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Effect of Kinetin and NAA on Regeneration of Carnation (*Dianthus caryophyllus* L.) from Shoot-tip Explants

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Abstract

Optimization of *in-vitro* culture condition of carnation bears high commercial interest for which identification of suitable regenerating media is an essential component. An experiment was undertaken involving 14 different regeneration media to identify effective media composition to get carnation plantlets *in-vitro* from shoot-tip explants. Results from two consecutive years revealed that MS medium supplemented with NAA (1 mg L^{-1}) + Kinetin (2.5 mg L^{-1}) and NAA (1 mg L^{-1}) + Kinetin (3.5 mg L^{-1}) were equally effective for production of proliferating cultures (99.45%) whereas response to other media were not satisfactory. A composition of MS medium supplemented with NAA (0.5 mg L^{-1}) + Kinetin (0.5 mg L^{-1}) and NAA (1 mg L^{-1}) + Kinetin (0.5 mg L^{-1}) showed inhibitory effect on proliferation. Earliest sign of proliferation (3.97 days) was noticed using a culture medium containing NAA (1 mg L^{-1}) and Kinetin (2.5 mg L^{-1}). Explants cultured on the same medium also produced longest shoots (6.60 cm) and highest number of average leaves culture^{-1} (33.07). Maximum number of shoots culture^{-1} (6.73) was obtained from the explants cultured on MS medium supplemented with NAA (1 mg L^{-1}) and Kinetin (3.5 mg L^{-1}). A study on root induction of regenerated shoots of carnation revealed that MS medium supplemented with IBA (4 mg L^{-1}) + NAA (1 mg L^{-1}) is the most effective rooting medium among four different media compositions, imparting rooting of 98.20% shoots and hence may be utilized for better root induction of carnation explants *in-vitro*.

1. Introduction

Flower production and consumption of floricultural products occur predominantly in the European countries and Japan. In 2006, the worldwide export of floricultural products was estimated at US $ 5051.04 million (Satya and Maitra, 2007). Netherlands plays the dominant role in the export of cut-flowers and potted plants. The other countries Israel, Kenya, Colombia, Italy, Thailand and India are becoming additional flower producing and consuming nations. The potential annual demand for planting material is 16 trillion units of propagules worldwide valued at US $ 4 trillion per year. Netherlands, USA, Germany, Japan, Russia and UK are the major importing countries from India (Reddy, 2003). The most important ornamental plant species like orchids, roses, carnation, gerbera, anthurium are now-a-days being used for commercialization at large scale levels. Production of disease-free elite plantlets is obligatory to meet the increasing demand of the Floriculture Industry (Jain et al., 1997). With a view to this, in this report, an attempt was made to develop an efficient plant regeneration protocol for carnation using tissue-culture technique.

2. Materials and methods

The experiment was conducted in the plant tissue culture laboratory of the Department of Botany, University of Kalyani, Kalyani, West Bengal, India. Healthy shoot-tips of explants of *Dianthus caryophyllus* cv. Chabaud Super Mix were collected from the 45-60 day old seedlings. Surface decontamination of the explants consisted of treatment with Carbendazim (2 g L^{-1}) and passage through 5% ($^{v}/_{v}$) Teepol, a commercial detergent,

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for 10 minutes and 0.1% ($^{w}/_{v}$) $HgCl_2$ for 4-5 minutes. After 5-6 minutes in sterile distilled water, shoot-tips (0.3-0.5 cm) were dissected out of the cuttings, rinsed in sterile distilled water for several times and blotted on sterile filter paper discs before planting them vertically on semi-solid Murashige and Skoog (1962) nutrient medium containing 0.8% ($^{w}/_{v}$) agar-agar and varied concentrations and combinations of Kinetin and NAA. Microshoots were transferred into rooting medium containing IBA and NAA in different concentrations and combinations. The different media along with notations are presented in Table 1. The pH of the medium was adjusted to 5.7 before

Table 1: Composition of the media used for *in-vitro* culture of carnation (concentration is expressed as mg L^{-1})

Media used	Basal medium	Kinetin	NAA	IBA	IBA
For direct regeneration					
K_1	MS	0.5	0.5	-	
K_2	MS	1.0	0.5	-	
K_3	MS	1.5	0.5	-	
K_4	MS	2.0	0.5	-	
K_5	MS	2.5	0.5	-	
K_6	MS	3.0	0.5	-	
K_7	MS	3.5	0.5	-	
K_8	MS	0.5	1.0	-	
K_9	MS	1.0	1.0	-	
K_{10}	MS	1.5	1.0	-	
K_{11}	MS	2.0	1.0	-	
K_{12}	MS	2.5	1.0	-	
K_{13}	MS	3.0	1.0	-	
K_{14}	MS	3.5	1.0	-	
For rooting					
CR_1	MS	-	1.0	1.0	
CR_2	MS	-	1.0	2.0	
CR_3	MS	-	1.0	3.0	
CR_4	MS	-	1.0	4.0	

adding agar-agar and sucrose and autoclaved at 121^{0}C for 15 minutes. All the cultures were incubated at 25 ± 1^{0}C under 12h photoperiod at a photon flux density of 20-30 μmol m^{-2} S^{-1} from daylight fluorescent tubes. Each treatment consisted of 15 explants. The experiment was laid following completely randomized design (CRD) with three replications.

Collected data regarding percentages were converted following arc-sine transformation procedure. For statistical significance testing both analysis of variance (F test) and Student's t-test were used.

3. Results and Discussion

The efficiency of culture media on direct regeneration of shoots from shoot-tip explants of carnation *in-vitro* was found statistically significant in both the individual years and in the pooled effect too. Two culture media, MS medium supplemented with NAA 1.0 mg L^{-1} and kinetin 2.5 mg L^{-1} (K_{12}) as well as NAA 1.0 mg L^{-1} and Kinetin 3.5 mg L^{-1} (K_{14}) were found to be the most efficient medium (Table 2) as observed from the percentage of regeneration of carnation plantlets from shoot-tip explants (99.45%). MS medium containing NAA 0.5 mg L^{-1} and Kinetin 0.5 mg L^{-1} (K_1) and NAA 1.0 mg L^{-1} and Kinetin 0.5 mg L^{-1} (K_8) were found inhibitory regarding the regeneration of carnation shoot-tip explants *in-vitro*.

Significant differences were observed between the effects of different culture media on the time period requirement for proliferation. The earliest sign of proliferation were noticed when the explants were cultured on K_{12} (3.87 days, 4.07 days and 3.97 days in the first year, second year and in pooled effect respectively). Delayed effect was noticed with the media K_9, K_2, K_3, K_{10} and K_4. Regeneration media like K_{14} (4.27 days), K_{13} (5.07 days), K_7 (6.87 days), K_{11} (6.90 days), K_5 (8.20 days) and K_6 (9.23 days) performed moderately well for the proliferation of carnation explants (Table 2). The two regeneration media K_1 and K_8 showed no response regarding the proliferation of carnation shoot-tip explants *in-vitro*.

Explants cultured on K_{14} produced maximum number of shoots $culture^{-1}$ (6.73 shoots $culture^{-1}$). Regeneration media - K_{12}, K_6, K_{13}, K_5 and K_{11} performed moderately well in this regard while other media were found less effective. Regeneration media like K_1 and K_8 were found non-responsive producing hardly any shoot in the carnation explants *in-vitro* (Table 3). K_{12} was established as the most effective regeneration medium producing longer microshoots (6.60 cm), which was equally statistically effective with K_{14} (6.27 cm), followed by the explants cultured on K_7, K_6, K_5, K_{13} and K_4. The rest of the culture media were found less effective in this regard. Number of leaves $culture^{-1}$ was found maximum from the explants cultured on K_{12} (33.07 leaves per culture) followed by K_{14} and K_7. Moderately fair number of leaves was produced by K_4, K_5, K_6, K_{11} and K_{13}. The rest of the culture media were found less effective in respect of leaf production of carnation *in-vitro*. Regeneration media K_1 and K_8 were found non-responsive.

From the results it is evident that for regeneration of carnation the two concentrations (2.5 and 3.5 mg L^{-1}) of kinetin were found equally effective regarding the percentage of proliferating cultures (99.45%), but kinetin @2.5mg L^{-1} showed earliness (3.97 days) in shoot proliferation than concentration of kinetin @3.5 mg L^{-1} (4.27 days). Shoot length and leaf number were also found better with this concentration. Regarding the number of shoots $culture^{-1}$, kinetin at 3.5 mg L^{-1} was found most satisfactory than the all other concentrations of kinetin. The beneficial effects of kinetin on the regeneration of carnation have been experienced by other workers (Ram and Zaidi, 1999; Mujib and Pal, 1994; Jagannatha et al., 2002). NAA in various concentration and combination has been found to be effective

Table 2: Effect of culture media on proliferation of carnation (cv. Chabaud Super Mix) shoot-tip explants *in-vitro*

Treatments	Percentage of proliferating cultures			Days required for proliferation		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled
K_1	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	-	-	-
K_2	44.44 (42.09)	50.00 (45.30)	47.22 (43.70)	27.27	26.73	27.00
K_3	46.67 (43.38)	42.22 (40.81)	44.44 (42.10)	18.93	19.27	19.10
K_4	67.78 (55.77)	75.55 (60.87)	71.67 (58.32)	11.87	12.27	12.07
K_5	98.89 (86.77)	97.78 (83.55)	98.34 (85.16)	7.47	8.93	8.20
K_6	94.45 (77.56)	92.22 (74.98)	93.34 (76.27)	9.60	8.87	9.23
K_7	94.44 (77.19)	95.56 (78.75)	95.00 (77.91)	6.60	7.13	6.87
K_8	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	-	-	-
K_9	51.11 (45.93)	48.89 (30.98)	50.00 (38.46)	29.20	26.73	27.97
K_{10}	54.45 (47.84)	56.67 (49.13)	55.56 (48.49)	16.07	16.60	16.33
K_{11}	70.00 (57.12)	72.22 (58.58)	71.11 (57.85)	6.53	7.27	6.90
K_{12}	100.00 (90.00)	98.89 (86.77)	99.45 (88.38)	3.87	4.07	3.97
K_{13}	82.22 (65.59)	83.33 (66.36)	82.78 (65.98)	4.93	5.20	5.07
K_{14}	98.89 (86.77)	100.00 (90.00)	99.45 (88.38)	4.13	4.40	4.27
SEm ±	1.92	4.13	2.28	0.71	0.84	0.55
CD (*p*=0.05)	5.56	11.96	6.47	2.08	2.44	1.56

Figures in the parenthesis are the transformed values

Table 3: Effect of culture media on shoot regeneration of carnation (cv. Chabaud Super Mix) from shoot-tip explants *in-vitro*

Treatments	No. of shoots culture^{-1} at the time of separation to individual shoots			Shoot length (cm) at the time of separation to individual shoots			No. of leaves culture^{-1} at the time of separation to individual shoots		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
K_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
K_2	0.47	0.36	0.41	0.43	0.63	0.53	2.07	3.60	2.83
K_3	0.87	1.07	0.97	1.50	1.41	1.46	4.53	5.33	4.93
K_4	1.67	2.13	1.90	2.64	3.17	2.90	12.53	10.93	11.73
K_5	2.73	3.93	3.33	3.89	4.93	4.41	24.60	25.60	25.10
K_6	3.27	4.07	3.67	4.39	4.72	4.56	25.53	27.60	26.57
K_7	4.53	5.07	4.80	5.65	5.83	5.74	30.53	32.67	31.60
K_8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
K_9	0.73	0.80	0.77	0.65	0.67	0.66	4.20	4.93	4.57
K_{10}	1.13	1.53	1.33	1.58	1.54	1.56	5.13	5.53	5.33
K_{11}	2.47	2.33	2.40	3.23	3.18	3.20	15.53	14.93	15.23
K_{12}	4.20	4.47	4.33	6.54	6.66	6.60	32.73	33.40	33.07
K_{13}	2.80	4.13	3.47	3.56	3.71	3.64	11.07	18.40	14.73
K_{14}	5.80	7.67	6.73	6.73	5.82	6.27	29.07	34.87	31.97
SEm ±	0.26	0.22	0.17	0.37	0.40	0.27	1.22	1.45	0.95
CD (*p*=0.05)	0.74	0.63	0.48	1.07	1.16	0.77	3.54	4.19	2.68

for shoot regeneration in carnation. Direct shoot formation from shoot-tip explants of *Dianthus caryophyllus* was possible with addition of NAA (0.5-1.0 mg L^{-1}), while NAA at 1.0 mg L^{-1} proved to be more suitable auxin for shoot regeneration in carnation. Increase in the concentration of NAA from 0.5 to 1.0 mg L^{-1} also pronounced the effect of kinetin. The effect of NAA on shoot regeneration of carnation was also noticed by Lubomski and Jerzy (1989), Choudhary and Mubarack (1991),

Mangal et al. (2001) and Pareek et al. (2004).

MS medium when supplemented with NAA (1 mg L^{-1}) + IBA (4 mg L^{-1}) [CR_4] showed maximum percentage of rooting of explants (98.20%) statistically at par with the MS medium (CR_2) supplemented with NAA (1 mg L^{-1}) + IBA (2 mg L^{-1}) (96.00%). However, microshoots cultured on CR_1 produced lowest percentage of rooting of explants (7.60%). Microshoots cultured on CR_2 struck roots earliest (11.80 days) followed by the microshoots cultured on CR_4 (14.32 days). The other media produced less marked effect (Table 4) but the most delayed effect was noticed with the rooting medium CR_1 (28.10 days).

Microshoots cultured on CR_2 and CR_4 showed better rooting and were ready for transplanting after 30 days of culture (Table 5 and Table 6). On the other hand, microshoots cultured on that medium produced 7.24±0.94 number of 0.35±0.04 cm long roots $shoot^{-1}$ (Table 7) at the time of transplanting (45 days after transfer to the rooting media).

Rooting of shoots may occur satisfactorily when cultures are allowed to stand for long period under illumination (Plate 1). Two different auxins – NAA and IBA were used for rooting of carnation *in-vitro* in different concentrations and combinations among them MS medium supplemented with NAA 1 mg L^{-1} and IBA 4 mg L^{-1} was found suitable for better rooting than the low hormone medium.

But, earliness in rooting (Table 4) and initially greater number of roots $shoot^{-1}$ (Table 5) were obtained from medium containing NAA 1 mg L^{-1} and IBA 2 mg L^{-1}. This medium produced an average number of 7.18 roots after 15 days of culture with good root length which was initiated only 12 days after culture. Afterwards root number (16.08) and length (1.31 cm) both were increased in the microshoots cultured on CR_4 (Table 6). Wankhade et al. (2006) also reported the use of IBA in the rooting of carnation microshoots.

The ultimate success of micropropagation is hardening and well establishment in the pot or field. It was observed that 80% establishment was possible at potting mix containing sand, soil and screened and sterile cowdung manure (Plate 1). During hardening a thin film of water was kept on the surface of leaf. It was facilitated to keep the plants cool and compensate the water loss by transpiration. Firstly the plantlets were kept in polyhouse under 100% shade condition. After 12 days water was reduced and plants were kept at 75% shade. The plant

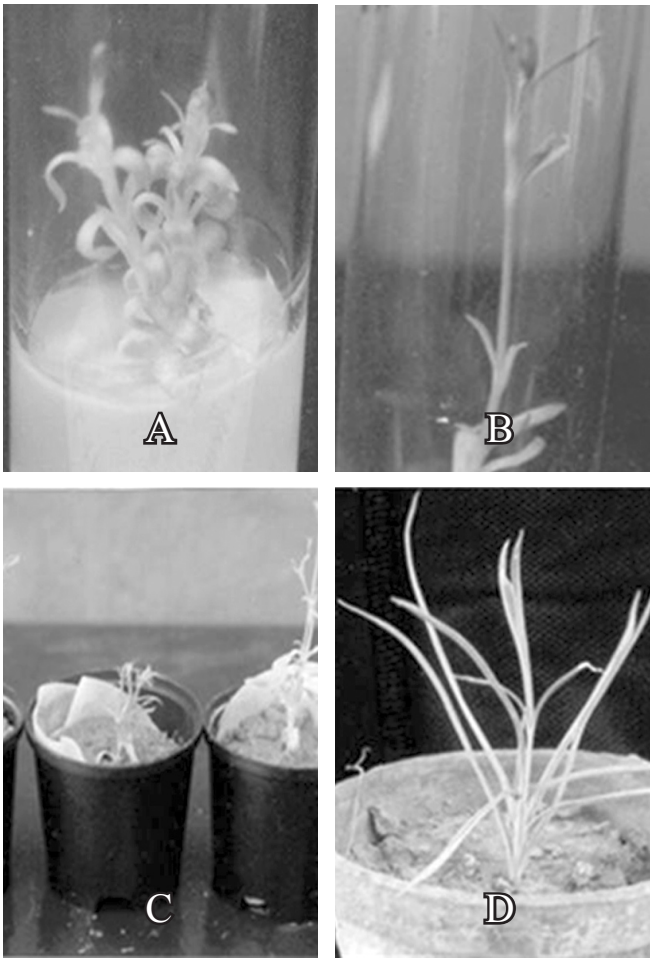

Plate 1: *In-vitro* regeneration and establishment of carnation. A. Initiation of microshoots, B. Development of shoots, C. Hardening of regenerated plants, D. Field establishment of regenerated plants

Table 4: Effect of culture media on the rooting of microshoots of carnation (cv. Chabaud Super Mix)

Treatments	Percent of microcuttings rooted			Days required for initiation of roots		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled
CR_1	7.20 (15.40)	8.00 (16.20)	7.60 (15.80)	28.90	27.30	28.10
CR_2	95.60 (79.54)	96.40 (80.40)	96.00 (79.97)	11.76	11.84	11.80
CR_3	80.40 (63.87)	83.60 (66.21)	82.00 (65.04)	24.24	22.20	21.72
CR_4	98.40 (84.44)	98.00 (83.76)	98.20 (84.10)	14.32	14.32	14.32
SEm ±	2.20	2.09	1.52	0.45	0.35	0.28
CD (*p*=0.05)	6.60	6.27	4.37	1.36	1.04	0.82
Figures in the parenthesis are the transformed values						

Table 5: Effect of media on the rooting of microshoots (after 15 days of culture) of carnation (cv. Chabaud Super Mix)						
Treatments	Root number (after 15 days)			Root length (cm) after 15 days		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled
CR_1	0.00	0.00	0.00	0.00	0.00	0.00
CR_2	7.52	6.84	7.18	0.48	0.46	0.47
CR_3	0.00	0.00	0.00	0.00	0.00	0.00
CR_4	5.28	5.16	5.22	0.54	0.50	0.52
SEm ±	0.13	0.22	0.13	0.01	0.01	0.01
CD (p=0.05)	0.40	0.66	0.37	0.03	0.03	0.03

Table 6: Effect of media on the rooting of microshoots (after 30 days of culture) of carnation (cv. Chabaud Super Mix)						
Treatments	Root number (after 30 days)			Root length (cm) after 30 days		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled
CR_1	0.00	0.00	0.00	0.00	0.00	0.00
CR_2	14.60	13.12	13.86	1.27	1.21	1.24
CR_3	5.32	5.20	5.26	0.47	0.66	0.56
CR_4	16.32	15.84	16.08	1.31	1.31	1.31
SEm ±	0.29	0.38	0.24	0.03	0.10	0.05
CD (p=0.05)	0.88	1.13	0.69	0.09	0.31	0.16

Table 7: Effect of media on the rooting of microshoots (after 45 days of culture) of carnation (cv. Chabaud Super Mix						
Treatments	Root number (after 45 days)			Root length (cm) after 45 days		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled
CR_1	8.24±1.24	6.24±1.30	7.24±0.94	0.36±0.07	0.35±0.05	0.35±0.04
CR_2	Transplanted	Transplanted	Transplanted	Transplanted	Transplanted	Transplanted
CR_3	15.56±1.44	14.28±1.66	14.92±1.22	1.18±0.08	1.18±0.05	1.18±0.05
CR_4	Transplanted	Transplanted	Transplanted	Transplanted	Transplanted	Transplanted
t value	19.31	19.21	25.14	38.66	58.69	65.22
t value (tabulated) = 2.0126						

growth was prominent after a month. A similar procedure was used by Rabindra and Thomas (1995) for hardening of grape plants.

4. Conclusion

MS medium supplemented with NAA (1 mg L^{-1}) and Kinetin (3.5 mg L^{-1}) produced maximum number of shoots culture^{-1} (6.73) during *in-vitro* regeneration of carnation from shoot tip explants. But earliness in the sign of proliferation was observed using a culture medium containing NAA (1 mg L^{-1}) and Kinetin (2.5 mg L^{-1}). The study on root induction of regenerated microshoots of carnation revealed that MS medium supplemented with IBA (4 mg L^{-1}) + NAA (1 mg L^{-1}) is the most effective rooting medium imparting rooting of 98.20% microshoots and hence may be utilized for better root induction of carnation explants *in-vitro*. Our findings from present research work provide a number of suitable media for *in-vitro* regeneration and plantlet establishment of carnation which will be helpful for the plant breeders and biotechnologists involved in the improvement of carnation.

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