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Influence of ethyl methane sulfonate (EMS) on M3 generations of two genotypes of tomato (*Solanum lycopersicum)*

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Abstract

Mutation breeding is considered to be economic and efficient in plant improvement, and the use of chemical mutagens such as ethyl methane sulfonate (EMS) can potentially address plant breeding challenges. The aim of this present study is to create variations from existing two genotypes of tomato (Cobra and Roma vf) using a chemical mutagen ethyl methane sulfonate (EMS) in the M3 generation. Variations like increased yield, increase in fruit size and weight, variations in leaf shape, growth habit and fruit colour were conspicuously observed in the two genotypes studied in the M3 generation. In Cobra, variant 3 recorded the highest fruit weight per plant of 1.118kg while 0.318kg was the least and it was recorded by variant 7. Variations in the fruit size are equally observed with some variants producing large fruits while others produce small fruits. Variant 2 had a single fruit weight of about 63g while variant 8 produced small fruit with a single fruit weighing about 27g. There are equally some remarkable variants in Roma vf, variant 2, had a fruit weight of 1.145kg with 56.4g fruits harvested with a single fruit weight of 20.3g. This variant produces lots of many small fruits with a creeping growth habit that makes it behave like an indeterminate variety.

Keywords: Mutation; Mutagens; Mutant; Variants; Varieties

1. Introduction

With increase in population, to eliminate hunger and overcome poverty, food security must be ensured. However, among other things food security to with stand population increase cannot be adequately addressed without crop improvement. Because increased crop production remains the only route through which food security and supply to the citizenry can be satisfactorily be achieved. Therefore, innovative and integrated approach need to be employed in ensuring sustainable food production as well as supply and consumption. As number of food insecure population in Africa and Nigeria in particular remains unacceptable. Crop production to increase yield face a lot of environmental stresses, both abiotic and biotic stresses. These stresses according to Ahmad et al. (2019ab) and Naikoo et al. (2019) requires alterations in the secondary metabolites such as phenolic. Hence induced mutation that can cause reliable change in the base sequence of dioxyribonucleic acid (DNA) can be very pivotal in the development of crops that can show reasonable increased tolerance to a range of these stresses to increase yield. In crop breeding program mutation breeding is coherent tool according to Bhat et al. (2007) and Khursheed et al. (2016) meant to understand genetic phenomena like inheritance, genetic advance, genotypic and phenotypic coefficient of variability, mutagenic effectiveness and efficiency. This technology has been employed world over for crop improvement programs of various crops (Bhat et al., 2005, Adamu and Aliyu, 2007, Khankwal and Shu, 2009). In breeding program mutation can be achieved through radiation (Traditional), chemical and biological means. The chemical and biological agents are referred to as mutagens. Mutagens cause changes to the composition of DNA that can affect the transcription and replication of the DNA. In severe cases this however can lead to death of crops. Many mutagens are however not

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mutagenic by themselves, they can form mutagenic metabolites through cellular process. In such cases they are called promutagens.

Ethyl methane sulfonate (EMS) is one of the chemical mutagens that have been found to be useful in crop breeding program because of its availability, easy to handle, relatively cost effective and milder effect on genetic constituents of the target crop. According to the report of Minocha and Amason, (1962), Hajira, (1979) the chemical usually causes higher proportion of point mutation in plants. Though loss of some parts of a chromosome or deletion do occur. EMS has proved effective in creating phenotypic variations like potato shaped leaves, reduced date to flowering, resistant to some diseases in tomato (Yudhvir, 1995). Induced mutation has been used to enhance yield, better nutritional quality and wider adaptability of most important crops such as wheat, rice, pulses, millets and oil seeds worldwide (Raina et al., 2016). Induced mutation can rapidly create variability in quantitatively and qualitatively inherited traits in crop (Bhat et al., 2006; Raina et al., 2018). Crop improvement through induced mutation has resulted in the development and release of about 3222 mutant varieties worldwide, with India alone having about 330 mutant varieties (Rafiul et al., 2016). Induced mutations are widely used to obtain mutants with desirable plant traits (Ahloowalia et al., 2004). Improvements in mutant varieties of different crop species have been used widely in cultivation (Kharkwal and Shu, 2009; Mohan Jian and Suprasanna, 2011). Thus induced mutation in tomato is important for tomato breeding and for tomato improvement. The use of chemical mutagens such as ethyl methane sulfonate (EMS) can potentially address plant breeding challenges in tomato production. Thus the aim of this present study is to create variations from existing two genotypes of tomato (Cobra and Roma vf) using a chemical mutagen ethyl methane sulfonate (EMS) in the M3 generation. Selection of the variants in the field will be done based on the useful agronomic traits exhibited by the variants.

2. Material and methods

2.1. Plant Materials

Two varieties of tomatoes used in this experiment are; Cobra and Roma vf

2.2. **Experimental materials**

2.2.1. Mutagenic agent used and the source

Ethyl methane sulfonate (EMS) was obtained from the United States of America.

Non chemicals

Petri-dishes, filter papers, masking tape, beakers, syringe, sieve, used water sachets for the nursery, compost manure, phyto-feeds, wooden stakes, and ropes.

2.3. Experimental procedure

A stock solution of EMS was prepared using distilled water. The stock solution was then used to prepare, 0.01%, 0.1%, 0.25% and 0.5% EMS solutions using 0.1 M phosphate buffer (pH7.2). These concentrations of ethyl methane sulfonate (EMS), (0.01%, 0.1%, 0.25% and 0.5%) was used to induce mutation on the two tomato varieties.

2.4. Field evaluation of Mo plants of two varieties of tomato soaked in Ethyl Methane Sulfonate

2.4.1. Soaking of the seeds in the mutagen

One hundred seeds each of the two varieties were soaked in water and in 0, 0.01, 0.1, 0.25 and 0.5% concentrations of ethyl methane sulfonate (EMS) for 3hrs, 6 hrs and 12 hrs respectively. At the end of each duration of soaking the seeds were washed five times with distilled water and air dried before planting in the petri dishes. Treated seeds were planted in petri dishes and placed in a dark room at 24 ± 2 ^{0}C until germinated, as described by Talebi et al. (2012) to enhance germination. The sprouted seeds were planted in plastic bags in the nursery. Seedlings were raised in the nursery growth medium of compost in plastic bags. Two seedlings were planted in a bag, watering was done when necessary until they mature for transplanting. The seedlings spent three weeks after planting before transplanting them to the field. The experimental field was ploughed, harrowed and ridged. The experiment was laid out in a 2x3x5 factorial in a randomized complete block design (RCBD) with three replications. The factors are; A=2 varieties, B=3 durations of soaking and C= 5 concentrations of EMS. Seedlings were planted at a spacing of 1m between ridges and 0.45m within ridges. Poultry manure was applied evenly to the ridges to enhance the nutrient capacity of the soil. Phyto- feed was applied in a ring form two weeks after transplanting to the plants to boast the nutrients of the soil. Weeding was carried out manually using hoe at a regular interval to ensure a weed free field. Harvesting was done when the fruits were fully ripe, that is when the fruits turn fully red. These fruits were harvested according to treatment and the seeds processed for use in the next experiment.

2.4.2. Field Evaluation of M1 seeds of the two genotypes

100 seeds from plants harvested from each treatment in experiment 1 were presoaked in water and then planted in petri dishes to enhance germination. The experiment was laid in a 3x5 factorial in a randomized complete block design with 3 replications. Factor A is the duration of soaking (3hrs, 6hrs and 12 hrs.) and Factor B is the concentrations of EMS (0, 0.01, 0.1, 0.25, and 0.5%). The matured fruits were harvested according to treatments. Selections were done based on the desired agronomic traits for further evaluation in the M2. Agronomic traits such as earliness to maturity, fruit yield and quality, fruit size and so on were some of the traits based for the selection. The selected fruits were processed and seeds planted in the next experiment for evaluation as M2 plants.

2.4.3. Field evaluation of selected M2 variants of Cobra and Roma vf

Ten selected variants of cobra and eleven variants of Roma vf were selected and evaluated in this experiment. Normal agronomic practices were carried out and the matured fruits harvested at full maturity and packed according to treatment for use in M3

2.4.4. Field evaluation of M3 variants of Cobra and Roma vf

Eight variants of cobra and four variants of Roma vf were evaluated in field using randomized complete block design with three replications. Agronomic parameters studied include; days to 50% flowering, days to maturity, fruit weight per plant, number of branches, number of fruits harvested per plant, number of flowers per truss, number of fruits per truss, number of truss per plant, number of fruits affected by blossom end rot, leaf length, leaf width, number of leaves and plant height.

2.5. Statistical Analysis

The analysis of variance was carried out using Genstat software version 12.1 (VSN International Ltd, 2009) as outlined in the design of the study. Treatment means were separated using least significant difference (LSD) as outlined in Obi (2002)

3. Results

3.1. Observations of M3 variants of Cobra

Table 1: shows mean number of days to 50% flowering, days to maturity, fruit weight per plant, number of fruits harvested per plant, number of flowers per truss, number of fruits per truss and number of fruits affected by blossom end rot.

3.1.1. Days to 50% flowering

The mean number of days to 50% flowering had a significant difference on the variants studied. The least number of days was recorded by variant 3 (42.2). The longest number of days was recorded by variant 8 (49.2). The number of days to 50% flowering of variants 2, 4, 5 and 6 were statistically similar ($P < 0.05$).

3.1.2. Days to maturity

There is a significant effect on the number of days to maturity of the variants. The least number of days was recorded by variant 3 (74.4), followed closely by variants 4, (75), 5 (75), and 6 (75.8). The longest number of days was recorded by variant 1 (84.4). Variants 7 and 8 are statistically the same ($P < 0.05$).

3.1.3. Fruit weight per plant

The fruit weight per plant had a significant effect ($P < 0.05$) on the variants. The highest fruit weight per plant was recorded by variant 3 (1.118kg), followed closely by variants 5 (1.078kg), and 2 (1.069kg). The least weight was recorded by variant 1 (318g). Variants 4, 6 and 8 are statistically the same ($P < 0.05$). Variant 3 recorded 71.55% and 63.5% increase against variant 7 and 6 respectively.

3.1.4. Number fruits and number of flowers per truss

The number of fruits actually harvested per plant had a significant effect ($P < 0.05$) on the variants. Variant 3 (23.6) had the highest number of fruits harvested. This is followed by variants 5 (22.6) and 8 (21.2). The least number of fruits harvested was recorded by variant 7. The decrease in the number of fruits harvested relative to variant 3 by variants; 7, 6, 1, 2 and 4 were; 174.42%, 78.79%, 59.46%, 38.82%, and 28.26% respectively. The number of flowers per truss had a significant difference ($P < 0.05$) on the variants. The highest number was recorded by variant 3 while variant 6 recorded the least number. The other variants are statistically the same ($P < 0.05$). The highest number of fruits per plant was recorded by variant 3 which is 70.27% significantly different ($P < 0.05$) from the least recorded by variant 6. Variants 2, 4, and 8 were statistically similar ($P < 0.05$). The number of fruits per truss equally had significant effect on the variants. The highest number was recorded by variant 4 while the least was recorded by variant 6.

3.1.5. Number of fruits affected by blossom end rot

The number of fruits affected by the blossom end rot had a significant effect ($P < 0.05$) on the variant. The highest number affected was recorded by variant 1 (control), while the rest are statistical the same ($P < 0.05$).

Table 1 Influence of EMS on yield and yield component of M3 variants of cobra

Mutants	D50%F	DM	FW/P	NFH	NFL/T	NFR/P	NFR/T	NBER
CV1 (control)	48.2	82.4	479	14.8	5.6	19.2	2.8	3.6
CV2	43.0	77.0	1069	17.0	6.4	24.8	3.8	0
CV3	42.2	74.4	1118	23.6	6.6	37.0	4.0	0
CV4	43.0	75.0	956	18.4	6.4	27.2	4.2	0
CV5	45.4	75.0	1078	22.6	6.0	33.8	3.2	0
CV6	45.0	75.8	412	13.2	4.8	11.0	2.2	0
CV7	48.6	82.8	318	8.6	5.8	14.6	3.6	0
CV8	49.2	84.2	574	21.2	5.4	21.6	3.2	0
LSD($P < 0.05$)	2.312	2.401	440.6	8.99	1.354	12.068	1.236	0.2509

D50% = Days to 50% flowering, DM = Days to maturity, FW/P = Fruit weight per plant; NFH = Number of fruits harvested per plant, NFL/T = Number of flowers per truss, NFR/P = Number of fruits per plant, NFR/T= Number of fruits per truss, NBER = Number of fruits affected by blossom end rot.

Table 2 shows leaf length, leaf width, number of branches, number of branches at maturity, number of truss per plant, plant height, plant height at maturity and plant girth

3.1.6. Leaf length and width

The mean leaf length and width had a significant effect ($P < 0.05$) on the variants. The longest leaf length and width was recorded by variant 1. The shortest was recorded by variant 8. Variants 4, 5, 6 and 7 are statistically similar ($P < 0.05$) in terms of leaf length and width.

3.1.7. Number of branches

The number of branches per plant had a significant difference ($P < 0.05$) on the variants. The highest number of branches at flower bud initiation was recorded by variant 4 while variant 2 had the least. Variants 3, 5, 6 and 8 recorded no significant difference ($P < 0.05$)

3.1.8. Number of leaves

The highest number of leaves per plant was recorded by 6 which is significantly different ($P < 0.05$) from the least (variant 2). The other variants are statistical the same ($P < 0.05$)

3.1.9. Number of truss per plant

There is a significant difference on the mean number of truss per plant of the variants. The highest number was recorded by variants 3 while variant 1 recorded the least. Variants 2, 4 and 5 are statistical the same ($P < 0.05$)

3.1.10. Plant girth

Variant 3 had the highest plant girth which is significantly different from variant 1 with the least mean plant girth. The other variants studied are statistical the same (P < 0.05)

3.1.11. Plant height

The plant height shows a significant effect (P < 0.05) on the variants. The highest mean plant height was recorded by variant 1 while variant 5 had the least. The other variants are statistical the same. The variant 5 recorded 31.06% decrease in height relative to variant 1

Table 2 Influence of EMS on growth components of M3 variants of cobra

Mutants	LL	LW	NB	NL	NT/P	PG	PH
CV1	21.46	17.56	3.8	23.8	11.4	3.94	38.4
CV2	20.0	16.84	3.6	18.2	18.6	4.86	32.5
CV3	21.28	17.04	5.4	26.2	19.6	4.92	30.9
CV4	18.94	14.54	6.8	32.4	19.4	4.8	36.1
CV5	18.02	12.96	4.8	23.6	19.2	4.66	29.3
CV6	15.66	12.7	5.8	39.6	8.2	4.5	31.0
CV7	15.5	12.7	6.2	30.8	12.2	4.84	30.4
CV8	15.38	12.12	5.2	22.6	12.8	4.8	33.8
LSD(P < 0.05)	3.764	3.582	2.146	10.55	5.139	0.503	8.71

LL = Leaf length, LW = Leaf width, NB = Number of branches, NL = Number of leaves, NT/P = Number of truss per plant, PG = Plant girth, PH = Plant height.

3.2. Observations of M3 variants of Roma vf

Table 3 shows number of days to 50% flowering, days to maturity, fruit weight per plant, number of fruits harvested per plant, number of flower per truss, number of fruits per truss and number of fruits affected by blossom end rot

3.2.1. Days to 50% flowering

The mean number of days to 50% flowering had a significant difference (P < 0.05) on the variants. The least number of days to 50% flowering was recorded by variant 2 while variant 1 recorded the longest number of days. Variant 3 and 4 are statistical the same (P < 0.05)

3.2.2. Number of days to maturity

The mean number of days to maturity had a significant difference (P < 0.05). Days of maturity among the variants range between 75.8-80.4 days of which variant 4 had longer days to maturity.

3.2.3. Fruit weight per plant

The mean fruit weight per plant had a significant difference on the variants. The highest weight was recorded by variant 2 (1.145kg) while the least was recorded by variant 4. The recorded fruit weight in variant 2 was 41.22% and 45.68% higher than variant 1 and 4 respectively. The highest mean number of fruits harvested was recorded by variant 2 (56.4) of which is 74.82% significantly different from variant 1 (14.2) which had the least. Variants 3 and 4 are statistical similar (P < 0.05)

3.2.4. Number of flowers and fruits per truss

The number of flowers per truss had a significant difference (P < 0.05) on the variants. The highest number of flowers was recorded by variant 2 while variant 1 had the least. Variant 3 and 4 are statistically the same (P < 0.05). The mean number of fruits per truss equally had a significant effect on the variants. The highest number was recorded by variant 2while variant 4 recorded the least.

3.2.5. Number of fruits affected by blossom end rot

The highest number of fruits affected by blossom end rot was recorded by variant 1 (control) which is statistically different from the rest of the variants.

Table 3 Influence of EMS on yield and yield component of M3 variants of Roma vf

Mutants	D50%F	DM	FW/P	NFH	NFL/T	NFR/P	NFR/T	NBER
RV1(control)	49.2	79.4	673	14.2	5.8	24.6	4.4	0.4
RV2	42.4	75.8	1145	56.4	8.4	77.0	6.2	0.0
RV3	45.4	76.8	1079	25.6	6.2	25.4	3.8	0.0
RV4	45.4	80.4	622	24.4	6.4	27.0	3.6	0.0
LSD($P < 0.05$)	2.885	7.73	410.7	12.4	1.075	15.95	1.349	0.67

D50%f = Days to 50% flowering, DM=Days to maturity, FW/P = Fruit weight per plant, NFH = Number of fruits harvested per plant, NFL/T = Number of flowers per truss, NFR/P = Number of fruits per plant, NFR/T = Number of fruits per truss, NBER = Number of fruits affected by blossom end rot.

Table 4: shows leaf length, leaf width, number of branches, number of branches at maturity, number of truss per plant, Plant height, plant height at maturity and plant girth.

3.2.6. Leaf Length and width

The leaf length and width had a significant effect ($P < 0.05$) on the variants. The shortest leaf length and width was recorded by variant 2 while variant 1 recorded the longest leaf length and width. Variant 3 and 4 are statistically the same.

3.2.7. Number of branches

The number of branches per plant had a significant difference ($P < 0.05$) on the variants. The highest number of branches was recorded by variant 4 while variant 1 had the least

3.2.8. Number of leaves

The number of leaves had a significant difference ($P < 0.05$) on the variant studied. The highest number of leaves was recorded by variant 4 while variant 1 had the least. Variant 2 and 3 show statistically similar results.

3.2.9. Number of truss per plant

The number of truss per plant had a significant difference ($P < 0.05$) effect on the variants. The highest number was recorded by variant 2 while variant 1 had the least. Variant 3 and 4 are statistically the same.

3.2.10. Plant girth

The plant girth equally had a significant effect on the variants. The highest girth was recorded by variant 2 while variant 1 had the least. Variant 3 and 4 are statistically the same.

3.2.11. Plant height

The plant height had a significant effect ($P < 0.05$) on the variants. Variants 3 recorded the least plant height that is 19.28% decrease in height relative to variant 1 that recorded the highest plant height. Variants 2 and 4 are statistically the same.

Table 4 Influence of EMS on growth components of M3 variants of Roma vf

Mutants	LL	LW	NB	NL	NT/P	PG	PH
RV1	23,22	17.28	3.2	13.4	11.2	3.84	36.38
RV2	16.1	11.1	4.8	28.4	26.6	5.12	35.5
RV3	18.6	14.98	5.6	27.6	16.8	5.02	30.5
RV4	20.48	16.32	8	38.2	16.4	4.94	35.4
FLSD(P<0.05)	3.827	3.154	2.37	9.19	4.976	0.67	4.31

LL = Leaf length, LW = Leaf width, NB = Number of branches, NL = Number of leaves, NT/P = Number of truss per plant, PG = Plant girth, PH = Plant height.

4. Discussion

Induced mutation using EMS created lots of variations in the two genotypes. Cobra variant 3, showed a remarkable difference from others as shown in Table 1. The performance of the variant was consistent as it recorded the shortest number of days to flowering, maturity and equally had the highest yield. It recorded the highest fruit weight of 1.118kg per plant with the highest number of fruits harvested (23.6) per plant giving a single fruit weight of 47.2g. Variant 5 is nest in rank with fruit weight per plant of 1.078kg and number of fruits harvested per plant is 22.6 (Table1). Fruit weight as it where, is an index of its size and is determined by the amount of photosynthesis which takes place in the plant as well as how efficiently those products of photosynthesis were transported or translocated to the fruits that represent the sink. Weight of fruits for the variants provided information on resource capture and conversion efficiency of the different variants over the period studied. There is a high tolerance of the variants studied to a physiological disease blossom end rot. The variants recorded zero incidence to the physiological disease as compared to the control that recorded incidence of 3.6 per plant. This probably enhanced the recorded yield result of the variants. Yudhvir, (1995) submitted that induced mutation using EMS produced variants which are resistant to diseases in tomato. In Roma vf genotype, variant 2 recorded the shortest number of days to flowering, to maturity and equally had the highest fruit yield per plant as shown in Table 3. It recorded a fruit weight per plant of 1.145kg with the number of fruits actually harvested of 56.4 giving a single fruit weight of 20.4g supporting Yudhvir, (1995) who submitted that mutation induction with EMS reduces fruit size in tomato. This reduction in fruit size agriculturally may be a welcome development as it will enable the tomato plant to survive environmental stress. To survive stress a crop may reduce the number of fruits/seeds generated by producing a smaller quantity of fruits and viable seeds. This reproductive adjustment attribute is differential among crops as have been created by EMS induced mutation in tomato. The genetic differences of the variants highlight the high genotypic plasticity for fruit weight yield per plant observed in the study, while the in consistent performance of the variants may have indicated the existence of genotype x environmental (G x E) interaction. This variant 2 of Roma vf produced lots of small cone shaped fruits. The fruits had a dark green shade around the calyx region when green which gradually turns red when it ripens. The variant equally had a creeping growth habit similar to cherry tomato. This makes it suitable for growth in green houses as well as the ability to overcome or prevent lodging. The leaf morphology is completely distinct from the control. Variations were created in the M3 generations of the two genotypes studied. The results presented is of evidence that EMS increased the genetic variability in the two varieties of tomato studied. Gulfishan et al. (2013) noted that EMS play an important role in increasing genetic variability in self-pollinated plants which possess narrow genetic variability. The success of this study lies in the dose of mutagen used and its effectiveness. Gulfishan et al. (2012) and Gnanamurty et al. (2012) reported that selection of an adequate mutagen concentration or dose determine the success of mutation breeding.

5. Conclusion

Yield is the major focus of breeders in crop improvement program and variants or mutants with this trait should be selected and recommended to the farmers. In the case of the present study variant 3, 5 and 2 of the tomato variety Cobra and variant 2 and 3 of Roma vf show remarkable improvement in yield as they had highest fruit weight among the other variants. Hence they are recommended to farmers for tomato production improvement and yield. Variant 2 of Roma vf produces lots of many small fruits though with a single fruit weighing 27g, it has a creeping growth habit that makes it behave like an indeterminate variety. Thus ideal for greenhouse production. All variants or mutants except the control were found to be tolerant to environmental disorders like the blossom end rot according to the results generated from this study. These mutants are also recommended to the farmers especially in areas prone to the tomato disease.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors agreed to publish the work on this journal, hence there is no conflict of interest regarding the publication of this article.

Statement of ethical approval

The present research work does not contain any studies performed on animals'/humans subjects by any of the authors.

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