Open Access
PSM Biological Research
ISSN (Online): 2517-9586

Research Article 2021 | Volume 6 | Issue 1 | 5-12

Article Information

Received: December 30, 2020

Accepted: January 23, 2021

Online first: January 30, 2021

Published: January 31, 2021

Keywords
Carnation,
Cultivars of *Dianthus caryophyllus*,
Micropropagation,
MS-salt strength,
Shoot multiplication.

Authors' Contribution
FMNS designed and performed the experiments. FAA and MAA wrote and revised the paper.

How to cite
Salam, F.M.N., Alhadi, F.A., Al-Thobhani, M.A., 2021. Effect of Full and Half MS-Salt Strength on Some Commercial Cultivars of Carnation (*Dianthus caryophyllus* L.). PSM Biol. Res., 6(1): 5-12.

***Correspondence**
Fathia Mohamed Noman Salam
Email:
malakplant@gmail.com

Effect of Full and Half MS-Salt Strength on Some Commercial Cultivars of Carnation (*Dianthus caryophyllus* L.)

Fathia Mohamed Noman Salam[1*], Fatima Ahmed Alhadi[2], Mansoor Abdalhaleem Al-Thobhani[3]

[1]Biology Department. Faculty of Science, Ibb University, Yemen.
[2]Biology Department, Faculty of Science, Sana'a University, Yemen.
[3]Department of Horticulture and Forestry, Faculty of Agriculture, Sana'a University, Yemen.

Abstract:
This study was conducted to evaluate the effect of full and half-salt concentrations in Murashige and Skoog (MS) medium containing cytokinin (1mg/L Kinetin), auxin (0.1mg/L NAA) and 20gm/L sucrose, on four commercial cultivars of carnation " Gigi, Saeb, Morgan, and Salem". Single nodes were used as a source of explants ≥ 1 cm long to determine the best MS-salt level that gives up the highest multiplication rate (Time of bud initiation, number of shoots, length of shoot, and number of leaves/shoot) and to determine the cultivar that will show the best proliferation rate. After four weeks of consecutive sub-culturing, the multiplication rate features were recorded. Full MS-salt strength gave a significantly higher number of shoots (1.9±0.14), length of shoots (2.69±0.20 cm), and the number of leaves/shoot (7.10±0.54) as compared to the half MS-salt strength (1.56±0.89, 2.00±0.12, and 4.29±0.26, respectively). However, the two salt levels showed no significant difference in the time of bud initiation. Moreover, the cultivar Salem exhibited maximum records in the length of shoots (4.25±0.36 cm), and the cultivar Gigi produced superior number of shoots (2.5±0.34), and they both exhibited the highest number of leaves/shoot (8.42±1.39; and 8.5±0.85, respectively) on full MS-salt strength. It is concluded that for micropropagation of carnation, full MS-salt strength is better than half MS-salt strength, and the cultivars Salem and Gigi are more responsive to the micropropagation on full MS-salt strength than the cultivars (Saeb and Morgan).

INTRODUCTION

Carnation (*Dianthus caryophyllus*) is one of the most important global floricultural crops. It belongs to the family Caryophyllaceae and the genus Dianthus (Muneer *et al*., 2016). The genus Dianthus involves about 300 reported species, most of which are annual or perennial herbs, and a few are shrubs (Al-Sahn *et al.,* 2020). Carnation is native to the Mediterranean region. Its name was derived from the Latin term "Carnatio" which means fleshness (Ali *et al*., 2008).

Carnations are proper for cut flowers and pots. They are also excellent for bedding, borders, and edging of different types of gardens. They give a singular softness to the rock gardens. Though cut carnations are widely traded in the world market year-round, they are particularly demanded for social and family functions, Valentine's Day, Easter, Mother's Day, and Christmas (Ashebre, 2016; Malik *et al.,* 2018). Carnation is widely cultivated due to its attractive properties, including the diversity of its single and multi-color florets, flower scent and size, and long life (Ali *et al*., 2008; Liu *et al.,* 2018). In addition, carnation can be used for perfumery, traditional medicinal, cosmetic, and culinary purposes (Office of the Gene Technology Regulator, 2020).

Carnation multiplication can be done by seed or vegetative methods of propagation (Kantia and Kothari, 2002). For commercial production, the common method of propagation of carnation is stem cutting using lateral young shoots. This technique has some problems such that many diseases like viruses or bacteria and fungi could be transferred from the field cultivated stock plants to the newly produced plants (Shibli *et al.,* 1999; Salehi, 2006). Furthermore, this process is strongly genetic dependent and it may lead to production losses in some commercial cultivars (Justamante *et al.,* 2019). Moreover, it needs high technical expertise to produce flowers of good commercial quality (Al-Sahn *et al.,* 2020). Besides, only four daughter plants could be produced from one mother plant by the ordinary propagation method (Khan *et al.,* 2004).

Tissue culture is a powerful technique that is used for the rapid propagation and production of carnation plantlets (Liu *et al*., 2018). It is a very effective technique for the production of many horticultural crops and cut flower crops in particular. Micropropagation is the most successful and most commonly adopted technique of plant tissue culture. Micropropagation techniques have been standardized for large-scale multiplication of many horticultural crops, mainly to meet the demand of crop producers with higher quality and disease-free plants. Further, plant tissue culture techniques are worthwhile for faster multiplication of the elite indigenous planting material. Micropropagation will enable rapid production of commercially valuable plants from small stem cuttings, axillary buds, etc. (Ahloowalia *et al*., 2002).

Several factors such as selection type of explants, genotype, collection season, the composition of the medium, and the physiological status of the mother plant are determinant factors that should be considered when tissue culture protocols are developed for clonal propagation of plant species (Nadgauda, 2002). MS medium (Murashige and Skoog, 1962) a common medium used in plant tissue culture, has been widely used for micropropagation of carnation by many workers such as (Thakur *et al*., 2018; Kharraz *et al*., 2011; Salehi, 2006).

The present study aimed to evaluate the effect of full and half salt-strength MS medium on some commercial cultivars (CVs) of carnation (*Dianthus caryophyllus* L.) as a part of standardizing an *in vitro* micropropagation protocol for some promising commercial carnation cultivars that have recently introduced to Yemen to facilitate the production of local healthy plant material to meet the needs of carnation cut flower producers.

MATERIALS AND METHODS

Selection of Plant Material

Four cultivars (cvs) of carnation (*Dianthus caryophyllus* L.). 'Saeb, Gigi, Morgan, and Salem' were selected for this study (Figure 1). Cultivars were transferred to the laboratory of tissue culture, Department of Horticulture, Faculty of Agriculture, Sana'a University from a commercial greenhouse in Sana'a, Yemen. The experiments were done on MS (Murashige and Skoog, 1962) medium containing various inorganic, organic components and other additives.

Preparation of Culture Media

Half and full salt concentrations MS media supplemented with cytokinin ((1mg/L Kinetin (Kin)), auxin ((0.1mg/L Naphthalene Acetic Acid (NAA)) and 20gm/L sucrose were prepared.

Different constituents of macro-nutrients, micro-nutrients, and vitamins were weighed individually by using an electronic balance. Each salt was dissolved separately in double-distilled water (DDW) and volume was made up using a volumetric flask. A stock solution of macro-nutrients was prepared at strengths of 10 times the final concentration required to make a liter of nutrient media, while micro-nutrients and vitamins were prepared at a strength of 100 times the final concentration required to make a liter of nutrient media.

Stock solutions of Kinetin (Kin) were prepared in a sterile container by dissolving them in few drops of 1N NaOH. The volume was then made up to the required concentration (1mg/L Kinetin) with double distilled water. The stock of 1-Naphthalene acetic acid, (NAA) was prepared by dissolving the required quantity of each in a few drops of absolute alcohol (ethanol) and diluting with double distilled water up to a known quantity (1mg/L Kinetin and 0.1mg/L NAA).

Sugar as a source of sucrose (20gm/L sucrose) was dissolved in double-distilled water that was then added to make up the final volume of the known quantity. The pH was adjusted to 5.5-5.8 by the addition of a buffer containing 1N HCL or 1N NaOH as required.

While boiling, the medium agar (7gm/L) was added gradually with continuous stirring to dissolves the agar completely and to prevents clumps formation in the prepared medium. Then the medium was dispensed into small glass bottles of 300 ml capacity. These bottles were then closed with polypropylene stoppers. The glass bottles, Petri dishes, and blades were autoclaved at 121˚C for 15 minutes at a pressure of 15 Ibs/In^2.

Preparation of Explant and Inoculation

Single nodes of the plant were used as a source of explants which were ≥ 1 cm long. Surface sterilization was performed as follows: The cuttings were thoroughly rinsed with soap under running tap water for 1 hour. The explants surfaces were then sterilized with 0.5% sodium hypochlorite solution mixed with Tween 20 (2 drops/100 ml solution) for 10 minutes. After that, explants were washed thoroughly 3 – 4 times with sterilized distilled water inside the Laminar Air Flow Cabinet.

The end of surface-sterilized explants was further cut and carefully inoculated onto the prepared medium near Bunsen burner flame using forceps and scalpel blades. The culture vessels were immediately closed and labeled. Then, they were transferred to the incubation room. Cultures were incubated at a temperature of 25 ± 2˚C in a room with an air-conditioned atmosphere under light and dark cycles of 16:8 h.

The growth features were evaluated weekly for 4 weeks. Data were noted for explant shooting after four weeks of inoculation. These data include the time of bud initiation, number of shoots proliferated, length of shoots, and number of leaf pairs per shoots.

Statistical analysis

The descriptive statistics of the data were expressed as a mean and standard error means. Two-way ANOVA followed by a multiple comparison test of LSD was conducted to examine the significant differences in the means using MSTATC program for LSD at $P< 0.05$.

RESULTS

In this study, the time of bud initiation, shoot proliferation, length of shoots, and the number of leaf pairs per shoots were estimated after 4 weeks of culture and the results were presented as shown in Table (1) and Figure (2).

It was clear that the effect of MS-salts strength on time of bud initiation (weeks) showed no significant difference between the full and half MS-salt strength media (2.73±0.11 and 2.92±0.13, respectively). On the other hand, the four cultivars showed a significant difference in time of bud initiation in which the cultivar 'Salem' exhibited faster growth on full and half MS-salt strength media (2.25± 0.13 and 2.33±0.14, respectively) as compared with the other cultivars (Table 1).

Furthermore, the number of shoots produced per explant was significantly greater in the full MS-salt strength medium (1.90±0.14) as compared with the half MS-salt strength medium (1.56±0.89). Regarding the four carnation cultivars, shoot proliferation differed significantly; the cultivar 'Gigi' showed the highest average number of shoots in full MS-salt strength medium (2.5±0.34) and the cultivar 'Salem' produced the highest average number of shoots in half MS-salt strength medium (1.84±0.17) (Table 1).

Moreover, a significant difference was shown between the two MS-salt levels on the length of shoots (Table 1). The longest shoot was found when using a full MS-salt strength medium (2.69±0.20 cm) as compared to half MS-salt strength medium (2.00±0.12 cm). Cultivars of carnation behaved significantly differently for the length of shoots. The cultivar 'Salem' produced a significantly higher average length of shoots (4.25±0.36 and 2.75±0.20, respectively) on full and on half MS-salt strength media than the other cultivars (Figure 2).

There was a significant difference between the two salt levels of MS medium on the number of leaf pairs per shoot. The highest number of leaves/shoot (7.10±0.54) was obtained when explants were cultured on full MS-salt strength, whereas, the minimum number of leaves/shoot (4.29±0.26) was recorded on half MS-salt strength medium.

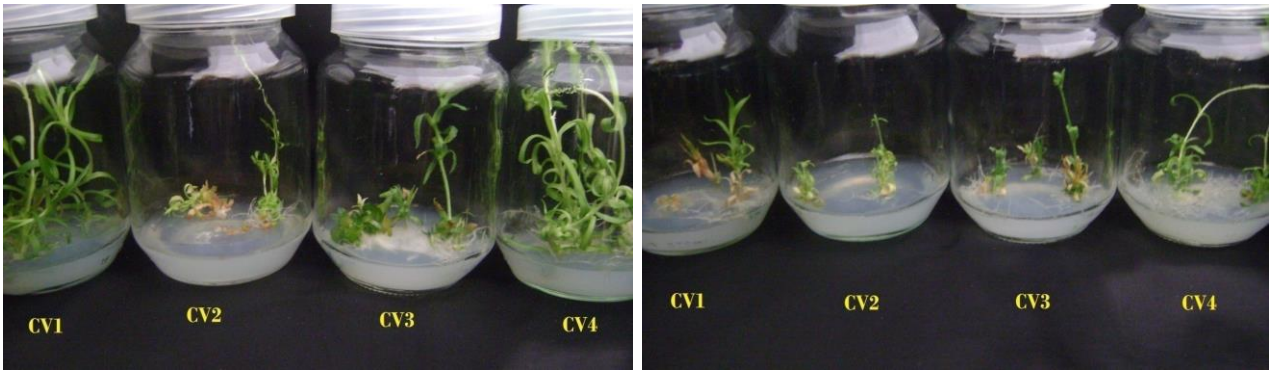
Table 1. Effect of full and half MS–salt on explant producing shoot of four cultivars of carnation after 4 weeks of inoculation.

Cultivars	Full MS				Half MS			
	Time of bud initiation	Number of shoots	Length of shoots (cm)	Number of leaves/shoots	Time of bud initiation	Number of shoots	Length of shoots (cm)	Number of leaves/ shoots
Gigi	2.67±0.19^{abc}	2.5±0.34^{a}	2.75±0.25^{b}	8.42±1.39^{a}	2.92±0.23^{ab}	1.5±0.19^{bc}	2.0±0.21^{cd}	4.5±0.45^{bc}
Saeb	3.0±0.30^{a}	1.5±0.26^{bc}	1.46±0.28^{d}	5.67±1.03^{b}	3.25±0.35^{a}	1.42±0.19^{c}	1.33±0.14^{d}	3.08±0.48^{c}
Morgan	3.0±0.17^{a}	1.5±0.15^{bc}	2.29±0.26^{bc}	5.83±0.79^{b}	3.17±0.17^{a}	1.5±0.15^{bc}	1.92±0.24^{cd}	4.42±0.60^{bc}
Salem	2.25±0.13^{c}	2.08±0.26^{ab}	4.25±0.36^{a}	8.5±0.85^{a}	2.33±0.14^{bc}	1.84±0.17^{bc}	2.75±0.20^{b}	5.17±0.42^{b}
Mean	2.73±0.11^{a}	1.90±0.14^{a}	2.69±0.20^{a}	7.1±0.54^{a}	2.92±0.13^{a}	1.56±0.89^{b}	2.00±0.12^{b}	4.29±0.26^{b}
F- Value	2.92*	3.50*	16.10***	2.26	3.07*	1.09	8.37***	3.13*

* $P < 0.05$; *** $P < 0.001$. Mean ± SEM followed by different letters in the same column show significant differences at the $P < 0.05$ based on Fisher's LSD test.

CV1: Gigi CV2: Saeb CV3: Morgan CV4: Salem

Fig. 1. Cultivars of carnation (*Dianthus caryophyllus* L.) 'Gigi, Saeb, Morgan, and Salem'.



(A) (B)

Fig. 2. *In vitro* growth of 4 cultivars of Carnation (*Dianthus caryophyllus* L.) after 4 weeks of inoculation on 2 salt levels MS medium. (A) Full salt levels MS medium. (B) Half salt levels MS medium. CV1= Gigi, CV2= Saeb, CV3= Morgan, CV4= Salem.

Furthermore, in the full MS-salt strength medium, a significantly higher average number of leaf pairs per shoot were produced by the cultivars 'Gigi' (8.42±1.39) and 'Salem' (8.5±0.85). However, in the half-strength MS medium, the cultivar 'Salem' showed the highest average number of leaf pairs per shoot (5.17±0.42) than the other cultivars (Table 1).

DISCUSSION

In the present study, the time of bud initiation showed no significant difference between the full and half MS-salt. The obtained data are in line with the studies of Siddiqui et al. (1993); Mangal et al. (2002); Onamu et al. (2003); Duhoky et al. (2009); and Hassan et al. (2011), who reported that the lowest time of initiation was recorded when carnation cultivar explants were cultured on MS medium. However, the four cultivars, in the present work, showed a significant difference in the time of bud initiation. This may be attributed to the differences in genotype (Salehi, 2006).

Furthermore, the current study showed that the number of shoots produced per explant was significantly greater in the full MS-salt strength medium, and shoot proliferation differed significantly as the cultivar 'Gigi' showed the highest average number of shoots in full MS-salt strength medium and the cultivar 'Salem' produced the highest average number of shoots in half MS-salt strength medium. These data are

in agreement with the results of Shibli et al. (1999); Mangal et al. (2002); Pareek et al. (2004); Biradar et al. (2007); Ali et al. (2008); Arici and Koç (2009); Hassan et al. (2011); and Lumbomski and Jerzy, (1989) who mentioned that the best shoot formation or multiplication was given on full MS-salt strength medium. However, Hoque et al. (1996); and Dharma, (2003) found that the best response of shoot regeneration was achieved in half-strength MS medium in carnation cultivars. The difference in response may be due to the sensitivity of the tested cultivars (genotype) to the concentrations of the media and hormones involved in the culture as mentioned by Frey and Janick, (1991); and Kallak et al. (1996).

Additionally, our study revealed a significant difference between the two MS-salt levels on the length of shoots as a full MS-salt strength medium exhibited the best results of the length of shoots. However, cultivars of carnation behaved significantly differently for the length of shoots. The difference in results may be attributed to the difference in the salt formulation of medium and the nature of the cultivars tested (Hameed et al. 2006). In this regard, Çetin et al. (2007); and Kanwar and Kumar, (2009) found that shoots of carnation were elongated on full strength MS medium. Thus, it can be concluded that the best treatment for increasing the shoot length of carnation cultivars was full MS-salt strength.

Moreover, the present study showed a higher number of leaves/shoots on full MS-salt strength, and the cultivars 'Gigi' and 'Salem' formed a significantly higher average number of leaf pairs per shoot than the other cultivars in the full MS-salt strength medium. However, in the half-strength MS medium, the cultivar 'Salem' showed a higher average number of leaf pairs per shoots than the other cultivars. In similar, Abou Dahab et al. (2004) found that the greatest number of leaves was produced in full strength medium at third subculture.

As mentioned by Khatun et al. (2016), the initial response to cytokinin may be mediated by increasing the concentration of cytosolic calcium via promoting calcium uptake from the medium. Calcium affects the cytoskeleton, which can regulate exocytosis (Hager *et al.,* 1991).

CONCLUSION

In conclusion, it was found that a full MS-salt strength medium supplemented with 20gm/L sugar, 1mg/L Kin and 0.1mg/L NAA is more effective medium for the propagation of carnation by tissue culture technique as compared with the half one. Moreover, the cultivars; Gigi and Salem are more responsive to the micropropagation on MS medium than the cultivars Saeb and Morgan.

ACKNOWLEDGMENT

The authors recognize and appreciate the great efforts of the late Dr. Taha Mokred, Department of Horticulture and Forestry, Faculty of Agriculture, Sana'a University, Yemen, to achieve this research. They also express their thanks to Mr. Ebraheem Al-Nawd, MSc. Biochemistry, Department of Chemistry, Faculty of Medical Laboratory Sciences, Jibla University for Medical and Health Sciences, Ibb, Yemen, for his role in conducting this paper.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

Funding

None.

REFERENCES

Abou Dahab, A.M., Habib, A.M.A., Hosni, Y.A., Gabr, A.M.M., 2004. Effect of MS-salt strength, sucrose and IBA concentration and acclimatization media on (*Ruscus*

hypoglossum L). Micropropagation. Arab J. Biotech., 8: 141-158.

Ahloowalia, B.S., Prakash, J., Savangikar, V.A., 2002. Low cost options for tissue culture technology in developing countries. In: Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, held at Vienna., 26–30 August 2002. IAEA, Austria., pp. 3-10.

Ali, A., Afrasiab, H., Naz, S., Rauf, M., Iqbal, J., 2008. An efficient protocol for *in vitro* propagation of carnation (*Dianthus caryophyllus* L.). Pak. J. Bot., 40(1): 111-121.

Al-Sahn, J.H.A., Abbass, J.A., Hussein, J.K., 2020. Study of bearing some carnation (*Dianthus caryophyllus* L.) cultivars for salt stress under Iraq conditions. Plant Arch., 20(1): 59-69.

Arici, Ş.E., Koç, N.K., 2009. Regeneration and Agrobacterium-mediated transformation studies in carnation (*Dianthus caryophyllus* L. cv. Turbo). Afr. J. Biotechnol., 8(22): 6094-6100.

Ashebre, K.M., 2016. Effect of Plant Growth Regulators and Different Agronomic Practices on Carnation (*Dianthus caryophyllus* L.) Cultivars Growth and Quality Parameters: A Term Paper. J. Nat. Sci. Res., 6(5): 8-15.

Biradar, K., Amita, J.S., Kenganal, S., Nadaf, M.Y., HLHanchinal, R.R., 2007. Regeneration of plantlets from shoot tip culture in carnation (*Dianthus caryophyllous* L.). Res. Crops., 8(1).

Çetin, E.S., Kazaz, S., Baydar, N.G., 2007. Effects of Different Media on Shoot Tip Culture of Carnation (*Dianthus caryophyllus* L.). Batı Akdeniz Tarımsal Araştırma Enstitüsü Derim Dergisi., 24(2): 01-08.

Dharma, S., 2003. Studies on Factors Influencing the Production of Hardened Glaucous Carnation Plantlets. M.Sc. Thesis. Department of Floriculture and Landscape Architecture, Dr. Y. S. Parmar University of Horticulture and Forestry, Solan., 120 p.

Duhoky, M.M.S., Salman, M.A., Amin, M.E.M., 2009. Micropropagation of Carantion. J. Duhok Univ., 12(1): 61-66.

Frey, L., Janick J., 1991. Organogenesis in carnation. J. Am. Soc. Horticult. Sci., 116: 1108–1112.

Hager, A., Debus, G., Edil, H.G., Strosky, H., Serrano, R., 1991. Auxin induces exocytosis and the rapid synthesis of a high turnover pool of plasma membrane H+ ATPase. Planta., 185: 527-537.

Hameed, N., Shabbir, A., Ali, A., Bajwa, R., 2006. *In vitro* micropropagation of disease free rose (*Rosa indica* L.). Mycopath., 4(2): 35-38.

Hassan, S.A., Munshi, J.L., Sultana, R., Jahan, M.A.A., Khatun, R., 2011. High Frequency *in vitro* Regeneration of (*Dianthus caryophyllus* L.) an Herbaceous Perennial Ornamental Plant. Bangladesh J. Sci. Ind. Res., 46(4): 495-498.

Hoque, M.I., Hashem, R., Khatum, M., Sarker, R.H., 1996. *In vitro* Multiple Shoot Regeneration in Carnation (*Dianthus caryophyllus* L.). Plant Tiss. Cult., 6(2): 99-106.

Justamante, M.S., Acosta-Motos, J.R., Cano, A., Villanova, J., Birlanga, V., Albacete, A., Cano, E.Á., Acosta, M., Pérez-Pérez, J.M., 2019. Integration of Phenotype and Hormone Data during Adventitious Rooting in Carnation (*Dianthus caryophyllus* L.) Stem Cuttings. Plants., 8(226) :12-19.

Kallak, H., Hilpus, I., Virumae, K., 1996. Influence of genotype and growth regulators on morphogenic processes in carnation shoot apex cultures. Sci. Hort., 65(2-3): 181-189.

Kantia, A., Kothari, S.L., 2002. High efficiency adventitious shoot bud formation and plant regeneration from leaf explants of (*Dianthus chinensis* L.). Sci. Hort., 96: 205-212.

Kanwar, J.K., Kumar, S., 2009. Influence of growth regulators and explants on shoot regeneration in carnation. Hort. Sci. (Prague)., 36(4): 140–146.

Khan, S., Naz, S., Saeed, B., 2004. *In vitro* production of (*Cordyline terminalis*) for commercialization. Pak. J. Botany., 36(4): 757-761.

Kharraz, M., Nemati, H., Tehranifar, A., Bagheri, A., Ahmad, S., 2011. *In vitro* Culture of Carnation (*Dianthus caryophyllus* L.) Focusing on the Problem of Vitrification. J. Biol. Environ. Sci., 5(13): 1-6.

Khatun, F., Huq, H., Hoque, M.E., Ali, M.R.**,** Adil, M., Ashraf-Uz-Zaman, K., 2016. *In vitro* rapid regeneration of sugar variety of banana. Int. J. Bus. Soc. Scient. Res., 4(2):132-139.

Liu, B.L., Wei, H., Park, J.E., Jeong, B.R., 2018. Medium Supplementation with Rare Earth Elements Reduces Hyperhydricity during Adventitious Shoot Multiplication *In vitro* of Carnation. Flower Res. J., 26(1): 1-10.

Lumbomski, M., Jerzy, M., 1989. *In vitro* propagation of pot carnation from stem intertnodes. Acta Hort., 251: 235-240.

Malik, A., Prince, Beniwal, V., Sehrawat, S.K., 2018. Influence of Auxins and Types of Cutting on Rooting Efficacy in Carnation (*Dianthus caryophyllus* L). Int. J. Pure App. Biosci., 6(3): 325-331.

Mangal, M., Bhardwaj, S.V., Sharma, D.R., Kaur, R., Mangal, A.K., 2002. Use of meristem tip culture to eliminate carnation latent virus from carnation plants. Ind. J. Exper. Biol., 40: 119-122.

Muneer, S., Soundararajan, P., Jeong, B.R., 2016. Proteomic and antioxidant analysis elucidates the underlying mechanism of tolerance to hyperhydricity stress in *in vitro* shoot cultures of (*Dianthus caryophyllus* L.) J. Plant Growth Regul., 35: 667-679.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture, Physiol. Plant., 15: 473-497.

Nadgauda, R., 2002. Micropropagation Technology Park Project. Final Report. National Chemical Laboratory Pune., 411 -008.

Office of the Gene Technology Regulator. Department of health. Australian government. The Biology of *Dianthus caryophyllus* L. (Carnation). Version 2.1, February 2020. https://www1.health.gov.au/internet/ogtr/publishing.nsf/Content/5DCF28AD2F3779C4CA257D4E001819B9/$File/Biology%20of%20Carnation%20%20v2.1%20February%202020.pdf

Onamu, R., Obukosia, S.D., Musembi, N., Hutchinson, M.J., 2003. Efficacy of Thidiazuron *in vitro* propagation of carnation shoot tips: Influence of dose and duration of exposure. Afr. Crop Sci. J., 11(2): 125-132.

Pareek, A., Kantia, A., Kothari, S.L., 2004. *In vitro* cloning of ornamental species of Dianthus. Ind. J. Biotechnol., 3: 263-266.

Salehi, H., 2006. Can a general shoot proliferation and rooting medium be used for a number of carnation cultivars. Afr. J. Biotechnol., 5: 25-30.

Shibli, R.A., Ajlouni, M.M., Shatnawi, M.A., Abu-Ein, A., 1999. An effective method for *in vitro* production of disease-free carnation (*Dianthus caryophyllus* L.) cv. Balady. Plant Tissue Cult. Biotech., 9(2): 159-166.

Siddiqui, F.A., Naz, S., Iqbal, J., 1993. *In vitro* propagation of carnation" In: Advances in plant tissue culture, I. Ilahi ed., University of Peshawar, Pakistan., 43-47.

Thakur, K., Kanwar, K., Rachappanavar, V., Pandey, H., 2018. Refinement of technology for micropropagation of carnation (*Dianthus caryophyllus* L. CV. ‘Master’). J. Pharmac. Phytochem., 7(4): 1767-1770.