Impact of physical and chemical mutagens on vegetative growth ofChina aster [*Callistephuschinensis* (l.) Nees] cv. Phule Ganesh Pink

ABSTRACT

The present study was carried out in two different experiments of mutation during September, 2021 to March, 2022 at Floriculture Research Farm, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat. The experiment was conducted for two generations and laid out in Randomized Block Design (RBD) with three and five replications. During 2021-22, seedlings of China aster var. Phule Ganesh Pink were treated with different doses of various mutagens which included four treatments of gamma rays (0.25 kR, 0.50 kR, 0.75 kR and 1.00 kR) and another four treatments of chemical mutagen (0.15 % DES, 0.20 % DES, 0.20 % EMS and 0.25 % EMS) and without mutagenic treatment as control. Effect of different doses of chemical mutagen on vegetative aspects were investigated. Fifteen mutants selected from the M_1 generation.Generally, lower doses of chemical mutagens *i.e.* 0.15 % DES showed positive effect on growth related attributes as compared to higher doses. Significantly, the maximum survival per cent *i.e.,* 95.08 was observed in T_9 (Control) while, the maximum plant height (55.95 cm) and number of branches (9.60) were recorded in T_5 (0.15 %). The highest number of variations were obtained in treatments of chemical mutagens in which maximum number of mutants were noted in T_6 (0.20 % EMS).

Keywords: China aster, Mutation, Mutagens, Vegetative attributes and Flowering parameters.

INTRODUCTION

"China aster [*Callistephus chinensis* (L.) Nees] is one of the commercially grown crops which is half hardy winter flowering annual crop. It is diploid with chromosome number 2n=18 and belongs to family Asteraceae. It is primarily originated from Northern China" (Desai, 1967). The evolution of China aster [*Callistephus chinensis* (L.) Nees] has a history of remarkable variations. According to Emsweller *et al.* (1937), the original plant had single flowers with two or more rows of blue, violet or white ray florets. The stature was medium tall and height of the plant ranged from 18 to 24 inches. The first change in the flower had been the prolongation or development of central florets and the production of quilled flowers. Germans developed double forms in aster during 18th century and hence, it is also called as German asters. Advancement of the aster evolution and large-scale seed production by Germans led to the introduction of branching types like, tall, medium tall and dwarf types and this contributed to evolutionary improvement in China aster.

"China aster is an important annual crop of our country and grown throughout the world due to existing of various vibrant colours ranging from violet, purple, magenta, pink and white; forms, sizes and pretty good post-harvest life" (Dilta *et al.*, 2007). "It is grown commercially as cut flower for flower arrangement, interior decoration and loose flower for garland making, worshipping, *etc*." (Munikrishnappa, 2013). "It can also be grown as bedding plant and potted plant in landscaping as well it is a richest source of natural pigments" (Bhargav *et al.*, 2016). "In India, China aster is commercially grown by marginal and small farmers of Karnataka, Tamil Nadu, Telangana, Andhra Pradesh, Maharashtra and West Bengal states" (Kumari *et al.*, 2017). There is a need to develop novel flower colours

and forms in China aster as the consumer preferences changes frequently. Although, China aster is being grown in considerable areas, its cultivation is concentrated around big cities and there is a need to popularize it under different agro climatic conditions.

"Mutation is a sudden heritable change in a characteristic of an organism and treating a biological material with a mutagen in order to induce mutations is known as mutagenesis. When mutations are induced for crop improvement, the entire operation of the induction and isolation *etc.* of mutants is termed as mutation breeding" (Singh, 2016)."Mutation occur constantly in nature and these spontaneous mutations are the natural genetic variation that promote the development of new varieties of enhancement of the prevailing ones. The decelerating natural mutation rate of 1×10^{-8} to 10^{-9}per generation in molecular organisms forces to choose induced mutation through physical and chemical agents" (Mba, 2012). "In past 80 years, physical mutagen, mostly ionizing radiations, have been used widely for inducing hereditary aberrations and more than 70 % of mutant varieties were developed using physical mutagenesis" (Mba *et al.,* 2013).

"Gamma radiation from radioactive cobalt-60 (^{60}Co) is widely used. It has shorter wave length and therefore, possess more energy than protons and X-rays which gives them ability to penetrate deeper into the tissue" (Amano, 2006). "The effect of chemical mutagens on plant materials is generally considered milder. An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. The ration of mutational to undesirable modifications is generally higher for chemical mutagens than for physical mutagens. A clear advantage of the point mutations created by chemical mutagen is their potential to generate not only loss of function but also gain of function phenotypes if the mutation leads to a modified protein activity or affinity" (Acquaah, 2006).

Materials and Methods

The present study entitled "Impact of physical and chemical mutagens on vegetative growth of China aster [*Callistephus chinensis* (l.) Nees] cv. Phule Ganesh Pink"was carried out in two different experiments of mutation during September, 2021 to March, 2022 at Floriculture Research Farm, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat. The experiment was conducted for two generations and laid out in Randomized Block Design (RBD) with three and five replications. During 2021-22, seedlings of China aster var. Phule Ganesh Pink were treated with different doses of various mutagens which included four treatments of gamma rays (0.25 kR, 0.50 kR, 0.75 kR and 1.00 kR) and another four treatments of chemical mutagen (0.15 % DES, 0.20 % DES, 0.20 % EMS and 0.25 % EMS) and without mutagenic treatment as control.

For physical mutagenic treatments, the seedlings were irradiated with gamma rays from Bhabha Atomic Research Center, Trombay, Maharashtra with different doses of 0.25 kR, 0.50 kR, 0.75 kR and 1.00 kR on 2nd November, 2021. Diethyl sulphate and Ethyl methyl sulphonate were used for chemical mutagenic treatments at different doses. Chemical solution was prepared by diluting 125 µl of chemical mutagens in 100 ml of water and then added more water to make solution of 250 ml of 0.15 %. Likewise, 500 µl and 625 µl of DES as well as EMS were diluted in 100 ml of water and then added remaining water to make it upto for making solutions of 250 ml of 0.15 %, 0.20 %, 0.20 % and 0.25 %, respectively.

Forty-eight seedlings were selected per treatment per replication and were treated with different concentrations of chemical mutagens by immersing their roots in the chemical solutions of 250 ml each for 3 hours. After the treatments, these seedlings were removed from the chemical solutions and washed in running tap water for 20 minutes to remove the chemical mutagens adhering to roots of the treated seedlings. The healthy seeds of China aster var. Phule Ganesh Pink were procured from Floriculture Research Farm, ASPEE College of Horticulture, Navsari Agricultural University, Navsari. Seedlings were kept in normal water to keep untreated for control.

The standard method of analysis of variance technique appropriate to the Randomized Block Design (RBD) as described by Panse and Sukhatme (1985) was used. The data were analyzed with the technical help received from computer centre, ASPEE College of Horticulture, N.A.U., Navsari. The treatment differences were tested by employing 'F' test at five per cent level of significance on the basis of null hypothesis. The appropriate standard errors (S.Em. ±) were calculated in each case and the Critical Difference (CD) at five per cent level of probability was worked out, where the treatment effects were found significant under 'F' test. The percentage co-efficient of variation (CV %) was also worked out for all the cases.

Result and Discussion

1. Survival percentage (%)

Data pertaining to survival percentage of plants at thirty days after transplanting are presented in Table 1 and graphically depicted in Fig. 1. An inquisition of the data showed that highest survival per cent *i.e.* 95.08 was observed in T_9 (Control) which was found at par with T_7 (93.87 %), T_5 (93.45 %) and T_8 (86.39 %) whereas, T_4 (1.00 kR) recorded minimum survival (60.96 %). Further, it was clear from the data that reduction in per cent survival was increased with increase in the concentrations of physical and chemical mutagenic treatment. However, seedling survival per cent in treatments of gamma rays is lower than in chemical mutagens, with the exception that concentrations of DES (0.20%) demonstrated lower survival than treatment of 0.50 kR.

The response of the survival percentage to the dose displayed a negative relationship, indicating that the increased dose of mutagen ceases the survival rate. The $LD_{50,}$ in particular, is predicted on the premise that they produce limited genome effects at lower doses of mutagens that induce phenotypical alterations, but higher dose may produce substantial genome effects that frequently lead to aberrations or abnormalities. Survival was reduced following gamma rays exposure due to inactivation and/or decrease in auxin content, which impacts cell division and ultimately results in poor establishment and survival (Gordon, 1957 and Mahure *et al.*, 2010) or deadly effect of gamma rays produced by chromosomal

abnormality (Datta and Benerji,1993). These results are parallel to findings of Banerji and Datta (2005), Kim *et al.* (2016), Patel *et al.* (2018) in gladiolus and Din *et al.* (2020) in chrysanthemum.

In chemical mutagenic treatment, the drop in plant survival may be attributed to the creation of some toxic compounds by specific biochemical process, which causes cell death, resulting in plant mortality (D'Amato and Ostenhof, 1956; Gordon, 1956). The reduction in survival was high in gamma radiation as compared to chemical treatment and this is evident that chemicals produce only point mutation whereas, radiations normally cause chromosomal rearrangement and deletions (Bhat *et al.*, 2007). Moreover, DES break definite chromosomes of the genome and sometimes also break definite region of a chromosome which induce more chromosomal fragments and fewer chromosome recombination (Gaul, 1970) which ultimately kill the growing portion and lead mortality. This finding is in conformity with Ghani *et al.* (2013) in barberton daisy, Arvind and Dhanavel (2022) in marigold and Chandana (2021) in China aster.

Table 1: Effect of different mutagens on plant height of China aster var. Phule Ganesh Pink

Treatment	Survival percentage
T_1: 0.25 kR gamma rays	68.08
T_2: 0.50 kR gamma rays	84.23
T_3: 0.75 kR gamma rays	73.81
T_4: 1.00 kR gamma rays	60.96
T_5: DES @ 0.15 %	93.45
T_6: DES @ 0.20 %	82.88
T_7: EMS @ 0.20 %	93.87
T_8: EMS @ 0.25 %	86.39
T_9: Control	95.08
SEm±	3.18
CD at 5 %	9.20
CV %	8.66

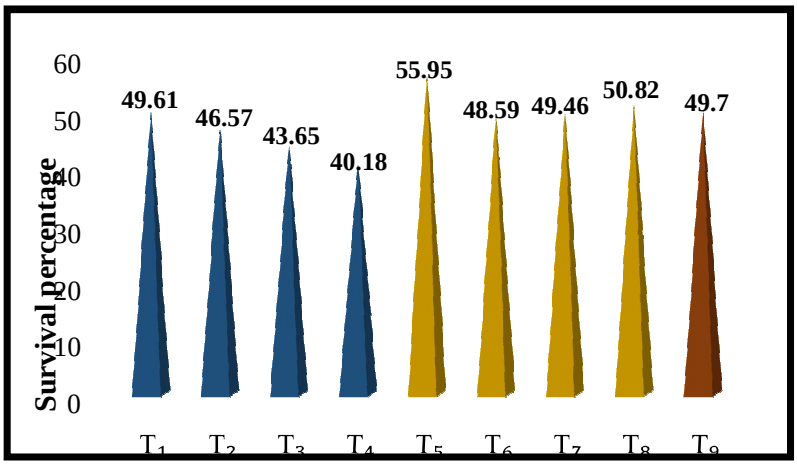

Fig. 1: Effect of different mutagens on survival percentage of Chinaaster var. Phule Ganesh Pink

B. Plant height (cm)

Plant height of China aster var. Phule Ganesh Pink was affected byvarious mutagens that have been summarized in Table 2 and graphically presented inFigure 2.

An appraisal of the data showed that, among different mutagenictreatments, significantly maximum plant height (55.95 cm) was recorded in T_5(DES @ 0.15 %) which was at par with T_8 (0.25 % EMS), T_9 (Control), T_1 (0.25 kR), T_7 (0.20 EMS) and T_6(0.20 % EMS) *i.e.*50.82 cm, 49.70 cm, 49.61 cm, 49.46 cm and 48.59 cm, respectively. While, plantremained dwarf (40.18 cm) in T_4 (1.00 kR).Plant height was positively increasedwith the increased doses of EMS whereas, in case of DES mutagenic treatments, plantheight was started declining with higher dose. In case of gamma ray treatments, plant height decreased with increased doses. It was apparent from the data presented that chemical mutagens have an increased effect than gamma rays on enhancing plant height.

"Plant height is quantitative trait which is predominantly controlled by polygenes and each gene contributes a small effect whichexternally expressed in plant morphology. Moreover, increases in the plant height may be due to the reason that certain chemical mutagens produce single base substitution with different mutation spectra, because of which broad variation occur in morphological parameters as compared to control" (Abdullah*et al.*, 2009). The stimulatory effect of the mutagen may be attributed to the increase in the rate of cell division or cell elongation as well as an activation of auxin as reported by (Zaka *et al.*, 2004 and Joshi *et al.*, 2011). This is in line with the findings of Banerji and Datta (2005) and Kim *et al.* (2016) in chrysanthemum.

"Reduction in vegetative characters by gamma rays treated plants depends on the nature and degree of chromosomal injury or morphological, cytological and physiological disturbance induced by irradiation and the decline in interior auxin manufacture, leading to plummeting growth of the plant" (Banerji and Datta, 2002). Also, it may be due to the inactivation of auxin synthesis, nature and degree of chromosomal aberration (Singh *et al.*, 2015). These results are parallel to findings of Purente *et al.* (2020) and Nasri *et al.* (2021) in chrysanthemum.

Table 2: Effect of different mutagens on plant height of Chinaaster var. Phule Ganesh Pink

Treatment	Plant height (cm)
T_1: 0.25 kR gamma rays	49.61
T_2: 0.50 kR gamma rays	46.57
T_3: 0.75 kR gamma rays	43.65
T_4: 1.00 kR gamma rays	40.18
T_5: DES @ 0.15 %	55.95
T_6: DES @ 0.20 %	48.59
T_7: EMS @ 0.20 %	49.46
T_8: EMS @ 0.25 %	50.82
T_9: Control	49.70
SEm±	2.85
CD at 5 %	8.25
CV %	13.20

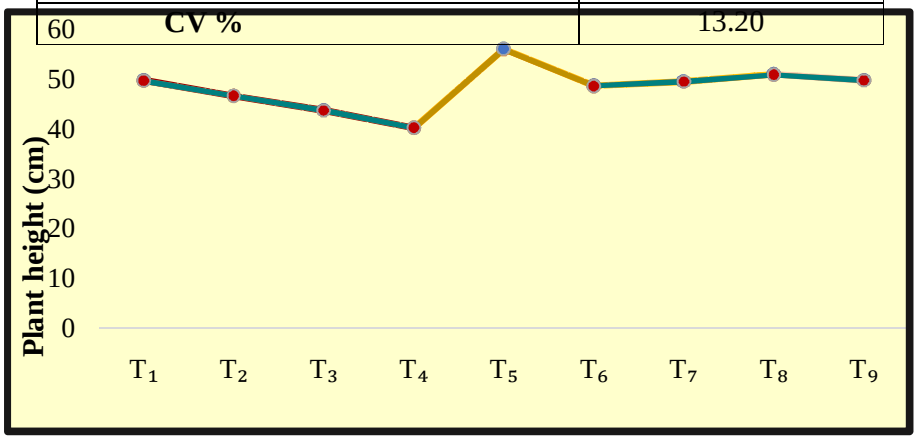

Fig. 2: Effect of different mutagens on plant height (cm) of Chinaaster var. Phule Ganesh Pink

C. Number of Primary Branches

The number of primary branches per plant was significantly affected dueto various mutagenic treatments and the results of the same are presented in Table 3and explained graphically in Figure 3.

Data revealed that the maximum number of primary branches(9.60) were found in T_5 (0.15 % DES) which was at par with treatment T_9 (Control), T_7 (0.20 % EMS) and T_8 (0.25 % EMS)*i.e.*9.28, 8.40 and 8.32, respectively in China aster var. Phule Ganesh Pink. The leastnumber of primary branches per plant (5.80) were observed in 0.75 kR and 1.00 kR. (T_3 and T_4). Thenumber of primary branches per plant were decreasing with increasing doses of gamma rays, DES and EMS.

"The increase in the production of branches implies a positive effect on the better framework and flower production of the plants. The physiological effects of DES and their hydrolysis products could also be the reason for increasing the number of branches. The lower and intermediary doses or concentrations of gamma rays, DES and EMS have a stimulatory effect on cell replication and elongation, yielding a biopositive vegetative effect in comparison to higher ones" (Chandrashekar, 2014). Decrease in primary branches in treatments of gamma rays is a result of proliferative capacity of cell. Above findings are in conformity with EI-Nashar and Asrar (2016) in calendula, Patel *et al.* (2018) in gladiolus and Chandana (2021) in China aster.

Table 3: Effect of different chemical mutagens on number of primary branches of Chinaaster var. Phule Ganesh Pink

Treatment	Number of primary branches
T_1: 0.25 kR gamma rays	6.84
T_2: 0.50 kR gamma rays	6.44
T_3: 0.75 kR gamma rays	5.80
T_4: 1.00 kR gamma rays	5.80
T_5: DES @ 0.15 %	9.60
T_6: DES @ 0.20 %	7.76
T_7: EMS @ 0.20 %	8.40
T_8: EMS @ 0.25 %	8.32
T_9: Control	9.28
SEm±	0.49
CD at 5 %	1.43
CV %	14.57

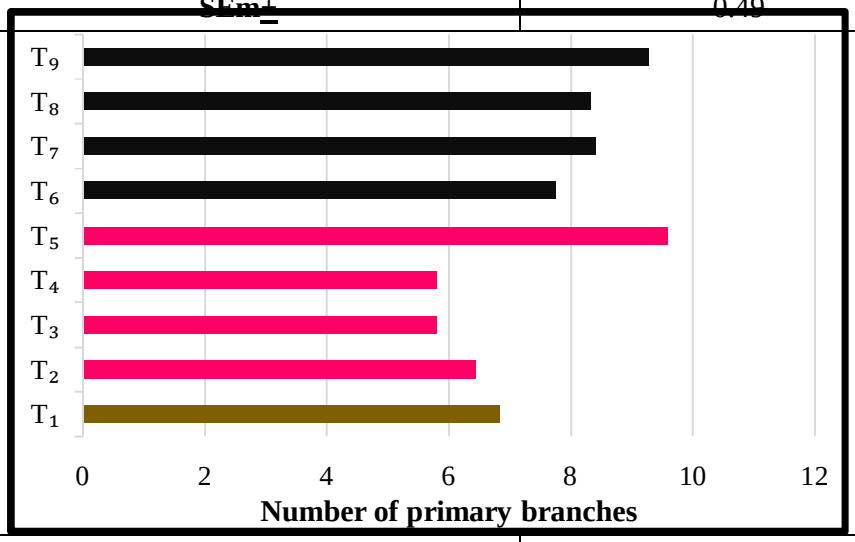

Fig. 3: Effect of different mutagens on number of primary branches of Chinaaster var. Phule Ganesh Pink

Selection of Putative Mutants for M_1 Generation

It's important to note that the specific criteria for selecting mutants in flower crops may vary depending on the type of flower crop, market demands, consumer preferences, and regional or cultural factors. The selection process often involves a combination of visual assessments and trait measurements to identify mutants with the desired traits.In the M_1 generation, a total of 4 mutants were found(Table 4 and Photo 1) displayedvariations in plant type and number of branches, respectively.

Table 4: Salient features of selected mutants of China aster for M_1 generations

Mutagenic treatments	Salient features	Genotypes
T_4 (1.00 kR)	Spreading, maximum number of flowers with small diameter, suitable for landscaping	ASTM 3
T_8 (0.20% EMS)	Erect, floriferous, deep purplish pink coloured flower	ASTM 5
T_5 (0.15% EMS)	Spreading and profuse flowering	ASTM 7
T_6 (0.20% DES)	Erect growing habit with red coloured flower	ASTM 9

ASTM 3 **ASTM 5**

After application of mutagens

Before application of mutagens (Control)

Photo 1: Performance of Phule Ganesh Pink with the application of mutagens

Conclusion

On the basis of the results obtained from the investigation, it can be concluded that application of physical and chemical mutagens gave good performance. DES @ 0.15 %gave the maximumin vegetative growth of China aster cv. Phule Ganesh Pink in case of plant height and number of primary branches.

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