Journal of Agricultural, Food and Environmental Sciences
UDC 635.64-152.86
Original scientific paper

ISOLATION OF PROMISING MUTANT OF ENHANCED FRUIT QUALITY IN TOMATO

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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, EC-620176, EC-620177 and Patharkutchi. Combination of gamma radiation and EMS caused more damage followed by EMS treatment and gamma radiation, mainly in M_1 generation. 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. One mutant "Dark green fruit" was isolated from the M_2 population of Patharkutchi treated by 150 Gy gamma radiation. Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll content in the leaf and immature fruit of this mutant was significantly higher compared to the parental genotype. Average total chlorophyll content in the leaf and immature fruit of the mutant over M_3 and M_4 generation was 318.52 mg/100 g fresh and 21.93 mg/100 g fresh, respectively in sharp contrast to 198.25 mg/100 g and 12.21 mg/100 g fresh, respectively in the leaf and immature fruit of the parental line. This "Dark green fruit" mutant with higher average lycopene (7.49 mg/100 g fresh) and ascorbic acid (35.86 mg/100 g fresh) contents in the ripe fruits emerged as a promising genetic resource for further utilization in tomato breeding for enhancement of lycopene and ascorbic acid content in the fruits.
Key words: Mutation frequency, Gamma rays, EMS, Dark green fruit, Tomato

Introduction

The cultivated tomato (*Solanum lycopersicum* L.) 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. Mutant alleles in tomato 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. The present investigation was undertaken to study the frequency and spectrum of macro-mutations 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 2010-2014. The gamma radiation was applied to dry seeds at National Botanical Research Institute, Lucknow, India where Cobalt- 60 served as source of gamma rays. Solutions of ethyl methane sulphonate (Sigma Chemical Company, USA) were made with 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 (EC-620176), Berika (EC-620177), a variety from Bulgaria and Patharkutchi, the highly adaptable and popular variety 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. Seeds from all 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 from 3 genotypes and all mutagenic treatments was 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, color and chlorophyll content over the set of characters specific for the three genotypes. Non-viable chlorophyll deficient mutants did not continue beyond 30 days after planting.

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. A number of viable macro-mutants with discernable morphological characters like, multiparous cyme, dwarf plant with pyriform fruit, irregular shaped fruit, and dark green fruit could be isolated in the M_2 generation from all three genotypes exposed to mutation. All macro-mutants were self-pollinated to produce the seeds and advanced up to M_4 generation.

The "*Dark green fruit*" mutant could be isolated from the M_2 population of the tropicalized and popular cultivar of West Bengal, Patharkutchi treated by 150 Gy gamma radiation. Selfed population of this mutant bred true in both M_3 and M_4 generation suggesting the involvement of single mutated gene for manifestation of the basic character "*Dark green fruit*" in this genotype. This putative mutant was characterized taking a number of morphological and fruit quality characters in the M_4 generation. The data was statistically compared with that of the parental genotype "Patharkutchi".

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 (reduction in seed germination), injury (reduction in length of seedlings) and sterility (reduction in pollen viability). 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 least occurred due to gamma radiation (29.82%).

Spectrum of mutation

The spectrum of mutation is essentially a parameter for the index of mutation frequency. Spectrum of mutation varied within 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 was isolated from EC-620176 in 0.1% EMS treatment. The spectrum of mutation as a whole considering both chlorophyll deficient and other macro mutations together decreased 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 EC-620176 (four) with 100Gy gamma radiation and 50 Gy gamma radiation + 0.15% EMS treatment; in EC-620177 (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. 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 (Walter *et al*. 1987; Sakin and Senkar, 2002; Prem *et al*. 2011).

Total mutation frequency

Average total mutation frequency combining three genotypes together differed significantly among the mutagens. The highest mutation frequency of 4.33 % resulted by gamma radiation followed by 3.52% in combination treatment and the least of 3.19 % in EMS treatment (Table 1). 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 sole EMS treatment. Combined treatment induced intermediate mutation frequency showing additivity because of independent action of two mutagens. Total mutation frequency also did not necessarily reflect the spectrum of mutation and for this reason, the highest of 6.96 % mutation frequency in EC-620176 with 250 Gy gamma radiation emerged from only two mutant types. 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.

Of the different putative macro-mutants that was isolated in the three genotypes in M_2 generation, five (exerted 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 EC-620177 and viable chlorophyll deficient mutant (0.10%EMS) from EC-620176 hold immense promise for their utilization in tomato breeding programme.

Table 1.Total mutation frequency in M_2 generation of three tomato genotypes

Mutagenic treatments	M_2 plants examined	Total mutants			Chlorophyll mutation frequency (%)	Total mutation frequency (Mf) %
		Viable chlorophyll mutants	Non-viable chlorophyll mutants	Viable macro-mutants		
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 Gyγ rays	154	0	2	3	1.30	3.25
100 Gyγ rays	156	0	5	1	3.21	3.84
150 Gyγ rays	171	0	5	2	2.92	4.09
200 Gyγ rays	155	0	5	1	3.23	3.87
250 Gyγ 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 Gyγ rays	172	0	2	3	1.16	2.91
100 Gyγ rays	152	0	4	1	2.63	3.29
150 Gyγ rays	157	0	4	2	2.54	3.82
200 Gyγ rays	173	0	5	3	2.89	4.04
250 Gyγ 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

Dark green fruit mutant

Selfed population of this mutant bred true in both M_3 and M_4 generation suggesting the involvement of single mutated gene for manifestation of the basic character "*Dark green fruit*" in this genotype.

Different qualitative characters as documented in this mutant and its parental genotype (Table 2) indicated deviation in 3 characters viz., twig color (dark green *vis a vis* medium green in the parent), leaf color (very dark green *vis a vis* dark green in the parent), and unripe fruit color (dark green *vis a vis* green in the parent).

Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll content in the leaf and immature fruit of this mutant was markedly high compared to the parental genotype. Average total chlorophyll content in the leaf and immature fruit of the mutant over M_3 and M_4 generation was 318.52 mg/100 g fresh and 21.93 mg/100 g fresh, respectively in sharp contrast to 198.25 mg/100 g and 12.21 mg/100 g fresh, respectively in the leaf and immature fruit of the parental line (Table 3). Isolation of high chlorophyll mutant in tomato was also reported earlier by 100 Gy gamma radiation (El-Sayed *et al*., 1994) and 24 Gy gamma radiation (Asmahan and Al-Twaty, 2006) which correspond to the present findings. Earlier reports also suggested that some useful induced mutant appeared to be allelic to some spontaneous mutations already known in tomato, such as dark green fruit (Kendrick *et al*., 1997) and jointless pedicel (Mao *et al*., 2000). This induced mutant *dark green fruit* resembled the already identified spontaneous mutant dark green fruit locus *dg* located in chromosome 1 (Levin *et al*., 2003) which enhanced fruit carotenoid content (Van Tuinen *et al*., 1997). Average lycopene content in the ripe fruits of this mutant was much higher (7.49 mg/100 g fresh) compared to moderate of 4.37 mg/100 g fresh in the parental genotype (Table 3) which supported the proposition that exaggerated photo-responsiveness as manifested by high chlorophyll content in fruit was responsible for enhanced carotenoid particularly lycopene content in the ripe fruits.

Different quantitative characters that markedly altered in this mutant as recorded in Table 3 for both M_3 and M_4 generation was fruit number per plant (average 43.40 fruits less than the parent), fruit weight (average 32.38 g more than the parent), total phenol content of immature fruit (average 4.85 mg/100 g fresh higher than the parent) and ascorbic acid content (average 8.19 mg/100 g fresh higher than the parent).

Table 2. Different qualitative characters of Patharkutchi and "Dark green fruit" mutant

Character	Patharkutchi	Dark green fruit mutant
Growth habit	Semi-determinate	Semi-determinate
Branching pattern	Upright	Upright
Twig colour	Medium green	Dark green
Leaf orientation	Pinnately compound	Pinnately compound
Leaf type	Serrated	Serrated
Leaf size	Narrow	Narrow
Leaf colour	**Dark green**	**Very dark green**
Inflorescence	Monoparous cyme	Monoparous cyme
Flower size	Medium	Medium
Sepal size	Medium	Medium
Anther dehiscence	Consistent	Consistent
Male sterility/fertility	Male fertile	Male fertile
Fruit shape	**Flattish-round**	**Flat round to heart**
Green shoulder	High	High
Pedicel attachment	Jointed	Jointed
Fruit pubescence	No	No
Fruit ribbing	High	High
Blossom end	Indented	Indented
Unripe fruit colour	**Green**	**Dark green**

Table 3. Different quantitative characters of Patharkutchi and "Dark green fruit" mutant in M_3 and M_4 generation

Character		Plant height (cm)	Primary branch/ plant	Days to first flowering	Flower per cluster	Fruit per cluster	Fruit per plant	Fruit weight (g)	Polar diameter (mm)	Equatorial diameter (mm)	Pericarp thickness (mm)	Locule No.	Test weight of seed (g)
Patharkutchi	M_3	141.23	9.13	31.25	7.87	6.87	51.67	77.33	50.16	54.34	6.52	4.51	3.31
	M_4	123.16	9.67	33.72	8.16	6.33	56.33	82.91	49.52	50.26	5.87	4.38	3.35
Dark green fruit mutant	M_3	93.44	9.31	47.24	6.67	3.17	9.33	107.45	58.71	61.25	7.16	4.83	3.31
	M_4	98.33	10.33	46.91	5.87	2.87	11.87	117.56	53.48	55.52	6.82	5.25	3.28
S.Em.(±)	M_3	0.85	0.53	0.60	0.23	0.24	0.35	0.67	1.04	0.85	0.31	0.16	0.16
	M_4	0.71	2.07	2.35	0.29	0.31	0.71	0.89	1.06	1.02	0.39	0.18	0.20
C.D.(5%)	M_3	3.32	0.51	0.94	0.91	0.96	1.35	2.61	4.06	3.33	1.19	0.62	NS
	M_4	2.78	1.96	3.71	1.15	1.19	2.76	3.51	4.15	4.00	1.53	0.73	NS

Character		Chlorophyll a of leaf (mg/100g)	Chlorophyll b of leaf (mg/100g)	Total Chlorophyll of leaf (mg/100g)	Total phenol of leaf (mg/100g)	Total Chlorophyll of immature fruit	Total phenol of immature fruit (mg/100g)	TSS (°B)	Lycopene (mg/100g)	β Carotene (mg/100g)	Reducing sugar (%)	Total sugar (%)	Ascorbic acid (mg/100g)	Acidity (%)	Seed protein content (mg/100g)
Patharkutchi	M_3	95.47	105.43	210.93	22.16	12.57	7.13	6.21	4.27	0.78	2.79	3.48	26.27	0.57	23.08
	M_4	105.26	95.23	185.57	27.22	11.86	6.65	6.35	4.48	0.65	2.58	3.26	28.35	0.64	21.56
Dark green fruit mutant	M_3	170.19	159.54	329.73	37.02	23.23	12.11	5.86	7.73	0.84	3.14	3.85	34.85	0.58	23.17
	M_4	157.16	162.24	307.42	34.25	20.63	11.37	5.73	7.26	0.86	3.08	3.64	36.16	0.66	20.82
Dwarf plant mutant	M_3	107.22	121.71	226.93	28.78	14.79	8.37	5.83	4.26	0.76	3.69	4.17	28.24	0.73	27.96
	M_4	111.42	118.35	216.43	31.57	13.28	7.51	5.66	4.18	0.69	3.28	3.84	27.57	0.78	26.71
S.Em.(±)	M_3	1.42	2.23	2.09	0.94	0.67	0.62	0.33	0.29	0.27	0.15	0.06	0.49	0.20	0.24
	M_4	1.52	1.91	2.16	1.27	0.81	0.51	0.37	0.23	0.29	0.19	0.05	0.44	0.22	0.16
C.D. (5%)	M_3	5.57	8.71	8.17	3.69	2.61	2.43	NS	1.15	NS	0.57	0.23	1.94	0.78	0.96
	M_4	5.94	7.48	8.46	4.98	3.14	1.97	NS	0.91	NS	0.74	0.19	1.72	0.84	0.64

Conclusion

It emerged conclusively from the present investigation on applied mutagenesis of tomato that lower doses of gamma radiation (50 – 250 Gy) was the most effective mutagenic treatment for inducing broad spectrum of viable mutation in tomato and induced mutation could alter a number of both qualitative and quantitative characters. The "dark green fruit" mutant emerged as a promising genetic resource for further utilization in tomato breeding for enhancement of lycopene and ascorbic acid content in the fruits.

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