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Estimation of mutagenic efficiency and effectiveness of ethyl methane sulphonate in two divergent lines of tomato (*Solanum lycopersicum* L.)

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Abstract

Present investigation was carried out to study the frequency and spectrum of macro-mutations along with mutagenic efficiency and effectiveness of ethyl methane sulphonate (EMS) in tomato. For this purpose, two tomato cultivars "Patharkutchi" and "Alisa Craig" were subjected to EMS treatments employing 10 concentrations which ranged between 0.05 to 0.50% solutions. Results showed that seed germination, seedling height and pollen fertility in M_1 generation reduced steadily with the increase in concentrations of EMS. The LD_{50} dose for Patharkutchi and Alisa Craig was 0.64 and 0.49%, respectively. Mutation frequency and mutagenic efficiency increased with the increasing concentration up to a certain level which there after started to decrease in both the genotypes. Mutagenic effectiveness generally decreased with the increase in the concentration of EMS solution. The most effective and efficient EMS treatment was 0.20 to 0.30% solution at which maximum number of desirable variation occurred in M_2 generation in both the genotypes and those variants can be utilized in further genetic study and breeding programme of tomato.

Keywords: Ethyl methane sulphonate, $LD_{50,}$ Chlorophyll mutants, Mutagenic efficiency and Effectiveness.

Introduction

Tomato (*Solanum lycopersicum* L.), member of the family Solanaceae, is one of the most important vegetable crop for both fresh market and food processing industry. Its consumption worldwide is second only to potato as a vegetable (Javanmardi and Kubota 2006). Tomato contains abundant and well balanced nutrients consisting of vitamins (A, B, C), minerals, dietary fiber and lycopene (Toor *et al.,* 2006). It is can be used as an excellent model plant for studying the fruit development, ripening and metabolism of novel metabolites (Carrari and Fernie 2006, Mochida and Shinozaki 2010).

Induced mutation is very effective tool for creating genetic variation in quantitatively and qualitatively inherited traits and thereby making crop improvement from economic point of view (Maduli and Mishra 2007, Khan and Goyal 2009, Kozgar *et al.,* 2011). Among different chemical mutagens, ethyl methane sulphonate (EMS) is an effective mutagen and has been used to induce genetic variability in a number of crop plants (Jabeen and Mirza 2002, Chopra 2005). It alkylates the guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C- to-A/T transitions (Bhat *et al.,* 2007). The present investigation was under taken to study the frequency and spectrum of macro-mutations along with mutagenic efficiency and effectiveness of ethyl methane sulphonate (EMS) in two genotypes of tomato with a view to induce genetic variation for further utilization in tomato breeding programme.

Materials and Methods

i. Study site

The present investigation was undertaken in the Department of Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, situated at 22°57'N lat and 88°20'E long with an average altitude of 9.75 m above the mean sea level during 2011-2013.

ii. Treatment with EMS

Pre-soaked seeds (6 h, in water) of Patharkutchi (highly adaptable and popular cultivar of West Bengal) and Alisa Craig (old and popular cultivar of England) were treated

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with freshly prepared 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50% (V/V) EMS solution (6 h at 25±2 C°) and treated seeds were rinsed thoroughly in running water for an hour and dried on filter paper before sowing.

iii. Study of M_1 generation

Sowing was done in three replications (100 seeds in each) for each treatment along with their parental controls (non-treated seeds). Germination reduction (%) in M_1 generation at 12 days after sowing over control was referred to as lethality (L). Reduction in seedling height recorded in the seed bed at 25 days after sowing over control plants was termed as injury (I). 50 M_1 seedlings of 25 days old per replication for each treatment were subsequently transplanted in the main field. Pollen viability determined by pollen stainability with 1% acetocarmin solution and its reduction (%) were recorded over the control was called sterility (S). LD_{50} dose (the dose required to kill 50% population) were determined by probit analysis (Finney 1971). Seeds of the M_1 generation for each treatment were bulked separately to raise the M_2 generation.

iv. Identification of mutants in M_2 generation

The M_2 generations of the respective treatment along with the parental control were evaluated in randomized block design with 3 replications. Data on different characters viz., growth habit, branching pattern, leaf orientation, leaf let type, leaf colour, inflorescence type, flower size, sepal size, anther déhiscence, exsertion of stigma, stigma length, anther length, pollen viability, fruit shape, green shoulder, pedicel attachment, fruit ribbing, fruit pubescence, blossom end of fruit, unripe fruit colour and ripe fruit colour were recorded to document the deviation with respect to parental control. Chlorophyll deficient mutants (both viable and non-viable) in M_2 progenies was recorded to determine the Chlorophyll mutation frequency.

v. Determination of mutagenic efficiency and effectiveness

Total mutation frequency (Mf) was determined as percentage of mutated M_2 progenies for both chlorophyll deficient and other viable macro-mutants. Mutagenic efficiency and effectiveness were determined using standard formula (Kozak *et al.,* 1965). The formulae used for mutagenic efficiency were (Mf/L); (Mf/I) and (Mf/S). The mutagenic effectiveness was determined by using the formula Mf x 100/C x T where, T and C indicates duration of treatment in hours and percent concentration of EMS solution, respectively.

Results and Discussion

Biological damage in M_1 generation

In both the genotypes, reduction in germination (%) was highest at 0.50% EMS dose i.e. 49.02% in Patharkutchi and 54.39% in Alisa Craig (Table 1). In both the cases, with the increase in EMS concentration there was sharp reduction in germination which might be due to a number of factors viz., adverse effect on cytochrome oxidase content, thus reducing the respiration (Swaminathan *et al.,* 1962), drastic distortion of the actively dividing phase (Singh and Singh, 1989), damage of cell constituents at molecular level (Khan and Goyal, 2009), interference in the synthesis of enzyme and acceleration in the degradation of existing enzyme (Yusuf and Nair, 1974) and/or altered enzyme activity (Khan and Goyal, 2009). Seedling height also reduced maximum at 0.50% EMS concentration in both the genotypes i.e., 62.04% in Patharkutchi and 60.33% in Alisa Craig (Table 1). This reduction in seedling height might have been due to abnormality in physiological systems (Gaul, 1977) and growth hormone (Gunckel and Sparrow, 1961). Pollen fertility was recorded to be inversely proportional to the dose and maximised at 0.50% EMS concentration in both Patharkutchi and Alisa Craig (Table 1). In most of the cases, meiotic abnormalities (Mathusamy and Jayabalan 2002, Khan

Table 1: Effect of EMS on seed germination, seedling height and pollen fertility in M_1 generation

Mutagenic treatments	Germination (%)	Seedling height (cm)	Pollen fertility (%)
Patharkutchi			
Control	84.81	23.13	83.95
0.05% EMS	71.22	12.24	71.84
0.10% EMS	63.31	12.03	69.86
0.15% EMS	59.25	11.57	68.23
0.20% EMS	58.84	11.36	65.52
0.25% EMS	56.34	10.98	61.37
0.30% EMS	54.34	10.56	61.03
0.35% EMS	51.23	10.07	57.01
0.40% EMS	47.84	9.84	56.23
0.45% EMS	47.76	9.31	55.36
0.50% EMS	43.24	8.78	52.77
SEM±	3.58	1.18	2.76
AlisaCraig			
Control	89.94	19.56	80.35
0.05% EMS	76.67	10.91	69.85
0.10% EMS	68.87	10.84	68.54
0.15% EMS	65.54	10.52	67.32
0.20% EMS	63.21	9.87	67.01
0.25% EMS	59.81	9.65	66.51
0.30% EMS	54.66	9.21	63.71
0.35% EMS	50.19	8.96	63.15
0.40% EMS	46.83	8.59	60.11
0.45% EMS	44.32	8.01	56.32
0.50% EMS	41.02	7.76	53.25
SEM±	4.47	0.97	2.18

and Wani 2005) and/or some genetic and physiological changes (Roychowdhury and Tah, 2011) are responsible for pollen sterility.

LD_{50} dose

Reduction in seed germination (%) was positively correlated with the increasing EMS concentration for both genotypes (r = + 0.986 in Patharkutchi and r = + 0.940 in Alisa Craig). LD50 dose was 0.64 % EMS for the Patharkutchi and 0.49% EMS for Alisa Craig. It indicated that the European cultivar, Alisa Craig was more susceptible to mutagenic treatments.

Chlorophyll mutation frequency

Although there is no economic value of chlorophyll mutants, but these provides the most dependable indices for estimating the genetic effect of mutagen (Gautam *et al.,* 1998). Chlorophyll mutation frequency was highest at 0.50% EMS for Patharkutchi (3.48%) and 0.50% and 0.45% EMS for Alisa Craig (3.33%), Table 2. There was increase in chlorophyll mutation frequencies with the increasing EMS concentration and this finding was supported by some earlier works (Sakin and Sencar 2002, Mangaiyarkarasi *et al.,* 2014). Single viable chlorophyll deficient mutant was recorded at each 0.05% and 0.10% EMS in Patharkutchi and 0.10% EMS in Alisa Craig. Irrespective of the dose, maximum number of chlorophyll mutants was recorded in Patharkutchi (43 nonviable and 2 viable) followed by in Alisa Craig (40 nonviable and 1 viable). Most of the nonviable mutants were "Albino" type which died within 12-19 days after sowing. Huge reduction in chlorophyll contents in the viable chlorophyll mutants caused yellow to yellowish green colour plants with reduced fertility. Occurrence of chlorophyll mutation might be due to differences in the chemical composition of the chromosomes near the centromere, making them more sensitive to chemical mutagens (Chopra, 2005).

Macro mutants

Among the different macro mutants, the fruit mutants (fruit shape, size, colour) were most frequent along with leaf mutants (leaf shape, size, orientation). In M_2 segregating population, maximum variation was recorded with the treatment of EMS at 0.25% and 0.30% concentration in Patharkutchi and 0.30% concentration in Alisa Craig (Table 2).

Total mutation frequency

There was a trend of increase in mutation frequency with the increasing concentration of EMS in both the genotypes. But, after a certain level it started to reduce (Table 2). In Patharkutchi, mutation frequency was highest at 0.35% EMS (4.71%) and reduced to 3.91% at 0.50% EMS concentration. In Alisa Craig also, mutation frequency increased to the highest value of 3.85% at 0.35% EMS concentration which there after reduced to 3.33%. This kind of trend might be attributed to chromosomal aberrations or saturation in the mutational events (Mehraj-ud-din, 1999).

Table 2: Chlorophyll mutation frequency and Total mutation frequency in M_2 generation

Mutagenic treatment	M2 plants examined	Viable chlorophyll mutant	Nonviable chlorophyll mutant	Macro mutants	Chlorophyll mutation frequency (%)	Total mutation frequency (%)
Patharkutchi						
0.05% EMS	300	1	0	5	0.33	2.00
0.10% EMS	300	1	1	5	0.67	2.33
0.15% EMS	300	0	2	6	0.67	2.67
0.20% EMS	280	0	3	5	1.07	2.86
0.25% EMS	270	0	5	7	1.85	4.44
0.30% EMS	270	0	5	7	1.85	4.44
0.35% EMS	255	0	7	5	2.75	4.71
0.40% EMS	245	0	5	5	2.04	4.08
0.45% EMS	230	0	7	2	3.04	3.91
0.50% EMS	230	0	8	1	3.48	3.91
Alisa Craig						
0.05% EMS	300	0	2	3	0.67	1.67
0.10% EMS	300	1	1	4	0.67	2.00
0.15% EMS	300	0	4	4	1.33	2.67
0.20% EMS	270	0	4	5	1.48	3.33
0.25% EMS	250	0	5	4	2.00	3.60
0.30% EMS	260	0	4	6	1.54	3.85
0.35% EMS	245	0	6	3	2.45	3.67
0.40% EMS	230	0	7	1	3.04	3.48
0.45% EMS	210	0	7	1	3.33	3.81
0.50% EMS	210	0	7	0	3.33	3.33

Table 3: Mutagenic efficiency and effectiveness in M_2 generation

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 effectiveness
Patharkutchi								
0.05% EMS	2.00	23.10	0.087	47.08	0.042	26.34	0.076	6.67
0.10% EMS	2.33	25.35	0.092	47.99	0.049	28.91	0.081	3.88
0.15% EMS	2.67	30.14	0.088	49.98	0.053	30.64	0.087	2.97
0.20% EMS	2.86	30.62	0.093	50.89	0.056	33.87	0.084	2.38
0.25% EMS	4.44	33.57	0.132	52.53	0.085	38.81	0.115	2.96
0.30% EMS	4.44	35.93	0.124	54.35	0.082	39.21	0.113	2.47
0.35% EMS	4.71	39.59	0.119	56.46	0.083	44.00	0.107	2.24
0.40% EMS	4.08	43.59	0.094	57.46	0.071	44.93	0.091	1.70
0.45% EMS	3.91	43.69	0.090	59.75	0.065	45.97	0.085	1.45
0.50% EMS	3.91	49.02	0.080	62.04	0.063	49.30	0.079	1.30
Alisa Craig								
0.05% EMS	1.67	25.87	0.077	44.22	0.045	25.51	0.078	5.57
0.10% EMS	2.00	26.82	0.087	44.58	0.052	27.14	0.086	3.33
0.15% EMS	2.67	27.13	0.098	46.22	0.058	28.66	0.093	2.97
0.20% EMS	3.33	29.72	0.096	49.54	0.058	29.05	0.098	2.78
0.25% EMS	3.60	33.50	0.133	50.66	0.088	29.67	0.150	2.40
0.30% EMS	3.85	39.23	0.113	52.91	0.084	33.15	0.134	2.14
0.35% EMS	3.67	44.20	0.106	54.19	0.087	33.85	0.139	1.75
0.40% EMS	3.48	47.93	0.085	56.08	0.073	37.64	0.108	1.45
0.45% EMS	3.81	50.72	0.077	59.05	0.066	42.35	0.092	1.41
0.50% EMS	3.33	54.39	0.072	60.33	0.065	46.17	0.085	1.11

Mutagenic efficiency

The higher efficiency of a mutagen indicates relatively less biological damage (seedling injury, pollen sterility, ovule sterility etc.) in relation to the mutagenic treatments (Shah *et al.,,* 2008). In mutation breeding programme such treatments are not desirable that does not produce useful mutants. In both the genotypes, Patharkutchi and Alisa Craig, mutagenic efficiency on the basis of lethality, injury and sterility was maximum at 0.25% EMS concentration (Table 3). At higher concentration of EMS there were sharp downfall in mutagenic efficiency which implied that lower doses of mutagenic treatment were more useful treatment with less biological damage and this finding agreed well to some earlier reports (Shah *et al.,* 2008, Shirsat *et al.,* 2010, Sharma *et al.,* 2006).

Mutagenic effectiveness

Mutagenic effectiveness usually means the rate of point mutations relative to the dose. In the present investigation, the mutagenic effectiveness was inversely proportionate to concentration of EMS as there was sharp decrease in effectiveness with the increase in dose of EMS. The highest value was at 0.05% EMS i.e. 6.67 in Patharkutchi and 5.57 in Alisa Craig. The lowest value was 1.30 in Patharkutchi and 1.11 in Alisa Craig at 0.50% EMS solution (Table 3). It may be concluded from the above discussion that the most effective and efficient treatment was 0.20 to 0.30 % EMS solution at which maximum number of desirable variation occurred in M_2 generation in both the genotypes and those variants can be utilized in further genetic study and breeding programme of tomato.

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सारांश

टमाटर में बारम्बारता एवं स्पेक्ट्रम ज्ञात करने के लिए मैक्रो–उभयधार्मिता के साथ उभयधर्मी प्रभाविता तथा इथाइल मेथेन सल्फोनेट (ई एम एस) की प्रभाविता का अध्ययन किया गया। इस उद्देश्य के लिए, टमाटर की दो प्रजातियों 'पाथरकुची' तथा 'एलिसा क्रैग' ई एम एस की 10 सान्द्रता का प्रयोग जो औसतन 0.05 से 0.50 प्रतिशत घोल के बीच था। परिणाम से प्राप्त हुआ कि बीज जमाव, अंकुर की ऊँचाई तथा परागकण उर्वरता एम, पीढ़ी में तेजी से घटते हुए, बढ़े ई एम सान्द्रता पर पाया गया। एत्न डी 50 की मात्रा प्रजाति 'पाथर कुची' तथा एलिसा क्रैग क्रमशः 0.64 तथा 0.49 प्रतिशत था। उभयधर्मी बारम्बारता तथा उभयधर्मिक प्रभाविता बढ़ते सान्द्रता के साथ एक निश्चित स्तर तक बढ़ता है उसके बाद घटते क्रम में दोनों प्रजातियों में पाया गया। उभयधर्मी प्रभाविता ई एम एस शोधन 0.20 से 0.30 प्रतिशत जो एम2 पीढी में अधिकतम संख्या में वांछित विविधता प्राप्त हुई और उन विविधताओं को आगे टमाटर के आनुवांशिक अध्ययन व प्रजनन कार्यक्रम के लिए उपयोग में किया जा सकता है।

References

Bhat R, Upadhyaya N, Chaudhury A, Raghavan C, Qiu F, Wang H, Wu J, McNally K, Leung H and Till B (2007) Chemical and irradiation induced mutants and TILLING. In: Upadhyaya NM (ed). Rice Functional Genomics-

Challenges, Progress and Prospects. Springer, NY 149-180.

Carrari F and Fernie AR (2006) Metabolic regulation underlying tomato fruit development. J. Exp. Bot. 57(9): 1883-1897.

Chopra VL(2005) Mutagenesis: Investigating the process and processing the outcome for crop improvement. Current Sci. 89: 353-359.

Finney DJ (1971) Probit Analysis, 3rdedn.Cambridge University Press, Cambridge, UK.

Gaul H (1977) Mutagen effects observable in the first generation. I. Plant injury and lethality, II. Cytological effects, II sterility In: Manual on Mutation Breeding, 2ndedn. IAEA technical report series No. 119, IAEA, Vienna, Austria 85-99.

Gautam AS, Sood KC and Mittal RK (1998) Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulfonate in rajmash (*Phaseolus vulgaris* L.). Legume Research 21: 3-4.

Gunckel JK and Sparrow AH (1961) Ionizing radiations: biochemical, physiological and morphological aspects of their effects on plants. Encyclopedia of Plant Physiology 16: 555-611.

Jabeen N and Mirza B (2002) Ethyl methane sulphonate enhances genetic variability in *Capsicum annuum*. Asian J. Pl. Sci. 1: 425-428.

Javanmardi J and Kubota C (2006) Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. Post harvest Bio. and Tech. 41: 151-155.

Khan S and Goyal S (2009) Improvement of mungbean varieties through induced mutations. African J. Pl. Sci. 3: 174-180.

Khan S and Wani MR (2005) Genetic variability and correlation studies in chickpea mutants. J. Cytol. and Gen. 6: 155-160.

Konzak CF, Nilan RA, Wagner J and Foster RJ (1965) Efficient chemical mutagenesis. Radiation Botany (Suppl) 5: 49-70.

Kozgar MI, Goyal S and Khan S (2011) EMS induced mutational variability in *Vigna radiata* and *Vigna mungo*. Research J. Bot. 6: 31-37.

Maduli KC and Mishra RC (2007) Efficacy of mutagenic treatments in producing useful mutants in finger millet (*Eleusine corocana* Gaertn.). Indian J. Gen. and Pl. Breed. 67: 232-237.

Mangaiyarkarasi R,Girija M and Gnanamurthy S (2014) Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in *Catharanthus roseus*. International J. Current Microb. and App. Sci. 3: 881-889.

Mathusamy A and Jayabalan N (2002) Effect of mutagens on pollen fertility of cotton (*Gossypium hirsutum* L.). Indian J. Gen. and Pl. Breed. 62(2): 187.

Mehraj-ud-din ABS, Khan S and Rehman M (1999) Induced mutations in mungbean (*Vignaradiata*(L.)Wilczek): Efficiency and effectiveness of chemical mutagens. Legume Res. 22 (4): 245-248.

Mochida K and Shinozaki K (2010) Genomics and bioinformatics resources for crop improvement. Plant and Cell Physio. 51: 497–523.

Roychowdhury R and Tah J (2011) Germination behaviours in M_2 Generation of Dianthus after Chemical Mutagenesis. International J. Adv. Sci. and Tech. Res. 2: 448-454.

Sakin MA and Sencar O (2002) The effects of different doses of gamma ray and EMS on formation of chlorophyll mutations in durum wheat (*Triticum durum* Desf.). Tarim Bilimleri Dergisi (Journal of Agricultural Sciences) 8: 15-21.

Shah TM, Mirza JI, Haq MA and Atta BM (2008) Induced genetic variability in chickpea (*Cicer arietinum* L.). Pakisthan J. Bot. 40: 605-613.

Sharma AK, Singh VP, Singh RM (2006) Efficiency and effectiveness of the gamma rays, EMS and their combinations in urdbean. Indian J. Pulses Res. 19: 111-112.

Shirsat RK, Mohrir MN, Kare MA and Bhuktar AS (2010) Induced mutations in horsegram : Mutagenic efficiency and effectiveness. Recent Res. in Sci. and Tech. 2: 20-23.

Singh RR and Singh J (1989) Genotypic variability relationship with EMS in a *Trigonella* species complex. Indian J. Horti. 46: 111-114.

Swaminathan MS, Chopra VL and Bhaskaran S (1962) Chromosome aberrations and the frequency and spectrum of mutations induced by ethyl methane sulphonate in barley and wheat. Indian J. Gen. and Pl. Breed. 22: 192-207.

Toor RK, Savage GP and Lister CE (2006) Seasonal variations in the anti-oxidant composition of greenhouse-grown tomatoes. J. Food Composition and Analysis 19: 1-10.

Yusuf KK and Nair PM (1974) Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. Radiation Bot. 14: 251-256.