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Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS

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Abstract

Induction of mutation by gamma rays, ethyl methane sulfonate (EMS) and their combined treatments was studied in three widely divergent genotypes of tomato, EC620176, EC620177 and Patharkutchi. A steady reduction in germination percentage, seedling height and pollen fertility occurred in M_1 generation with the increasing doses / concentrations of mutagens. Combination of gamma radiation and EMS caused more damage followed by EMS treatment and gamma radiation alone in M_1 generation. The LD_{50} dose for EC620176, EC620177 and Patharkutchi corresponded to 67.3 Gy, 290.9 Gy and 303.8 Gy gamma radiation, and 0.10%, 0.17 % and 0.38 % EMS treatment, respectively. Highest mutation frequency was resulted by gamma radiation followed by the combined mutagens and EMS treatment. Genotype and mutagen, both, influenced the production of mutants. Mutagenic efficiency of lower doses/concentrations was more compared to higher doses in producing desirable mutants. Mutagenic effectiveness of gamma radiation was the highest followed by sole EMS and combined mutagens. Gamma irradiation (50-150 Gy) was most efficient followed by 0.05-0.10 % EMS and their combination treatment in inducing wide array of macro-mutation in tomato. Five putative mutants with exserted stigma flower, dark green fruit, dwarf plant having pyriform fruit from Patharkutchi, multiparous cyme from EC620177 and chlorophyll deficient mutant from EC620176 that could be isolated in M_2 generation hold promise for their utilization in tomato breeding programme.

Key words: Mutagens, effectiveness, efficiency, gamma rays, EMS, mutants, tomato

Introduction

The cultivated tomato (*Solanum lycopersicum*) is the second most consumed vegetable after potato and contributes greatly to agro-based industry in the world. Spontaneous or induced mutants, with desirable changes in particular characters have been a key material for gene discovery, mapping, functional genomics and breeding in many crops including tomato. Mutation assisted improvement of crops has currently been strengthened by the doubled haploids and molecular markers. Tomato is a good example of a successful use of mutations affecting major genes for plant breeding however, mutant alleles are only currently known for an insignificant fraction of the about 35,000 genes in the tomato genome hence, large scale mutagenesis and introgression of natural genetic variation can be useful to fill this gap [1]. Induced mutagensis as a breeding strategy for improvement of tomato has been explored through different studies [2-5]. The present investigation was undertaken to study the frequency and spectrum of macro-mutations along with mutagenic efficiency and effectiveness of gamma rays, ethyl methane sulphonate (EMS) and their combinations in three genotypes of tomato.

Materials and methods

The present investigation was undertaken in the Department of Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal during 2010-2012. The gamma radiation was given to the dry seeds at National Botanical Research Institute, Lucknow, where Cobalt- 60 served as source of gamma rays. Solutions of ethyl methane sulphonate (Sigma Chemical Company, USA) were prepared in freshly prepared phosphate buffer (pH 7.0) for treating the seeds. Dry seeds of three widely divergent genotypes of tomato *viz*., *ps-2* functional male sterile line

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(EC620176), Berika (EC620177), a variety from Bulgaria and Patharkutchi, the highly adaptable and popular cultivar of West Bengal were irradiated with 50, 100, 150, 200 and 250 Gy gamma rays. Pre-soaked seeds of these genotypes (6 h, in water) were treated with 0.05, 0.10, 0.15, 0.20 and 0.25% ethyl methane sulphonate (EMS) for 8 h at 25±2°C. Gamma irradiated (50, 100, 150, 200 and 250 Gy) seeds of these three genotypes were also pre-soaked (6 h, in water) before treating with 0.15 % EMS solution for 8 h at 25±2°C as combination treatment. The EMS treated seeds were washed thoroughly in running water at least for an hour before sowing.

Treated seeds of three genotypes were sown separately in the trial using 100 seeds each in three replications along with parental controls (non-treated seeds) in well prepared seed beds. The percentage of M_1 plants emerging in the seedbed were averaged over three replications and reduction in germination was referred to as lethality. In the main field at Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya situated at 22º57'N latitude and 88 º 20' E longitudes with average altitude of 9.75 m above the mean sea level, 25 M_1 plants per replication of three in each treatment were grown. Reduction in seedling height recorded in seed bed at 25 days after sowing over control plants was termed as injury. Pollen viability determined by stainability with 1% acetocarmin solution and its reduction compared to that of control plants was called sterility. Seed germination % was employed to calculate the LD_{50} dose of the mutagen (dose required to kill 50% of the tested population) following probit analysis [6].

Seeds from all the plants of each of the three genotypes from respective treatment in M_1 generation were bulked to raise the M_2 generation. Total of maximum 180 plants of the M_2 generation in each treatment were grown keeping 60 plants at 60 x 50 cm spacing (5.0 x 3.6 m bed) in each replication along with the parents in well prepared field. A range of 150 to 173 M_2 plants in 3 genotypes in each mutagenic treatment could be examined for segregation. The spectrum of mutation in the M_2 progeny comprising chlorophyll deficient mutations (both viable and non-viable) and other macro mutations could be detected based on altered plant stature, leaf morphology, inflorescence type, floral morphology, pedicel character, fruit morphology, colour and chlorophyll content over the set of characters specific for the three genotypes. Non-viable chlorophyll deficient mutants did not survive beyond 30 days after transplanting.

Chlorophyll mutation frequency was determined as percentage of mutated M_2 progenies for chlorophyll deficiency (both viable and non-viable). Total mutation frequency (Mf) was determined as % of mutated M_2 progenies for both chlorophyll deficient and other viable macro-mutants. Data on biological damage (lethality, injury and sterility) in M_1 generation and total mutation frequencies were used to calculate the mutagenic efficiency and effectiveness according to standard formula [7]. The formulae used for mutagenic efficiency were (Mf/L); (Mf/I) and (Mf/S) where Mf, L, I and S denote total mutation frequency in M_2 generation, lethality in seed germination, seedling injury and pollen sterility, respectively in M_1 generation. The mutagenic effectiveness was determined by using the formula Mf x 100 / KR or Mf x 100/C x T where, Mf, KR, T and C indicates total mutation frequency, dose of radiation in kilo rad, duration of treatment in hours and percent concentration of EMS solution, respectively.

Results and discussion

Damage in M_1 generation

The impact and tolerance level of the tomato genotypes to the mutagen were manifested in M_1 generation itself in terms of lethality, injury and sterility which was documented earlier in details [8]. All the mutagenic treatments showed inhibitory effect on seed germination which might have happened due to a number of factors *viz*., adverse effect on cytochrome oxidase content, thus reducing the respiration [9], drastic distortion of the actively dividing phase [10], damage of cell constituents at molecular level [11], interference in the synthesis of enzyme and acceleration in the degradation of existing enzyme [12] and/or altered enzyme activity [11].

Considering three genotypes together, percent reduction in germination over control was maximum in combination treatment (42.50%) followed by sole EMS treatment (31.91%) and gamma radiation (29.82%). The germination percentage showed a sharp dose-rate relationship, which decreased with the increase in dose/concentration of the mutagenic treatments.

Effect of mutagens was found inhibitory to the length of seedlings, the combination treatment was most drastic in reducing the seedling height (58.81%) while reduction due to gamma radiation alone was the

least (43.87%) and that in EMS treatment came in between (47.69%). Such reduction in shoot length which might have been due to adverse effect on physiological systems [8] and growth hormone [13] was also reported earlier in different crops.

Pollen fertility reduced steadily with the increasing doses/concentrations of mutagens in all the three genotypes which agreed well to the earlier findings in different crops.

Further, there were significant genotypic differences with respect to pollen fertility. The European genotype, EC620176 was highly vulnerable and Patharkutchi was the most tolerant in this regard. Irrespective of the genotypes, reduction in pollen fertility was highest with combination treatment followed by EMS treatment and gamma radiation. Radiation induced sterility has been attributed to chromosomal aberrations and cryptic deficiencies [14] while in EMS and other chemical mutagens, sterility has been ascribed to cryptic deletions and specific gene mutation [15]. Considering three parameters of biological damage together, combination treatment caused the most damage and gamma radiation, alone produced the least damage (Table 1).

LD_{50} dose

Seed germination % was significantly and negatively correlated with both gamma radiation dose (r = –0.935 in EC620176; r = –0.949 in EC620177; r = –0.928 in Patharkutchi) and EMS concentration (r = –0.927 in EC620176; r = –0.911 in EC620177; r = –0.915 in Patharkutchi). The LD_{50} for EC620176, EC620177 and Patharkutchi corresponded to 67.3 Gy, 290.9 Gy and 303.8 Gy for gamma radiation, and 0.10 %, 0.17 % and 0.38 % for EMS treatment, respectively.The differences occurred in fertility of M_1 plants were considered as the measure of sensitivity of a genotype to mutagen [17]. The tropicalized cultivar Patharkutchi emerged as the most tolerant genotype to mutagen compared to the other two genotypes of European origin.

Spectrum of mutation

The spectrum of mutation is essentially a parameter for the index of mutation frequency. Spectrum of mutation varied with the genotypes and it was the highest of twelve in Patharkutch followed by eight in EC-620177 and the least of five in EC-620176 indicating variation in allelic mutability of different genotypes. Chlorophyll deficient mutants were mostly “Albino” type which perished within 30 days after planting. The only viable chlorophyll mutant could be isolated from EC620176 in 0.1% EMS treatment. The spectrum of mutation as a whole considering both chlorophyll deficient and other macro mutations together did not necessarily increase with the increasing doses of both gamma radiation and EMS concentration in two genotypes of Europe, but it did so in Patharkutchi. Spectrum of mutation varied with the mutagen and dose. Widest mutation spectrum was obtained in EC620176 (four) with 100Gy gamma radiation and 50 Gy gamma radiation + 0.15% EMS treatment; in EC620177 (four) with 50 Gy gamma radiation and in Patharkutchi (four) with 50, 200 and 250 Gy gamma radiation and 0.05% EMS treatment.

Among the macro-mutants, fruit mutants (shape, size, high chlorophyll content, etc.) were more frequently occurring followed by leaf mutants. Higher doses of gamma radiation produced more non-viable chlorophyll deficient mutants whereas EMS at higher concentration produced comparatively lesser in number and in combination treatments, occurrence of non-viable chlorophyll deficient mutants increased with the increase in dosage of gamma rays. It was clearly evident that the physical and chemical mutagens induced different mutation spectrum and the type of mutant depended not only on the type of mutagen but also on the genotype used as recorded in several earlier studies [7, 16-19].

Chlorophyll mutation frequency

Chlorophyll mutation frequency varied conspicuously among the genotypes, the highest of 3.93% was found in EC620176 followed by 2.79 % in EC620177 and the least of 2.51 % in Patharkutchi. However, average chlorophyll mutation frequency taking three genotypes together did not vary much among the mutagens although, it was the highest of 2.96 % in gamma radiation followed by 2.79 % in treatment and the least of 2.49 % in sole EMS treatment (Table 2).

Frequency of chlorophyll deficient mutants increased with the increasing doses of both gamma radiation and EMS concentration which agreed well to the earlier findings [18, 20]. In EC620176, highest of 6.33 % frequency was recorded with 250 Gy gamma radiation followed by 4.63% with 0.25% EMS treatment; in EC620177, 0.25% EMS treatment produced the highest of 4.19% frequency followed by 3.68% with 250 Gy gamma radiation and in Patharkutchi also, 0.25% EMS treatment produced

Table 1. Effect of mutagens on germination, seedling height and pollen fertility in M_1 generation

Mutagenic treatments	Germination (%)	Seedling height (cm)	Pollen fertility (%)
***Positional sterile-2* line (EC620176)**			
Control	66.40 (0.00)	13.25 (0.00)	78.57 (0.00)
50 Gy γ rays	41.48 (-37.53)	8.47 (-36.08)	53.07(-32.46)
100 Gy γ rays	41.28 (-37.83)	8.07 (-39.09)	52.57(-33.09)
150 Gy γ rays	40.70 (-38.70)	7.49 (-43.47)	47.17 (-39.96)
200 Gy γ rays	38.05 (-42.70)	6.98 (-47.32)	45.17 (-42.51)
250 Gy γ rays	35.82 (-46.05)	6.58 (-50.34)	41.26 (-47.49)
0.05% EMS	48.81 (-26.49)	8.39 (-36.68)	46.23 (-41.16)
0.1% EMS	44.52 (-32.95)	8.28 (-37.51)	45.28 (-42.37)
0.15% EMS	42.68 (-35.72)	8.22 (-37.96)	38.16 (-51.43)
0.20% EMS	41.53 (-37.45)	7.04 (-46.87)	42.68 (-45.68)
0.25% EMS	41.14 (-38.04)	6.69 (-49.51)	42.39 (-46.05)
50 Gy + 0.15% EMS	38.10 (-42.62)	7.92 (-40.23)	41.66 (-46.98)
100 Gy + 0.15% EMS	35.28 (-46.87)	6.40 (-51.70)	40.46 (-48.50)
150 Gy + 0.15% EMS	36.07 (-45.68)	5.25 (-60.38)	36.46 (-53.60)
200 Gy + 0.15% EMS	35.76 (-46.14)	4.87 (-63.25)	37.33 (-52.49)
250 Gy + 0.15% EMS	33.67 (-49.29)	4.05 (-69.43)	32.16 (-59.07)
SEM ±	0.91	0.59	2.58
Berika (EC620177)			
Control	85.57 (0.00)	19.83 (0.00)	81.36 (0.00)
50 Gy γ rays	74.03 (-13.49)	11.32 (-42.91)	76.14 (-6.42)
100 Gy γ rays	65.77 (-23.14)	10.16 (-48.76)	77.28 (-5.01)
150 Gy γ rays	63.80 (-25.44)	9.28 (-53.20)	75.34 (-7.40)
200 Gy γ rays	60.34 (-29.48)	8.52 (-57.03)	71.33 (-12.33)
250 Gy γ rays	58.52 (-31.61)	8.04 (-59.46)	69.27 (-14.86)
0.05%EMS	62.07 (-27.46)	10.63 (-46.39)	74.33 (-8.64)
0.1% EMS	53.92 (-36.99)	10.19 (-48.61)	74.18 (-8.82)
0.15% EMS	46.62 (-45.52)	9.62 (-51.49)	75.67 (-6.99)
0.20% EMS	45.34 (-47.01)	9.31 (-53.05)	70.25 (-13.66)
0.25% EMS	45.19 (-47.19)	8.95 (-54.87)	70.17 (-13.75)
50 Gy + 0.15% EMS	56.08 (-34.46)	9.45 (-52.34)	72.67 (-10.68)
100 Gy + 0.15% EMS	54.23 (-36.62)	8.94 (-54.92)	72.33 (-11.10)
150 Gy + 0.15% EMS	52.14 (-39.07)	7.74 (-60.97)	71.23 (-12.45)
200 Gy + 0.15% EMS	50.32 (-41.19)	7.62 (-61.57)	71.67 (-11.91)
250 Gy + 0.15% EMS	42.62 (-50.19)	5.73 (-71.10)	68.14 (-16.25)
SEM ±	0.35	0.58	4.31
Patharkutchi			
Control	88.43(0.00)	25.27 (0.00)	80.23 (0.00)
50 Gy γ rays	76.48 (-13.51)	21.48 (-17.67)	76.33 (-4.86)
100 Gy γ rays	68.78 (-22.22)	18.31 (-29.82)	76.67 (-4.44)
150 Gy γ rays	64.37 (-27.21)	16.08 (-38.37)	74.67 (-6.93)
200 Gy γ rays	63.78 (-27.88)	14.17 (-45.69)	72.33 (-9.85)
250 Gy γ rays	61.37 (-30.60)	13.34 (-48.87)	70.67 (-11.92)
0.05%EMS	74.62 (-15.62)	15.78 (-39.52)	74.33 (-7.35)
0.1% EMS	72.34 (-18.20)	15.26 (-41.51)	75.36 (-6.07)
0.15% EMS	73.16 (-17.27)	13.85 (-46.91)	70.16 (-12.55)
0.20% EMS	68.12 (-22.97)	10.77 (-58.72)	72.33 (-9.85)
0.25% EMS	62.08 (-29.80)	8.92 (-65.81)	66.00 (-17.74)
50 Gy + 0.15% EMS	58.26 (-34.12)	13.68 (-47.57)	74.28 (-7.42)
100 Gy + 0.15% EMS	56.17 (-36.48)	11.14 (-57.30)	73.67 (-8.18)
150 Gy + 0.15% EMS	52.28 (-40.88)	10.59 (-59.41)	73.82 (-7.99)
200 Gy + 0.15% EMS	50.49 (-42.90)	9.93 (-61.94)	68.67 (-14.41)
250 Gy + 0.15% EMS	42.49 (-51.95)	7.82 (-70.03)	65.33 (-18.57)
SEM ±	1.12	0.63	4.73

Figures in parenthesis show percent reduction over respective control plants

the highest frequency of 3.73 % followed by 3.61% with 250 Gy gamma radiation + 0.15% EMS treatment. Conversely, all the three genotypes showed lower frequency of chlorophyll mutation at 50 Gy gamma rays (1.96 % in EC-620176, 1.30 % in EC-620177 and 1.16 % in Patharkutchi). Similarly in EMS treatment also, lower frequency of chlorophyll mutation was also noticed at 0.05% EMS in all the three genotypes (1.85 % in EC-620176, 1.30 % in EC-620177 and 0.58 % in Patharkutchi).

Chlorophyll development seems to be controlled by many genes located near the centromere and proximal segments of the chromosomes [20]. Localized chromosome breakage by EMS treatment as reported by might be the reason of getting chlorophyll deficient mutants in EMS treatments [21]. The frequency of chlorophyll mutation in M_2 generation is generally regarded as the most reliable index of mutation rate because of greater accuracy of scoring however, in the present investigation it indicated neither the spectrum of mutation nor the probability of isolating promising macro-mutants.

Total mutation frequency

Average total mutation frequency taking three genotypes together did vary markedly among the mutagens and the highest of 4.33 % was resulted by gamma radiation followed by 3.52% in combination treatment and the least of 3.19 % in EMS treatment (Table 2). Maximum efficiency of gamma radiation in inducing the highest mutation frequency might have been due to its high penetrating power of causing more chromosomal aberration as compared to EMS in alone treatment. Efficiency of combined treatment for inducing mutation frequency came in between that of gamma radiation and EMS showing additivity because of independent action of these two mutagens. [22]. Total mutation frequency also did not necessarily reflect the spectrum of mutation and for this reason, the highest of 6.96 % mutation frequency in EC620176 with 250 Gy gamma radiation emerged from only two mutant types (Table 3). Hence, both mutation spectrum and frequency are important to ascertain the genetic variation that is available for selection in M_2 or M_3 generations.

Mutagenic efficiency

The efficient mutagenesis is the production of desirable changes free from association with undesirable ones [7]. It has been generally observed that the mutagen dose that gives the higher mutation rates also induces lethality, sterility and other undesirable effects. In mutation breeding programme, such treatments are not desirable as they result in multiple mutations and other drastic effects reducing the yield potentials of the mutants. It is therefore, desirable to induce high mutation rates with least accompanying deleterious effects. Hence, mutagenic efficiency gives an idea of the proportion of mutation in relation to deleterious effects of the mutagen [7]. The higher efficiency of a mutagen indicates relatively less biological damage in relation to mutations induced [23]. In EC620177 and Patharkutchi, mutagenic efficiency decreased with an increase in the dose of mutagens because lethality increased with the mutagen level (Table 3). So, lower concentration of mutagens caused relatively less damage enabling the genotypes to manifest the induced mutations more frequently. However, this trend was not established in EC620176 because higher dose/ concentration of mutagen caused both damage and high mutation frequency concomitantly in this genotype. It seemed that strong mutagens reach their saturation point even at a lower dose in the genotypes having highly mutable allelic sites, and any further increase in the mutagen dose add to their mutation frequency.

The average mutagenic efficiency in three genotypes based on pollen sterility was the maximum over all the treatments (0.283) as compared to that based on lethality (0.115) and seedling injury (0.076). Considering three genotypes together, gamma radiation showed the highest mutagenic efficiency (0.215) as compared to that of EMS (0.136) and combination treatments (0.122). However, all treatments which yielded the highest mutagenic frequency might not confer the highest mutagenic efficiency which was in line of earlier finding [18]. In all the three genotypes, the efficiency of lower doses/ concentrations was more compared to higher doses in producing desirable mutants.

Mutagenic effectiveness

Mutagenic effectiveness is defined as a measure of frequency of mutation induced by a unit of mutagen. The degree of effectiveness of mutagen and the response of the genotypes was varying. However, irrespective of the genotypes mutagenic effectiveness of gamma radiation was the highest followed by EMS and combination treatment (Table 3). Mutagenic effectiveness in all the three genotypes decreased with increasing doses/concentrations of mutagens.

Table 2. Total mutation frequency in M_2 generation of three tomato genotypes

Mutagenic treatments	M_2 plants examined	Viable chlorophyll mutants	Total mutants Non-viable chlorophyll mutants	Viable macro-mutants	Chlorophyll mutation frequency (%)	Total mutation frequency (Mf) %
			***Positional sterile-2* line (EC-620176)**			
50 Gy γ rays	153	0	3	3	1.96	3.92
100 Gy γ rays	157	0	3	4	1.91	4.46
150 Gy γ rays	159	0	5	3	3.14	5.03
200 Gy γ rays	167	0	7	3	4.19	5.98
250 Gy γ rays	158	0	10	1	6.33	6.96
0.05%EMS	162	0	3	1	1.85	2.47
0.1%	167	1	2	2	1.79	2.99
0.15%	157	0	4	0	2.54	2.54
0.20%	158	0	5	0	3.16	3.16
0.25%	151	0	7	0	4.63	4.63
50 Gy + 0.15% EMS	172	0	2	3	1.16	2.91
100 Gy + 0.15% EMS	152	0	4	2	2.63	3.94
150 Gy + 0.15% EMS	153	0	3	2	1.96	3.26
200 Gy + 0.15% EMS	158	0	6	0	3.79	3.79
250 Gy + 0.15% EMS	164	0	5	2	3.05	4.26
			Berika (EC-620177)			
50 Gy γ rays	154	0	2	3	1.30	3.25
100 Gy γ rays	156	0	5	1	3.21	3.84
150 Gy γ rays	171	0	5	2	2.92	4.09
200 Gy γ rays	155	0	5	1	3.23	3.87
250 Gy γ rays	163	0	6	1	3.68	4.29
0.05%EMS	154	0	2	1	1.30	1.94
0.1%	153	0	2	2	1.31	2.61
0.15%	154	0	4	1	2.60	3.25
0.20%	151	0	5	0	3.31	3.31
0.25%	167	0	7	0	4.19	4.19
50 Gy + 0.15% EMS	168	0	4	1	2.38	2.98
100 Gy + 0.15% EMS	159	0	3	2	1.89	3.14
150 Gy + 0.15% EMS	165	0	6	0	3.63	3.63
200 Gy + 0.15% EMS	152	0	5	0	3.28	3.28
250 Gy + 0.15% EMS	166	0	6	1	3.61	4.21
			Patharkutchi			
50 Gy γ rays	172	0	2	3	1.16	2.91
100 Gy γ rays	152	0	4	1	2.63	3.29
150 Gy γ rays	157	0	4	2	2.54	3.82
200 Gy γ rays	173	0	5	3	2.89	4.04
250 Gy γ rays	153	0	5	3	3.27	5.22
0.05%EMS	172	0	1	3	0.58	2.32
0.1%	164	0	3	1	1.83	2.43
0.15%	154	0	3	3	1.95	3.89
0.20%	151	0	4	2	2.65	3.72
0.25%	161	0	6	1	3.73	4.34
50 Gy + 0.15% EMS	154	0	3	1	1.95	2.59
100 Gy + 0.15% EMS	151	0	4	0	2.65	2.65
150 Gy + 0.15% EMS	167	0	4	2	2.39	3.59
200 Gy + 0.15% EMS	154	0	6	0	3.89	3.89
250 Gy + 0.15% EMS	166	0	6	2	3.61	4.82

Table 3. Effectiveness and efficiency of mutagenic treatments in the three genotypes

Mutagenic treatments	Total mutation frequency (Mf)	Lethality (L)	Mutagen efficiency (Mf/L)	Injury (I)	Mutagen efficiency (Mf/I)	Pollen sterility (S)	Mutagen efficiency (Mf/S)	Mutagenic effective-ness
***Positional sterile-2* line (EC-620176)**								
50 Gy ৲ rays	3.92	37.53	0.104	36.08	0.109	32.46	0.121	78.42
100 Gy ৲ rays	4.46	37.83	0.118	39.09	0.114	33.09	0.135	44.63
150 Gy ৲ rays	5.03	38.70	0.129	43.47	0.115	39.96	0.125	33.53
200 Gy ৲ rays	5.98	42.70	0.140	47.32	0.126	42.51	0.141	29.90
250 Gy ৲ rays	6.96	46.05	0.151	50.34	0.138	47.49	0.146	27.84
Mean	**5.26**	**40.56**	**0.128**	**43.26**	**0.120**	**39.10**	**0.134**	**42.85**
0.05%EMS	2.47	26.49	0.093	36.68	0.067	41.16	0.060	8.17
0.1%	2.99	32.95	0.091	37.51	0.079	42.37	0.071	4.95
0.15%	2.54	35.72	0.071	37.96	0.067	51.43	0.049	2.80
0.20%	3.16	37.45	0.084	46.87	0.067	45.68	0.069	2.61
0.25%	4.63	38.04	0.121	49.51	0.093	46.05	0.101	3.06
Mean	**3.16**	**34.13**	**0.092**	**41.71**	**0.075**	**45.34**	**0.071**	**4.32**
50 Gy + 0.15% EMS	2.91	42.62	0.068	40.23	0.072	46.98	0.061	0.65
100 Gy + 0.15% EMS	3.94	46.87	0.084	51.70	0.076	48.50	0.081	0.43
150 Gy + 0.15% EMS	3.26	45.68	0.071	60.38	0.053	53.60	0.061	0.24
200 Gy + 0.15% EMS	3.79	46.14	0.082	63.25	0.059	52.49	0.072	0.21
250 Gy + 0.15% EMS	4.26	49.29	0.086	69.43	0.061	59.07	0.072	0.19
Mean	**3.63**	**46.12**	**0.078**	**57.00**	**0.064**	**52.13**	**0.069**	**0.34**
Berika (EC-620177)								
50 Gy ৲ rays	3.25	13.49	0.241	42.91	0.075	6.42	0.506	65.00
100 Gy ৲ rays	3.84	23.14	0.165	48.76	0.078	5.01	0.766	38.41
150 Gy ৲ rays	4.09	25.44	0.161	53.20	0.077	7.40	0.553	27.26
200 Gy ৲ rays	3.87	29.48	0.131	57.03	0.067	12.33	0.313	19.35
250 Gy ৲ rays	4.29	31.61	0.136	59.46	0.072	14.86	0.288	17.16
Mean	**3.87**	**24.63**	**0.167**	**52.27**	**0.074**	**9.20**	**0.485**	**33.43**
0.05%EMS	1.94	27.46	0.071	46.39	0.042	8.64	0.224	6.42
0.1%	2.61	36.99	0.071	48.61	0.054	8.82	0.295	4.32
0.15%	3.25	45.52	0.072	51.49	0.063	6.99	0.464	3.58
0.20%	3.31	47.01	0.071	53.05	0.062	13.66	0.242	2.74
0.25%	4.19	47.19	0.089	54.87	0.076	13.75	0.304	2.77
Mean	**3.06**	**40.83**	**0.075**	**50.88**	**0.059**	**10.37**	**0.306**	**3.96**
50 Gy + 0.15% EMS	2.98	34.46	0.086	52.34	0.056	10.68	0.279	0.66
100 Gy + 0.15% EMS	3.14	36.62	0.085	54.92	0.057	11.10	0.282	0.35
150 Gy + 0.15% EMS	3.63	39.07	0.093	60.97	0.059	12.45	0.291	0.27
200 Gy + 0.15% EMS	3.28	41.19	0.079	61.57	0.053	11.91	0.275	0.18
250 Gy + 0.15% EMS	4.21	50.19	0.083	71.10	0.059	16.25	0.259	0.19
Mean	**3.45**	**40.31**	**0.085**	**60.18**	**0.057**	**12.48**	**0.277**	**0.33**
Patharkutchi								
50 Gy ৲ rays	2.91	13.51	0.215	17.67	0.165	4.86	0.598	58.23
100 Gy ৲ rays	3.29	22.22	0.148	29.82	0.110	4.44	0.739	32.91
150 Gy ৲ rays	3.82	27.21	0.140	38.37	0.100	6.93	0.551	25.46
200 Gy ৲ rays	4.04	27.88	0.145	45.69	0.088	9.85	0.410	20.21
250 Gy ৲ rays	5.22	30.60	0.171	48.87	0.107	11.92	0.438	20.88
Mean	**3.85**	**24.28**	**0.164**	**36.08**	**0.114**	**7.60**	**0.547**	**31.54**
0.05%EMS	2.31	15.62	0.148	39.52	0.058	7.35	0.316	7.67
0.1%	2.43	18.20	0.134	41.51	0.059	6.07	0.402	4.03
0.15%	3.89	17.27	0.225	46.91	0.082	12.55	0.309	4.29
0.20%	3.72	22.97	0.161	58.72	0.063	9.85	0.377	3.08
0.25%	4.34	29.80	0.145	65.81	0.065	17.74	0.244	2.87
Mean	**3.34**	**20.77**	**0.162**	**50.49**	**0.065**	**10.71**	**0.329**	**4.39**
50 Gy + 0.15% EMS	2.59	34.12	0.076	47.57	0.055	7.42	0.349	0.57
100 Gy + 0.15% EMS	2.65	36.48	0.073	57.30	0.046	8.18	0.323	0.29
150 Gy + 0.15% EMS	3.59	40.88	0.087	59.41	0.059	7.99	0.449	0.26
200 Gy + 0.15% EMS	3.89	42.90	0.091	61.94	0.062	14.41	0.269	0.21
250 Gy + 0.15% EMS	4.82	51.95	0.092	70.03	0.068	18.57	0.259	0.21
Mean	3.51	41.27	0.083	59.25	0.058	11.31	0.329	0.31

The tropicalized genotype, Patharkutchi responded most in producing viable macro-mutants. Lower doses of gamma radiation (50-150 Gy) proved to be the most effective than EMS (0.05-0.10 %) and combined mutagens at this dose/ concentration for inducing broad spectrum of viable mutation in tomato.

Of the different putative macro-mutants that could be isolated in the three genotypes in M_2 generation, five (exserted stigma flower (50 Gy gamma rays), dark green fruit (150 Gy gamma rays), dwarf plant having pyriform fruit (200 Gy gamma rays) from Patharkutchi; multiparous cyme (50 Gy gamma rays) from EC620177 and viable chlorophyll deficient mutant (0.10%EMS) from EC620176 hold immense promise for their utilization in tomato breeding programme.

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