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Evaluation of Tip Culture and Thermotherapy for Elimination of Carnation Latent Virus and Carnation Vein Mottle Virus from Carnation Plants

Amal A. Ahmed, Eman A.H. Khatab, Rehab A. Dawood and Amira M. Ismeil

Virus and Phytoplasma Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

Corresponding Author: Amal A. Ahmed, Virus and Phytoplasma Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

ABSTRACT

Carnation Latent Virus (CLV) and Carnation Vein Mottle Virus (CarVMV) were of the most important virus affecting carnation plants. The two viruses were detected serologically by DAS-ELISA using specific antibodies. Shoots from plants infected with (CLV and CarVMV) which maintained in green house were used for tip culture. Murashige and Skoog Medium (MS) supplemented with (0.2 mg L⁻¹ BA) and (1 mg L⁻¹ BA and 0.5 mg L⁻¹ kinetin) were used for proliferation and micropropagation of the infected shoot clumps, respectively. The plantlets were rooted in MS medium supplemented with (1.5 mg L⁻¹ NAA). Plantlets regenerated from meristem measuring 0.1 and 0.2 mm yielded (90 and 80%) for CLV and (92 and 80) for CarVMV virus-free tested plantlets respectively, while larger meristems (0.3 and 0.4 mm) were not effective. As well as, the plantlets survival was (20, 35, 65 and 80%) for meristem measuring (0.1, 0.2, 0.3 and 0.4 mm), respectively. Where as the exposure of carnation plantlets infected with CLV and CarVMV to three temperature regimes (36, 38 and 40±1°C for 3 and 4 weeks). It was found that (38±1°C/3 weeks) gave (65 and 70%) for CLV and CarVMV virus-tested plantlets, respectively and plantlets survival were (67 and 40%). While (38 and 40±1°C for 4 weeks) gave higher percentage of virus-free plantlets but has a negative correlation with the survival of the plants. However, because of better survival rate, the temperature regime 38±1°C/3 weeks is recommended for virus-free tested carnation plants.

Key words: Benzyl amino purine (BA), elimination, naphthalene acetic acid (NAA), tissue culture and viruses

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is a member of Carpophyllaceae and anative of the Mediterranean area (Dole and Wilkins, 1999). This plant is one of the world's most popular, economic and important cut flowers due to perpetual flowering (Mii *et al.*, 1990) and presence of new single and multi-color cultivars (Dole and Wilkins, 1999). Carnations are divided to two groups, standard and miniature (spray), that produce in each stem, one large terminal flower and several smaller lateral flowers, respectively (Dole and Wilkins, 1999). Carnation cultivars, have high heterogosity and must be vegetatively propagated (Mii *et al.*, 1990). Generally, one or more viruses (Ram and Zaidi, 1999) infect stem cuttings of this plant. Tissue culture and micropropagation

techniques can aid us in producing uniform and pathogen-free plants suitable for export to other countries around the world. Micropropagation methods of this plant have been reviewed (Mii *et al.*, 1990) and some other reports are also available (Choudhury and Garg, 1999; Jagannatha *et al.*, 2001).

Carnation are affected by over 15 virus disease. The most important viruses are Carnation Mottle Virus (CarMV), Carnation Vien Mottle Virus (CarVMV) Carnation Latent Virus (CLV), Carnation Necrotic Fleck Virus (CNFV), Carnation Etched Ring Virus (CERV).

CLV and CarVMV are from the most determinal viruses affecting carnation crop, causes significant economic losses to farmers.

CLV causes no distinct symptoms on carnation plants, however, it can affect crop production and has been demonstrated to impair flower quality. And it transmitted from plant to plant by aphid (Mangal *et al.*, 2001a).

CarVMV leads to mild symptoms, it causes sometimes severe infection in all types of carnations. This virus is responsible for the poor quality of cut-flower in terms of size, split calyces and reduced vigour, in addition to lesser yields in terms of lateral shoots, total number of flowers and fresh weight (Lisa, 1995).

Since there is no chemical sprays of virus control once a plant is infected with a virus there is little the lower grower can do. The only current method for controlling virus diseases is through the use of virus tested materials in conjugation with strict hygienic practices. To prevent infection of this virus-tested material, the carnation grower should enforce hygiene regulations for example, washing hands with hot soapy water before handling plants, aphid-proof glasshouses, strelization of cutting and propagating instruments, etc. (Lisa, 1995).

It was found that the yield of a "virus-tested" Carnation crop was compared to that of a virus infected crop, indicated that the yield was 39% greater and the profit was 20% greater in the case of the virus tested carnations. Also the virus-tested carnation were of a superior quality (Salehi and Khosh-Khui, 1996).

So, tissue culture and micro propagation techniques can aid us in producing uniform and pathogen-free plants suitable for local industry and export to other countries around the world.

The present study aims to produce and evaluate the efficiency of different *in vitro* techniques for CLV and CarVMV as mixed infection from carnation plants.

MATERIALS AND METHODS

Plant material: Carnation plants (*Dianthus caryophyllus* L.) were used as a source for CLV and CarVMV. The plants were tested by ELISA. From the plants tested positive by DAS-ELISA and show mixed infection, some of them were used for meristem tip culture directly and some used for *in vitro* propagation to produce proliferating shoot clumps for therapeutic purposes.

Meristem tip culture: Apical meristem tips measuring 0.1, 0.2, 0.3 and 0.4 mm (domes with 1-2 primordia) were excised in sterile conditions and transferred to glass tubes containing Murashige and Skoog's Medium (MS) supplemented with 0.1 mg L⁻¹ NAA and 2 mg L⁻¹ kin (Mangal *et al.*, 2002a)

Production of proliferating shoot clumps: Single-node or shoot-tip explants with about 0.5 cm length were cultured on the basic MS medium supplemented with 30 g L⁻¹ sucrose, 8 g L⁻¹ agar and 0.2 mg L⁻¹ benzyl amino purine BA.

Multiplication (micropropagation) of infected shoot cultures: Plant cultures were micropropagated by subculturing shoots at 3-week intervals onto a multiplication medium consisting of MS supplemented with 1 mg L⁻¹ BA and 0.5 kinetin. Cultures were incubated at 25±1°C under a 16:8 h photoperiod.

***In vitro* thermotherapy:** Infected shoot cultures were kept for one day in a growth cabinet under artificial light with diurnal alternating periods (16 h light/8 h dark). Day temperature was initially 28±1°C and was increased at 2±1°C per day until (36-38 and 40±1°C) were obtained. Then, the plant materials were kept at the previous temperatures for 3 and 4 weeks.

Plant regeneration: Shoots produced at the end of each assay were rooted on (MS) medium supplemented with 1.5 mg L⁻¹ (NAA).

Virus screening: The health status of regenerated materials was checked on *in vitro* plantlets through DAS-ELISA.

RESULTS

Plant material: DAS-ELISA test was done to detect CLV and CarMV in carnation plants which were used to produce carnation free plants that's revealed in Table 1.

Meristem tip culture: Plants were regenerated from 0.1 to 0.4 mm were tested by DAS-ELISA test. The result showed that meristem measuring 0.1 and 0.2 mm Fig. 1 yielded virus-free tested plantlets and larger meristems were not effective as shown in Table 2.

Production of proliferating shoot clumps: The combined effect of BA and NAA produced successful adventitious shoot regeneration from bud explants as shown in Fig. 2a, b.

Micro propagation of infected shoot cultures: Micro propagated Plants were recultured periodically every 21 days until the desired shoots were obtained Fig. 2c.

In vitro thermotherapy

Effect of heat treatment on elimination of carnation viruses: On giving heat treatment, it was observed that the plants tolerate 36±1°C for 4 weeks but do not tolerate well 38±1°C for

Table 1: Detection of CLV and CarMV in carnation plants using ELISA test

Sample	Optical density at 405 nm		
	Infected	Positive ontrol	Negative control
CLV	0.270	0.498	0.089
CarVMV	0.379	0.521	0.089

Table 2: Effect of meristem size on Elimination of carnation viruses

Meristem size (mm)	% survival	% CLV elimination	% CarMV elimination
0.1	20	90	92
0.2	35	80	80
0.3	65	0	0
0.4	80	0	0

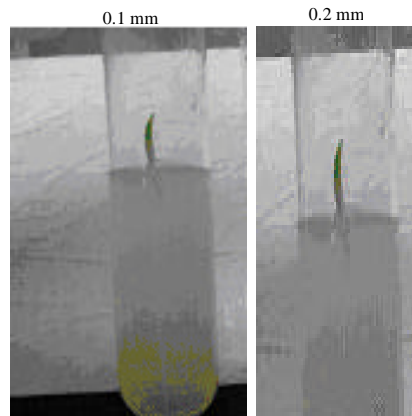


Fig. 1: Regeneration of meristem

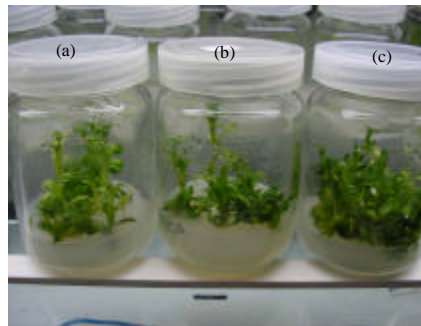


Fig. 2(a-c): (a, b) Proliferating shoot clumps and (c) micropropagation of shoot culture



Fig. 3: Regeneration of shoots after transferring to the ambient temperature

more than 3 weeks and $40\pm 1^\circ\text{C}$ for more than 2 weeks. Though the plants also dry up at $38\pm 1^\circ\text{C}$ for 4 weeks and $40\pm 1^\circ\text{C}$ for 2 weeks. As is clear from the data in Table 3. It was found that a good percentage of these (85% at $36\pm 1^\circ\text{C}/3$ weeks and 70% at $38\pm 1^\circ\text{C}/3$ weeks) remained alive and showed fresh bud sprouting once these were transferred to the ambient temperature Fig. 3. After

Table 3: Effect of heat treatment on elimination of carnation viruses

Treatment	% CLV elimination	% CarMV elimination	% survival
36±1°C/3 weeks	30	42	85
36±1°C/4 weeks	50	56	65
38±1°C/3 weeks	65	70	67
38±1°C/4 weeks	75	79	40
40±1°C/3 weeks	90	95	5
40±1°C/4 weeks	-	-	0

heat treatment of 4 weeks, however, there were only 65 and 40% plants which had a few sprouting buds after exposure to 36±1°C and 38±1°C, respectively. All the plants died when these were exposed to 40±1°C.

DISCUSSION

Regeneration through *in vitro* culture has now become a viable alternative to conventional propagation methods. The formation of healthy shoots and higher rates of multiplication is one of the prerequisite of an economically viable micro-propagation protocol (Salehi and Khosh-Khui, 1996; Waseem *et al.*, 2009).

Tissue culture techniques for carnations are already extensively used for rapid micro-propagation and for virus elimination by meristem culture (Bhardwaj and Garg, 1996; Mangal *et al.*, 2001b).

In vitro propagation of apical meristems is an important part of virus-elimination therapy for improving the health of plant collection. A successful protocol for meristem tip culture (0.1 and -0.2 mm) were used to eliminate carnation latent virus and CarMV from carnation plants. These results were in agreement with Bhardwaj and Garg (1996), Mangal *et al.* (2002b) and Ram and Zaidi (1999). They found that Meristems measuring 0.1 and -0.2 mm yielded virus free plants and larger meristems were not effective.

Enzyme-linked immunosorbent assay of heat-treated plants revealed that the nodal cuttings from the plants exposed to 38±1°C/3 weeks or 40±1°C for 3 weeks were free from carnation latent virus and carnation vien mottle virus by percentage (65 and 70%), respectively. Lower temperatures and shorter duration of heat treatment could not eliminate the virus from the plants. The results of the present study are in concurrent with those Bhardwaj and Garg (1996) and Lisa (1995) who reported that The effect of exposure of carnation plants infected with Carnation Latent Virus (CLV) and Carnation Vien Mottle Virus (CarVMV) to different temperature regimes (35 to 40±1°C) for different periods (1 to 4 weeks) revealed that the exposure to different temperatures for different periods has a negative correlation with the survival of plants. Whereas 85.33 per cent plants survived after 4 weeks at 35±1°C, the plants when exposed to 40±1°C for the same period could not tolerate the heat shock. However, only those plants which were exposed to 36±1°C for weeks and those exposed to 40±1°C for 3 weeks were free from viruses. However, because of better survival rate, the higher temperature regime of 38±1°C/3 weeks or 40±1°C/2 weeks is recommended for production of virus-tested carnation plants. Though, similar-virus status was inhibited by plants developed through direct sprouting and *in vitro* culture technique.

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