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A Research on Obtain Virus Free Plant by Means of Meristem Culture in Lena and Scania Carnation (*Dianthus caryophyllus* L.) Cultivars

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Abstract. This study was carried out to obtain viruses (Mottle, Vein mottle, Ring spot) free plants to carnation cultivars (Lena, Scania) by means of meristem culture. In the result of experiment carried out *in vitro* conditions, the MS basal medium added 1.0 mg/l of BAP was found to be appropriate for meristem development and shoot formation when 0.2-0.4 mm long meristem tips were cultured. For rooting of shoots, the best results were obtained with MS basal medium containing 1.0 mg/l IBA. It was determine tahat the plants obtained from *in vitro* conditions established and developed in a mixture of two parts chestnut soil+one part sand+one part sheep manure. Plants were inspected by ELISA to identify whether they were infected by viruses. The viruses were completly eliminated testing plants from meristem culture.

Key Words: Carnation, meristem culture, virus

1. Inroduction

Carnation *(Dianthus caryophyllus* L.) is most famous for its use as a cut flower in the florist trade, but also perform well in the garden as a bedding plant. Carnation a member of the family Caryophyllous has 88 genera and 1750 species. Carnations were cultivated over 2000 years ago. Modern varieties were developed first in France in 1840. The name carnation is derived from the latin tern *"Carnatio"* meaning fleshness. Caryophyllous means pink refers to the colour of blooms of the original species.

Its crop succeeds in natural spread area and outside areas of natural spread area because of new varieties which improved throught breeding [1].

Carnation production is 63% of the total cut flower production and carnation export is 99% of total cut flowers export in Turkey. It is the most important flower in Turkey [2].

One of the most viable methods for obtaining virus-free stocks from propagative material that comes from infected plants is viral eradication by using tissue culturetechniques, aided or not by thermo-and/or chemotherapies [3]. These methodologies allow quick propagation of plant material, producing healthy plants from a single individual in a short period of time, regardless of location or season of the year.

Meristem is active growth points formed by a very fast-growing cells in a dome 0.1 mm. İn diameter and 0.25 mm in lenght in plants. Meristems in these dimensions are culture based on the virus and virus-like disease-free plants are obtained [4].

The most successful and most widely used discipline of plant tissue culture technique is micrpopropagation which 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 [5,6,7,8]. They used

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meristem culture method and obtain virus free plants from different carnation varieties [9]. MS medium supplemented with combination of 1.0 mg/l NAA for shoot induction and virus free materials [10].

Repoted the obtain virus-free materieal and the best shoot formation on MS mediun with NAA and Kinetin [11]. The highest rooting response was obtained in MS medium containing 5 mg/l. IBA. The best results in initiation stage were occurred when the explants of carnation cultivar cultured on MS medium supplemented with a high concentration of BA and low concentration of IAA. The highest multiplication rate were obtained by culturing the explants on MS medium supplemented with 2.0 mg/l+0.1 mg/l IAA [12].

Also reported developed shoots and callus to MS medium containing different concentratins of NAA ana Kinetins. It ws observed that good rooting to MS media with NAA and BAP [13].

Researchers used modified MS media for meristem culture to three different carnation cultivars. They isolated 0.2 and 0.8 mm size meristems from plants have made aplication. They reported beter results Lena and Scania cultivars than White sim cultivar [14].

Best shoot formation response was obtained from nodal meristem on MS medium supplemented with 4.0 mg/l BAP. Apical meristem showed more pronounced effect for shoot formation than nodal meristem. Maximum number of multiple shoots were obtained on MS medium containing 1.0 mg/l BAP. Shoots after attaining the size of 5.0 cm were shifted for rooting. Best rooting response was obtained on MS medium containing 1.0 mg/l NAA [15].

In this research, to obtain viruses (Mottle, Vein mottle, Ring spot) free plants to carnation cultivars (Lena and Scania) by means of meristem culture for obtaining virus-free material.

2. Material and Methods

In the research, Carnation *(Dianthus caryophyllus* L.) cultivars (scania and Lena) and viruses (Mottle, Vein mottle, Ring spot) were use.

Apical meristems of 0.2-0.4 mm size were used as explants. Explants were obtained from field grown plants. They were washed thoroughly with tap water. The single node cuttings

were immersed in ethyl alcohol 70% for 30 seconds, then transferred to a 2% hypochlorite solution for 10 minutes and washed three times, sequentially in sterilized distilled water. Inoculation was carried out in laminar air flow cabinet. It was cleaned with 50% ethanol solution and was irradiated with UV irradiations for 30 minutes before use.

MS medium [16] supplemented with different concentrations of auxin and cytokinin along with 3% sucrose was used. pH of the medium was adjusted to 5.8. Agar (6 g/l) was used for solidification of media. The media was autoclaved at 121 °C for 15 minutes at 15 lbs/In^2 pressure.

For shoot induction and proliferation, MS media containing different concentration and combinations of BAP (0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/l) was used. For rooting MS media was supplemented with different concentration of IBA (0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/l). Six explants were cultured in each test jar and four jar were used for each cultivar. Explants were cultivated and maintained in a growth room under light intensity of 3000 lux having temperature of 23±1.0°C and photoperiod was 16 hour with 8 hours dark period in every 24 hour cycle.

Developed *In vitro* plants were shifted for rooting. These plants were acclimated in pots containing a mixture of chestnut soil+sand+sheep manure in the 2:1:1 proportion, and kept at 90% humidity level for four weeks. The plants remained in an environment without incidence of direct sunlight during this period.

The leaf samples were tested by Enzeyme-linked immunosorbent assey (ELISA) for the presence of virusses [17].

3. Results and Discussion

The meristems of various sizes (0.2-0.4 mm diameter) were excised under binocular microscope and cultured on the Murashige/Skoog (MS) media containing Benzyl Amino Purine (BAP). Highly significant differences were observed in the mean of plant height for different hormones and their levels.

It was observed that when BAP was used alone at 1.0 mg/l concentration for Lena cv.and Scania cv. total 16.3 and 18.8 shoots were obtained respectively in all the cultures. By increase in the concentration of BAP the rate of shoot multiplication was decreased and at 6.0 mg/l BAP only 3.2 and 2.9 shoots per culture were formed (Table 1). No good response of shoot multiplication was obtained at basal MS. Some researchers reported similar results in carnation in which vigorous shoots were produced [13,15,18,19]. Kovac also reported highest shoot multiplication in carnation in MS medium containing 1.0 mg/l BAP [20].

Table 1. Effects of different concentrations of BAP on *In vitro* shoot multiplication.

Media	Number of multiple shoot formed		Average shoot lenght (cm)		Days for shoot multiplication	
	Lena	Scania	Lena	Scania	Lena	Scania
MS Basal	2.4	2.6	1.3	1.6	32	30
MS 0.5 mg/l BAP	9.8	10.5	3.7	4.4	28	27
MS 1.0 mg/l BAP	16.3	18.7	5.4	5.9	25	23
MS 2.0 mg/l BAP	7.5	8.2	3.5	4.2	27	25
MS 4.0 mg/l BAP	4.1	5.0	2.1	2.8	30	29
MS 6.0 mg/l BAP	3.2	2.9	1.6	1.7	35	32

Reported the best shoot formation response of carnation on MS medium supplemented with BAP [21].However, another mentioned the best shoot induction response in MS medium containing 5.0 mg/l Kinetin while used MS medium supplemented with combination of NAA and Kinetin for shoot induction from meristem, but in present study it was observed that addition of kinetin failed to stimulate shoot induction response. On the other hand there was a decline in shoot induction with increase in the concentration of Kinetin [22,23].

Well developed *In vitro* plants after attaining the size of about 5.0 cm. were shifted for rooting. For *In vitro* rooting MS medium was supplemented with NAA ranging from 1.0 mg/l to 6.0 mg/l. Largest roots were recorded onIBA at 1.0 mg/l. Followed by 2.0 mg/l IBA, whereas the shortest roots were observed at basal MS and 6.0 mg/l.BAP for the two carnation cultivars. By increasing the concentration of IBA, root induction response was decreased and at 6.0 mg/l of IBA very poor results were obtained (Table 2). Similar results were founded by [11,13,15,20]researchers also reported highest shoot multiplication in carnation in MS medium containing 1.0 mg/l BAP. Although 2.0 mg/l. BAP also showed good shoot multiplication response but time taken for shoot multiplication was more and number of shoots formed were comparatively less than 1.0 mg/l BAP alone. However they reported highest numbers of shoots per explant on MS medium containing 0.9 mg/l BA and 0.3 mg/l NAA [24,25].

Table 2. Effects of different concentrations of IBA on *In vitro* rooting.

Media	Root induction response*		Days for root induction	
	Lena	Scania	Lena	Scania
MS Basal	1	1	23	23
MS 0.5 mg/l IBA	3	3	21	20
MS 1.0 mg/l IBA	5	5	19	18
MS 2.0 mg/l IBA	4	4	22	20
MS 4.0 mg/l IBA	2	2	24	22
MS 6.0 mg/l IBA	1	1	28	28

*1 poor, 2 fair, 3medium, 4 good, 5 very good

Rooted plantlet were acclimated in pots containing a mixture of chestnut soil+sand+sheep manure in the 2:1:1 proportion, and kept at 90% humidity level for four weeks. The plants remained in an environment without incidence of direct sunlight during this period in glasshouse.

All plants in glasshouse tested for virus detection by (ELISA) technique were free of all three viruses (Mottle, Vein mottle, Ring spot).

The pathogens as virus, viroid and mycoplasma cause serious economic losses in plants. To obtain virus-free material in plants, the methods such as nuceller embryony, meristem cultur, shoot-tip grafting and thermotherapy are used alone or in combination.

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