SKUAST Journal of Research 25(2): 214-220; (2023)
(*10.5958/2349-297X.2023.00029.6*); NASS rating: 4.17

Research Paper

Effect of gamma radiations and ethyl methyl sulphonate on vegetative and Floral characters of chrysanthemum (*Dandrenthema grandiflora* L.)

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(Received March 16 2023; accepted May 11, 2023)

ABSTRACT

Induced mutation is an important and fast tool of breeding in ornamental plants as the variation in phenotypic characters like flower colour, shape and size, and leaf variegation can be easily detected. In addition, the heterozygous nature of chrysanthemum offers high mutation frequency. An experiment to determine the optimum dose of mutagens required for inducing variability and to determine the level of LD_{50} dose of mutagens in chrysanthemum cultivar Dolly Pink. The experiment comprised of two types of mutagens viz., ethyl methyl sulphonate (0.10, 0.15, 0.20 and 0.25%), and gamma rays (10, 20, 30 and 40 Gy) including control (no mutagenic treatment), laid out in randomized complete block design (RCBD) with three replications. Gamma rays (10 Gy) significantly improved most of the vegetative and flowering parameters by recording maximum plant height (40.54 cm), leaf length (5.62cm), leaf width (4.03 cm), number of branches per plant (30.80), stem thickness (3.74 mm), diameter of flower head (5.43cm), flower weight (1.71 g), pedicel length (4.50 cm), number of flowers per plant (42.33), flower yield per plant (57.31 g) and minimum days taken to appearance of first flower bud (49.63 days) and opening of first flower bud (77.59 days) as compared to control. On the other hand, EMS (0.10%) showed maximum plant spread (27.50 cm), branch length (26.90 cm) as compared to control. EMS was found to create more morphological variation in chrysanthemum than gamma rays. Two mutants with altered flower colour and one mutant with altered leaf morphology were obtained at 0.10 per cent and 0.15 per cent EMS, respectively. Whereas one leaf mutant was produced from gamma rays applied at 30 Gy. Based on probit analysis, LD_{50} dose for gamma rays and EMS was found to be 26.57 Gy and 0.137 per cent, respectively.

Key words: Chrysanthemum, ethyl methyl sulphonate, flowering, gamma rays

Chrysanthemum is a popular flowering plant that holds a prominent position in decorative horticulture and has a high commercial value as cut flower. It is economically exploited and successfully grown in various regions of the world due to its gorgeous blossoms and long keeping quality. Its cultivation is mainly done in Karnataka, Maharashtra, Tamil Nadu, Andhra Pradesh, and West Bengal (Anonymous, 2017). Chrysanthemum has gained tremendous popularity in most of the flower industries in Southeast Asia and European countries because of the shape of the crown, beautiful colours, long lasting flower life, diversity in height and growth, reasonable prices etc. Its flowers are important in garland and wreath making, religious offering, hall decorations etc. apart from its commercial value. From time to time, several breeding procedures have been used to improve the chrysanthemum among which mutation breeding has been found to be the fastest breeding strategy to induce heritable changes, particularly in phenotypic features such as flower shape, flower head size, ray floret, and leaf variation. Mutagens produce gene chromosomal or genomic alterations by causing random changes in nuclear DNA or cytoplasmic organelles. Induced mutagenesis is a well-established method for plant improvement in which plant genes are altered by using chemical or physical mutagens on suckers or other plant components. Mutation breeding is an important technique in a breeder's toolbox for creating heritable differences in plants, and it has been effectively applied to a variety of crops. Physical and chemical mutagens have effectively developed a huge number of new and promising types in number of ornamental plants, and are widely regarded as one of the most effective breeding tools for improving ornamental plants. Although mutation breeding is a random process, there has been research on the causes of distinct flower colour mutations with specific colours. Gamma rays have been discovered to act as an efficient mutagen for inducing changes in plant morphology among various physical and chemical mutagens. Mutation breeding, as a result of a permanent heritable change in the structure of the genetic material results in altered phenotypes (Rego and Faria, 2001), is already well established as a time-saving and low-cost method for flower crop development. Therefore, the present study was undertaken to standardize the optimum dose of mutagens for inducing variability in growth and flower characters of chrysanthemum cultivar Dolly Pink and to determine the LD_{50} dose of mutagens.

MATERIALS AND METHODS

The present investigation was carried out during the year 2020-2021, at the experimental farm of the Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agricultural Sciences and Technology (K), Shalimar campus. The healthy terminal stem cuttings (5-7 cm) of chrysanthemum cv. Dolly Pink free from symptoms of any disease or insect pest were taken and on a raised bed having sand as rooting media. Bed was kept moist by sprinkling water with the help of watering cane to ensure satisfactory rooting of cutting. The rooted cuttings were then treated with four doses of EMS (0.10, 0.15, 0.20 and 0.25%) for 3 hours and after the treatment, these rooted cuttings were washed in running tap water for 1 hour to remove the chemical residues. Another set of rooted cuttings were treated with four doses of gamma rays (10, 20, 30 and 40Gy) at Bhabha Atomic Research Institute, Zakura Srinagar, Jammu and Kashmir. The treated plants along with un-treated plants as control were then planted in field in randomized completely block design. Cultural operations like weeding, irrigation, and management of insect pest and diseases were performed well in time according to recommended package of practices except pinching and disbudding operations. Staking was also done in plants to avoid lodging. Observations were recorded on five randomly selected plants in each treatment for various growth and flower characters. Mean value for all the characters were worked out. Desirable variants with change in plant morphology and flower colour was also recorded as mutation spectrum. LD_{50} values were determined based on survival rates of the rooted plants using probit analysis (Sharma, 1998) as well as the growth rate. The rooted cuttings were counted 30 days after planting and the survival percentage was worked out for both gamma irradiation and EMS treatments.
The data of all characters recorded was statistically analyzed with Statistical Analysis System software (SASs) V. 9.1 (June 2006), SAS Institute.

RESULTS AND DISCUSSION

Mutagenic effect on vegetative parameters: The analysed data as evident from Fig. 1 indicates that mutagenic treatments had a significant impact on all morphological features related to plant survival and growth. In gamma irradiated population, the survival percentage ranged from 76.66 percent in 10 Gy to 30.00 percent in 40 Gy treatment. Similarly the survival percentages for EMS treatments ranged from 66.66 percent in 0.10% EMS to 56.66 percent in 0.15% EMS, with none of the plants survived at a concentration exceeding 0.15 percent. It was observed that survival percentage of rooted plants showed a decreasing trend with increase in dosage of both the mutagens. Kapadiya *et al.* (2016) also detected a considerable decline in the survival of the chrysanthemum cultivar 'Maghi' after being exposed to higher doses of gamma rays.

Control 0.10 % EM 0.10 % EMS

Fig. 1: Colour mutants in chrysanthemum variety dolly pink

According to Dilta *et al.* (2003), higher EMS concentrations reduced the percentage of survived plants in chrysanthemum. On the other hand lower doses of gamma rays (10 Gy) significantly improved most of the vegetative parameters (Table 1) by recording maximum plant height (40.54 cm), leaf length (5.62cm), leaf width (4.03 cm), number of branches per plant (30.80), stem thickness (3.74 mm) as compared to control. However, higher doses of gamma rays negatively affected all these parameters. Earlier studies (Kim *et al*. 2004; Wi *et al*. 2007) also reported that the low dose of irradiation induce growth stimulation by changing the hormonal signalling network in plant cells or by increasing the antioxidative capacity of cells to easily overcome daily stress factors such as fluctuation of light intensity and temperature in growth conditions.

Table1: Effect of different mutagens on vegetative growth parameters of chrysanthemum cultivar dolly pink

Mutagenic treatment	Plant height (cm)	Plant spread (cm)	Leaf length (cm)	Leaf width (cm)	Number of branches per plant	Branch length (cm)	Stem thicknes s (mm)
EMS (0.10%)	28.92	27.50	5.27	3.80	26.78	26.90	3.52
EMS (0.10%)	26.94	25.49	4.64	3.69	23.53	22.32	3.43
Gamma rays (`10 Gy)	40.54	25.69	5.62	4.03	30.80	21.52	3.74
Gamma rays (20 Gy)	33.20	25.03	5.21	3.84	25.57	21.18	3.38
Gamma rays (30 Gy)	27.31	24.01	4.86	3.51	23.63	19.86	3.19
Gamma rays (40 Gy)	25.47	19.91	4.46	3.40	22.59	19.20	3.10
Control	36.06	26.62	5.39	3.88	28.07	23.85	3.66
$CD_{0.05}$	**1.446**	**0.975**	**0.206**	**0.229**	**1.362**	**2.363**	**0.172**

According to Kole and Meher (2005), increasing effects of gamma rays on plant height in zinnia could be due to its stimulatory impact at lower dosages; while as lowering effects could be due to its physiological harm at higher dosage. Kumari *et al*. (2013), in chrysanthemum variety 'Otome Pink', reported reduction in leaf size in terms of length and width in plants treated with greater doses of gamma radiation. Behera *et al*. (2012) showed that induction of mutagenesis through EMS treatment in *Asteracantha longifolia* affected plant height, morphology, and even leaf size. Priya Misra *et al*. (2009) recorded greater number of branches in plants treated with gamma rays as compared to control in chrysanthemum. Similar results were also observed by Banerji and Datta (2005) while they irradiated rooting cuttings of chrysanthemum cv. 'Khumaini'.

All doses of EMS caused reduction in vegetative parameters as compared to control. Latado *et al.* (2004) recorded drastic reduction in plant height of various gladiolus varieties with EMS, DES, MNH and gamma rays. They also explained that higher dose of chemical mutagens proved to be injurious as promote physiological disturbances, retarded cell division by arresting the mitotic division and had ill-effect on auxin thereby resulting in reduction in plant height and other growth parameters.

All doses of gamma rays resulted in reduction of both plant spread as well as branch length as compared to control. Gordon (1957) and Gunckel and Sparrow (1961) explained that the retardation/reduction in plant spread could be due to physiological, morphological and cytological disturbances caused by irradiation. On the other hand, lower dose of EMS (0.15%) resulted in increase of plant spread and branch length as compared to control. However higher doses significantly reduced both of these parameters. This might be due to the ill effects of EMS on auxin production thereby stimulating the side growth and resulting in increased plant spread.

Mutagenic effect on flowering and yield parameter : In Fig. 3, lower dose of gamma rays (10 Gy) resulted in significant decrease in the number of days taken to appearance of first flower bud (49.63 days) and opening of first flower (77.59 days) whereas higher doses delayed these parameters as compared to control. Rajasekar (2019) stated that lower dosage of gamma radiations coupled with a rise in auxin and gibberellin activity, as well as absence of inhibitors, may be attributed to rapid flower bud initiation. These findings are similar to those of Kapadiya *et al*. (2016), who discovered that lower doses of gamma rays positively influenced early flowering in chrysanthemum (*Chrysanthemum morifolium* Ramat.) var. Jaya. The analysed data as evident from Table 2 indicates that gamma rays (10 Gy) recorded maximum diameter of flower head (5.43cm), flower weight

(1.71 g), pedicel length (4.50 cm), number of flowers per plant (42.33), flower yield per plant (57.31 g) as compared to control. But all these parameters showed a decreasing trend with increase in dosage of gamma rays. EMS resulted in significant reduction of all flowering as well as yield parameters as compared to control. The plants having no mutagenic treatment produced maximum number of ray florets per head (233.72) followed by 10 Gy of gamma rays (232.85).

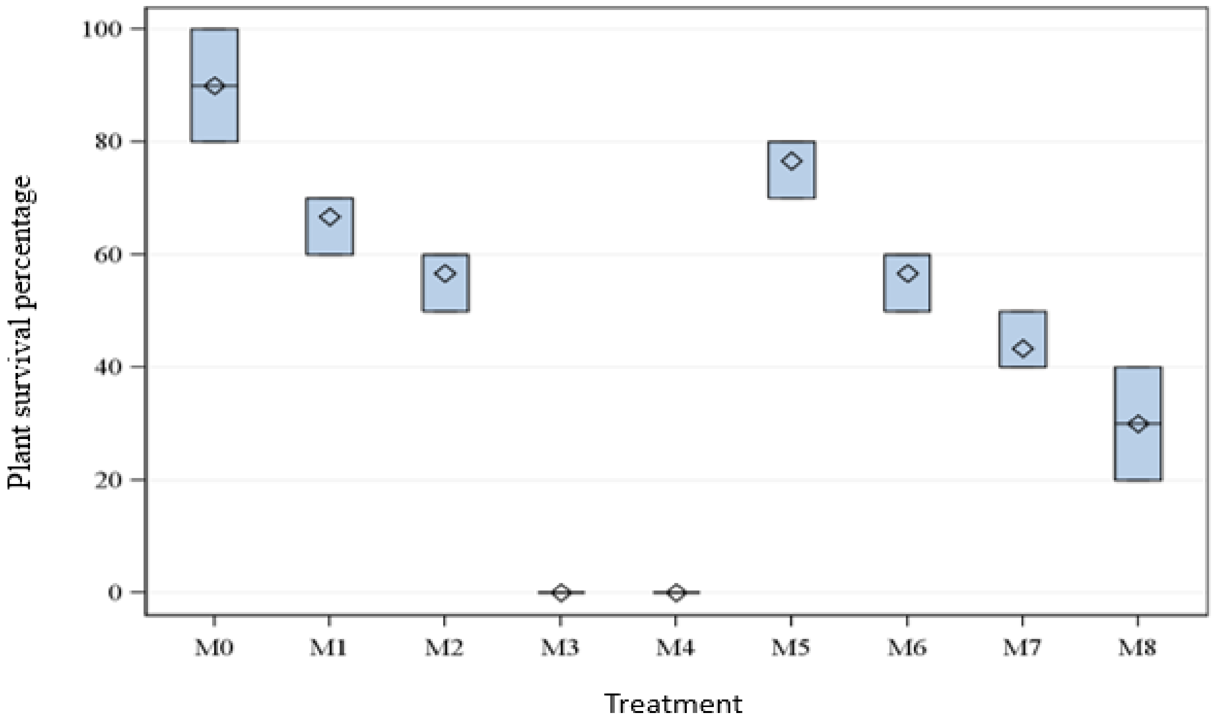

Fig. 2. Distribution of plant survival percentage with treatment

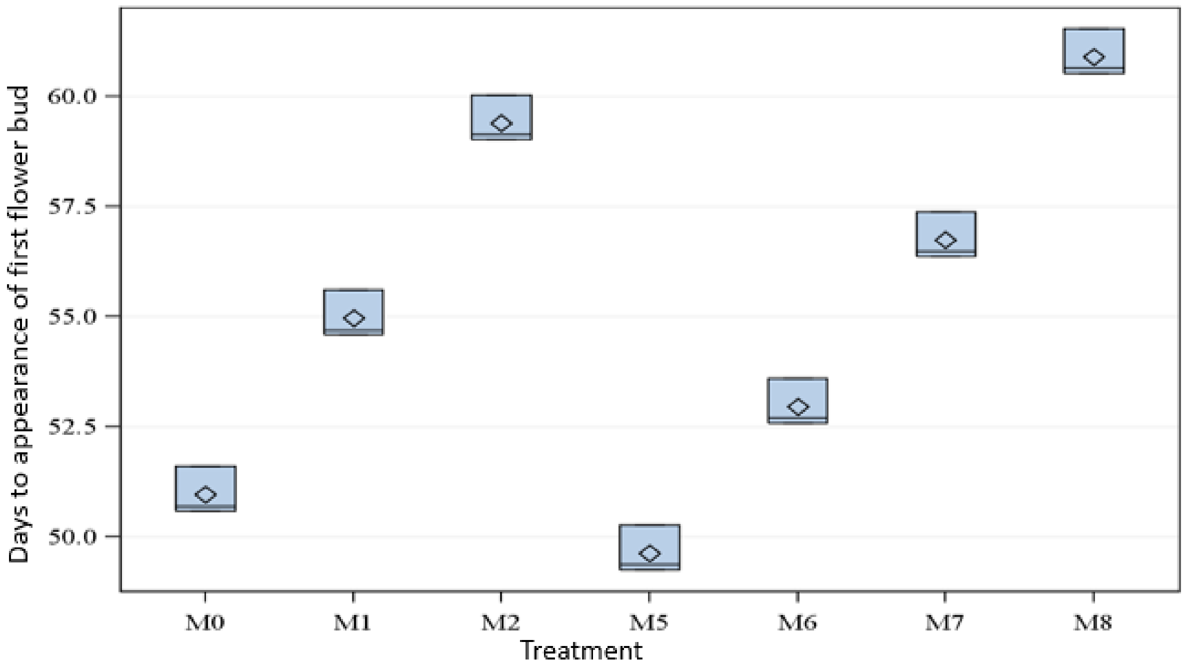

Fig. 3. Distribution of days to appearance of first flower bud with treatment

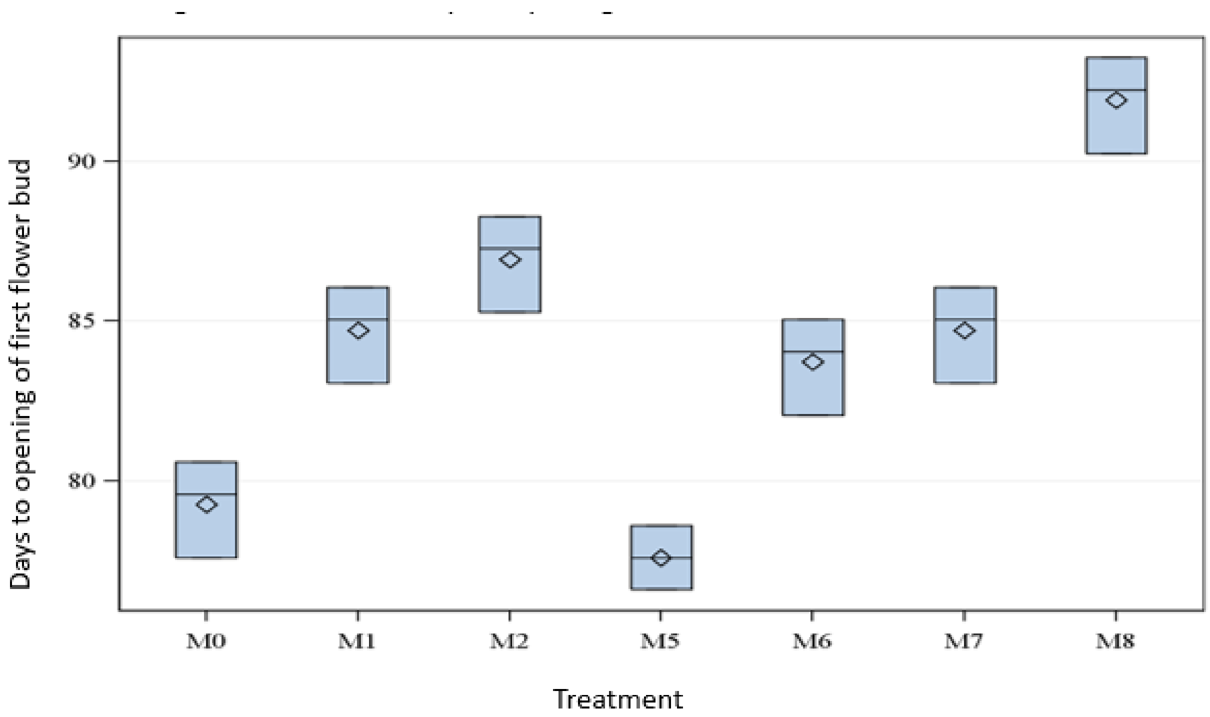

Fig. 4. Distribution of days to opening of first flower bud with treatment

Table 2: Effect of different mutagens on flowering parameters of chrysanthemum cultivar dolly pink

Mutagenic treatment	Days to appearance of first flower bud	Days to opening of first flower bud	Diameter of flower head (cm)	Number of ray florets per head	Weight of flower (g)	Length of pedicel (cm)	Number of flowers per plant	Flower yield per plant (g)
EMS (0.10%)	54.97	83.72	4.51	228.53	1.55	4.12	38.73	52.58
EMS (0.10%)	59.39	84.72	3.98	219.85	1.46	3.99	37.46	47.49
Gamma rays (`10 Gy)	49.63	77.59	5.43	232.85	1.71	4.50	42.33	57.31
Gamma rays (20 Gy)	52.96	84.72	4.95	224.61	1.65	4.18	39.00	52.22
Gamma rays (30 Gy)	56.74	86.94	4.14	222.68	1.58	3.67	34.73	44.31
Gamma rays (40 Gy)	60.89	91.92	4.02	211.60	1.37	3.51	30.00	29.16
Control	50.96	79.26	5.08	233.72	1.70	4.27	41.06	55.90
$CD_{0.05}$	**0.033**	**0.789**	**0.149**	**0.384**	**0.105**	**0.081**	**1.266**	**4.541**

Kapadiya *et al.*, (2014) observed increase in flower weight with lower doses of gamma rays in variety "Maghi". Similar findings were previously reported by Mahure *et al.* (2010) in cultivar 'Red Gold'. The reduced size and fewer petals of the flower head may account for the reduction in flower head weight generated by EMS treatments. Zargar *et al.* (1998) discovered that gamma irradiation increased the pedicel length of chrysanthemum cv. 'Satish Modi' by up to 1.0 kR, but that higher gamma ray dosages drastically reduced the pedicel length above control. Soliman *et al.* (2014) also found that the number of flowers per plants was higher in the lower concentration (10 Gy) of gamma rays than in the control. These findings could be explained by the fact that plants exposed to lower doses of gamma rays developed sufficient food reserves as a result of increased plant height and spread, as well as an increase in the number of branches, resulting in higher photosynthate production. It could be owing to enhanced biomass mobilisation from leaves to flowers.

Table 3: Probit analysis for calculating LD_{50} for Gamma rays and EMS on 30th day after planting

Treatment	Dosage	Survival %	LD 50 Value
M_1	EMS (0.1%)	66.66	
M_2	EMS (0.15%)	56.66	0.137%
M_3	EMS (0.20%)	0	
M_4	EMS (025%)	0	
M_5	Gamma Rays(10Gy)	76.66	
M_6	Gamma Rays(20Gy)	56.66	26.57 Gys
M_7	Gamma Rays(30Gy)	43.33	
M_8	Gamma Rays(40Gy)	30.00	

LD_{50} value: The data presented in table 3 shows that the LD_{50} value for EMS was 0.137 percent, and the LD_{50} value for gamma rays was 26.57 Gy on the basis Probit analysis. Because gamma rays are more intense and penetrating in nature, they may have caused damage to cells undergoing meiotic division in the bud region. Lamseejan *et al*. (2000) investigated the effects of gamma rays on a purple colour clone of spray type chrysanthemum and the LD50 dose was determined to be 14 Gy based on the survival of treated cuttings. For rooted cuttings, 10–25 Gy has been proposed as a suitable dose for mutation induction in chrysanthemum (Thinh *et al*., 2011; Broertjes and Van Harten, 1978) and 30 - 40 Gy for *Heliconiabihai* (Rodrigues *et al*., 2005). According to Berenschot *et al*. (2008) in Petunia, the LD_{50} corresponded to 100 Gy for gamma radiation and 0.1% for EMS treatment. Higher EMS concentrations, according to Dilta (2003), lowered the percentage of chrysanthemum plants that survived. The drastic loss in plant survival could be related to the creation of poisonous compounds result in chemical mutants such as EMS and DES, as well as varying gamma ray dosages (Cobalt-60) via certain biochemical components, which eventually induce cell death.

CONCLUSION

Findings of the present study clearly shows that among the mutagens ethyl methyl sulphonate caused more damage than gamma rays. Lower dose of Gamma radiation (10Gy) resulted in improvement of most of the vegetative and floral characters.

ACKNOWLEDGMENT

The authors are highly thankful to Bhabha Atomic Research Station Zakura for providing facilities to irradiate the plants with gamma rays.

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