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Short Research Article

Effects of Genotype, Explants Type and Growth Regulators on Organogenesis in Carnation

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Abstract

In vitro organogenesis and shoot multiplication protocol of carnation (*Dianthus caryophyllus* L.) cultivars Madras, Tempo, Dark Rendez Vous and Raggio de Sole was developed using apical and nodal sections of shoots as explants. It has been found that *in vitro* shoot multiplication is highly variable and genotype dependant. The earliest establishment of shoot sections (8.24 days) with maximum number of shoots explants^{-1} (2.16) was recorded in MS medium supplemented Benzyl-6-adenine 2 mg l^{-1}. Nodal sections as explants produced more number of shoots as compared to apical portion when inoculated on MS medium supplemented with Benzyl-6-adenine (2 mg l^{-1}). In case of cultivars, earliest shoot establishment (8.97 days) was noticed in cv. Raggio de Sole. On the other hand, number of shoots explant^{-1} was found to be maximum (2.00) in cv. Dark Rendez Vous. MS medium supplemented with BA 2 mg l^{-1}, GA_3 1 mg l^{-1} and NAA 0.1 mg l^{-1} was found to be the most effective medium for shoot multiplication, producing 6.74 shoots shoot^{-1} with longer shoots (3.16 cm). Shoot proliferation was recorded maximum (6.92 shoots shoot^{-1}) in cv. Raggio de Sole, whereas, maximum length of shoots in multiplication medium (3.51 cm) was observed in cv. Tempo. The regenerated shoots were rooted on MS medium added with NAA 2 mg l^{-1}. The rooted plantlets were hardened in medium containing cocopeat and sand (1:1, v/v) and successfully transferred to field conditions.

1. Introduction

Carnation (*Dianthus caryophyllus* L.) is among the top cut flowers of the World. It is being cultivated commercially in many parts of India. As per the estimates of National Horticulture Board, in India area under carnation cultivation was 210 ha and in Himachal Pradesh it was cultivated in an area of 50 ha in the year 2013 (National Horticulture Board, 2014). For quality flower production in carnation, disease free and healthy planting material is pre-requisite. The availability of quality planting material, however, remains to be major limitation. Carnations can be propagated through cuttings; however, multiplication by cuttings is too slow to be exploited commercially. For commercialization of this crop, planting material is required in large scale, which further needs the development of an easier, quicker and economically viable method of propagation. Production of carnation through micropropagation technique has been explained by various workers [Yadav et al. (2013 and 2012), Maitra et al. (2011) and Gutierrez-Miceli et al. (2010)]. Plant tissue culture for carnation propagation enables a million fold expansions year^{-1} of a desired plant in addition to disease free, healthy and vigorous planting material. It involves rapid multiplication of explants by repeated sub culturing and production of plantlets for transfer to field. *In vitro* propagation protocol is, however, genotype dependant (Mubarack et al., 1991; Kallak et al., 1997; Kharrazi et al., 2011; Kang et al., 2011). Hence, the present investigation was carried out with the objective to develop a rapid mass multiplication of selected cultivars for production of carnation plantlets *in vitro.*

2. Materials and Methods

The experiments were conducted during 2014−2015 in plant tissue culture laboratory of department of floriculture and landscape architecture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan.

2.1. Explant

Nodal and inter-nodal explants of 0.5–0.7 cm size were excised and used as explants from carnation cultivars Madras, Tempo, Dark Rendez Vous and Raggio de Sole. Healthy and disease free plants grown under protected conditions were selected as source of explants.

2.2. Surface sterilization

The explants after thorough washing and treatment with

fungicides were surface sterilized with CaOCl (5%) solution for 15–20 minutes followed by washing thrice with autoclaved distilled water before inoculation. These were cultured on MS medium (Murashige and Skoog, 1962) containing 30 g l^{-1} sucrose having pH adjusted to 5.8, solidified with 7.5 g l^{-1} Agar-agar which was autoclaved at a temperature of 12 °C for 20 minutes at 15 lbs in^{-2} pressure.

2.3. Organogenesis

For shoot induction, MS media containing different concentrations and combination of auxins (Naphthalene Acetic Acid) and cytokinin (Benzyl Adenine) was used. The cultures were incubated at 24 °C±1 °C and photoperiod was 16 hours with 8 hours dark period in every 24 hours cycle. Various observations on culture establishment were recorded. In the second experiment, *in vitro* regenerated shoots were separated and placed on basal MS medium having different combinations of BA (1 and 2 mg l^{-1}) either alone or in combination with GA_3 (1 mg l^{-1}) NAA (0.1 mg l^{-1}) for multiplication. Observations were recorded after four weeks.

2.4. Acclimatization

In vitro multiplied shoots approximately 3 cm in length were transferred on MS medium containing NAA 2 mg l^{-1} for *in vitro* rooting. The *in vitro* rooted plantlets were transferred hardening medium containing coco peat: sand (1:1, v/v). All treatments were replicated four times, with 10 explants $treatment^{-1}$. The data were subjected to statistical analysis employing a completely randomized design and were analyzed by two-way ANOVA using OP STAT Statistical Software, of HAU, Hisar (India).

3. Results and Discussion

3.1. Shoot induction

Production of carnation plants from shoot tip culture (apical meristem) has been described by many scientists (Yadav et al., 2003; Saher et al., 2004). It is evident from (Table 1) that among cultivars, earliest shoot establishment (8.97 days) was noticed in cv. Raggio de Sole, whereas maximum days for shoot establishment (9.78 days) were recorded in cv. Madras. The difference among cultivars for establishment could be attributed to variation in their genetic makeup. The optimum growth in carnation cultivars Yellowdotcom, Jakarta and Polartessino from single cell was recorded in the MS medium supplemented with sucrose 3%, NAA 1.0 mg l^{-1} and TDZ 1.0 mg l^{-1} except cv. Belmonte, in which, BA 1.0 mg l^{-1} was found to be best in place of TDZ, other ingredients were same (Kang et al., 2011). Mubarack et al. (1991); Kallak et al. (1997); Kharrazi et al. (2011) also confirmed that *in vitro* propagation in carnation is genotype dependant. Apical explants resulted in earlier culture establishment (9.14 days) as compared to nodal sections (9.89 days). Earlier establishment in apical explants might have resulted because of early cell division in meristematic region as compared to nodal sections.

Table 1: Effect of establishment medium on number of days taken for establishment of shoot explants

Cultivars / Treatments	Madras	Tempo	RDS	DRV	Apical	Nodal	Mean
MS	11.30	11.67	10.03	10.72	10.69	11.16	10.93
MS+BA (1 mg l^{-1})	9.81	9.37	9.03	9.35	8.86	9.91	9.39
MS+BA (2 mg l^{-1})	8.22	8.28	7.86	8.58	7.87	8.60	8.24
Mean	9.78	9.77	8.97	9.55	9.14	9.89	-
Apical	9.52	9.58	8.35	9.13			
Nodal	10.04	9.96	9.59	9.98			

CD (p=0.05) for

Treatment: 0.48 treatment×cultivars: NS; Cultivars: 0.55 treatment×explants: NS; Explants: 0.39 cultivars×explants: NS; RDS: Raggio de Sole; DRV: Dark Rendez Vous

Medium composition has also significantly affected establishment of cultures with earliest establishment of shoots (8.24 days) recorded in MS medium supplemented with 2 mg l^{-1} BA followed by medium supplemented with 1 mg l^{-1} BA (9.39 days). Maximum delay in culture establishment (10.93 days) was, however, recorded in basal MS medium.

Data in (Table 2) reveals that in case of cultivars, number of shoots $explant^{-1}$ was found to be maximum (2.00) in cv. Dark Rendez Vous followed by cv. Raggio de Sole (1.87). Variation in shoot proliferation among cultivars could again be attributed to their genotypic constitution. More number of shoots $explant^{-1}$ was recorded in nodal sections (2.21) as compared to apical sections (1.48). Illahi et al. (1995) also induced rapid propagation from nodal segments of carnation

Table 2: Effect of establishment medium on number of shoots $explants^{-1}$

Cultivars / Treatments	Madras	Tempo	RDS	DRV	Apical	Nodal	Mean
MS	1.25	1.42	1.66	1.78	1.29	1.76	1.53
MS+BA (1 mg l^{-1})	2.08	1.61	1.75	1.94	1.32	2.37	1.85
MS+BA (2 mg l^{-1})	2.02	2.17	2.19	2.27	1.82	2.49	2.16
Mean	1.78	1.73	1.87	2.00	1.48	2.21	-
Apical	1.27	1.50	1.61	1.53			
Nodal	1.29	1.96	2.13	2.47			

CD (p=0.05) for

Treatment: NS treatment×cultivars: NS; Cultivars: 0.21 treatment×explants: NS; Explants: 0.17 cultivars×explants: 0.30; RDS: Raggio de Sole; DRV: Dark Rendez Vous

on MS medium supplemented with 1, 2 or 3 mg l^{-1} BA. As the concentration of BA was increased in MS medium corresponding increase in number of shoots explant^{-1} was also noted. Maximum number of shoots explant^{-1} (2.16) was observed in MS medium supplemented with 2 mg l^{-1} BA. Number of shoots explant^{-1} was however, recorded minimum (1.53) in MS medium without BA. However, it was recorded minimum (1.73) in case of cv. Tempo. The role of cytokinins for cell division and shoot proliferation is well established. Dharma (2003) observed that maximum number of shoots was observed on MS medium supplemented with 5 µM BAP in carnation cv. Tempo and Diplomat. Kharrazi et al. (2011) observed that lower concentrations of BAP (1 mg l^{-1} for Eskimo Mogr and 2 mg l^{-1} for Innove Orange Bogr) is more suitable for obtaining normal plantlets with minimum vitrification. Yadav et al. (2013) also reported similar results in cv. White Sim, and found that concentration combination of plant growth regulators Kinetin (1.0 ppm)+NAA (0.15 ppm) resulted in excellent shoot proliferation. Abu-Qaoud (2013) also observed high frequency of multiplication of carnation cv. White Sim on MS medium supplemented with 0. 5 µM NAA in combination with 4.4 µM or 8.8 µM BA. Interaction effect of treatment and cultivars, and treatment and explants was found to be non-significant. Maximum number of shoots explant^{-1} (2.47) was, however, recorded in nodal sections of cv. Dark Rendez Vous and minimum (1.27) in apical sections of cv. Madras.

3.2. Shoot multiplication

As regards shoot multiplication (Table 3), among cultivars it was observed that maximum number of shoots original shoot^{-1} (6.92) was recorded in cv. Raggio de Sole whereas, it was recorded minimum (4.93) in Tempo. It is clear that genotypic differences are responsible for this variation. The inter-varietal variation in response to *in vitro* cloning of carnation was also explained by Mujib and Pal (1995) in carnation.

MS medium supplemented with BA 2 mg l^{-1}, GA_3 1 mg l^{-1} and NAA 0.1 mg l^{-1} was found to be the more effective for shoot multiplication producing 6.74 shoots original shoots^{-1} compared to MS medium supplemented with BA 1 mg l^{-1}, GA_3 1 mg l^{-1} and NAA 0.1 mg l^{-1} which produced 4.77 shoots shoot^{-1}. Usually, hormonal combinations with high concentrations of cytokinins (2–10 µM) and low concentration of auxins (0.1–0.5 µM) are effective for multiple shoot formation from shoot apex and are in line with findings of present investigations (Hempel, 1979 and Dabski et al., 1979). MS medium supplemented with Kinetin (3.5 mg l^{-1}) and NAA (1 mg l^{-1}) produced maximum number of shoots culture^{-1} (6.73) during *in vitro* regeneration of carnation cv. Chabaud Super Mix from shoot tip explants (Maitra et al., 2011). Similar results were reported by Iantcheva et al. (2005) who studied that optimum regeneration in carnation was noticed in medium supplemented with 0.9 mg l^{-1} BAP and 0.9 mg l^{-1} NAA.

Table 3: Effect of multiplication medium on number of shoots original shoot^{-1}

Cultivars / Treatments	Madras	Tempo	RDS	DRV	Apical
MS+BA (1 mg l^{-1})+GA_3 (1 mg l^{-1})+NAA (0.1 mg l^{-1})	4.64	3.71	6.30	4.77	4.77
MS+BA (2 mg l^{-1})+GA_3 (1 mg l^{-1})+NAA (0.1 mg l^{-1})	7.05	6.15	7.55	6.74	6.74
Mean	5.85	4.93	6.92	5.32	-

CD (p=0.05) for Cultivar: 1.09; Multiplication medium: 0.77; Cultivar× multiplication medium: NS

In case of cultivars, maximum length of shoots (3.51 cm) was observed in Tempo and minimum (2.39 cm) in Raggio de Sole (Table 4). Longer shoots (3.16 cm) were observed in MS medium supplemented with BA 2 mg l^{-1}, GA_3 1 mg l^{-1} and NAA 0.1 mg l^{-1}. In contrast, shoots were shorter (2.67 cm) in MS medium supplemented BA 1 mg l^{-1}, GA_3 1 mg l^{-1} and NAA 0.1 mg l^{-1}. These results are in conformity with results obtained by Mujib et al. (1993) who used shoot tips and node cuttings as explants for *in vitro* regeneration of shoots in carnation cv. William Sim.

3.3. Acclimatization

The multiplied shoots of the selected cultivars were rooted in MS medium supplemented with NAA 2 mg l^{-1}. The rooted plantlets were successfully transferred to polyhouse after hardening in a medium containing cocopeat and sand (1:1, v/v).

Table 4: Effect of multiplication medium on length of shoots

Cultivars / Treatments	Madras	Tempo	RDS	DRV	Apical
MS+BA (1 mg l^{-1})+GA_3 (1 mg l^{-1}) +NAA (0.1 mg l^{-1})	2.48	3.26	2.01	2.91	2.67
MS+BA (2 mg l^{-1})+GA_3 (1 mg l^{-1})+ NAA (0.1 mg l^{-1})	3.24	3.76	2.77	3.61	3.16
Mean	2.86	3.51	2.39	2.89	-

CD (p=0.05) for Cultivar: 0.55; Multiplication medium: 0.34; Cultivar× multiplication medium : NS

4. Conclusion

Multiplication in carnation is genotype dependant, in present studies cv. Raggio de Sole produced more number of shoots as compared to other cultivars. Nodal sections of shoots produced more number of shoots as compared to shoot apical explants. For shoot induction MS medium supplemented with BA 2 mg l^{-1} resulted in earlier shoot establishment and higher proliferation. MS medium supplemented with BA 2 mg l^{-1}, GA_3 1 mg l^{-1} and NAA 0.1 mg l^{-1} was found to be the optimum for shoot multiplication.

5. References

Abu-Qaoud, H., 2013. Adventitious shoot formation and plant regenerationfrom leaf explants of carnation (*Dianthus caryophyllus* L.). African Journal of Biotechnology 12 (21), 3244–3249.

Dabski, M., Malinowska, B., Hempel, M., 1979. Studies on *in vitro* multiplication of carnation. IV. The multiplication and rooting of shoots. Acta Horticulturae 91, 339–344.

Dharma, S., 2003. 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, Himachal Pradesh.

Gutierrez-Miceli, F.A., Arias, L., Juarez-Rodriguez, N., Abud-Archila, M., Amaro-Reyes, A., Dendooven, L., 2010. Optimization of growth regulators and silver nitrate for micropropagation of *Dianthus caryophyllus* L. with the aid of a response surface experimental design. *In vitro* Cellular and Developmental Biology- Plant 46, 57–63.

Hempel, M., 1979. Studies on *in vitro* multiplications of carnations. I. The influence of some kinetins on the differentiation of shoot apices. Acta Horticulturae 91, 317–321.

Iantcheva, A., Vlahova, M., Atanassova, B., Atanassov, A., 2005. Plant regeneration via direct organogenesis and somatic embryogenesis of two Bulgarian spray carnation cultivars. Biotechnology and Biotechnological Equipment 19(3), 15–19.

Illahi, I., Aziz, F., Jabeen, M., 1995. Tissue culture studies for micropropagation of carnation (*Dianthus caryophyllus* L.). Pakistan Journal of Botany 27, 411–415.

Kallak, H., Reidla, M., Hilpus, I., Virumae, K., 1997. Effects of genotype, explants source and growth regulators on organogenesis in carnation callus. Plant Cell, Tissue and Organ Culture 51(2), 125–127.

Kang, C.H., Han, B.S., Han, S.G., Kown, S.H., Song, Y.J., 2011. Selection of suitable varieties of carnation (*Dianthus caryophyllus* L.) and optimization of culture conditions for efficient tissue culture. Korean Journal of Plant Resources 24(2) 121–129.

Kharrazi, M., Nemati, H., Tehranifar, A., Bagheri, A., Sharifi, A., 2011. *In vitro* culture of carnation (*Dianthus caryophyllus* L.) focusing on the problem of vitrification. Journal of Biodiversity and Environmental Sciences 5(13), 1–6.

Maitra, S., Roychowdhury, N., Ghosh, P.D., Satya, P., 2011. Effect of kinetin and NAA on regeneration of carnation (*Dianthus caryophyllus* L.) from shoot-tip explants. International Journal of Bio-resource and Stress Management 2(3), 287–292.

Mubarack, M.M., Choudhary, M.L., Prakash, P., 1991. Varietal response on plant regeneration from leaf callus of carnation (*Dianthus caryophyllus* L.). South Indian Horticulture 39(4), 210–213.

Mujib, A., Pal, A.K., 1995. Inter varietal variation in response to *in vitro* cloning of carnation. Crop Research-Hisar 32(4), 127–128.

Mujib, A., Pal, A.K., Jana, B.K., 1993. Effect of growth regulators on plantlet regeneration of carnation from shoot tip and node cuttings *in vitro*. Maharashtra Journal of Horticulture 7(1), 96–98.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 473–497.

National Horticulture Board, 2014. Ministry of Agriculture, Govt. of India, Gurgaon, 286.

Saher, S., Piqueras, A., Hellin, E., Olmos, E., 2004. Hyperhydricity in micropropagated carnation shoots: the role of oxidative stress. Physiologia Plantarum 120, 152.

Yadav, M.K., Gaur, A.K., Garg, G.K., 2003. Development of suitable protocol to overcome hyperhydricity in carnation during micro propagation. Plant Cell, Tissue and Organ Culture 72, 153–156.

Yadav, M.K., Singh, R.K., Yadav, D., Tripathi, M.K., Rana, B., Sirohi, A., Kumar, R., Garg, G.K., 2012. 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 12(1), 11–18.

Yadav, M.K., Singh, R.K., Yadav, D., Tripathi, M.K., Tomar, A., Rana, B., Bansal, S., Garg, G.K., 2013. Evaluation of shoot proliferation, height and vitrification in carnation during micropropagation of selected ideotypes. Progressive Agriculture 13(1), 196–198.