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Refinement of technology for micropropagation of carnation (*Dianthus caryophyllus* L. CV. 'Master')

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Abstract

The present investigation was conducted on "Refinement of technology for Micropropagation of carnation (*Dianthus caryophyllus* L. cv. 'Master')". Surface sterilized nodal segments were established on solid MS medium containing 2.0 mg/ BA. Multiplication of established buds was carried out on both control and low cost medium (50 g/l starch+ 30 g/l table sugar and Aquaguard water) supplemented with 2.0 mg/l Kinetin. *In vitro* rooting was carried out on solid half strength MS medium supplemented with 0.04% activated charcoal as well as on low cost medium consisting of half strength MS + 50 g/l starch, 15 g/l table sugar and 0.04% activated charcoal. However, multiplication rate and rooting percentage of *in vitro* shoots was lower on low cost medium containing table sugar and starch than control medium containing sucrose and agar-agar but when the cost per plant was calculated it was reduced by 50% on low cost medium in comparison to control medium. Thus, by substituting the components of standard tissue culture medium with low cost components we can reduce the cost per plant for commercial production of the carnation.

Keywords: refinement technology, micropropagation, carnation, *Dianthus caryophyllus*

Introduction

Carnation belongs to family Caryophyllaceae, the genus *Dianthus* and species *caryophyllus*. It is native to the Mediterranean region. The name carnation is derived from the latin term "Carnatio" meaning fleshness (Ali *et al.*, 2008) [1]. Carnations were cultivated over 2000 years ago. The three most common kinds of carnations are: annual carnations, border carnations, and perpetual-flowering carnations in which *D. caryophyllus* L. belongs to perpetual-flowering kind. This plant is one of the world's most important cut flowers due to perpetual flowering (Mii *et al.*, 1990) [7] and single and multi-color cultivars.

The natural climate for carnation occurs near 30° N or S latitudes of equator and on the western edges of the continents. Places having congenial climate like Kalimpong, Shimla, Solan, Kullu, Ooty, Nainital of Himalayan region etc. are most suitable areas for the production of cut flowers. From Himachal Pradesh and Ooty, only rooted cuttings are supplied by the growers to different parts of the country (Singh, 2006) [13]. The importance of this ornamental flower is due to its beauty, diversity of colors, excellent keeping quality, and wide range of different forms (Ali *et al.*, 2008) [2]; In the United States, it ranks next to rose in popularity (Laurie *et al.*, 1968;) [6].

The most successful and most widely used discipline of plant tissue culture technique is micropropagation which is a technique for growing plants from seed or small pieces of plant tissue under aseptic/sterile conditions *in vitro* in controlled laboratory conditions on specially selected nutrient growth media. It also refers to the propagation of plants by using meristem tip culture which is the transfer of apical buds and surrounding leaf primordia to sterile culture conditions (Villabobos, 1986) [15].

Material and Method

The present investigation on "Refinement of technology for micropropagation of carnation (*Dianthus caryophyllus* L. cv. 'Master')" was carried out in the Department of Biotechnology of Dr. YSP UHF, Nauni, Solan (H.P.). The plant material in the form of stem cuttings of carnation (*Dianthus caryophyllus* L. cv. 'Master') was obtained from the Dept. of Floriculture and Landscaping, Dr. YSP UHF, Nauni, Solan (H.P.). Nodal segments were isolated from cuttings and used as explant.

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1. *In vitro* establishment of cultures

For *in vitro* establishment of cultures surface sterilized nodal segments were cultured on solid MS medium supplemented with different concentrations of BA (0.5 mg/l-2.5 mg/l). The cultures were incubated at temperature of 25±2 °C under 16 hour photoperiod for four weeks. The observation was recorded for establishment of shoot buds of nodal segments of carnation

2. *In vitro* multiplication on control medium

Multiplication of established shoots was carried out on MS medium (8 g/l agar-agar+30 g/l sucrose) supplemented with BA (1.0 mg/l-2.0 mg/l) and kinetin (1.0 mg/l - 3.0 mg/l) alone or in combination with each other. The cultures were incubated at 25±2 °C under 16 hour photoperiod for four weeks. After four weeks of incubation observations were recorded.

3. *In vitro* multiplication on low cost medium

For standardization of low cost protocol *in vitro* multiplication of established shoots was carried out on MS medium standardized, where sucrose is replaced with different carbon sources (table sugar (30 g/l), sugarcane jaggery (30 g/l)), agar-agar is replaced with different solidifying agents (50 g/l starch, 100 g/l tapioca pearls, 30 g/l isabgol, cotton) and distilled water is replaced with different water sources (Aquaguard water, tap water). The cultures were incubated at 25±2 °C under 16 hour photoperiod for four weeks. After four weeks of incubation observations were recorded.

4. Subculturing of microshoots

Subculturing of multiplied shoots was done on solid MS multiplication medium (8 g/l agar-agar + 30 g/l sucrose) and low cost medium (50 g/l starch and 30 g/l table sugar) at an interval of 4 weeks. Clumps of shoots from multiplied explants were separated singly and subcultured to fresh multiplication medium after four weeks of previous subculture. The observations were recorded.

5. *In vitro* rooting of microshoots on control and low cost medium

In vitro raised shoots of 2.5-3.0 cm length were excised at different stages of subculturing and transferred to half strength MS medium and low cost MS medium with activated charcoal (200 mg/l, 400 mg/l) and IBA (1.0 mg/l-2.5 mg/l) for root induction. Experiment consisted of 24 microshoots in three replications followed by completely randomized design. The cultures were incubated at 25±2°C under 16 hour's photoperiod. After three weeks of incubation observations were recorded.

6. Hardening

1. Preparation of potting mixture

Potting medium (sand: cocopeat in ratio 1:1) was brought to homogenous consistency and was sterilized in an autoclave at a pressure of 15 lbs per inch² at 121 °C for half an hour. After sterilization, mixture was transferred to small plastic pots.

2. Washing of roots

In vitro raised microshoots of length 3.0 to 4.0 cm along with 3 to 4 leaves were taken out from the tubes in such a way that roots were not damaged. Roots were then washed for 10-15 minutes under running tap water to remove the adhering medium. After that, plants were kept in water for about 30

minutes, so that they do not wilt after transferring to soil. Thereafter, roots of the *in vitro* raised shoots were dipped in 0.2% bavistin solution for 15 minutes to avoid any fungal attack.

3. Transplantation into pots

Well-developed plantlets were transferred to plastic cups of diameter 7.0 cm. Plantlets were then placed in the potting medium. The plants were covered with polythene bags to maintain the relative humidity. The plants were watered at every alternate day and observed.

Result and Discussion

Growth regulators play a very important role in shoot regeneration and multiplication. Nodal segments were established on control media comprising MS medium supplemented with 3% sucrose, 0.8% agar- agar and 2.0 mg/l BA induced maximum shoot establishment of 77.77 per cent. Similarly, Mizory *et al.* (2014) [8] also obtained *in vitro* shoot regeneration in carnation from nodal segment on same medium containing 2.0 mg/l BA. It was also observed that with increase or decrease in concentration of BA shoot formation response was decreased.

MS medium supplemented with 2.0 mg/l BA was found to be best resulting in 4.50 numbers of shoots per explant with shoot length of 2.44 cm. Similar result showing *in vitro* shoot multiplication of *Dianthus caryophyllus* L. on medium containing BA were also observed by Kharrazi *et al.*, (2011) [5] obtained 5.0 number of shoots on medium supplemented with 4.0 mg/l BAP. A very high incidence of hyperhydricity showing thick stem and leaf with stunted growth was observed during *in vitro* multiplication of microshoots on medium containing BA alone and in combination with Kn. Hyperhydricity is a major physiological disorder encountered during *in vitro* cultures of carnation resulting in total loss of cultures.

With the objective of reducing this problem another experiment was conducted where MS medium supplemented with kinetin (1.0 mg-3.0mg/l) alone was used as the shoot obtained in medium containing BA in combination with kinetin were less hyperhydric than those multiplying on medium containing BA alone. Normal shoots with no signs of hyperhydricity were produced in the medium supplemented with 2.0 mg/l kn alone resulting in 3.67 number of shoots per explant and 4.55 cm shoot length. It may attribute to the concentration of growth regulators in the medium. It was possible that endogenous growth regulators were synthesized in the culture resulting in inhibitory effect. Thus, replacing the growth regulators may play a positive role in overcoming the problem. Similarly, Tsay and Ku (1994) [14] also observed that shoot vitrification increased with increasing concentration of BA during *in vitro* culture of *Dianthus caryophyllus* L. and lower down the concentration of BA in the medium for further multiplication of shoots.

In vitro multiplication on low cost medium

In the present investigation 0.8% agar-agar was substituted by 5% starch, 3% sucrose replaced by 3% table sugar and distilled water was replaced with aqua-guard water along with 2.0 mg/l BA. The carbon source such as sucrose contributes about 34% of the production cost (Demo *et al.*, 2008) [4]. Also the replacement of medium components such as agar-agar can reduce the cost to medium preparation, hence, the cost of plantlet production which will in turn lower the cost of the plantlets. In case of low cost medium average number of

microshoots was 3.52 per explant and highest shoot length of 3.01 cm was obtained with 3 per cent table sugar and 5% starch Mohamed *et al.* (2010) ^[7] obtained highest number of microshoots per explant (6.8) on medium supplemented with 50.0 g/l potato starch and 1.0 g/l agar.

Subculturing of Microshoots

Highest number of shoot frequency with maximum 5.58 number of microshoots per explant and maximum shoot length of 4.66 cm was observed after 5th subculturing on control medium. Whereas, on low cost medium maximum 5.27 number of microshoots per explant with maximum shoot length of 3.53 cm was obtained after fifth subculturing. The number of microshoots per explant was statistically at par with control but the shoot length per explant was more in case of control as compared to the low cost medium. Bora *et al.* (2007) ^[3] reported high rates of multiplication in carnation cv. Lipstick on the MS medium supplemented with 0.5 mg/l NAA, 1.5 mg/l kinetin and 0.5 mg/l GA₃.

Rooting of microshoots

Out of seven media used, half strength MS medium supplemented with 0.04% activated charcoal resulted in highest rooting percentage of 62.49 with 4.50 number of roots per shoot and 1.61 cm root length. It was found that on half strength media highest root formation can occur as compare to MS medium indicating that a higher concentration of nutrients inhibited the formation of roots. The rooting was also done on best combination of low cost substitutes (table sugar+ starch+ Aquaguard water). For this half strength MS medium supplemented with 50 g/l starch+ 15 g/l table sugar+0.04% activated charcoal resulted in 48.58% rooting with 3.50 number of roots per shoot and 1.75 cm root length. Similar results were observed by Erst *et al.* (2014) ^[5] where maximum rooting on half strength MS basal medium in *Dianthus mainensis*.

In the present investigation it was observed that half strength MS medium supplemented with different concentrations of IBA (1.0-2.5 mg/l) resulted in low rooting percentage. It was due to the reason that microshoots have enough amount of hormones and do not require any extra amount of exogenous growth hormones for their regeneration and thus showed inhibitory effect for rooting percentage. In contrary to our results, Rahman *et al.* (2014) ^[10] obtained highest percentage of rooting on MS medium supplemented with 0.3 mg/l IBA in carnation.

In comparison rooting percentage in control medium was highest (62.49%) as compared to low cost medium (48.58%). Also, number of roots per explant and root length showed variation in our investigation. The number of roots per shoot in control medium was 4.50 whereas in low cost medium it was 3.50. The root length was more in case of low cost medium (1.75cm) and in control medium 1.61 cm root length was obtained. Similar results were obtained by Ogero *et al.* (2012) ^[9] in sweet potato. They found higher number of roots on the control medium as compared to low cost medium.

Comparative cost of control and low cost medium

As micropropagation technology is more expensive than the conventional methods of plant propagation therefore sometimes the unit cost per plant becomes unaffordable, despite its obvious advantages. It is major concern limiting its wide application. The major factors which account for the cost of micropropagated plants is media preparation (Savangikar, 2002) ^[11]. Mostly for media preparation two

important components sucrose (carbon source) and agar-agar (gelling agent) account for 34% (Demo *et al.*, 2008) ^[4] and 70% (Savangikar, 2002) ^[11], respectively increase in the cost. The cost analysis showed that average cost of one liter of control medium with agar (0.8%) and sucrose (3.0%) was Rs. 118.27 whereas the cost of one litre of low cost medium with starch (5%) and table sugar (3%) was Rs. 42.05. Also, when per plant cost was replacing these two components it was very low Rs. 3.13 as compared to per plant cost with sucrose and agar which was Rs. 7.61. Likewise, Sharma (2012) ^[12] observed that when tapioca pearls and table sugar were replaced with agar and sucrose, respectively, per plant cost was low Rs. 4.28 as compared to per plant cost with sucrose and agar-agar which was Rs. 9.05.

Conclusion

The present investigation was conducted on "Refinement of technology for Micropropagation of carnation (*Dianthus caryophyllus* L. cv. 'Master')". Surface sterilized nodal segments were established on solid MS medium containing 2.0 mg/ BA. Multiplication of established buds was carried out on both control and low cost medium (50 g/l starch+ 30 g/l table sugar and Aquaguard water) supplemented with 2.0 mg/l Kinetin. *In vitro* rooting was carried out on solid half strength MS medium supplemented with 0.04% activated charcoal as well as on low cost medium consisting of half strength MS + 50 g/l starch, 15 g/l table sugar and 0.04% activated charcoal. However, multiplication rate and rooting percentage of *in vitro* shoots was lower on low cost medium containing table sugar and starch than control medium containing sucrose and agar-agar but when the cost per plant was calculated it was reduced by 50% on low cost medium in comparison to control medium. Thus, by substituting the components of standard tissue culture medium with low cost components we can reduce the cost per plant for commercial production of the carnation.

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