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Induction of Mutation in Chrysanthemum (*Dandranthema grandiflora* L.) cv. Yellow Stone through Gamma Radiations and Ethyl Methyl Sulphonate

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Abstract: This study investigates the effects of gamma rays and ethyl methyl sulphonate (EMS) on chrysanthemum variety yellow stone which is renowned for resistance to disease to enhance its growth and floral characteristics. Four EMS concentrations (0.10, 0.15, 0.20, and 0.25%) and four gamma-ray doses (10, 20, 30, and 40 Gray) were applied to rooted cuttings in order to take advantage of variability and assess heritable impacts on a number of characteristics, including survival rate, vegetative growth, and blooming. Reduction in survival rate and various morphological parameters was observed with chemical mutagens and higher doses of gamma rays. Gamma rays (10 Gy) significantly improved most of the vegetative and flowering parameters by recording maximum plant height, leaf length, leaf width, number of branches per plant, stem thickness, diameter of flower head, flower weight, pedicel length, number of flowers per plant, flower yield per plant, minimum days taken to appearance of first flower bud and opening of first flower bud as compared to control. More morphological variations in vegetative and floral characters were observed with EMS treatments exhibiting three flower mutants at 0.10% EMS. One leaf mutant was produced from gamma rays applied at 30 Gy. LD_{50} dose for gamma rays and EMS was 25 Gy and 0.112 per cent, respectively.

Keywords: Morphological variations, Gamma rays, Ethyl methyl sulphonate, Mutation

Chrysanthemums (*Dendranthema grandiflora* L.), is a perennial herb plant belonging to the family Asteraceae. It is referred to as the "Queen of the East" and the "Autumn Queen" because of its vivid blossoms, which make it a beloved flower, especially in November. The chrysanthemum is a deeply rooted cultural institution and a symbol of monarchy in Japan. As one of the most exquisite and maybe the oldest flowering plants, chrysanthemums have a long history of cultural interchange and are grown commercially all over the world. Several cultivars that rank among the top 10 cut, potted, and garden plants in the world have been created during the years of breeding (Kishi-Kaboshi et al., 2017, Shahrajabian et al., 2019, Din et al., 2020). Two common techniques used in chrysanthemum breeding programs are crossbreeding and mutation breeding. Many cultivars have long been based on crossbreeding and naturally occurring sexual and asexual reproduction. The selection of superior hybrid progenies is hampered by the complex genetic variables and self-incompatibility of chrysanthemums (Anderson et al., 2017, Zhang et al., 2018, Kumari et al., 2019, Baghele 2021).Su et al. (2019) and Suprasanna and Jain (2022) observed that mutation breeding prove to be a successful breeding strategy for generating genetic variety in ornamental plants, such as chrysanthemums. Mutations can be caused spontaneously or artificially by chemical mutagens like EMS or physical agents like X-rays or gamma-rays (Kharkwall, 2017). Chrysanthemums have benefited greatly from mutation breeding (Patil et al., 2017, Kumari et al., 2019, Datta 2020), especially when paired with *in vitro* culture methods, which are occasionally the only means of enhancing an existing cultivar in vegetatively propagated species (Kumari et al., 2019). Gamma rays are electromagnetic radiation with a high energy level, deep penetration, and no particles (Anne and Lim 2020). They are essential for mutant breeding programs. EMS has proven to be highly successful in causing point mutations in plant genomes. EMS is a useful tool in the chrysanthemum mutation program because, in addition to producing significant levels of gene mutations and also induces low rates of chromosomal abnormalities during mutagenesis (Jankowicz-Cieslak et al., 2012, Luan et al., 2006).

There are 3377 authorised mutant varieties, of which 285 are mutant chrysanthemum varieties and 728 are ornamental plant mutants, according to the mutant variety database (IAEA 2022). 400 mutants were dispersed among vegetatively generated plants, with a small number of fruit trees and the bulk being floricultural plants (Kumari et al., 2019, Melsen et al., 2021). This demonstrates how well mutant breeding has worked in horticulture, particularly in floriculture. Chrysanthemum research in recent years has concentrated on in vitro methods for producing new varieties. Purente et al. (2020) created leaf and stem mutants with variable lignin and cellulose content, leaf size, and plant height in C. *indicum* var. *Aromaticum* using different EMS doses. Chrysanthemums have also been successfully mutated by physical irradiation; the ideal dosage varies from 1.0 to 3.0 Krads, depending on the genotype (Dilta et al., 2003). Cultivars with their original yellow flower colour are thought to be resistant to mutation including both radio and chemo mutagens, however chrysanthemum mutants have been successfully created (Schum 2003, Miler et al., 2020). Therefore, the creation of mutants with unique inflorescence shapes and colours may result from the efficient mutation of yellow-colored chrysanthemum types. Mutation breeding is already widely accepted for producing changed phenotypes because it creates a permanent heritable change in the structure of the genetic material, making it a cost-effective and time-efficient method for generating flower harvests (Rego and Faria 2001). Consequently, the goal of the current study was to determine the LD_{50} dose of mutagens and standardise the ideal amount of mutagens that would cause variation in the chrysanthemum cultivar Yellow Stone growth and floral features.

MATERIAL AND METHODS

The research was carried out with the Chrysanthemum (*Dendranthema x grandiflora* L.) spray-cut yellow flower cultivar Yellow Stone, during the 2020–21 growing season, the rooted cuttings of the chrysanthemum cultivar Yellow Stone were procured from Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agricultural Sciences and Technology (K), Shalimar campus. A set of rooted cuttings was exposed to four doses of gamma rays (10, 20, 30 and 40Gy) at Baba Atomic Research Institute, Zakura Srinagar, Jammu and Kashmir. Another set was treated with four dosages of EMS (0.10, 0.15, 0.20, and 0.25%) over duration of three hours. Following the treatments, the rooted cuttings were immersed in a 0.3% solution of sodium thio-sulphate (STS) for fifteen minutes in order to alleviate any solution-related stress on the plant sections. The rooted cuttings were ringed for an hour-long under running water to get rid of any remaining particles of chemical residue. One set of rooted cuttings that was not treated with any mutagen served as control. Both treated and control plants were planted in raised beds prepared in the unheated polyethylene-covered greenhouse in July. Cultural operations like weeding, irrigation, and pest and diseases management, were performed well in time according to recommended package of practices except pinching and disbudding. Beds were drenched with 0.1% Bavistin 10 days before planting to prevent soil borne disease like root rot. Staking was also done in plants to avoid lodging. Observations on vegetative growth and flower characters were recorded on five randomly selected plants from each treatment. Desirable variants with change in plant morphology and flower colour was also recorded as mutation spectrum (Table 1). The ratio of variegated useful plants to irradiated plants was called the mutation frequency (MF).

LD_{50} value: Sensitivity tests were conducted to determine LD_{50} which is the safe dose at which half of the planting materials survive with maximum recovery of viable plant materials. LD_{50} values were determined with the help of probit analysis based on the survival rate of the rooted cuttings after treatment with varying doses of gamma rays and EMS, compared with untreated control, to minimize loss of experimental material. Thirty days after planting, the rooted cuttings were counted, and the survival rate for both gamma irradiation and EMS treatments was estimated.

Statistical analysis: The system software (SASs) V. 9.1 (June 2006), SAS Institute, was used to statistically assess the data of all characters that were collected

RESULTS AND DISCUSSION

Mutagenic effect on vegetative parameters: The dose-dependent negative linear relationship between applied doses of EMS and gamma rays and survival percent of plants

Table 1. Effect of different mutagens on vegetative growth parameters of chrysanthemum cultivar yellow stone

Mutagenic treatment	Survival (%)	Plant height (cm)	Plant spread (cm)	Leaf length (cm)	Leaf width (cm)	Number of branches per plant	Branch length (cm)	Stem thickness (mm)
EMS (0.10%)	66.66 (53.81)	41.53	28.09	5.56	4.42	33.04	34.66	4.14
EMS (0.15%)	56.66 (48.86)	39.31	21.20	5.32	4.33	31.89	30.45	4.04
Gamma rays (10 Gy)	0.00 (0.00)	53.18	23.68	6.06	4.87	39.25	31.64	4.40
Gamma rays (20 Gy)	0.00 (0.00)	50.91	22.07	5.70	4.63	34.81	30.95	4.16
Gamma rays (30 Gy)	76.66 (61.25)	42.45	21.23	5.50	4.52	33.51	30.14	4.04
Gamma rays (40 Gy)	56.66 (48.86)	39.79	20.22	5.23	4.36	30.96	29.43	3.92
Control	43.33 (41.17)	47.56	23.92	5.87	4.73	35.25	32.98	4.29
CD (p-0.05)	(0.73)	1.78	1.47	0.20	0.18	2.21	2.15	0.17

(Table 1). The survival rate of the plants decreased consistently with increasing doses of both mutagens. The chrysanthemum cultivar Yellow Stone responded differently to different doses of EMS with survival rate of 53.33% at 0.10% EMS concentration. The concentration of 0.15% EMS reduced the survival rate, with none of the plants survived at concentrations beyond this concentration. Similarly gamma rays treated plants showed a decreasing trend of survival percentage with increasing dosage. Untreated rooted cuttings had the highest survival rate of 81.66%, followed by a survival rate of 71.66% at 10 Gys of gamma rays. Kiran Kumari et al. (2013) observed significant decrease in survival following gamma ray exposure. Poor establishment and survival following gamma-ray exposure were attributed to auxin depletion and/or inactivation, which impair cell division (Gordon 1957, Mahure et al., 2010) or lethal effect of gamma rays caused due to chromosomal aberration (Datta and Banerji 1993). Dilta et al. (2003) concluded that higher concentrations of EMS reduced the plant survival per cent in chrysanthemum. Lower doses of gamma rays (10 Gy) has been proved to significantly improve most of the vegetative parameters by recording maximum plant height (53.18 cm), leaf length (6.06cm), leaf width (4.87 cm), number of branches per plant (39.25), stem thickness (4.40 mm) as compared to control. Plants exposed to gamma rays may exhibit a decrease in vegetative characteristics depending on the type and degree of chromosomal damage or irradiation-induced physiological, morphological, and cytological disruption (Banerji and Datta 2002). EMS treated plants showed drastic reduction as compared to gamma radiation. All doses of gamma rays resulted in reduction of both plant spread as well as branch length as compared to control. The lower dose of EMS (0.10%) resulted in increase of plant spread and branch length as compared to control. However higher doses significantly reduced both of these parameters.

Mutagenic effect on flowering and yield parameter: Lower doses of gamma rays (10 Gy) significantly shortened the time for the first flower bud to appearance (56.59 days) and first flower to open (86.99 days) in comparison to the control (Table 2). Higher doses, on the other hand, delayed the bud formation. Dobanda (2004) also observed that gamma ray at lower doses induce earliness with respect to opening of first floret. The initiation of flowering may be affected by mutagenic treatments due to alterations in various biosynthetic pathways, which are believed to be directly or indirectly associated with the flowering physiology (Mahure et al., 2010, Ismael and Mohmoud 2015). Lower doses of gamma rays (10 Gy) recorded maximum flower head diameter (6.50 cm), flower weight (4.19 g), pedicel length (6.41 cm), number of flowers per plant (48.40), and flower yield per plant (98.47 g) in comparison to the control. However, all of these characteristics exhibited a declining trend with increasing gamma rays doses. Conversely, EMS caused significant reduction in flowering and yield parameters compared to control. These results are in conformity with Kapadiya et al (2014). Mahure et al. (2010)

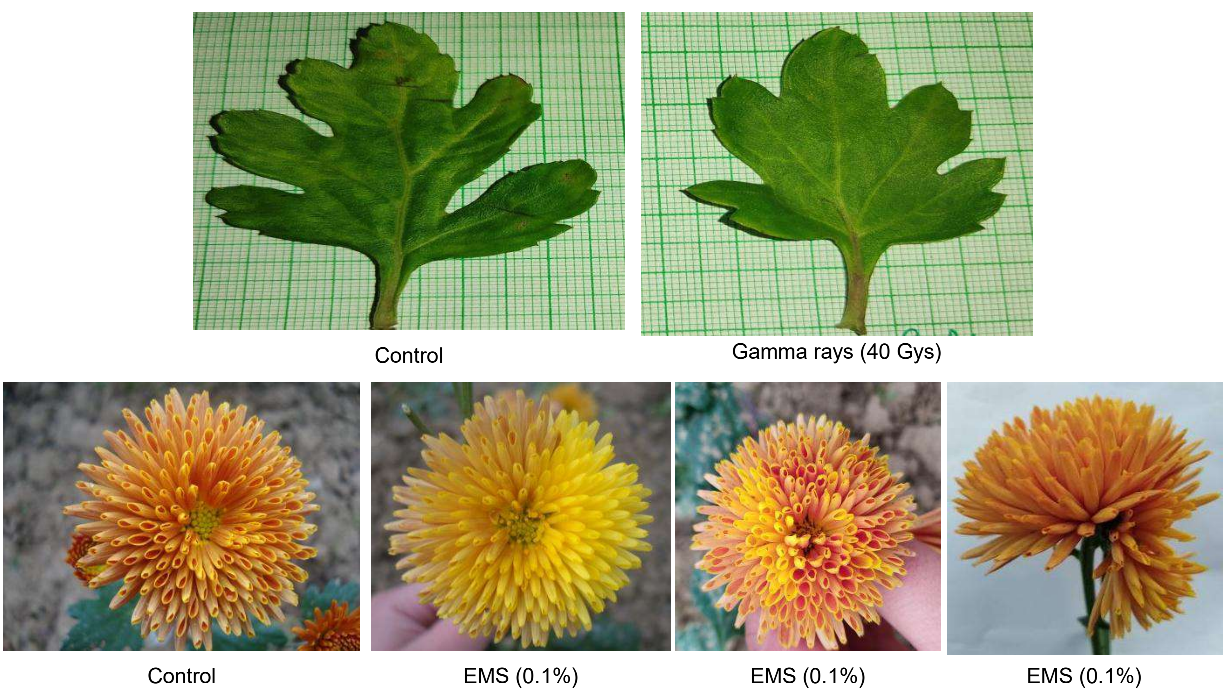

Fig. 1. Mutants in chrysanthemum cv. yellow stone

Table 2. Effect of different mutagens on flowering parameters of chrysanthemum cultivar yellow stone

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%)	61.83	92.32	6.25	241.58	3.42	6.12	42.13	67.80
EMS (0.15%)	64.98	101.62	6.16	230.86	3.32	6.05	41.20	60.74
Gamma rays (`10 Gy)	56.59	86.99	6.50	247.80	4.19	6.41	48.40	98.47
Gamma rays (20 Gy)	60.78	94.00	6.20	241.79	3.64	6.22	42.20	69.55
Gamma rays (30 Gy)	68.72	94.99	6.18	230.60	3.58	5.79	41.53	59.36
Gamma rays (40 Gy)	71.72	102.19	6.10	224.62	3.31	5.61	40.40	53.26
Control	59.87	91.66	6.42	248.47	3.88	6.28	43.93	82.02
CD (p-0.05)	0.06	0.61	0.12	0.40	0.07	0.05	2.23	6.46

Table 3. LD_{50} for gamma rays and EMS on 30th day after planting

Treatment	Survival (%)	LD 50 value
EMS (0.1%)	53.33	0.112%
EMS (0.15%)	40.00	
EMS (0.20%)	0,00	
EMS (025%)	0,00	
Gamma Rays (10Gy)	66.66	25 Gys
Gamma Rays (20Gy)	56.66	
Gamma Rays (30Gy)	40.00	
Gamma Rays (40Gy)	30.00	

also observed that in cultivar Red Gold flower size was smaller in plants treated with EMS and DES compared to gamma ray treated plants. Yellow Stone variety exhibited three flower mutants with 0.1 per cent EMS and only one foliage mutant with 40 Gy gamma rays of mutation frequency of 5.62 and 3.33 per cent, respectively.

LD_{50} value: LD_{50} for EMS was 0.112 percent, and the LD_{50} for gamma rays 25 Gy on the basis probit analysis (Table 3). Because gamma rays are more intense and penetrating in nature, they may have caused damage to cells undergoing meiotic division in the bud region.

CONCLUSION

Most of the floral and vegetative characteristics improved with lower dose of gamma radiation (10Gy). The number of morphological mutants decreased with increase in dosage of EMS but vice versa in gamma rays. Maximum morphological mutant were observed in EMS as compared to gamma rays.

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