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MUTAGENIC IMPACT OF ETHYL METHANE SULPHONATE ON THE MORPHOLOGY AND MICROSPOROGENESIS OF *Artemisia annua* L.

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ABSTRACT

Malaria is a global concern and for its cure the suffering people mostly depend on herbal remedies, with currently the most preferred cure being artemisinin combination therapy. *Artemisia* species possess varying content of artemisinin and ethyl methane sulphonate (EMS) mutagenesis is important tool for creating unique genetic variation or mutants in a shorter period. The present study was aimed to assess the effects of EMS doses on the morphology of *Artemisia* plant as well as on its micro-sporogenesis and biochemical content. The experimental procedure for EMS treatment followed was as per Williams method where triple technical repeats of three different EMS doses (0.1, 0.3, and 0.5%) and a control (0% EMS) were applied. The process of screening mutants frequently revealed the presence of several allelic mutants generated by EMS. In present study the germination and survival percentage were higher at the doses of 0.1 and 0.3% of 3 h and 5 h of EMS treated seeds. The LD_{50} dose was 0.3% EMS (3 and 5 h). At these doses, germination, survival, plant height, and pollen fertility were high and maturity was early. Some leaf variants were also observed at 0.5% (5 h) and 0.3% (3 h). A wide spectrum of cyto-morphological abnormalities was noted in EMS treated seeds. Lower doses proved to be a revolutionary mutant for creating new variation as EMS mutagenesis enables cost-effective and high-throughput generation of mutations, greatly accelerating plant genomes research.

Keywords: Abiotic stress, *Artemisia annua*, chemical mutagen, ethyl methyl sulphonate

INTRODUCTION

Medicinal plants have been integral to human health for millennia, serving as foundation for traditional healing practices across the globe. Historical records have revealed the extensive use of plant-derived remedies, underscoring their significance in the evolution of modern pharmacotherapy (Kotnis *et al.*, 2004). Medicinal plants are major source of biodynamic compounds of therapeutic value, and different varieties of plants have variable therapeutic properties (Harsha *et al.*, 2002).

Malaria is still a global concern with around 214 million annual cases and 430,000 annual deaths, mainly among of the children of < 5 year age (WHO, 2016). The disease is caused by *Plasmodium* sp., particularly *P. falciparum*, that proliferate in female Anopheles mosquitoes (Cox, 2010). There has been a continuous effort to control the malaria spread and the disease has almost been eradicated from Europe, North America, and parts of Asia and Latin America, however, in Sub-Saharan Africa almost 80% of global annual malarial patients are found (Carter and Mendis, 2002). Besides vector control and insecticide-treated nets, research has focused on developing new drugs and vaccines. Currently the preferred cure is artemisinin combination therapy (ACT) which is derived from *Artemisia* plant

(Banek *et al.*, 2014; Lalloo *et al.*, 2016). It is important to improve the potential of plant for artemisinin production through plant breeding. However, improving the quality through breeding efforts have been a challenging task due to the heterozygous nature of *A. annua*, which results in transgenic plants with varying artemisinin content even though generated in the same laboratory (Larson *et al.*, 2013).

Artemisia annua L., one of the largest genera belongs to Compositae family, comprises of about 350 species. *A. annua* is predominantly distributed in the northern temperate region of the world in 0-50 cm precipitation areas. Many *Artemisia* species have been used since ancient times as folk remedies for reducing phlegm, relieving cough, invigorating blood circulation, stopping pain, inducing sweat, diuresis, antihypertension, anthelminthic, antitoxic, and antiallergy (Tan *et al.*, 1998). *Artemisia* species possess secondary metabolites like terpenoids, flavonoids, coumarins, glycosides, sterols, and polyacetylenes. Artemisinin, an endoperoxide sesquiterpene lactone isolated from *A. annua*, has proved highly effective antimalarials. Artemisinin-based combination therapies are considered best treatment for uncomplicated *P. falciparum* malaria (Hong *et al*., 2009). Besides, the essential oil has antioxidant activity equivalent to 18% of reference compound (α-tocopherol).

Mutagenesis is an important breeding tool due to its easy use, low cost, and wide applicability. The frequency of mutations can be matched by adjusting the mutagen dose; and saturation can easily be reached. For *in vitro* mutagenesis, a variety of physical and chemical mutagens have been used which include γ- and X-ray radiations, fast neutrons, colchicine, dimethyl sulphate, diethyl sulphate, ethyl methane-sulfonate (EMS), MNU 1-methyl-1-nitrosourea, and SA sodium azide (Ibrahim *et al.*, 2018). EMS, inducing many point mutations, is one of the most effective chemical mutagens for developing new cultivars in ornamental plants. EMS advantageously leads to low chromosomal aberrations during mutagenesis, in addition to high levels of gene mutations (Jankowicz-Cieslak *et al.*, 2012). EMS is a non-transgenic chemical mutagen and quite useful in discovering new genes for plants. Special protocols have been developed for many plant species (Unan *et al.*, 2021). EMS, a mono-functional alkylating agent with one reactive group that react with DNA, causes extensive cross linkage of DNA, chromosome breakage, and chromosome mutations. EMS alkylate DNA at 7-N and 6-0 position causes depurination which leads to backbone breaks. When 7-ethyl guanine is produced, its base pairs with thymine causing G:C→A:T transitions. These alkylating agents cause TILLING effect that leads to the changes in DNA structure. This special property of EMS targets only one point of DNA and affects only that site leading to the alteration in DNA sequence through frameshift mutations. Earlier studies identified various mutagenic effects of EMS in many plants like *Arabidopsis* (Greene *et al.*, 2003), *Lycopersicon esculentum* (Saba and Mirza, 2002), soybean (Karthika and Subbalakshmi, 2006), *Jatropha curcas* (Dhakshanamoorthy *et al.*, 2010), *Vigna* sp. (Kozgar *et al.*, 2011), etc. The first commercial rice varieties, CL112 and CL141, which were M2 from Clearfield rice varieties AS350, promoted imidazolinone resistant commercial progress and has great significance in rice research (Sudianto *et al*., 2013). EMS treatment is inexpensive, easy to implement, induces point mutations at high rates (Gillmor and Lukowitz, 2020). Mutagenesis studies necessitate extensive plant populations due to infrequent occurrence of mutations in plant cells. EMS mutagenesis can achieve a large number of mutants in a short period. It is necessary to strengthen research of phenomics, transcriptomics, proteomics, metabolomics, and other methods in EMS mutant screening and gene function analysis. The present study was aimed to assess the effects of EMS doses on the morphology of *Artemisia* plant as well as on the micro-sporogenesis and biochemical content, so that the most effective conditions can be determined which could later on can be applied for successful mutagenesis in basic research studies such as knock-out of genes for confirming particular functions.

MATERIALS AND METHODS

The present study was conducted in 2020 in the Department of Botany, University of Allahabad (India). The seeds of *Artemisia annua* genotype EC-415012 were procured from NBPGR, Bhowali,

Nainital (India). Artemisia seeds were raised in field (25º27'43.01"N; 81º51'10.42"E) in a randomized complete block design. The net plot size was 4 m x 4 m, with nine rows (each 4 m long) with row to row distance of 45 cm and plant to plant spacing of 20 cm. The untreated seeds (control) were planted in the first row of each plot. The plots were irrigated during vegetative growth as and when required; and the plants were harvested individually at full maturity. Germination (%) was recorded 7 days after sowing (DAS) and plant survival (%) 14 DAS.

Mutagenic treatment and observations

Certified, healthy, uniform and dry Artemisia seeds (genotype EC-415012) were surface sterilized with 0.1% $HgCl_2$ solution for 1 min and thoroughly washed with distilled water. Induction of EMS mutagenesis in seeds was done as per Williams *et al*. (1992). Triple technical repeats of three EMS doses *viz*., 0.1, 0.3, and 0.5% along with a control (0% EMS) were applied. The seeds were pre-soaked in distilled water for 5 h and later immersed in EMS solution for two different durations i.e. 3 h; and 5 h after the treatment to seeds were washed. All the chemical mutagenic treatments were given at 25±2°C. The seeds soaked in distilled water for 10 h served as control. The different concentrations used for chemical mutagenic treatment were 0.1, 0.3 and 0.5%. The seeds were immediately washed thoroughly under running tap water to remove the excessive mutagens. Later on treated seeds were post-soaked in distilled water for 1 h. The post-soaked seeds were dried in folds of filter paper. The treated seeds were kept on moist blotting paper in triplicate petriplates to record germination percentage and sown in field in a randomized block design with three replications along with control as M_1 generation. The entire M_1 generation was thoroughly studied for various biological parameters. Proper agronomic practices were followed for raising healthy crop and grown strictly under rainfed conditions. The data on M_1 seed germination and subsequent survival of M_1 plants till maturity were recorded. Growth pattern of M_1 plants were recorded for the selection of morphological mutations i.e. germination, dwarf, taller variant and vigorous plant types, together with agronomic and yield related mutants for early maturity, higher yield, more number of branches, achenes, plant height, etc.

Meiotic analysis

The young capitula of suspected plants were fixed in Carnoy's fixative *viz*., glacial acetic acid: ethyl alcohol (1:3, v/v) and then transferred to 70% alcohol after 24 h and stored at 4°C until use. These buds were used for cytological assessment by screening the pollen mother cells. Meiotic slides were prepared by using anther smear technique with 2% standard acetocarmine stain; and observed and micro-photographed under Nikon phase contrast microscope (Nikon Eclipse, E200, Japan) at 40X magnification. For pollen fertility, the anthers of mature capitula were subjected to acetocarmine–glycerine stainability test (Ordóñez, 2014). Fertile pollen grains were recorded with stained cytoplasm whereas undersized and unstained pollen grains without nuclei were considered sterile.

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen}}{\text{Total number of pollen}} \text{ x } 100$$

Statistical analysis

All the experiments were conducted three replicates. The data was analysed was using SPSS 16.0 software. One-way analysis of variance and Duncan's multiple range test (DMRT, $p < 0.05$) were performed to test the significance of treatments and the graph were plotted by using sigma plot 10.0 software. Actual mean and standard error were calculated.

RESULTS AND DISCUSSION

Mutation breeding is used to elevate the variability in quantitative characters. EMS is one of the most popular mutagens used in creating variability in plants. New varieties of desired traits have been successfully produced in many crops like cucumber (Shah *et al.*, 2015), cowpea (Gnanamurthy and Dhanavel, 2014), rice (Wu *et al.*, 2005), wheat (Feiz *et al.*, 2009), etc.

Seed germination and plant survival

Table 1 shows variations in morphological parameters in presence of EMS. Artemisia seed germination and seedling survival was highly affected in presence of EMS. In control, the seed germination was in the range of 95.42-98.23%. But in 0.5% EMS dose the germination declined upto 80.56 and 76.60% in 3 and 5 h, respectively. In control, the plant survivability was in the range of 97.23-95.24%. But at higher EMS dose, it declined with lowest survivability of 24.66% in 5 h duration. EMS showed differential effect on seed germination of three clones of *A. annua* (E-Shuen Leow *et al*., 2020). In present study the seed germination and plant survival decreased with increase in EMS concentrations. Similar pattern of effectiveness of mutagenesis was observed in sunflower (Sabetta *et al*., 2011). The EMS is absorbed by the treated samples which show a cytotoxic effect, and can induce either DNA damage or inhibit physiological processes that block/reduce enzyme activity and RNA synthesis (Kumar *et al*., 2013).

Plant height, internodal length, and primary number of branches

In control the mean plant height was 93-94 cm but in presence of EMS it was reduced to 89.30 cm (3 h duration of 0.01%) and 85.6 cm (5 h duration of 0.01%) to 69.60 cm (3 h duration of 0.05%) and 48.3 cm (5 h duration of 0.05%), respectively (Table 1). Internodal length and primary branches were also affected and declined upto 4.3 cm (3 h), 3.4 cm (5 h) and 18.0 (3 h), 12.0 (5 h) at 0.05%, respectively. Plant height and internodal length were negatively affected by higher EMS doses. However, at lower EMS dose it was not effected much. At higher dose the plant height decreased drastically which may be because of the delayed inhibition of physiological and biological pathways essential for cell division such as enzyme activity, imbalance of hormonal changes and inhibition of mitotic process which resulted in the lessening of length. Talebi *et al.* (2012) reported that EMS reduced seedling height due to cell cycle arrest at G2/M phase during somatic cell division or various kinds of damage in the entire genome. Also, a study on *Artemisia vulgaris* has shown similar result wherein NaCl-stressed calluses were successfully regenerated with 0.5% EMS treatment for 60 min, exhibiting the most robust salt tolerance (Kumar and Kumari, 2021). Possibly the 60 min exposure was the best length for 0.5% EMS treatment which could stimulate maximum frequency of mutagenesis for salt tolerance.

Table 1: Effect of EMS on different morphological parameters of *Artemisia annua*

Duration	Doses (%)	Germin-ation (%)	Survival (%)	Plant height (cm)	Inter-nodal length (cm)	Primary No. of branches	Leaf area (cm^2)	Days to 50/% flowering	Days to maturity	Pollen fertility (%)
3 h	Control	98.23 ± 0.95^{a}	97.23 ± 0.20^{a}	94.70 ± 0.90^{a}	6.80 ± 0.06^{a}	28.00 ± 0.85^{a}	32.40 ± 0.51^{a}	104.00 ± 1.87^{b}	151.00 ± 1.87^{a}	96.12 ± 0.75^{a}
	0.1	93.12 ± 1.26^{ab}	92.12 ± 1.53^{b}	89.30 ± 1.78^{b}	6.40 ± 0.12^{b}	27.00 ± 0.86^{ab}	24.50 ± 0.42^{b}	122.00 ± 2.35^{a}	144.00 ± 2.95^{ab}	90.39 ± 1.76^{b}
	0.3	90.44 ± 2.16^{b}	84.29 ± 1.57^{c}	80.30 ± 2.43^{c}	6.20 ± 0.20^{c}	25.00 ± 0.89^{b}	18.60 ± 0.39^{c}	120.00 ± 2.56^{a}	141.00 ± 3.47^{ab}	88.78 ± 1.98^{b}
	0.5	80.56 ± 2.47^{c}	63.75 ± 1.60^{d}	69.60 ± 1.94^{d}	4.30 ± 0.14^{d}	18.00 ± 0.94^{c}	13.40 ± 0.33^{d}	108.00 ± 3.51^{b}	139.00 ± 3.85^{b}	80.09 ± 2.03^{c}
5 h	Control	95.42 ± 1.29^{a}	95.24 ± 1.30^{a}	93.30 ± 0.97^{a}	7.40 ± 0.07^{a}	25.00 ± 0.86^{a}	30.40 ± 0.32^{a}	101.00 ± 1.57^{c}	154.00 ± 0.10^{b}	95.68 ± 0.77^{a}
	0.1	92.30 ± 1.27^{a}	70.72 ± 1.39^{b}	85.60 ± 1.91^{b}	6.20 ± 0.16^{b}	21.00 ± 0.83^{b}	22.40 ± 0.50^{b}	128.00 ± 1.71^{a}	175.00 ± 3.01^{a}	88.41 ± 1.42^{b}
	0.3	86.20 ± 1.77^{b}	62.21 ± 1.60^{c}	66.10 ± 1.69^{c}	5.80 ± 0.16^{c}	19.00 ± 0.76^{b}	11.20 ± 0.29^{c}	117.00 ± 3.48^{b}	162.00 ± 5.49^{b}	76.23 ± 1.62^{c}
	0.5	76.60 ± 1.90^{c}	24.66 ± 0.74^{d}	48.30 ± 1.50^{c}	3.40 ± 0.09^{d}	12.00 ± 0.51^{c}	6.80 ± 0.21^{d}	101.00 ± 4.16^{c}	160.00 ± 4.84^{b}	63.76 ± 1.71^{d}

Abbreviations: S.E.- Standard error and means are followed by lower case letter is statistically significant at $p<0.05$

Leaf area, days to 50% flowering, days to maturity, and pollen fertility

All the studied morphological parameters were affected variably by EMS treatment and at higher EMS doses it declined sharply. The morphological data decreased with increase in mutagenic concentrations. In control, leaf area (30.4-32.4 cm^2), days to 50% flowering (101-104 days), days to maturity (152-154 days) and pollen fertility (95.68-96.89%) were observed. But maximum decrease was noted at 0.05% EMS in 5 h EMS exposure. At 0.05% EMS of 5 h exposure the pollen fertility) decreased upto 63.76%. Different types of leaf shape also observed at high EMS concentration in *Artemisia* plant (Fig. 1). EMS affected leaf shape and the changes in leaf shape and size due to various mutagens have been reported by Khursheed and Khan (2014) in *Vicia faba* bean. They attributed the alteration in size to the cellular damage by EMS which effects the metabolism of plant. The occurrence of micronuclei, along with other abnormalities in chromosomes significantly affected the pollen fertility. The decrease in pollen fertility in mutagenic treated plants can be attributed to meiotic abnormalities generated by mutagens, resulting in the production of abnormal pollen grains (Mathusamy and Jayabalan, 2002; Khan and Wani, 2005).

Fig. 1: Different shapes of leaves in *Artemisia annua* due to the effect of EMS: A. Control; B, C, and D) Whorled or bunched leaf

Microsporogenesis

Control sets exemplified perfectly normal meiotic behaviour with 9 bivalents at diakinesis and metaphase I and 9:9 separations at anaphase I (Fig. 2). TAB% in EMS treated PMCs depicted an elevation from 5.33 (at 0.1% EMS) to 18.16% (at 0.5% EMS) in 3 hr duration (Table 2). Multivalent

Table 2: A comparative assessment of chromosomal anomalies induced in *Artemisia annua* L. by EMS during microsporogenesis

EMS treat-ment (%)	PMC's observed (No.)	Metaphasic abnormalities (%)							Anaphasic abnormalities (%)				Telo-phasic abnormalities (%)	Oth (%)	T.Ab. (%)
		Sc	Pm	St	Un	Mv	Sa	Asy	Br	Lg	Mn	St	Dp		
EMS (3 h)															
0.1	372	0.36 ± 0.08	0.27 ± 0.03	0.45 ± 0.09	0.27 ± 0.03	0.36 ± 0.09	0.18 ± 0.09	0.36 ± 0.15	0.00 -	0.54 ± 0.09	0.00 -	0.18 ± 0.09	0.18 ± 0.09	0.27 ± 0.16	3.40 ± 0.05
0.3	384	0.43 ± 0.09	0.70 ± 0.18	0.52 ± 0.15	0.61 ± 0.08	1.04 ± 0.26	0.78 ± 0.15	0.69 ± 0.17	0.52 ± 0.09	0.70 ± 0.18	0.78 ± 0.08	0.43 ± 0.08	0.69 ± 0.08	0.61 ± 0.09	8.51 ± 0.05
0.5	351	0.95 ± 0.09	1.42 ± 0.16	0.76 ± 0.09	1.14 ± 0.16	1.52 ± 0.25	0.85 ± 0.16	0.67 ± 0.10	0.76 ± 0.15	1.24 ± 0.19	0.95 ± 0.09	0.95 ± 0.10	0.85 ± 0.16	1.04 ± 0.09	13.10 ± 0.14
EMS (5 h)															
0.1	350	0.57 ± 0.10	0.38 ± 0.09	0.38 ± 0.10	0.48 ± 0.10	0.76 ± 0.09	0.38 ± 0.09	0.38 ± 0.10	0.00 -	0.38 ± 0.10	0.00 -	0.47 ± 0.09	0.19 ± 0.10	0.94 ± 0.09	5.33 ± 0.06
0.3	362	0.92 ± 0.08	1.10 ± 015	0.74 ± 0.10	0.83 ± 0.15	1.38 ± 016	0.93 ± 0.19	0.92 ± 0.15	0.82 ± 0.15	1.20 ± 0.08	1.10 ± 0.07	1.11 ± 0.17	0.83 ± 0.17	1.11 ± 0.17	12.98 ± 0.07
0.5	388	1.32 ± 014	1.51 ± 020	1.14 ± 0.23	1.40 ± 0.21	2.28 ± 0.27	1.58 ± 0.27	1.15 ± 0.14	1.06 ± 0.17	1.07 ± 0.17	1.59 ± 0.17	1.23 ± 0.22	1.50 ± 0.25	1.33 ± 0.18	18.16 ± 0.05

Abbreviations: Sc - Scattering; Pm - Precocious movement of chromosomes; St - Stickiness; Un - Unorientaion; Mv - Multivalent; Sa - Secondary associations of bivalents; Asy - Asynchronous division; Br - Bridge formation; Lg - Laggard; Dp - Disturbed polarity; Oth - Others; T.Ab – Total abnormalities

formation was the most predominant abnormality observed with percentage frequency ranging from 0.76 (at 0.1% EMS) to 2.28% (at 0.5% EMS concentration). Other chromosomal abnormalities noted were precocious movement, secondary association and bridges which were more evident at 0.5% EMS. Besides, various abnormalities like disturbed polarity and micronuclei were observed with the frequency of 0.19 (0.1% EMS), 0.83 (0.3% EMS), 1.50 (0.5% EMS) and 1.10 (0.3% EMS), 1.59% (0.5% EMS) in 5 hr duration respectively. The elevation in TAB% by EMS (5 h) treated set was recorded as 3.4 (at 0.1% EMS) to 13.10% (at 0.5% EMS) (Table 2). Multivalent formation was the most prevalent abnormality observed in all the treated sets. Specific mutagens exhibit specific response of different genotype to provide significant evidence for the selection of desirable traits in cytological investigation (Avijeet *et al.*, 2011). In present study, the screened mutant showed chromosomal lesion like disturbed metaphase with two stray chromosomes, sticky metaphase I, laggard at telophase I, disturbed metaphase II non-synchronization, and disturbed polarity at anaphase

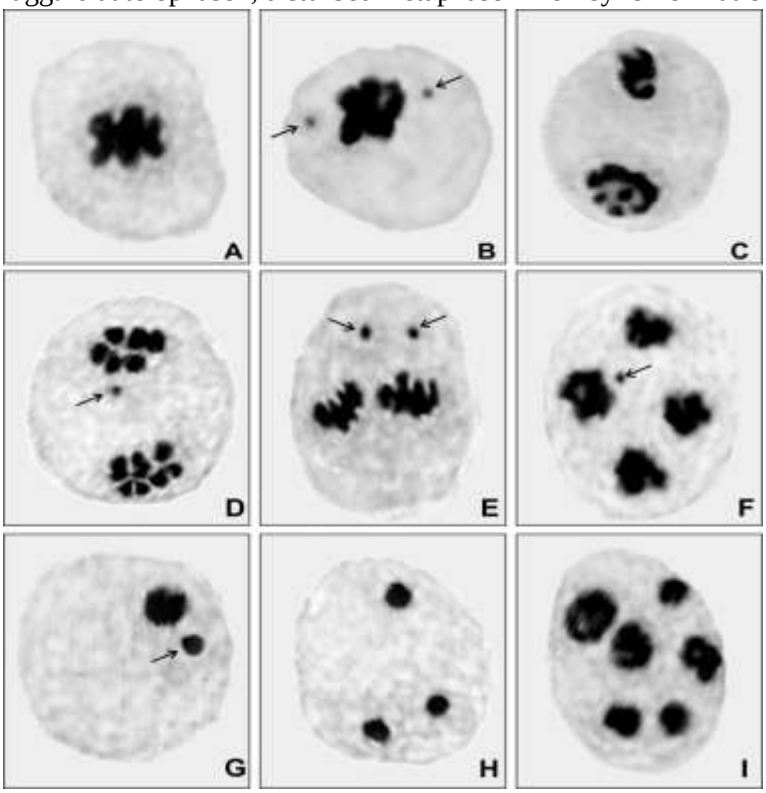

Fig. 2: Chromosomal aberrations induced by EMS A) Stickiness at metaphase I; B) Two precocious chromosome at sticky metaphase I; C) Stickiness with unorientation at anaphase I; D) One laggard chromosome at anaphase I; E) Two precocious chromosome at metaphase II; F) One laggard chromosome at anaphase II; G) Binucleate PMC with single micronuclei; H) Trinucleate condition with two micronuclei; I) Multinucleate condition

II. Sticky chromosomes at metaphase-I seem to be caused by spindle dysfunction and clumping of chromosomes. Chromosome migration to the poles causes premature movement of chromosomes during metaphase which can lead to early chaisma terminalization in diakinesis or metaphase I (Srivastava and Kapoor, 2008) or because of spindle disruption development (Dwivedi and Kumar, 2015). Formation of laggards (Fig. 1) is caused by chromosomal stickiness, delayed terminalization, or chromosome movement failure (Reddy and Munirajappa, 2012). EMS's potential to cause nuclear modification in the form of nuclear buds may be connected to its mutagenic effects (Chaudhary *et al.*, 2015). EMS is the source of chromosomal breakdown in guanine-rich regions additionally further alters DNA bases, which result in the production of micronuclei (Kumar and Singh, 2018). The existence or lack of nuclear pore complex in conjunction with the content of micronuclear DNA could be one of the critical elements influencing the transcriptional activity of tiny nuclei (Hoffelder *et al.*, 2004). It is speculated that these nuclear buds could have changed during interphase into micronuclei during cellular cycle phase (Shimizu *et al*., 1998). Micronuclei paved the way for understanding submissively the process that underlies the rebuilding of nucleus following meiosis or mitosis. The occurrence of micronuclei, along with other abnormalities in chromosomes, significantly affects the

fertility of pollen. The decrease in pollen fertility, seen in mutagenic treatment plants, can be attributed to meiotic abnormalities generated by mutagens, resulting in the production of abnormal pollen grains (Mathusamy and Jayabalan, 2002; Khan and Wani, 2005).

Conclusion: Increased EMS doses resulted in a decrease in every feature that was observed, including germination, survival, plant height, internodal length, leaf area, days to flowering, and days to maturity. Conversely, this decrease in seed germination only occurred on the first day of the greatest dose of EMS; in the days that followed, the mutants were still able to germinate, even at the maximum dose of EMS treatment (0.5%). But the pollen fertility was decreased very much at this dose. Thus, we believe that a 0.1% EMS dose treatment is high enough to yield variability as demonstrated by the other attribute, but low enough to achieve a degree of germination that is comparable to the untreated control samples in the following days. Therefore, we think that when a large number of mutants are available, our statically derived data on some features affected by EMS mutation would be helpful. And also Present study revealed that lower EMS doses may prove revolutionary mutant for creating new variation as EMS mutagenesis enables cost-effective and high-throughput generation of mutations, greatly accelerating the plant genomes research and facilitating the advancement of molecular breeding for plants with enhanced tolerance to abiotic stress.

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Authors' contribution: Conceptualization of research (GK, RS); designing of experiments (RS); execution of field/ lab experiments and data collection (GK, RS); analysis of data and interpretation (GK, RS); and preparation of manuscript (RS).

REFERENCES

Avijeet, C., Shukla, S., Rastogi, A., Mishra, B.K., Ohri, D. and Singh, S.P. 2011. Impact of mutagenesis on cytological behavior in relation to specific alkaloids in opium poppy (*Papaver somniferum* L.). *Caryologia*, **64**(1): 14-24.

Banek, K., Lalani, M., Staedke, S.G. and Chandramohan, D. 2014. Adherence to artemisinin-based combination therapy for the treatment of malaria: A systematic review of the evidence. *Malaria Journal*, **13**: 1-14.

Carter, R. and Mendis, K.N. 2002. Evolutionary and historical aspects of the burden of malaria. *Clinical Microbiology Reviews*, **15**(4): 564-594.

Çavaş, T. and Ergene-Gözükara, S. 2005. Induction of micronuclei and nuclear abnormalities in *Oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents. *Aquatic Toxicology*, **74**(3): 264-271.

Cox, F.E. 2010. History of the discovery of the malaria parasites and their vectors. *Parasites & Vectors*, **3**: 1-9.

Dhakshanamoorthy, D., Selvaraj, R. and Chidambaram, A. 2010. Physical and chemical mutagenesis in *Jatropha curcas* L. to induce variability in seed germination, growth and yield traits. *Romanian Journal of Biology Plant Biology*, **55**(2): 113-125.

Feiz, L., Beecher, B.S., Martin, J.M. and Giroux, M.J. 2009. In planta mutagenesis determines the functional regions of the wheat puroindoline proteins. *Genetics*, **183**(3): 853-860.

Gillmor, C.S. and Lukowitz, W. 2020. EMS mutagenesis of arabidopsis seeds. pp. 15-23. **In**: *Plant Embryogenesis: Methods and Protocols*. (*https://doi.org/10.1007/978-1-0716-0342-0_2*).

Gnanamurthy, S. and Dhanavel, D. 2014. Effect of EMS on induced morphological mutants and chromosomal variation in cowpea (*Vigna unguiculata* (L.) Walp). *International Letters of Natural Sciences*, (17). (*DOI: 10.18052/www.scipress.com/ILNS.22.33*).

Greene, E.A., Codomo, C.A., Taylor, N.E., Henikoff, J.G., Till, B.J., Reynolds, S.H. *et al.* 2003. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in *Arabidopsis*. *Genetics*, **164**(2): 731-740.

Gustafsson, Å. 1940. The mutation system of the chlorophyll apparatus. **In**: *Kungliga Fysiografiska Sallskapets i Lund Handlingar*, **51**(11): [*doi/full/10.5555/19431601472*].

Harsha, V.H., Hebbar, S.S., Hegde, G.R. and Shripathi, V. 2002. Ethnomedical knowledge of plants used by Kunabi tribe of Karnataka in India. *Fitoterapia*, ***73***(4): 281-287.

Hoffelder, D.R., Luo, L., Burke, N.A., Watkins, S.C., Gollin, S.M. and Saunders, W.S. 2004. Resolution of anaphase bridges in cancer cells. *Chromosoma*, **112**: 389-397.

Hong, J.H. and Lee, I.S. 2009. Effects of *Artemisia capillaris* ethyl acetate fraction on oxidative stress and antioxidant enzyme in high-fat diet induced obese mice. *Chemico-Biological Interactions*, **179**(2-3): 88-93.

Ibrahim, R., Ahmad, Z., Salleh, S., Hassan, A.A. and Ariffin, S. 2018. Mutation breeding in ornamentals. *Ornamental Crops*, **11:** 175-211.

Jankowicz-Cieslak, J., Huynh, O.A., Brozynska, M., Nakitandwe, J. and Till, B.J. 2012. Induction, rapid fixation and retention of mutations in vegetatively propagated banana. *Plant Biotechnology Journal*, **10**(9): 1056-1066.

Karthika, I.R. and Subba, B. 2006. Effect of gamma rays and EMS on two varieties of soybean. *Asian Journal of Biological Sciences*, **5**(4): 721-724.

Khursheed, S. and Khan, S. 2014. Cytology of morphological mutants of *Vicia faba* L. var. Vikrant. *Annual Research & Review in Biology*, **5**(4): 366-371.

Kotnis, M.S., Patel, P., Menon, S.N. and Sane, R.T. 2004. Renoprotective effect of *Hemidesmus indicus*, a herbal drug used in gentamicin-induced renal toxicity. *Nephrology*, **9**(3): 142-152

Kozgar, M.I., Goyal, S. and Khan, S. 2011. EMS induced mutational variability in *Vigna radiata* and *Vigna mungo*. *Research Journal of Botany*, **6**(1): 31-37.

Kumar, S.P. and Kumari, B.R. 2021. Impact of ethyl methane sulphonate mutagenesis in *Artemisia vulgaris* L. under NaCl stress. *BioTech*, **10**(3): 18. (*https://doi.org/10.3390/biotech10030018*).

Kumar, A.P., Boualem, A., Bhattacharya, A., Parikh, S., Desai, N., Zambelli, A. *et al.* 2013. SMART - Sunflower mutant population and reverse genetic tool for crop improvement. *BMC Plant Biology*, **13**: 1-8.

Kumar, G. and Dwivedi, K. 2012. Ionizing radiation mediated cytological manifestation in microsporogenesis of *Brassica campestris* L.(Brassicaceae). *Journal of Central European Agriculture*, **13**(4): 805-813.

Kumar, G. and Singh, S. 2018. Nuclear polymorphism induced genomic instability in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] (Fabaceae). *Cytologia*, **83**(2): 137-141.

Lalloo, D.G., Shingadia, D., Bell, D.J., Beeching, N.J., Whitty, C.J. and Chiodini, P.L. 2016. UK malaria treatment guidelines. *Journal of Infection*, **72***:* 635-649.

Larson, T.R., Branigan, C., Harvey, D., Penfield, T., Bowles, D. and Graham, I.A. 2013. A survey of artemisinic and dihydroartemisinic acid contents in glasshouse and global field-grown populations of the artemisinin-producing plant *Artemisia annua* L. *Industrial Crops and Products*, **45**: 1-6.

Leow, E.S., Jelodar, N.B. and Chan, L.K. 2020. Ethyl methane sulfonate (EMS) enhanced the formation of leaf glandular trichomes and the production of artemisinin in *Artemisia annua* L. *Asia Pacific Journal of Scienc & Technology*, **25**: 1-9.

Lichtenthaler, H.K. and Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans*, **11**(5): 591-592.

Muniswamy Reddy, P.M. 2012. Gamma ray induced meiotic abnormalities in S13 mulberry. *International Journal of Science and Nature*, **3**(1): 170-172.

Muthusamy, A. and Jayabalan, N. 2002. Effect of mutagens on pollen fertility of cotton (*Gossypium hirsutum* L.). *Indian Journal of Genetics*, **62**(2): 187. (*links/55ff9d6708aec948c4f9b520*).

Muthusamy, A. and Jayabalan, N. 2014. Radiation and chemical mutagen induced somaclonal variations through in vitro organogenesis of cotton (*Gossypium hirsutum* L.). *International Journal of Radiation Biology*, **90**(12): 1229-1239.

Ordóñez, M. 2014. Nueva comunicación interna en la empresa. *Madrid: Estudio de comunicación SA*. (*www.estudiodecomunicacion.com/wp-content/uploads/2018/01/140801ARF*).

Saba, N. and Mirza, B. 2002. Ethyl methane sulfonate induced genetic variability in *Lycopersicon esculentum*. *International Journal of Agriculture Biology*, **4**: 89-92.

Sabetta, W., Alba, V., Blanco, A. and Montemurro, C. 2011. sunTILL: A TILLING resource for gene function analysis in sunflower. *Plant Methods*, **7**: 1-13.

Shah, I., Shah, M., Khan, A. and Usman, A. 2015. Response of insect pollinators to different cucumber, *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae) varieties and their impact on yield. *Journal of Entomology and Zoology Studies*, **3**(5): 374-378.

Shimizu, N., Itoh, N., Utiyama, H. and Wahl, G.M. 1998. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. *The Journal of Cell Biology*, **140**(6): 1307-1320.

Singh, R. and Kumar, G. 2022. Analyzing frequency and spectrum of chlorophyll mutation induced through gamma ray and combination treatment (Gamma+ EMS) on genetic paradigm of *Artemisia annua* L.: Effect of gamma and gamma + EMS on the frequency and spectrum of chlorophyll mutation in *Artemisia annua* L. *Caryologia*, **75**(1): 15-27.

Srivastava, A. and Kapoor, K. 2008. Seed yield is not impaired by chromosome stickiness in sodium azide treated *Trigonella foenum-graecum*. *Cytologia*, **73**(2): 115-121.

Sudianto, E., Beng-Kah, S., Ting-Xiang, N., Saldain, N.E., Scott, R.C. and Burgos, N.R. 2013. Clearfield® rice: Its development, success, and key challenges on a global perspective. *Crop Protection*, **49**: 40-51.

Talebi, A.B., Talebi, A.B. and Shahrokhifar, B., 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *American Journal of Plant Science*, **3**(12): 1661-1665.

Tan, R.X., Zheng, W.F. and Tang, H.Q. 1998. Biologically active substances from the genus *Artemisia*. *Planta Medica*, **64**(4): 295-302.

Unan, R., Deligoz, I., Al-Khatib, K. and Mennan, H. 2021. Protocol for ethyl methanesulphonate (EMS) mutagenesis application in rice. *Open Research Europe*, **1**: 19. (*DOI: 10.12688/openreseurope.13317.3*).

Wani, A.A., 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combination treatments in chickpea (*Cicer arietinum* L.). *Asian Journal of Plant Sciences*, **8**(4): 318-321.

Williams, N.D., Miller, J.D. and Klindworth, D.L. 1992. Induced mutations of genetic suppressor of resistance to wheat stem rust. *Crop Science*, **32**: 612-616.

World Health Organization [WHO]. 2016. *World Malaria Report*. WHO, Geneva. Switzerland. (*https://www.who.int/publications/i/item/9789241511711*).

Wu, J.L., Wu, C., Lei, C., Baraoidan, M., Bordeos, A., Madamba, M.R.S., Ramos-Pamplona, M. *et al.* 2005. Chemical-and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics. *Plant Molecular Biology*, **59**: 85-97.