INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2002/04–1–89–92
http://www.ijab.org

Ethyl Methane Sulfonate Induced Genetic Variability in *Lycopersicon esculentum*

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ABSTRACT

In order to determine the optimal conditions for mutagenesis of *Lycopersicon esculentum* cv. Roma, seeds were treated with 0.1, 0.5 and 1% ethyl methane sulfonate (EMS) and were exposed for 3 and 6 h. After treatment, the effect of EMS on seed germination was studied. An over all decrease in seed germination was observed with increasing concentration and exposure of EMS. The treated and untreated plants as control were grown to observe 12 different characters in M1 generation. The characters studied include root and shoot length of seedlings, leaf area, flowering age, plant height, number of branches, number of leaves, fruiting age, ripening age, number of fruits, weight of fruits and chlorophyll content. In general, the variance was increased for all the characters under study in the treated populations compared to the control.

Key Words: Induced mutagenesis; EMS; Genetic variability; *Lycopersicon esculentum;* Mutation breeding

INTRODUCTION

Mutation is the ultimate source of all genetic variation. It provides the raw material for evolution. Mutations can be induced artificially. Induced mutations are the plant breeder's one hope for freedom from complete dependence on nature as the only source of the genetic variants necessary in plant improvement (Hayes *et al*., 1955). These mutations typically occur at much higher frequencies than spontaneous mutations do (Satoh *et al*., 1982). Recent addition of chemical mutagens opened up new era in mutation research. Ethyl methane sulfonate (EMS), a compound of the alkaline sulfonate series is widely known to induce a higher frequency of mutations in both microorganisms (Kolmark, 1957) and higher plants (Minocha & Arnason, 1962; Hajra, 1979). In plants, EMS usually causes point mutations, but loss of a chromosome segment or deletion can also occur (Okagaki *et al*., 1991). Therefore, EMS has the potential of altering loci of particular interest without inducing a great number of closely linked mutations.

In the present study, *L. esculentum* cv. Roma (a variety of tomato) seeds were used for EMS mutagenesis because its high productivity in Pakistan (Burney, 1996). The purpose of the present study was to determine the optimal conditions for seed mutagenesis of *L. esculentum,* to observe the effect of EMS on germination of seeds, and to study different characters of control and mutagenized plants.

MATERIALS AND METHODS

L. esculentum cv. Roma seeds were subjected to three treatment levels of EMS (0.1, 0.5 and 1% V/V) and two durations of exposures (3 and 6 h) resulting in six treatment combinations along with one control. The treated and control seeds were taken and germinated in wet filter papered petriplates. Before that, the seeds were surface sterilized with 95% ethanol and 0.3% mercuric chloride (Somasegaran *et al*., 1982). These seeds were observed daily and percentage of seed germination was calculated by counting the number of seeds showing germination. Lengths of root and shoot were also measured by means of scale.

Tomato seeds were sown in pots. After sowing of seeds, plants were routinely observed for any morphological change. Number of leaves, number of branches, height of plant, flowering age, leaf area, fruiting age, ripening age, number and weight of fruit was recorded for each plant. Chlorophyll was extracted in 80% acetone and was assayed as described by Lichtenthaler and Wellburn, (1983).

RESULTS AND DISCUSSION

An overall increase in EMS concentration reduced the germination in the M1 generation, (Fig. 1). Seeds treated with 1% EMS for 6 h had the lowest germination percentage among all the treatments. Similar adverse effect of increasing EMS concentrations on the germination has been reported previously on seeds of *Capsicum annuum,* which was attributed to the toxic nature (Alcantara *et al*., 1996). However, the intensity of effects may vary due to structural differences among seeds of different plants. Root and shoot length of seedlings were measured on 12^{th} day of seed placement and averages are shown in Fig. 2a and 2b, respectively. It showed that 1% EMS for 6 h retarded the growth of seedling with an average root and shoot length of 1.83 cm and 1.11 cm, respectively.

The average leaf area (Fig. 2c), flowering age (Fig. 2d), fruiting age (Fig. 2e) and ripening age (Fig. 2f) of the treated plants have not shown much difference from each other and from the control plants. The averages of plant height (Fig. 3a), number of branches (Fig. 3b) and number of leaves (Fig. 3c) of treated plants have also not shown

Fig. 1. Number of germinating seeds in control and treated plants of *Lycopersicon esculentum*

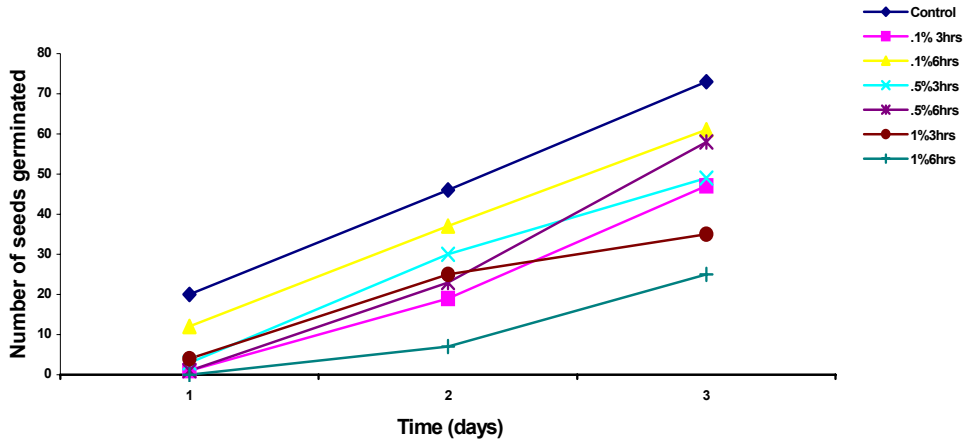
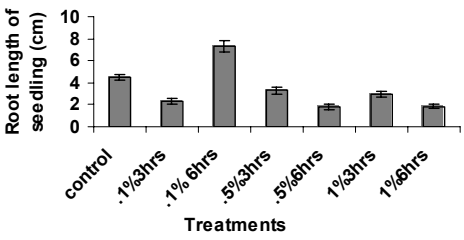
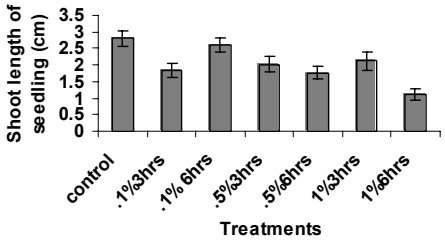


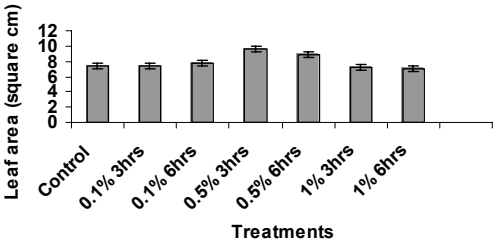
Fig. 2. Averages of (a) Root length of seedling, (b) Stem length of seedling, (c) Leaf area, (d) Flowering age, (e) Fruiting age and (f) Ripening age in control and EMS treated plants of *Lycopersicon esculentum*



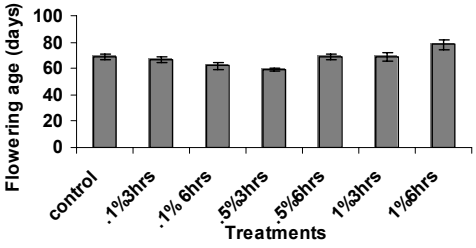
(2a)



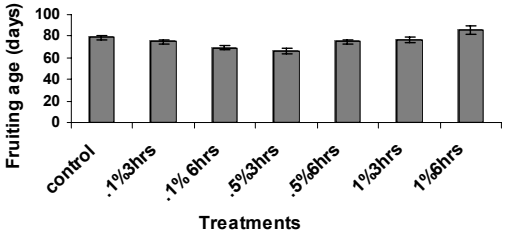
(2b)



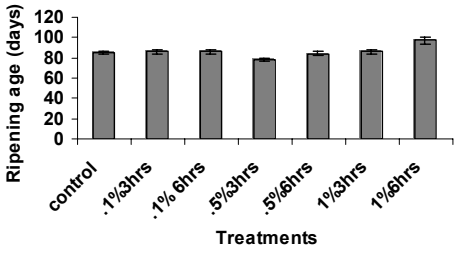
(2c)



(2d)



(2e)

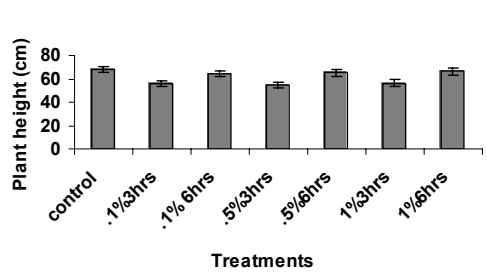


(2f)

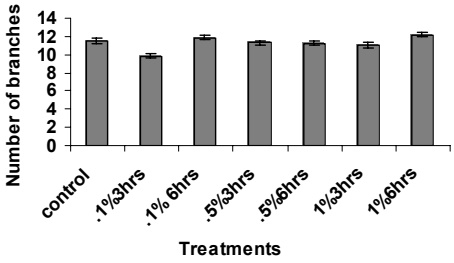
much difference from the control plants. However, as far as average number of fruits and average fruit weight is concerned (Fig. 3d & Fig. 3e, respectively), it was observed that control have highest number (5 per plant) and weight (165 g) of fruits. As the EMS concentration increased, the number and weight of fruit was decreased. The least number (2.5) and weight (51 g) of fruit was observed in 1% EMS for 6 h. The highest chlorophyll content (0.8337 mg/g of tissue; Fig. 3f) was observed in 0.5% EMS for 3 h. In 1% EMS for 3 h, chlorophyll content was 0.2715 mg/g of tissue which was the lowest value among all treatments and control.

For most of the characters, i.e., root length of seedlings, shoot length of seedlings, plant height, number of branches, number of leaves, leaf area, flowering age, fruiting age, ripening age, number of fruits, weight of fruits and chlorophyll content, the minimum variances were observed in the control plants and the maximum in the maximum treatment categories (Table I). Similar results have also been reported for *Capsicum annuum* (Patil *et al*., 1997) and *Avena sativa* (Krishna-Murthy & Vasudevan, 1984), when treated with EMS and dimethyl sulfate; and azide, EMS and gamma rays, respectively. It is obvious from the current findings that with an increase in the chemical concentration, there was also increase in the rate of mutation leading to higher variances. The maximum value of variance in any character is because of some distinct mutants, which showed a considerable difference from the mean value of that character. So, more variances in general means more mutants in that treatment. In the highest exposure categories (1% EMS for 3 and 6 h), the variances were lower than other treatments. This is due to the reason that at 1% concentration EMS is toxic to plants and it retarded the germination of seeds so resulting in less number of viable plants.

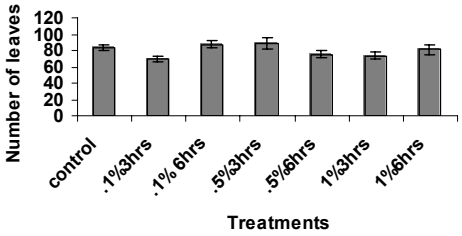
Fig 3. Averages of (a) Plant height (b) Number of branches (c) Number of leaves (d) Number of fruits (e) Weight of fruits (f) Chlorophyll content in control and EMS treated plants of *Lycopersicon esculentum*



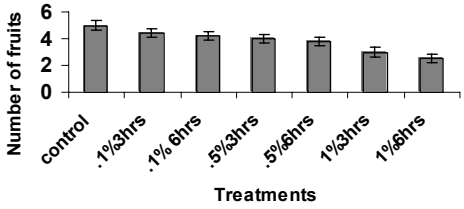
(3a)



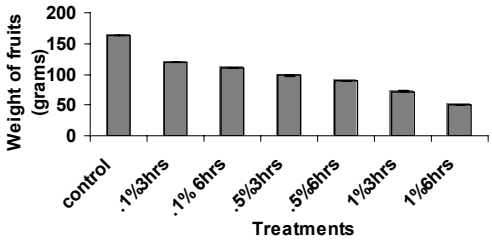
(3b)



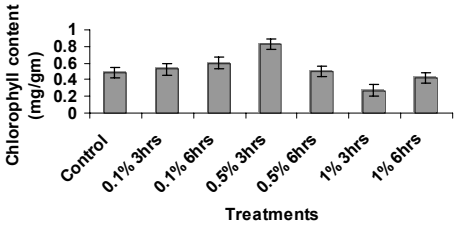
(3c)



(3d)



(3e)



(3f)

Table I. Variances of different characters in the control and the Ethyle Methane Sulfonate treated tomato plants

Treatments	Seedling root length	Seedling shoot length	Leaf area	Flowering age	Plant height	Fruiting age	Ripening age	No. of branches	No. of leaves	No. of fruits	Wt. of fruits	Chlorophyll
Control	0.9113	0.6658	3.31	33	82.3	37.65	32	5.5	184.9	0.4	3.8	24.42
.1% 3 h	2.524	1.44	3.83	116.36	120.7	119.75	116.36	2.13	234.7	1	4.2	60.59
.1% 6 h	11.39	2.15	4.12	158	211.2	105.46	158	10.25	443.5	1.2	4.7	100.4
.5% 3 h	4.62	2.16	3.34	36	133.27	38.19	36	13.03	852.68	2	6.2	154.3
.5% 6 h	3.73	1.64	8.073	84.4	211.13	98.27	84.4	5.67	468	2.8	6.8	159.1
1% 3 h	2.71	2.66	5.026	256.4	173.28	135.62	256.4	6.48	381.68	1.3	5.3	62.42
1% 6 h	1.15	0.546	8.718	197.69	103.4	160.8	197.69	6.15	561.6	1	4.3	60

h = Hours

It may still be possible to increase the concentration and duration of treatment to induce more mutations. However, the higher concentration of the chemical will cause serious damage to the developing plant as a result of cells, other than germ cells, being affected by the chemical treatment.

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(Received 02 November 2001; Accepted 22 November 2001)