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Induction of variability in China aster [*Callistephus chinensis* (L.) Nees] by chemical mutagens

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ABSTRACT

The study was conducted at the Floriculture Research Farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, during 2020-21. Seedlings of China aster variety Arka Kamini were treated with different doses of chemical mutagens, namely Diethyl Sulfonate (DES) and Ethyl Methane Sulfonate (EMS), comprising 11 treatments: 0.05% DES (T_1), 0.10% DES (T_2), 0.15% DES (T_3), 0.20% DES (T_4), 0.25% DES (T_5), 0.05% EMS (T_6), 0.10% EMS (T_7), 0.15% EMS (T_8), 0.20% EMS (T_9), 0.25% EMS (T_{10}) and a control (T_{11}). The effects of the mutagens were evaluated, focusing on the induction of mutants and the occurrence of vegetative and floral abnormalities. A total of 24 mutants with desirable colour characteristics were isolated from the treatments. Additionally, three vegetative chimeras were observed in the 0.20% DES, 0.15% EMS, and 0.20% EMS treatments. Notably, a mutant (T_5M_1) exhibiting strong red coloured flowers was identified in the 0.25% DES treatment.

Key words: Chemical mutagens, China aster, diethyl sulphonate, Ethyl Methane Sulfonate (EMS).

INTRODUCTION

China aster (*Callistephus chinensis* (L.) Nees) is a significant commercial flower crop belonging to the family Asteraceae, with its origin in both China and Europe. The genus *Callistephus* derives its name from the Greek words *kalistos,* meaning "most beautiful", and *stephos,* meaning "a crown". China aster is a versatile flower crop cultivated for use as cut flowers, loose flowers, bedding plants and pot plants. The flowers of China aster are commonly used for floral arrangements, interior decoration, garland making, worship, and other purposes. (Munikrishnappa *et al.,* 2013).

Hybridization and mutation are two promising breeding methods used for developing new cultivars in flower crops and to improve the varietal wealth, by adding new types having improved flower quality, yield and yield attributing traits (Azadi *et al.*, 2016; Fu *et al.*, 2016; Rout and Jain, 2020). Mutation is sudden heritable changes in the DNA sequence that are not derived from genetic segregation or recombination. Spontaneous mutation occurs at

a very low frequency *i.e.* 1 in 10 lacs or 10^{-6} (Durland and Moghadam, 2022). Since, mutations bring variation, they provide the ultimate basis for evolution of new forms, varieties or species. Mutations may result into deletion, inversion, translocation of chromosome and nucleotide base substitutions (Berdan, 2020). Mutation can be induced artificially with the help of various physical and chemical agents which are called mutagens (Nakatsuka and Koishi, 2018). Most commonly used mutagens are gamma rays, EMS (Ethyl methane sulphonate), DES (Diethyl sulphonate), Colchicine, *etc*. Dose inducing 25 to 50% lethality (LD_{25}-LD_{50}) among mutated plants will result in the highest mutation rates (Susrama *et al.*, 2022). Mutagenesis can be exploited experimentally (experimental mutagenesis) by physical, chemical and biological means (Mullins, 2021). Induced mutations in ornamental plants are known for insertion of unique traits, such as altered flower characters (colour, size, morphology, fragrance); leaf characters (form, size, pigmentation); growth habit (compact, climbing, branching) and physiological traits such as changes in photoperiodic response, early flowering, free flowering, flower keeping quality and tolerance to biotic and abiotic stresses (Datta, 2018; Suprasanna *et al.*, 2022).

Chemical mutagenesis is a simple and effective method for generating valuable starting material in plant breeding (Kumar, 2024). Chemical mutagens can induce a high frequency of non-lethal point DNA mutations, thereby creating genetic diversity in various crops (Abdelhameed, 2024). Among chemical mutagens, alkylating agents such as ethyl methyl sulfonate (EMS) and diethyl sulfonate (DES) are widely used in various flower and other crops. These agents induce a high frequency of nucleotide substitution variations and have the potential to generate numerous new mutants with desirable traits. Therefore, the present study was conducted with a focus on identifying desirable variations induced by chemical mutagens in China aster cultivar Arka Kamini.

MATERIALS AND METHODS

The present experiment was conducted at the Floriculture Research Farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, during the winter season of 2020-21. A total of 11 treatments, including diethyl sulfonate (DES) and ethyl methyl sulfonate (EMS), were utilized: 0.05% DES (T_1), 0.10% DES (T_2), 0.15% DES (T_3), 0.20% DES (T_4), 0.25% DES (T_5), 0.05% EMS (T_6), 0.10% EMS (T_7), 0.15% EMS (T_8), 0.20% EMS (T_9), 0.25% EMS (T_{10}) and a control (T_{11}). Forty seedlings were selected for each treatment and replication, and their roots were immersed in 250 ml of chemical solutions at different concentrations of mutagens for 3 hours. After the treatments, the seedlings were removed from the chemical solutions and rinsed in running tap water for 20 minutes to remove any chemical mutagens adhering to the roots. The seedlings were kept in normal water to remain untreated as the control. They were then planted on raised beds in a randomized block design (RBD) with three replications. Observations were recorded on various vegetative, flowering, and yield parameters to evaluate the effects of the mutagens, focusing on the induction of mutants and the occurrence of vegetative and floral abnormalities. The observed data were statistically analysed as per Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Isolated Mutants : The results of the mutagenic treatments on China aster var. Arka Kamini

Table 1: Mutants of China aster var. Arka Kamini isolated from different chemical mutagenic treatments and their characteristics.

Sr. No.	Mutants	Plant height (cm)	Number of primary branches/ plant at (60 DATP)	Plant spread (cm) (60 DATP)		Days to first flower bud initiation	Days to first flower opening	Duration of flowering (days)	Diameter of flower (cm)	Stalk length (cm)	Number of flowers per plant	Flower head colour (RHS colour chart)	
				E-W	N-S								
1.	T_1M_1	25.5	2	16.3	19.2	49	59	29	5.89	15.3	13	Strong purplish pink	Red purple group 68 B
2.	T_1M_2	23.6	3	15.3	16.2	43	55	30	5.36	17.3	24	Moderate purplish pink	Red purple group 68 C
3.	T_1M_3	38.1	4	30.2	29.5	42	53	34	5.58	29.2	23	Very light purple	Purple group 75 C
4.	T_2M_1	31.5	2	22.4	24.3	46	58	29	5.63	24.1	21	Very pale purple	Purple group 75 D
5.	T_2M_2	37.9	4	25.6	22.3	48	58	24	4.55	19.3	14	Strong purplish red	Red purple group 59 D
6.	T_3M_1	29.9	4	20.3	19.6	45	56	30	6.05	15.6	33	Deep purplish pink	Red purple group 73 A
7.	T_3M_2	33.7	3	25.8	24.1	44	56	34	5.05	23.7	13	Strong purplish pink	Red purple group 73 B
8.	T_3M_3	38.7	2	29.5	27.2	42	57	35	5.05	17.3	17	Light purplish pink	Red purple group 73 C
9.	T_3M_4	28.6	2	19.5	17.3	46	59	34	5.73	19.5	12	Strong purplish red	Red purple group 60 D
10.	T_4M_1	23.9	3	15.3	14.2	51	62	26	5.69	14.5	13	Strong purple	Purple violet group N80 A
11.	T_4M_2	24.6	4	14.3	16.3	53	65	27	5.36	16.5	24	Strong purplish red	Red purple group 67 A
12.	T_5M_1	21.3	3	13.6	12.1	54	67	24	6.15	14.8	13	Strong red	Red group 53 B
13.	T_6M_1	29.4	4	16.3	17.5	45	57	29	5.83	16.5	15	Red	Red purple group 60 A
14.	T_6M_2	25.3	2	18.3	19.1	41	54	28	5.44	11.2	13	Strong purplish pink	Red purple group 73 B
15.	T_6M_3	20.3	3	13.1	13.6	47	57	30	4.71	11.9	14	Moderate purplish pink	Red purple group 65 A
16.	T_7M_1	32.7	2	25.6	23.4	49	59	27	5.77	25.2	17	Light purplish pink	Red purple group 73 C

Conted. ....

17.	T_7M_2	25.3	2	18.3	17.3	43	56	31	4.84	23.5	17	Very pale purple	Purple group 76 C
18.	T_7M_3	28.1	4	19.8	18.6	44	55	34	4.05	17.3	12	Very pale purple	Purple group 75 D
19.	T_7M_4	23.6	3	15.6	14.9	46	57	26	4.07	13.9	12	Strong purplish pink	Red purple group 73 B
20.	T_8M_1	28.4	4	20.1	22.3	49	58	27	5.20	17.7	24	Moderate purplish pink	Red purple group 65 A
21.	T_9M_1	41.3	4	29.2	30.1	46	58	29	6.28	29.8	21	Deep purplish pink	Red purple group 73 A
22.	T_9M_2	35.6	3	25.3	23.6	47	59	31	5.75	18.9	23	Light purplish pink	Red purple group 73 C
23.	$T_{10}M_1$	35.8	3	23.8	22.5	52	65	35	5.86	24.3	18	Deep purple	Purple violet group N81 A
24.	$T_{10}M_2$	29.3	4	19.6	20.1	55	68	33	5.27	20.3	33	Deep purplish pink	Red purple group 73 A

revealed the isolation of several desirable mutants, which are listed in Table 1, along with their respective characteristics. Total 24 mutants were isolated from various treatments which included mostly in flower colour over control (Plate 1). The highest number of mutants (4) was observed in both T_3 (0.15% DES) and T_7 (0.15% EMS). Additionally, T_5 (0.25% DES), which received the highest dosage of diethyl sulfonate, produced a mutant with distinct and attractive flowers of strong red color (Plate 2). The frequency of color mutations rose with increasing doses up to a certain threshold, after which it began to decrease.

Plate 1: Flower of China aster var. Arka Kamini.

Plate 2: Flower of T_5M_1 (0.25% DES) with strong red color.

Changes in flower color may result from mutations in the biosynthetic pathway of structural or regulatory genes (Kapadiya, 2014).

Changes in flower color may result from quantitative and/or qualitative alterations in the color pigments, which could be caused by mutations in their biosynthetic pathways or induced at independent loci that regulate flower color (Kolar *et al.*, 2015). Ethyl methanesulfonate (EMS) induces mutations by alkylating guanine bases, leading to (mis)matches with thymine instead of cytosine, which results in G/C to A/T transitions (Banerji, 2016). Less frequently, EMS can cause G/C to C/G or G/C to T/A transversions through the hydrolysis of 7-ethylguanine, or A/T to G/C transitions due to mismatches with 3-ethyladenine (Samatadze *et al.,* 2019). Banerji (2017) reported that 55% of the records on induced mutations in ornamental plants were related to changes in flower color, while 15% involved changes in flower morphology.

Vegetative Abnormalities (%) : Vegetative variations and abnormalities observed in China aster var. Arka Kamini due to various mutagenic treatments with chemical mutagens are presented in Table 2. Vegetative variants with a multiple branching habit were obtained in T_3 (0.15% DES), T_5 (0.25% DES), T_7 (0.10% EMS), and T_{10} (0.25% EMS), showing a branching pattern distinct from that of the untreated plants. Three vegetative chimeras were obtained in T_4 (0.25% DES), T_8 (0.15% EMS) and T_9 (0.20% EMS). In the case of treatment T_5 (0.25% DES), one plant exhibited purple leaf venation, but the yield was the lowest, thus it is considered a vegetative abnormality.

Vegetative abnormalities increased significantly compared to the control as a result of the mutagenic treatments with chemicals. The variations observed were not solely due to genetic mutations but also due to somatic mutations. Somatic mutation occurs when a mutant cell continues to divide, resulting in an individual with a patch of tissue that has a genotype different from the rest of the body. These mutations can include karyotypic changes, point mutations, somatic gene amplification, insertion or excision of transposable elements, and the segregation of pre-existing chimeric tissues (Patil *et al.*, 2017). Vegetative abnormalities included changes in plant morphology such as branching habit, leaf shape, size, margin, apex, fission, and fusion. The frequency of these abnormalities increased with

Table 2: Vegetative abnormalities observed in China aster var. Arka Kamini due to different chemical mutagens.

Treatments	Number of vegetatively abnormal plants	Vegetative abnormality percentage (%)	Form
T_1	0	0	-
T_2	0	0	-
T_3	1	0.83	Multiple branching
T_4	1	0.83	Chimera
T_5	2	1.67	Multiple branching
	1	0.83	Purple leaf venation
T_6	0	0	-
T_7	1	0.83	Multiple branching
T_8	1	0.83	Chimera
T_9	1	0.83	Chimera
T_{10}	2	1.67	Multiple branching
T_{11}	0	0	-

*Percentage has been calculated from 120 plants.

higher doses of mutagens. Vegetative abnormalities in the treated plants may be due to the inactivation and/or disruption of auxin synthesis (Choudhary *et al.*, 2018) and the extent of chromosomal aberrations (Singh *et al.*, 2018).

Floral Abnormalities : Floral abnormalities observed in various treatments with chemical mutagens on China aster var. Arka Kamini are presented in Table 3. Floral abnormalities included changes in flower head size, deformed petals, flower type, and other variations such as changes in size, shape, type, color, and asymmetrical development of disc and ray florets. The maximum floral abnormalities were recorded in T_5, followed by T_4. Chemical mutagens also significantly increased floral abnormalities compared to the control. Floral abnormalities were observed at 0.15% DES (deformed flowers), 0.20% DES (single whorled flowers), 0.25% DES (double whorled and deformed flowers), 0.15% EMS (deformed flowers), 0.20% EMS (uneven floret length), and 0.25% EMS (deformed flowers). Additionally, the mutation frequency increased with higher doses, but only up to a certain range.

The variations might be caused by chromosomal aberrations, disturbances in the production and distribution of growth substances, which are induced by physical and chemical mutagens (Singh *et al.*, 2018). Furthermore, some chromosomal aberrations induced by mutagenic chemicals can be attributed to insufficient penetration of the chemicals and their poor ability to break chromosomes in plants (Ghani *et al.*, 2013).

CONCLUSION

A total of 24 mutants were isolated from various treatments in the M_1 generation, with the majority showing changes in flower colour compared to the control. The frequency of color mutations increased with the dosage up to a certain point, after which it declined. The highest number of mutants (4) was observed in both T_3 (0.15% DES) and T_7 (0.15% EMS). Furthermore, T_5 (0.25% DES), yielded a plant with flowers that were distinct and characterized by a strong red colour. The application of lower concentrations of chemical mutagens resulted in the development of higher numbers of desirable

Table 3: Floral abnormalities observed in China aster var. Arka Kamini due to different chemical mutagens.

Treatments	Number of floral abnormal plants	Floral abnormality percentage (%)	Form
T_1	0	0	-
T_2	0	0	-
T_3	1	0.83	Deformed flower
T_4	3	2.5	Single whorl of petals
T_5	2	1.67	Double whorl of petals
	2	1.67	Deformed petals
T_6	0	0	-
T_7	0	0	-
T_8	1	0.83	Uneven florets length
T_9	1	0.83	Deformed flower
T_{10}	2	1.67	Deformed flower
T_{11}	0	0	-

*Percentage has been calculated from 120 plants.

mutants with minimal abnormalities. Notably, vegetative variants with multiple branching habits were obtained in T_3 (0.15% DES), T_5 (0.25% DES), T_7 (0.10% EMS), and T_{10} (0.25% EMS). Three vegetative chimeras were observed in T_4 (0.25% DES), T_8 (0.15% EMS), and T_9 (0.20% EMS), indicating positive mutagenic effects. While higher concentrations, such as 0.25% DES and 0.25% EMS, led to increased floral abnormalities, lower concentrations (0.15% DES and 0.10% EMS) produced fewer abnormalities, demonstrating the potential of these lower doses to induce beneficial mutations with minimal negative impact on plant morphology and flower development.

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