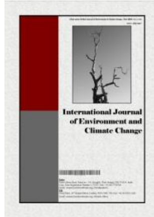

International Journal of Environment and Climate Change

11(11): 230-238, 2021; Article no.IJECC.77234
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

An Effective Protocol for Carnation (*Dianthus caryophyllus* L.) cv. 'Geolei' Explants Sterilization for Successful Callusing and Shoot Regeneration

Ujjwal Sirohi [a*], Swati Sharma [a], Mukesh Kumar [b], R. S. Sengar [a], L. K. Gangwar [c], Akash Tomar [d], Vyankatesh Dhanraj Bagul [a] and M. K. Yadav [a]

[a] Department of Agricultural Biotechnology,Sardar Vallabhbhai Patel University of Agricultureand Technology, Meerut, 250110, Uttar Pradesh, India.
[b] Department of Horticulture,Sardar Vallabhbhai Patel University of Agricultureand Technology, Meerut, 250110, Uttar Pradesh, India.
[c] Department of Genetics and Plant Breeding,Sardar Vallabhbhai Patel University of Agricultureand Technology, Meerut, 250110, Uttar Pradesh, India.
[d] College of Biotechnology, Sardar Vallabhbhai Patel University of Agricultureand Technology, Meerut, 250110, Uttar Pradesh, India.

Authors' contributions

This work was carried out in collaboration among all authors. The present research has been equally contributed by all the authors. Authors MKY, AT, MK, RSS and LKG designed the study, and US performed the experiments and recorded the data. US, SS and VDB did the statistical analysis. US has prepared the draft and MKY has checked the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2021/v11i1130538
Editor(s):
(1) Dr. Fang Xiang, University of International and Business Economics, China.
Reviewers:
(1) ABA-TOUMNOU Lucie, University of Bangui, Central African Republic.
(2) Ahmed Asaad Zaeen, University of Baghdad, Iraq.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available in this link:
https://www.sdiarticle5.com/review-history/77234

Original Research Article

Received 11 September 2021
Accepted 22 November 2021
Published 24 November 2021

ABSTRACT

Carnation is a popular floricultural crop grown widely for its attractive cut flowers. Micro-propagation can be used to create large-scale carnation output. For growth and development, plants require some necessary nutrients as well as growth regulators. Due to the importance of carnation, the present work is carried out using leaf and nodal segments to examine the potential of several plant growth regulators for *in vitro* callus formation and adventitious shoot regeneration. Explants were

**Corresponding author: E-mail: sirohiujjwal@gmail.com;*

sterilized properly with bavistin, sodium hypochlorite and mercuric chloride. The minor contaminated cultures were created by consecutively treating the explants with 0.25% bavistin, 0.50% sodium hypochlorite, and 0.1% mercuric chloride for ten, fifteen, and two minutes.
MS media with 2.5 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) in combination with 0.75 mg/l naphthalene acetic acid (NAA) resulted in the maximum callus induction (90.47%) from leaf explants. Maximum shoots (76.47%) were produced in MS media supplemented with 2.0 mg/l Thidiazuron (TDZ) + 0.25 mg/l NAA. NAA at 1.25 mg/l was most efficient for maximum root induction (83.32%). In the present study, an effective protocol of carnation explants sterilization was optimized for successful callusing and shoot regeneration.

Keywords: Bavistin, callus induction; 2,4-dichlorophenoxy acetic; naphthalene acetic acid; mercuric chloride; regeneration; sodium hypochlorite; thidiazuron.

ABBREVIATIONS

MS: Murashige and Skoog

1. INTRODUCTION

Carnation is a member of the *Caryophyllaceae* family, derived from the Latin phrase "Carnation," which means "fleshness" [1]. The term 'Dianthus' comes from the Greek words 'dios' which means' God' or 'divine,' and 'anthos,' which means 'flower,' and is thus known as 'Divine Flower.' The name 'caryophyllus' comes from the Greek words 'caryan' which means 'nut,' and 'phyllon,' which means 'leaf.' Due to the clove-like aroma of carnation, Linnaeus selected the name 'caryophyllus' after the genus name of clove [2]. *Dianthus* species are adapted to cold alpine areas of Europe and Asia. However, they are also found along the Mediterranean coast. *D. caryophyllus* is thought to have commenced in the Mediterranean regions of Greece and Italy (including Sicily and Sardinia). However, its precise origin is difficult to determine due to its lengthy period in cultivation [3]. *Dianthus* species can be cultivated through seeds in the spring and cuttings in late summer [4]. Seeds, stem cuttings, and layering are the most common methods of propagation for carnations [5]. However, these approaches are not suitable for commercial development due to the possibility of genetic variation and slow multiplication rates. As a result, tissue culture techniques have been effectively used for quick plant replication, breeding cycle reduction, and the generation of superior cultivars [6,7]. However, propagation can be done at any time of year in a glasshouse setting. For large-scale replication of disease-free plants, *in vitro* propagation procedures have also been standardized.Carnation's heterozygous makeup makes it difficult to developusing traditional breeding procedures [8]. As a result, combining plant tissue culture technology and genetic transformation approaches to improve it could be a more promising strategy. It is generally recognized that to conduct effective genetic transformation research the plant must regenerate successfully through *in vitro* methods. The response of plants to *in vitro* regeneration conditions is critical for genetic manipulation strategy. Establishing effective *in vitro* regeneration methods is also necessary condition for establishing genetic transformation methods for introducing novel features or studying gene expression regulation *in vitro*. This report describes the optimization of efficient sterilization methods using suitable explants (leaf and nodal explants) for successful callus induction and direct shoot regeneration in carnation.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

The current study was conducted in the Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology in Meerut,(U.P., India). The plant material was collected from the Department of Floriculture and Landscaping, Dr. YSP University of Horticulture & Forestry, Nauni, Solan, (H.P., India), in rooted cuttings of carnation (*Dianthus caryophyllus* L. cv. 'Geolei'). Leaf and nodal segments were used as explants.

2.2 Explants Surface Sterilization

The leaf and nodal segments were washed thoroughly under running tap water for 15-20minutes. Subsequent steps were conducted under laminar airflow (LAF). The leaf explants were then surface sterilized with different concentrations of bavistin (w/v) (A systemic

fungicide, Carbendazim) for 10 minutes and after that the same explants were treated with sodium hypochlorite (NaOCl) (v/v) solution for fifteen minutes. These combinations were also tried with 0.1% mercuric chloride ($HgCl_2$) to check the surface sterilization efficacy on nodal explants for two minutes. After every step, the explants were washed with autoclaved water four to five times. Effects of different concentration were recorded as uncontaminated cultures after four weeks of inoculation.

2.3 Culture Conditions and Callusing

Surface sterilized leaf segments were inoculated on solid Murashige and Skoog [9] medium containing sucrose (3% w/v), agar (0.8%w/v) and various concentrations of growth regulators for *in vitro* culture establishment. Before autoclaving at 121ºC and 1.06 kg/cm^2 for 20 minutes, the pH of the culture media was adjusted to 5.8±0.2 [7]. All cultures were incubated at 25±1ºCfor 16 hours light and 8 hours dark photoperiods under cool white fluorescent tubes (Philips, India). Except for rooting, all trials were performed using full Murashige and Skoog-salt strength [10]. Murashige and Skoog media was supplemented with different concentrations of 2,4-D and NAA for callus induction. The cultures were kept in darkness for seven days to induce callus before being transferred to a 16-hour photoperiod for three weeks. Following four weeks of inoculation, observations were recorded about the percentage of explants producing callus [callus induction frequency (CIF)], callus weight, induction response, as well as the type of callus. Each treatment had six repetitions (Petri dishes), with four to five (1-1.5cm) leaf explants in each Petri dish [11], with slight modifications.

2.4 *In vitro* Direct Shoot Regeneration

Surface disinfected nodal segments were cultured onto solid MS media supplemented with varied quantities of 6-Benzylaminopurine (BAP) and different combinations of TDZ and NAA for *in vitro* culture establishment. The cultures were incubated for four weeks. Observations were recorded for percent shoot induction, average shoot length (cm), and shoot induction response.

2.5 *In vitro* Rooting of Plantlets

Regenerated adventitious shoots from the nodal segment were tweaked off and inoculated for rooting, because the section of the explants in touch with the media gets contaminated in most cases, but the shoots remain unaffected. Half strength MS-media with 0.8% agar supplemented with various concentrations of NAA was used as rooting media. Observations were recorded on root induction (%) and average root length. After that, the rooted plants were withdrawn from the culture tubes, appropriately cleaned to remove agar gel that stuck to the roots, and transferred into small plastic cups filled with sterilized soil and compost (1:1) for hardening [12]. For acclimatization, the plantlets were housed in a green house facility.

2.6 Statistical Analysis

All the experiments were set up in complete randomized design (CRD) with a minimum of three replications per treatment. All the experiments were studied through statistical software OPSTAT (http:// 14.139.232.166/opstat/index.asp) [13,14].

3. RESULTS

3.1 Explants Surface Sterilization

The effects of different doses of bavistin and sodium hypochlorite applied for surface sterilization of leaf explants were observed for 10 and 15 minutes, respectively. After four weeks, the concentration combination of 0.25% (w/v) bavistin and 0.50% (v/v) sodium hypochlorite proved to be the most effective, but it caused minor callus contamination (Fig. 1). A 0.25% (w/v) bavistin and 0.50% (v/v) sodium hypochlorite with 0.1% $HgCl_2$ produced the least contamination for nodal segments.

3.2 Callusing

A total of six culture media combinations with control were tested (Table 1). In the present study, all different culture media compositions induced callusing through leaf segments differently. On culture media supplemented with 2.5 mg/l 2,4-D and 0.75 mg/l NAA showed the maximum callus induction frequency (CIF) (90.47%) (Fig. 3b), followed by 88.95% CIF on culture media supplemented with 2.0 mg/l 2,4-D + 0.75 mg/l NAA. Callus induction (CIF) was minimum (23.80%) in media when supplemented with 0.5 mg/l 2,4-D + 0.50 mg/l NAA. On the other hand, no response was seen in media with no growth regulator (Fig. 2).

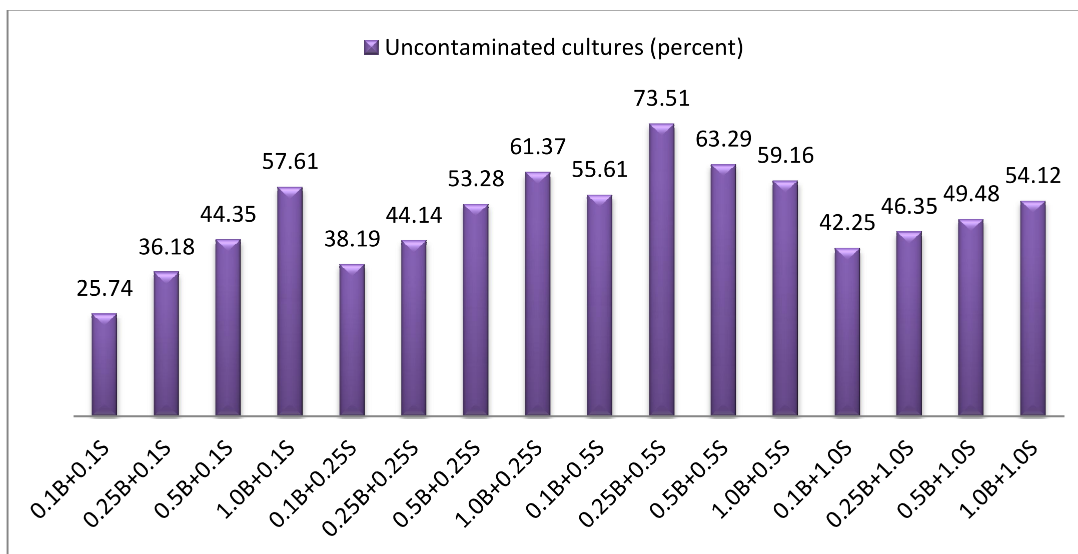

Fig. 1. The effect of different concentrations of bavistin (B) and sodium hypochlorite (S) solution treated for 10 and 15 minutes, respectively, on surface sterilization of explants after four weeks of incubationof cultures of carnation cv. Geolei

Table 1. Effect of growth regulators added in MS medium on callus induction from leaf explants after four weeks old culture of carnation cv. Geolei

SNo.	Growth Regulator (mg/l)	Callus weight/ explants (gm)	Callus induction Response	Type of Callus
T0	Control	0.45	--	NR
T1	0.5 24D+ 0.50 NAA	0.65	+	Less proliferated, soft
T2	1.0 24D+ 0.50 NAA	1.06	++	Greenish white, friable
T3	1.5 24D+ 0.50 NAA	1.29	+++	Yellowish-white, friable
T4	2.0 24D+ 0.75 NAA	2.15	++++	Greenish white, well proliferated
T5	2.5 24D+ 0.75 NAA	2.43	++++	Greenish, well proliferated
T6	3.0 24D+ 0.75 NAA	1.93	+++	Brownish whiteproliferated

Symbol; --: no response (NR), +: poor response, ++: moderate, +++: good, ++++: excellent

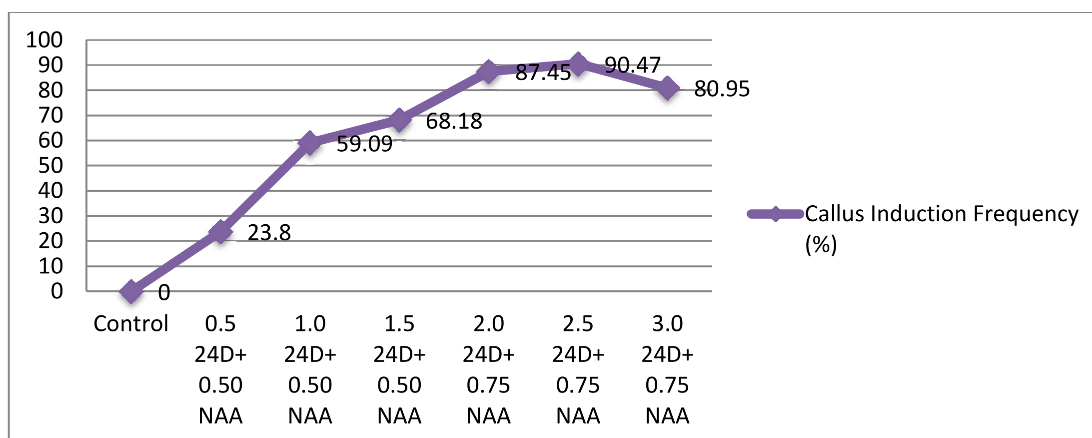

Fig. 2. Effect of plant growth regulators (2,4-D and NAA) supplemented in MS medium on callus induction frequency from leaf explants after four weeks old culture of carnation

Fig. 3. (a): Leaf segments inoculated for callus induction; (b) proliferated callus after four weeks old culture; (c) callus showing no response towards shooting of carnation cv. Geolei

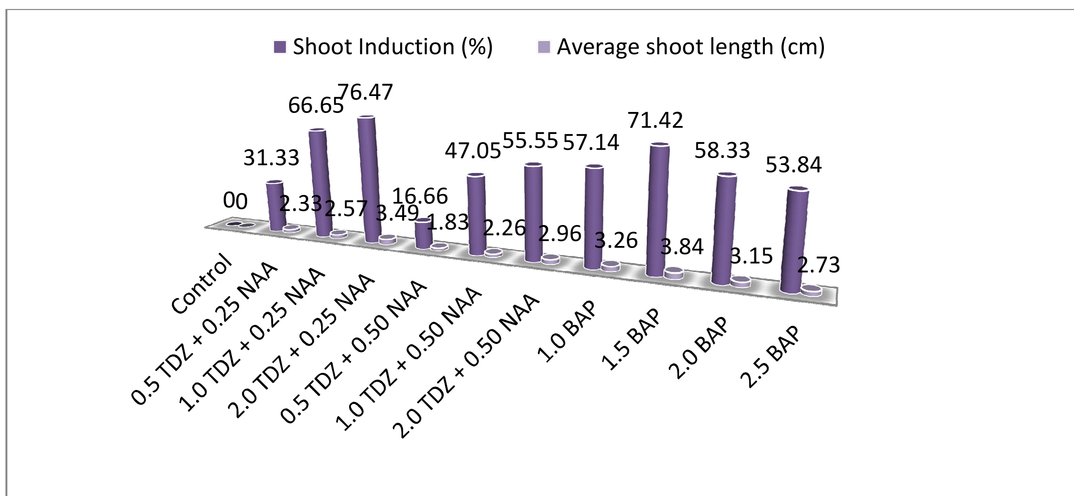

Fig. 4. Effect of plant growth regulators supplemented in MS medium on adventitious shoot induction and average shoot length from nodal segment explants after four weeks of the culture of carnation cv. Geolei

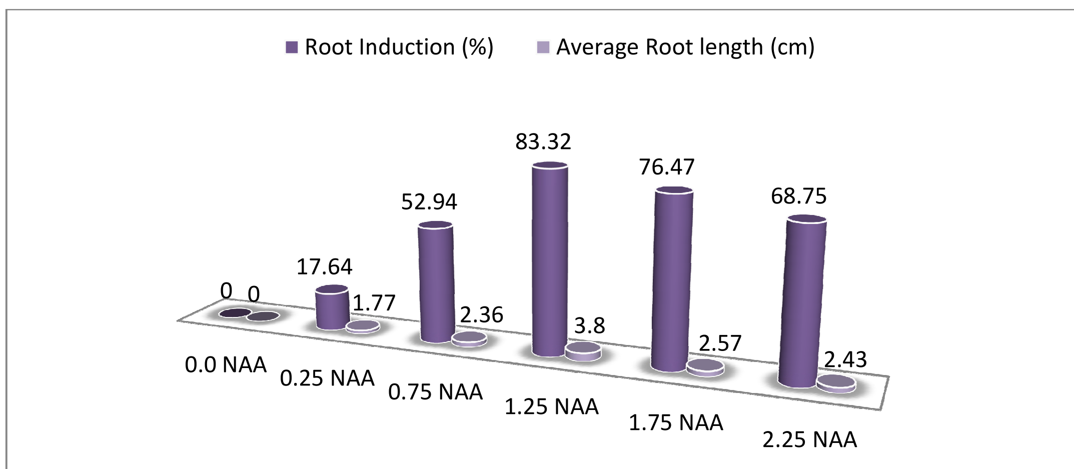

Fig. 5. Effect of plant growth regulators added in MS medium on root induction and average root length after four weeks of the culture of carnation cv. Geolei

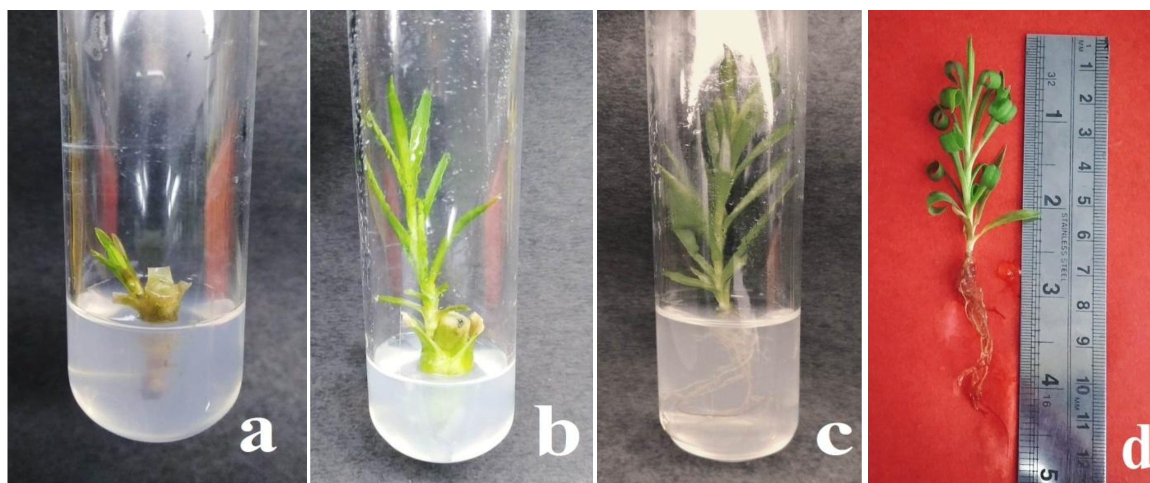

Fig. 6. (a)Adventitious shoot induction and regeneration from nodal segment; (b) shooting after fourweeks in shooting media; (c)elongated roots after four weeks in rooting media; (d) fully developed plantlet

3.3 Adventitious Shoot Induction

The four-week-old callus taken from leaf explants was transferred to shoot induction media. Still, no differentiation towards shooting was seen (Fig. 3c). As a result, we have decided to pursue regeneration employing nodal segments through adventitious shoot induction. Nodal segment explants were inoculated on MS media supplemented with differing proportions of BAP alone and TDZ + NAA. In seven to ten days, the explants began eliciting adventitious shoots. However, only one to two shoots were produced on each explant (Fig. 3 a,b). After four weeks of culture, the last observations for data collecting were taken (Fig. 3). Maximum shoots (76.47%) and (71.42%) were produced by nodal segments maintained on MS medium supplemented with 2.0 mg/l TDZ + 0.25 mg/l NAA and 1.5 mg/l BAP, respectively. Using 1.5 mg/l BAP in MS media, the average shoot length was recorded 3.84 cm, followed by 3.49 cm with concentration combination of 2.0 mg/l TDZ + 0.25 mg/l NAA.

3.4 Rooting and Acclimatization

In the present study, half-strength MS media was used for rooting which was supplemented with 0.25 to 2.25 mg/l NAA including control and media with 1.25 mg/l NAA resulted in the highest response in terms of maximal root induction (83.32%) and most extended root length (3.8 cm) (Figs. 4, 3c). The rooted shoots were withdrawn from the culture vessels after four weeks of culture (Fig. 3d), cleaned carefully to remove the culture media, and dipped in 0.01% carbendazim (bavistin) solution for 10-15 minutes before being transplanted to small plastic cups containing sterilized soil and vermicompost (1:1). To maintain relative humidity, the cups were covered with polythene bags. The combination was then saturated with 1/10th MS medium to maintain the moisture and nutrition, allowing the plant to grow well [11].

4. DISCUSSION

In the present study we tried to check the effectiveness of sterilizing agents Sodium Hypochlorite (NaOCl) with bavistin and $HgCl_2$ in combination and alone and their sterilizing efficacy were tested on Leaf and Nodal segment explants. Two different time combinations were used to make the explants (leaf and nodal segment) contamination-free. Our finding showed that the leaf and nodal segments are more susceptible to fungal contamination, thus increasing the bavistin concentration found more effective; however, increasing the sodium hypochlorite concentration makes the plants less sensitive in the culture. The reason for this is that the NaOCl has a high oxidation capability, which is extremely powerful against bacteria, fungi, and viruses present on the surface of the explants and infest the deep tissues of the explants. The reaction between amino acids and NaOCl yields the aldehyde, NH_4Cl, and CO_2. As a result, direct contact of the tissue with NaOCl during sterilizing process, depending on the concentration,

application time, and temperature, may damage the tissue's health [15].

Several authors have also reported the effectiveness of bavistin and sodium hypochlorite [11,16,17,18,19]. Plant materials were sterilized by treating them for five minutes with an aqueous solution of 0.1% $HgCl_2$ and two drops of Tween 20 under aseptic conditions, followed by five rinses with autoclaved distilled water to remove any residual of $HgCl_2$ [18]. Leaf explants were surface-sterilized for bavistin (w/v) and 5–20 minutes with 0.5% sodium hypochlorite (v/v) [11]. Leaf explants were surface-sterilized by soaking them in 70% ethanol for a minute was useful. In 0.1% $HgCl_2$ containing one drop of Tween 20, for 12 minutes, then washed six times with sterile distilled water [19]. Our study showed clearly that the leaf and nodal segment explants sterilized with the various concentration of NaOCl, bavistin and $HgCl_2$ were effective and reduced contamination in the culture.

In our study the best callusing (90.47%) was observed when media was supplemented with 2.5 mg/l 2,4-D + 0.75 mg/l NAA after effective sterilization of explants. Our study supported by many researchers such as Quadri [20] supplemented MS media with BAP with 2,4-D in combination for callus induction, resulting in 91.66 percent callus induction from leaf and nodal segment explants. Thakur and Kanwar [11] found highest callus induction when MS medium supplemented with 2,4-D and NAA. Jorapur [8] obtained cent percent percent callusing with MS supplemented with 2,4-D and NAA. When MS medium supplemented with NAA and kinetin, Mehta [21] obtained 92 percent callus induction.

Growth regulators play a critical function in the proliferation of shoots and roots. Present study was an effort to get good callus and shoot regeneration with less contamination. Hyperhydricity is a serious physiological condition that develops during carnation *in vitro* cultures and causes excessive hydration and decreased mechanical strength, leading to culture failure. Culture media containing 0.15 mM $FeSO_4.7H_2O$ and 2.25 mM $MgSO_47H_2O$ [22], were used to overcome this situation. The regenerated plantlets showed substantially less hyperhydricity in the carnation.

After callus induction shoot regeneration were achieved using nodal explants on the MS medium supplemented with TDZ, NAA and BAP in combination. This combination gave the maximum shoot regeneration with good shoot length. We also observed that BAP alone at 1.5 mg/l showed the good shoot regeneration but this did not generate good shoot length. It is evident from the present study that TDZ in combination with NAA produced better results for shoot regeneration while BAP alone also proved better but not in terms of shoot length. Therefore combinations of PGRs may prove a better option for shoot regeneration because they have the synergistic effects on the growth of the cell and induce the cell division faster. The 6-Benzylaminopurine, often known as benzyl adenine, BAP, or BA, is a synthetic cytokinin that stimulates the growth and development of plants.

Our study was supported the work of several researchers such as Khatun [17] who reported producing the highest number of shoots (25±0.4) with modified MS media which was supplemented with 1.0 mg/l TDZ and 1.0 mg/l TIBA, the explants demonstrated adventitious shoot regeneration (12.33%). In comparison the maximum frequency of shoot regeneration (18.33 percent) was observed with 1.0 mg/l TDZ and 0.5 mg/l TIBA [23]. When inoculated on MS media supplemented with 2.0 mg/l BA, nodal sections produced more shoots-, than the apical segment [24]. Average shoot regeneration (80.56 percent), average number of shoots (6.01), and average shoot length (1.93 cm) were found to be highest in MS medium supplemented with 1.5 mg/l TDZ, 0.25 mg/l Kinetin, and 0.25 mg/l NAA [11]. In another study, MS medium was used with 10% coconut water + 1.0mg/l BAP, the maximum percentage of shoot induction was recorded [18]. Jorapur [8] reported that the number of shoots dropped when BAP concentration was increased from 2 mg/l to 3 mg/l. Thakur [2] used 2.0 mg/l BAP to achieve maximum shoots (77.77%) with a shoot length of 2.44 cm.On the same media containing 2.0 mg/l BA, Al-Mizory [25] demonstrated *in vitro* shoot regeneration from nodal segment.

For healthy growth and successful commercial production, regenerated shoots must form the root. Because of its effect on fast cell division, auxin plays a critical function in root induction. In the present study, half-strength MS media with 1.25 mg/l NAA proved better and resulted in the highest response in terms of maximal root induction and extended and healthy roots were obtained. Regenerated microshoots were survived well with soil and vermicompost (1:1) mixture under glasshouse conditions. Several researchers have reported that auxins alone or in

combination for rooting in *Dianthus caryophyllus* L. In half MS medium enhanced with combination of IBA and NAA, rapid roots induction and a maximum number of roots per culture were attained [26]. Esmaiel [19] noticed various responses of ten carnation cultivars on MS medium without adding any growth regulator. When cultivated individually on half-strength MS medium with 0.5 mg/l IBA + 0.5 mg/l NAA, 78.2% of regenerated shoots rooted [12]. For 15 days, Casas [27] placed the proliferating shoots on a rooting media of half-strength MS medium with 0.7% agar + 5.4 µM NAA. The shoots were also rooted well in half-strength MS medium supplemented with 2 mg/l indoles butyric acid (IBA) and 0.2% activated charcoal [28].

5. CONCLUSION

The finding of the present investigation has developed an effective surface sterilization protocol. A high frequency *in vitro* callus induction procedure for carnation cv. Geolei was developed in this study. The possible reasons for callusing may be that they are not differentiating towards shooting might be the culture conditions, certain lab practices which went overlooked, or endogenous growth regulators that might have been generated in the culture, which produced an inhibitory impact. A suitable concentration combination of TDZ, NAA and BAP for direct adventitious shooting using nodal segment produced the best results. For rooting, NAA gave the optimum response.The present research indicated that the use of explants sterilized with bavistin, sodium hypochlorite in combination with mercuric chloride is most effective.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ali A, Afrasiab H, Naz S, Rauf M, Iqbal J. An efficient protocol for in vitro propagation of carnation (Dianthus caryophyllus). Pakistan Journal of Botany. 2008;40(1):111.
2. Thakur K, Kanwar K, Rachappanavar V, Pandey H. Refinement of technology for micropropagation of carnation (*Dianthus caryophyllus* L. CV.'Master'). Journal of Pharmacognosy and Phytochemistry. 2018;7(4):1767-1770.
3. Tutin TG, Walters SM, Dianthus L. In Flora Europea TG, Tutin NA, Burges AO, Chater JR, Edmondson VH, Heywood DM, Moore DH, Valentine, et al., eds. Cambridge: Cambridge University Press. 1993;227-246.
4. Galbally J, Galbally E. Carnation and pinks for garden and greenhouse. Timber Press, Portland; 1997.
5. Ahmadian M, Babaei A, Shokri S, Hessami S. Micropropagation of carnation (*Dianthus caryophyllus* L.) in liquid medium by temporary immersion bioreactor in comparison with solid culture. Journal of Genetic Engineering and Biotechnology. 2017;15:309-315.
6. Zhu X, Li X, Ding W, Jin S, Wang Y. Callus induction and plant regeneration from leaves of peony.Horticulture, Environment, and Biotechnology. 2018;59(4):575-582.
7. Maurya RL, Sharma MK, Yadav MK, Kumar G, Kumar M. *In vitro* high-frequency callus induction in carnation (*Dianthus caryophyllus* L.) cultivar "Irene". Plant Cell Biotechnology and Molecular Biology. 2019:1363-8.
8. Jorapur S, Jogdande N, Dhumale D. Petal callus mediated de novo regeneration of shoots in carnation (*Dianthus caryophyllus* L.). The Pharma Innovation Journal. 2018;7(1):218-22.
9. Murashige I, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Plant Physiology. 1962;15: 473-487.
10. Salam FMN, Alhadi FA, AlThobhani MA. Effect of full and half ms-salt strength on some commercial cultivars of carnation (*Dianthus caryophyllus* L.). PSM Biological Research. 2021;6(1):5-12.
11. Mustafa Yildiz, Fatih Ozcan S, Cansu T Kahramanogullari, Ege Tuna. The effect of sodium hypochlorite solutions on the viability and *In vitro* regeneration capacity of the tissue. The Natural Products Journal. 2012;2(4).
12. Yadav MK, Gaur AK, Garg GK. Development of suitable protocol to overcome hyperhydricity in carnation during micropropagation. Plant cell, tissue and Organ Culture. 2003;72(2):153-6.
13. Thakur K, Kanwar K. *In vitro* plant regeneration by organogenesis from leaf callus of carnation, *Dianthus caryophyllus* L. cv.'Master'. Proceedings of the National Academy of Sciences, India Section B:

Biological Sciences. 2017;88(3):1147-1155.
14. Sheoran OP. Online statistical analysis (OPSTAT) software developed by Chaudhary Charan Singh Haryana Agricultural University, Hisar, India; (2010). Available:http://www.hau.ernet.in/opstat.html
15. Qadri ZA, Masoodi NH, Din A, Wani MA. *In vitro* callussing of carnation (*Dianthus caryophyllus* L.) cv. Scania and Indios. *In vitro*. 2018;27(1).
16. Kanwar JK, Kumar S. Influence of growth regulators and explants on shoot regeneration in carnation. Horticultural Science. 2009;36(4):140-146.
17. Kumar A, Verma A, Singh SK, Raghava SPS, Kumar PA. *In vitro* shoot regeneration from leaf segments of carnation (Dianthus caryophyllusL.) via indirect organogenesis. Plant Cell Biotechnology and Molecular Biology. 2006;7:65–68.
18. Mehta R, Sharma S, Nath A. *In vitro* selection and biochemical characterization of carnation (Dianthus caryophyllusL.) callus tolerant to Alternaria dianthi. Indian Journal of Plant Physiology. 2011;12(2):120-126.
19. Maurya RL, Kumar M, Sirohi U, Chaudhary V, Sharma VR, Datta SK, Yadav MK. An effective micropropagation protocol and determination of the clonal fidelity of in vitro developed microshoots of carnation (*Dianthus caryophyllus* L.) using SSR markers. The Nucleus. 2021;1-7.
20. Kharrazi M, Nemati H, Tehranifar A, Bagheri A, Sharifi A. In vitro culture of carnation (*Dianthus caryophyllus* L.) focusing on the problem of vitrification. Journal of Biological & Environmental Scie nces. 2011; 5(13):1-6.
21. Khatun MM, Rahman MM, Roy PK. *In vitro* regeneration and field evaluation of Carnation (*Dianthus caryophyllus* L.) through shoot tip and node culture. Journal of Applied Science and Technology. 2013;9(1):93-99.
22. Ranade R, Kanwar K. Examining synergistic effects of TDZ and TIBA on adventitious shoot induction in *Dianthus caryophyllus* L. leaf explants. The International Journal of Agricultural Science Research. 2014;4(2):17-26.
23. Sharma P, Dhiman SR, Gupta YC, Sharma P, Gautam B. Effects of genotype, explants type and growth regulators on organogenesis in carnation. International Journal of Bio-resource and Stress Management. 2016;7(5):1152-1155.
24. Khatun M, Roy PK, Razzak MA. Additive effect of coconut water with various hormones on in vitro regeneration of carnation (*Dianthus caryophyllus* L.). The Journal of Animal and Plant Sciences. 2018;28(2):589-596.
25. Al-Mizory LS. Effect of different concentration of cytokinins, carbon source and agar on in vitro propagation of Dahlia sp. through one single node. Journal of Life Science. 2013 Oct 1;7(10):1103-12.
26. Esmaiel NN, Al-Doss AA, Barakat MN. An assessment of in vitro culture and plant regeneration from leaf base explants in carnation (*Dianthus caryophyllus* L.). Journal of Food, Agriculture and Environment. 2013;11:1113-1117.
27. Hassan AS, Munshi JL, Sultana R, Jahan MA. A, Khatun R. High frequency in vitro regeneration of *Dianthus caryophyllus* L., a herbaceous perennial ornamental plant. Bangladesh Journal of Scientific and Industrial Research. 2011;46(4):495-498.
28. Casas J, Olmos E, Piqueras A. *In vitro* propagation of carnation (*Dianthus caryophyllus* L.) In: Protocols for *In vitro* propagation of ornamental plants, methods in molecular biology. 2010;589: 109-116.

© 2021 Sirohi et al; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/77234