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Gamma Ray-Induced *in Vitro* Mutations in Flower Colour in *Dendranthema grandiflora* Tzelev

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ABSTRACT

Aseptic culture of *Dendranthema grandiflora* Tzelev cv. 'Snow Ball' was used as a source of explant. Isolated nodal segments were established and shoot were multiplied on Murashige and Skoog (MS) medium supplemented with 0.5 mg L^{-1} 6-benzyladenine (BA), 0.1 mg L^{-1} indole-3-aceticacid (IAA) and 1 mg L^{-1} gibberellic acid (GA_3). *In vitro* raised shoots (2-3 cm) were treated with 5, 10, 20 and 30 Gy of gamma rays and multiplied on the same medium. 3-4 cm long shoots were rooted on half-strength MS medium supplemented with 0.3 mg L^{-1} indole-3-butyricacid (IBA) and 0.2% activated charcoal. The rooted shoots were hardened and observed for morphological characters. *In vitro* mutation in flower colour was detected in one branch of the same plant with 10 Gy irradiation. The original floral colour of 'Snow Ball' is white with flat and incurving florets. The mutant floret colour was yellow with flat and incurving florets. All ray florets in each flower head and all flower heads in mutated branch were of the same colour and shape. The plants regenerated from mutated branch produced flowers, which were of the same colour/shape, indicating the development of solid mutant in relatively short period of time.

Keywords: chrysanthemum, genetic variability, growth regulators, *in vitro* regeneration, mutation breeding, shoots, 'Snow Ball'

INTRODUCTION

Chrysanthemums are very popular cut flowers and ornamental pot plants of high economic value. It is the second largest cut flower after rose among the ornamental plants in the global flower market (Kumar *et al*. 2006). Ornamental plant market demands new cultivars showing different characteristics. The new cultivars are difficult to obtain by crossing due to self-incompatibility problems present in the species (Miñano *et al*. 2009). All present cultivars are the result of spontaneous mutations, hybridization, induced mutations and selections. Washer (1956) reported that about one-third of commercial varieties are thought to have arisen spontaneously as sports or somatic mutations. A large number of commercial varieties have been developed through induced mutations and seedling selections (Broetjes and van Harten 1988; Datta 1988; Datta *et al.* 2001; Schum 2003; Chan 2006; Zalewska *et al*. 2007). Mutation breeding by radiation, an agricultural application of nuclear technology has been widely utilized to improve the well-adapted plant varieties by one or few important traits (Kumar *et al*. 2006; Chatterjee *et al*. 2006; Jain and Spencer 2006; Jain 2010).

Although extensive work has been done for developing novelties in chrysanthemum through induced mutations using physical and chemical mutagens (Broetjes and van Harten 1988), there is always a need to explore the possibility of new variety for floriculture trade. About 2335 varieties were released through mutagenesis in the world, in which ornamental crops and decorative crops are 552 varieties (Mba *et al*. 2005). India has commercially released 46 mutant cultivars in chrysanthemum in the year 2004 (Chopra 2005). Gamma radiation has been very successfully used for the development of new flower-colour/shape mutants in chrysanthemum. Mutations mostly appear as chimeras (Wolff 1996; Chakrabarty *et al*. 1999; Mandal *et al*. 2000; Misra *et al*. 2004). Mutation sectors varied from a small sector of the floret to the entire flower and a portion of a branch (Mandal *et al*. 2000). Isolation of mutant tissue is

Fig. 1 ***Dendranthema grandiflora*** **cv. 'Snow Ball' irradiated with 10 Gy gamma rays.** (**A**) Mutated branch (yellow, flat and incurving florets). (**B**) Control (original colour white with flat and incurving florets).

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Short Communication

possible through conventional methods when an entire branch is mutated however, it is difficult to isolate when a sector of a flower is mutated (Datta *et al*. 2001). Chrysanthemum is generally considered a crop with high frequency of somaclonal variations due the fact that many cultivars are periclinal chimeras (Dowrick and El-Bayoumi 1965; Shibata *et al*. 1998; Mińano *et al*. 2009). However, the origin and the nature of the explant may affect the occurrence of variation (Zalewska *et al*. 2007). Mandal *et al*. (2000) reported that the ability to regenerate plants from a single cell of florets is a useful approach to establish a mutant in pure form and facilitate the production of a wide range of new flower cultivars. In the present report *in vitro* shoots were used to develop mutations through gamma ray treatment in chrysanthemum cv. 'Snow Ball' with the aim of isolating mutations and developing new varieties.

MATERIALS AND METHODS

Explant culture

Aseptic cultures of chrysanthemum (*Dendranthema grandiflora* Tzelev) cv. 'Snow Ball' maintained in the Department of Biotechnology, University of Horticulture and Forestry, Solan were used for developing shoots from nodal segments. The shoots were multiplied on Murashige and Skoog medium (MS, Murashige and Skoog 1962) supplemented with 8 g L^{-1} (w/v) Difco bacto agar (LobaChemie, Mumbai, India), 30 g L^{-1} (w/v) sucrose (LobaChemie, Mumbai, India), 0.5 mg L^{-1} 6-benzyladenine (BA), 0.1 mg L^{-1} indole-3-aceticacid (IAA) and 1 mg L^{-1} gibberellic acid (GA_3). The cultures without plant growth regulators (PGRs) served as control. All the PGRs were purchased from Sigma-Aldrich, Bangalore, India. The explants were cultured in 100 mL Erlenmeyer flasks (Borosil, Mumbai, India) containing 30 mL of the medium. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C at a pressure of 1.1 Kg cm^{-2} for 15 min. The culture were kept at 24 ± 2°C under a16-h photoperiod with a photosynthetic photon flux density (PPFD) of 50-60 µmol m^{-2} s^{-1} provided by cool white fluorescent lamps (40 W each, Philips, India). The cultures were transferred to fresh medium at 4-weeks interval. Multiplied shoots (20 shoots for each treatment) were treated with 0, 5, 10, 20 and 30 Gy of gamma rays (Cobalt 60 radiation source) and maintained in the multiplication medium for 4 weeks.

Rooting and hardening of shoots

A total of 200 shoots (40 shoots treatment $^{-1}$) about 3-4 cm long were rooted in half-strength MS medium supplemented with 0.3 mg L^{-1} indole-3-butyric acid (IBA) and 0.2% activated charcoal (E. Merck (India) Ltd., Mumbai, India). Shoots with well developed roots were transferred to plastic pots filled with a mixture of soil: sand: farm-yard manure (FYM, 1:1:1) and kept in the glasshouse with 80-90% relative humidity for 4 weeks. Data for morphological traits were recorded at regular intervals till the termination of experiment.

Statistical analysis

Data recorded for different parameters were subjected to completely randomized design (Gomez and Gomez 1984). The statistical analysis based on mean values per treatment was made using analysis of variance (ANOVA). The LSD multiple range test ($P \leq 0.05$) was used to determine difference between treatments.

RESULTS AND DISCUSSION

Significant effects of radiation treatments were observed for morphological traits (**Table 1**). A significant ($P > 0.05$) reduction in survival rate and rooting of shoots after gamma radiation was observed with the increase in dose rate. No rooting was observed in shoots treated with 20 and 30 Gy gamma radiation. There was no significant difference in plant height, number of leaves, number of flower buds/ flowers and flower size among the control and gamma radiation treated plants. Leaf area (cm^2) increased with gamma radiation. No morphological abnormalities in leaf shape, flower heads and branching pattern were observed in treated plants.

Table 1 Effect of gamma radiation on morphological characters of chrysanthemum.

Characters	Gamma radiation (Gy)				
	0	**5**	**10**	**20**	**30**
Survival (%)	90.4	66.6	52.3	42.8	9.0*
	(71.95)	(59.2)	(46.3)	(40.8)	(20.1)
Rooting (%)	100.0	82.6	82.3*	0	0
	(90.0)	(65.4)	(65.1)	(0)	(0)
Height (cm)	62.1	58.7	58.4	0	0
Leaf number	38.7	38.3	31.6	0	0
Leaf area (cm^2)	8.6	13.0*	10.9	0	0
No. of buds	15.1	11.9	11.7	0	0
No. of flowers	11.4	9.4	8.1	0	0
Flower size (cm)	6.6	5.3	6.5	0	0

Figures within parentheses are arc sine transformed values
*Significant at $P \leq 0.05$

Chimeric mutation in flower colour was detected in one complete branch of a single plant mutated with10 Gy. All the plants died with 20 and 30 Gy irradiation in culture. No variation was observed in the hardened plants with 5 Gy irradiation. The colour of the flowers in mutated branch was matched with the Royal Horticultural Colour Chart (Anon 1938). The original colour of the 'Snow Ball' is white with ray florets flat and incurving (**Fig. 1B**). Only one mutation was observed in flower colour i.e. yellow (Group 5C, Fan 1) with flat and incurving florets (**Fig. 1A**). The mutated branch produced 5-6 flower heads with ray florets in each head and all the flower heads were of the same colour and shape. All the survived plants treated with 5 Gy irradiation were growing vigorously and flowered true to the mother floret colour/shape in glasshouse. The mutant branch was propagated to develop shoots. *In vitro* shoots were rooted and hardened in the next generation, where they expressed the same colour/shape. Datta *et al.* (2005) reported that after mutagen treatment in chrysanthemum cuttings, generally abnormal leaves/flower heads are observed in the first generation, which is not observed in the present experiment.

Many reports appeared in literature on gamma-ray induced flower colour/shape mutations in chrysanthemum during the last decades (Broetjes *et al*. 1988; Misra *et al*. 2004; Lema-Rumińska *et al*. 2004; Datta *et al*. 2005; Kumar *et al*. 2006; Yamaguchi *et al.* 2009). D'Amato (1965) reported formation of chimera after mutagen treatment in mutation breeding of vegetatively propagated crops. Isolation of chimera in pure form is possible through available conventional techniques when the entire branch is mutated. In the present study, one entire branch was mutated and in the plants regenerated from mutated branch, all the flower heads were of same colour and shape, indicating the development of solid mutant. Barakat *et al*. (2010) reported non-chimeric mutations in flower shape during *in vitro* mutagenesis in *Chrysanthemum morifolium* 'Delistar White'. Flower colour change may be due to either quantitative and/or qualitative changes in pigments as a result of gamma-ray induced mutations in the pigment biosynthesis pathway or was perhaps induced at an independent loci controlling flower colour (Lema-Rumińska and Zalewska 2005). The induction of flower colour mutations with gamma ray irradiation are in agreement with the results reported earlier (Datta *et al*. 2001; Misra and Datta 2007; Nencheva 2010). Chan (2006) developed a protocol for *in vitro* propagation and mutation induction through callus obtained from ray florets in *D. grandiflora*. Datta *et al*. (2001) reported gamma ray induced genetic manipulations in flower colour and shape in *D. grandiflorum* `Puja' by treating rooted cuttings with gamma radiation and isolated solid mutants from sectorial chimeric tissue, which is a lengthy and a two-step process. However, the technique used in the present investigation is relatively simple, involves single step, where *in vitro* shoots were irradiated with gamma rays to get solid

mutant in short time. Datta *et al*. (2005) also developed solid mutants from ray florets with gamma radiation in *Chrysanthemum morifolium* cvs. 'Flirt', 'Puja', 'Maghi' and 'Sunil'. This technique can be used for establishment of new varieties in chrysanthemum.

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