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Research Article

Identification and Assessment of Elite Heat Tolerant Tomato EMS Mutant Lines

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ABSTRACT

Mutagenized populations have become indispensable resources for introducing variation. This study is conducted in Department of Horticulture, Arid Agriculture University Rawalpindi, Pakistan in which thirty-six hundred seeds were treated with chemical mutagen Ethyl Methane Sulphonate (EMS) viz. 4mM, 8mM, 16mM, 24mM, 32mM, 40mM, 48mM, 56Mm and 64mM to induce mutations in tomato Rio Grande. Here, we demonstrate the utility of this population for physiological screening under heat stress conditions by using basic thermotolerant screening tools. Populations were also observed for morphological and reproductive traits. Plant variation was cataloged for seed germination, membrane stability index %, cell viability (Tetrazolium Trichloride Analysis, TTC), relative water content of leaves, proline content of leaves, plant height, leaf area, internodal distance, chlorophyll contents, no. of branches, flower and fruit per cluster, cluster/ plant and fruit set percentage. From all the results obtained during screening it's concluded that EMS **8mM** and **16mM** are suitable concentrations to develop tomato variants and usage of chemical mutagen increases thermotolerance in tomato plants by increasing thermo stability of plants by means of vegetative and reproductive traits when temperature rises above 45°C for identification of suitable variants as tomato breeding material.

Key Words: Tomato, Mutagenesis, Heat stress, EMS, Thermotolerance

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is second most economically important vegetable crop next to Potato and known as a benchmark in Horticulture worldwide. Area under tomato cultivation is increasing every year because of its economic importance. According to FAOSTAT (2013), the estimated world production of tomato is about 164 thousand tons with average yield of 34.7 thousand tons/ha and 473 thousand hectare area under cultivation. The popularity of tomato as fresh and processed food has made it an important source of vitamin A and C in diets. Tomatoes are being consumed throughout the world on a large scale and serve many benefits for heart and other organs. They contain the carotene lycopene, one of the most powerful natural antioxidants (Mourvaki *et al*., 2005). The average yield of tomato in Pakistan is very low as compared to other countries of the world. Although, there are many factors causing low yield including failure to ripe, uneven or blotchy ripening and tomato yellow shoulder disorder but the major problem in different regions of Pakistan is the failure to set fruit or poor fruit set due to day temperatures above 45°C-50°C combined with low humidity and/or drought night temperatures above 21°C or below 10°C. Hot drying winds can add to the problem. Dry soil can cause blossoms to dry up and drop. Cold soils at planting time can stunt growth and delay or eliminate flowering. Lack of

air circulation can inhibit the movement of pollen to the flower pistils (Singh *et al.*, 2007)

A temporary increase of 10˚–15˚C in temperature above normal can lead to heat stress thus resulting in poor growth, reproductive development and yield (Wahid *et al*., 2007). Shortage of tomatoes occurs during summer season due to high temperatures (Hall, 1992; Hussain *et al.*, 2006; Singh *et al*., 2007). High temperature causes cellular injury within minutes that could be related to a disturbance of cellular structure and may lead to cell death (Schoffl*et al*., 1999). Direct damages caused by high temperatures may include denaturation and aggregation of protein, and enhanced membrane lipids liquidity leading to electrolyte. Indirect or slower heat damages can also occurred in terms of enzyme inactivation in chloroplast and mitochondria, limitation in protein production, degradation of protein (Howarth, 2005). In general, it is obvious that high temperature influences plants anatomy markedly at the tissue, cellular, and sub-cellular levels. The additional impacts of all these alterations in temperature may lead to reduced crop growth and yield (Wahid *et al*., 2007). Plants have developed strategy adapted mechanism to respond to stress which lead them to tolerance. Mutation breeding is one of the major techniques to develop stress resistant plants. The chances of survival of mutants are much higher under rapidly fluctuating climatic conditions. The use of nuclear techniques for developing new varieties under the changing climatic conditions would be the most ideal approach as compared to any new cost effective techniques developed which are freely available without too many regulations. The purpose of induced mutations is to enhance the mutation frequency rate in order to select appropriate variants for plant breeding. Mutations are also induced by chemical mutagen (e.g., EMS) treatment of both seed and vegetatively propagated crops.

Ethyl Methane Sulfonate (EMS) is an effective and widely used chemical mutagen to induce point mutation. It can generate sublethal and substerile alleles, thus an EMS generated mutant population is best suited for identifying more useful alleles of a specific gene of interest. Considering the need of mutants for basic and applied research, a public resource is required.

The current study was planned to generate EMS derived tomato mutant library and screen mutants for heat stress tolerance. The materials developed and identified in the current study could be used as a key source for developing heat tolerant tomato cultivar as well as to understand loss/gain of function phenomenon of heat tolerance in plants.

MATERIALS AND METHODS

The experiment was conducted in field area and laboratory of the Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi. **S**eeds of tomato variety “Rio Grande” were obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Seed Treatment with Ethyl Methane Sulphonate (EMS)

Tomato seeds were subjected to EMS (Ethyl Methane Sulphonate) mutagenesis. A set of thirty six hundred seeds was imbibed in 200 ml distilled water for 24 hours at room temperature. Nine treatments with different concentrations having four hundred seeds each were then incubated in 100 ml solution of EMS (Sigma-Aldrich, St. Louis) (Table 2) with gentle shaking for 12 hours. After EMS treatment, the seeds were washed twice in 200 ml of 3% sodium thiosulfate buffer for 20 minutes at room temperature, with gentle shaking, followed by three washings in 200 ml distilled water. Decanted EMS solution after washing was deactivated by mixing them with equal volume of "inactivating solution" [0.1M NaOH, 20% w/v $Na_2S_2O_3$] for 24 hours. All pipits/tubes contaminated with EMS were soaked in inactivating solution for 24 hours prior to disposal (Lima *et al.*, 2004).

Sowing of Treated Seed and Transplantation of Seedlings

The seeds treated with EMS along with control were sown in germination trays. The sowing

medium comprised of soil, farm yard manure and sand (2:1:1). The seeds were covered with coconut husk. After three weeks of sowing, all treatments were assessed for germination percentage to find out the optimized lethal dose of chemical mutagen. Percent germination and lethal dose were calculated by using the following formulas (Laskar*et al.*, 2016):

$$\text{GerminationPercentage}\ (\%) = \frac{\text{Numaber of seeds germinated}}{\text{Total number of seeds}} \text{x100}$$

$$\text{Lethal Dose} = 100 - \frac{\text{The frequency of M1 seedling}}{\text{The frequency of control seedling}}$$

One month after sowing, nursery of surviving treatments was transplanted into open field conditions to raise M_1 generation following Randomized Complete Block Design (RCBD) with four replications. Plants were spaced 60cm × 40cm apart and were staked. Soil was fertilized by Farm Yard Manure (FYM) at the time of bed preparation and three irrigations per week were done during the growing period. Data regarding climatic conditions (temperature minimum, maximum, rainfall and sunshine hours) during the experimental period were obtained from metrological department, Rawalpindi, Pakistan and presented in (Table 1). All irrigation and management practices necessary for normal crop growth were carried out. Seed of each plant was harvested separately and used to generate M2 populations following similar conditions as used for M1 generation. Following parameters were recorded in M2 generation for the assessment of tolerant lines.

Thermotolerance Related Traits

Membrane stability index (MSI%)

The membrane stability of the leaves was assayed following procedures outlined by Tripathy *et al.* (2000). Leaf segments (1cm diameter) were collected from fully expended fourth leaf from top of plants at flowering stage. Electrolytes adhering to the leaf surface were removed by washing collected samples thrice with deionized water. The collected samples were then placed in capped vial (20 ml) containing 10 ml of deionized water and subjected to induction temperature of 33°C for 2 hours followed by challenging temperature 40°C for 2 hours. All vials were then incubated for 24 hours in the dark at room temperature. Electrical conductance was measured using conductivity meter. The vials were autoclaved for 15 minutes at 121°C after the first measurement, to kill the leaf tissue and release all the electrolytes. The second electrical conductivity reading was taken after cooling. For all the samples, these two measurements were carried out individually. The conductance of stressed samples was a measure of electrolyte leakage due to heat stress and assumed to be proportional to the degree of injury to membranes. The membrane stability index was calculated as reported by Blum and Ebercon (1981)

$$\text{Membrane stability index}\ (\%) = \left[1 - \left(\frac{\text{electrolyte leakage after 24 hrs.of incubation}}{\text{total electrolyte leakage}}\right)\right] \times 100$$

Cell viability % (Triphenyl Tetrazolium Chloride) assay

Ten randomly selected plants from each replication were labeled and leaf disks were excised were collected from fully expended fourth leaf from top of plants at flowering stage. After exposing the leaf disks to induction temperature of 33°C for 2 hours followed by high temperature of 40°C for 2 hours, they were incubated in 0.2% TTC solution for 4 hours and cell viability was measured by taking the absorbance at 485 nm (Senthil-kumar*et al.*, 2002a; 2002b)

Relative water content of leaves (%)

The relative water content (RWC) of leaves was determined in the fully expanded uppermost leaf of the main shoot (Pirzad *et al.*, 2011). The weight of fresh leaf samples were recorded followed by their immersion in distilled water in a Petri dish. After two hours, the leaves were removed, the turgid weight recorded after blotting of surface water. Samples were then dried out at 70°C in an oven to obtain dry weight. Leaf RWC was calculated using the following formula (Turner, 1981):

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} x100/$$

Proline contents of leaves (µmole/g fresh weight)

Proline contents of leaves were estimated at 50% flowering stage by using methodology of Bates *et al*. (1973). Fresh plant leaves (1g) were homogenized with 5ml 3.0% Sulfosalicyclic acid in mortar. Samples were centrifuged at 3000 rpm for 5 min. Supernatant was adjusted to 5 ml with distilled water and mixed with 5 ml glacial acetic acid and 5 ml ninhydrin (1.25g of ninhydrin dissolved in a solvent prepared by mixing 30ml of glacial acetic acid, 8ml ortho-phosphoric acid and 12 ml distilled water) . The reaction mixture was shaken and the contents in the tubes were boiled in water bath for 1 hour. Tubes were then cooled and the mixture was extracted with 10 ml of toluene in separating funnel. Absorbance of toluene layer was recorded at 520 nm.

A calibration curve consisting of a dilution series 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg of proline was prepared. The standard curve was used as a reference to calculate the concentrations of unknown samples.

Morphological and reproductive parameters

Morphological and reproductive parameters including plant height, leaf area, and chlorophyll content of leaves; number of branches, Internodal distances, number of flowers per cluster, number of fruits per cluster, the number of cluster per plant and fruit set percentage were recorded.

RESULTS AND DISCUSSION

DEVELOPMENT OF MUTANT POPULATION

Mean performance of M1 plants showed that high concentrations of chemical mutagens proved lethal as seeds treated with EMS (4, 8, 16, 24 and 32mM) showed significantly lower germinated percentage as compared to control (Table 4). Concentrations of EMS above 24 mM worked out to be more lethal and concentration of EMS 4mM the least (Table 4). Seed germination is the initial step of plant development. Subsequently the inhibitory impact of mutagenic treatments on seed germination could be unambiguously seen. Mutagenic treatments uncovered a progressive diminishing pattern in germination from lower to higher doses (Sunil *et al*., 2011).

. Table 4 depicts that EMS concentration of 40mM may be considered as lethal dose for tomato variety "Rio Grande" seeds. Hence, it is concluded that chemical mutagen EMS concentrations (8, 16mM) are suitable to create variants in tomato CV "Rio Grande". The subsequent selection was therefore made from the plants of 16 mM treatment and called M2 populations hereafter. The plants of M1 generation of 16 mM EMS treatment were screened by membrane stability index. A set of twenty four (24) plants showing variability as compared to control were selected and their seed was utilized for the development of M2 population (unpublished data).

The selected EMS mutant lines 16 mM) were used to raise M_2 population, which was subjected to different screening analysis to assessment of thermo-tolerance, morphological and reproductive traits.

Thermo-Tolerance Related Traits

Cell membrane stability is the critical tool to measure the heat stress tolerance. Cell membrane stability of M_2 population revealed that differences were statistically significant among various levels. Wild type plants (Rio Grande, unmutated) showed less membrane stability index as compared to all plants developed through mutagenic treatment (Table 5). It was also revealed that MSI % was significantly higher in M_2 population (EMS concentration; 16mM) at elevated temperature as compared to wild type plants.

Cell viability analysis was performed by using Tetrazolium trichloride salt. Cell viability of M_2 populations depicted that differences were statistically significant among various levels (Table 5). Wild type plants showed significantly lower cell viability from the rest of M_2 population.

Relative water contents of M_2 population revealed that differences were statistically significant as compared to wild type at elevated temperature (40 $^{\circ}$C) (Table 5).

Proline contents of M_2 population revealed statistically significant differences as compared to wild type at elevated temperature (40 $^{\circ}$C) (Table 5). Proline contents of leaves from were found higher in M_2 population as compared to wild type. Chlorophyll contents of leaves also showed significantly higher values in M_2 population as compared to wild type plants.

Morphological Traits

Plant height, leaf area, number of branches and internodal distance showed significantly higher values in M_2 population as compared to wild type plants. Number of flowers, number fruits, number of cluster/plant and fruit set percentage were also observed significantly higher in M_2 plants as compared to wild type.

DISCUSSION

The conductivity of electrolytes spilling from leaf discs at extreme temperatures is considered as a measure of cell membrane thermostability. It has been proposed as a screening system for heat tolerance in plants (Sullivan, 1972). This method depends on the perception that when leaf tissue is harmed by exposure to extreme temperatures, cell film porousness is expanded and cell electrolytes diffuse out into bathing solution. The measure of electrolytes has measured as they are spilled from injured cells can be assessed the electrical conductivity of the solution. The viability of this method in recognizing hereditary variability in heat stress has been accounted for in warm season crops. Illustrations incorporate soybean and tomato (Chen *et al*., 2010, Shen and Li, 1982). This system is straight forward, speedier and less costly than entire plant screening. Plant breeders can apply this system at an early vegetative stage for plants developed in field nursery for selecting plants, propelling eras and quickening reproducing programs.

The progressions in surrounding temperature are detected by plants with a scrambled arrangement of sensors situated in different cellular compartments. The expanded ease of the layer stimulates actuation of lipid-based bilayers and to an expanded $Ca2^{+}$ influx and cytoskeletal revamping. Movement between these courses leads to the generation of osmolytes and antioxidants in response of heat stress. A particular impact of high temperatures on photosynthetic membranes incorporates the swelling of grana stacks and such auxiliary changes are joined by particle spillage from leaf cells and changes in vitality designation to upkeep of cell layer capacity under high temperature stress.

The early impacts of thermal stress contain auxiliary adjustments in protein of chloroplast edifices and diminished movement of catalysts (Ahmad *et al*., 2010). Furthermore, by making wounds the cell film, association of microtubules and at last to the cytoskeleton, heat stress changes layer porousness and modifies cell separation, stretching, and extension (Smertenko*et al*., 1997; Potters *et al*., 2008; Rasheed, 2009).

Populations raised through mutagen (EMS) concentration are observed to show increased stability of membrane at higher temperatures by increasing heat shock proteins in cell membranes (Chen *et al*., 2010). In present study highly stable membrane was observed in populations which were developed through EMS as compared to control. The mutagenic treatment mainly sustained the function of cellular membranes for processes such as photosynthesis and respiration under stress (Blum, 1988).

Heat stress quickens the active vitality and development of atoms crosswise over layers in this way slackening chemical bonds inside of molecules of biological membranes. This makes the lipid bilayer of natural layers more liquid by either denaturation of proteins or an expansion in unsaturated fats (Savchenko *et al*., 2002). The stability and functions of biological membranes are delicate to high temperature, as heat stress

adjusts the tertiary and quaternary structures of membrane proteins. Such adjustments upgrade the penetrability of membranes, as obvious from expanded loss of electrolytes.

The Triphenyl Tetrazolium Chloride (TTC) viability assay has been used to study the basic thermal responses of different plants tissues and demonstrate the role of heat shock proteins in plant thermotolerance. The cell viability quantifies by the relative level of TTC reduction by using the spectrophotometric assay of the red formazon (Towill and Mazur, 1975). Reduced TTC in tissues is indication of more viable cells. Modification of TTC reduction in temperature is considered to be the indicative of the cellular and tissue response to temperature. The ability of viable cells to reduce various tetrazolium salts has been reported for a variety of organisms. The decrease in cell viability due to extreme temperature is attributed to an uncoupling of the electron transport chain through disruption of the inner mitochondrial membrane and/or inactivation of enzymes of the respiratory pathway (Porter *et al*., 1994).

In the present study Triphenyl Tetrazolium Chloride (TTC) assay was carried out to identify cell viability. The test revealed that cell viability tremendously increased in populations developed through EMS as compared to control.

Loss of cell water content occurs due to high temperature for which the cell size and in end the growth is diminished. Deterioration in net absorption rate (NAR) is likewise another purpose behind lessened relative growth rate (RGR) under high temperature however mutagen treatments enhances the capacity of more intakes of water and supplements from soil to keep up turgidity and general plant life in anxiety conditions. Plant water status is the utmost imperative variable under changing encompassing temperatures (Mazorra *et al*., 2002). All in all, plants have a tendency to keep up stable tissue water status paying little mind to temperature when humidity is sufficient; be that as it may, high temperatures extremely hinder this tendency when water is constrained under field conditions, high temperature stress is every now and again connected with reduced water accessibility (Machado and Paulsen, 2001; Simoes-Araujo *et al*., 2003). As the heat stress vulnerable the leaf water content, plants presented to heat stress demonstrated a buildup of a few heat stable proteins which had all earmarks of being connected with heat resilience.

Results in present study depicted that the plants which were treated with mutagens have more ability to absorb water when the transpiration rate was higher in heat stress conditions as compared to non mutant plants (control).

A gathering of osmo-protectants is a critical versatile component in plants subjected to extreme temperatures, as essential metabolites take an interest directly in the osmotic change (Sakamoto and Murata, 2002). For example, a collection of proline is important to direct osmotic adjustments and shield cell structures from expanded temperatures by maintaining the cellular water equalization, membrane stability, and by buffering the cellular redox potential (Farooq *et al*., 2008).

On the grounds that proline is thought to be a perfect solute. It ensures collapsed protein structures against denaturation, balances out cell layers by associating with phospholipids, capacities as a hydroxyl radical ion scavenger, or serves as a vital and nitrogen source. Recently, Rivero*et al*. (2004) demonstrated that, in tomatoes under heat stress (35ºC), the proline content of leaves increased significantly with respect to the optimum growth temperature (25ºC) which relates with the results of present study in which mutagenic populations showed increased levels of proline accumulation. This was due to an induction of the main enzymes involved in proline synthesis (P5CS and OAT), as well as to an inhibition of the enzyme responsible for degrading proline (PDH), thereby causing a overall stimulation of the stress-resistance machinery of the plant (Delauney and Verma, 1993; Claussen, 2005).

In present study the mutant populations exhibited elevated levels of proline as compared to control counterpart thus providing the scope for selection of desirable variants (i.e. thermotolerant individuals).

The results of the present study showed decreased plant height with higher concentration of chemical mutagen might be due to reduced mitotic division in merisemetic tissues and reduced moisture content. A research study conducted by Jabeen and Mirza (2002) on *Capsicum annum* to create genetic variability in plant height by using EMS, also support the results of the present study. Dhakshanamoorthy (2010) conducted research on *Jatropha curcas* L. to induce variability in growth parameters and identified that maximum plant height 105 cm at maturity was recorded in 1% EMS while minimum height was observed by using 4% EMS (81.33 cm). Which proves that lower concentration has shown the stimulatory effect of plant height whereas EMS treatment in higher concentration has an inhibitory effect as compared to control.

Increasing leaf area does not mean in only increasing the ability of plants to capture light but also changing its growth and maintenance process. The results of present study regarding leaf area were in concurrence with the results of (Pakorn*et al*., 2009) who confirmed that leaf area in *Anubiascongensis* reduced with the increasing concentrations of mutagens due to damage of genetic material and decline of cell division and ultimately stunted growth. Moreover, Jabeen and Mirza (2002) were performed an experiment and recorded that, leaf area decreased with the increase in concentrations of ethyl methane sulphonate (EMS).

The consequences were also in harmony with the findings of Jitendra *et al*. (2012) who using EMS in Safflower reported increased chlorophyll a and b contents with decrease in mutagens dose. Introduction to high temperature affected chlorophyll loss, lead to checked decrease in the photochemical effectiveness of PSII and cause significant degradation of Rubisco in plant clears out.

In the present study, with increasing level of EMS there was a decrease in the internodal length and ultimately plant height. The findings of (Motilal*et al*., 2012) are harmonized with the results of the current study. He explained that there was a decrease in inter node length with the increase dose level of EMS in *Asteracanthalongifolia* L. Significant effect of high temperatures on shoot development is an extreme reduction in the main internode length bringing about premature plant death (Hall, 1992). In the present study tall plants than control (Rio Grande) were observed which can be the source of variants with increased plant height (plant growth) under elevated temperature.

Reproductive growth of plants is more delicate to high temperature because fertility of plant is significantly reduced when the temperature increases. Temperature variances occurred before the blooming period can activate morphological changes in blossoming flowers. These progressions are relying on the power (temperature degrees) length of time and degree of the temperature increment. A higher temperature can potentially interrupt tomato fruit set by causing injury to developing pollen grains (Mckee and Richard, 1998). Hazra*et al*. (2007) summarized the causes of fruit set stoppage at high temperature in tomato; it comprises bud drop, irregular development of flower, reduced pollen production, dehiscence and viability, ovule abortion and meager viability, less availability carbohydrate and other reproductive abnormalities occur. Sato *et al*. (2006) reported that under moderately elevated temperature the reduction of sink and source strength in tomato leads to a depletion in available carbohydrates at critical stages of plant growth. This leads to reduced fruit set and other yield related parameters. Morphological traits which includes fruits and flowers number per plant, percentage of fruit set and fruit fresh weight were varied in heat resistant and heat susceptible tomato lines (Abdelmageed and Gruda, 2009). A number of factors contribute

to reduced tomato fruit set under high temperatures and can be considered as potential selection criteria. These factors include reduced flower production, ovule and pollen viability, and pollen dehiscence (ElAhmadi and Stevens, 1979).

In present study mutants produced higher number of flowers, clusters and fruits per plant than control population. It appears that incidence of bud drop, irregular flower development, reduced pollen production, dehiscence and viability, ovule abortion and insufficient viability, less availability of carbohydrates and other reproductive abnormalities which are the possible outcome of elevated temperature were cooped in better way by mutants and thus resulting in better fruit set as compared to parental population proving previous reports (ElAhmadi and Stevens 1979; Sato *et al*. 2009; Abdelmageed and Gruda, 2009).

Poor fruit set of tomato induced by high temperature stress is a major source of low yield in tropical and subtropical areas of the world. Use of chemical mutagens (EMS) may help to create mutants which not only has better thermostability but also depict enhance economic traits and eventually the crop yield.

So, the use of chemical mutagen may prove a useful tool for creating variations and provide a pool for future selections to improve yield and yield components in tomato crop even under elevated temperatures.

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TABLES

Table 1: Meteorological data during the crop period (March-July 2013).

	Year2013			
	Temperatures (Mean)		**Rain fall**	**R.H**
	Max.(^{0}C)	**Min.(^{0}C)**	**(mm)**	**(%)**
March	25.7	11.7	2.18	57.5
April	30.5	16.1	1.63	48
May	37.4	20.8	0.4	34.9
June	39	24.8	3.45	44.5
July	35.3	24	8.4	65.1

Table 2: Treatments description for chemical mutagen (Ethyl Methane Sulphonate.

S. No	**Treatment**	**Concentration of EMS**
1	T_1	Control (0)
2	T_2	4Mm
3	T_3	8Mm
4	T_4	16Mm
5	T_5	24Mm
6	T_6	32mM
7	T_7	40mM
8	T_8	48mM
9	T_9	56mM
10	T_{10}	64mM

Table 3: Mean performance for germination percentage of M_1 population raised from seeds treated with various levels of chemical (EMS) levels.

Treatment	Germination %
Control	85.25a
EMS 4mM	80.75b
EMS 8mM	77.00c
EMS 16mM	76.25c
EMS 24mM	43.75d
EMS 32mM	22.75e
EMS 40mM	0.00f
EMS 48mM	0.00f
EMS 56mM	0.00f
EMS 64mM	0.00f

LSD (0.05) = 1.4365 cm

Table 4: Effects of mutagen treatments on the survival and lethal dose in M_1 plants of tomato (*Solanum lycopersicum* L.) "Rio Grande".

Treatment	M_1 seeds treated	Surviving M_1 plants	Lethal Dose
Control	400	(329) 85.25	-
EMS 4mM	400	(323) 80.75	1.82
EMS 8mM	400	(308) 77.00	6.38
EMS 16mM	400	(305) 76.25	7.29
EMS 24mM	400	(175) 43.75	46.80
EMS 32mM	400	(91) 22.75	72.34
EMS 40mM	400	0.00	100
EMS 48mM	400	0.00	100
EMS 56mM	400	0.00	100
EMS 64mM	400	0.00	100

Table 5: Mean performance of mutants and wild type plants. Means were compared by two sample T-Test

	MEAN (± SE)	
	Wild Type	Mutant
CELL VIABILTY %	46.737 ± 0.2632	80.24 ± 0.2725
CHLOROPHYLL CONTENT	55.453 ± 0.105	61.462 ± 0.4178
NO.OF FLOWERS/CLUSTER	4.6842 ± 0.1096	5.48 ± 0.1744
NO.OF FRUITS/CLUSTER	3.3684 ± 0.1137	4.56 ± 0.2386
FRUIT SET %	72.105 ± 1.9984	83.157 ± 3.7398
INTERNODAL DISTANCE	4.5316 ± 0.011	5.828 ± 0.1612
LEAF AREA	95.959 ± 0.1422	143.53 ± 0.4248
MSI%	31.422 ± 0.1884	92.32 ± 0.2752
PLANT HEIGHT	55.632 ± 0.1137	69.28 ± 1.3058
PROLINE CONTENT	0.5119 ± 2.42E-03	1.5135 ± 0.0484
RWC	0.4863 ± 1.13E-03	0.5208 ± 4.83E-03