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Effect of gamma rays on per cent survival of calli in carnation cultivars 'Tempo' and 'Raggio-de Sole'

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Abstract
The present study was conducted at Department of Floriculture and Landscaping, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) to analyse radiosensivity in calli of carnation for the development mutants. Calli of carnation culivars 'Tempo' and 'Raggio de Sole' were exposed to different doses of gamma rays (10 Gy, 20 Gy, 30 Gy, 40 Gy and 50 Gy). Lowest survival rate (53.42 per cent) was obtained at the highest dose of gamma irradiation (50 Gy). Survival rate of callus was more in cultivar 'Tempo' over 'Raggio- de- Sole'. Among interactions, lowest survival rate of callus was observed in cultivar 'Tempo' at 50 Gy gamma rays.

Keywords: carnation, tissue culture, gamma irradiation, mutation

Introduction
During the last two decades, a lot of interest has been generated in cell mutagenesis (Gavazzi *et al.*, 1987; Crino *et al.*, 1990) [6, 2]. The major advantage of *in vitro* mutagenesis is that much higher number of mutants could be recovered by treating large number of population in a small *in vitro* space than *in vivo* mutagenesis. Furthermore, optimal conditions for survival and propagation after irradiation are controlled. Therefore, the breeding programme is shortened which is of outstanding economical importance (Walther and Sauer, 1985) [16]. Induction of variation depends upon the kind and dose of mutagenic treatment. Chemicals as well as radiations have been utilized for mutation induction. However, radiation is the preferred method to make genetic changes and the problem of handling and disposing of chemicals (Ahloowalia, 1998) [1]. In the mutation studies in vegetatively reproducing plants, LD_{50} values were accepted as an upper limit to prevent the unwanted mutations (Sananda and Amano, 1998) [8]. Determination of survival of explants after irradiation is an essential step to find out LD_{50}. Thus the present investigation was conducted to study the effect of different doses of gamma rays on per cent survival of calli in carnation cultivars 'Tempo' and 'Raggio de Sole'.

Material and methods
To study the effect of different doses of gamma rays on per cent survival of callus and callus type in carnation, the *in vitro* multiplied calli of cultivars 'Tempo' and 'Raggio-de-Sole' grown on standardized callus multiplication medium was cut into uniform size pieces (80 mg) under aseptic conditions in a laminar flow cabinet and put into polypropylene tissue culture bottles (3 calli pieces/culture bottle). The culture bottles were then closed with plastic caps followed by sealing with parafilm. These culture bottles were exposed to gamma rays.
The gamma ray treatment was performed in the Radiation Therapy Department of Post Graduate Institution of Medical Education and Research (PGI), Chandigarh, using Theatron 750C in which cobalt 60 was used as the source of irradiation. The source to subject distance chosen was 80 cm while the field of view was 25x25 cm^2.
The irradiation was done at the rate of 77.28 rads/minute. Hence, the treatments of 10, 20, 30, 40 and 50 Gy were of 13, 26, 39, 52 and 65 minutes, respectively. After irradiation, the culture bottles were maintained as such in the tissue culture laboratory of the department upto 21 days. The surviving calli after 21 days were transferred on callus multiplication medium.

Result
Per cent survival of calli
Per cent survival of calli was observed after 21 days of irradiation. Data on per cent survival presented in Table 1. Indicates that increase in the doses of gamma rays resulted in significant decreased per cent survival of calli which was minimum at 50 Gy (54.32 %). Even the lowest dose of 10 Gy (98.41%) resulted in significant decrease in per cent survival over control where

It was 100 per cent. In general, per cent survival of calli was obtained more in cultivar 'Raggio-de-Sole' (83.85 %) than 'Tempo' (80.50 %). The interaction between doses of gamma rays and cultivars revealed that minimum per cent survival was observed at 50 Gy gamma irradiation dose in cultivar 'Tempo' (52.44 %) followed by 'Raggio-de-Sole' (56.20 %). In contrast, maximum survival (100.00 %) was observed at 10 Gy in cultivar 'Raggio-de-Sole' which was at par with control in both cultivars.

Callus type

After 21 days of irradiation, surviving calli were sub-cultured onto freshly prepared MS medium supplemented with 10µM each of NAA and kinetin. After 28 days of sub-culturing, callus type was studied based on colour and texture. In both cultivars 'Tempo' and 'Raggio-de-Sole' light green coloured calli of friable texture were observed in all treatments including control.

Table 1: Effect of different doses of gamma rays on per cent survival of calli in carnation cultivars 'Tempo' and 'Raggio-de-Sole' after 21 days of irradiation

Doses of Gamma Rays (Gy)	Tempo	Raggio-de-Sole	Mean
Control	100.00 (9.99)	100.00 (9.99)	100.00 (9.99)
10	96.83 (9.84)	100.00 (9.99)	98.41 (9.92)
20	90.64 (9,52)	95.67 (9.78)	93.15 (9.65)
30	77.61 (8.81)	83.79 (9.19)	80.70 (9.00)
40	62.79 (7.92)	67.47 (8.21)	65.13 (8.07)
50	52.44 (7.24)	56.20 (7.50)	54.32 (7.37)
Mean	80.50 (8.89)	83.85 (9.11)	

$CD_{0.05}$:

Doses of gamma rays = (0.06)
Cultivars = (0.03)
Doses of gamma rays x Cultivars = (0.08)

Discussion

Per cent survival of calli

Present studies on *in vitro* mutagenesis or gamma rays revealed that there was decrease in per cent survival of calli with increased gamma irradiation doses in both cultivars. These results are in accordance with the results of Fereol *et al.* (1996) [5] in *Alpinia purpurata*, Mangal (1999) [11] in cauliflower and Pathania (2000) [15] in gladiolus. Paramesh and Choudhary (2005) [14] reported the decreased percentage of *in vitro* shootlets of carnation with increased doses of gamma rays. Similar reports were given by Sobhana and Rajeevan (2004) [9] in dendrobium. Lower survival of calli at higher doses can be attributed to the fact that when explants composed of various cell stages are irradiated, the dormant cells less sensitive to radiation could survive, but the more sensitive dividing cells inactivated and much higher doses led to physiological damage and the ultimate death of cells according to their stage of the cell cycle (Nagatomi *et al.*, 1992) [12]. In cv. 'Raggio-de-Sole' there was not significant difference between at lowest dosage (10 Gy) and control on per cent survival of calli. It is known that cells that were affected by radiation might self repair and restore their cellular functions (Ozalpan, 2001) [13].

In these results, highest gamma treatment was not lethal for calli, but it gave lowest per cent survival. The estimated LD_{50} regarding gamma irradiation for survival of calli *in vitro* lies beyond 50 Gy. Another no attempt has been made for estimating LD_{50} in these studies. 50 Gy as an optimal dosage was also reported by Singh *et al.* (1999) [10] in carnation and Pathania (2000) [15] in gladiolus. Walther and Sauer (1986) reported that radiosensitivity varied with species.

Cultivars differed significantly with respect to per cent survival of calli. The response against the different irradiation dosage varies in different genetic varieties of the plants (Conger *et al.*, 1977; Gupta and Nath, 1977; Dilta *et al.*, 2003) [3, 7, 4]. More per cent survival was obtained in 'Raggio-de-Sole' than 'Tempo'. It indicates that 'Tempo' is more sensitive to radiation than 'Raggio-de-Sole', it is likely that more harmful effects were produced in the cells of 'Tempo' after gamma radiation which inhibited cell proliferation.

Callus type

No adverse effects on the callus type could be observed at any of the gamma treatment and the calli was friable like unirradiated calli. Similar reports were given by Mangal (1999) [11] in cauliflower.

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