



## Studies on effect of induced mutagenesis on *Lycopersicon esculentum* mill. Var. PKM- 1 in M<sub>1</sub> generation

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### Abstract

Tomato (*Lycopersicon esculentum* Mill.) is one of the most commercial and nutritional important vegetable crops belongs to the night shade family Solanaceae, and it originates in South America but first domesticated in Mexico and now widely cultivated in tropical and sub-tropical regions. Tomato is a protective food because it provides large amount of nutrients like  $\beta$ -carotene, lycopene, vitamin C and flavonoids. In addition to that tomato very familiar, because the presence of lycopene's and it have good anti-oxidative activities and anti-cancer functions. In this present investigation, to assess the efficiency and effectiveness of chemical mutagens; Ethyl Methane Sulphonate (EMS), Diethyl Sulphate (DES) and Sodium Azide (SA) using different concentration. The parameters in M<sub>1</sub> generation like 15th day Plant seedlings, 30th-day plant height, Number of Branches per plant, Days to first flowering, No. of Leaves per plant, No. of flowers per plant, No. of branches per plant, No. of Cluster per plant and Number of fruits per plant were observed that, all the parameters except days to first flowering show a concentrations dependent decreased in treatments. From this observation, all the parameters (except days to first flowering) gradually decreased in all the concentration of EMS, DES and SA from 10 MM to 50 MM.

**Keywords:** *Lycopersicon esculentum*, EMS, DES, sodium azide and M<sub>1</sub> generation

### Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most commercial and nutritional important vegetable crops belongs to the night shade family Solanaceae, and it originates in South America (Jayabalan and Rao, 1987; Melo *et al.*, 2015) [1, 2] but first domesticated in Mexico and now widely cultivated in tropical and sub-tropical regions (Paran and Fallik, 2011; Sharma *et al.*, 2019) [3, 13].

Tomato (*Solanum lycopersicum* L.) is the greater conspicuously grown vegetable crop and consider as a conservative food in the world. Being its tremendous nutritive value and all over the place production in several agro-climatic conditions, it has an important role in the economy of human societies (Meena *et al.*, 2014). Commercially and dietaries it is a very important crop. Its area is developing day by day because of its short-span growing period and high-yielding vegetables (Pedapati *et al.*, 2013) [6]. Tomato has a very prestigious position from the plate of poor to the kitchen of rich men due to its value added products and diversified nutritious values. It is label as poor man's orange due to its abundance in vitamin 'C' and nutritive measure (Giovannoni, 2001) [7].

The manufacturing of tomatoes is extremely determined by environmental aspects such as temperature, relative humidity, light, and CO<sub>2</sub> level in the atmosphere. Being a warm- season crop it can be grown in a dimension area of soil and temperature but the most sufficient range of temperature for its record yield is 20 to 24°C. The median temperature below 16°C and above 27°C is not desirable for its planting. Tomato is a rich authority of certain fundamental and beneficial aspects in the human diet such as antioxidants, vitamins, and minerals. The crop

determination is exclusive among vegetables due to its high nutritive values and numerous uses and is treated as 'protective food' for some antioxidant properties due to the presence of lycopene and flavonoids (Sepat *et al.*, 2013) [8]. Plant genetic assets facilitate plant breeders to create innovatively genetic recombination and selected crop cultivars higher appropriate to the diversified systems of agriculture (Glaszmann *et al.*, 2010) [9]. Furthermore, the tomato has a approximately short lifespan, a small scale genome, and elementary diploid genetics, and is gladly and strongly mutate by genetic engineering technology, which produce the species an excellent plant model for both basic and applied research (Ranjan *et al.* 2012; Gerszberg *et al.* 2015) [10, 11].

Tomato has the richest source of vitamin A, B1 and C; and also the minerals like Fe, K, S, and P as well as flavonoids, carbohydrates and phenolic compounds (Hobson and Grierson, 1993; Campbell *et al.*, 2004) [23]. In addition to that tomato very familiar, because the presence of lycopene's and it have good anti-oxidative activities and anti-cancer functions (Fentik *et al.*, 2017).

Mutation was first introduced by Hugo De Vries in 1901. Mutation is the sudden heritable change in the organism. Mutation breeding is a traditional breeding method for crop improvement. The mutation causes lots of beneficial variation to the plants. Mutation breeding is one of the good tools for plant breeders in now a day. In the past nine decades through mutation breeding 2252 mutant varieties are officially released in the world (Maluszynski *et al.*, 2000) [12]. Globally, 3346 mutant plant varieties were officially released through mutation breeding (FAO/IAEA Mutant Varieties Database, 2021). Mutation is adequately known as

a variation. On the basis of the causes, mutations are of two types namely spontaneous and induced mutation. Spontaneous mutations are naturally occurred in the organism but the induced mutations are synthetically caused by the factors. The agents of the induced mutation are called induced mutation. Plants created using mutagenesis are sometimes called mutagenic plants or mutagenic seeds.

Mutation breeding is one of the advanced breeding methods in plant production. Mutation breeding is an essential tool for the development of new varieties by using physical and chemical agents of crop improvement for cultivars (Sharma *et al.*, 2019) <sup>[13]</sup>. In addition to that mutation, breeding is one of the encouraging options to increase genetic diversity and achieve speedily crop improvement (Chaudhary *et al.*, 2019) <sup>[14]</sup>.

Chemical mutagenesis is one of the highest powerful and comfortable approaches used in distinct plant species. In tomatoes, EMS, DES and sodium azide have been used for chemical mutagenesis. The Ethyl Methane Sulphonate (EMS) is the maximum cosmically used chemical mutagens in plants. Because of its extensive capability of inducing point mutations and deletions in the chromosomal segments (Kostov *et al.*, 2007) <sup>[15]</sup>. The chemical mutagen Ethyl methane sulfonate (EMS) is one of the most powerful chemical mutagens and it main causes a point mutation. The chemical mutagenesis is frequently produced in induced mutations and resulting in the amino acid changes (GC – AT). These chemical mutagens also can induce a lot of mutation and alternation in a level of genetical and morphological. (Bhat *et al.*, 2007) <sup>[16]</sup>.

## Materials and Methods

The healthy seeds of *Lycopersicon esculentum* Mill. (PKM-1 Variety) were collected from Tamil Nadu Agricultural Research University (TNAU) Coimbatore.

### Chemical mutagen treatment

Mutagens employed chemical mutagens namely Ethyl Methane Sulphonate (EMS), Diethyl Sulphate (DES) and Sodium Azide (SA) were used at various concentrations to induced mutagenesis.

For EMS, DES and SA treatment hundred healthy seeds were pre-soaked in distilled water for 6h and were treated with different concentrations (10, 20,30,40,50 MM) freshly prepared aqueous solution of Ethyl Methane Sulphonate for 6 h, Diethyl Sulphate 6h and of another set 100 seeds were soaked in Sodium Azide solution in different concentrations of 10, 20, 30, 40 and 50 MM and the same set of 100 seeds were soaked in distilled water and used as control. After treatment, seeds were thoroughly washed in running water for 3 to 4 times to leach out the residual of chemicals.

### Raising M1 generation

To raise M1 generation the seeds treated with Different Concentrations of EMS, DES and SA were sowing along with control at the Botanical Garden, of Annamalai University in a Complete Randomized Block Design (CRBD). The fruits were harvested detached from healthy individuals of M1 plants; the spacing was maintained at 20cm (Plant to plant in a row) and 60cm (between rows) in the field. Plant protection methods are regulated during the growth period of plant like weeding and irrigation.

The mutagenic treated seeds were grown in the field with control seed, while the following Characters were observed

and recorded in M1 generations Viz., 15th day Plant seedlings, 30th-day plant height, Number of Branches per plant, Days to first flowering, No. of Leaves per plant, No. of flowers per plant, No. of branches per plant, No. of Cluster per plant and Number of fruits per plant. Data of yield and yield attributes were collected and all the data were statistically analysed for each character separately. The mean data of each genotype for different characters were used for statistical analysis.

## Results and Discussion

The effect of chemical mutagenesis on Tomato (*Lycopersicon esculentum* Mill.) PKM-1 Variety quantitative studies with following treatments Viz., (10 MM, 20 MM, 30 MM 40 MM and 50 MM) and morphological parameters 15th day plant seedlings, 30th day plant height, Number of branches, Days to first flowering, Number of flowers per plant, Number of cluster per plant and Number of fruits per plant. When compared to the control all the morphological parameters are significantly reduced.

The performance of mean and standard error was respectively presented in Table 1 to 7.

### 15th day seedlings: cm/ per plant

The 15th day seedlings were measured at the time of seedlings from the bottom to the top of the plant and expressed in centimeters.

In M1 generation, chemical mutagens concentrations into 15th day seedlings were decreased ( $6.09 \pm 0.17$ ) in 50 MM of EMS, ( $5.6 \pm 0.15$ ) 50 MM of DES, ( $4.33 \pm 0.23$ ) and 50MM of SA ( $4.33 \pm 0.23$ ). When compared to other concentration of mutagen and control ( $11.5 \pm 0.25$ ) in Table 1.

### 30th day plant height: cm/ per plant

Among the different concentrations of mutagens, plant height showed a decreasing trend of mean values that control in M1 generation. The highest reduction was recorded at 50 MM of EMS ( $9.13 \pm 0.29$ ), 50 MM of DES ( $9.61 \pm 0.47$ ), 50 MM of SA ( $8.48 \pm 0.46$ ) and Other concentration of mutagens and control plant ( $18.29 \pm 0.47$ ) Table 2.

### Number of branches per plant

The number of branches per plant that arise from the main axis is calculated at the time of harvesting and standard error performance respectively presented in table 3.

The minimum number of branches per plant was observed ( $14.1 \pm 0.41$ ) in 50 MM of EMS, ( $18.1 \pm 0.38$ ) in 50 MM of DES, ( $12.8 \pm 0.38$ ) in 50 MM of SA and the highest number of branches per plant was observed in control ( $28.5 \pm 0.64$ ) when compared to all the concentration of mutagens is Table 3.

### Days to first flowering

A Gradual increase of days to first flowering was observed in all the concentration of mutagens than the control. Among them, 50 MM of (56.5 days) SA was taken more days for flowering, whereas in 50 MM of EMS (53.6 days), 50 MM of DES (51.4 days) and the control it was 44.6 days in Table 4.

### Number of flowers per plant

The effect of chemical mutagen treated plants number of flowers per plant was reduced compared to the control.

Minimum number of flowers were noted ( $13 \pm 0.45$ ) in 50 MM of EMS, ( $9.6 \pm 0.34$ ) 50 MM of DES, ( $8 \pm 0.54$ ) 50 MM of SA and highest number of flowers was reported in control ( $23 \pm 0.61$ ) in Table 5.

#### Number of cluster per plant

The mean performance of a number of clusters per plant was presented in Table 6. The highest concentration of chemical mutagens was decreased in cluster per plant ( $5.7 \pm 0.24$ ) 50 MM of EMS, ( $4.1 \pm 0.34$ ) 50 MM of DES, ( $3 \pm 0.42$ ) 50 MM of SA and cluster is observed in control ( $7.7 \pm 0.21$ ).

#### Number of fruits per plant

The number of fruits per plant showed a gradual declination among the different concentrations of mutagens when compared to the control. The highest reduction of fruits per plant recorded at 50 MM of EMS ( $6.6 \pm 0.30$ ), 50 MM of DES ( $4.5 \pm 0.26$ ), 50 MM of SA ( $3.4 \pm 0.31$ ) and highest number of fruits per plant was observed in control ( $11.3 \pm 0.39$ ) in Table 7.

In the present investigation the data on seven quantitative traits namely 15th day plant seedlings, 30th day plant height, Number of branches, Days to first flowering, Number of flowers per plant, Number of cluster per plant, Number of fruits per plant and yield /plant were analysed to assess the expansion of induced variability in M1 generation

of Tomato. The exclude variation in M1 generation of the treated population in all the characters in both negative/positive directions. In the M1 generation, all the quantitative characters were decreased with increasing concentration of chemical mutagen EMS, DES and SA except days to first flowering. From this observation, all the parameters (except days to first flowering) gradually decreased in each concentration of EMS, DES and SA in 50 MM.

The same results were found in *Setaria italica* (Anitha and Mullainathan, 2018) [17], *Capsicum annuum* (Aruldoss and Mullainathan, 2015) [18] and *Pennisetum typhoides* (Ambli and Mullainathan, 2014) [19].

The plant height decreased significantly in all the three treatments with an increase in concentration. The reasonable answer for the declining rate in the seven traits may be due to the damage imposed by mutagenic induction, a similar report was observed in marigold (Aravind and Dhanavel, 2021) [20].

The further decrease in the number of branches per plant in all the three treatments. The relative observation was observed in EMS under heat stress conditions revealed much lower branches in tomato (Akhtar, 2014) [21].

The effect of sodium azide on tomato revealed that a number of fruits per plant showed declined trend which can be concentration-dependent (Aliyu and Adamu, 2007) [22].

**Table 1:** Effects of EMS, DES and SA on 15th day plant seedlings (cm/plant) of *L. esculentum* mill. (Var. PKM 1) in M1 generation

Mutagens	Treatments Conc.	15th Day plant seedlings (cm)		
		Range	Mean $\pm$ S.E	% of Reduction over control
	Control	10.5-12.6	$11.5 \pm 0.25$	-
EMS	10 mm	9.5-11.2	$10.31 \pm 0.17$	-10.34
	20 mm	8.4-10.8	$9.52 \pm 0.25$	-17.21
	30 mm	7.5-9.6	$8.62 \pm 0.24$	-25.04
	40 mm	6.5-8.4	$7.39 \pm 0.22$	-35.74
	50 mm	5.4-7.2	$6.09 \pm 0.17$	-47.04
DES	10 mm	9.5-11.2	$10.3 \pm 0.20$	-10.43
	20 mm	8.5-9.5	$9.02 \pm 0.10$	-21.56
	30 mm	7.2-8.6	$8 \pm 0.16$	-30.43
	40 mm	5.8-7.4	$6.4 \pm 0.17$	-44.34
	50 mm	5 - 6.4	$5.6 \pm 0.15$	-51.30
SA	10 mm	9-10.2	$9.53 \pm 0.13$	-17.13
	20 mm	7.5-9.2	$8.5 \pm 0.20$	-26.08
	30 mm	6.8-8	$7.39 \pm 0.11$	-35.74
	40 mm	5-7.2	$5.95 \pm 0.23$	-48.26
	50 mm	3.2-5.5	$4.33 \pm 0.23$	-62.34

**Table 2:** Effects of EMS, DES and SA on 30th day plant height at maturity (cm/plant) of *L. esculentum* Mill. (Var. PKM - 1) in M1 generation

Mutagens	Treatments Conc.	30th Day plant height at maturity (cm)		
		Range	Mean $\pm$ S.E	% of Reduction over control
	Control	15.3-20.2	$18.29 \pm 0.47$	-
EMS	10 mm	12.4-18.4	$14.79 \pm 0.61$	-19.13
	20 mm	10.2-16.6	$14.12 \pm 0.69$	-22.79
	30 mm	9.5-12.8	$10.99 \pm 0.31$	-39.91
	40 mm	8.2-11.5	$9.81 \pm 0.35$	-46.36
	50 mm	7.8-10.6	$9.13 \pm 0.29$	-50.08
DES	10 mm	13.6-19.2	$16.47 \pm 0.55$	-9.950
	20 mm	12.5-18.8	$15.85 \pm 0.69$	-13.34
	30 mm	11.5-15.8	$13.71 \pm 0.49$	-25.04
	40 mm	9.5-13.8	$11.51 \pm 0.45$	-37.06
	50 mm	7.6-11.8	$9.61 \pm 0.49$	-47.45
SA	10 mm	10.5-16.8	$13.46 \pm 0.68$	-26.40
	20 mm	10.2-14.8	$11.92 \pm 0.43$	-34.82

	30 mm	9.4-13.6	11.27 ± 0.47	-38.38
	40 mm	7.6-11.8	9.57 ± 0.46	-47.67
	50 mm	6.4-10.4	8.48 ± 0.46	-53.63

**Table 3:** Effects of EMS, DES and SA on number of branches per plant of *L. esculentum* Mill. (Var. PKM - 1) in M1 generation

Mutagens	Treatments Conc.	Branches per plant		
		Range	Mean ± S.E	% of Reduction over control
	Control	25-31	28.5 ± 0.64	-
EMS	10 mm	23-29	26.2 ± 0.65	-8.070
	20 mm	20-26	23 ± 0.56	-19.29
	30 mm	17-23	20.2 ± 0.63	-29.12
	40 mm	15-19	17.3 ± 0.40	-39.29
	50 mm	12-16	14.1 ± 0.41	-50.52
DES	10 mm	24-30	27.5 ± 0.65	-3.508
	20 mm	23-29	26 ± 0.66	-8.771
	30 mm	22-26	24 ± 0.42	-15.78
	40 mm	19-23	20.8 ± 0.44	-27.01
	50 mm	16-20	18.1 ± 0.41	-36.49
SA	10 mm	22-27	24.6 ± 0.50	-13.68
	20 mm	21-25	23.1 ± 0.53	-18.94
	30 mm	18-22	20.2 ± 0.39	-29.12
	40 mm	14-18	16.2 ± 0.50	-43.15
	50 mm	11-15	12.8 ± 0.38	-55.08

**Table 4:** Effects of EMS, DES and SA on days to first flowering of *Lycopersicon esculentum* Mill. (Var. PKM - 1) in M1 generation

Mutagens	Treatments Conc.	Days to first flowering		
		Range	Mean ± S.E	% of Reduction over control
	Control	40-48	44.6 ± 0.79	-
EMS	10 mm	38-50	45.5 ± 1.34	-2.071
	20 mm	44-52	48.8 ± 0.86	-9.417
	30 mm	47-54	50.2 ± 0.76	-12.55
	40 mm	47-55	51 ± 0.92	-14.34
	50 mm	49-58	53.6 ± 0.96	-20.18
DES	10 mm	40-48	44.3 ± 0.90	-0.672
	20 mm	42-50	46.3 ± 0.87	-3.811
	30 mm	43-52	47.8 ± 0.99	-7.174
	40 mm	45-54	49.4 ± 0.92	-10.76
	50 mm	48-56	51.4 ± 0.82	-15.24
SA	10 mm	43-51	47.2 ± 0.81	-5.830
	20 mm	44-52	48.5 ± 0.83	-8.744
	30 mm	46-54	49.9 ± 0.81	-11.88
	40 mm	48-56	51.9 ± 0.85	-16.36
	50 mm	54-59	56.5 ± 0.56	-26.68

**Table 5:** Effects of EMS, DES and SA on number of flowers per plant of *L. esculentum* Mill. (Var. PKM - 1) in M1 generation

Mutagens	Treatments Conc.	Flowers per plant		
		Range	Mean ± S.E	% of Reduction over control
	Control	21-27	23 ± 0.61	-
EMS	10 mm	18-26	21.8 ± 0.81	-5.217
	20 mm	16-22	19.6 ± 0.58	-14.78
	30 mm	15-14	17.0 ± 0.47	-26.08
	40 mm	12-17	14.9 ± 0.50	-35.21
	50 mm	11-15	13.0 ± 0.45	-43.47
DES	10 mm	20-25	22.3 ± 0.54	-3.043
	20 mm	18-23	20.2 ± 0.51	-12.17
	30 mm	15-19	17.4 ± 0.43	-24.34
	40 mm	12-16	14.1 ± 0.41	-38.76
	50 mm	8-11	9.6 ± 0.34	-58.26
SA	10 mm	17-23	19.6 ± 0.58	-14.78
	20 mm	15-21	17.9 ± 0.71	-22.17
	30 mm	12-18	15.2 ± 0.61	-33.91
	40 mm	9-13	10.9 ± 0.41	-52.60
	50 mm	3-8	8 ± 0.54	-65.22

**Table 6:** Effects of EMS, DES and SA on number of cluster per of *L. esculentum* Mill. (Var. PKM - 1) in M1 generation

Mutagens	Treatments Conc.	Cluster per plant		
		Range	Mean $\pm$ S.E	% of Reduction over control
	Control	7- 9	7.7 $\pm$ 0.21	-
EMS	10 mm	6-8	7.1 $\pm$ 0.23	-7.792
	20 mm	6-8	6.9 $\pm$ 0.23	-10.38
	30 mm	5-7	6.1 $\pm$ 0.23	-20.78
	40 mm	5-7	6 $\pm$ 0.25	-22.08
	50 mm	5-7	5.7 $\pm$ 0.24	-25.97
DES	10 mm	6-9	7.3 $\pm$ 0.3	-5.195
	20 mm	5-9	7 $\pm$ 0.36	-9.090
	30 mm	5-8	6 $\pm$ 0.29	-22.08
	40 mm	7-4	5.2 $\pm$ 0.32	-32.46
	50 mm	6-3	4.1 $\pm$ 0.34	-46.75
SA	10 mm	6-9	7.3 $\pm$ 0.3	-5.195
	20 mm	5-7	5.9 $\pm$ 0.28	-23.38
	30 mm	4-7	5.3 $\pm$ 0.3	-31.17
	40 mm	3-5	4 $\pm$ 0.26	-48.05
	50 mm	1-5	3 $\pm$ 0.42	-61.04

**Table 7:** Effects of EMS, DES and SA number of fruits per plant of *L. esculentum* mill. (Var. PKM - 1) in M1 generation

Mutagens	Treatments Conc.	Fruits per plant		
		Range	Mean $\pm$ S.E	% Reduction over control
	Control	9-13	11.3 $\pm$ 0.39	-
EMS	10 mm	8-12	9.6 $\pm$ 0.45	-15.04
	20 mm	7-11	8.8 $\pm$ 0.35	-22.12
	30 mm	7-9	8.3 $\pm$ 0.26	-26.55
	40 mm	6-9	7.4 $\pm$ 0.33	-34.51
	50 mm	5-8	6.6 $\pm$ 0.30	-41.59
DES	10 mm	8-12	9.9 $\pm$ 0.34	-12.39
	20 mm	10-8	8.9 $\pm$ 0.23	-21.24
	30 mm	6-9	7.6 $\pm$ 0.30	-32.74
	40 mm	5-8	6.7 $\pm$ 0.26	-40.71
	50 mm	3-6	4.5 $\pm$ 0.26	-60.17
SA	10 mm	7-10	8.3 $\pm$ 0.3	-26.54
	20 mm	6-9	7.5 $\pm$ 0.27	-33.62
	30 mm	5-7	5.7 $\pm$ 0.26	-49.56
	40 mm	3-6	4.6 $\pm$ 0.34	-59.29
	50 mm	2-5	3.4 $\pm$ 0.31	-69.91

### Conclusion

The study exposed the characteristic among chemical mutagenesis treatment in M<sub>1</sub> generation was decreased in increasing concentrations of treatment.

The mean achievement/performance of different quantitative traits was good in control when compared to the treated plants, induced mutagenesis in the best method to develop genetic variability within a short time.

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