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SHORT COMMUNICATION

Production of Virus-free Carnation Plants through Heat Therapy

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ABSTRACT

The effect of exposure of carnation plants infected with carnation latent virus (CLV) to two temperature regimes (35 ± 2 °C and 40 ± 2 °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 only 33.33 per cent plants survived after 4 weeks at 35 ± 2 °C, the plants when exposed to 40 ± 2 °C for the same period could not tolerate the heat shock. However, only those plants which were exposed to 35 ± 2 °C for 4 weeks and those exposed to 40 ± 2 °C for 3 weeks were free from CLV. However, because of better survival rate, the higher temperature regime of 40 ± 2 °C is recommended for production of virus-tested carnation plants.

Keywords: Enzyme-linked immunosorbent assay, ELISA, carnation latent virus, heat therapy, micro-propagation, carnation plants

1. INTRODUCTION

The floriculture industry is in exponential growth phase in the Himachal Pradesh and the farmers are becoming more quality conscious. Viruses have been posing problems to carnation cultivation with significant loss in yield and quality. The need to develop methods to produce virus-free plant material is therefore obvious. Carnation latent virus (CLV) though remains latent in many carnation varieties, is capable of producing distinct chlorotic spotting and/or mosaic mottling symptoms on a popular variety, namely Scania. The present communication reports on the elimination of this virus by exposing infected plants to dry heat.

2. MATERIAL & METHODS

Serological detection of infected plants of carnation *Dianthus caryophyllus* cv. Scania was carried out using alkaline phosphatase-based DAC indirect enzyme-linked immunosorbent assay (ELISA) system against CLV. Antisera against carnation latent virus was procured from the Institute of Himalayan Bioresource Technology (IHBT), Palampur. Antigen at 10^{-2} dilutions, and antiserum and conjugate both at 10^{-3} dilutions were used. Laxbro brand microtitre plates were incubated at 37 °C for 3 h, 2 h, 90 min, and 30-60 min after coating of antigen, antiserum, conjugate (anti Fc-type), and substrate, respectively[1,2]. Sap from already maintained cultures of carnation latent virus on carnations was used as positive control. The plants were also indexed biologically on *Chenopodium quinoa* Wild., *C. amaranticolor*, Coste and Reyn., *Dianthus barbatus* L., *Seponaria vaccaria* L. and *Gomphrena globosa*. Two to three months old plants showing positive titers were transferred to heat therapy chamber (198.36 cm × 182.88 cm weighing 2.5 kg), installed at the Modern Scientific Instruments Industries, Meerut), where desired temperature and light conditions could be

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maintained. The infected plants were exposed to 35 ± 2 °C and 40 ± 2 °C for 1, 2, 3 weeks and more than 3 weeks. At least 20 pots of 22.86 cm size having three plants each, were taken as replications in each treatment.

Axillary bud explants from these heat-treated as well as untreated plants were cultured[3] on MS medium supplemented with NAA (α-naphthyle acetic acid) 1μM and Kinetin 20 μM. The resultant shoots were multiplied on the same medium and rooted on the MS medium supplemented with IBA(indole-3-butyric acid) 5μM and regenerated plants were subsequently reindexed serologically and biologically as before, and treatments resulting in the successful elimination of the virus were recorded.

3. RESULTS

3.1 Effect of Heat Treatment on Survival of Plants

On giving heat treatment, it was observed that the plants do not tolerate 35 ± 2 °C for more than 4 weeks and 40 ± 2 °C for more than 3 weeks. Though the plants also dry up at 35 ± 2 °C for 4 weeks and 40 ± 2 °C for 3 weeks, yet after shifting to ambient temperature, new shoots were thrown up. As is clear from the data in Table 1 that there is no significant difference among the mean survival percentage under two temperature regimes, though means of the two treatments for different time intervals differ significantly. Hundred per cent survival of plants was observed when these were subjected to either of the two temperatures for 2 weeks. After 3 weeks of heat treatment, though most of the plants had already dried or were drying up, yet a good percentage of these (66.66 % at 35 ± 2 °C and 55.55 % at 40 ± 2 °C) remained alive and showed fresh bud sprouting once these were transferred to the ambient temperature. After heat treatment of 4 weeks, however, there were only 33.33 per cent plants which had a few sprouting buds after exposure to 35 ± 2 °C. All the plants died when these were exposed to 40 ± 2 °C. Though the virus retrieval was similar as exhibited by *in vitro* grown plants, percentage survival was extremely low.

3.2 Effect of Heat Treatment on Elimination of Virus

The surviving plants after heat treatment were tested for the presence of the virus. It was observed that only those plants which were exposed to either 35 ± 2 °C for 4 weeks or to 40 ± 2 °C for 3 weeks were found free from the virus, whereas shorter durations of heat treatment could not eliminate the virus from the plants (Table 1). Further, all the plants regenerated from the axillary buds taken from plants which were heat treated at 35 ± 2 °C

Table 1. Effect of heat treatment on survival of potted plants

	Percentage plants supporting bud sprouting				
	Period of growth (weeks)				
Temperature °C	1	2	3	4	Mean
35 ± 2	100.00 (90.00)	100.00 (90.00)	66.66 (60.00)	33.33 (30.00) *	75.00 (67.50)
40 ± 2	100.00 (90.00)	100.00 (90.00)	55.55 (48.24)*	0.00 (0.00)	63.88 (57.06)
Mean	100.00 (90.00)	100.00 (90.00)	61.10 (54.12)	16.66 (15.00)	

	Temperature	Weeks
S.E.(m)	(4.166)	(5.891)
CD(0.05)	NS	(17.662)

+ Values expressed in parenthesis are arc sine transformed values

* Plants tested free from carnation latent virus as indicated by ELISA

for 4 weeks or at 40 ± 2 °C for 3 weeks were found free from the virus under test, whereas those regenerated from axillary buds taken from untreated infected plants still contained the virus.

4. DISCUSSION

The effect of dry heat treatment on survival of carnation plants is well documented[4]. Higher temperatures impose severe strain on the plant, gaseous solubility is reduced and plant survival is affected adversely[5]. The results of the present study are in concurrent with those of Korematsu and Furuyu[4], who reported that 36 per cent plants of carnation died at 35-38 °C after 3 weeks and 58 per cent died after 1 week at 45 °C. It has also been reported that different cultivars of carnation respond differently to heat treatment. In addition, these also show marked seasonal periodicity in their response[6]. During the present study, heat treatment was given during winters which might be the reason for poor survival of the plants after 4 weeks of heat treatment. Quak[7,8], Brierley[9] and Hollings[10], on the contrary, successfully heat- treated carnation plants for 6-8 weeks at 38-40 °C. However, information regarding season of the heat treatment and the cultivars used is not available.

Enzyme-linked immunosorbent assay of heat-treated plants revealed that the axilary buds from the plants exposed to 35 ± 2 °C for 4 weeks or 40 ± 2 °C for 3 weeks were free from carnation latent virus. Lower temperatures and shorter duration of heat treatment could not eliminate the virus from the plants. Though, similar -virus status was inhibited by plants developed through direct sprouting and *in vitro* culturing, the former method cannot be recommended because of very poor recoverability. However, because of higher survival rate and the shorter duration of the heat treatment, treating carnation latent virus infected plants at 40 ± 2 °C for 3 weeks is recommended to get rid of the virus, using *in vitro* culture technique.

REFERENCES

1. Bhardwaj, S.V.; Handa, A. & Vaid, M.K. Occurence and serology of a mosaic disease of broad bean in Himachal Pradesh, India. *FABIS Newsletter*, 1993, **33**, 27-30.

2. Bhardwaj, S.V.; Thakur, P.D.; Khosla, K. & Sharma, D.R. Detection of plum pox virus in India. *Acta Horticulture*,1995, **386**, 237-40.

3. Murashige, T. & Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 1962, **15**, 473-79.

4. Korematsu, H. & Furuyu, H. Studies on the production of virus-free plants of carnation 1. Effect of high temperature treatment of mother plants. *Bull. Hiroshima Perfect. Agric. Exp. Stn.*,1976, **37**, 69-74, 277-86.

5. Hollings, M. Disease control through virus-free stock. *Annu. Rev. Phytopathol.*,1965, **3**, 367-96.

6. Stone, O.M. Factors affecting the growth of carnation plants from shoot apices. *Ann. Appl. Biol.*, 1963, **52**, 199-09.

7. Quak, F. Meristem culture, gecombineerd met warmte behandeling, Vor het verkrygen van virus-virje anjerplanten. *Tijdschr. Plizekt.*, 1957, **63**, 13-14. (German)

8. Quak, F. Heat treatment and substances inhibiting virus multipliction in meristem culture to obtain virus-free plants. *In* Proceedings of the 15th International Horticulture Congress.,1961, **1**, pp. 144-48.

9. Brierley, P. Heat cure of carnation viruses. *Pl. Dis. Rep.*,1964, **48**, 143.

10. Hollings, M. Heat treatment in the production of virus-free ornamental plants. *Natl. Agr. Advis. Serv. Quart. Rev.*,1962, **57**, 31-34.