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Applied mutagenesis in tomato for the isolation of mutants of enhanced fruit quality

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Abstract
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. The present investigation was undertaken to study the frequency and spectrum of macro-mutations of gamma rays, EMS and their combinations in "Patharkutchi," the highly adaptable and popular cultivar of West Bengal. The dry seeds were irradiated with 50, 100, 150, 200 and 250 Gy gamma rays. Pre-soaked seeds of this genotype (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 ºC. Gamma irradiated seeds of this genotype were also pre-soaked (6 h, in water) before treating with 0.15 % EMS solution for 8 h at 25 ± 2 ºC as combination treatment.
Combination of gamma radiation and EMS caused more damage followed by EMS treatment and gamma radiation, alone 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" could be isolated from the M_2 population of Patharkutchi treated by 150 Gy gamma radiation. 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 breeding tomato for enhancement of lycopene and ascorbic acid content in the fruits.

Keywords: Mutation frequency, gamma rays, dark green fruit, tomato

Introduction
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 (*Solanum lycopersicum*). 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
In this investigation 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 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 ºC. 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 ºC as combination treatment. The EMS treated seeds were washed thoroughly in running water at least for an hour before sowing.
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 the three genotypes exposed to mutation. All the macro-mutants were selfed and advanced up to M_4 generation.

Results and Discussion
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%).

The spectrum of mutation clearly suggested 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 which was also recorded in several earlier studies (Sakin and Senkar, 2002; Prem *et al.* 2011) [6, 5].

Dark green fruit mutant

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 compared with that of the parental genotypes "Patharkutchi" statistically.

Different qualitative characters as documented in this mutant and its parental genotype (Table 1) indicated deviation in 3 characters viz., twig colour (dark green *vis a vis* medium green in the parent), leaf colour (very dark green *vis a vis* dark green in the parent), and unripe fruit colour (dark green *vis a vis* green in the parent).

Different quantitative characters that markedly altered in this mutant as recorded in Table 2 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).

Average total chlorophyll content in the leaf and immature fruit of the mutant over in 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 2). Isolation of high chlorophyll mutant in tomato was also reported earlier by 100 Gy gamma radiation and 24 Gy gamma radiation (Asmahan and Al-Twaty, 2006) [1] which agreed well 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) [4] and jointless pedicel (Mao *et al.*, 2000) [4]. 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) [3] which enhanced fruit carotenoid content (Van Tuinen *et al.*, 1997) [2-7]. Average lycopene content in the ripe fruits of this mutant was much high (7.49 mg/100 g fresh) compared to moderate of 4.37 mg/100 g fresh in the parental genotype (Table 2) 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.

Conclusion

It emerged conclusively from the present investigation on applied mutagenesis of tomato that lower doses of gamma radiation (150 – 250 Gy) was the most effective mutagenic treatment for inducing broad spectrum of viable mutation in tomato. Induced mutation could alter a number of both qualitative and quantitative characters. The "dark green fruit" mutant induced in Patharkutchi with 150 Gy gamma radiation emerged as a promising genetic resource for further utilization in breeding tomato for enhancement of lycopene and ascorbic acid content in the fruits.

Table 1: 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 2: Different fruit characters of Patharkutchi and "Dark green fruit" mutant

Character	Patharkutchi	"Dark green fruit" mutant
Fruit per plant	56.33	9.33
Fruit weight (g)	82.91	109.45
Total Chlorophyll content of leaf ((mg/100g)	198.25	318.52
Total Chlorophyll content of immature fruit (mg/100g)	12.21	21.93
Total phenol of immature fruit (mg/100g)	6.89	11.74
Lycopene content of ripe fruit (mg/100g)	4.37	7.49
β Carotene content of ripe fruit (mg/100g)	0.72	0.85
Ascorbic acid of ripe fruit (mg/100g)	27.31	35.51

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