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FULL LENGTH ARTICLE

Induction of mutagenized tomato populations for investigation on agronomic traits and mutant phenotyping

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Abstract Global demand for tomato production increased tremendously due to its diverse utility in raw, cooked and processed form of food. This necessitates the continued supply of highly nutritious and better yielding improved cultivars to the producers, considering the rapid changing agro-climatic condition. In this study, induced mutant tomato populations of widely recommended tomato genotype Arka Vikas (Sel-22) were generated using chemical mutagen ethyl methane sulfonate (EMS), hydrazine hydrates (HZ) and their combined treatments. In the *in vitro* study, a gradual reduction in germination percentage and seedling height occurred with the increasing concentrations of mutagens. Combination of EMS and HZ caused maximum biological inhibition followed by EMS and HZ treatments alone in M_1 generation. The rate of survival and fertility in M_1 plants of tomato was found highly affected due to mutagenic treatment, in which sensitivity toward combined treatment was found highest followed by EMS and HZ. Inspection on induced phenotypic variations in individual plants of M_2 population resulted in identification and isolation of wide range of mutants with altered phenotypes. Highest mutation frequency was resulted by combined mutagens followed by the EMS and HZ treatment. Agronomic trait analyses showed intra and inter treatment variations in three quantitative traits (Plant height, fertile branch per plant and fruits per plant) of M_2 mutagenized population. Assessment on rate of mutant recovery in M_2 population showed highest mutant recovery is possible with combination treatments and then 0.02% HZ followed by 0.02% EMS. In the present study, phenotyping of the mutants revealed that vegetative organs ('plant size', 'plant habit' and 'leaf morphology') was the most sensitive category (69.33%) to which most of the mutant belongs, followed by 'fruit color and size' (20.27%) and

Abbreviations: EMS, ethyl methane sulfonate; HZ, hydrazine hydrates; LD, lethal dose; S.E., standard errors; S.D., standard deviation; C.V., coefficient of variations; DMRT, Duncan multiple range test.

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'germination' (9.79%). Comparative investigation on number of mutants and phenotype category in M_2 mutant plants confirmed that the number of pleiotropic mutants was more than the non-pleiotropic mutants in all the treatment conditions. Heritability and reproducibility of the 30 putative mutants were checked in M_3 generation based on the observed agro-economical traits; results showed 17 complete and 5 partial heritable mutants.

1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the night shade family solanaceae, originated in the South American and as a food in Mexico (Paran and Fallik, 2011). It is one of the most popular and widely cultivated vegetable crops in the world (Srinivasan, 2010) and its fruit as a vegetable in trade is also considered to be prominent ''protective food'' (Alam et al., 2007). Tomato is a richest source of nutrient dietary fibers, antioxidant and beta-Carotene (Hobson and Grierson, 1993; Beecher, 1998); Vitamins A and C mineral such as iron, phosphorus (Kalloo, 1991); as well as Carbohydrates and phenolic compound such as flavonoids, polyphenols (Campbell et al., 2004) and organic acid such as naringenin and chloragenic acid (Knekt et al., 2002); therefore, Tomato is among the most nutritious vegetable eaten directly as raw vegetable or consumed in a variety of product such as ketchup, sauce, chutney, juice, soup, and puree. Tomato genus *Solanum* is a diploid, with $2n = 24$ chromosomes and genome size of 950 Mb which is composed of 77% heterochromatin, 33% Euchromatin (Peterson et al., 1996). Tomato is a warm season crop and requires a relatively cool, dry climate for high yield and better quality; it is adapted to a wide range of climate condition from temperate to hot humid tropics (Srinivasan, 2010). India is the second largest producer of tomato (Table 1). The world dedicated 4.8 million hectors in 2012 for tomato cultivation and total production was about 161.8 million tones. The average world farm yield was 33.6 tones/hectors in 2012 (FAOSTAT production crops, 2012 data).

The sustained efforts for the crop improvement through induced mutations around the world have resulted in the development and release of about 3222 mutant varieties, of which India contributed 330 mutant varieties. Overall 20 tomato mutant varieties released till now and India alone shared 4 tomato mutant varieties (Joint FAO/IAEA Mutant Variety Database, 2015). The mutagen used in the present investigation, EMS and HZ were selected based on their mode of action and their meager reports on tomato. Ethyl methanesulfonate (EMS) is a monofunctional ethylating agent that may produce both GC to AT and AT to GC transition mutations and can cause base-pair insertions or deletions as well as more extensive intragenic deletions and also there is clear-cut evidence that EMS is able to break chromosomes (Sega, 1984). Hydrazine produces mainly single locus mutations rather than chromosomal aberrations, however some of the derivatives of hydrazine especially methylhydrazine produce chromosomal aberrations and other chromosomal and nuclear effects. Hydrazine can react with the pyrimidines in DNA to saturate the 5, 6 double bond, especially thymine, to form N4-aminocytosine, and to open up the pyrimidine ring with consequent loss of pyrimidines from DNA. The mutations produced by hydrazine are probably produced by direct mispairing at replication rather than by error-prone repair (Kimball, 1977). Therefore, keeping in view the nutritional and economic values of Tomato (*Solanum lycopersicum* L.) and continued demand of improved varieties in the changing agro-climatic conditions, an attempt has been made to explore the possibilities of inducing genomic alteration in tomato by employing doses of EMS and HZ, for improvement of quantitative as well as qualitative traits and assessments were also made on the isolated mutant phenotypes. Different concentrations of chemical mutagen EMS, HZ and their combination were used for induction of mutation and optimization of doses.

Table 1 World's top ten countries of tomato producers in 2012. Source: FAOSTAT, 2014.

Rank	Country	Production (MT)
1	China	50,000,000
2	India	17,500,000
3	United States	13,206,950
4	Turkey	11,350,000
5	Egypt	8,625,219
6	Iran	6,000,000
7	Italy	513,977
8	Spain	4,007,000
9	Brazil	3,873,985
10	Mexico	3,433,567

2. Material and methods

The healthy seeds of tomato (*Solanum lycopersicum* L.) var. Arka vikas (Sel-22) were procured from Government Seed Store, Aligarh, India. The improved tomato variety ''Arka vikas'' was developed through a selection from American variety ''Tip Top'' and recommended for cultivation in agro-climatic zone of the present site of study. Chemical mutagen EMS and HZ, manufactured by Sisco Research Laboratories Pvt. Ltd., Mumbai, India, were used for mutation induction. The pH of the solution was maintained using buffer tablets (MERCK manufactures, Mumbai, India). To optimize the chemical mutagenesis, tomato seeds were treated with different EMS and HZ concentrations (0.01%, 0.02%, 0.03% and 0.04%) for incubation time of 9 h at room temperature, pH 7.0. Similarly combination treatment of equal concentrations (0.01 + 0.01%, 0.02 + 0.02%, 0.03 + 0.03% and 0.04 + 0.04%) of both EMS and HZ was also employed. Thoroughly washed 150 seeds were sown in three replications for each treatment of the mutagen used as well as untreated to serve as control and kept in the Net House of the Department of Botany, Aligarh Muslim University, Aligarh, India, to raise M_1 generation during winter season of 2011–12. Another 15