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Gamma radiation and ethyl methyl sulfonate induced mutagenesis in China aster

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Abstract

The objective of this study was to determine the change in flower morphology and the proper dose of mutagens for inducing mutagenesis in China aster (*Callistephuschinensis* L.). The seeds of china aster were irradiated with six doses of gamma-rays (^{60}Co) *viz*., 20 Gy, 40 Gy, 60 Gy, 80 and 100 Gy at BARC, Zakura Srinagar and treated with six doses of Ethyl methyl sulphonate *viz*., 0.125%, 0.25%, 0.5%, 0.75 and 1.0% in the laboratory. In order to determine the proper dose and morphological changes; germination percentage, survival rates, growth and flowering characteristics of M_1 plants were evaluated. The results revealed that germination percentage and survival rate of seedlings were decreased with increase in dose of gamma irradiation as well as ethyl methyl sulphonate during field study. It was found that low doses gamma irradiation and ethyle methyl sulphonate had stimulatory effect number of branches per plant, stem thickness and flowering parameters. A negative correlation were obtained between all plant growth and flowering with increase in gamma and EMS doses. In M_1 generation, one type of dark colour mutant at 0.75% EMS and one type of morphological mutant at 80Gy irradiation was observed. The appropriate dose for inducing mutation was found to be 93.0 Gy and 0.84% in gamma radiation and EMS, respectively.

Keywords: Gamma radiation, ethyl methyl sulphonate, mutagenesis, China aster

Introduction

China aster [*Callistephus chinensis* L. (Nees)] belongs to family Asteraceae, is native to China. The basic chromosome number of aster is 9 and most species are diploid i.e. 2n=18. (Kunthe, 2005) [5]. The inflorescence is called as capitulum or head. It has two types of florets, central and peripheral. The central florets are called as disc florets while as peripheral florets are termed as ray florets (Bose, 1999) [2]. Aster is available in variety of vibrant colors like violet, purple, pink and white. The single and semi-double varieties are predominantly cross pollinated where as double varieties are self pollinated. There is an increasing demand in the market to produce variants with novel colours and plant forms. There are number of breeding methods available for creating variation within the existing germplasm. Mutation breeding is an effective tool to create variability in crop population within a short period of time and to make selection in the population with the view to bring about further improvement in flower crop. In general mutation breeding has been playing a key role in self-pollinated crop with limit variability. Mutations can be induced in the crop by using physical or chemical mutagens. The physical mutagens include X-rays, gamma rays and ultraviolet rays (Tharek *et al*. 2021) [10], while chemical mutagens commonly used in plants are Ethyl Methane Sulfonate (Purente *et al*. 2020) [7]. Physical mutagens like gamma rays and chemical mutagens like EMS have been widely used to create genetic diversity. Thus the present study was undertaken to identify the appropriate dose of both physical and chemical mutagens to be used for creating variation in germplasm and in development of mutants having varied colour and plant architecture in China aster.

Materials and Methods

The material for the present study comprised of seeds of china aster. The seed lot of china aster was divided into samples each comprised of 200 seeds. One set of samples were exposed to gamma irradiation with doses of 20, 40, 60, 80 and 100 Gy from ^{60}Co source at Baba Atomic Research Centre Srinagar, while as other set of samples were soaked in EMS solution with concentration of 0.125, 0.25, 0.5, 1.0, 1.25 in the laboratory. One sample kept as control was maintained which was neither treated with irradiations nor with EMS.

The seed samples were then sown in field for raising M_1 generation. A total of 13 treatments in M_1 generation including untreated dry seeds as control were used. Plants in each treatment of M_1 population were observed for mutations at regular intervals from seedling to crop maturity stage. Data on germination and seedling survival percentage were recorded 10 days and 25 days after sowing, respectively under ambient condition. Germination percentage was calculated using the formula as per ISTA, 1985 [4]. The experiment was designed according to completely randomized with three replications and data analysis was carried out using the analysis of variance and SAS statistical computer package ($p \leq 0.5$) (SAS, 1985) [9].

Results and Discussion

The treatment of china aster with gamma radiations and EMS significantly affected the vegetative growth. Gamma ray and EMS were effective in inducing variations in china aster seeds as compared to the control (Table 1). The germination percentage and survival of seedlings showed a negative correlation with dosage of gamma radiations and EMS. The germination percentage and seedling survival obtained from seeds irradiated with 20 to 120Gy and treated with EMS 0.125% to 1.25% was significantly lower as compared to the control. Ambavane *et al.* (2015) [1] also reported reduced germination percentage and survival rate at higher doses of gamma radiations. The lowest germination percentage (49.04%) and seedling survival (44.50%) was obtained from seeds treated with 1.25% EMS. Purente *et al.* (2020) [7] also reported that sample germination rate significantly decreased with increasing of EMS doses in chrysanthemum. The vegetative growth of plants remained more or less unaffected at lower doses of gamma radiations and got adversely affected at higher doses. However EMS at lower concentrations showed a stimulatory effect of plant height and stem thickness as compared to control. The higher levels of gamma radiations and EMS were found to have inhibitory effect on plant height, number of branches per plant and stem thickness. The lowest values of plant height (43.40 cm), number of branches per plant (11.25) and stem thickness (1.86 cm) was recorded by plants irradiated with 120 Gy gamma rays as compared to control.

The mutagens were found to have no significant effect on induction of flowering and crop duration in china aster. However, number of flowers per plant, flower diameter and flower duration was significantly affected by application of both physical and chemical mutagens. The number of flowers per plant, flower diameter and flower duration were significantly reduced with increase in the dosage of gamma radiations as compared to control. The lowest number of flowers per plant (18.25), flower diameter (5.46 cm) and flower duration (24.40 days) were recorded under 120 Gy gamma radiations. Pallavi *et al.* (2017) [6] obtained phenotypical variations such as plant height, the number of flowers and flower diameter in zinnia. EMS on the other hand had stimulatory effect on number of flowers per plant, flower diameter and flower duration at lower concentrations as compared to control. While as EMS at higher dosage showed negative correlation with number of flowers per plant, flower diameter and flower duration as compared to control. Physical and chemical mutagenic treatments at higher dosage caused decrease in plant height, primary branches per plant, days required for first flowering (Sadashiv *et al.*, 2012) [8].

Based on the probit analysis, the LD $_{50}$ dose for gamma radiation and EMS was found to be 93.0 Gy and 0.84%, respectively. EMS (0.75%) produced plants showing dark colouration in comparison to control which revealed the formation of colour mutant. The gamma radiations at 80 Gy produced plants having altered flower morphology while as further increase in the dosage (120 Gy) showed development of abnormal flower shapes in plants. Floral variations were also obtained by Pallavi *et al* (2017) [6] in zinnia having novel form and colour. Mutants induced by EMS that differed in various phenotypic traits were also obtained by Chen *et al.* (2020) [3] in pea nut.

In conclusion, lower doses of mutagens particularly EMS promoted most of the characters while as higher doses of both physical and chemical mutagens were found to inhibit both growth and flowering. LD $_{50}$ dose was found to be 93.0 Gy in case of gamma radiation and 0.84% in case of EMS. Colour mutant having dark colouration was developed due to application of 0.75% EMS. 80 Gy radiation produced a mutant having altered flower morphology while as 120 Gy gamma radiation produced abnormal flower shapes

Table 1: Effect of physical and chemical mutagens on survival percentage and vegetative growth of China Aster

Symbol	Treatment	% germination	% Survival of seedlings	Plant height	No. of branches/plant	Stem thickness/diameter (cm)
T_1	Control	85.33	86.32	48.80	18. 50	3.03
T_2	20 Gy	82.65	83.07	47.20	18.75	3.08
T_3	40 Gy	80.25	73.81	47.60	18.06	2.90
T_4	60 Gy	78.67	61.63	46.66	17.33	2.60
T_5	80 Gy	64.06	58.03	46.42	15.67	1.98
T_6	100 Gy	56.33	46.06	45.40	12.33	1.95
T_7	120 Gy	50.03	43.42	43.40	11.25	1.86
T_8	EMS 0.125%	80.04	83.01	51.60	17.25	3.40
T_9	EMS 0.25%	80.07	69.50	56.22	18.88	3.41
T_{10}	EMS 0.5%	79.33	54.40	57.50	19.75	3.42
T_{11}	EMS 0.75%	70.43	52.03	56.40	18.75	3.04
T_{12}	EMS 1.0%	60.23	47.06	53.34	14.75	2.90
T_{13}	EMS 1.25%	49.04	44.50	47.44	13.88	2.70
C.d.		1.832	1.234	0.958	0.641	0.058

Table 2: Effect of physical and chemical mutagens on flowering of China Aster

Symbol	Treatment	Days taken to opening of first flower (day)	No. of flowers/plant	Flower diameter (cm)	Duration of flowering (day)	Crop duration (day)
T_1	Control	91.33	28.22	6.40	32.33	142.21
T_2	20 Gy	91.30	29.41	6.38	32.16	142.20
T_3	40 Gy	91.21	27.23	6.26	31.50	143.18
T_4	60 Gy	92.33	27.07	6.15	31.46	142.58
T_5	80 Gy	92.32	24.50	6.04	28.70	140.95
T_6	100 Gy	93.43	20.40	5.60	26.80	139.66
T_7	120 Gy	93.33	18.25	5.46	24.40	139.33
T_8	EMS 0.125%	91.66	28.50	6.88	32.32	143.03
T_9	EMS 0.25%	91.67	30.25	6.94	33.63	141.46
T_{10}	EMS 0.5%	92.50	30.65	6.63	33.74	142.21
T_{11}	EMS 0.75%	92.43	28.50	6.44	34.06	141.33
T_{12}	EMS 1.0%	92.33	27.07	5.80	30.12	139.67
T_{13}	EMS 1.25%	93.03	25.28	5.25	29.08	138.85
C.d.		NS	0.810	0.315	0.754	NS


Parent


Change in morphology (80 Gy)


Colour mutant (0.75% EMS)


Abnormal Flower morphology (120 Gy)

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