13-16°C in summer. Sandy loam soil rich in organic matter contain with pH of 5.5-6.5 are most ideal for carnation cultivation. Carnation flowers from July to August. Flowers are attractive to moths and butterflies and are pollinated by them [4]. The carnation flower is a wonderful accent to bouquets and carnation home floral arrangements. It can be used as substitute for rose petals in making syrup. An essential oil is also obtained from its flowers, which is used in perfumery. The flowers are considered to be alexiteric, antispasmodic, cardiotonic,

diaphoretic and nervine. In traditional European herbal medicine, carnation is prescribed to treat coronary and nervous disorders [5]. The demand of carnation is increasing among the flower lovers in the country day by day. It has also great export potential.

Carnations are attracted by a number of pathogens like fungi, bacteria and viruses which decrease their yield and quality. Fusarium wilt, bacterial wilt and ring spot are major diseases of carnation. Vegetative propagation cannot eliminate the pathogen from the new plants. Plant tissue culture technique can play a key role to produce large number disease free plantlets which are true-to parental type. Plant tissue culture technique has been used in this plant for commercial micropropagation and also virus elimination [3,6,7,8,9,10,11]. In recent years this technique has gained greater momentum on commercial application in the field of plant biotechnology and floriculture.

Considering the importance of this flower plant and to overcome the problems like fungal and bacterial diseases, attempt has been made to develop an efficient and reproducible protocol for the production of large number plantlets through *in vitro* culture.

MATERIALS AND METHODS

Shoot tips and nodal segments were collected from garden grown plants of Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka in winter season. The explant materials were kept in water, brought to the laboratory and washed thoroughly under running tap water for one hour in order to remove dirt on the stem surface. These were then cut into small pieces and surface sterilization was done with an aqueous solution of 0.1% $HgCl_2$ with two drops of tween 20 for six minutes under aseptic condition and rinsed five times with autoclaved distilled water to wash out any trace of $HgCl_2$. Shoot tips and nodal segments, approximately 1.5 cm in length were cut from surface sterilized stem for explants. MS basal medium was used for regeneration of plantlets through *in vitro* culture. Shoot tip and nodal segment explants were cultured on MS supplemented with different concentrations of cytokinins (Zeatin, BAP, Kn) and auxin (NAA) singly or in combination for shoot regeneration. For rooting, half-strength MS supplemented with auxins such as IBA, IAA and NAA were used. Coconut water (CW) (5-25% v/v) and urea (50-250mg/l) were added to the medium for shoot multiplication and elongation of shoots, respectively. The pH of the media was adjusted to 5.8 before adding agar. All media were gelled with 0.7% agar. The cultures were maintained at 25±2°C under cool white fluorescent light for a daily 16h photoperiod. Sub-culturing was done every three weeks interval. The well rooted *in vitro* regenerated plantlets were taken out from the test tubes and gently washed them at laboratory with tap water to free from agar. They were then transplanted to small earthen pots containing a mixture of soil and compost (2:1) and covered with transparent polyethylene lid to maintain high humidity for 7 days. After 7 days polyethylene lid was removed and after 20 days the plants were planted in the experimental field.

RESULTS AND DISCUSSION

At first step, we used very low concentrations of Zeatin and Zeatin+ NAA in MS medium to observe primary response of shoot tips and nodal segment explants (Table 1). In these experiments, results were not satisfactory. Next step, explants were cultured on MS supplemented with different concentrations of BAP, Kn and NAA alone or in various combinations for multiple shoot induction. Results of these experiments are shown in Table 2. All explants comprising shoot tips and nodal segments were cultured for direct multiple shoot regeneration. Initiation of multiple shoots in most of the explants was observed within four weeks of culture. In both shoot tips and nodal segment explants, the highest percentage of shoot

induction was observed in MS+1.0 mg/l BAP (Table 2). In the case of nodal segments, 80% of cultures were found to regenerate shoots and the number of regenerated shoots per culture was 25±0.4 on MS+1.0 mg/l BAP (Table 2, Fig.1b). In the same medium, multiple shoot induction from the shoot tip explant was 20±0.4 per culture (Table 2, Fig. 1a).

Table 1: Effect of different concentrations and combinations of growth regulators in MS medium on primary response of shoot tip and nodal segments explants of Carnation (*Dianthus caryophyllus* L.).

Growth regulators (mg/l)	% explants showing shoot regeneration		Average number of shoots/ explant	
	Shoot tip	Nodal segment	Shoot tip	Nodal segment
Zeatin				
0.25	-	-	-	-
0.5	-	-	-	-
0.75	-	-	-	-
1.0	-	-	-	-
1.25	20	30	4±0.2	5±0.2
1.5	28	40	6±0.2	8±0.3
1.75	-	-	-	-
2.0	-	-	-	-
Zeatin +NAA				
0.5+0.25	-	-	-	-
0.75+0.25	-	-	-	-
1.0+0.25	10	15	5±0.2	6±0.2
1.25+0.25	30	44	8±0.3	10±0.3
1.5+0.25	20	22	6±0.2	8±0.2

Many authors reported that MS supplemented with 1.0 mg/l BAP was more effective to highest number of shoot regeneration of carnation [2,10,12,13,14,15], which was similar with our results. In an attempt to enhance shoot proliferation CW (5-25% v/v) was added to the medium. Addition of 10% CW to the medium increased the number of shoots [nodal explant=155 (Fig. 1d), shoot tip explant= 105 (Fig. 1c)] per culture. We got a report that addition of 10% CW in the medium increased the number of shoots in Carnation culture [16]. Fruitful effect of urea on shoot elongation was also observed. Addition of 100 mg/l urea to the medium increased the length of shoots (Fig. 1e). Thus the more effective medium determined for multiplication of shoots with proper length was MS+1.0 mg/l BAP +10% CW+100 mg/l urea. A scientist reported that urea was important media factor in the *in vitro* culture of *Dianthus caryophyllus* L.[17]. Also some other scientists reported that urea was fruitful media supplement for *in vitro* shoot elongation [16, 18]. *In vitro* well developed shoots were excised from the culture vessel and implanted onto the rooting medium containing half-strength MS with different concentrations of IBA, IAA and NAA. Best response was observed when 1.0 mg/l NAA was added to half-strength MS medium (Table 3). In this combination, it was observed that 80% shoots rooted well within 8-9 days of culture and each microcutting produced 10-12 roots (Fig. 1f). A group of researchers reported that NAA was more effective for *in vitro* rooting of Carnation [2]. They observed that 1.0 mg/l NAA combination was

suitable for best rooting, which is similar with our result (Table 3). But another group of scientists reported that 0.5 mg/l NAA was best combination with half-strength MS for root formation in *Dianthus caryophyllus* [19]. For hardening, the well rooted plantlets were transferred to small earthen pots containing a mixture of soil and compost (2:1) (Fig. 1g). After hardening, the plantlets were transferred in the experimental field, where they were survived with 80% survival rate. The present work demonstrates a simple and successful protocol for *in vitro* regeneration and field evaluation of Carnation (*Dianthus caryophyllus* L.)

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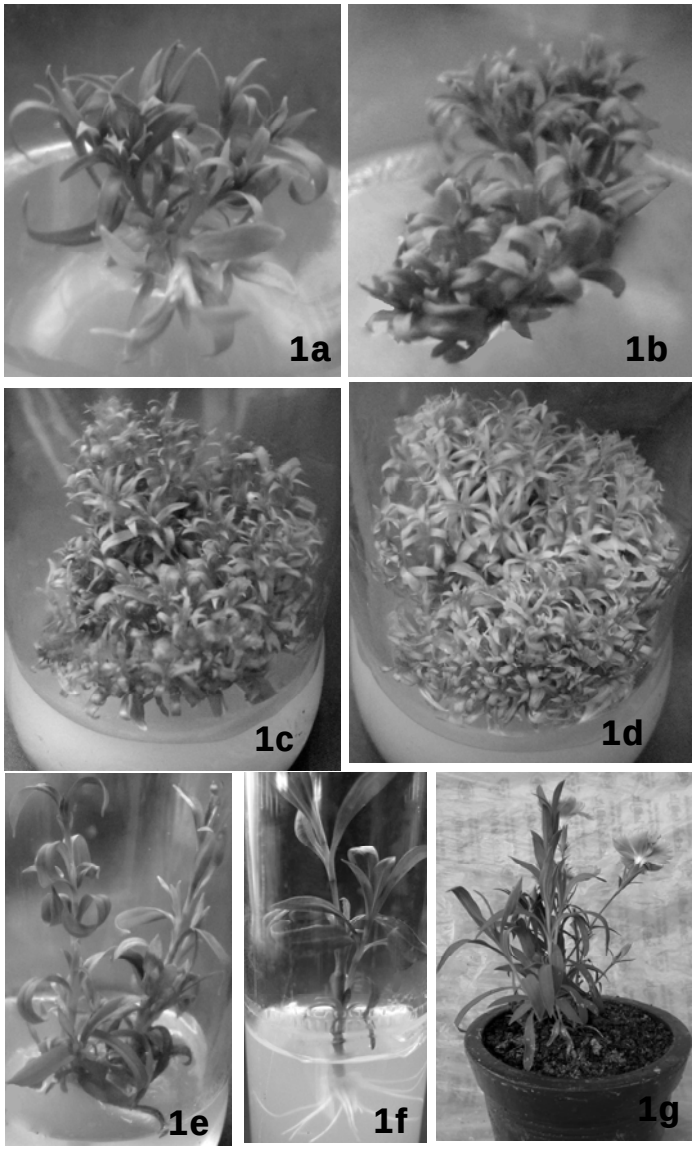
Table 2. Effect of different concentrations and combinations of growth regulators in MS on shoot proliferation from shoot tip and nodal segment explants of carnation (*Dianthus caryophyllus* L.) Data were taken after six weeks of culture.

Growth regulators (mg/l)	% explants showing shoot regeneration		Average number of shoots/ explant	
	Shoot tip	Nodal segment	Shoot tip	Nodal segment
BAP				
0.25	30	38	6±0.2	8±0.2
0.5	50	55	12±0.3	14±0.2
1.0	80	80	20±0.4	25±0.4
1.5	60	70	14±0.2	15±0.3
2.0	58	60	10±0.2	10±0.3
2.5	55	50	6±0.2	6±0.3
Kn				
0.25	30	38	5±0.3	6±0.3
0.5	40	42	6±0.2	6±0.2
1.0	45	48	5±0.3	6±0.3
1.5	50	52	7±0.3	8±0.2
2.0	40	44	6±0.3	8±0.3
2.5	45	48	5±0.2	6±0.2
BAP+NAA				
0.5 + 0.25	40	42	8±0.3	10±0.3
1.0 + 0.25	50	50	12±0.3	14±0.4
1.5 + 0.25	44	48	6±0.2	8±0.3
2.0 + 0.25	40	45	5±0.2	6±0.2
2.5 + 0.25	40	42	5±0.2	5±0.2
1.0 + 0.5	45	45	6±0.2	8±0.3
1.5 + 0.5	50	52	6±0.2	8±0.3
2.0 + 0.5	58	60	7±0.3	10±0.3
2.5 + 0.5	40	48	6±0.2	8±0.2
Kn+NAA				
0.5 + 0.25	40	42	8±0.3	10±0.3
1.0 + 0.25	42	45	10±0.3	12±0.2
1.5 + 0.25	50	58	12±0.3	14±0.3
2.0 + 0.25	44	44	10±0.3	12±0.2
1.0 + 0.5	40	42	8±0.2	10±0.2
1.5 + 0.5	50	55	10±0.2	12±0.3
2.0 + 0.5	48	50	6±0.3	8±0.2
2.5 + 0.5	42	48	6±0.2	8±0.2

Table 3. Effect of IBA, IAA and NAA in half-strength MS on root formation from regenerated shoots of *Dianthus caryophyllus* L. Data were taken after four weeks of culture.

Growth regulators (mg/l)	Rooted shoot (%)	Days required for rooting	Number of roots/ culture	Average root length (cm)
IBA				
0.5	30	10-12	5-7	1.5±0.12
1.0	48	10-12	5-7	2.2±0.14
1.5	54	9-11	7-8	2.5±0.12
2.0	40	12-14	6-8	2.4±0.14
2.5	34	12-14	4-6	2.2±0.12
NAA				
0.5	50	10-12	5-6	1.8±0.22
1.0	**80**	**8-9**	**10-12**	**3.2±0.16**
1.5	70	9-10	6-8	2.5±0.22
2.0	60	10-12	5-7	2.0±0.12
2.5	28	10-12	5-6	1.5±0.22
IAA				
0.5	30	10-12	4-6	2.4±0.14
1.0	40	9-10	5-7	2.5±0.24
1.5	50	10-12	6-8	2.5±0.22
2.0	44	10-12	5-7	1.5±0.12
2.5	28	12-14	4-6	1.8±0.16
IBA+IAA				
1.0 + 0.5	60	15-18	6-8	2.2±0.12
1.5 + 0.5	50	15-18	5-7	2.4±0.22
2.0 + 0.5	50	14-16	6-8	2.8±0.12
1.5 + 1.0	38	14-16	6-7	2.2±0.16
2.0 + 1.0	20	15-17	5-7	2.2±0.14
IBA+IAA+NAA				
1.0 + 0.5 + 0.5	34	14-16	4-5	1.8±0.22
1.0 + 1.0 + 0.5	48	12-14	5-7	2.5±0.14
1.0 + 1.0 + 1.0	60	12-14	6-8	2.8±0.16
1.5 + 1.5 + 1.5	38	14-16	4-6	1.5±0.06



Figs.1a-g. *In vitro* regeneration of *Dianthus caryophyllus*. 1a. Multiple shoot regeneration from shoot tip explant on MS+1.0 mg/l BAP. 1b. Multiple shoot regeneration from nodal segment explant on the same medium. 1c-d. Positive effect of coconut water (10%) on shoot proliferation in shoot tip explant (Fig. 1c) and nodal segment explant (Fig.1d). 1e. Elongated shoots on MS+1.0 mg/l BAP +100 mg/l urea. 1f. Root induction on half-strength of MS +1.0 mg/l NAA. 1g. Two months old *in vitro* grown plantlet in earthen pot containing a mixture of soil and compost (2:1).