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Studies on rooting and hardening of *in vitro* plantlets of carnation (*Dianthus caryophyllus* L.)

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Abstract
The present research work involves rooting and hardening in carnation (*Dianthus caryophyllous*). The experiments were laid out in a Completely Randomized Design (factorial) consisting of two cultivars i.e. 'Parendillo' and 'Yellow Star'. The *in vitro* multiplied shoots were separated and individual shoots were transferred to MS medium supplemented with different levels of auxins (NAA/IBA) with or without 0.1% activated charcoal for rooting of *in vitro* multiplied shoots. The concentration of both NAA and IBA varied from 1-2 mgl^{-1}. Per cent rooting, days taken for visible root formation, length of the longest root (cm) and number of root/shoot were recorded 4 weeks after culturing. MS medium containing 2 mgl^{-1} NAA and 0.1% activated charcoal was found to be the best for *in vitro* rooting of multiplied shoots. The rooted plantlets were then planted in different hardening media in disposable cups and watered immediately with fine mist of spray. These cups were then covered with the misted pricked transparent plastic bag to ensure high humidity. Pricking of plastic bag was done to maintain aeration. The *in vitro* rooted plantlets showed 100% survival and maximum growth of plantlets hardened in a mixture of cocopeat : sand : perlite (2:1:1, v/v).

Keywords: Rooting, hardening, MS medium, *in vitro*

Introduction
Carnation (*Dianthus caryophyllus* L.) is one of the most important and popular cut flower crops in the world and commands a respectable status in the world floriculture trade. It is one of the major floricultural crop in many countries with high ornamental and commercial value. The importance of this cut flower lies in its beauty, diversity of colours and excellent keeping quality (Ali *et al.* 2008, Kanwar and Kumar 2009) [2, 11]. It occupies prime position in the international cut flower trade and is a good commercial cut flower for internal market as well. Due to its remarkable ability to rehydrate after continuous shipping, carnation is preferred by growers to roses and chrysanthemums in flower exporting countries. The global cut flower market is maintained by the introduction of the new improved cultivars. The most extensive and viable application of plant tissue culture is micropropagation, which produces disease free planting material in a shorter period from the single individual. It has been a useful tool for the mass production of ornamental crops. This method is most suitable for the quick multiplication of new and elite varieties in vegetatively propagated crops. Its utility in raising true to type plants, and propagating disease and virus free clones has been a special advantage. Carnation is conventionally propagated vegetatively through shoot tip cuttings, however recently tissue culture techniques have become very popular for the production of disease free planting material. Successful micropropagation depends on the ability to transfer *in vitro* raised plantlets to potting mixture and acclimatize them successfully to the field conditions. Studies on the hardening of *in vitro* grown plantlets reveal that media play an important role on hardening success as well as in producing plantlets of the intrinsic quality. The application of tissue culture for regeneration and commercial propagation of whole plants is an established technique. The *in vitro* regenerated plants are used for the establishment of mother block which further acts as a source of cuttings for mass multiplication of carnation.

Materials and Methods
Two commercial standard cultivars of carnation viz. 'Parendillo' and 'Yellow Star' were selected for conducting different rooting and hardening experiments. Two explants namely, shoot tips and nodal sections were used for the establishment of cultures. Shoot tips and nodes were excised from stem cuttings taken from healthy plants and were cultured.

All the cultures were kept in the culture trollies of culture room maintained at a temperature of 25±2 °C was maintained under artificial light (16 hours light and 8 hours dark period daily) having intensity of 1.5 Klux at plant level. The multiplied shoots were separated and individual shoots were transferred to MS medium supplemented with different levels of auxins (NAA/IBA) with or without 0.1% activated charcoal for rooting of *in vitro* multiplied shoots. The concentration of both NAA and IBA varied from 1-2 mgl^{-1}. The *in vitro* cultures were incubated in culture room during rooting. Per cent rooting, days taken for visible root formation, length of the longest root (cm) and number of root/shoot were recorded 4 weeks after culturing. After complete development of roots the plantlets were carefully taken out of culture vessel. The roots were washed gently under running tap water for few minutes so that they may not wilt after their transfer to respective growing medium. The rooted plantlets were then planted in five different hardening media i.e. Cocopeat + Vermicompost (1:0.5, v/v), Sand + Vermicompost (1:0.5, v/v), Sand + Soil + FYM (1:1:1, v/v), Sawdust + Sand (1:1, v/v) and Cocopeat + Sand + Perlite (2:2:1, v/v) in disposable cups and watered immediately with fine mist of spray. These cups were then covered with the misted pricked transparent plastic bag to ensure high humidity. Pricking of plastic bag was done to maintain aeration. These plants were kept under low light intensity about (1 Klux). High humidity was maintained during initial one week and later it was gradually lowered. After 15-20 days of hardening the plants were finally transplanted to well prepared beds (Sand: Soil : FYM; 1 : 1 : 1, v/v) under polyhouse. Observations like per cent survival of *in vitro* rooted plantlets after 30 days of transplanting and per cent increase in height of plantlets 20 days after transplanting were recorded.

Results and Discussion

In vitro rooting:

Higher per cent rooting was observed in cv. 'Parendillo' than in 'Yellow Star'. According to Kallak *et al* (1997) [10] rooting percentage depends upon genotype. Another report suggests that rooting medium in carnation is highly genotype dependent, it confirms the differential behaviour of cultivars (Salehi 2005) [16] s. Among the growth regulator combinations 100% rooting was effected in cv. 'Parendillo' when MS medium was supplemented with 2 mgl^{-1} NAA and 0.1% activated charcoal (Table 1). NAA was found to be more efficient in inducing rooting as compared to IBA. These results are in confirmatory with the results of Kadu (2013) [9] who obtained 96% rooting with MS medium containing 2 mgl^{-1} NAA and 0.2% activated charcoal. Profuse rooting was observed in carnation cvs 'White Sim', 'Exquisite' and 'Scania' on MS medium supplemented with different concentrations of NAA i.e. 1 mgl^{-1}, 1.5 mgl^{-1} and 2 mgl^{-1} (Yadav *et al* 2012) [18]. Ali *et al* (2008) [2] also reported best rooting response on MS medium containing 1 mgl^{-1} NAA. Dharma (2003) [5] also obtained 100% rooting in carnation when MS full salt medium was supplemented with 5 µM NAA or 5 µM NAA + 1 µM B-9.

Table 1: Effect of NAA, IBA and activated charcoal in MS medium on per cent rooting of *in vitro* regenerated shoots of carnation (4 weeks after culture)

MS medium supplements			Cultivars of carnation		Mean
NAA (mgl^{-1})	IBA (mgl^{-1})	Activated charcoal (%)	Parendillo	Yellow Star	
2.0	-	-	35.53 (36.59)*	22.17 (28.03)	28.85 (32.31)
1.0	-	0.1	75.53 (60.36)	66.63 (54.73)	71.08 (57.54)
1.5	-	0.1	91.07 (72.62)	82.17 (65.12)	86.62 (68.87)
2.0	-	0.1	100.00 (90.00)	95.53 (82.14)	97.77 (86.42)
-	1.0	-	13.07 (21.19)	13.07 (21.19)	13.07 (21.19)
-	1.5	-	24.40 (29.59)	22.17 (27.83)	23.28 (28.71)
-	2.0	-	51.07 (45.61)	48.83 (44.33)	49.95 (44.97)
-	1.0	0.1	55.53 (48.17)	55.53 (48.14)	55.53 (48.18)
-	1.5	0.1	66.63 (54.72)	64.40 (53.39)	65.52 (54.05)
-	2.0	0.1	91.06 (72.84)	82.17 (65.05)	86.62 (68.94)
Mean			60.39 (53.17)	55.27 (49.07)	-

*values in parenthesis are arc sine transformed values

$CD_{0.05}$ for:	Cultivars	1.83
	MS medium supplements	4.10
	Cultivars x MS medium supplements	NS

In our studies superiority of NAA was found over IBA for inducing rooting. However, several other workers have advocated use of IBA for rooting of *in vitro* regenerated shoots. Dogra (2007) [6] observed maximum per cent rooting (99.15) with 5 µM IBA. Qaoud (2013) [15] reported 100% rooting with 5.4 µM NAA. Pathania (1988) [14] also obtained best rooting response with 5 µM IBA and Bora *et al* (2007) [3] and Ahmad *et al* (2008) with 0.5 mgl^{-1} IBA. It is evident from the results that cv. 'Parendillo' proved better over 'Yellow Star' for other rooting parameters including days taken for visible root formation, length of longest root and number of roots/shoot (Table 2). The genotypic superiority of

'Parendillo' has already been established. Among different combinations, MS medium containing 2 mgl^{-1} NAA and 0.1% activated charcoal was most effective to induce early rooting, length of longest root and number of roots/shoot irrespective of cultivar. Jagannatha *et al* (2002) recorded earlier rooting with NAA in comparison to IBA but they further found that IBA was best for rooting because NAA induced callusing alongwith root formation. Similar reports were given by Veichin *et al* (1998) [17]. Dharma (2003) [5] reveals that maximum number of roots/shoot were observed on MS full salts medium containing 5 µM NAA (7.68) and 5 µM NAA + 1 µM B-9 (6.55). From the present study, it has also been concluded that rooting was significantly increased with the addition of 0.1% activated charcoal. These results are in close confirmatory with the results of Kadu (2013) [9] who obtained 96% rooting with MS medium containing 2 mgl^{-1} NAA and 0.2% activated charcoal.

Table 2: Effect of NAA, IBA and activated charcoal in MS medium on days taken for visible root formation, length of longest root and number of roots/shoot of *in vitro* regenerated shoots of carnation

MS medium supplements			Days taken for visible root formation			Length of the longest root (cm)			Number of roots/shoot		
			Cultivars of carnation		Mean	Cultivars of carnation		Mean	Cultivars of carnation		Mean
NAA (mgl^{-1})	IBA (mgl^{-1})	Activated charcoal (%)	Parendillo	Yellow Star		Parendillo	Yellow Star		Parendillo	Yellow Star	
2.0	-	-	13.25	15.17	14.21	2.43	2.26	2.35	4.40	4.04	4.22
1.0	-	0.1	11.70	10.93	11.31	2.31	2.33	2.27	6.12	5.02	5.57
1.5	-	0.1	9.68	10.09	9.89	2.35	2.26	2.31	6.53	6.23	6.37
2.0	-	0.1	8.06	7.97	8.02	3.28	2.73	3.01	7.86	6.83	7.35
-	1.0	-	13.83	15.50	14.67	0.94	0.48	0.71	3.00	1.32	2.16
-	1.5	-	11.93	15.50	13.71	2.08	2.05	2.07	4.59	4.29	4.44
-	2.0	-	10.12	12.96	11.54	2.26	2.12	2.19	4.83	4.86	4.85
-	1.0	0.1	12.58	12.58	12.57	2.58	2.38	2.48	4.66	4.92	4.79
-	1.5	0.1	11.83	12.50	12.17	2.69	2.50	2.60	5.21	5.00	5.11
-	2.0	0.1	9.04	10.59	9.82	2.95	2.84	2.90	6.66	5.94	6.30
Mean			11.20	12.38	-	2.39	2.19	-	5.39	4.85	-
$CD_{0.05}$ for:		Cultivars			0.90						
		MS medium supplements			0.40						
		Cultivars x MS medium supplements			1.27						
		Cultivars			0.17						
		MS medium supplements			0.08						
		Cultivars x MS medium supplements			0.24						
		Cultivars			0.22						
		MS medium supplements			0.49						
		Cultivars x MS medium supplements			0.69						

Hardening

Successful micropropagation depends on the ability to transfer *in vitro* raised plantlets to potting mixture and acclimatize them successfully to the field conditions. Studies on the hardening of *in vitro* grown plantlets reveal that media play an important role on hardening success as well as in producing plantlets of the intrinsic quality. In the present studies, different hardening media had a variable difference on per cent survival of plantlets whereas genotypes did not have a significant difference. Besides this, different hardening media and cultivars had a variable difference on per cent increase in height of plantlets (%) also.

100% survival of *in vitro* raised plantlets was observed in both the cultivars when cocopeat, sand and perlite (2:1:1, v/v) were used as hardening medium (Table 3). Optimum porosity, aeration and moisture holding capacity of this medium provided excellent conditions for establishment of hardened plantlets in the field. Dharma (2003) [5] observed 96.30% survival of carnation plantlets on hardening media (sand: soil: FYM; 1:1:1, v/v) and 92.59% on cocopeat and perlite (1:1, v/v). Dogra (2007) [6] also obtained successful results when carnation plantlets were hardened on cocopeat : perlite in the ratio (1:1, v/v).

Ghosh and Mohan Ram (1986) [7] concluded that vermiculite and garden soil in the ratio 1:1, v/v was the most suitable substrate whereas from the studies carried out by Mirzaev (1988) peat : perlite (1:1 or 1:2, v/v) followed by peat : perlite : sand (1:1:1, v/v) proved to be the best hardening media. Besides this, there are several reports related to hardening of *in vitro* rooted plantlets by Mehta (2004) [12] and Bora *et al* (2006, 2007) [3, 4]. Maximum per cent increase in height of plantlets (8.51%) was also recorded when cocopeat, sand and perlite in the ratio 2:1:1, v/v were used as hardening media, irrespective of cultivar (Table 3). Better rooting parameters obtained in this medium helped in better establishment of hardened plants to field conditions. These plants showed quick adaption and hence more per cent increase in height after transplantation.

Table 3: Effect of different hardening media concentrations on a) per cent survival of *in vitro* rooted plantlets of carnation 30 days after transplanting and b) per cent increase in height of plantlets (cm) before and after 20 days of transplanting in carnation

Hardening media	Per cent survival of *in vitro* rooted plantlets of carnation 30 days after transplanting			Per cent increase in height of plantlets (cm) before and after 20 days of transplanting in carnation		
	Cultivars of carnation		Mean	Cultivars of carnation		Mean
	Parendillo	Yellow Star		Parendillo	Yellow Star	
Cocopeat+ Vermicompost (1:0.5, v/v)	86.50 (68.48)*	82.50 (65.32)	84.50 (66.90)	5.55 (2.60)*	5.64 (2.58)	5.59 (2.57)
Sand + Vermicompost	78.25	77.50	77.88	5.18	4.89	5.02

(1:0.5, v/v)	(62.22)	(61.72)	(61.97)	(2.49)	(2.42)	(2.45)
Sand + Soil + FYM (1:1:1, v/v)	36.00 (36.86)	33.25 (35.19)	34.63 (36.03)	3.99 (2.23)	3.92 (2.22)	3.95 (2.23)
Cocopeat + Sand +Vermicompost (1;1;0.5, v/v)	93.75 (77.55)	95.00 (88.78)	94.38 (79.17)	8.22 (3.04)	7.99 (3.00)	8.10 (3.02)
Sawdust + Sand (1:1, v/v)	66.25 (54.52)	66.50 (54.67)	66.38 (54.59)	5.26 (2.50)	4.99 (2.45)	5.13 (2.48)
Cocopeat + Sand + Perlite (2:2:1, v/v)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	8.51 (3.08)	8.46 (3.07)	8.48 (3.08)
Mean	76.79 (64.94)	75.79 (64.61)	-	6.12 (2.65)	5.98 (2.62)	-

*values in parenthesis are square root transformed values

$CD_{0.05}$ for:	Cultivars	NS
	Hardening media	4.42
	Cultivars x Hardening media	NS
	Cultivars	0.02
	Hardening media	0.03
	Cultivars x Hardening media	NS

References

1. Ahmad F, Mandal BB, Gupta S. Effect of some growth regulators viz., NAA, IAA and IBA on rooting of carnation (*Dianthus caryophyllus* L.) under *in vitro* conditions. Applied Biological Research. 2008; 10:44-46.
2. Ali A, Afrasiab H, Naz S, Rauf M, Iqhbal J. An efficient protocol for *in vitro* propagation of carnation (*Dianthus caryophyllus* L.). Pakistan Journal of Botany 2008; 40(1):111-121.
3. Bora A, Singh S, Talukdar MC, Hazarika BN. An efficient method for *in vitro* plant regeneration in carnation. Indian Journal of Horticulture. 2007; 64(4):439-443.
4. Bora A, Talukdar MC, Hazarika BN. Direct organogenesis in *Dianthus caryophyllus.* Journal of Ornamental Horticulture. 2006; 9(3):164-168.
5. Dharma S. Studies on factors influencing the production of hardened glaucous carnation plantlets. M.Sc. Thesis, submitted to Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), 2003.
6. Dogra S. *In vitro* mutagenesis in carnation (*Dianthus caryophyllus* L.) for induction of resistance to *Fusarium oxysporum* f. sp. Dianthi. Ph. D. thesis, submitted to Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), 2007.
7. Ghosh S, Mohan Ram YH. Multiplication of spray carnation by axillary bud culture. Current Science. 1986; 55(19):966-97.
8. Jagannatha J, Ashok TH, Sathyanarayana BN. In vitro micro-propagation studies in carnation. Karnataka Journal of Agricultural Sciences. 2012; 15(1):109-114.
9. Kadu AR *In vitro* micropropagation of gerbera using axillary bud. Asian Journal of Bio Science. 2013; 8(1):15-18.
10. Kallak H, Reidla M, Hilpus I and Virumae K. Effects of genotype, explant source and growth regulators on organogenesis in carnation callus. Plant Cell, Tissue and Organic Culture. 1997; 51(2):125-127.
11. Kanwar JK, Kumar S. Influence of growth regulators and explants on shoot regeneration in carnation. Horticultural Science. 2009; 36:140-146.
12. Mehta R. *In vitro* selection and biochemical characterization of carnation (*Dianthus caryophyllus* L.) callus culture tolerant of *Alternaria dianthi*. M.Sc. Thesis, submitted to Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), 2004.
13. Mirzaev MM. The effectiveness of methods of raising meristemic carnation plants in soilless substrates. Biotechnologia v Sadovodstve I Vinogradarstve. 1988; 31-34.
14. Pathania NS. Propagation of carnation (*Dianthus caryophyllus* L.) through tissue culture. M. Sc. Thesis, submitted to Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), 1988.
15. Qaoud HA. Adventitious shoot formation and plant regeneration from leaf explants of carnation (*Dianthus caryophyllus* L.). African Journal of Biotechnology. 2013; 12(21):3244-3249.
16. Salehi H. Can a general shoot proliferation and rooting medium be used for a number of carnation cultivars? African Journal of Biotechnology. 2005; 5(1):25-30.
17. Veichin C, Yihjuh S, Chienchou L, Hsinsheng T. Effects of medium composition and vessel closure on the hyperhydricity and rooting of carnation *in vitro* culture. Journal of Agricultural Research of China. 1998; 47(4):364-376.
18. Yadav MK, Singh RK, Yadav D, Tripathi MK, Rana B, Sirohi A *et al.* Effect of ABA and GA_3 on hyperhydricity and assessment of *in vitro* and *ex vitro* rooting pattern for suitability of acclimatization in carnation. Progressive Agriculture. 2012; 12(1):11-18.