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Ethyl Methane Sulfonate Enhances Genetic Variability in *Capsicum annuum*

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Abstract: The study was carried out to enhance the genetic variability in *Capsicum annuum* cultivar Longhi and to determine the optimal conditions for the induction of genetic variability by using ethyl methane sulfonate (EMS). Increase in EMS concentration resulted in gradual decrease of seed germination. The treated and untreated plants as controls were grown to observe eight different characters in M₁ generation including leaf area, number of leaves, number of branches, height of plants, days to flowering, days to fruiting, number of fruits per plant and chlorophyll content. In general, the variance was increased for all the characters under study in the treated populations compared with control suggesting an increase in genetic variability. However the 0.5% concentration for 6 hrs was highly toxic and resulted in an adverse effect on germination of seed.

Key words: Induced mutagenesis, EMS, *Capsicum annuum*, mutation breeding

Introduction

Breeding is the most commonly used method for crop improvement and genetic variability is the basis of any breeding program. Genetic variability is also important to adopt a population to the inevitable changes in the environment and to promote the survival of the species. Mutation breeding is an important method used for the improvement of crops through the induction of mutations at loci controlling economically important traits and/or eliminates undesirable genes from elite breeding lines (Lippert *et al.*, 1964). Mutations may arise spontaneously or they may be induced by radioactive or chemical mutagens. Among the chemical mutagens EMS is reported to be the most effective one (Minocha and 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).

A lot of work has been done to induce mutations artificially by EMS in crop plants. Seed mutagenesis has been used for induction of early flowering in spring rape (Thurling and Depittayan, 1992), herbicide tolerance in soybean (Sebastian *et al.*, 1989), male sterility in wheat (Maan and Williams, 1984) and cucumber (Robinson, 1978), increased pollen viability and fruit rot resistance in bell pepper (Ashok *et al.*, 1995) as well as quantitative variations in different yield traits in *Avena sativa* L. (Krishna and Vasudevan, 1984). High efficiency of EMS for creating variability, phenotypic variation like potato shaped leaves, reduced fruit size, and maximum disease resistance have been observed in tomato (Yudhvir, 1996).

In *Capsicum annuum* a high frequency of plastid-encoded antibiotic-resistant variants (Rao *et al.*, 1997) and mutants with high yield and increased vitamin C (ascorbic acid) have been isolated (Pillai and Abraham, 1996). All these studies suggest that EMS is an effective mutagen and can be used to generate mutants in a variety of plants including *Capsicum annuum* (Munyon, 1985; Zubrzycki and Vonder, 1972). In Pakistan the use of EMS in enhancing the genetic variability has received little attention.

The study, was carried out to enhance the genetic variability by EMS in *Capsicum annuum* cultivar Longhi and to determine the optimal conditions of the treatment for the induction of this genetic variability.

Materials and Methods

Seeds of *Capsicum annuum* CV. Longhi were subjected to different treatment levels of ethyl methane sulfonate (EMS). Treatment parameters were three concentrations (0.01, 0.1, 0.5% V/V) and two durations of exposure (3 and 6 hrs.) resulting in six treatments combinations along with one control. Seeds were pre-soaked in distilled water for 12 hrs. The seeds were then dried, weighed and counted so that for each treatment and control 5 gm of seeds were taken. Treated and untreated seeds were sown in the field at Department of Biological Sciences, Quaid-i-Azam University, Islamabad on 1st March, 2001. These plants were observed routinely for any sort of change in them. Different morphological and biochemical characters were studied. The morphophysiological traits studied included germination percentage, leaf area, days to flowering, plant height, number of

branches, number of leaves, days to fruiting, number of fruits, shape of leaves, shape of fruits, arrangement of leaves, pattern of branches, number of petals and number of sepals. The biochemical parameter studied was total chlorophyll content per gram of tissue.

Germination of seedlings in petri plates: For this purpose seeds were first of all surface sterilized by using the method of Somasegaran *et al.* (1982). Seeds were placed in 95% ethanol for one minute. Then in 0.03% mercuric chlorate for four minutes. The seeds were then washed six times with distilled water in order to remove the traces of above two sterilizing agents. Seven medium sized petri plates carrying a filter paper and small amount of moisture were autoclaved. 100 surface sterilized seeds from different treatment concentrations were allowed to germinate on separate petri plates in this way total six petri plates were prepared. 100 untreated surface sterilized seeds were placed on separate petri plate as a control.

Plants grown in the pots: Manure and fertilizers are required for heavy and healthy crop of *Capsicum annuum*. About 70 pots of equal sizes were taken and were filled with a well prepared growth media of farm yard manure, soil and sand with a ratio of 1:1:1. Seven pots were selected. 100 seeds from six different treatments were sown in six different prepared pots. Seeds were placed in pots at a depth of 1-2 cm and then these seeds were covered with the same soil media. 100 untreated seeds were sown in a pot and in this way there were total seven pots carrying sown seeds. These pots were irrigated well in a spraying manner. Germination was started in about 7-14 days. After 25-30 days seedlings at 4 leaf stage were shifted to pots in a manner that there was one plant per pot and for each treatment 10 pots were taken. In 10 pots control plants were transplanted. Urea and Potash were mixed in the ratio of 1:1 and two treatments of 5 gm of the mixture was given to each pot after 40 days of transplantation of seedlings respectively. To avoid aphids and other insects 2 ml of monitor (insecticide) was added to 1 litre of water and was sprayed twice to plants.

Extraction and assay of chlorophyll from plant tissues: Chlorophyll was extracted and assayed according to the method of Lichtenthaler and Well burn (1983). Tissue samples of 100 mg were ground in a pestle and mortar with 3 ml of 80% acetone. The cell debris was pelleted and the optical density of supernatant was recorded at 663 and 646 nm. The concentration of chlorophyll (mg g⁻¹) in the extract was then calculated:

$$\text{Total chlorophyll} = 7.18OD_{663} + 17.32OD_{646}$$

Results

Germination percentage: In case of control, germination %age was 54%. Among treated seeds maximum germination percentage was observed in 0.01% 3hrs, i.e., 40% seeds were germinated (Table 1). Seeds treated with highest concentration of EMS, i.e., 0.5% for 6 hrs, had the lowest germination percentage

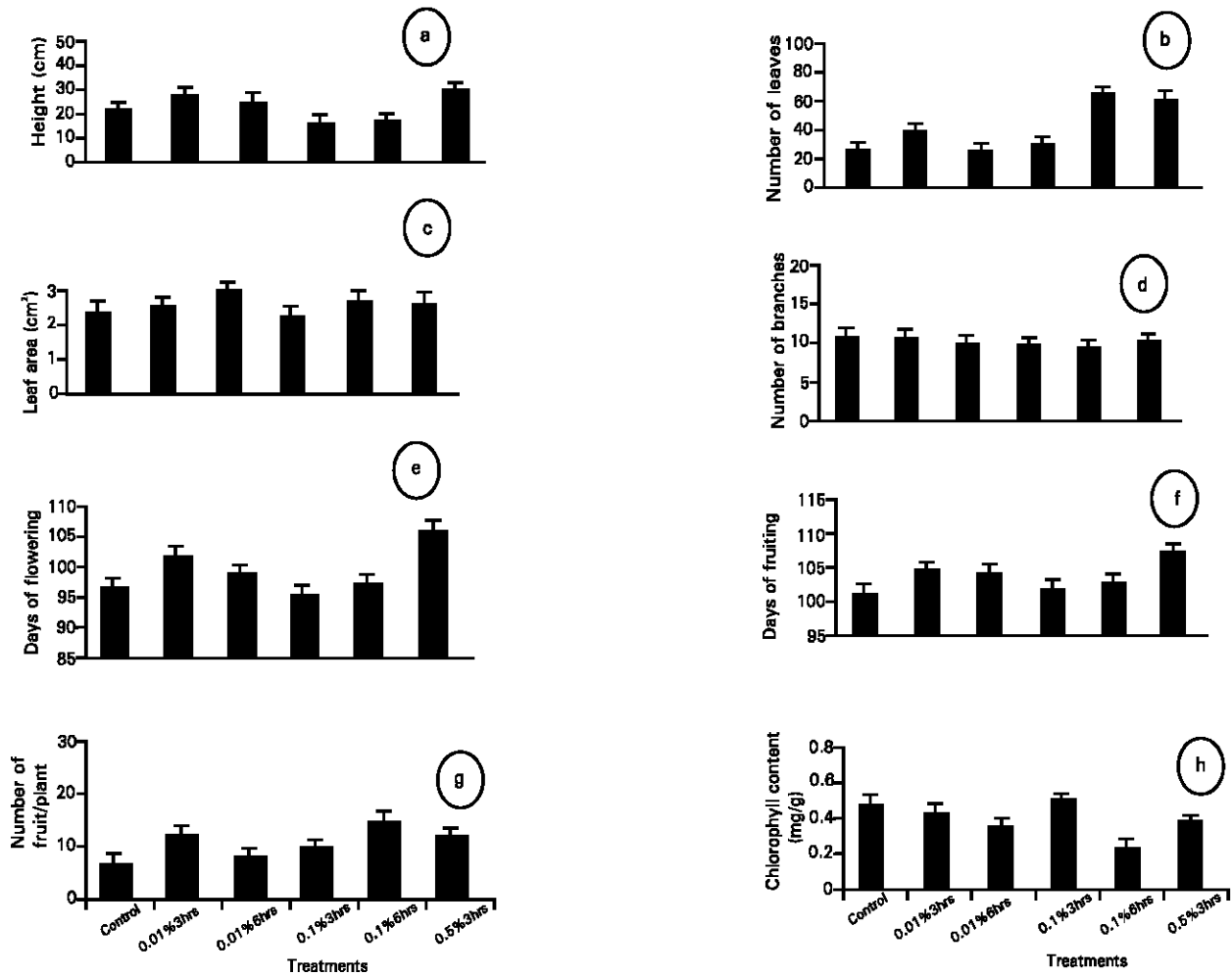


Fig.1: Average of the different characters in control and treated population (a) height (b) number of leaves (c) leaf area (d) number of branches (e) days to flowering (f) days to fruiting (g) number of fruit/plant (h) chlorophyll content

Table 1: Germination percentage in control and EMS treated seeds of *Capsicum annum*

Treatments (%)	Germination %age
Control	54
0.01 3hrs	40
0.01 6hrs	30
0.1 3hrs	19
0.1 6hrs	15
0.5 3hrs	8
0.5 6hrs	5

(only 5%) among all the treatments. Ethyl methane sulfonate is a toxic compound and its treatment has an adverse effect on seed germination. Increase in concentration of EMS resulted in gradual decrease in percent germination of seedlings.

Height of the plants: In the control plants less variation was observed. Maximum variance was observed in plants treated with 0.5% EMS, for 3 hrs (152.17) and minimum variation was observed in plants treated with 0.01% EMS, for 3 hrs (61.28) (Table 2). In case of control group average height was 2.06 cm. Maximum average height was observed in 0.01% EMS treatment for 6 hrs (37.2 cm) and minimum average height was observed (Fig. 1a) in plants treated with 0.1% EMS for 3 hrs (18.9).

Number of leaves: The variance was increased in treated populations as compared with control plants. Among the treatment groups, maximum variance was observed in plants treated with 0.5% EMS, for 3 hrs (1712.84) and minimum variation was observed in plants treated with 0.01% EMS, for 3 hrs (620.9) (Table 2). In case of control group average number of leaves was 249.7. Maximum average number of leaves was observed in 0.1% EMS treatment for 6 hrs (77.8) and minimum average number of leaves was observed (Fig. 1b) in plants treated with 0.01% EMS for 6 hrs (27.8)

Leaf area: There was more variation in the leaf area of the treated plants as compared with control plants (Table 2). Maximum variance was observed in plants treated with 0.5% EMS, for 3 hrs (0.90) and minimum variation was observed in plants treated with 0.01% EMS, for 3 hrs (0.49). In case of control group average leaf area was 2.06 cm². Maximum average leaf area was observed in 0.01% EMS treatment for 6 hrs (2.62 cm²) and minimum average leaf area was observed (Fig. 1c) in plants treated with 0.1% EMS for 3 hrs.

Number of branches: There was more variation in the number of branches of the treated plants as compared with control plants (Table 2). Maximum variance was observed in plants treated with

Table 2: Variances of different morphological and bio-chemical characters in treated and untreated plants of *Capsicum annuum*.

Treatment (%)	Height of plants	Number of branches	Number of Leaves	Leaf area	Days to flowering	Days to fruiting	Number of fruits/plant	Chlorophyll content
Control	020.72	12.88	249.7	0.28	18.23	020.94	012.62	0.002
0.01 3hrs	061.28	16.71	620.9	0.49	98.01	103.98	053.92	0.013
0.01 6hrs	150.60	23.28	752.62	0.82	47.388	045.69	018.25	0.018
0.1 3hrs	086.76	16.23	373.51	0.92	27.611	023.194	024.111	0.016
0.1 6hrs	104.72	68.04	1712.84	0.57	41.11	052.00	120.361	0.013
0.5 3hrs	152.17	15.12	939.12	0.90	120.488	112.00	102.111	0.027
0.5 6hrs	-	-	-	-	-	-	-	-

At 0.5% 6 hrs no viable seedling was observed

0.1% EMS, for 6 hrs (68.04) and minimum variation was observed in plants treated with 0.5% EMS, for 3 hrs (15.12) (Table 2). In case of control group average number of branches was 14. Maximum average number of branches was observed in 0.5% EMS treatment for 3 hrs (14.3) and minimum average number of branches was observed (Fig. 1d) in plants treated with 0.1% EMS for 6 hrs (12.4).

Days to flowering: There was more variation in the days taken to flowering of the treated plants as compared with control plants (Fig. 1e). Maximum variance was observed in plants treated with 0.5% EMS, for 3 hrs (120.48) and minimum variation was observed in plants treated with 0.1% EMS, for 3 hrs (27.61) (Table 2). In case of control group, average days taken to flowering were 97.7. Maximum average days taken to flowering were observed in 0.5% EMS treatment for 3 hrs (106.6) and minimum average days taken to flowering were observed (Fig. 1e) in plants treated with 0.1% EMS for 3 hrs (96.88).

Days to fruiting: Maximum variance was observed in plants treated with 0.5% EMS, for 3 hrs (112) and minimum variation was observed in plants treated with 0.1% EMS, for 3 hrs (23.19) (Table 2). In case of control group, average days taken to fruiting were 103.5. Maximum average number of days taken to fruiting was observed in 0.5% EMS treatment for 3 hrs (112) and minimum average days taken to fruiting were observed (Fig. 1f) in plants treated with 0.1% EMS for 3 hrs (103.77).

Number of fruits/plant: Number of fruits of all treated and untreated plants were counted. It was found that, variation was more in the number of fruits of the treated plants as compared to control plants. Maximum variance was observed in plants treated with 0.1% EMS, for 6 hrs (120.36) and minimum variation was observed in plants treated with 0.01% EMS, for 6 hrs (18.25) (Table 2). In case of control group average number of fruit was 7.8. Maximum average number of fruit (Fig. 1g) was observed in 0.1% EMS treatment for 6 hrs (19.11) and minimum average number of fruit was observed in plants treated with 0.01% EMS for 6 hrs (11.88).

Chlorophyll content: Maximum variance was observed in plants treated with 0.5% EMS, for 3 hrs (0.027) and minimum variation was observed in plants treated with 0.01% EMS, for 3 hrs (0.013) (Table 2). In case of control group, average chlorophyll content was 0.56 mg g⁻¹. Maximum average chlorophyll content was observed in 0.1% EMS treatment for 3 hrs (0.613 mg g⁻¹) and minimum average chlorophyll content was observed (Fig. 1h) in plants treated with 0.1% EMS for 6 hrs (0.26 mg g⁻¹).

Discussion

In the study seeds of *Capsicum annuum* were treated with 0.01, 0.1 and 0.5% EMS for 3 and 6 hrs. In the laboratory germination test, it was observed that increase in concentration of EMS had adverse effect on seed germination. Similar results have been reported earlier (Alcantara *et al.*, 1996). In that study seeds of *Capsicum annuum* were treated with 0.5, 1 and 1.5% EMS and exposed for 3, 6 and 9 hrs. In the M1 generation, seeds treated with 1.5% EMS for 9 hrs had the lowest germination percentage

(84%) among all treatments. Although in this report, at highest concentration of EMS, seeds had the lowest germination percentage but was still much higher than observed in our investigation (Table 1). In our study, when these treated seeds were planted in the field, at maximum concentration and longest exposure that is 0.5% EMS, for 6hrs no viable seedling was observed. While in another report when seeds of the *Capsicum annuum* treated with three concentrations (0.5, 1 and 5%) for 3, 6 and 9 hrs. were planted in the field no detectable differences in germination percentage were observed compared to the results in the laboratory (Alcantara *et al.*, 1996). These results and results of the present study suggest that toxic level of EMS concentration and exposure depends on the experimental conditions and on the variety used.

In most of the characters, i.e., plant height, number of branches, number of leaves, leaf area, days to flowering, days to fruiting, number of fruits/ plant and chlorophyll content the minimum variances were observed in control plants and the maximum variances were observed in the treated plants. These results support an earlier report (Patil *et al.*, 1997). Seeds of *Capsicum annuum* were treated with 0.1, 0.2 and 0.5% EMS and Dimethyl Sulfate for 12 and 18 hrs. Data on Plant height, number of branches, number of fruits and fruit yield/plant were recorded. Variances for all characters under study were increased in the treated plants. Similar results have been reported in *Avena sativa* (Krishna and Vasudevan, 1984). The observed variation in the treated population was more than that in the control population. This is the expected result because the control plants are supposed to be genetically similar and any kind of difference observed in the control plants is only due to environment. The results of the present study suggest that particular dosage of EMS treatment below the toxic level, (that is 0.5% 6 hrs in our conditions) can be used to increase the genetic variability in *Capsicum annuum*, which is the basis for any breeding program.

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