Effect of Cytokinins and Auxins on *In-vitro* Regeneration of Carnation (*Dianthus caryophyllus* L.) through Callusing

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Abstract

An attempt was made to identify suitable regeneration protocol for establishment of carnation plantlets through callusing. 'Chabaud Super Mix', a commercial carnation cultivar, was used for callus induction in twelve different callusing media followed by seven regeneration media in two consecutive years. The study indicated that auxin (2, 4-D & NAA) and cytokinin (kinetin & BA) are responsible for the callus production of carnation from terminal young unfurled leaf explants. Basal MS medium along with 2,4-D (10 mg l^{-1}) and NAA (1 mg l^{-1}) and combination of BA (3 mg l^{-1})+NAA (2 mg l^{-1}) produced nodular, greenish yellow to greenish profuse callus within 8-10 days of inoculation. MS medium having BA (3 mg l^{-1}) and NAA (2 mg l^{-1}) initiated callus earlier. Profuse amount of nodular greenish callus was found on the MS medium having NAA (1 mg l^{-1}) and 2,4-D (5 mg l^{-1}). Supplementation with kinetin (2.5 mg l^{-1})+NAA (1 mg l^{-1}) in MS medium resulted into earliest initiation of shoots. MS medium supplemented with kinetin (4 mg l^{-1})+NAA (1 mg l^{-1}) helped in more rapid shoot development. Therefore, to get early plantlet establishment MS medium having NAA (1 mg l^{-1}) and 2,4-D (5 mg l^{-1}) followed by kinetin (4 mg l^{-1})+NAA (1 mg l^{-1}) might be an effective choice in carnation *in-vitro* culture. 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

Carnation (*Dianthus caryophyllus* L.) is one of the important export-oriented cut flowers having commercial value worldwide. It is an attractive ornamental flower valued for high keeping quality and diversity of color (Kharrazi et al., 2011). Only in USA the total production of standard carnations reached 20.58 million stems in 2002 with a market value of $ 3.24 million (USDA, 2003). With the increase in day to day use and market competition, production of large number of elite planting materials at shortest possible time is an essential prerequisite for carnation cultivation. Production of carnation through conventional soft wood cuttings requires large number of mother stocks, demands involvement of skilled manpower as well as produces fewer amounts of saplings per unit of time. Micropropagation, on the other hand offers a better opportunity for generating enormous number of plantlet clones within a very short period. Although few protocols are available for *in-vitro* regeneration and micropropagation of carnation, the processes of callusing, shoot regeneration and root establishment is affected by genotype used, culture environment, plant growth regulators and kind of explant (Pareek et al., 2004; Kanwar and Kumar, 2009). Little information is available on the regeneration ability of carnation varieties propagated through seeds. The present experiment, therefore, was taken up to explore the possibility of developing an effective regeneration protocol for a popular carnation cultivar, Chabaud Super Mix, through induction of callus.

2. Materials and Methods

Young unfurled leaves of *Dianthus caryophyllus* cv. Chabaud

Super Mix were used for induction of calli. Explants were cultured on semi-solid Murashige and Skoog (1962) nutrient medium containing 0.8% ($^{w}/_{v}$) agar agar and varied concentrations and combinations of BA (N^6-Benzyl Adenine), Kinetin (6-Furfuryl aminopurine), NAA (α-Naphthalene Acetic Acid) and 2,4-D (2,4-Dichlorophenoxy Acetic Acid) for callus induction. The developed calli were then grown on the same basal medium supplemented with NAA and different levels of kinetin for shoot regeneration. For rooting, MS medium having different supplements of IBA (Indole-3-Butyric Acid) and NAA were tried. All the cultures were incubated at 25±1^{0}C under 12 h photoperiod at a photon flux density of 20-30 μ mol m^{-2} S^{-1}. Collected data regarding percentages were converted following arc-sine transformation procedure. For testing of significance Completely Randomized Design (CRD) was adopted. The composition of different callusing, regeneration and rooting media along with notations are presented in Table 1.

Table 1: Composition of the media used for in-vitro culture of carnation through callus induction (mg l^{-1})

Notation used	Basal medium	Kinetin	BA	NAA	2,4-D	IBA
Callusing of carnation						
C_0	MS	-	-	-	-	-
C_1	MS	-	-	1.0	1.0	-
C_2	MS	-	-	1.0	2.0	-
C_3	MS	-	-	1.0	3.0	-
C_4	MS	-	-	1.0	4.0	-
C_5	MS	-	-	1.0	5.0	-
C_6	MS	-	-	1.0	10.0	-
C_7	MS	1.0	-	-	4.0	-
C_8	MS	1.0	-	-	5.0	-
C_9	MS	-	1.0	2.0	-	-
C_{10}	MS	-	2.0	2.0	-	-
C_{11}	MS	-	3.0	2.0	-	-
Regeneration of carnation (from callus)						
CRG_1	MS	1.0	-	1.0	-	-
CRG_2	MS	1.5	-	1.0	-	-
CRG_3	MS	2.0	-	1.0	-	-
CRG_4	MS	2.5	-	1.0	-	-
CRG_5	MS	3.0	-	1.0	-	-
CRG_6	MS	3.5	-	1.0	-	-
CRG_7	MS	4.0	-	1.0	-	-
Rooting of regenerated micro-shoots of carnation						
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

3. Results and Discussion

3.1. Physical characteristics of the calli developed

Profuse and large calli were obtained using media C_{11}, C_6 and C_5, respectively (Plate 1). Callusing media like C_4, C_7, C_8 and C_{10} were found to produce medium amount of calli while C_2 and C_3 produced small amount. On the other hand C_0, C_1 and C_9 were found non-responsive to callusing (Table 2).

Malczewska et al. (1979) reported positive effect of different auxins like NAA, 2,4-D and cytokinins like BA in different combinations on callus formation of carnation. It was clear from the results that the media C_0, C_1 and C_9 were non-responsive. Among the other responsive callusing media C_{11} and C_6 reported earlier response to callusing (Table 2). The effect of 2,4-D on callus formation at the basal end of the explants was also noticed by several workers (Debergh, 1972; Sankhla et al., 1995). Calli obtained from the explants cultured on the medium C_2, C_3 and C_{10} were yellowish in color (Plate 1). Whitish yellow and creamy yellow calli were noticed with C_7 and C_8. Yellowish green callus was obtained from the explants cultured on C_4. Greenish yellow calli developed in case of C_6 and C_{11} although completely greenish callus was obtained only from the explants cultured on C_5. Callusing medium C_0, C_1 and C_9 exerted hardly any effect (Table 2). Highly friable to friable calli were obtained from the explants cultured on C_3, C_4, C_2 and C_{10} respectively. Slightly nodular calli were noticed with C_5, C_7 and C_8. But compact nodular calli were developed in two callusing media, C_6 and C_{11} (Table 2). It was observed that calli developed on C_2, C_5 and C_{10} produced small roots.

3.2. Days to callus initiation

The use of culture media on callus formation differed significantly in their effect. Results revealed MS medium supplemented with BA 3.0 mg l^{-1} and NAA 2.0 mg l^{-1} (C_{11}) was the most effective callusing medium requiring only 7.77 days to obtain callus, followed by C_6 (9.80 days) and C_5 (11.03 days). The most delayed effect was obtained from the explants cultured on C_2 that required 22.96 days to produce callus (Table 2). Media like C_0, C_1 and C_9 were found non-responsive.

3.3. Days required for initiation and collection of shoots

Development of young plantlets from undifferentiated calli is the penultimate expression of cells totipotency. Pooled analysis indicated CRG_4 (7.81 days) and CRG_7 (8.06 days) as the most effective regeneration medium in terms of earliness (Table 3) while CRG_2 (12.00 days) delayed regeneration. The medium CRG_1 was found to be ineffective for regeneration from callus. Lubomski and Jerzy (1989) as well as Choudhary

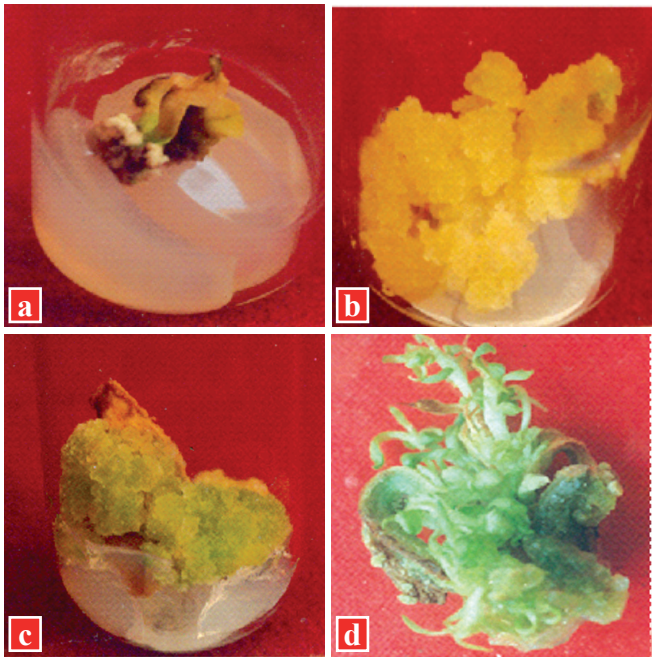

Plate 1: Callus induction and regeneration of shoots of carnation via caulogenesis. a) Callus initiation in young unfurled leaf, b) Young yellowish calli, c) Initiation of greenish calli, d) Regeneration of shoots

CRG_2 produced shoots last of all (20.44 days). Calli grown on culture media CRG_1 failed to produce any shoot (Table 3).

3.4. Rooting of carnation micro-shoots

Use of auxins for better root development was earlier reported by Can and Koo (1992). In the present experiment, regenerated micro-shoots were transferred to four different rooting media containing MS medium supplemented with NAA (1 mg l^{-1}) and IBA in different concentrations for studying their effect on initiation of roots. The observations suggested that (Table 4) MS medium when supplemented with NAA (1 mg l^{-1})+IBA (4 mg l^{-1}) showed maximum percentage of rooting (98.20%).

The medium CR_2 (MS medium supplemented with NAA @ 1 mg l^{-1}+IBA @ 2 mg l^{-1}) produced next best result with 96% rooting of regenerated micro-shoots. However, micro-shoots cultured on CR_1 produced lowest percentage of rooting (7.6%). Earliest roots from the micro-shoots were recorded in CR_2 (11.80 days) followed by CR_4 (14.32 days). Hardening of the plantlets and subsequent establishment in the pot or field is the ultimate objective of micropropagation. Establishment of 80% of the regenerated plantlets was noticed with a potting mixture containing sand, soil and screened and sterile cow dung manure. A thin film of water was kept on the surface of leaf during hardening. It facilitated cooling of plants and

Table 2: Effect of culture media on the physical characteristics of the callus of carnation (cv. Chabaud Super Mix)

Treatments	Response	Callus type/ texture	Size of callus	Rooting occurred	Color of callus	Days to callus initiation		
						1st year	2nd year	Pooled
C_0	X	-	x	-	-	-	-	-
C_1	X	-	x	-	-	-	-	-
C_2	✓	Friable	Small	✓	Yellowish	23.68	22.23	22.96
C_3	✓	Highly friable	Small	-	Yellowish	18.90	18.50	18.70
C_4	✓	Highly friable	Medium	-	Yellowish green	14.83	15.67	15.25
C_5	✓	Slightly nodular	Large	✓	Greenish	11.50	10.57	11.03
C_6	✓	Nodular	Large	-	Greenish yellow	9.40	10.20	9.80
C_7	✓	Slightly nodular	Medium	-	Whitish yellow	17.73	17.27	17.50
C_8	✓	Slightly nodular	Medium	-	Creamy yellow	16.27	16.93	16.60
C_9	x	-	x	-	-	-	-	-
C_{10}	✓	Friable	Medium	✓	Yellowish	14.60	15.33	14.97
C_{11}	✓	Nodular	Profuse	-	Greenish yellow	6.93	8.60	7.77
SEm±	-	-	-	-	-	0.72	0.52	0.44
CD (p=0.05)	-	-	-	-	-	2.12	1.53	1.26

and Mubarack (1991) also used kinetin along with NAA for regeneration of carnation. CRG_7 was the most effective regeneration medium regarding shoot production of carnation *in-vitro*, required 10.30 days followed by CRG_4 (11.48 days). The other media showed delayed effect and the culture media compensated the water loss by transpiration. Initially, the plantlets were kept in poly house under 100% shade condition. Application of water was reduced and plants were kept at 75% shade after 12 days. The plant growth was found prominent after a month. Rabindra and Thomas (1995) followed a similar

Table 3: Effect of culture media on the regeneration of shoots from the callus of carnation (cv. Chabaud Super Mix)

Treatments	Days required for initiation of shoots			Days required to collect individual shoots		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled
CRG_1	-	-	-	-	-	-
CRG_2	12.13	11.88	12.00	20.63	20.25	20.44
CRG_3	10.00	10.38	10.19	17.63	17.00	17.31
CRG_4	8.25	7.38	7.81	11.18	11.78	11.48
CRG_5	10.50	10.25	10.38	14.63	15.05	14.84
CRG_6	9.88	10.13	10.00	14.13	14.88	14.50
CRG_7	8.50	7.63	8.06	10.48	10.13	10.30
SEm±	0.17	0.26	0.15	0.32	0.26	0.21
CD (p=0.05)	0.51	0.76	0.44	0.94	0.77	0.59

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

Treatment	% of micro-cuttings 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*	6.60	6.27	4.37	1.36	1.04	0.82

Figures in the parentheses are the angular values; *(p=0.05)

procedure for hardening of grape plantlets.

4. Conclusion

In carnation cv. Chabaud Super Mix, MS medium having BA (3 mg l^{-1}) and NAA (2 mg l^{-1}) may be used for earlier callusing but to get early plantlet establishment MS medium having NAA (1 mg l^{-1}) and 2,4-D (5 mg l^{-1}) followed by kinetin (4 mg l^{-1}) + NAA (1 mg l^{-1}) might be an effective choice in carnation *in-vitro* culture through callus induction. For better root initiation in the developed micro-shoots, MS medium supplemented with NAA (1 mg l^{-1}) and IBA (4 mg l^{-1}) may be used. The current protocol developed is expected to be suitable for facilitating regeneration of other carnation genotypes.

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