EFFECT OF X AND GAMMA RAYS ON *In vitro* ADVENTITIOUS BUD PRODUCTION OF POT CARNATION (*Dianthus gratianopolitanus* Vill.)

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SUMMARY

Internodal segments of pot cultivar Mini Pinky were exposed to ionozing radiation. Six doses of X- and gamma rays in the range of 5-30 Gy were applied. For the regeneration of adventitious shoots, 2 to 3 mm stem segments were cultured on MS medium with 0.02 mg·liter^{-1} BA, 1.75 mg·liter^{-1} IAA and macroelements reduced to one half. Adventitious shoots were growing up directly from epidermal tissue of explants and sporadically with an intermediate callus phase. Number of adventitious shoots developed from one irradiated explant ranged from 0 to 5. Most of internodal segments regenerated only one adventitious shoot. Dose 30 Gy of X-rays completely reduced the regeneration ability of internodal segments.

KEY WORDS: Stem internodes, ionizing radiation, ornamental species.

EFECTO DE LOS RAYOS X Y GAMMA SOBRE LA PRODUCCIÓN DE YEMAS ADVENTICIAS *In vitro* DE CLAVEL DE MACETA (*Dianthus gratianopolitanus* Vill.)

RESUMEN

Los segmentos entrenodales del clavel de maceta cultivar Mini Pinky fueron sometidos a la radiación ionizante. Seis dosis de rayos X y de radiación gama en el intervalo de 5 a 30 Gy fueron aplicados. Para la generación de los vástagos adventicios, los segmentos del tallo de 2 a 3 mm fueron puestos sobre el medio MS con 0.02 mg·litro $^{-1}$ de BA, 1.75 mg·litro^{-1} de IAA y 50% de microelementos. Los vástagos adventicios aparecieron directamente del tejido epidermal de los explantes y esporádico con la fase intermedia del callo. El número de vástagos adventicios generados de un explante, expuesto a la radiación variaba de 0 a 5. Los segmentos entrenodales, en su mayoría, generaron sólo un vástago adventicio. La dosis 30 Gy de la radiación X eliminó la habilidad regenerativa de los segmentos entrenodales.

PALABRAS CLAVE: entrenudos del tallo, radiación ionizante, especie ornamental.

INTRODUCTION

In carnation (*Dianthus caryophyllus* L.), adventitious bud techniques may be used for several purposes, such as mutation breeding (Leshem, 1986; Mii *et al*., 1990; Radojevic *et al*., 1990) and *Agrobacterium* - mediated gene transfer (Lu *et al*., 1991; Vainstein *et al*., 1992; Robinson and Firoozabady, 1993; Messeguer and Mele, 1994; Van Altvoorst *et al*., 1995).

Regeneration of adventitious shoots from stem segments derived from *ex vivo* and *ex vitro* grown carnation plants was obtained by Dommergues and Gillot (1973), Lubomski and Jerzy (1989), Frey and Janick (1991) and Nugent *et al*. (1991). Adventitious shoot formation has also been obtained using leaf explants (Van Altvorst *et al*., 1992; Messeguer *et al*., 1993), petals (Kakehi, 1979; Gimelli *et al*., 1984; Frey and Janick, 1991; Vainstein *et al*., 1992; Messeguer *et al*., 1993; Fisher *et al*., 1993), ovules (Demming *et al*., 1987), anthers (Villalobos, 1981) and macerated shoot tips (Johnson, 1980).

Irradiation of such explants can significantly restrict the production of adventitious shoots. Nevertheless, the irradiation is indispensable for mutation induction. Therefore in the present paper, *in vitro* formation of adventitious shoots from internodal segments treated with X- and gamma rays was evaluated.

Recibido: 30 de noviembre de 1998.
Aceptado: 4 de julio de 1999.

MATERIAL AND METHODS

Stock plants of pot carnation (*Dianthus gratianopolitanus* Vill., syn. *D. caesius* Sm.) cv. Mini Pinky, growing in a glasshouse, were used as the source of stem internodes for subsequent *in vitro* procedure.

Vegetative shoot fragments were removed from stock plants in winter when internodes were longer than those in summer. After shortening of leaves, shoot fragments were sterilized in 2.6% of NaClO. Next the stems were excised and divided into 2 to 3 mm internodal segments without axillary buds and placed in sterile flasks, 100 segments in each one.

Isolated internodal segments were exposed to ionizing radiation. X-radiation was supplied by THX-250 Medicor type apparatus with the parameters: 250 KV, 15 mA, 1 mm Cu filter. Gamma rays were supplied by ^{60}Co source, Theratron 780. Six doses of irradiation in the range of 5 to 30 Gy, with dose rate of 2 Gy·min^{-1}, were applied.

For the production of adventitious shoots, isolated explants (internodal segments) were cultured on Murashige and Skoog (1962) medium which included macroelements reduced to one half and the following constituents: 0.02 mg·liter^{-1} benzylamino-purine (BA), 1.75 mg·liter^{-1} indole-3-acetic acid (IAA), 30 g·liter^{-1} sucrose and 8 g·liter^{-1} Bacto Bacto-agar.

After 3 weeks of culture, the number of explants showing shoot formation was counted and the explants which formed adventitious shoots were transferred to a medium containing 0.5 mg liter^{-1} 6-furfuryl amino-purine (kinetin). Number of developed adventitious shoots was counted after 3 weeks of culture on medium with kinetin.

The cultures were incubated at 24^{o}±2^{o}C in 16 hours light/8 hours dark cycle, and were illuminated with LF-40 fluorescent tubes providing a photon flux density (PFD) of 35 µmol·m^{-2}·s^{-1} in the photosynthetic active radiation (PAR) range (400-700 nm). The pH was adjusted to 5.6 before sterilization (107.9 KPa pressure for 20 min). Contamination of explants was not observed.

The data from experiment were statistically elaborated with analysis of variance and significance of differences were evaluated by the Student t test. Each treatment comprised 10 replicates of 10 explants each.

RESULTS

Adventitious shoot regeneration from *in vitro* isolated internodal segments of pot carnation was dependent on kind of ionizing radiation and doses of irradiation (Table 1).

The irradiation clearly reduced the regeneration ability of explants; the higher the dose of irradiation, lower was the number of explants showing adventitious shoot formation. Negative influence of irradiation on shoot regeneration was observed in X-ray treatment as well as in gamma ray treatment. The number of irradiated explants forming adventitious shoots was lower in X-ray treatment. Dose 30 Gy of X-rays completely reduced the regeneration ability of internodal segments.

Number of adventitious shoots developed from one incubated explant ranged from 0 to 5 (Table 2). Most of internodal segments regenerated only one adventitious shoot.

Adventitious shoots were growing up directly from epidermal tissue of stem, near to the cut surface of explants, and sporadically from callus developed earlier from explant.

TABLE 1. Effect of ionizing radiation on adventitious shoot formation from *in vitro* isolated internodal segments of pot carnation

Irradiation dose	(Gy)	Number of explants forming adventitious shoots	Number of developed adventitious shoots	Average number of shoots per one regenerating explant
	0	76	130	1.7
X	5	54	90	1.6
	10	50	69	1.4
	15	42	60	1.4
	20	23	36	1.6
	25	11	17	1.5
	30	0	0	0.0
gamma	5	66	111	1.7
	10	59	108	1.8
	15	51	82	1.6
	20	34	62	1.8
	25	18	31	1.7
	30	8	12	1.5
$LSD_{0.05}$		6.6	8.9	0.41

for interaction (kind of radiation x irradiation dose)

TABLE 2. Number of adventitious shoots developed from irradiated internodal segments

Irradiation dose		Number of shoots from one incubated explants						Average number of shoots per one incubated explants
	(Gy)	0	1	2	3	4	5	
	0	24	46	13	12	3	2	1.3
X	5	46	36	7	7	1	3	0.9
	10	50	39	7	0	4	0	0.7
	15	58	30	9	1	1	1	0.6
	20	77	19	0	1	1	2	0.4
	25	89	8	1	1	1	0	0.2
	30	100	0	0	0	0	0	0.0
gamma	5	34	40	11	12	2	1	1.1
	10	41	33	16	0	7	3	1.1
	15	49	37	5	3	4	2	0.8
	20	66	22	1	8	1	2	0.6
	25	82	10	4	3	1	0	0.3
	30	92	5	0	1	1	0	0.1

DISCUSSION

Pot carnation is a very important crop on the European market. It has been a good reason for the breeding of new cultivars. Techniques involving mutation breeding or the selection of spontaneous mutants are very easy and more time-effective than classical methods. Genetic transformation is still in its developmental stage today.

From the breeder's point of view, *in vitro* regeneration of adventitious shoots on plant organs seems to be very useful. Adventitious shoots developing from explants most probably originate from one or a few genetically identical cells. According to stochastic model of Broertjes and Keen (1980), the apex of adventitious shoot ultimately originates from a single cell only. Therefore solid, non-chimeric mutants which are genetically uniform in all cell layers can be produced after irradiation. The results of investigation on mutagenesis in many plant species seem to be promising enough to introduce adventitious bud techniques to breeding procedure of pot carnation.

Radiosensitivity of carnation explants is very adventageous for mutation breeding purposes. It should be stressed that even 30 Gy acute dose of gamma rays did not restrict the production of adventitious shoots entirely.

This result enables us to use high doses of gamma radiation to induce somatic mutagenesis in pot carnation. For instance in gerbera (Jerzy and Lubomski, 1992) adventitious shoots are still formed from *ex vitro* derived leaf explants after application of 25 Gy dose of gamma rays while in chrysanthemum (Jerzy *et al*., 1993) dose of 25 Gy reduced adventitious shoot formation to 0.

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