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Bangladesh J. Sci. Ind. Res. **46(4)**, 495-498, 2011

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

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High Frequency *In Vitro* Regeneration of *Dianthus caryophyllus* L., a Herbaceous Perennial Ornamental Plant

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Abstract

High frequency plant regeneration was established from shoot tips and nodal explants of a perennial ornamental plant, *Dianthus caryophyllus* L. Best shoot induction was observed on MS basal medium supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA, in which 82% of the explants responded to produce maximum number of shoots (38) per culture. *In vitro* raised healthy shoots were rooted on half strength MS medium with 0.5 mg/l IBA +0.5 mg/l NAA. For acclimatization and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for 7 days before transplanting in pots where plantlets were reared for three weeks. The survival rate of regenerated plantlets was 78%.

Key words: *Dianthus caryophyllus,* Shoot proliferation, Micropropagation, Acclimatization

Introduction

Dianthus caryophyllus L., commonly known as 'Carnation' belongs to the family Caryophyllaceae. It is a herbaceous perennial plant growing up to 80 cm tall (Fig. 1a). Carnations are often worn on special occasions, especially Mother's day, Weddings, Parents Day, Father's Day and also on Teacher's Day to express love, fascination, distinction, admiration and gratitude. The plant is most popular throughout the world and economically important for cut flowers due to perpetual flowering (Mii *et al.*, 1990) and presence of single and multicolor cultivars (Dole and Wilkins, 1999). Due to high heterozygosity, for propagation of carnation negative methods are commonly used; which are not efficient because many diseases like viruses (Bierly, 1964; Bierly and Smith, 1957; Kassanin, 1955; Ram and Zaidi, 1999) or bacteria and fungi (Forsberg, 1963; Holley and Baker, 1963; Thakur *et al.,* 2002) transfer from mother stock plants to new propagules (Shibli *et al.,* 1999) also infect stem cutting of this plant (Salehi, 2006).

Tissue culture technique is a very effective method for production of plants that are free of diseases (Leshem, 1986; Pierik, 1987; Shibli *et al.,* 1997). Shoot tip and adventitious shoot culture from stem culture is not only used for producing disease free plant but it is also able to produce in large scale true to type mother plants (Leshem, 1986) that are suitable for export to other countries around the world. One such propagation system is developed that can be used in our commercial horticulture nurseries in the country.

With all these considerations the present investigation has been undertaken in order to develop a reliable repeatable and efficient method for rapid disease free micropropagation of *Dianthus caryophyllus* L.

Materials and Methods

The experiment was conducted at Biological Research Division in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Healthy and profusely growing vine of *Dianthus caryophyllus* L.was collected from the nursery of BCSIR Campus, Dhaka and used as source of explants. Shoot tips and nodal explants were used for this purpose. The explants were washed thoroughly under running tap water, pre-soaked in liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface-sterilized with 0.1% (w/v) mercuric chloride for 5 min, followed by five times rinse with sterile distilled water under laminar air flow cabinet. The surface-sterilized explants were sized to 1-1.5 cm length containing a single node with an axillary bud or a shoot tip with an apical bud. The explants were placed vertically on the culture medium. The new shoots induced from the *in vitro* cultures were further used as an explants for adventitious shoot regeneration.

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MS (Murashige and Skoog, 1962) basal medium was used for shoot proliferation and adventitious shoot regeneration and half strength MS was used for *in vitro* root induction. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 15x150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 121°C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2°C under a fluorescent light.

Shoot induction and proliferation from Shoot tips and nodal explants was obtained in two separate sets of experiments. In the first experiment 0-2.0 mg/l BAP and 0-2.0 mg/l Kn were incorporated into MS media for shoot induction. In the second set, combination of BAP (0-2.0 mg/l) with NAA (0.1-0.5 mg/l) and BAP (0-2.0 mg/l) with IAA (0.1-0.5 mg/l) were assessed for shoot multiplication. Number of new shoot proliferation of each culture was recorded every week after inoculation.

For *in vitro* rooting, individual shoots (3-5 cm) were excised from the proliferated shoot cultures and implanted onto half strength MS media with different concentrations and combinations of NAA, IBA and IAA.

The rooted plants were taken out from the culture tubes, washed to remove agar gel adhered to the roots and trans planted to plastic pots with soil and compost (1: 1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2°C under a 12 h photoperiod, for acclimation. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tips and nodal explants of *Dianthus caryophyllus* L. were cultured on MS media supplemented with BAP alone and various concentration of BAP with NAA or IAA for multiple shoot regeneration. The explants were found to be swollen and they produced two to three shoots within three-four weeks after inoculation (Fig. 1b and c) on MS media containing BAP alone but the number of shoots increased up to 16 when the explants were cultured in MS with 0.5 mg/l BAP + 0.1 mg/l NAA (Fig. 1d). Both the explants responded in the same medium but highest numbers of micro shoots were observed to be induced from nodal explants (Table I, Fig.1e). Combinations of BAP alone and BAP with IAA were not found to be more suitable than BAP with NAA for shoot induction (Table I) and combinations of Kn alone and Kn with NAA or IAA were also not found to be more suitable for shoot induction (Data were not shown). Newly initiated shoots were separated and subcultured repeatedly in fresh MS with 0.5 mg/l BAP + 0.1 mg/l NAA , where the number of shoots increased up to 38.2 ± 2.35 per culture (Fig. 1f). Salehi (2006) reported that the best shoot proliferation media were MS containing 3.0 mg/l Kn and 0.5 mg/l NAA or 1.0 mg/l BAP and 1.0 mg/l NAA; in *Dianthus caryophyllus* L., average of shoot numbers produced in the establishment media was 2 to 3, which increased to 30 or higher with some cultivers in subsequent subcultures. In *Dianthus caryophyllus* L., it was also observed by other researchers that multiple shoots were developed by using different concentration of cytokinin with auxins (Shibli *et al.* 1999; Jain *et al.* 2001; Ghani *et al.* 2008).

Table I: Effect of growth regulators in MS on morphogenic response of *Dianthus caryophyllus* L. shoot tips and nodalexplants

Growth regulators (mg/l)			Shoot tips		Nodal segments	
BAP	NAA	IAA	% of explants forming shoots	Mean No. of Shoot/explant	% of explants forming shoots	Mean No. of Shoot/explant
Without	growth	regulators	-	-	-	-
0.5			68.8 ± 1.35	26.2 ± 1.56	71.2 ± 1.35	32.4 ± 1.63
1.0			62.2 ± 1.88	22.8 ± 2.15	68.2 ± 1.06	24.4 ± 1.14
1.5			57.2 ± 1.96	18.4 ± 0.50	62.6 ± 1.84	20.4 ± 0.82
2.0			52.0 ± 1.14	14.6 ± 0.81	59.6 ± 1.36	16.6 ± 0.77
0.5	0.1		74.4 ± 1.50	32.8 ± 1.65	82.2 ± 3.07	38.2 ± 2.35
1.0	0.2		62.6 ± 2.16	26.0 ± 1.30	72.6 ± 1.70	32.4 ± 1.63
1.5	0.5		58.8 ± 1.77	22.4 ± 1.36	63.8 ± 2.14	27.8 ± 0.99
2.0	0.5		52.6 ± 0.87	18.0 ± 1.14	56.6 ± 2.10	23.2 ± 0.59
0.5		0.1	69.4 ± 1.57	24.6 ± 0.50	71.4 ± 2.38	30.6 ± 1.08
1.0		0.2	63.4 ± 2.87	21.4 ± 0.92	67.6 ± 2.16	26.4 ± 0.45
1.5		0.5	51.2 ± 0.86	18.2 ± 0.86	18.4 ± 0.93	22.2 ± 0.76
2.0		0.5	48.6 ± 1.66	14.4 ± 0.72	32.6 ± 1.63	18.4 ± 0.45

Results are mean ± SE of three experiments with 15 replications.

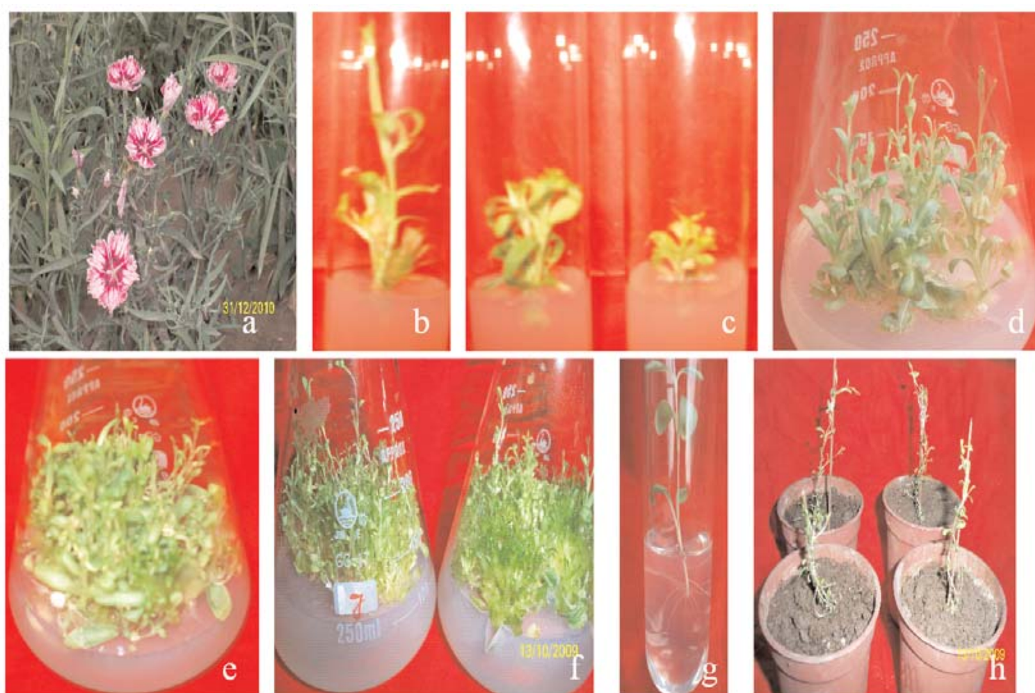

Fig. 1: *In vitro* regeneration of *Dianthus caryophyllus* L. from shoot tips and nodal explants

(a) Explants collected from field grown mature ornamental plants.

(b) Induction of shoots in third weeks of culture on MS + 0.5 mg/l BAP + 0.1 mg/l NAA from shoot tips.

(c) Induction of shoots in third weeks of culture on MS + 0.5 mg/l BAP + 0.1 mg/l NAA from nodal explants.

(d) Development and multiplication of shoots on MS + 0.5 mg/l BAP + 0.1 mg/l NAA from nodal explants after six weeks of culture.

(e) Development and multiplication of shoots on MS + 0.5 mg/l BAP + 0.1mg/l NAA from nodal explants after nine weeks of culture.

(f) Development and multiplication of shoots on MS + 0.5 mg/l BAP + 0.1 mg/l NAA from nodal explants after twelve weeks of culture.

(g) Rooting of *in vitro* regenerated shoots cultured on half strength MS + 0.5 mg/l IBA in third weeks.

(h) Acclimatized regenerated plants (two months old).

In the present study, 78.2% regenerated shoots rooted (Fig. 1g) when cultured individually on root induction medium, consisting of half-strength MS medium with 0.5 mg/l IBA + 0.5 mg/l NAA (Table II). Use of auxins singly or in combination for rooting was also reported by different authors in *Dianthus caryophyllus* L. and other ornamental plants (Ilahi *et al.* 1995; Salehi and Khosh-Khui 1996; Jagannatha *et al.* 2001; Ghani *et al*. 2008).

After four weeks, plantlets with fully expanded healthy leaflets and well developed roots were removed from the culture tubes and washed thoroughly to remove all traces of agar and then transferred to pots. None of the plantlets survived when directly transferred from the rooting medium to the pot, under natural conditions. About 85 percent of the transplanted plantlets of *Dianthus caryophyllus* L. survived, when the plantlets in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and reared for three weeks. The plantlets were reared under semi-controlled temperature (30±2°C) and light (2000 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded, turned deep green and became healthier (Fig. 1h).

After three weeks, the experimental plants were transferred to an open space and gradually acclimated to outdoor conditions, where 78 percent plants were survived. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation. This could be considered for conservation and commercial propagation of *Dianthus caryophyllus* L., an economically important cut flower plant in the country.

Table II: Effect of auxin(s) on root induction in regenerated shoots of *Dianthus caryophyllus* L. on half strength MS

Growth regulators (mg/l)			% of shoots producing roots (±SE)	No. of roots /shoot (±SE)
IBA	NAA	IAA		
0.5			72.2±1.08	6.4±0.78
0.75			67.2±1.53	5.8±0.59
1.0			63.2±1.46	4.2±0.76
	0.5		71.0±0.10	5.6±0.72
	0.75		57.8±1.85	4.2±0.76
	1.0		54.2±1.53	3.0±0.63
0.5	0.5		78.2±1.28	7.4±0.67
1.0	1.0		59.4±1.08	6.2±0.71
0.5		0.5	65.2±1.16	5.8±0.65
1.0		1.0	61.4±0.75	4.6±0.96
0.5	0.5	0.5	62.6±0.93	4.6±0.72
1.0	1.0	1.0	54.4±1.63	3.2±0.76

Data were recorded after four weeks of culture. Results are mean ± SE of 15 replications.

Acknowledgement

The authors are indebted to Mr. Jasim Uddin Chowdhury, Director, BCSIR Laboratories, Dhaka and to Professor, Samir Ali, Vice-Principal, Savar College, Savar, Dhaka for their kind permission, sincere suggestions and moral support during the research work.

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Received : April, 04, 2010;
Accepted : March 03, 2011