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Mutagenic Effects of Ethyl Methane Sulphonate (EMS) on Quantitative Traits in M_1 Generation and the Effectiveness and Efficiency of Different Doses in Garden Pea (*Pisum sativum* L.)

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ABSTRACT

Background: The investigation on "Mutation induction in garden pea (*Pisum sativum* L.)" was carried out to study the mutagenic effects of ethyl methane sulphonate (EMS) on quantitative traits in M_1 generation and determine the effectiveness and efficiency of different doses of EMS.

Methods: The two varieties of garden peas, *Kashi Nandini* and *Kashi Uday*, were subjected to mutagenic treatments with the EMS at 0.05, 0.10, 0.15, 0.20, 0.30 and 0.40%. The M_1 generation was raised in RBD with two replications during *Rabi* 2019-20. Plants from three concentrations, 0.10, 0.15 and 0.20% EMS, were evaluated in M_1 plant progenies (M_2) during *Rabi* 2020-21.

Result: Two varieties of garden pea exposed to EMS showed varied responses to different concentrations. The inhibitory effects were apparent in germination, survival and pollen sterility in both varieties. The mean performances in the M_1 generation showed differential responses to different concentrations of EMS. Mutagenic effectiveness decreased with increased EMS concentrations. Mutagenic efficiency based on pollen sterility decreased and based on lethality increased with increased EMS concentrations. In subsequent generations of mutagenized populations, the plants with altered phenotypes might produce mutants with higher yields.

Key words: Chlorophyll mutants, EMS, Garden pea, LD_{50}, Mutagenic effectiveness, Mutagenic efficiency, Pollen sterility.

INTRODUCTION

Pea (*Pisum sativum* L.) is a protein-rich, self-pollinated nutritious vegetable grown worldwide, having the chromosome number 2n=2×=14 and has a genome size of 5000 Mb. People use peas for fresh consumption or as a processed vegetable. Protein (23%) and digestible starch (50%) are abundant in pea seeds. The limited natural genetic diversity in pea has plateaued the production potential; thus, traditional breeding cannot boost yield potential anymore. To break the production plateau, plant breeders are always searching for distinctive genetic variations. Mutation breeding is one of the possible alternatives to conventional breeding in crop improvement (Sharma *et al*., 2010). Exposing plant genetic material to mutagens increases the chance of isolating unique genetic material. The induced mutation is one of the best methods to evolve new cultivars by producing variability at the gene level. Brock (1970) considered that induced mutations are alternative to naturally occurring variations as the source of germplasm for plant improvement and as an alternative to hybridization and recombination in plant breeding. Therefore, mutation breeding is one of the most desirable to create variability in peas. Chemical mutagens provide handy tools to augment the natural mutation rate, thereby enlarging the genetic variability and increasing the scope of obtaining desired mutants. EMS is a common and potent chemical mutagen to induce mutations and can be applied quickly and to easily monitor its effects. In plants, EMS usually causes point mutations to influence plant growth and development by inducing genetic, cytological, biochemical, physiological and morphogenetic changes in cells and tissues. Mutagenic effectiveness and efficiency of mutagens are essential to identify the valuable mutagens and doses/concentrations for effective mutation breeding programs. Therefore, selecting effective and efficient mutagen is essential to recover a high frequency of desirable mutations in any mutation breeding studies (Kharkwal, 1998). A study of induced variability for chlorophyll and viable morphological mutations in the M_2 generation was the most dependable

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tool to utilize beneficial mutations for efficient crop improvement programmes (Kumar *et al*., 2007). Hence, the traditional breeding methods have limited application in pea and other pulses mutation breeding appears to play an essential role in improving this vital crop. Therefore, keeping this in view, the present investigation took up to determine the effectiveness and efficiency of different doses of ethyl methane sulphonate (EMS) and to study the mutagenic effects of EMS on quantitative traits in M_1 generation.

MATERIALS AND METHODS

The experiment was conducted at the Field Experimentation Center, Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam, India. The seeds of the two varieties *viz*., *Kashi Nandini* and *Kashi Uday* were obtained from IIVR, Varanasi (UP) for the investigation. For the preparation of the EMS solution, an appropriate quantity of EMS was thoroughly mixed in distilled water with repeated stirring with help of a glass rod. The EMS solution was prepared just before the treatment of the seeds. The base solution of the chemical was diluted by adding an appropriate quantity of distilled water to prepare solutions for different concentrations. The following six different fresh solutions (by volume) of EMS *viz*., 0.05, 0.1, 0.15, 0.20, 0.30 and 0.40% were prepared from 1 per cent EMS solution.

Treatment of experimental material

Healthy, uniform and dry seeds of two garden pea varieties, Kashi Nandini and Kashi Uday were pre-soaked in distilled water for 6 hours. The soaked seeds were treated with different EMS concentrations of 0.05, 0.1, 0.15, 0.20, 0.30 and 0.40% for 6 hours. The treated seeds were washed in running tap water for 2 hours to eliminate the residual effect of EMS. The control sample consisted of an equal number of seeds soaked in distilled water alone under identical conditions. One hundred was considered per treatment. The treated seeds along with the controls comprising 14 treatments were sown in a randomized block design (RBD) with two replications in the M_1 generation during *Rabi* 2019-20. A uniform spacing of 30x10cm was used. Seeds were harvested from all the treatments of M_1 generation including controls of both varieties. Seeds from only three doses were selected for growing the M_2 generation in both the varieties and were sown as M_2 families. The observations were recorded on germination, survival, pollen sterility and seedling height, plant height at maturity, pods/plant, seeds/ pod, 100-seed weights and days to 50% flowering and maturity in the M_1 generation. The LD_{50} based on germination was determined in the M_1 generation. Mutagenic effectiveness and efficiency were calculated based on chlorophyll mutants in M_2 generation and biological injury (pollen sterility and survival percentage) in M_1 generation.

Pollen sterility (%)

Pollen sterility was analysed in 10 randomly selected plants belonging to each mutagenic treatment and control. Acetocarmine (1%) test was used to determine pollen fertility. Pollen grains that stained fully and had a regular outline was considered fertile, while partially stained, shrunken and empty were considered sterile. The values were expressed in percentages. The pollen sterility was calculated as follows:

$$\text{Pollen sterility (\%)} = \frac{\text{Number of sterile pollen}}{\text{Total numbre of pollens}} \times 100$$

Lethal dose 50 (LD_{50})

Through the preliminary experiments, the LD_{50} value was determined based on the germination and survival percentage of the treated seeds using probit analysis. The sprouted seeds were evaluated from the 7th day to the 15th day after sowing in all the treatments and the germination and survival percentage were worked out based on the probit analysis.

Chlorophyll mutants

The detection of various chlorophyll mutations was made as per the classification of Gustaffson (1940). The seedlings were scored for chlorophyll mutations in the field from the first day of emergence to the fourth week in the M_2 generation.

Mutagenic effectiveness and efficiency

The M_1 and M_2 generations were screened for various mutants during the entire growth period and mutation frequency was calculated by the formula given below:

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutant plants}}{\text{Total Number of plants}} \times 100$$

The mutation frequency was used to calculate the mutagenic effectiveness and efficiency by using the formula suggested by Konzak *et al*. (1965).

$$\text{Effectiveness} = \frac{M_f}{\text{Concentration} \times \text{Time}} \times 100$$

$$\text{Efficiency} = \frac{M_f}{\text{\% Biological damage}} \times 100$$

Where,
M_f = Mutation frequency (plant basis).
% Biological damage = Pollen sterility (%) or % Lethality.

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance indicated that the variation due to genotypes was significant for days to 50% flowering and maturity and pods/plant. The variation due to treatment (EMS) was significant for all the characters-germination, survival, seedling height, days to 50% flowering, plant height at maturity, pods/plant, seeds/pod and 100-seed weights. The genotypes × doses interaction was significant for the characters-seedling height, pods/plant and the seeds/ pod (Table 1). These results were similar to the previous findings of Dinkar (2020).

Mean performances in M_1 generation

In the present investigation, the per cent germination decreased with increasing the concentrations of mutagen

in both varieties. Nevertheless, it varied depending on the variety. A more significant reduction in germination compared to the control was observed at higher concentrations in both varieties. The germination percentage was the highest in controls of both the varieties (94.50%) in *Kashi Nandini* and (94.0%) in *Kashi Uday*. The lowest germination percentage was at higher concentrations in the varieties, 46.5% in *Kashi Nandini* and *Kashi Uday* at 0.40% EMS, indicating a more biological injury to seed germination with an increase in concentrations of EMS. The maximum reduction in germination over control occurred at 0.40% EMS, while the minimum reduction was at 0.01% EMS in both varieties (Table 2). A similar inhibitory effect on seed germination was evident in several earlier reports of (Ciftci *et al.* 2006) in pea. A progressive decrease in the survival till maturity was evident with an increase in concentrations of mutagen (EMS) in both varieties. The decrease in survival was more (40.5 and 41.0% in *Kashi Nandini* and *Kashi Uday*, respectively) at 0.40% EMS concentration compared with both the controls 85.5% in *Kashi Nandini* and 85.0% in *Kashi Uday* (Table 2). These findings are in close agreement with the earlier reports of Sharma *et al.* (2010) and Govardhan and Lal (2013) in field pea (*Pisum sativum* L). The rapid infusion of chemical mutagens and their ability to produce damage to genetic material could be one of the causes of reducing survival percentage.

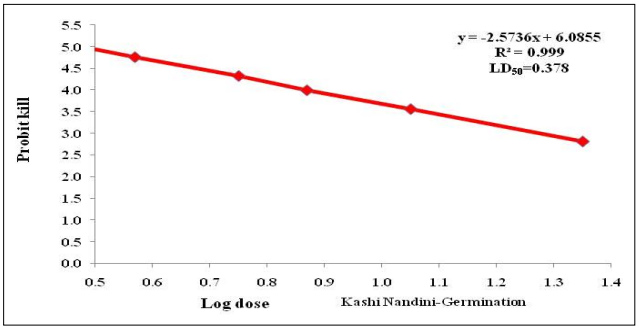

Fig 1: Probit analysis for calculation of LD_{50} for *Kashi Nandini* based on germination%.

Lethal dose (LD_{50})

The LD_{50} value varies with biological materials, nature of treatment and subsequent environmental conditions (Babaei *et al.*, 2010). The LD_{50} values were at 0.40% EMS in both the varieties based on germination percentage (0.378% in *Kashi Nandini* (Fig 1) and 0.376% in *Kashi Uday* (Fig 3).

Table 1: ANOVA for the characters in M_1 generation of the two garden pea (*Pisum sativum* L.) varieties exposed to various concentrations of EMS.

Source of variations	DF	GP	SP	SH	DFF	PHM	DM	NPP	NSP	HSW
Replicates	1	4.32	9.14	0.14*	0.32	29.71**	7.00	0.02	0.12	1.24
Genotype (G)	1	2.89	0.14	0.00	170.04**	0.03	28.00**	3.67**	0.16	0.44
EMS doses (D)	6	1395.82**	1212.89**	1.88**	438.99**	192.98**	132.98**	19.73**	13.85**	19.01**
G x D	6	1.39	0.89	0.28**	3.54	0.79	2.83	0.42**	0.31**	0.96
Error	13	2.32	5.14	0.03	5.94	2.11	2.31	0.07	0.06	0.58
Total	27	311.88	272.55	0.50	107.51	45.18	32.59	4.65	3.18	4.78
CV (%)		1.99	3.27	1.62	5.46	2.82	1.71	4.41	5.53	2.57

GP=Germination (%); SP=Survival (%); SH=Seedling height (cm); DFF=Days to 50% flowering; PHM=Plant height at maturity (cm); DM=Days to maturity; NPP=Number of pods/plant; NSP=Number of seeds/pod; HSW=100-seed weights (g).
**Significant at 5% and 1% level, respectively.

Table 2: Mean performances for germination%, survival% and seedling height (cm) in M_1 generation.

EMS concentrations (%)	Germination (%)			Survival (%)			Seedling height (cm)		
	Kashi Nandini	*Kashi Uday*	Mean	*Kashi Nandini*	*Kashi Uday*	Mean	*Kashi Nandini*	*Kashi Uday*	Mean
Control	94.5	94.0	94.3	85.5	85.0	85.3	11.1	11.50	11.30
0.05	92.0	90.5	91.3	85.0	84.0	84.5	10.4	9.91	10.16
0.10	88.5	90.0	89.3	80.0	80.0	80.0	8.9	9.76	9.35
0.15	86.5	84.5	85.5	79.0	79.0	79.0	9.3	9.59	9.46
0.20	72.0	70.5	71.3	64.0	64.0	64.0	10.3	9.56	9.90
0.30	57.0	56.5	56.8	51.5	53.5	52.5	9.7	9.48	9.61
0.40	46.5	46.5	46.5	40.5	41.0	40.8	9.5	9.40	9.44
Mean	76.7	76.1	76.4	69.4	69.5	69.4	9.9	9.90	9.90
± SE_m	7.1	7.0	7.1	6.7	6.5	6.6	0.3	0.27	0.25
CD (5%)									
Variety	1.2			1.8			0.1		
Dose			2.3			3.4			0.24
Interaction	3.3			4.9			0.34		

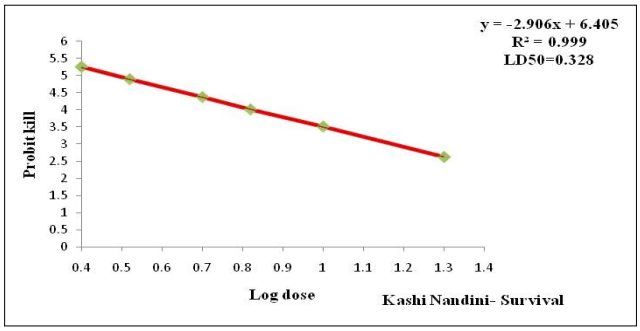

Fig 2: Probit analysis for calculation of LD_{50} *Kashi Nandini* based on survival%.

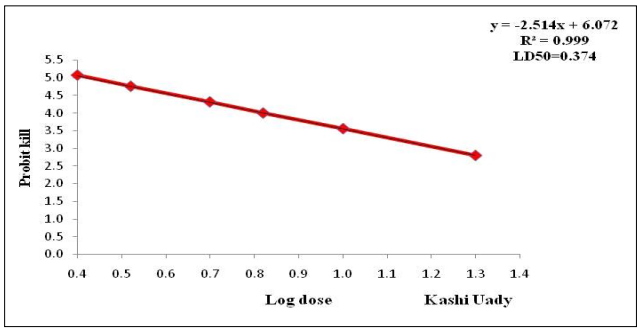

Fig 3: Probit analysis for calculation of LD_{50} *Kashi Uday* based on germination %.

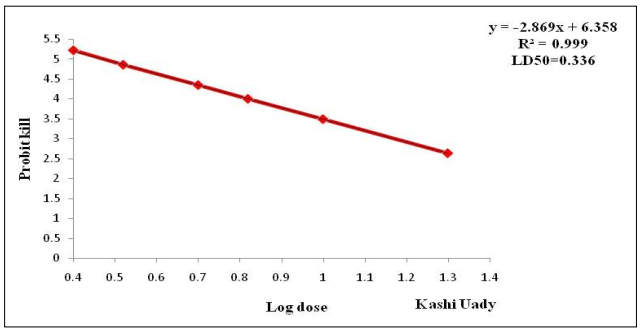

Fig 4: Probit analysis for calculation of LD_{50} *Kashi Uday* based on survival %.

The LD_{50} values based on survival percentage were 0.328% in *Kashi Nandini* (Fig 2) and 0.336% in *Kashi Uday* (Fig 4). The LD_{50} values vary from genotype to genotype, depending on the differences in their genetic constitutions, same results were also reported by Karthika and Laxmi (2006) in Soybean, Jain and Khandelwal (2008) in mungbean.

Pollen sterility

An increase in pollen sterility in correspondence with the increase in EMS concentrations was evident in both varieties. The mutagenic treatments caused a high level of sterility in the M_1 generation of both varieties compared to control. The minimum per cent pollen sterility was 1.79 in *Kashi Nandini* and 2.19 in *Kashi Uday* (Plate 1). The maximum pollen sterility was 40.49 and 38.67% in *Kashi Nandini* and *Kashi Uday*, respectively, observed at higher concentrations (0.40% EMS). Higher doses of chemical mutagen (EMS) were more effective, showing an inhibitory effect on pollen fertility (Plate 2 and 3), which is in tune with the results of Satpute and Fultambkar (2012) and Sangle *et al.* (2011) in red gram.

Mean performance for quantitative characters in M_1 generation

In the present experiment, the response of the two varieties of garden pea was variable for the seedling height at different concentrations of EMS (Table 2). Seedling height was the highest in controls. At concentrations, 0.05% and 0.20% EMS, the seedling heights were 10.4 cm and 10.3 cm, respectively, above the mean (9.9 cm) and the lowest value observed at 0.10% EMS (8.9 cm) in *Kashi Nandini*. At the same time, in *Kashi Uday*, the highest seedling height was at 0.01% EMS (9.91 cm) and the lowest at 0.40% (9.4 cm). Govardhan and Lal (2013), Umavathi and Mullainathan (2015) and Deshpande and Malone (2019), obtained similar results on seedling height. Plant height at maturity had the highest value at 0.20% EMS (58.2 cm) above the control (57.7 cm) and the lowest value at 0.40% EMS (40.3 cm) in *Kashi Nandini* (Table 3). *Kashi Uday* showed the highest plant height at 0.2% EMS (59.2 cm), also above the control (57.9 cm) and the minimum at 0.40% (39.2 cm). Bolbhat and Dhumal (2009) in horse gram reported similar results on plant height. Days to 50% flowering and maturity showed

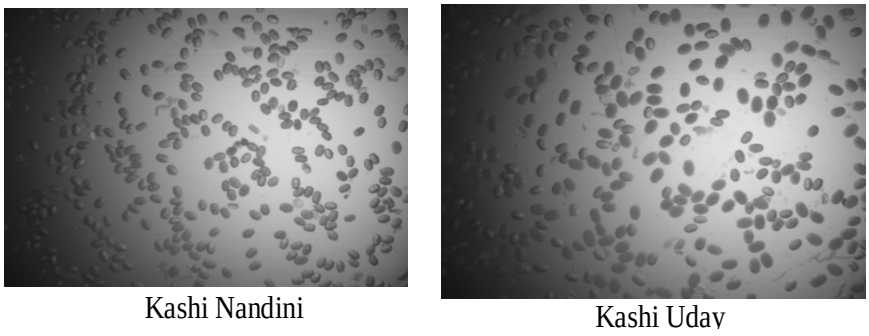

Plate 1: Results of the pollen viability test for controls of both the varieties.

invariably delay in all the mutagen treated populations of both varieties (Table 3). The maximum delay in days to 50% flowering was at higher concentrations 0.30 and 0.40% EMS in both the varieties and the minimum days to 50% flowering was in controls. Late flowering might be due to insufficient production of hormones required for flowering (Tambe 2009). Treatments with higher concentrations took the maximum days to maturity. Days to maturity was maximum at 0.40% EMS (97 days in *Kashi Nandini* and 97.5 days in *Kashi Uday*), while the minimum was in controls, 79 84 days in *Kashi Nandini* and *Kashi Uday*, respectively (Table 3). These results were similar to Dhanavel *et al*. (2008). EMS at higher concentrations showed a decrease in pods/plant and seeds/pod in both varieties, the lowest at 0.30 and 0.40% EMS. Treatment with 0.2% EMS showed more pods per plant in *Kashi Nandini* and was above the control. In *Kashi Uday*, 0.2% of EMS exhibited a greater number of pods per plant, which is a close agreement with the findings of Mullainathan and Umavathi (2018), Dhanavel *et al.* (2008).

The seeds/pod were the highest in controls (6.5 and 6.75 in *Kashi Nandini* and *Kashi Uday*, respectively). Among the treatments, the seeds/pod were the highest at 0.10%

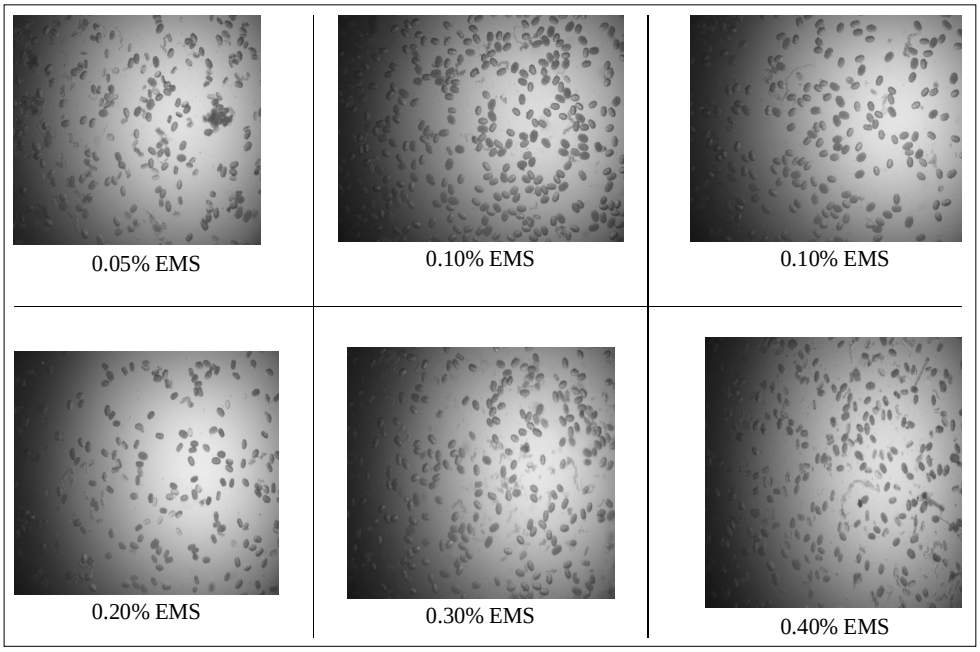

Plate 2: Per cent pollen sterility at different concentrations of EMS in *Kashi Nandini.*

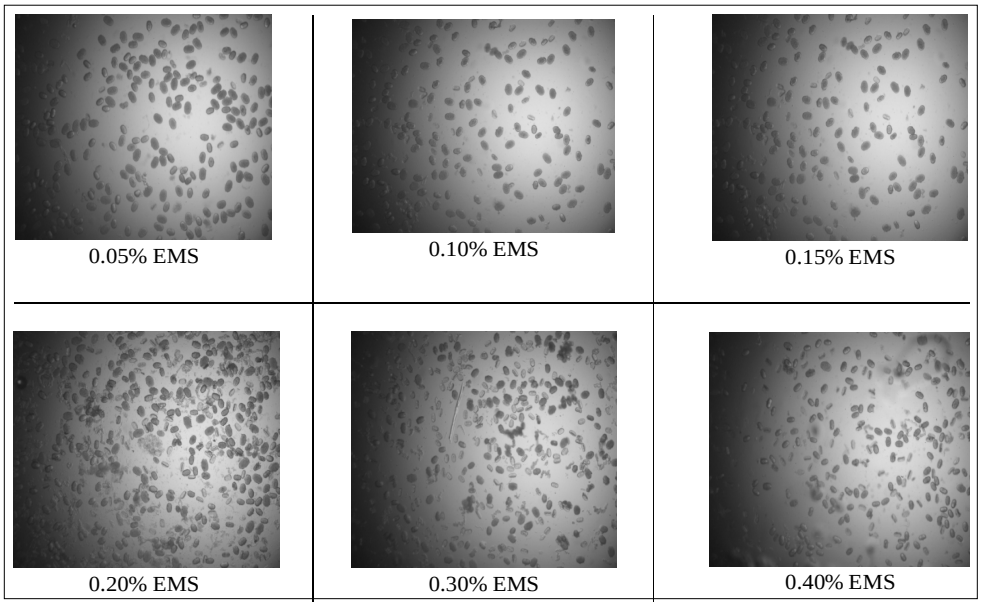

Plate 3: Per cent pollen sterility at different concentrations of EMS in *Kashi Uday.*

EMS followed by 0.05% EMS. The minimum number of seeds/pods was at higher concentrations, 0.30 and 0.40% EMS in *Kashi Nandini*. In the case of *Kashi Uday*, the seeds/pod among treatments was the highest at 0.05% EMS, followed by 0.10% and 0.15% EMS, while the minimum was at higher concentrations, 0.30 and 0.40% EMS. The controls registered the highest 100-seed weights. The 100-seed weights were the highest among the treatments with 0.20% EMS, followed by 0.05 and 0.15% EMS. The lowest 100-seed weights were for the highest concentrations 0.30 EMS and 0.40% EMS in *Kashi Nandini*. At the same time, in *Kashi Uday*, the 100-seed weights had the highest value at 0.20% EMS (31.7), followed by 0.10% EMS (30.8),0.15% EMS (30.5) and 0.01% EMS (29.6); the lowest values recorded

Table 3: Mean performances for days to 50% flowering, days to maturity and plant height at maturity (cm) in M_1 generation.

EMS	Days to 50% flowering			Plant height at maturity (cm)			Days to maturity		
concentrations	*Kashi*	*Kashi*	Mean	*Kashi*	*Kashi*	Mean	*Kashi*	*Kashi*	Mean
(%)	*Nandini*	*Uday*		*Nandini*	*Uday*		*Nandini*	*Uday*	
Control	31.0	36.5	33.8	57.7	57.9	57.8	79.0	84.0	81.50
0.05	32.5	38.0	35.3	52.5	52.3	52.4	81.0	84.5	82.75
0.10	36.5	41.0	38.8	53.4	52.8	53.1	86.5	87.0	86.75
0.15	40.0	45.0	42.5	55.0	54.7	54.8	89.0	91.0	90.00
0.20	42.5	49.0	45.8	58.2	59.2	58.7	90.5	92.0	91.25
0.30	50.0	56.5	53.3	44.0	45.4	44.7	93.5	94.5	94.00
0.40	62.5	63.5	63.0	40.3	39.2	39.8	97.0	97.5	97.25
Mean	42.1	47.1	44 .6	51.6	51.6	51.6	88.1	90.1	89.10
± SE_m	4.2	3.8	4.0	2.6	2.7	2.6	2.4	1.9	2.20
CD (5%)									
Variety	2.0			1.2			1.2		
Dose			3.7			2.21			2.32
Interaction	5.3			3.1			3.3		

Table 4: Mean performances for no. of pods/plant, no. of seeds/pod and test weight in M_1 generation.

EMS	No. of pods/plant			No. of seeds/pod			Test weight		
concentrations	*Kashi*	*Kashi*		*Kashi*	*Kashi*		*Kashi*	*Kashi*	
(%)	*Nandini*	*Uday*	Mean	*Nandini*	*Uday*	Mean	*Nandini*	*Uday*	Mean
Control	7.6	8.4	8.0	6.5	6.8	6.6	32.2	32.1	32.2
0.05%(EMS)	6.7	7.1	6.9	5.0	6.0	5.5	30.2	29.6	29.9
0.10%(EMS)	5.2	7.1	6.2	5.6	5.7	5.7	28.5	30.8	29.6
0.15%(EMS)	6.1	7.0	6.5	4.6	4.4	4.5	30.0	30.5	30.3
0.20%(EMS)	8.2	8.0	8.1	3.9	3.2	3.5	31.3	31.7	31.5
0.30%(EMS)	3.0	3.5	3.3	1.9	2.6	2.2	27.3	27.0	27.1
0.40%(EMS)	2.1	2.9	2.5	1.6	1.6	1.6	26.4	26.0	26.2
Mean	5.6	6.3	5.9	4.2	4.3	4.2	29.4	29.7	29.5
± SE_m	0.9	0.8	0.8	0.7	0.7	0.7	0.8	0.9	0.8
CD (5%)									
Variety	0.2			0.2			0.6		
Treatment			0.4			0.4			1.2
Interaction	0.6			0.5			1.6		

Table 5: Frequency of different chlorophyll mutants in M_2 generation and mutagenic effectiveness of EMS.

Treatments	EMS concentration (%)	No. of seedlings	Chlorina	Xantha	Viridis	Albino	Total	M_f	$M_e=(M_f/C)$ *Time
V1T2	0.10	320	1	1	1	2	5	1.563	2.6042
V1T3	0.15	287	1	1	1	2	5	1.742	1.9357
V1T4	0.20	280	1	2	1	2	6	2.143	1.7857
V2T2	0.10	350	2	1	1	2	6	1.714	2.8571
V2T3	0.15	295	1	2	1	2	6	2.034	2.2599
V2T4	0.20	285	1	1	2	2	6	2.105	1.7544

at 0.30% EMS (27.0) and 0.4% EMS (26.0) (Table 4). Similar results of decreasing 100-seed weights at higher concentrations support the findings of Biradar (2004).

Frequency of chlorophyll mutants

The frequency of occurrence of chlorophyll mutants increased with increasing concentrations of EMS (Table 5). Albino mutants were more frequent than the other three chlorophyll mutants, Viridis, Xantha and Chlorina (Plate 4). In both *Kashi Nandini* and *Kashi Uday*, the high concentration (0.2% EMS) induced a high frequency of chlorophyll mutants (2.143%) and (2.105%), respectively, whereas the lowest frequency of chlorophyll mutants was at 0.1%EMS, 1.563% in *Kashi Nandini* and 1.714% in *Kashi Uday*. In the two varieties, *Kashi Uday* showed the highest mutation frequency. The two varieties of garden pea differed in their response to EMS concerning the frequency of chlorophyll mutations, which was in agreement with Basvaraj (2002) in soybean and Dhulgande *et al.* (2010) pea conclusively stated that various concentrations of mutagenic treatments had independent responses towards the frequency and spectrum of chlorophyll mutations.

Table 6: Mutation frequency and mutagenic efficiency of EMS on *Kashi Nandini* and *Kashi Uday* based on per cent lethality in M_1 generation.

Treatments	EMS concentrations (%)	% Lethality (L)	M_f	$M_e=M_f/L$
V1C	0.00	8	0.000	0.000
V1T1	0.05	15	8.152	0.543
V1T2	0.10	18	10.000	0.556
V1T3	0.15	22	12.500	0.568
V1T4	0.20	25	14.710	0.588
V1T5	0.30	38	27.140	0.714
V1T6	0.40	55	39.290	0.714
V2C	0.00	10	0.000	0.000
V2T1	0.05	13	7.143	0.549
V2T2	0.10	25	13.890	0.556
V2T3	0.15	30	17.860	0.595
V2T4	0.20	32	20.510	0.641
V2T5	0.30	46	35.380	0.769
V2T6	0.40	62	51.670	0.833

Mutagenic effectiveness of EMS for chlorophyll mutations

The effectiveness of chlorophyll mutations was the maximum at 0.10% EMS (2.604) and the minimum at 0.20% EMS (1.785) in *Kashi Nandini*. On the other hand, in *Kashi Uday*, 0.10% EMS (2.857) showed the maximum mutagenic effectiveness, while the minimum was at 0.20% EMS (1.754), indicating that 0.10% EMS is the most effective in inducing chlorophyll mutants in both varieties, followed by 0.15 and 0.20% EMS. The mutagenic effectiveness of EMS decreased with an increase in the concentrations of mutagen in both the varieties (Table 5), which agreed with the findings of Sharma *et al*. (2010).

Mutagenic efficiency based on pollen sterility and per cent lethality

The mutagenic efficiency based on pollen sterility was the maximum at 0.05% EMS (1.60), while the minimum was at 0.4% EMS (0.24) in *Kashi Nandini*. In *Kashi Uday*, the efficiency of mutagen was the maximum at 0.05% EMS (0.72) and the minimum at 0.4% (0.26), indicating that in both varieties, the mutagenic efficiency differed at different concentrations of EMS. Also, mutagenic efficiency decreased at higher concentrations of EMS (Table 6), which aligned with Kousar and Babu (2010) reporting the highest mutagenic efficiency at lower doses. The mutagenic efficiency based on per cent lethality was the maximum at 0.30 and 0.40% EMS (0.714), while the minimum was at 0.05% EMS (0.543) in

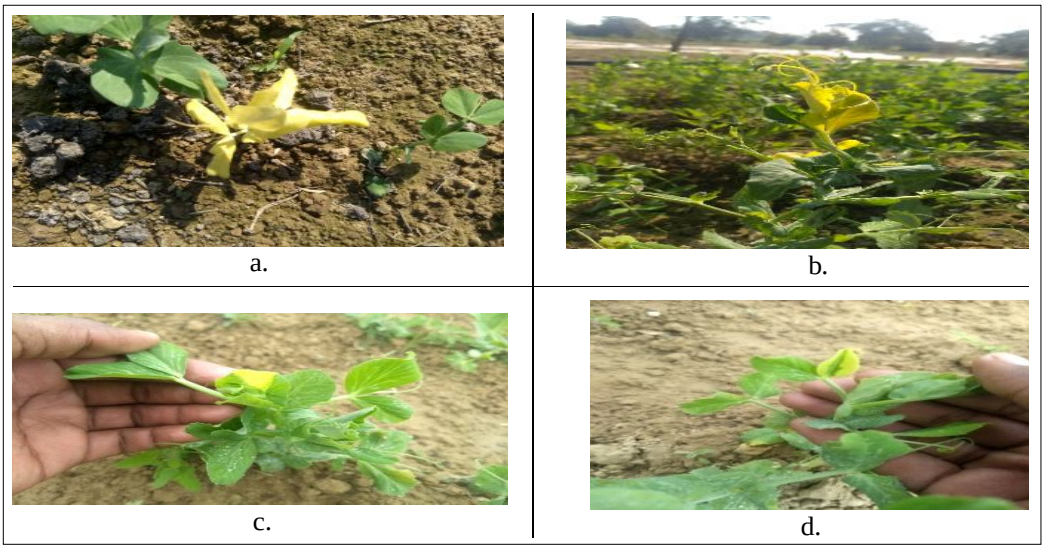

Plate 4: Chlorophyll mutants observed in M_2 generation at different concentrations of EMS in both the varieties *Kashi Nandini* and *Kashi Uday* (a. Albina b. Viridis c. Xantha d. Chlorina).

Table 7: Mutation frequency and mutagenic efficiency of EMS on *Kashi Nandini* and *Kashi Uday* based on pollen sterility in M_1 generation.

Treatments	EMS concentrations	% Sterility (S)	M_f	$M_e=M_f/S$
V1C	0.00	1.79	0.00	0.00
V1T1	0.05	3.39	5.43	1.60
V1T2	0.10	8.85	5.56	0.63
V1T3	0.15	12.56	5.68	0.45
V1T4	0.20	13.48	5.88	0.44
V1T5	0.30	27.78	7.14	0.26
V1T6	0.40	40.49	9.62	0.24
V2C	0.00	2.19	0.00	0.00
V2T1	0.05	7.60	5.49	0.72
V2T2	0.10	9.67	5.56	0.57
V2T3	0.15	11.07	5.95	0.54
V2T4	0.20	13.34	6.41	0.48
V2T5	0.30	28.34	7.69	0.27
V2T6	0.40	38.67	10.00	0.26

Kashi Nandini. In *Kashi Uday*, the highest efficiency of mutagen was at 0.4% EMS (0.833) and the minimum was at 0.05% EMS (0.549). The results from the present experiment indicated that in both the varieties, the efficiency of chemical mutagen (EMS) based on the lethality in M_1 generation showed an increase with increasing concentrations of mutagen (Table 7), which was in agreement with Sharma *et al*. (2010) and Ariraman *et al*. (2015).

CONCLUSION

The two varieties of garden pea exposed to EMS showed varied responses to different concentrations of the mutagen. The inhibitory effects were apparent in germination, survival and pollen sterility in both varieties. The mutagenic effectiveness of EMS decreased with increasing concentrations of the mutagen. The mutagenic efficiency based on pollen sterility decreased with increasing the concentration of the mutagen, while based on per cent lethality it increased. The mean performances in the M_1 generation showed differential responses to different concentrations of EMS. In the M_1 generation, higher doses of the mutagen caused a decrease in all quantitative characters. The plants with altered phenotypes might result in superior-yielding mutants in subsequent generations of mutagenized populations. Further, cross-breeding involving these variants of both varieties might evolve better recombinants with improved yield.

Conflict of interest: None.

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