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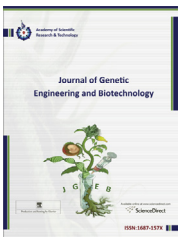

ORIGINAL ARTICLE

Micropropagation of carnation (*Dianthus caryophyllus* L.) in liquid medium by temporary immersion bioreactor in comparison with solid culture

Marzieh Ahmadian [a], **Alireza Babaei** [a,*], **Saber Shokri** [b], **Shahriar Hessami** [c]

[a] *Department of Horticultural Science, School of Agriculture, Tarbiat Modares University, Tehran, Iran*
[b] *Department of Horticultural Science, School of Agriculture, Urmia University, Urmia, Iran*
[c] *HPTCL (Hessami Plant Tissue Culture Laboratory), Karaj, Iran*

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Abstract Developing scale-up system and automation of micropropagation in a bioreactor has been a possible way of cost reduction and intensive manual handling. We report a comparison between the results of experiments aimed at improving carnation micropropagation using new bioreactor according to Temporary Immersion Bioreactor (TIB) and solid culture. By applying different levels of BAP, at the concentration of 3 mg L^{-1}, we observed 14.3 new shoots in TIB, but the number of new shoots on solid medium reached to 5.7 at the same treatment. Our results also showed that with 3 mg L^{-1} BAP in TIB, the initial fresh weight of plant material increased from 10 g to 450 g after 15 days. It is concluded that TIB showed more than 10 times shoot production of carnation. Shoot elongation and rooting induction was successfully stimulated in TIB by applying 1 mg L^{-1} IBA. Rooting of proliferated plantlets from TIB and solid culture were successfully happened, and led to highest number of roots (4.6 cm) and highest length of roots (6.87 cm) in TIB. More than 90% of plantlet was acclimatized to *ex vitro*. Our results suggested that mass production of carnation shoots in our simple TIB, with effective result, can be considered as a critical first step toward large scale production of carnation.

1. Introduction

Carnation (*Dianthus caryophyllus* L.) from dicotyledonous Caryophyllaceae family is one of the most popular commercial flowers all over the world [9]. As an important floricultural crop, carnation is cultivated all-year-round in temperate areas

* Corresponding author at: Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, P.O. Box: 14115-336, Tehran, Iran. Fax: +98 2148292200.
E-mail addresses: m.ahmadian@modares.ac.ir (M. Ahmadian), arbabaei@modares.ac.ir (A. Babaei), sa.shokri@urmia.ac.ir (S. Shokri), shhessami@gmail.com (S. Hessami).

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[22]. Carnation can be propagated through different ways of propagations like, seed, cutting, layering and tissue culture. Among the cut flowers and pot plants commercialized in the Alsmeer market, which is located in Netherlands, nearly 60% of them are produced by micropropagations [4]. Micropropagation is a modern system for production of healthy and virus free stock plants and supports breeding activities in agriculture and forestry [2,4]. Producing of plants through *in vitro* technique is labor intensive and expensive [17]. Since solid medium does not make the full automation of plant propagation possible, scientists shift to moving liquid culture for automation of tissue culture system without using solidifier. These movements to liquid culture make all tissues to be connect with nutrient solution and resulting in growing faster [12]. However, there is still a great obstacle, which is called hyperhydricity. Under precise conditions, hyperhydricity can irreversibly lead to loss of the regenerative ability of the tissue [33]. Vitreous appearance and wrinkled leaves are secondary events resulting from waterlogging of the apoplast [19]. For decreasing unpleasant effects of this physiological disorder, instead of permanent contact of explants with liquid nutrient medium, the times and the frequency of contact should be controlled. In this regard, bioreactors which work according to temporary immersion have been used [20]. Bioreactor technology can be readily adapted for the growth of cells and tissues in liquid media, as demonstrated by the successful mass propagation of *Phalaenopsis* Orchid [28], pistachio [11], *Dendrobium* Orchid [5], pineapple [30], grape rootstock [25], *Oncidium* Orchid [14], *Gerbera jamesonii* [27], and many other plants. Mass production of plants and automation of culture through culture of cells, tissues, somatic embryos and tubers by bioreactors is one of the suitable methods to reduce the cost of industrial propagation in many of plants [17]. The main purpose of applying bioreactors is to provide suitable growth conditions through adjusting physical and chemical parameters in order to get maximum quality and quantity of desired plants [36]. In some cases, bioreactor cultures have many advantages compared with solid cultures, with a better control of the contact of the plant with the culture medium, and optimal nutrient and growth regulator supply, as well as aeration and medium circulation [23]. Nowadays, commercializing and mass propagating of many ornamental species according to somatic embryogenesis and shoot proliferation by using temporary immersion bioreactor is one of the promising ways of rapid and continues multiplication [20,12]. In order to introduce an efficient micropropagation method for carnation micro shoots, our newly designed bioreactor with the supplementation of plant growth regulators (PGRs) to the medium were assessed and compared to solid culture.

2. Material and methods

2.1. Preparation of explants

Carnation (*Dianthus caryophyllus*) "Tessino Cherry" cultivar (a cultivar with important market value in Iran) was transferred to tissue culture laboratory of Tarbiat Modares University from a commercial green house in Pakdasht, Iran. Axillary buds were selected as explants, and they were isolated and were washed under running tap water and surface sterilized by 70% ethanol for 70 s and 2.5% sodium hypochlorite solution for 2 min followed by immersing in Nano Silver solution for 10 min by the concentration of 200 ppm. Disinfected explants were placed in vessels containing 25 mL of MS [35] medium in 250 mL jar supplemented with 30% sucrose and 8 g L^{-1} agar. The pH of medium was adjustment to 5.8 ± 0/2 and explant was culture in establishment medium without any plant grow regulators. Cultures was carried to growth room at light intensity of 30 μmol m^{-2} s^{-1} PPFD emitted by two cool white fluorescent lamps at 25/18 °C (day and night) with 16 h' photoperiod and 40% relative humidity. One month later, we followed two strategies: first, adventitious shoots from this solid medium were cut into 1.5 cm length single nodes with the average weight of 0.2 g for each shoots (10 g total weight of incoming shoots in TIB), and placed in 3 l translucent glass containers containing 1.5 L multiplication medium of carnation according to HIMEDIA™[1] laboratories protocol (control treatment), and other multiplication medium which is supplemented with different level of BAP 1, 2 and 3 mg L^{-1} instead of Kinetin in HIMEDIA™ protocol, and at the second strategy, explants were entered in solid multiplication medium with the same composition of the medium of TIB.

2.2. Preparation and adjustment of TIB

This bioreactor has been invented in a joint project by Tarbiat Modares University and HPTCL. For construction of TIB, we used two translucent glass containers of 3 l capacity, two timers TEBAN®, two pumps HAILEA® ACO-5501, silicone hoses 1/4″ high temperature resistant which is equipped with micro pore filters of Poly tetrafluoroethylene 0.22 μm and two solenoid valves ASCO® which is shown in both Fig. 1A and B. The required power for performing the immersion is produced by the pumps. By pumps, air was distributed through silicone hoses. The culture system (TIB) was placed on aluminium shelves. Aluminium shelves were equipped with white fluorescent lamps to provide the systems with 30 μmol m^{-2} s^{-1} PPFD, and other environmental condition was same as solid culture. Our designed TIB was equipped with timers, which make the adjustment of the numbers of immersion in a day in each immersion. When one of the solenoid valve become opened, air pushes culture medium from its container to plant material container which was controlled via timer based on its program. Then, the second solenoid valve became opened to make the culture passing back to medium container.

In order to find the best frequency of immersion in a day, three treatments of 2, 4 and 6 times of immersion per day (with duration of 2 min) with three replications for each treatment were carried out. After four weeks, the rate of hyperhydricity and the numbers of new shoots were evaluated. Assessment of hyperhydricity was performed via visual observation presented in Fig. 2.

2.3. Multiplication phase

For assessment of the PGRs efficiency on multiplication, plantlets obtained from axillary buds in solid medium were inoculated in bioreactor containing different levels of BAP

[1] HiMedia Laboratories Pvt. Ltd. A-516, Swastik Disha Business Park, Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com.