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Research Article

Isolation of induced mutants using gamma ray and ethyl methane sulphonate in Tomato (*Solanum lycopersicum* L.)

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Abstract
Present investigation was undertaken to compare the mutagenic efficiency and effectiveness of gamma ray and ethyl methane sulphonate (EMS) and to study the frequency and spectrum of macro-mutations in tomato. For this purpose, two cultivars of tomato with wide divergent in origin namely Patharkutchi of West Bengal, India and Alisa Craig of England were treated with 50, 100, 150, 200 and 250 Gy gamma rays and 0.05, 0.10, 0.15, 0.20 and 0.25% (V/V) EMS solution. Results showed that seed germination, seedling height and pollen fertility in M_1 generation reduced steadily with the increasing doses of both mutagens. The LD_{50} dose for Patharkutchi and Alisa Craig was 310.7 Gy and 229.7 Gy gamma ray, 0.30% and 0.20% EMS concentration, respectively. Gamma ray (50 Gy to 150 Gy) proved to be more efficient and effective mutagen followed by 0.05% to 10% EMS treatment. Five true breeding mutants hold promise for their utilization in tomato breeding programme.

Key words: Tomato, Gamma Ray, Ethyl methane sulphonate, Macro-mutants

Introduction

Induced mutagenesis has great potential as a complimentary approach in genetic improvement of crops. Exposing the genetic materials to mutagenic agents bring changes in nuclear DNA and/or cytoplasmic organelles which results in genomic or chromosomal mutations enabling the plant breeders to select useful mutants. Among the physical mutagens, gamma rays is the most widely used ionizing radiation that produces several useful mutants (Hitoshi, 2008) due to the property of large-scale deletions (Naito *et al.,* 2005) and occasionally, chromosome reconstitution. On the other hand, ethyl methane sulphonate (EMS) is considered as the most effective chemical mutagenic agent to induce genetic variability in a number of crop plants through primarily G/C- to-A/T transitions (Greene *et al.,* 2003; Devi and Mullainathan, 2011). The present investigation was undertaken to compare the mutagenic efficiency and effectiveness of gamma ray and EMS and to study the frequency and spectrum of macro-mutations in two widely divergent genotypes of tomato (*Solanum lycopersicum* L.).

Materials and methods

The present investigation was undertaken during autumn-winter season of 2010-2013 at the Department of Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal. Pre-soaked seeds (6 h, in water) of Patharkutchi (highly adaptable local cultivar of West Bengal, India) and Alisa Craig (old and popular cultivar of England) were treated with freshly prepared 0.05, 0.10, 0.15, 0.20 and 0.25% (V/V) EMS solution (Sigma Chemical Company, USA) in phosphate buffer (pH 7.0) for 6 h at 25±2 C° and rinsed thoroughly in running water for an hour and dried before sowing. The dry seeds of above cultivars were subjected to the gamma irradiation at National Botanical Research Institute, Lucknow, India with 50, 100, 150, 200 and 250 Gy gamma ray using Co^{60} as source. The crop was raised with three replications (100 seeds in each) along with control (non-treated seeds) at Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya situated at 22°57′N lat and 88°20′E long with an average altitude of 9.75 m above the mean sea level. Germination % at 12 days after sowing and seedling height at 25 days after sowing and pollen fertility (pollen stainability with 1% acetocarmin solution) were determined in M_1 generation over control and were referred to as lethality (L), injury (I) and sterility (S), respectively. LD_{50} dose (the dose required to kill 50% population) were determined by probit analysis (Finney, 1971). Seeds of the M_1 generation from each treatment were advanced to M_2 generation. Chlorophyll deficient mutants (both viable and non-viable) in M_2 progenies were recorded to determine the chlorophyll mutation frequency. Total mutation frequency (Mf) was determined as % of mutated M_2 progenies. Mutagenic efficiency was determined using standard formula i.e., (Mf/L); (Mf/I) and (Mf/S)

(Konzak *et al.,* 1965). The mutagenic effectiveness was determined by using the formula Mf x 100/KR or (Mf x 100)/(C x T) where, KR, T and C indicates dose of radiation in kilorad, duration of

treatment in hours and percent concentration of EMS solution, respectively (Konzak *et al.,* 1965). Useful promising mutants were identified in M_2 generation and were confirmed in M_3 and M_4 generations. Observations on different quantitative traits namely plant height, days to first flowering, fruit per plant, fruit weight and qualitative traits namely total leaf chlorophyll, lycopene, β-carotene, ascorbic acid as per Sadasivam and Manickam (1996), total sugar as per Dubois *et al.* (1956), total phenol content as per Singleton *et al.* (1999) were recorded. To study the degree of tolerance under the natural infection of *Alternaria solani*, infected leaf sample was collected and surface-sterilized with NaClO solution (2 min) diluted to give 2% (v/v) available chlorine, rinsed with sterile distilled water, placed on potato dextrose agar (PDA) and incubated at 27±1°C. The fungus was identified based on morphological characteristics. The pathogenecity study through Koch's postulates was completed by detach leaf technique. *Alternaria solani* was re-isolated from the lesions. Randomly collected 10 leaves from each 10 plants and each treatment were considered as sample. The percent disease index (PDI) was calculated using the standard formula (Wheeler, 1969) and percentage of the infected area of leaves was measured using the disease rating 0-4 scale given by Peteira *et al*. (2002) (0 = no symptoms, 1 = 0-10%, 2=10-25%, 3 = 25-50%, and 4 = 50-100%). Data were processed by analysis of variance and Duncan's multiple range test of the SPSS program version 17 was used for the comparison among treatment means.

Results and discussion

Biological damages in M_1 generation: In both the genotypes, all biological damage sharply increased with the increasing doses of both mutagens (Table 1). Trend of reduction in germination percentage might be due to damage of cell constituents at molecular level and/or altered enzyme activity (Khan and Goyal, 2009). Similar results were obtained in case of seedling height which might be due to physiological abnormality or hormonal imbalances (Gunckel and Sparrow, 1961). Whereas, increasing pollen sterility might be the result of meiotic abnormalities (Khan and Wani, 2005) and/or chromosomal aberrations (Roychowdhury and Tah, 2011). However, results clearly showed that EMS caused more biological damage than the gamma ray in both the genotypes, particularly for seed germination. In the genotype Patharkutchi, seed germination percentage of 57.67 due to 0.05% EMS treatment was significantly lower than 71.0% due to 50 Gy γ radiation and similar was the trend in Alisa Craig (Table 1).

LD_{50} dose: Reduction in seed germination percentage was significantly and positively correlated with the increasing doses of both gamma rays (r = 0.909 in Patharkutchi and r= 0.968 in Alisa Craig) and EMS concentration (r= 0.922 in Patharkutchi and r = 0.935 in Alisa Craig). The LD_{50} value for Patharkutchi and Alisa Craig corresponded to 310.7 Gy and 229.7 Gy gamma ray, 0.30% and 0.20% EMS concentration, respectively. Well adapted local cultivar Patharkutchi clearly showed less sensitivity to both mutagens than the European cultivar Alisa Craig indicating that effect of mutagen largely varied with the genotype differences, which was supported by our previous work (Sikder *et al.,* 2013).

Chlorophyll mutation frequency and total mutation frequency: In the present investigation, we did not get any viable chlorophyll mutants (Table 2). The chlorophyll mutants were mostly "Albino" and "Xantha" type of white to yellow coloured leaf which died within 10 to 15 days after sowing. Chlorophyll and total mutation frequency was maximum with the exposure of 250 Gy gamma irradiation i.e., 4.00 % and 7.33% in Patharkutchi and 3.00% and 6.67% in Alisa Craig, respectively (Table 2). Frequency of chlorophyll mutation and total mutation were increased with the increasing doses of both gamma radiation and EMS (Table 2). Irrespective of doses, highest chlorophyll mutants of 43 in number were found in Patharkutchi by treating with gamma radiation and least of 17 in Alisa Craig by EMS treatment. Localized chromosome breakage (Natarajan and Upadhya, 1964) and/or differences in the chemical composition of the chromosomes near the centromere (Chopra 2005) might be the reason of getting chlorophyll deficient mutants by induced mutagenesis.

Macro-mutants in M_2 generation: Highest number of macro-mutants, 38 in Alisa Craig and 33 in Patharkutchi could be isolated due to treatment with gamma radiation compared to EMS treatment (Table 2). Sato *et al.* (2006) opined that gamma ray induced mutations involve gene truncation and allowed for more efficient screening of knockout mutants than EMS mutagenesis and the present results supported this view. Among the different macro mutants, the fruit mutants (fruit shape, size, colour, shape) were most frequent along with leaf mutants (leaf shape, size, and orientation).

Mutagenic efficiency and effectiveness: Mutagenic efficiency is the proportion of the desirable mutation frequency in relation to damages associated with mutation **(**Konzak *et al.*, 1965**).** In

the present investigation, mutagenic efficiency increased with the increasing doses that due to increasing biological damages were associated with high degree of morphological mutant. However, in both the genotypes mutagenic effectiveness decreased with the increasing doses of both gamma irradiation and EMS (Table 3). It indicated that both the mutagens were most effective at lower doses as observed in the previous work (Shah *et al.,* 2008). The results also suggested that Patharkutchi was more vulnerable than the European cultivar Alisa Craig to mutagenic treatments. According to Blixt (1970), effectiveness of any mutagen depends not only on its dose or concentration but also its specificity to act on gene and genetic make-up of the cultivars. However, gamma ray (50 Gy to 150 Gy) proved to be most efficient and effective mutagen followed by 0.05% to 10% EMS treatment in inducing wide array of macro-mutants.

Characterization of useful macro-mutants in M_3 and M_4 generations: In the present investigation, out of total 131 macro-mutants (including chlorophyll mutants), only 3 mutants isolated from Patharkutchi and 2 mutants from Alisa Craig were promising. In Patharkutchi, mutant P150Gy11 (dwarf plant with pyriform shaped fruit), P100Gy6 (early flowering with high yield), P200Gy21 (dark green fruit) were isolated through 150Gy, 100Gy and 200Gy gamma radiations, respectively and in Alisa Craig, the mutant A100Gy7 (high yielding) and A200Gy26 (dwarf plant with high yield) were obtained through 100Gy and 200Gy gamma radiation, respectively. Very little variation in the performance of all the mutants over two consecutive years indicated the stability in their performances (Table 4). Mutant P150Gy11 produced "Pyriform" shaped fruit in contrast to flat-round fruit in Patharkutchi and plant height reduced significantly (52.79% over the control). Early works also reported "Dwarf mutant" through applied mutagenesis in chilli and sweet pepper (Honda, 2006; Devi and Mullainathan, 2011). Among the other traits, fruit weight, total leaf chlorophyll and ascorbic acid content were significantly increased as 66.45%, 6.6% and 30.14% over the control, respectively. Whereas, fruit per plant and total sugar content were reduced to 29.4% and 19.1% over the control, respectively. In P100Gy6, fruit per plant and total sugar significantly increased over the parent i.e., 21.44% and 5.51% respectively. But, days to flowering was reduced by 16.11% attributing to earliness which is also a desirable character. Mutant P200Gy21 was less branched with dark green leaf and dark green colour fruit. In this mutant, fruit weight (21.29%), total chlorophyll (26.76%), lycopene (45.86%), β-carotene (28.99%), ascorbic acid (28.54%) and total phenol (42.54%) were significantly increased over the parent. But, this mutant was associated with some undesirable traits like shy fruiting and delayed flowering. This induced mutant with dark green fruit resembled the already identified spontaneous mutant with dark green fruit locus *dg* located in chromosome 1 (Levin *et al*., 2003) which enhanced fruit carotenoid content (Van Tuinen *et al*., 1997). In Alisa Craig, mutant A100Gy7 had increased fruit weight (19.53%) and total leaf phenol content (30.44%) over the parents. But, plant height, fruit per plant and total sugar were significantly reduced. Whereas, the high yielding and dwarf mutant A200Gy26 was characterized by reduced plant height (41.79%) and increased fruit weight (19.62%) and fruits per plant (17.36%) with respect to the parent.

Screening of macro-mutants against Alternaria solani: The PDI clearly indicated that the mutants showed relatively more tolerance against *A. solani* under natural infection at field level than their respective parents (Table 5). Mutants P200Gy21 of Patharkutchi and A100Gy7 of Alisa Craig showed significantly lower reduction in PDI (Table 5). Phenolic compounds have immense role in defence mechanism against stress by plant pathogens (Khatun *et al.,* 2009). Enhanced phenolic content in leaf of P200Gy21 and A100Gy7 supported our PDI results. Reduced total sugar level in these genotypes may be attributed to the water stress developed through carbohydrate hydrolytic enzymes during fungal infection of plant (Otani *et al.,* 1995) which play stimulatory effect on carbohydrate hydrolytic enzymes of host plant (Abdalla and El-Khoshiban, 2007) and increase sugar contents in diseased plants compared to control plants.

It may be concluded that gamma ray mutagenesis can effectively be utilized in the development of desirable economical and quality traits along with some degree of tolerance against biotic stresses in tomato.

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

Mutagenic treatments	Germination (%)	Seedling height (cm)	Pollen fertility (%)
Patharkutchi			
Parent	84.67	14.17	86.23
50 Gy γ ray	71.00 (-16.15)	9.22 (-34.93)	58.97 (-31.61)
100 Gy γ ray	68.67 (-18.90)	8.54 (-39.73)	55.67 (-35.44)
150 Gy γ ray	55.67 (-34.25)	8.11 (-42.77)	49.07 (-43.09)
200 Gy γ ray	52.00 (-38.59)	7.51 (-47.00)	48.69 (-43.53)
250 Gy γ ray	44.33 (-47.64)	6.99 (-50.67)	46.23 (-46.39)
0.05% EMS	57.67 (-31.89)	8.97 (-36.70)	55.92 (-35.15)
0.10% EMS	55.00 (-35.04)	8.01 (-43.47)	54.78 (-36.47)
0.15% EMS	51.00 (-39.77)	7.65 (-46.01)	50.23 (-41.75)
0.20% EMS	46.33 (-45.28)	6.98 (-50.74)	48.27 (-44.02)
0.25% EMS	43.00 (-49.21)	6.23 (-56.03)	45.67 (-47.04)
SEM±	3.43	0.64	3.43
CD at 5%	11.39	2.13	11.39
AlisaCraig			
Parent	83.00	17.21	85.67
50 Gy γ ray	71.67 (-13.65)	10.45(-39.28)	57.36 (-33.05)
100 Gy γ ray	64.33 (-22.49)	9.33 (-45.79)	54.76 (-36.08)
150 Gy γ ray	51.67 (-37.75)	8.21 (-52.30)	50.34 (-41.24)
200 Gy γ ray	45.67 (-44.98)	7.56 (-56.07)	46.71 (-45.48)
250 Gy γ ray	37.33 (-55.02)	7.03 (-59.15)	43.22 (-49.55)
0.05% EMS	58.67 (-29.32)	9.37 (-45.55)	54.65 (-36.21)
0.10% EMS	54.00 (-34.94)	8.64 (-49.80)	51.98 (-39.33)
0.15% EMS	47.67 (-42.57)	7.96 (-53.75)	47.68 (-44.34)
0.20% EMS	42.33 (-49.00)	7.04 (-59.09)	44.61 (-47.93)
0.25% EMS	35.00 (-57.83)	6.75 (-60.78)	41.74 (-51.28)
SEM±	4.44	0.89	3.64
CD at 5%	14.75	2.96	12.09

Figure in parenthesis show percent reduction over respective parents

Table 2. Chlorophyll mutation frequency and total mutation frequency in M_2 generation of tomato

Mutagenic treatment	M2 plants examined	Nonviable chlorophyll mutant	Viable chlorophyll mutant	Macro mutants	Chlorophyll mutation frequency (%)	Total mutation frequency (%)
Patharkutchi						
50 Gy γ ray	300	5	0	4	1.67	3.00
100 Gy γ ray	300	6	0	5	2.00	3.67
150 Gy γ ray	300	10	0	6	3.33	5.33
200 Gy γ ray	300	10	0	8	3.33	6.00
250 Gy γ ray	300	12	0	10	4.00	7.33
0.05% EMS	300	3	0	2	1.00	1.67
0.10% EMS	300	4	0	5	1.33	3.00
0.15% EMS	300	5	0	7	1.67	4.00
0.20% EMS	300	7	0	8	2.33	5.00
0.25% EMS	300	9	0	8	3.00	5.67
AlisaCraig						
50 Gy γ ray	300	3	0	3	1.00	2.00
100 Gy γ ray	300	5	0	5	1.67	3.33
150 Gy γ ray	300	5	0	10	1.67	5.00
200 Gy γ ray	300	8	0	9	2.67	5.67
250 Gy γ ray	300	9	0	11	3.00	6.67
0.05% EMS	300	2	0	1	0.67	1.00
0.10% EMS	300	4	0	1	1.33	1.67
0.15% EMS	300	3	0	4	1.00	2.33
0.20% EMS	300	3	0	6	1.00	3.00
0.25% EMS	300	5	0	6	1.67	3.67

Table 3. Efficiency and effectiveness of gamma ray and EMS in Patharkutchi and Alisa Craig of tomato

Mutagenic treatment	Total mutation frequency (Mf)	Lethality (L)	Mutagenic efficiency (Mf/L)	Injury (I)	Mutagenic efficiency (Mf/I)	Pollen sterility (S)	Mutagenic efficiency (Mf/S)	Mutagenic effective-ness
Patharkutchi								
50 Gy γ ray	3.00	16.15	0.186	34.93	0.086	31.61	0.095	60.00
100 Gy γ ray	3.67	18.9	0.194	39.73	0.092	35.44	0.103	36.67
150 Gy γ ray	5.33	34.25	0.156	42.77	0.125	43.09	0.124	35.56
200 Gy γ ray	6.00	38.59	0.155	47.00	0.128	43.53	0.138	30.00
250 Gy γ ray	7.33	47.64	0.154	50.67	0.145	46.39	0.158	29.33
0.05% EMS	1.67	31.89	0.052	36.7	0.045	35.15	0.047	5.56
0.10% EMS	3.00	35.04	0.086	43.47	0.069	36.47	0.082	5.00
0.15% EMS	4.00	39.77	0.101	46.01	0.087	41.75	0.096	4.44
0.20% EMS	5.00	45.28	0.110	50.74	0.099	44.02	0.114	4.17
0.25% EMS	5.67	49.21	0.115	56.03	0.101	47.04	0.120	3.78
Alisa Craig								
50 Gy γ ray	2.00	13.65	0.147	39.28	0.051	33.05	0.061	40.00
100 Gy γ ray	3.33	22.49	0.148	45.79	0.073	36.08	0.092	33.33
150 Gy γ ray	5.00	37.75	0.132	52.3	0.096	41.24	0.121	33.33
200 Gy γ ray	5.67	44.98	0.126	56.07	0.101	45.48	0.125	28.33
250 Gy γ ray	6.67	55.02	0.121	59.15	0.113	49.55	0.135	26.67
0.05% EMS	1.00	29.32	0.034	45.55	0.022	36.21	0.028	3.33
0.10% EMS	1.67	34.94	0.048	49.8	0.033	39.33	0.042	2.78
0.15% EMS	2.33	42.57	0.055	53.75	0.043	44.34	0.053	2.59
0.20% EMS	3.00	49	0.061	59.09	0.051	47.93	0.063	2.50
0.25% EMS	3.67	57.83	0.063	60.78	0.060	51.28	0.072	2.44

Table 4. Observations on quantitative characters of promising macro-mutants of Patharkutchi and AlisaCraig in M_3 and M_4 generations of tomato

Mutants	Plant height (cm)			Days to 1st flower			Fruit per plant			Fruit weight (g)			Total chlorophyll (mg/100g)		
	M_3	M_4	Pooled	M_3	M_4	Pooled	M_3	M_4	Pooled	M_3	M_4	Pooled	M_3	M_4	Pooled
Patharkutchi															
Parent	141.04	134.70	137.87^{c}	38.36	36.12	37.24^{b}	51.63	44.81	48.22^{c}	75.14	68.88	72.01^{a}	208.58	195.68	202.13^{a}
P150Gy11	58.10	65.00	61.55^{a}	39.91	44.99	42.45b^{c}	33.22	35.66	34.44^{b}	120.76	110.52	115.64^{c}	223.04	207.90	215.47^{b}
P100Gy6	136.12	126.36	131.24^{c}	29.67	32.81	31.24^{a}	61.57	55.55	58.56^{d}	65.08	70.60	67.84^{a}	203.52	193.78	198.65^{a}
P200Gy21	106.67	96.17	101.42^{b}	47.78	43.74	45.76^{c}	13.68	11.00	12.34^{a}	90.99	83.69	87.34^{b}	260.58	251.86	256.22^{c}
Alisa Craig															
Parent	118.26	127.60	122.93^{c}	36.35	32.67	34.51^{a}	59.94	51.20	55.57^{b}	43.60	36.44	40.02^{a}	179.81	168.73	174.27^{a}
A100Gy7	94.35	100.57	97.46^{b}	36.95	34.27	35.61^{a}	45.84	50.74	48.29^{a}	113.62	122.12	117.87^{c}	186.35	176.13	181.24^{a}
A200Gy26	69.57	73.53	71.55^{a}	32.48	34.42	33.45^{a}	61.34	69.10	65.22^{c}	45.25	50.49	47.87^{b}	188.55	176.11	182.33^{a}

Mutants	Lycopene (mg/100g)			β-Carotene (mg/100g)			Total sugar (%)			Ascorbic acid (mg/100g)			Total leaf phenol (mg/100g)		
	M_3	M_4	Pooled	M_3	M_4	Pooled	M_3	M_4	Pooled	M_3	M_4	Pooled	M_3	M_4	Pooled
Patharkutchi															
Control	4.06	4.40	4.23a	0.74	0.64	0.69a	3.56	3.34	3.45b	24.76	28.98	26.87a	26.54	22.36	24.45ab
P150Gy11	4.04	4.32	4.18a	0.79	0.63	0.71a	2.89	2.79	2.84a	31.96	37.12	34.54b	29.49	25.79	27.64b
P100Gy6	4.20	4.34	4.27a	0.68	0.60	0.64a	3.71	3.57	3.64c	20.91	24.65	22.78a	22.46	20.24	21.35a
P200Gy21	6.06	6.28	6.17b	0.98	0.80	0.89b	3.46	3.28	3.37b	34.27	40.35	37.31b	37.56	32.14	34.85c
Alisa Craig															
Control	4.16	4.06	4.11a	0.77	0.65	0.71a	3.26	3.18	3.22b	25.67	29.69	27.68a	23.82	21.32	22.57a
A100Gy7	4.26	4.08	4.17a	0.78	0.70	0.74a	3.02	2.94	2.98a	23.54	26.90	25.22a	31.48	27.40	29.44b
A200Gy26	4.03	4.25	4.14a	0.79	0.69	0.74a	3.36	3.18	3.27b	28.28	31.38	29.83a	22.34	20.40	21.37a

Means followed by the same letters are not significant at p=0.05

Table 5. Percent disease index (PDI) in macro-mutants of Patharkutchi and Alisa Craig of tomato

Genotype	PDI
P200Gy21	31
P150Gy11	37
P100Gy6	50
Patharkutchi	62
A100Gy7	34
A200Gy26	44
Alisa Craig	64
SEM ±	0.68
CD at 5%	2.09