INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0984/2022/27–5–332–340
DOI: 10.17957/IJAB/15.1934
http://www.fspublishers.org

Full Length Article

Ethyl Methanesulfonate Induced Mutagenesis Enhances Transgenerational Stress Tolerance in *Solanum lycopersicum* Cultivar 'Moneymaker'

Yu-Jing Liu†, Guo-Hua Li[1†], Sheng-Yi Bai, Fu-Shun Zheng, Peng-Ze Zhou, Hong-Lei Li, Xiao-Min Wang and Guo-Xin Cheng*

School of Agriculture, Ningxia University, Yinchuan, Ningxia 750021, P. R. China

*For correspondence: lvge2011@126.com

†Contributed equally to this work and are co-first authors

Received 20 November 2021; Accepted 29 April 2022; Published 26 May 2022

Abstract

Several studies report mutagenic effect of ethyl methane sulfonate (EMS) on seed germination, while its promotive influence on seed development are neglected. This study explored the double impacts of EMS on seed and further excavate mutations in tomato cultivar 'Moneymaker'. Different EMS doses (low: 0 ~ 0.50%; moderate: 0.75 ~ 1.25%; high: 1.50 ~ 2.00%, v/v) were used to mutagenize tomato seeds. As a result, both moderate and high EMS dose possessed higher germinated seeds each day and low or moderate EMS dose (ranged from 0.75 to 1.00%) resulted in a slight reduction in percentage of germinated seeds as comparison to the control. The antioxidants and key genes expression in these treatments were also higher than control. This suggested that low or moderate EMS dose can promote the capacity for seed germination while 1.25% EMS causing 47.33% lethality was considered as a crucial indicator for constructing a library for tomato mutants. Three mutations observed in the M_2 generation such as cold-resistant mutant ('*cr-1*') and drought ('*dr-1*') resistant mutants exhibited purple tissues/organs and dwarfism, respectively; the salt ('*sr-3*') showed high yit eld than the control. These mutants provide a good resource for breeding program and findings provide a basis for germplasm innovation in tomatoes.

Keywords: Ethyl-methane-sulfonate; Moneymaker; Mutation; Dose; Tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is an important crop in terms of production and consumption (Maharatha *et al.* 2019). It is widely cultivated in tropical and subtropical regions of the world (Sualeh *et al.* 2016; Yao *et al.* 2019). Conventional breeding has been applied to improve the yield and quality of tomatoes for many years, severely limiting its genetic diversity (Das *et al.* 2019). One solution for breaking the limitation is inducing effective mutations in the tomato genome (Cheng *et al.* 2019). Mutation breeding in crop plants is an effective tool for introducing novel traits, especially in crops having narrow genetic base (Liman *et al.* 2018). The effectiveness of ethyl methane sulfonate (EMS) as a mutagen has been demonstrated in many horticulture crops, including pepper (Cheng *et al.* 2019), cucumber (Xue *et al.* 2016) and cabbage (Huang *et al.* 2016). It can induce random point mutations with significant effects on the coding or regulatory domain of key genes in plant tissues/organs by converting complementary base pairing (G:C to A:T) (Shirasawa *et al.* 2016), which is important to overcome barriers of conventional breeding and broaden the genetic background of crop plants.

Generally, EMS dose resulting in the death of 50% seeds (50% lethality, LD_{50}) is an important indicator for evaluating the balance between plant variation and seed germinability (Shah *et al.* 2015; Ke *et al.* 2019). Higher EMS doses raise the genetic variations in plant tissues while inhibiting plant development. In contrast, lower EMS doses result in a lower mutation frequency, but ensures seed germinability (Shah *et al.* 2015). The effective dose of EMS varies depending on the active level, presoaking time and method, plant species, and temperature (Sayed *et al.* 2012; Ruicheng *et al.* 2017). Therefore, it is challenging to identify the effective LD_{50} dose of EMS.

Several genetic studies have verified that all mutations cannot be identified with standard molecular analysis in the first generation due to no segregation of allelic genes (M_1 generation) (Pasternak 2005; Shah *et al.* 2015). However, the mutation site is isolated during meiosis in the M_2

To cite this paper: Liu YJ, GH Li, SY Bai, FS Zheng, PZ Zhou, HL Li, XM Wang, GX Cheng (2022). Ethyl methanesulfonate induced mutagenesis enhances transgenerational stress tolerance in *Solanum lycopersicum* cultivar 'moneymaker'. *Intl J Agric Biol* 27:332–340

generation, creating recessive homozygotes (Dicenta *et al.* 2007). At same time, some mutants with stress resistance or phenotypic mutation can be identified by stress treatments or phenotype screening. Mutants with desired characters like stress resistance, plant height, leaf-color changes, or growth period can be selected in the M_2 generation (Espina *et al.* 2018; Cheng *et al.* 2019).

A few studies have identified many causal mutations in tomato EMS populations. However, the response of the M_1 generation *Solanum lycopersicum* L. to EMS during seed germination remains unknown. This study aimed to explore physiological and biological changes in seeds when exposed to EMS and optimize EMS dose for inducing new mutants in the tomato cultivar 'Moneymaker'. Further, the induced effective mutations were screened in the M_2 generation. This study will provide a basis for germplasm innovation in tomatoes in the future.

Materials and Methods

Plant material

The experiment was performed using the seeds of tomato cultivar 'Moneymaker' provided by the School of Agriculture, Ningxia University (W 106.1' N 38.5'), Yinchuan, Ningxia 750021, P. R. China.

Experimental procedures

The experiment was conducted as described by Arisha *et al.* (2014) with a few modifications. The untreated tomato seeds (M_0 seeds) were soaked in water for 4 h at 28℃ and then treated with different doses of EMS (Table 1).

The treated seeds (M1 seeds) were incubated at 28℃ on a shaker (110 rpm) for 12 h to infiltrate EMS into seeds. The M1 seeds were transferred to fume cupboard and washed with running water for 4 h to remove residual EMS on seed surface. M1 seeds without residual EMS were incubated at 28℃ for germination and then sown into pots placed in a chamber with a cycle of 16 h light/8 h dark. The control seeds were subjected to the same conditions as the treated seeds except treated with water instead of EMS. Each treatment consisted of three replicates, containing 200 seeds. Standard cultural practices were performed uniformly during the growth of plants.

Generation analysis

M_1 generation: The number of germinated M_1 seeds was counted daily until the 7th day. Seeds with visible radicles (1 mm in length) were considered to have germinated. The number of surviving seedlings was recorded 7 days after the M_1 seeds sown in the pots. Germination percentage and survival ratio were calculated according to formula:

$$\text{Germination percentage}(\%) = \frac{\text{No. of germinated seeds each day}}{\text{Total seeds sown}} \times 100$$

$$\text{Survival ratio}\ (\%) = \frac{\text{No. of survival ratio}}{\text{Total seeds sown}} \times 100$$

The abnormal plants were counted 3 days after survival seedlings were planted in greenhouse and the frequency of abnormal plants was calculated by the formula:

$$\text{Frequency of abnormal plants}\ (\%) = \frac{\text{No. of anbnormal seedlings}}{\text{Surviving seedlings}} \times 100$$

Furthermore, owing to the importance of LD_{50} for mutations observed in M_2. The experiment was performed following a completely randomized design with three replicates.

M_2 generation: All M_2 seeds from the M_1 individuals were harvested, mixed and germinated in a chamber at 28℃ for 7 days. After germination, the survival M_2 seedlings were distributed averagely into three groups. To evaluate the stress resistance of the seedlings (4-leafed-6-leafed stage), each group was subjected to either cold (4ºC), salt (NaCl, 300 mM), or drought stress (polyethylene glycol, PEG 15%, g/v) for 12 h. These seedlings were put in triangular flasks with the above-mentioned solution. All mutations observed were recorded throughout the entire period of life. In addition, the frequency and segregation ratio of the mutant plants out of total number of seedlings in the same M_2 group was calculated according to the formula.

Measurement of physiological parameters

Antioxidants activities and the malonaldehyde (MDA) content: A total of 0.2 g seeds were sampled and ground in a mortar with 5 mL phosphate buffer to determine malondialdehyde (MDA) content and the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). These substances were quantified spectrophotometrically at optical densities (ODs) and calculated as described by Dionisio-Sese and Tobita (1998).

Seed electrical conductivity: A total of 0.2 g seeds were sampled and immersed in 10 mL ddH_2O for 24 h. Afterwards, the seed conductivity was measured using a Radiometer (Copenhagen) CDM80 conductivity meter with automatic temperature correction to 25°C. The conductivity value was calculated according to the method described by Sun *et al.* (2019).

RNA extraction and gene expression

Total RNA was isolated from the study of Meng and Feldman (2010) using the Trizol method. Then, the first strand of cDNA was synthesized using the SYBRGreenPCR Master Mix (Takara, Bio, Japan). Primers were designed using PRIMER5 software (version 5.0, Premier Company, Canada). qRT-PCR was performed on a qRT-PCR equipment (qTOWER3, Germany). The ubiquitin-conjugating gene *SlUbi3* was used as the reference gene (Hoffman *et al.* 1991). Relative gene expression was calculated using the $2^{-\triangle\triangle CT}$ method (Xiaojie 2012).

Statistical analysis

Data were analyzed using the SPSS software (version 19.0, SPSS, Inc., USA). The analyzed data are presented as means ± SE (standard error) of two replicates in all measured parameters except for physiological and biological parameters, which was done using three replicates. Statistical significance was inferred at $P < 0.05$.

Results

Germination percentage (%) and LD_{50} of M_1 generation

The EMS dose of 0.25 and 0.5% decreased the number of germinated seed each day in comparison to control (Fig. 1A–C). Interestingly, the number of germinated seed each day was enhanced significantly along with increase of EMS dose, and reached to peak at 3rd or 4th days (Fig. 1D–I).

The results of seed lethality under different EMS doses were also observed in the study (Table 2). The EMS dose of 1.25% was an ideal concentration to screen tomato mutant due to 47.33% seed lethality (LD_{50}) The moderate or high EMS dose promoted the capacity for seed germination, no significant difference was observed between the control and EMS treatments of 0.25, 0.50, 0.75 and 1.00%. Only higher doses of EMS (> 1.25%) resulted in the reduction in germination significantly, with the seeds treated with 2.00% EMS dose presenting the worst germinating capacity (17.33%). These findings show that EMS toxicity only play a key role in inhibition of tomato seeds at high dose.

Physiological responses and gene expression

The activities of antioxidants (SOD, POD and CAT) in seeds treated with lower or moderate EMS doses (0.25, 0.50, 0.75, 1.00 and 1.25%) were higher or equal to those treated with higher EMS doses (1.50, 1.75 and 2.00%) (Fig. 2A–D). An opposite trend was observed in the MDA content (Fig. 2E). Interestingly, no significant difference in the electrical conductivity of seeds was observed among the treatment, even though it ranged from 61 to 72%. These data revealed that EMS do not damage cells severely, and oppositely induce synthesis of antioxidants.

The qRT-PCR assay showed that expression of *SlGID1b* was upregulated in seeds treated with low or moderate EMS dose as comparison to the control (Fig. 3A). Similar result was also observed in the expression of *SlGID1ac* gene (Fig. 3B). However, contrary results were observed in the expression levels of *SlABA1* gene: The treatments with > 1.25 EMS dose possess the higher transcriptional levels of *SlABA1* gene than the control (Fig. 3C).

Survival and phenotype of the M_1 generation

It was found that EMS injury to seed passed on to seedlings. The survival ratio of seedlings only had a slight decrease, but frequency in abnormal seedlings was increased significantly among these treatments with EMS compared with the control (Table 3). The tendency was more and more obvious along with the increase in EMS dose, and reached to optimal effect when EMS dose was up to 1.25% which ensured not only > 80% survival ratio but also > 20% the frequency of abnormal plants observed. Some abnormal seedlings or plants observed in the M_1 population, including some types such as cotyledon deformity (Fig. 4A), purple stem, yellow leaf (Fig. 4B), abnormal lateral branching and fascicular terminal bud (Fig. 4C).

Table 1: EMS treatments and its classification of tomato seeds

EMS concentration (%, v/v)	Classification
0	Low
0.25	
0.50	
0.75	Moderate
1.00	
1.25	
1.50	High
1.75	
2.00	

Table 2: Percentage of germinated seeds with EMS treatments

EMS concentration (%, v/v)	Percentage of germinated seeds (%)
0	90.00 ± 17.32a
0.25	89.33 ± 5.50a
0.50	94.67 ± 9.23a
0.75	89.67 ± 8.96a
1.00	85.67 ± 4.16a
1.25	47.33 ± 12.70b
1.50	41.00 ± 3.46b
1.75	21.00 ± 6.24c
2.00	17.33 ± 9.29c

The error represents SD for three biological replicates, and the lowercases showed the significant level at $P < 0.05$.

Table 3: Survival ratio and the frequency of abnormal plants observed in M_1 as comparison to the control

EMS dose (%)	Survival ratio (%)	Frequency (%)
0.00	91.21	0.00
0.25	100.00	0.00
0.50	92.20	0.00
0.75	87.53	10.08
1.00	100.00	11.30
1.25	83.11	23.21
1.50	100.00	10.69
1.75	80.30	12.11
2.00	70.05	13.09

M_1: the first generation

M_2 generation observation

EMS induced point mutation did not cease in the M_1 generation but inherited to M_2 generation because under normal conditions the growth in the M_2 generation was significantly worse than control. To obtain stress-resistant mutants from the M_2 generation, all individuals distributed in the three groups with either cold, salt, or drought stress,

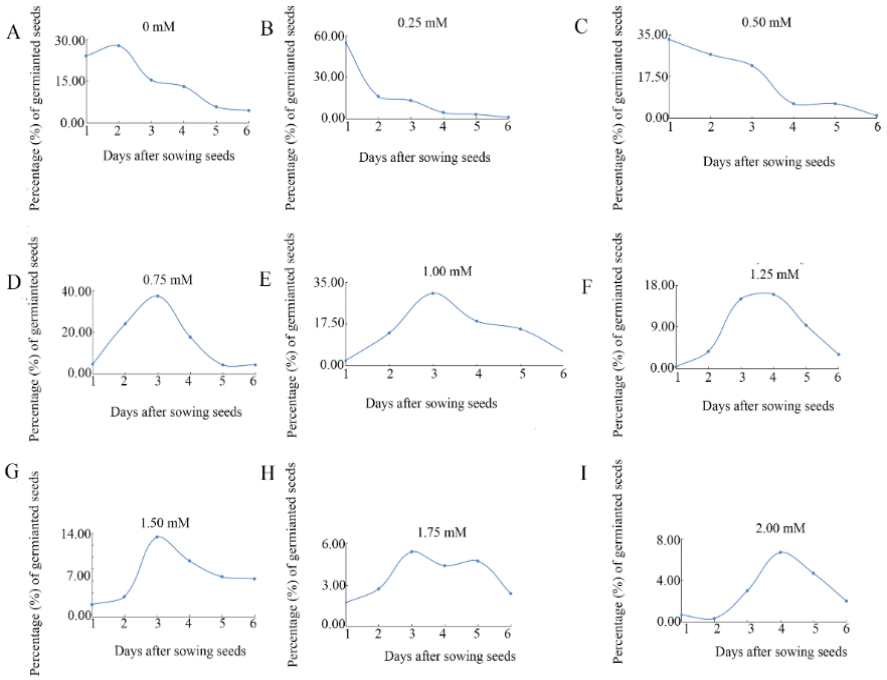

Fig. 1: The speed of seed germination in tomato as comparison to the control in the 7 days. Percentage of germinated seeds treated by EMS (**A**) 0, (**B**) 0.25 %, (**C**) 0.50 %, (**D**) 0.75 %, (**E**) 1.00 %, (**F**) 1.25 %, (**G**) 1.50 %, (**H**) 1.75 %, and (**I**) 2.00 %Tomato seeds were treated with different EMS doses in phosphate buffer solution (pH7.0) after soaked in water for 4 h at 20℃

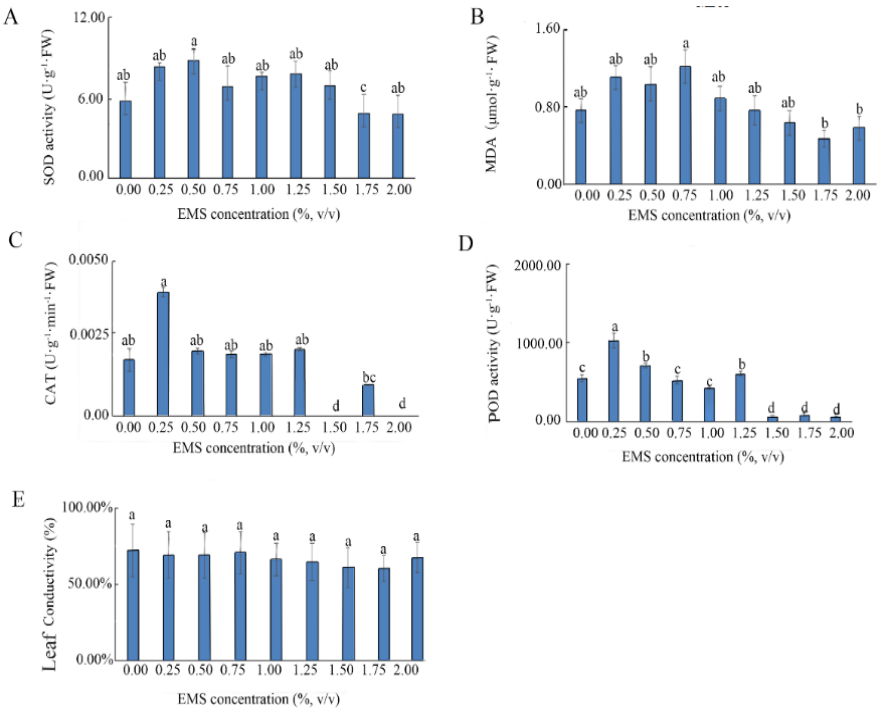

Fig. 2: Measurement of physiological parameters in the treatments treated by EMS dose. (**A**) SOD: superoxide diamutase; (**B**) POD: peroxidase; (**C**) CAT: catalase and (**D**) seed conductivity. The experiment was conducted with three biological replicates and each replicate contained 0.2 g tomato seeds. The error bars represent SD for three biological replicates, and the lowercases showed the significant level at $P < 0.05$

Table 4: Mutation frequency and segregation in mutants observed

	Types of mutants	No. of mutants	Mutation frequency (%)	Segregation ratio
The first family	Cold resistance	4	11.1	8:1
The second family	Salt resistance	3	4.1	23:1
The third family	Drought resistance	1	1.3	72:1

All M_2 seedlings are distributed in three families, and these individuals were treated by cold, salt and drought respectively at the 4-leafed-6-leafed stage

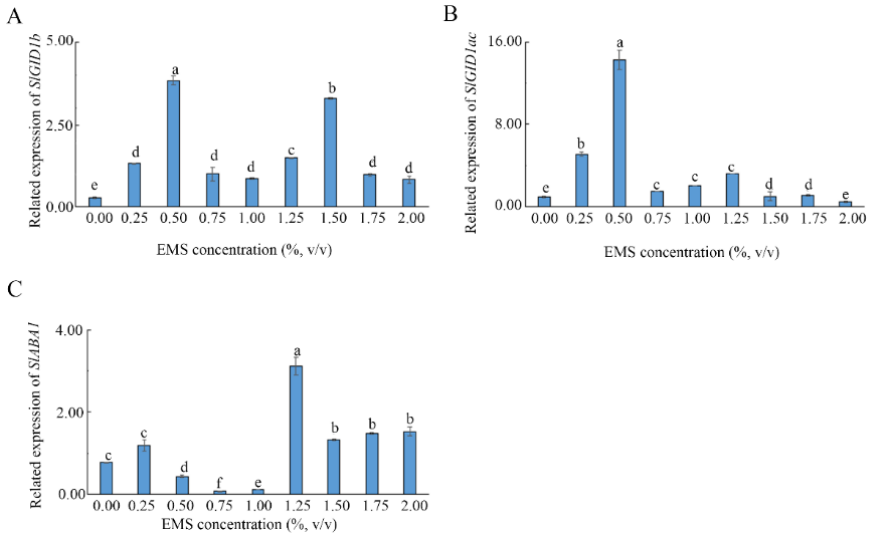

Fig. 3: Analysis of qRT-PCR in expression of key genes involved in hormone levels. Transcriptional levels of (**A**) *SlGID1b*, (**B**) *SlGID1ac* and (**C**) *SlABA1* genes in seeds of different treatments. The expression of these genes were normalized by that of ubiquitin-conjugating protein gene SlUBI3. The experiment was conducted with three biological replicates and each replicate contained 0.2 g seeds. The error bars represent ± SD for three biological replicates, and the lowercases showed the significant level at $P < 0.05$

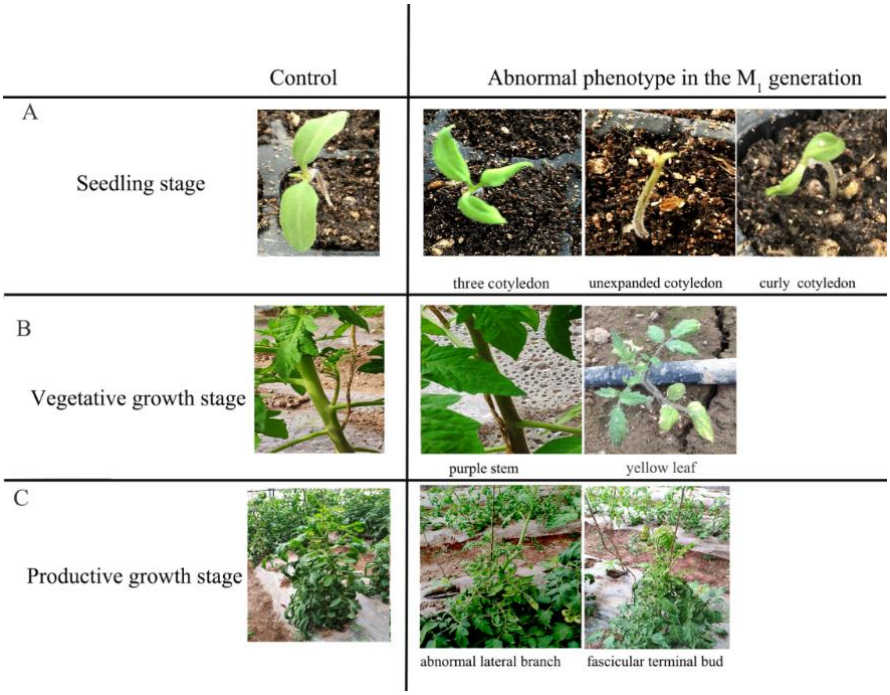

Fig. 4: Abnormal growth in the first generation (M_1) as comparison to the control (Normal seedling). Observation of phenotype in the M_1 generation at the stage of (**A**) seedling, (**B**) vegetative growth and (**C**) productive growth. The phenotype observed was recorded at the cotyledon stage, 10 days and 30 days after tomatoes were planted in greenhouse, respectively

respectively. Among the 72 plants in the cold treatment group, eight individuals resisting to cold stress was observed with segregation ratio 8:1 (Table 4). Among salt treatment group, only three salt-resistant seedlings were observed, which exhibited a mutation frequency of 4.1% and a segregation ratio of 23:1. In addition, one plant with a distinct mutant phenotype exhibited drought resistance in the drought-stress treatment group which showed a 1.3% mutation frequency and segregation ratio of 1:72 (Named '*dr-1*').

Most of the seedlings treated by cold stress died after being planted in the greenhouse, while only one seedling (named '*cr-1*') with purple leaf and stem phenotype survived to maturity stage as comparison to the control (Fig. 5A). Although, there was no difference in phenotype between '*cr-1*' and the control at maturity stage, but '*cr-1*' has less fruits than the control 120 days after sown (Fig. 6A). Furthermore, in the salt treatment, three types of phenotypic mutants with salt resistance were observed, including '*sr-1*', '*sr-2*', and '*sr-3*'. Only '*sr-3*' exhibited better growth at the seedling stage when compared with the control (complete wilting leaves) (Fig. 5B). The three mutants can complete their life cycle well, but '*sr-3*' possessed more fruits than the control 120 days after sown (Fig. 6B). In the drought treatment group, only one mutant (*dr-1*') survived. At the seedling stage, its leaves not wilted, and its growth was significantly better than the control under drought stress (Fig. 5C). At maturity, the surviving plant also exhibited differences in growth behavior, distinguishing it from the control. Especially, '*dr-1*'showed a phenotype of dwarfism with the characteristic of < 100 cm height and less fruit number (Table 5 and Fig. 6C).

Discussion

'Moneymaker' is a high-yielding tomato cultivar with little resistance with origin of the Netherlands and bred for field production (Koornneef and Hanhart 1990). However, the cultivar lacks essential stress resistance genes in its genome, making it easily threatened by various adverse factors. Given its high fruit number and susceptibility to adverse environmental conditions, 'Moneymaker' is a suitable candidate for large-scale mutant screening for stress resistance. Thus, it was selected as a model plant for studying EMS-induced mutagenesis in this study.

Many studies have focused on the mutagenesis effect of EMS but not the promotive influence on seed germination. Previous studies reported that the mutagen must permeate the germinating embryo and reach the meristemic region if it works during seed germination (Arisha *et al.* 2014). Therefore, it is no doubt that the toxic impacts of EMS on the entire seed biology result in low seed germination by damage of cell constituents, alteration of enzyme activity or delay, and inhibition of other physiological and biological processes (Talebi 2012; Kumar *et al.* 2013). However, in present study, seeds treated by lower or moderate EMS dose exhibited a good germinability as comparison to the control by promoting accumulation of antioxidants or expression of key genes. The immune and defense system of plants are triggered under environmental stress, promoting the metabolism of substances that react to the injury caused by the adverse factors, but this is not main factors. The phenomenon may primarily be attributed to EMS inducing point-mutation of negative regulatory genes such as *DAG1* (Gabriele *et al*. 2010), *RGL2* (Lee *et al.* 2002), *ACC* (Naing *et al.* 2021) related during seed germination. Mutation of these negative regulatory genes loss their inhibited function during seed germination, thereby increasing levels of antioxidants activity or activating expression of key genes like *SlGID1b* and *SlGID1ac*. However, this speculation based on the results of present experiment. The mechanism remains to be unclear and should be studied further.

Previous studies postulate that LD_{50} is a key factor in building mutant library of plant (Nascimento *et al.* 2015). Selecting an effective and efficient LD_{50} in mutation breeding programs is essential in producing a high frequency of desirable mutations. Yong *et al.* (2021) evaluated the effects of 0.5% EMS on tomato 'Improved Apollo' cultivar and found that it could enhance the mutation frequency. Similar results were also reported by Just *et al.* (2013), who found that 0.5% EMS could enhance the mean mutation frequency (one mutation per 1,710 kb) in tomato cultivar 'Micro-Tom'. However, in this study, the ideal EMS dose for mutation breeding in tomato cultivar 'Moneymaker' was 1.25% and not 0.5%, which contradicts the previous reports in tomato (Gavazzi *et al.* 1987; Shalaby and El-Banna 2013). The inconsistency in the results could be due to differences in genetic background and the methods employed by various studies, including variations in presoaking, treatment time, temperature, and the pH of the tomato seeds (Arisha *et al.* 2014). The findings of present study will inform future efforts geared towards the building of mutant library of tomato 'Moneymaker' cultivar.

Mutations in the M_2 generation are usually considered more genetically stable. In this study, three mutation types for specific stress resistance (cold, salt and drought) were observed during the M_2 generation. The visible mutation observed in one tomato plant that exhibited a uniform purple color in the leaf and stem, characterizing cold resistance. The plant (named '*cr-1*') with this mutation survived to maturity. Previous investigations have shown that a significant change in anthocyanin accumulation typically results in plant color variation (Cheng *et al.* 2019), consistent with our study. Although its growth is not better than the control, it is significant and crucial for identifying genes that regulate cold stress or anthocyanins synthesis. Furthermore, three tomato mutants with superior salt resistance than the control were also observed in the M_2 generation, and only one mutant '*sr-3*'possessed more fruits than the control. '*sr-3*'is a positive mutation caused by EMS, different from negative mutation which has to be

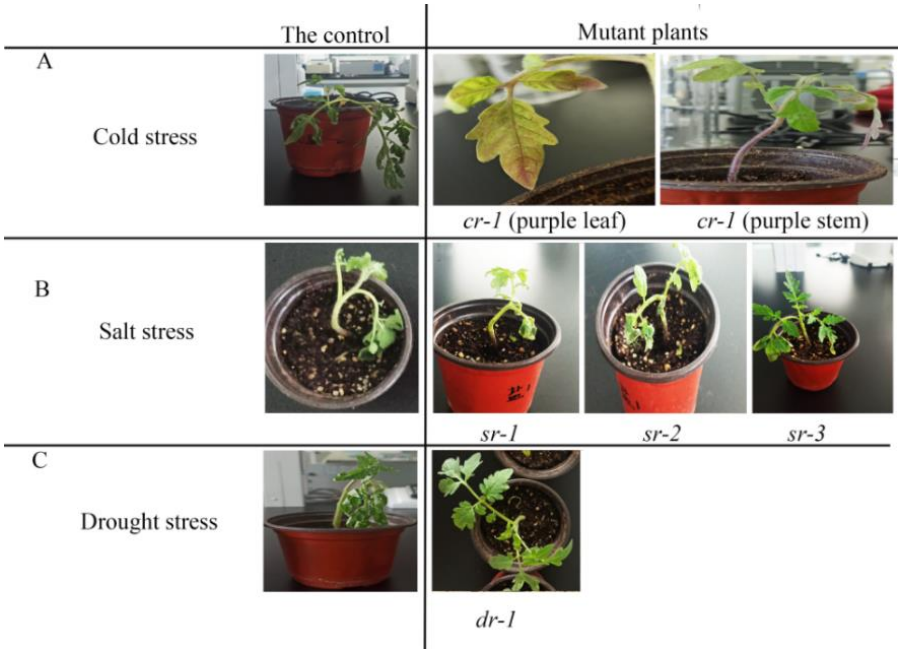

Fig. 5: Mutants with resistance stress screened in the M_2 generation. (**A**) '*cr-1*' mutant with cold resistance, (**B**) mutants with salt resistance, and (**C**) '*dr-1*' mutant with drought resistance. All M2 individuals were distributed into 3 families, and individuals from each family were treated by 4℃, 300 m*M* NaCl and 0.5 % polyethylene glycol (PEG) at the seedling stage, and planted in greenhouse until maturity

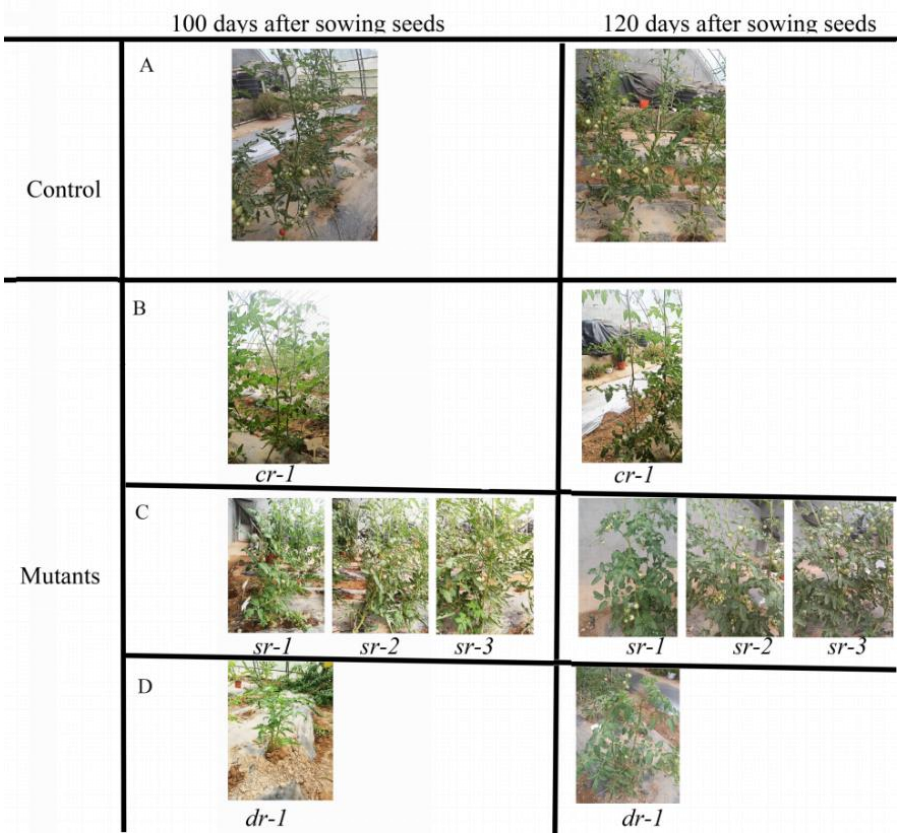

Fig. 6: Phenotype in mutants screened in the M_2 generation at different growth stages (**A**) The control, (**B**) '*cr-1*', (**C**) '*sr-1*', '*sr-2*', '*sr-3*'and (**D**) '*dr-1*'. These mutants were investigated at 100 and 120 days after sown

Table 5: Growth behavior at maturity stage in M_2 generation

Mutant	100 days after sowing			120 days after sowing	
	Plant height (cm)	Fruit number	Location of first fruit	Plant height (cm)	Fruit number
Control	108	5	4	160	40
cr-1	107	2	8	170	13
sr-1	110	7	8	175	24
sr-2	115	7	8	163	31
sr-3	115	4	9	137	25
dr-1	37	0	/	76	27

'/' presents no data

crossed or modified genetically to be utilized in breeding program. This finding will provide an important genetic resource for improvement of tomato yield in the future. Also, one mutant exhibited drought resistance and survived until maturity under drought stress. Mutants with drought resistance in tomatoes are crucial to understanding the regulatory mechanisms for plant growth and development (Lamin-Samu *et al.* 2021). All mutations observed in this study are not consistent with the classic Mendelian model. The unusual segregation ratio might be because the altered traits are controlled by multiple genes, interacting to give the observed mutation. Besides, the unusual segregation ratios may be because of the fewer number of individuals in the mutant population, which was not enough to result in the usual segregation ratio.

Conclusion

This study showed the regulatory effect of EMS on seed germination in tomato cultivar 'Moneymaker' and found that low or moderate EMS dose enhanced the capacity for seed germination by accumulation of antioxidants and expression of key genes during seed germination. The EMS dose of 1.25% yielded 50% lethality (LD_{50}) of seeds, serving as a crucial indicator for building mutant library of tomatoes. The effect of EMS on 'Moneymaker' did not stop in the M_1 generation but continued to the M_2 generation. Various stress resistance-related mutants with heritable changes, including '*cr-1*', '*sr-3*', '*dr-1*', were observed in the M_2 generation. A detailed understanding of the molecular basis underlying the desired agronomic traits of tomatoes is necessary for broadening the genetic background of tomatoes.

Acknowledgments

This work was supported through funding from the National Key Research and Development Program of China (No. 2021YFD1600302), the Natural Science Foundation of Ningxia (No. 2020AAC03092 and No. 2022AAC05023), the Major science and technology projects of Ningxia (No. NXNYYZ202001 and No. 2021BBF02024) and the National Natural Science Foundation of China (No. 31860561).

Author Contributions

Guo-Xin Cheng contributed to the conception of the study; Yu-Jing Liu performed the experiment; Guo-Hua Li, Sheng-Yi Bai, and Fu-Shun Zheng contributed significantly to analysis and manuscript preparation; Peng-Ze Zhou, and Hong-Lei Li performed the data analyses and wrote the manuscript; Xiao-Min Wang helped perform the analysis with constructive discussions.

Conflicts of Interest

No competing interest

Data Availability

All data was generated or used during the study appear in the submitted article.

Ethics Approvals

None

References

Arisha MH, BK Liang, NMS Sayed, ZH Gong, DW Li (2014). Kill curve analysis and response of first generation *Capsicum annuum* L. B12 cultivar to ethyl methane sulfonate. *Genet Mol Res* 13:10049–10061

Cheng GX, RX Zhang, S Liu, YM He, XH Feng, SU Haq, DX Luo, ZH Gong (2019). Leaf-color mutation induced by ethyl methane sulfonate and genetic and physio-biochemical characterization of leaf-color mutants in pepper (*Capsicum annuum* L.). *Sci Hortic* 257:108709

Das I, P Hazra, M Longjam, T Bhattacharjee, A Chattopadhyay (2019). Characterization of induced mutants and their hybrids of tomato (*Solanum lycopersicum* L.) for growth, yield and fruit quality traits to explore the feasibility in future breeding. *Genet Resour Crop Evol* 66:1421–1441

Dicenta F, E Ortega, P Martinez-Gomez (2007). Use of recessive homozygous genotypes to assess genetic control of kernel bitterness in almond. *Euphytica* 153:221–225

Dionisio-Sese ML, S Tobita (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Sci* 135:1–9

Espina MJ, CMS Ahmed, A Bernardini, E Adeleke, Z Yadegari, P Arelli, V Pantalone, A Taheri (2018). Development and phenotypic screening of an ethyl methane sulfonate mutant population in Soybean. *Front Plant Sci* 9:394–405

Gabriele S, A Rizza, J Martone, P Circelli, P Costantino, P Vittorioso (2010). The Dof protein DAG1 mediates PIL5 activity on seed germination by negatively regulating GA biosynthetic gene *AtGA3ox1*. *Plant J* 61:312–323

Gavazzi G, C Tonelli, G Todesco, E Arreghini, F Raffaldi, F Vecchio, G Barbuzzi, MG Biasini, F Sala (1987). Somaclonal variation versus chemically induced mutagenesis in tomato (*Lycopersicon esculentum* L.). *Theor Appl Genet* 74:733–738

Hoffman NE, K Ko, D Milkowski, E Pichersky (1991). Isolation and characterization of tomato cDNA and genomic clones encoding the ubiquitin gene ubi3. *Plant Mol Biol* 17:1189–1201

Huang S, Z Liu, D Li, R Yao, H Feng (2016). A new method for generation and screening of Chinese cabbage mutants using isolated microspore culturing and EMS mutagenesis. *Euphytica* 207:23–33

Just D, V Garcia, L Fernandez, C Bres, C Rothan (2013). Micro-Tom mutants for functional analysis of target genes and discovery of new alleles in tomato. *Plant Biotechnol* 30:225–231

Ke C, W Guan, S Bu, X Li, Y Deng, Z Wei, W Wu, Y Zheng, DA Lightfoot (2019). Determination of absorption dose in chemical mutagenesis in plants. *PLoS One* 14:2–9

Koornneef M, CJ Hanhart (1990). The genetics of regeneration capacity in tomato. *In: Abstract of 7th International Congress of Plant tissue cell Culture*, p:300. Amsterdam, The Netherlands

KumarAP, A Boualem, A Bhattacharya, S Parikh, N Desai, A Zambelli, A Leon, M Chatterjee, A Bendahmane (2013). SMART – sunflower mutant population and reverse genetic tool for crop improvement. *BMC Plant Biol* 13:38–45

Lamin-Samu A, M Farghal, M Ali, G Lu (2021). Morpho-physiological and transcriptome changes in tomato anthers of different developmental stages under drought stress. *Cells* 10:1–24

Lee SC, H Cheng, KE King, W Wang, J Peng (2002). Gibberellin regulates Arabidopsis seed germination *via* RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. *Genes Dev* 16:646–658

Liman M, O Falusi, A Gado (2018). *Status of Mutation Breeding of Crop Plants in Nigeria.* Lambert Academic Publishing, Sunnyvale, California, USA

Maharatha S, B Dahal, N Acharya, S Devkota (2019). Price behavior, marketing and consumption pattern of tomato in selected region of western Nepal. *Arch Agric Environ Sci* 4:472–477

Meng L, L Feldman (2010). A rapid TRIzol-based two-step method for DNA-free RNA extraction from *Arabidopsis siliques* and dry seeds. *Biotechnol J* 5:183–186

Naing AH, J Xu, CK Kim (2021). Editing of *1-aminocyclopropane-1-carboxylate oxidase* genes negatively affects petunia seed germination. *Plant Cell Rep* 41:209–220

Nascimento KS, MM Rêgo, AMM Nascimento, ER Rêgo (2015). Ethyl methanesulfonate in the generation of genetic variability in Capsicum. *Acta Hortic* 1087:357–363

Pasternak J (2005). *The Genetic System: Mendel's Laws of Inheritance and Genetic Linkage*, pp:37–74. John Wiley and Sons, Inc., New York, USA

Ruicheng Z, L Wei, P Sujun, L Dai, S Liu (2017). Application of chemical mutagenesis in improving germplasm resource. *Mol Plant Breed* 15:5189–5196

Sayed S, FAE Lil, SS Saleh (2012). Effects Of chemical mutagenic agent in phytoconsti, tuents on *Gardenia Jasminoids*, ellis, through tissue culture technique. *J Appl Sci Res* 8:1392–1397

Shah S, ZH Gong, MH Arisha, A Khan, SL Tian (2015). Effect of ethyl methyl sulfonate concentration and different treatment conditions on germination and seedling growth of the cucumber cultivar Chinese long (9930). *Genet Mol Res* 14:2440–2449

Shalaby TA, A El-Banna (2013). Molecular and horticultural characteristics of *in vitro* induced tomato mutants. *J Agric Sci* 5:155–163

Shirasawa K, H Hirakawa, T Nunome, S Tabata, S Isobe (2016). Genome-wide survey of artificial mutations induced by ethyl methanesulfonate and gamma rays in tomato. *Plant Biotechnol J* 14:51–60

Sualeh A, A Daba, S Kiflu, A Mohammed (2016). Effect of storage conditions and packing materials on shelf life of tomato. *Food Sci Qual Manage* 56:60–67

Sun J, G Cheng, L Huang, S Liu, M Ali, A Khan, Q Yu, SB Yang, DX Luo, ZH Gong (2019). Modified expression of a heat shock protein gene, CaHSP22. 0, results in high sensitivity to heat and salt stress in pepper (*Capsicum annuum* L.). *Sci Hortic* 249:364–373

Talebi AB (2012). Ethyl Methane Sulphonate (EMS) Induced mutagenesis in malaysian rice (cv. mr219) for lethal dose determination. *Amer J Plant Sci* 3:1661–1665

Xiaojie Y (2012). Analysis of the copy number of exogenous genes in transgenic cotton using real-time quantitative PCR and the $2^{-\triangle\triangle CT}$ method. *Afr J Biotechnol* 11:6226–6233

Xue C, C Wang, L Fan, N Hao, T Wu (2016). Ethylmethanesulfonate mutagenesis of cucumber for large-scale mutant screens. *Pak J Bot* 48:2261–2266

Yao Q, Z Peng, H Tong, F Yang, Q Su (2019). Tomato plant flavonoids increase whitefly resistance and reduce spread of tomato yellow leaf curl virus. *J Econ Entomol* 112:1–7

Yong WTL, AK Aswandy, BE Cheong, KF Rodrigues (2021). Mutagenic effects of ethyl methanesulfonate on nine protein coding genes in tomato (*Solanum lycopersicum* L.). *Sci Hortic* 276:109739