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COMPARATIVE SPECTRUM OF CELLULAR DAMAGE CAUSED BY TWO MAJOR ALKYLATING AGENTS (EMS AND MMS) IN *LYCOPERSICON ESCULENTUM* MILL.

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ABSTRACT: The present investigation was aimed to study a comparative mutagenic impact of two alkylating agents, ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS)) on seed germination, pollen fertility and meiotic cell division. Seeds of Tomato (*Lycopersicon esculentum* Mill.) were subjected to five different doses (0.02%, 0.04%, 0.06%,0.08%,0.10%) of EMS and MMS. A dose dependent reduction in seed germination and pollen fertility was seen while an increase in meiotic abnormalities with increasing doses were noticed in mutagenic population of both mutagens in M_1 generation. Various types of abnormal pollen mother cells (PMCs) such as univalents, multivalents, bridges, stickiness, stray chromosomes, laggards, unequal separation, disturbed polarity, etc were also recorded at different stages of meiotic cell division. Study revealed that the lower doses of the two mutagens causes less pollen sterility and chromosomal damage as compared to the higher doses. However, MMS is found to be more toxic and effective than EMS treatments. Therefore, the lower concentrations of the mutagens could be used by breeders for inducing desirable mutations and to improve genetic base in tomato based on the above cytological studies.
Key words: *Lycopersicon esculentum***,** Meiotic abnormalities, EMS, MMS, pollen fertility.

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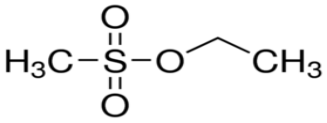
INTRODUCTION

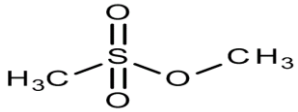
Lycopersicon esculentum Mill. (family-Solanaceae, 2n=24) commonly known as tomato, is considered as one of the most popular and extensively cultivated vegetable crops in the world [35] having various economic values as it is a richest source of nutrient dietary fibres, antioxidant and beta carotene [17]. Tomato is one of the most nutritious vegetable and consumed in different form like souce, ketchup etc. With the increasing human population food uncertainty is becoming the major constraint for the development of the nation, in various countries including India leading to the undernourishment that affects the lives of millions of people all around [12]. Genetic variation of valuable traits is mandatory for crop improvement. Mutation has been used to produce various cultivars with enhanced commercial value and study of genetics and plant developmental process [36, 6]. Induced mutagenesis has been considered as the most capable method for inducing variations in morphological and genetical traits of plants. Chemical mutagens, like an implement offer a better possibility for screening changes in the genotype to increase the variability of preferred traits and their direct or indirect utilization in various breeding programmes.

Ethyl methanesulfonate (EMS) is one of the alkylating agents which induces chemical changes of nucleotides leading to mispairing and base change [15]. It is a monofunctional ethylating agent that produces transition mutations and may cause base pair insertions or deletions as well as intragenic deletions and it was also reported to being capable of breaking chromosomes [31].



Structure of Ethyl methanesulfonate ($C_3H_8O_3S$)

Methyl methanesulfonate (MMS) is also an alkylating agent and may be also a reproductive, skin or organ toxicant. A MMS methylates DNA mainly on N7-deoxyguanosine and N3-deoxyadenosine, and may also methylates at other oxygen and nitrogen atoms in DNA bases or the phosphodiester linkage. Actually, this action was supposed to cause double-stranded DNA breaks directly and it is now believed that MMS stalls replication forks [24].



Structure of Methyl methanesulfonate ($C_2H_6O_3S$)

Study of mitotic and meiotic aberrations and their genetic outcomes form an integral part of most mutational studies as the cytological aberrations are found to be among the most dependable indices to estimate mutagenic potential affecting various genotypes [26]. Cytological studies play an important role in breeding programme involving the development of new varieties with the help of mutagenesis experiments [34]. Due to the great economic value of tomato, it is mandatory to extend its genotype and phenotype range and optimize some preferred characters and to obtain new significant genotypes by various means and chemical mutagenesis is one of these methods. Hence, the main purpose of the study is to determine the potential of mutagens in inducing genetic variability in tomato for enhancing the desired characters.

MATERIALS AND METHODS

Certified**,** healthy and dried seeds of tomato were subjected to 24 hours treatment with aqueous solution of five different concentrations (0.02%, 0.04%, 0.06%, 0.08%, 1.0%) of EMS and MMS after presoaking of 12 hours. One set of seeds were soaked in distilled water to use as control. Mutagenic solutions were prepared in phosphate buffer of PH 7. For each treatment along with control, 100 seeds were used. Treated seeds were sown in the earthen pots to raise the M_1 generation.

Pollen fertility: Pollen fertility was estimated by taking fresh pollen samples collected from mature anthers and dusting them on a drop of 2% acetocarmine solution placed on a clean slide. Pollen grains hat took stain and had regular outline were considered as fertile while sterile pollen grains were empty and remain unstained. Pollen fertility and sterility/reduction can be calculated by using the formula as follow:

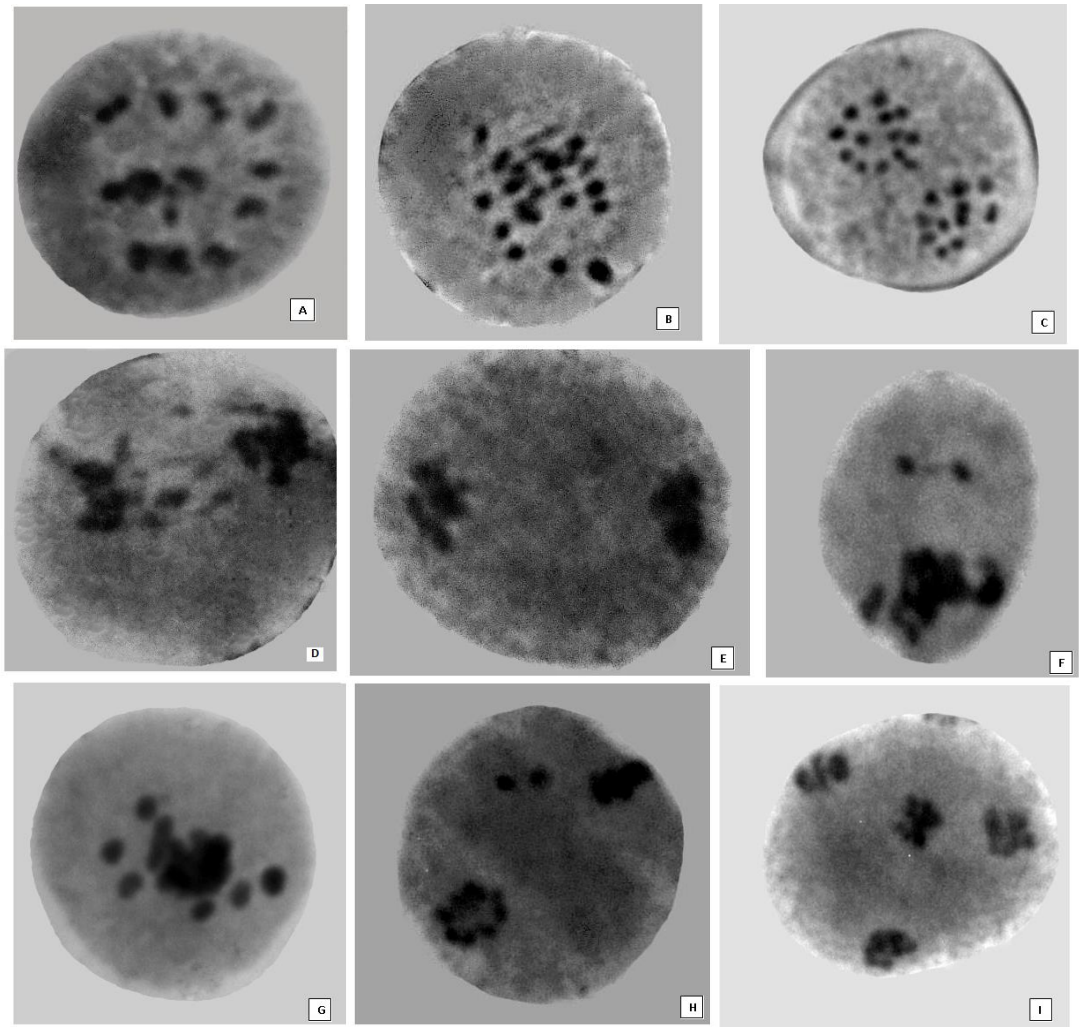
$$\text{Fertility (\%)} = \frac{\text{No. of fertile pollen}}{\text{Total no. of pollen}} \times 100$$

$$\text{Sterility/Reduction (\%)} = \frac{\text{Control-treated}}{\text{Control}} \times 100$$

Meiotic studies: Meiotic study is an effective tool for estimating the mutagenic potentials of chemical mutagens in the form of chromosomal aberrations at different stages of meiotic cell division. For this young flower buds from 20-30 randomly selected plants of M1 population were fixed in Cornoy's solution (Absolute alcohol: chloroform: acetic acid in 6:3:1) for 24 hours and washed then preserved in 70% alcohol. Anthers were smeared in 2% acetocarmine and Pollen mother cells were examined at various stages of microsporogenesis. Micrographs were taken from temporary preparations using X30 Olympus research photomicroscope.

RESULTS

Seed germination was quite normal and recorded 94.00% in control plants. Germination was found to decrease with increasing dose of both the mutagens (from 92.00% to 82.00% in 0.02% to 0.10% EMS and from 90.00% to 80.00% in 0.02% to 0.10% MMS). Higher doses of both the mutagens were shown to inhibit germination, however, MMS caused more inhibition to that of EMS. Pollen fertility and meiotic activities in M_1 generation are generally used to assess the mutagenic sensitivity of plants. Pollen fertility was found to decreased from 95.20% to 87.60% in EMS and 94.40% to 86.00% in MMS. Maximum reduction in pollen fertility was recorded in higher dose of MMS followed by EMS. Hence**,** both seed germination and pollen fertility were negatively correlated with mutagenic dose in both EMS and MMS (Table 1,2; Graph 1,2).



Figures : Showing Chromosomal abnormalities caused by different doses of EMS and MMS in *Lycopersicon esculentum* Mill. (M_1 Generation.)

Fig. A. PMC showing diakinesis (12 rod bivalents) (Control)
Fig. B. PMC showing 24 univalents at metaphase I (0.04 EMS)
Fig. C. PMC showing unequal separation at anaphase I (0.10 MMS)
Fig. D. PMC showing multiple bridge with 2 laggards at anaphase I (0.04MMS)
Fig. E. PMC showing stickiness at anaphase I (0.10 EMS)
Fig. F. PMC showing polar multivalents with 2 stray chromosomes at metaphase I (0.08MMS)
Fig. G. PMC showing stickiness with stray chromosomes at metaphase I (0.06 EMS)
Fig. H. PMC showing unsynchronization with 2 stray chromosomes at metaphase II (0.10 MMS) Fig. I. PMC showing disturbed polarity at telophase II (0.08 EMS)

Meiosis was completely regular in control plants with 12 bivalents (2n=24) at diakinesis and metaphase I stage and chromosomes were seen separating usually at anaphase and telophase stages without any abnormality. Various types of chromosomal aberrations like multivalent, precocious movement of chromosomes, stray chromosomes, stickiness, laggards, bridges, disturbed polarity etc. were noticed at different stages of meiotic cell division in mutagenic population. Meiotic abnormalities were found to be dose-dependent i.e. positively correlated with mutagen dose and recorded to increase with increasing dose (table III, graph 3). However, the overall maximum aberrations were recorded at highest dose (22.38% in EMS and 26.44% in MMS) of both the mutagens (Table-3) and MMS was found to be more toxic and effective than EMS.

Table 1: Mutagenic effects of EMS and MMS on Germination (%) in *Lycopersicon esculentum* Mill. (M_1 Generation)

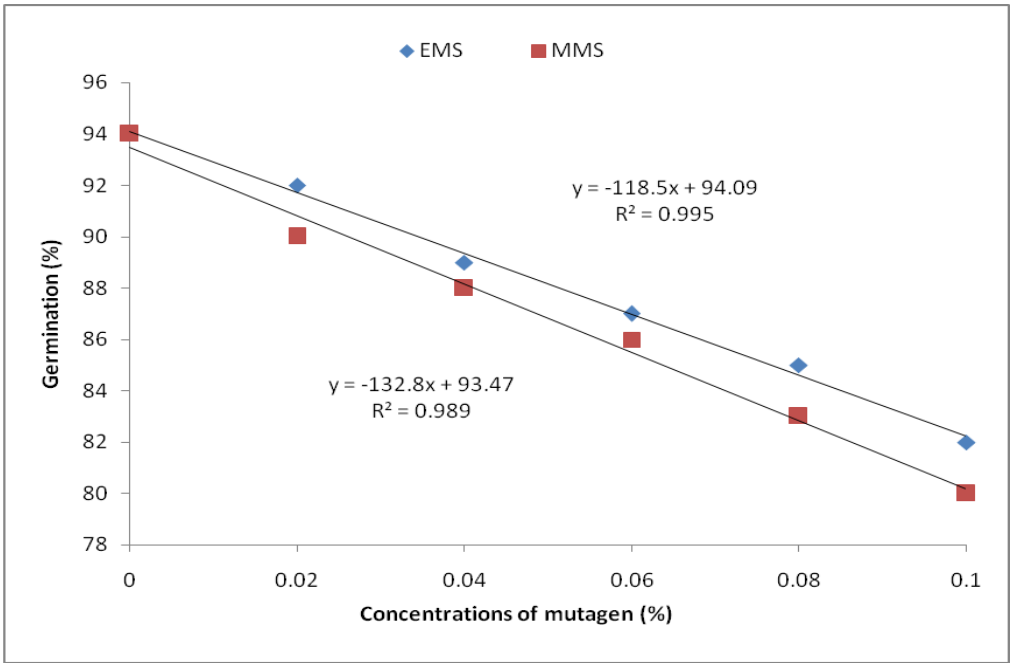
Mutagens	Concentration (%)	Germination (%)	Inhibition (%)
Control	0.00	94.00	06.00
EMS	0.02	92.00	08.00
	0.04	89.00	11.00
	0.06	87.00	13.00
	0.08	85.00	15.00
	0.10	82.00	18.00
MMS	0.02	90.00	10.00
	0.04	88.00	12.00
	0.06	86. 00	14.00
	0.08	83.00	17.00
	0.10	80.00	20.00

Table-2: Mutagenic effects of EMS and MMS on Pollen fertility (%) in *Lycopersicon esculentum* Mill. (M_1 Generation)

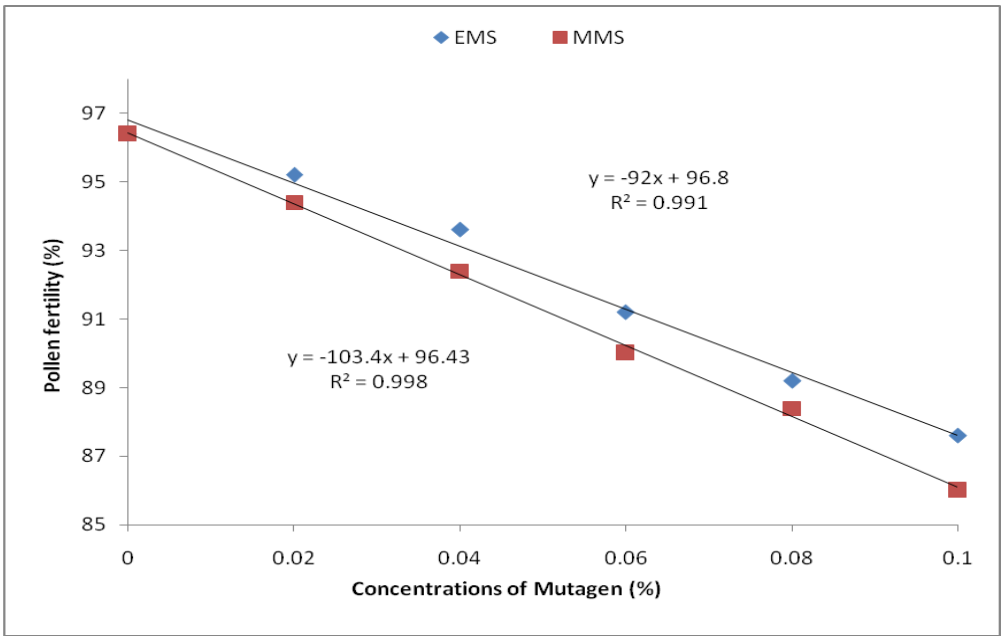
Mutagens	Concentration (%)	Pollen fertility	Reduction (%)
Control	0.00	96.40	00.00
EMS	0.02	95.20	01.24
	0.04	93.60	02.90
	0.06	91.20	05.39
	0.08	89.20	07.46
	0.10	87.60	09.12
MMS	0.02	94.40	02.07
	0.04	92.40	04.14
	0.06	90.00	06.63
	0.08	88.40	08.29
	0.10	86.00	10.78

Table-3: Frequency of Chromosomal Abnormalities (%) induced by EMS and MMS in *Lycopersicon esculentum* Mill. (M_1 Generation)

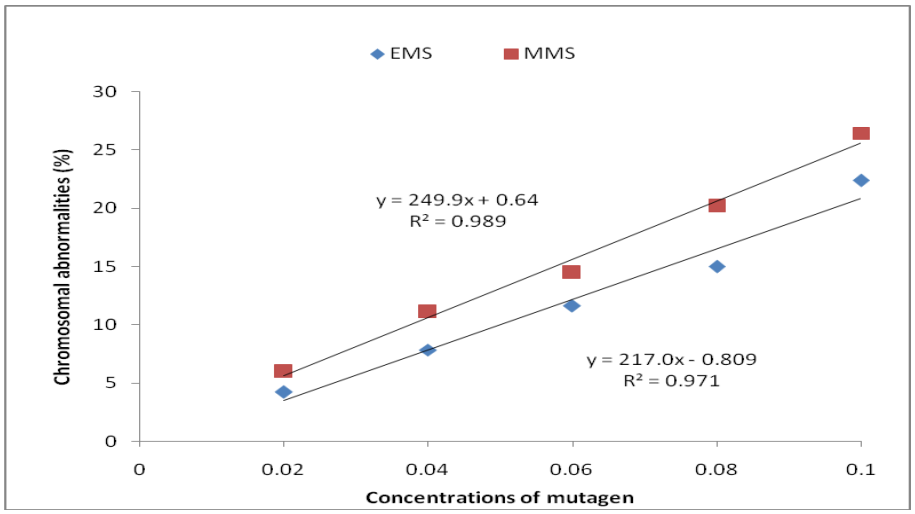
Conc. of Mutagens (%)	Total No. of PMCs Obser.	Metaphase-I/II						Anaphase-I/II				Telophase-I/II					Total % ofAbn. PMCs
		Univalents	Multivalents	Precocious Mov of chromosomes	Stray chromosome	Stickiness	% of Abn. PMCs	Laggards	Bridges	Unequal Sep. of chromosomes	% of Abn. PMCs	Bridges	Micro nucleate PMC	Disturbed polarity	% of Abn. PMCs	Total No. of Abn. PMCs	
Control	220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EMS																	
0.02	236	-	0.42	0.84	0.42	0.84	**2.54**	0.42	-	0.42	**0.84**	0.42	-	0.42	**0.84**	10	**4.23**
0.04	229	0.43	0.43	0.87	0.87	1.31	**3.93**	0.43	0.43	0.87	**1.74**	0.87	0.43	0.87	**2.18**	18	**7.86**
0.06	232	0.86	0.86	1.29	0.86	1.72	**5.60**	0.86	0.86	1.29	**3.01**	0.86	0.86	1.29	**3.01**	27	**11.63**
0.08	227	1.32	0.88	1.32	1.76	2.20	**7.48**	1.32	0.88	1.76	**3.96**	1.32	0.88	1.76	**3.96**	34	**14.97**
0.10	210	1.90	1.42	1.90	2.85	2.85	**10.95**	1.90	0.95	2.85	**5.71**	1.42	0.95	2.38	**4.76**	47	**22.38**
MMS																	
0.02	234	0.42	0.42	0.85	0.42	0.85	**2.99**	0.42	0.42	0.85	**1.70**	0.42	-	0.85	**1.28**	14	**5.98**
0.04	225	0.44	0.44	1.33	0.88	1.77	**4.88**	0.88	0.88	1.33	**2.66**	0.88	0.44	1.77	**3.11**	25	**11.11**
0.06	228	0.87	0.87	1.31	1.75	1.75	**6.57**	0.87	1.31	1.31	**3.50**	0.87	1.31	2.19	**4.38**	33	**14.47**
0.08	223	1.34	1.79	1.34	2.24	2.69	**9.41**	1.34	1.34	1.79	**4.48**	1.34	1.79	3.13	**6.27**	45	**20.17**
0.10	208	1.44	1.92	1.92	2.88	3.36	**11.53**	2.40	2.88	2.88	**8.17**	1.92	1.44	3.84	**7.21**	55	**26.44**



Graph 1: Frequency of Seed germination caused by EMS and MMS treatments in *Lycopersicon esculentum* Mill. (M_1 generation)



Graph 2: Frequency of Pollen fertility caused by EMS and MMS treatments in *Lycopersicon esculentum* Mill. (M_1 generation)



Graph 3: Frequency of Chromosomal abnormalities caused by EMS and MMS treatments in *Lycopersicon esculentum* Mill. (M_1 generation)

DISCUSSION

Seed germination in treated population was found to decrease with increasing concentrations of both the mutagens. Similar results were also observed Aliyu et al. [3], Saba and Mirza [29] Gandhi et al. [14]. Pollen fertility was found to decrease and sterility increased according to the concentrations of the both the mutagens, similar findings were also reported by Bhat et al. [4] and Chowdhary et al. [8]. Deviation in karyokinesis or cytokinesis can cause production of nonviable microspores [28]. The divergent response in the extent of induced meiotic chromosomal aberrations may be accredited to the differences in the genotypic constitution of the plant and the mechanism of action of these mutagens. Cytological studies have shown the enhanced, meiotic abnormalities with increasing doses of applied mutagens (EMS and MMS), confirming the observations of earlier investigators like Ahmad [2], Dhamyanti and Reddy [9] Bhat et al. [5] Khan et al. [20].

The formation of univalents and multivalents at metaphase I has been reported in different plants such as barley [21] and broad beans [4]. Occurrence of univalents may be due to the failure of pairing among homologous chromosomes indicating non-homology between chromosomes. Multivalents may be recognized as pairing due to translocation and inversion [10, 23]. Presence of multivalents with increasing doses of various mutagens (EMS, MES, and MMS) in *Lens culinaris* revealed that terminal affinities of chromosomes cause translocations [7]. Precocious movement of chromosomes may have resulted due to disturbed homology for chromosomal pairing or disturbed spindle tackle [1,20] or possibly due to breaking of nucleoprotein backbone by the effect of chemical treatment [27].

It was reported by Bhat et al. [5] stray bivalents are formed due to spindle dysfunctioning of chromosome clumping. Stickiness of chromosomes arise due to the inappropriate folding of chromosome fibres and partial dissociation and altered pattern of organization of nucleoproteins

[11,25] or caused by polymerization of nucleic acid due to effect of mutagenic treatment. Laggards may also be attributed to the failure of the multivalents to separate accurately [13]. Chromosome breakage and reunion of its broken ends may results in formation of bridges [18]. As reported by Saylor and Smith [30], formation of bridges occur when chiasmata in bivalents fails to terminalize due to which chromosomes get stretched between the poles. According to Sinha and Godward [33], paracentric-inversion may cause the formation of chromatin bridges at anaphase and telophase stages.

Mutation in the gene controlling normal spindle formation and proper separation of chromosome, may results in the unusual spindle formation and spindle dysfunction leading to the improper and uneven separation of chromosomes to opposite poles [19]. Occurrence of micronuclei may be associated with the fragments and lagging chromosomes that could not reach the pole get involved with the daughter nuclei as recognized by Kumar and Dubey [22]. Disturbed polarity at telophase may caused due to disturbed spindle formation, also investigated by several workers as Gulfishan et al. [16] in *Vicia faba* and Sharma and Kumar [32] in Chickpea.

CONCLUSION

Cytological analysis has been considered as one of the reliable approach to evaluate the potential of a mutagen by assessing the frequency of the cellular damage it causes in plants and provides, the better prospects of selecting the appropriate mutagenic dose and by using which the positive mutations with desired characters could be induced in order to improve the plant. The present investigation revealed that lower doses of the two mutagens caused less cellular damage hence, can be optimize for inducing desired agronomic traits in tomato.

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