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Induction of radiomutants in *Chrysanthemum morifolium* Ramat. cv. Gul-e-Sahir for novel traits

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Chrysanthemum is one of the most popular ornamental group of plants worldover. Its wide range of brilliant colour, shapes, size, long lasting flower life, etc makes it quite competitive in commercial floriculture. Here, we tried to estimate the efficiency of different doses of gamma rays to induce novel mutations in chrysanthemum cv. "Gul-e-Sahir" using terminal rooted cuttings as experimental material. The rooted cuttings were exposed to different doses of γ-rays (0, 10, 20 or 30 Gy) using a ^{60}Co source and transplanted in earthen pots (8"). Marked variations were recorded in leaf colour, leaf shape, flower size, shape and colour between the mutated and control populations. Four new flower colour variants with altered or novel flower colours were isolated that were unique and different from original flower colour. The original flower colour of Gul-e-Sahir is yellow whereas, flower colour of mutated population was of nearest shades of green yellow group as per RHS colour chart. The ray florets were normal in control whereas, in mutated population ray florets were spoon shaped, narrow, broad, flat and tubular in shape. These mutants were further multiplied on large scale and evaluated to check their stability. This study developed a mutagenesis protocol that could be used to develop novel colour mutants in chrysanthemum.

Keywords: Floriculture, Mutagenesis, Ornamental flowers

Chrysanthemum is one of the most beautiful ornamental group of plants in the world known as a commercial flower crop and as a popular exhibition flower[1]. In India chrysanthemum cultivation covers 20.55 thousand hectare area with loose flower production of 184.31 million tonnes and cut flower production of 14.64 lakh cut flowers concentrated mainly in Karnataka, Tamil Nadu, West Bengal, Madhya Pradesh and Himachal Pradesh[2]. Chrysanthemum is gaining popularity due to its wide range of brilliant colour, shapes, size, long lasting flower life and diversity in height and growth.

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Being a cross pollinated crop, Chrysanthemum generally possess high degree of heterozygosity, which causes a complex inheritance of genetic factors, coupled with frequent polyploidy which pose serious handicap in conventional breeding are taken to advantage of mutation breeding. The introduction of induced mutation has also attracted considerable attention in chrysanthemums due to the fact that any mutation in dominant genes is easily expressed in the first generation, and thus the selection of mutations of directly perceptible characters like flower colour, shape and size, etc. is generally easy and can be put in commercial use[3]. The main advantage of mutagenesis in chrysanthemum is the ability to alter one or few characters of an outstanding cultivar without changing the rest of the genotypes[3].

Mutation breeding induced by physical means is one of the efficient methods to evolve new cultivars for the floriculture industry. When irradiations were employed as a mean of mutagenesis in chrysanthemum, large number of promising mutants emerged in the form of solid mutants as well as partial chimeras. In the present experiment, we tried to find out the optimum dose of gamma rays for induction of variability in pot chrysanthemum cultivar "Gul-e-Sahir" under *in vivo* conditions and thereby develop a mutagenesis protocol for novel colour variants.

Materials and Methods

Plant material

Chrysanthemum cv. 'Gul-e-Sahir' was selected as the experimental material which is an early flowering variety with yellow coloured spray type of flowers and developed by PAU, Ludhiana. The experiment was conducted during the year 2013-14 and the variants induced during the study were multiplied and evaluated to check the stability. The healthy terminal stem cuttings (5-7 cm) free from symptoms of any disease or insect pest were taken in 2nd week of July. Basal 2-3 leaves were clipped off and cuttings were planted in propagation trays containing burnt rice husk as rooting medium as it is porous, possesses, good water holding capacity and facilitates growth of new roots. However, it is partially sterilized and hence free from most of the disease causing organisms. Propagation trays were kept moist by sprinkling water with the help of watering can to

ensure satisfactory rooting of cutting. New roots were developed after 10-15 days and rooted cutting were ready for gamma irradiation treatment.

Irradiation treatment and maintenance of irradiated plants

Terminal rooted cuttings were treated with different doses of γ-rays (10, 20 or 30 Gy) in gamma chamber at the Department of Fruit Science, PAU Ludhiana (^{60}Co radiation source). The treated cuttings were transferred in to earthen pots (8 inch) and cultural operations like weeding, irrigation and management of insect pest and diseases were performed well in time as recommended by PAU, Ludhiana. Pinching was done–at 4th and 7th week after transplanting. Staking was also done using sticks to keep the plants erect and maintain the proper shape of plant and bloom.

Data collection

The data were recorded on vegetative and flowering characters which includes percent survival, plant height (cm) at the time of bud appearance, number of branches per plant, internodal length (cm), number of leaves per plant, leaf size (cm), leaf colour, leaf abnormalities, plant spread (cm), days to bud initiation, days to colour shown stage, days to flower opening, number of flowers per plant, flower size (cm), flower weight (g), number of ray florets, longevity of bloom (days), flower colour variation i.e. flower form variation and chimera if any. Flower and leaf colour of control and mutants were compared with Royal Horticulture Society colour chart.

Statistical analysis

Data were analysed based on a simple, completely randomized design. Percentage data were subjected to arc sine √% transformation before carrying out ANOVA.

Results and Discussion

Estimation of LD_{50} dose

In this study, radiosensetivity was tested to investigate the most effective dose of gamma irradiation for induction of mutations under *in vivo* conditions. The LD_{50} dose varies for each plant species and depends, among other things, on the type of explants tissue and the growth stage. In the present investigation, LD_{50} values were determined based on the percentage of surviving of terminal rooted cuttings after γ-irradiation. The 30 Gy γ-ray treatment was determined to be the LD_{50} dose based on explant survival and survival rate was found to decrease linearly with increasing exposure to γ-rays (Fig. 1). The highest rate of plant survival was observed in control (untreated) plants. The lowest rate of plant survival was observed after 30 Gy treatment. In the present investigation, the rates of survival of rooted cuttings was found to decrease linearly with increasing γ-ray dose. The LD_{50} value was estimated to be 30 Gy. Reduction in survival after exposure to gamma rays was explained due to inactivation and/or decreases in auxin content that affect cell division resulting in poor establishment and survival[3,4].

Effect of γ-irradiation on morphological characteristics

Plant height and plant spread

There was a significant reduction in plant height and plant spread on terminal rooted cuttings exposed to higher doses of γ-rays (Table 1). The highest rate of mean plant height (40.5 cm), and plant spread (51.9 cm)

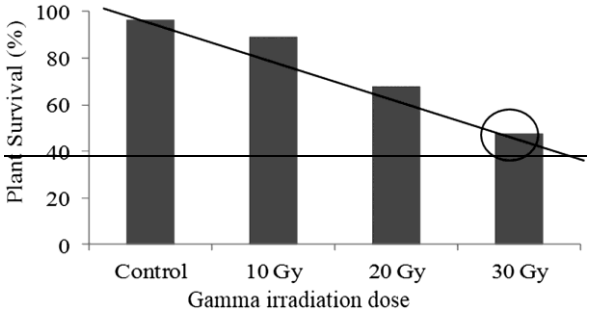

Fig.1 — Percent survival to estimate LD_{50} value in irradiated rooted cuttings of chrysanthemum cv. Gul-e-Sahir

Table 1—Effect of gamma-irradiation on vegetative characters of chrysanthemum (*Chrysanthemum morifolium* Ramat.) cv. Gul-e-Sahir

Gamma-ray dose (Gy)	Plant survival (%)	Plant height at first bud appearance	Plant spread (cm)	Number of branches per plant	Internode length (cm)	Number of leaves per plant	Leaf size (cm)	Leaf colour	Leaf abnormalities
Control (0)	96.40 (79.37)*	40.56	51.90	5.60	1.70	116.50	4.63	Dark Green	Normal
10	89.23 (70.82)	33.40	47.16	5.30	1.13	97.70	4.18	Dark Green	Normal
20	68.10 (50.59)	25.10	35.13	4.20	0.77	86.90	3.57	Light Green	Small
30	47.43 (43.51)	15.86	27.90	3.23	0.51	72.83	3.10	Light Green	Small and Fused
CD (5 %)	2.49	5.61	3.63	0.48	0.53	2.55	0.68		
SEM±	2.61	5.32	5.48	0.58	0.56	2.73	0.20		

*Arc sine √% transformed data. † All values are means (n=25)

was recorded in the untreated controls, whereas the minimum rate of plant height (15.9 cm), and plant spread (27.9 cm) was observed in the 30 Gy treatment. The reduction in survival rate at higher doses might attribute to genetic loss due chromosomal aberrations and gene mutation[4]. Standardisation of optimal doses for ionizing radiation mutagens has already been reported in chrysanthemum and other ornamental crops[5]. The plant height at the time of first bud appearance decreased significantly with increasing doses of gamma rays. The similar trend of reduction in plant height in rose has also been reported due to gamma irradiation[4]. In the present study, there was a significant reduction in vegetative growth parameters at higher doses of γ-rays. This decrease in number of branches per plant has also been repoted earlier[6]. The reduction in plant growth parameters at higher doses of gamma rays may be due to consequence of somatic competition[7].

Number of branches, internode length, number of leaves and leaf size

The maximum increases in mean number of branches (5.6), internode length (1.7 cm), number of leaves per plant (116.5), and leaf size (4.7 cm) were observed in the control treatment, followed by the 10 Gy treatment with number of branches (5.3), internode length (1.1 cm), number of leaves per plant (97.7), and leaf size (4.2 cm). Number of branches, internode length, number of leaves, and leaf size markedly reduced at higher γ-ray doses. At 30 Gy, the mean number of branches (3.2), internode length (0.5 cm), number of leaves (72.8), and leaf size (3.1 cm) were lowest (Table 1). The less number of branches may be due to inhibitory effect of higher mutagenic doses of gamma rays. The reduction in internodal length may be due to decrease in plant height with increase in gamma ray doses. The higher doses of gamma rays minimized the leaf size significantly. The reduction in leaf size of plants treated with higher doses of gamma rays may be due to inactivation or decrease in auxin content or disturbance in auxin synthesis[6,8].

Leaf colour and variation

All leaves were normal, with a dark-green colour, in control (untreated) plants and in terminal rooted cuttings treated at lower doses of γ-rays (10 Gy), while light green coloured leaves were observed at higher γ-ray doses (20 or 30 Gy). Morphological abnormalities in the foliage, which included changes in leaf shape and size, and fused leaves were also induced at higher doses of γ- rays (Table 1). Based on the visual observations leaf colour variations were recorded. Leaf colour variation may be due to adverse effect of radiation on chlorophyll synthesis process or might be due to reduced levels of substrates affecting biochemical pathways. Abnormalities in the colour, form, and texture of leaves following irradiation have been reported in chrysanthemum[5]. Variations, such as yellowish or light-green leaves, narrow leaves with small leaflets, wider leaves, lanceolate leaves, serrated margins, chlorophyll-deficient leaves, leaflets fused together, and unequal development of lamina from γ-irradiated nodal explants have been reported in rose and other ornamental crops[8].

Effect of γ-irradiation on floral characteristics

Flowering was significantly delayed following higher doses of γ-rays. The maximum delay in bud initiation (91.2 days), colour break stage (111.7 days), and days to flowering (130.03 days) was observed after the 30 Gy treatment. In control, without γ-irradiation, minimum days for bud initiation (61.4 days), days to colour break stage (77.7 days), and days to flowering (91.4 days) were observed (Table 2). Varying response of irradiated plants to number of flower per plant was observed to be significant. The number of flowers significantly reduced from (84.0) floweres in untreated control to (30.5) flowers in plants developed after 30 Gy doses of γ-irradition.

The maximum flower size (5.6 cm) was obtained in non-irradiated plants whereas, minimum flower size (3.2 cm) was recorded for 30 Gy γ-ray dose. Flower weight and number of ray florets per plant, and longevity of bloom also reduced significantly with increasing dose of γ-rays. Untreated plants had the maximum flower weight (50.1 g), number of ray florets (98.2), and longevity of bloom (28.7 days) (Table 2). The minimum flower weight (21.3 g), number of ray florets (65.4), and longevity of bloom (23.5 days) were recorded following the 30 Gy treatment. Each treatment showed significant differences from the others.

Flowering characters were also affected by the different doses of γ-rays. Treatment with higher doses delayed flowering and other flowering characters, depending on the dose. This may be an indirect effect of γ-rays through delayed sprouting and slower growth, or may be due to the deleterious effects of γ-irradiation on plant growth hormones such as auxins and gibberellins, or the induction of photo-insensitivity following irradiation and delay in flowering in various γ- irradiated ornamental crops

Table 2—Effect of gamma-irradiation on floral characters of chrysanthemum (*Chrysanthemum morifolium* Ramat.) cv. Gul-e-Sahir

Gamma-ray dose (Gy)	Days to bud initiation	Days to colour break stage	Days to flower opening	Number of flowers per plant	Flower size (cm)	Flower weight per plant (g)	Number of ray florets per flower	Longevity of bloom (days)	Flower colour variation	Flower form variation
Control (0)	61.40	77.76	91.40	84.06	5.56	50.09	98.19	28.76	Green yellow group (1 A)	Normal ray florets
10	64.33	83.00	94.06	75.93	5.10	42.13	90.26	25.13	Green yellow group (1 B) Yellow group (12 A)	Spoon shaped narrow ray Spoon Shaped broad ray florets
20	75.53	92.73	106.66	47.10	3.90	35.93	78.73	22.63	Yellow group (12 B) Yellow group (12 B)	Flat ray florets Ray florets tubular near to disc
30	91.16	111.70	130.03	30.53	3.20	21.30	65.43	23.56		
CD (5 %)	3.66	3.33	4.21	5.87	0.38	3.15	1.32	1.58		
SEM±	5.64	4.67	7.46	9.98	0.53	4.18	0.73	1.05		

[All values are means (n=25)]

has already been reported[7,8]. The probable cause of the reductions in flower diameter, the length of the flower bud, and the number of petals might have been the reduced size of petals and/or abnormalities and variations in floral characters due to hindered development by irradiation. The flowering characters decreased inversely with increasing doses of gamma rays. Reduction in weight was recorded but was not overly affected by gamma rays. Reduction in flower weight may be due to reduced size of flower head and reduced number of petals in flower head. Similar results were observed in rose as well[7].

Variations in flower colour, and flower form

Based on the visual observations flower colour and form variations were recorded. Plants without any variation in floral traits were observed in the untreated control plants. Gamma irradiation induced changes in flower colour, form, petal shape and other modifications of floral parts. Four new flower colour and form variants were isolated and further multiplied at large scale (Fig. 2). These were coded: GS-1 (Light yellow variant) induced at 10 Gy. All flowers were pure homozygote (solid mutant) producing spoon shaped, narrow ray florets with light yellow coloured flowers (green-yellow group 1-B) as compared to yellow colour flowers in original cv. Gul-e-Sahir (control). GS-2 (Dark yellow variant) the flower colour variant was spoon shaped, broad ray florets and attractive with dark yellow flowers (yellow group 12-A) as compared to yellow colour in original cv. Gul-e-Sahir (control) and isolated at 10 Gy dose of

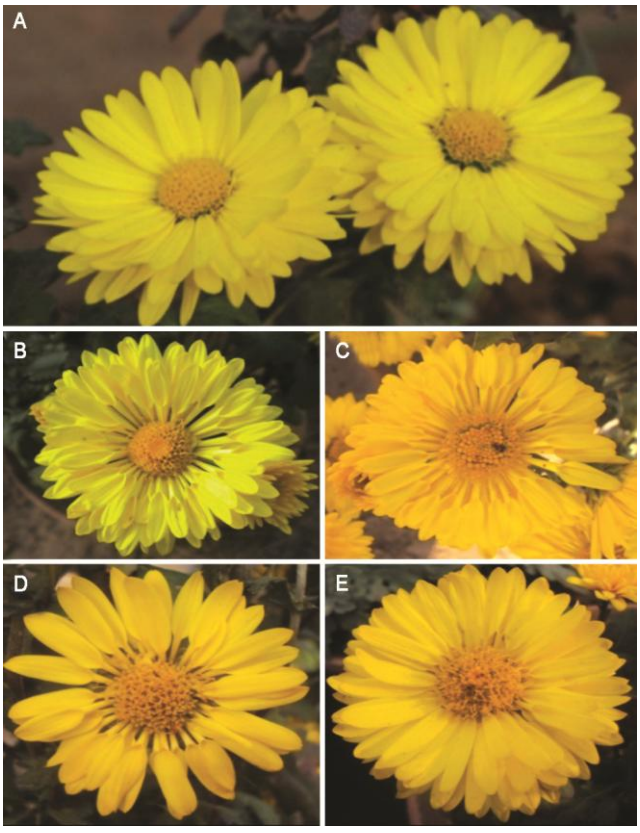

Fig.2 — Flower colour and form mutations induced by γ-irradiation in *Chrysanthemum morifolium* Ramat. cv. 'Gul-e-Sahir'. Untreated control (Panel A) and mutated flowers coded GS-1 (green yellow group 1 B with spoon shaped narrow ray florets) and GS-2 (yellow group 12 A with spoon shaped broad ray florets) at 10 Gy (Panel B & C), GS-3 (Yellow group 12 B, with flat ray florets) and GS-4 (Yellow group 12 B, with ray florets tubular near to disc) at 20 Gy (Panel D & E) .

Fig. 3 — Flower form abnormalities in *Chrysanthemum morifolium* Ramat. cv. 'Gul-e-Sahir' at 30 Gy (Panel A & B).

gamma rays. GS-3 (Yellow variant) induced at 20 Gy dose of gamma rays in which ray florets were flat as compared to normal ray florets in original cultivar.

There were little differences in floral characters between variant and original cultivars, i.e. ray florets were flat in the mutant as compared to normal ray florets in original cultivar. The colour of flower of variant was yellow (yellow group 12-B) as compared to yellow colour in original cultivar (control). GS-4 (Yellow variant) was induced at 20 Gy dose having tubular ray florets near to disc. Flower colour in yellow induced mutant, was different in shade (yellow group 12-B) from flower colour in original cv. Gul-e-Sahir. γ-ray doses (10 or 20 Gy) induced changes in flower colour and flower form, whereas the higher dose 30 Gy resulted in production of abnormal flower forms (Fig. 3). The change in flower form was also recorded in chrysanthemum with altered ray florets spoon shaped, tubular and irregular in induced variants were observed[8,9]. Radiation induced floral colour changes may be due to chromosomal aberrations, changes in chromosome number, genetic mutations, rearrangements of different histogenic layers, and/or altered biochemical pathways. The latter can increase and/or decrease the concentrations of one or more pigments by inhibiting their synthesis, or synthesising new pigments. The induction of colour mutations after γ-irradiation agrees with previous results in ornamental crops[10]. Flower colour variations and change in flower form and shape of ray florets after gamma irradiations in present study are in close conformity with the results obtained earlier for stability of flower colour mutants in chrysanthemum[6].

Conclusion

A protocol for mutagenesis of chrysanthemum has been established using γ-rays and may be used in future breeding programmes. In the present study it can be concluded that10 or 20 Gy γ-ray doses were effective for induction of novel flower colour variants in chrysanthemum. Induced mutations are desirable for developing new cultivars for the floriculture industry and the pot culture trade due to more urbanization and also to fulfill the demands of both domestic and international markets.

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