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Induced morphological mutants by gamma rays and EMS in chilli (*Capsicum annum* L.)

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Abstract
A study was conducted to examine variation in morphological characteristics in M2 plants of chilli (*Capsicum annum* L) variety Kashi Anmol (KA-2) at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during Rabi 2017 (M_1 generation) and Rabi 2018 (M_2 Generation).The development of M1 generation was done by treating the seeds with five different dosses of gamma rays namely 10 KR, 20KR, 30KR, 40KR and 50KR and five concentrations of ethyl methane sulphonate (EMS), namely, 10mM, 20mM, 30mM, 40mM and 50mM were used individually as well as in five combinations of 10KR+20mM, 20wasKR+20mM, 30KR+20Mm, 40KR+20Mm, 50KR+20Mm along with control, without any treatment were used during the course of study. In the agricultural plot, the seedlings of the M1 generation were planted and the seeds obtained from them were harvested and transplanted for the analysis of morphological characteristics. The combination dose of gamma gay and EMS (10KR + 20mM) produced highest frequency (66.66) of viable mutations followed by 50KR+ 20mM (60.77). Maximum number of morphological mutants belonged to dwarf plant followed by early maturing plants, bushy plants hexamerons plants, narrow leaf, tall plants, high yielding plant. The viable mutants obtained for earliness, higher fresh fruit yield per plant, slender fruit and short plant height may be used in future breeding programmes.

Keywords: Ethyl methane sulphonate, Hexamerous plants, Fresh fruit yield, and Future breeding programmes.

Introduction
The breeding of chilli is vital to improving its characteristics and increasing its genetic diversity. Using conventional breeding and artificial crosses to breed chilli pepper can produce high quality plants. A mutation breeding method is one way to improve diversity and to produce better quality plants through selection. Apart from conventional breeding, mutation breeding can improve diversity and produce new varieties (Adamu *et al.*, 2004) [1].
Generally, gamma radiation is used as Physical and chemicals mutagens, or in combination of both i.e., physical and chemical mutagens (Mondal *et al.*, 2007; Singh *et al.*, 2021) [9, 14].
The study was conducted to evaluate mutagens that induce mutations in chillies and to observe the variations in morphological characteristic induced in M2 series and in this generation homozygote recessive or dominant alleles will be produced due to segregate mutation (Page and Grossniklaus, 2002; Pharmawati *et al.*, 2018) [11, 12] During this generation of homozygotes, there will be either homozygotes recessive or homozygotes dominant, and visual screening is the most effective way to observe phenotypic mutations (Østergaard and Yanofsky, 2004) [10]. A variation of chilli can be developed through induced mutation, and this variation will play a vital role to overcome the problem of cultivation of chilli pepper in the future.

Materials and Methods
The objective of this study was to determine the degree of variation of morphological characteristics in M2 plants of the chilli (*capsicum annum* L.) variety Kashi Anmol (KA-2) at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during rabi 2017 (M1 generation) and Rabi 2018 (M2 generation)**.** Pure, uniform, healthy and dry 500 seeds per treatment of Kashi Anmol were irradiated with five different doses of gamma rays *viz.*, 10, 20, 30, 40 and 50 KR at NBRI, Lucknow**.** The Molar solution of EMS with different concentration was prepared in phosphate buffer (pH 7.0) for 10mM, 20mM, 30mM, 40mM and 50Mm as well as for combination. EMS treatments were done with 500 seeds for each and were merged in distilled H20 for about twelve hours at room temp. and

thus dehydrated with blotting paper and ultimately emerged in ethyl methane sulphonate (EMS) at five different concentrations *viz*., 10mM, 20mM, 30mM, 40mM and 50mM in phosphate buffer (pH 7.0) for 6 hours then cleaned in running tap water, thoroughly to remove the residual effect of the chemical. The combination treatment was performed by treating 500 seeds that had already been irradiated with five different doses (10KR, 20KR, 30KR, 40KR and 50KR) of gamma rays with 20mM EMS solution, followed by washing in running tap water in the same manner as described above for EMS treatment.

Seedlings for all treatments were raised and transplanted in randomized block design in three replications, at Agriculture Research Farm, Institute of Agricultural Sciences, B.H.U. during Rabi 2017 to rise M_1 generation with spacing of row to row 60 cm and plant to plant 45cm. The standard agronomic practices for chilly were followed to raise a good crop. The individual M_1 plants from each treatment were harvested separately to rise M_2 generation.

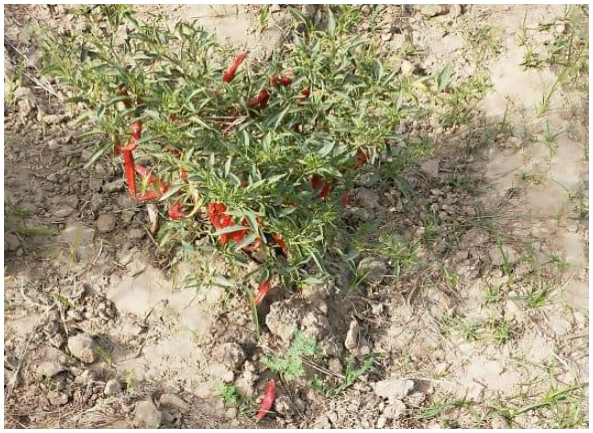
Results and Discussion

The characters of interest to the plant breeder can be amended or altered by mutation the frequencies of morphological deviants with respect to earliness, leaf inflorescence and stature are described as viable mutants.

In the present investigation a total of 13 types of morphological mutants were recognized these are early maturing, tall plants dwarf plants, bushy plants, narrow leaf, variegated leaf, increased pod size, hexamerous flower, small fruit, slender fruit, late fruiting, high yielding and sterile. The combination dose of gamma gay and EMS (10KR + 20mM) produced highest frequency (66.66) of viable mutations followed by 50KR+ 20mM (60.77). Lowest frequency of viable Mutants was produced at 30mM EMS (16.12) followed by 20mM EMS (16.66) and 40 KR gamma ray (16.48) Maximum number of morphological mutants belonged to dwarf plant followed by early maturing plants, bushy plants hexamerous plants, narrow leaf, tall plants, high yielding plant (Plates -1 and 2). Different workers have reported the isolation of a wide spectrum of morphological and viable mutants through mutation Ichiro (Honda *et al*., 2006; Devi and Mullainathan, 2011; Daudu and Falusi, 2011, Jegadeesan and Reddy, 2021) [3, 4, 7, 8].

As a result of EMS mutagenesis in *Capsicum annuum*, fascinating morphological characters have been generated that are different from control plants and that may be used in improvement program in chilli. 1% of EMS treatment in the M2 generation generated tall plants, small plants with pale green leaves, dwarf plants mutants, and plants with two stems. Plants grown with 0.75% EMS had short and many branches, but those grown with 0.5% EMS had pale green leaves (Pharmawati *et al.*, 2018) [12]. Height of the plant less than 20cm plant is categorized as a dwarf plant (Arisha *et al.*, 2015) [2]. In the M1 generation, treated seed cell genomes were damaged randomly since mutation is a random process. Different mutations are obtained in dissimilar cells of identical seed. (Greene *et al.*, 2003) [6] in the present study, the treatments with EMS caused abnormality in small plants and hence die. Thus, mutation creates negative value. Though, however, mutagenesis with EMS treatment resulted in tall plants with two main stems that are beneficial for agriculture (Khursheed *et al*., 2017) [15] Dwarf plant growth may result from inhibition of epidermal cells (Fridborg *et al.*, 1999) [5]. In addition to this, dwarf mutant occurred due to reduced levels of gibberellic acid (GA) (Fridborg *et al.*, 1999; Sikora *et al.*, 2011) [5, 13]. Treatment with EMS may lead to the damage of GA biosynthesis (Arisha *et al*., 2015) [2].

Slender Fruit

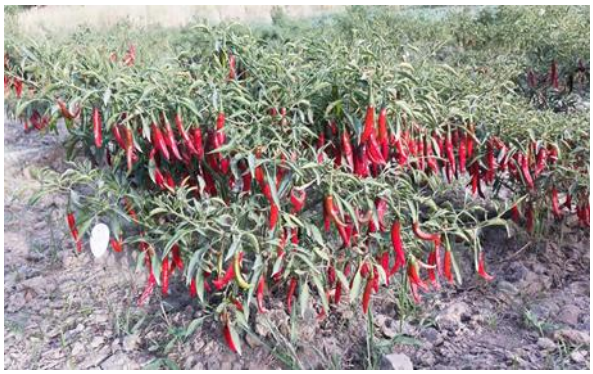


Narrow leaf Plant

Small Fruits

Tall Plant

Dawarf Plant



High yielding plant

Variegated Leaf

Sterile Plant

Bushy Plant

Hexamerous Flower

Table 1: Frequency and spectrum of induced mutation in M_2 generation in chilli Var 'Kashi Anmol' (KA-2)

Particulars	Gamma rays (kR)					EMS (M)					Gamma rays (kR) + EMS (20mM)				
	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50
Total M2 Plant studied	188	184	190	182	191	189	180	186	182	190	195	188	178	189	181
Spectrum of mutation															
Early maturing	2	2	-	-	-	2	-	1	-	1	2	2	-	-	-
Tall plants	2	-	-	-	-	1	-	1	-	1	2	-	-	-	-
Dwarf plants	-	-	-	2	-	-	1	-	2	-	3	-	-	2	3
Bushy Plants	-	-	-	1	1	-	2	1	1		1	-	1	2	2
Narrow leaf	-	-	3	-	-	-	-	-	-	-	-	-	1	2	1
Yellow Variegated leaf	1			-	-	-	-	-	-	-	-	-	-	-	-
Increased fruit size	1		1	-	-	-	-	-	-	2	-	-	-	1	1
Hexamerous flowers	-	2	-	-	2	2	-	-	1	-	-	1	1	-	2
Small fruit	-	1	-	-	-	-	-	-	-	-	2	-	-	-	-
Slender fruit	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Late fruiting	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-
High yielding	1	-	-	-	-	2	-	-	-	1	2	1	-	-	-

Sterile plants			2	-	1		-	-	-	-	-	-	1	2	2
Total No. of mutants	7	5	7	3	4	7	3	3	4	5	13	5	4	10	11
Frequency/1000M_2Plants	37.23	27.17	36.84	16.48	20.94	37.03	16.66	16.12	21.97	26.31	66.66	22.47	22.47	52.91	60.77

Conclusions

The investigation expressed a total of 13 types of morphological mutants identified as early maturing, tall plants, dwarf plants, bushy plants, narrow leaf, variegated leaf, increased pod size, hexamerous flower, small fruit, slender fruit, late fruiting, high yielding and sterile. The combination dose of gamma ray and EMS (10KR + 20mM) produced highest frequency (66.66) of viable mutations followed by 50KR+ 20mM (60.77). The lowest frequency of viable mutants was produced at 30mM EMS (16.12) followed by 20mM EMS (16.66) and 40 KR gamma ray (16.48). Maximum number of morphological mutants belonged to dwarf plant followed by early maturing plants, bushy plants, hexamerous flower, narrow leaf, tall plants, and high yielding plant which may be further used in future breeding programmes.

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