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Ethyl Methane Sulfonate Induces Morphological Mutations in *Capsicum annuum*

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ABSTRACT

The present study was carried out to induce morphological mutations in *Capsicum annuum* cultivar Longhi. Seeds were subjected to different treatment levels of ethyl methane sulfonate (EMS). The treated and untreated plants were self-fertilized for two generations to observe different morphological characters in M_3 generation. Several unique and interesting mutants were isolated in this investigation. These independent mutants have different phenotypes from control. The most distinct mutants included tall, dwarf, sterile, early maturing and late maturing. The change in leaf area, leaf arrangement, shape of leaves, pattern of branching and symmetry of flower was also observed.

Key Words: *Capsicum annuum*; Induced mutagenesis; EMS

INTRODUCTION

Mutation breeding is used to induce 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. Mutations can be induced by using radioactive or chemical mutagen. Among the chemical mutagens, chemical EMS induces a vastly higher proportion of point mutations (Minocha & Arnason, 1962; Hajra, 1979). In plants, EMS usually causes point mutations but loss of a chromosome segment or deletion can also occur. Mutations were induced artificially by EMS seed mutagenesis for early flowering in spring rape (Thurling & Depittayanan, 1992), herbicide tolerance in soybean (Sebastian *et al*., 1989), male sterility in wheat (Maan & Williams, 1984). High efficiency of EMS for creating phenotypic variation like potato shaped leaves, reduced fruit size, and maximum disease resistance were observed in tomato (Yudhvir, 1995). High frequencies of plastid-encoded antibiotic-resistant variants were isolated in *Capsicum annuum* (Rao *et al*., 1997). Optimal conditions for ethyl methane sulfonate induced mutagenesis of seeds were studied in *Capsicum annuum* (Alcantara *et al*., 1996). Mutants with high yield and increased vitamin C (ascorbic acid) were observed in *Capsicum annuum* (Pillai & Abraham, 1996). Increased pollen viability and fruit rot resistance were also observed in bell pepper (Ashok *et al.*, 1995). All these studies suggested that EMS is an effective mutagen and can be used to generate mutants in a variety of plants including *Capsicum annuum* (Zubrzycki & Vonder Pahlen, 1972; Munyan, 1985).

In Pakistan induced mutation breeding, which has been recognized, as a valuable supplement to coventional breeding in crop improvement (Allard, 1960) has been least applied in *Capsicum annuum.* The present investigation was under taken to induce viable mutations, which could be utilized directly or introduced in to our Capsicum improvement programe. We report here some of our results on the use of chemically induced mutations for crop improvement in *Capsicum annuum.*

MATERIALS AND METHODS

Plant material and treatments applied. 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 i.e. (3 and 6hrs.) resulting in six treatment combinations (T_0 = control, T_1 = 0.01% 3 h, T_2 = 0.01% 6 h, T_3 = 0.1% 3 h, T_4 = 0.1% 6 h, T_5 = 0.5% 3 h and T_6 = 0.5% 6 h). Before treatment, seeds were pre-soaked in distilled water for 12hrs at room temperature. Later on these seeds were dried on filter paper. For each treatment 5 g (approximately 800-1000) seeds were used. All seeds were uniformly exposed to EMS solution by stirring with a glass rod. After treatment seeds were rinsed thoroughly with distilled water, air-dried and stored for later studies.

Cultivation of plants in pots. Plants were cultivated in a well-prepared growth media of farmyard manure, soil and sand with a ratio of 1:1:1. 100 seeds of each treatment as well as control were sown in seven separate pots (six for treated seeds and one for untreated seeds). These pots were irrigated well in spraying manner. Germination started in about 7-14 days. After 25-30 days, seedlings (at 4-leaf stage) were shifted to new pots as one plant per pot. Plants were irrigated on alternate days. 5 g of the mixture of Urea and Potash in the ratio of 1:1 was applied to each pot after 20 and 40 days of transplanting seedlings. To avoid aphids, plants were sprayed twice with 0.2% solution of Monitor (insecticide) at 30 and 40 days stage.

Parameters studied. Treated and untreated plants were self fertilized for two generations and in the third generation they were observed routinely for any sort of change in them. The morphological traits studied included leaf area, days taken to flowering, plant height, number of branches, number of leaves, days taken to fruiting, number of fruits, shape of leaves, shape of fruits, arrangement of leaves, pattern of branches, number of petals, number of sepals and chlorophyll content.

Characterized mutants were assigned names on the basis of treatments and number of individual plant in that treatment i.e 5a represents first plant of T_5 treatment. Mutants from different treatments resembled morphologically so were discussed together.

Extraction and assay of chlorophyll. Chlorophyll was extracted and assayed from each treated and untreated plant according to the method described by Lichtenthaler and Well burn, (1983). Leaf tissue samples of 100 mg were ground in a pestle and mortar with 3 mL of 80% acetone and centrifuged at 3000 rpm for 2 minutes. The cell debris was pelleted and the optical density of supernatant was recorded at 663 and 646 nm. The concentration of Chlorophyll (mg/L) in the extract was then calculated according to the following formula.

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

Table I. Individual mutants and the changes observed

Plant	Mutations
5a	Tall, late maturing, four petalled
5b	Tall, late maturing, prolific with more number of leaves
5c	Tall, late maturing, prolific with more number of leaves and branches, flower smaller in size
2a	Tall, late maturing with more number of leaves and branches.
3a	Tall, late maturing, stiff stem, more number of leaves, originally stem was divided into two branches of equal size each was acting as a main stem.
1a	Tall, sterile, large glossy spinach like leaves.
5d	Tall, more number of leaves, originally stem was divided into two branches of equal size each was acting as main stem, produced flower but did not produce fruit..
5e	Tall, sterile, large glossy spinach like leaves, more number of leaves, more leaf area
5f	Tall, sterile, large glossy spinach like leaves forming a whorl mass at the apex, more number of leaves, more leaf area, chlorotic
2b	Dwarf, sterile, seedling like appearance
4b	Dwarf, fertile, more number of leaves.
4c	Dwarf fertile, whorl mass of leaves at the apex.
3d	Dwarf sterile
1b	Early maturing, more number of leaves, different shape of leaves.
4e	Late maturing with more number of leaves and different branching pattern, prolific.
4f	More number of leaves, more number of branches, bushy habit, prolific.

1,2…6 number of treatments; a,b…z number of plants

RESULTS AND DISCUSSION

Mutants were characterized on morphological basis. Overall 16 mutants were isolated. Two (1a, 1b) from T_1, Two from T_2 (2a, 2b), Two from T_3 (3a, 3d), Four (4b, 4c, 4e, 4f) from T_4 and six (5a, 5b, 5c, 5d, 5e, 5f) from T_5. Seeds of T6 treatment did not germinate. As reported earlier 0.5% concentration of EMS with 6hr exposure is highly toxic and has drastic effects on seed germination (Jabeen & Mirza, 2002).

Individual mutants and the changes observed in them are summarized in Table I. The morphological mutants isolated were categorized as tall, dwarf and of normal height. Total 10 tall mutants were isolated. These were, mutant 5a having height 39 cm, mutant 5b having height 50 cm, mutant 5c having height 50 cm, mutant 2a having height 47 cm, mutant 3a having height 39 cm, mutant 1a having height 47 cm, mutant 5d having height 46 cm, mutant 5e having height 48 cm and mutant 5f having height 46 cm (Fig 1a-i). While in controls height ranged from 21-34 cm. Out of these 9 tall mutants, 4 were fertile and late maturing (mutant 5a time taken to maturity was 130 days, mutant 5b time taken to maturity was 123 days, mutant 5c time taken to maturity was 121 days, mutant 3a time taken to maturity was 118 days) while in controls time taken to maturity ranged from 99-110 days. All these mutants had flowers with 5-lobed calyx and corolla, except from mutant 5a where flower had 4 petals (Fig. 1j) and mutant 5c that had flowers smaller in size as compared to control (Fig. 1k). De Haro and Del Rio (1998) also reported mutants with change in number of petals, sepals and ovules than normal in borage (*Borago officinalis*). Other 5 tall mutants were sterile, while in control no sterile plant was observed. Two types of sterile plants were observed. One type of sterile plants did not produce flower at all (mutants 5e, 5f and 1a) and the second type of sterile plants produced flower but did not produce fruit (mutant 5d and mutant 2a). These both types of plants were recorded in treated populations only and were not observed in control plants. This is most probable because of mutations in any one or more than one genes involved in flowering and subsequently fruit development. Several genes like Leafy and Ap1 involved in flowering have been isolated from model plants like Arabidopsis and Tomato (Leandro *et al*., 1990). Further studies would reveal weather these genes mutated in these lines are comparable with the reported genes or not. However such lines can be very helpful to understand the mechanism of flowering and fruit development and have been used to study genes involved in crop maturation (Odeigah *et al.*, 1996).

Some of these tall mutants had Spinach like leaves (mutant 5c, 5e, 5f). These types of mutants with variable leaves were also reported earlier in *Capsicum annuum* cultivar Keystone Resistant Giant no 3 (Alcantara *et al.*, 1996). Leaf variegation is a common mutation which can be either a nuclear or cytoplasmic mutation EMS may have a high specificity for mitochondrial and plastid genomes (Miller *et al*., 1984).

Fig. I. Comparison of the control and tall mutants of *Capsicum annuum* (a) mutant 5a (b) mutant 5b (c) 5c (d) 2a (e) 3a (f) 1a (g) 5d (h) 5e (i) 5f (j) 4 petalled flower of the mutant 5a (k) Comparison of the large sized flower of the control and small sized flower of the mutant

a

b

c

d

e

f

g

h

Continued to next page

Continued Fig 1.

i

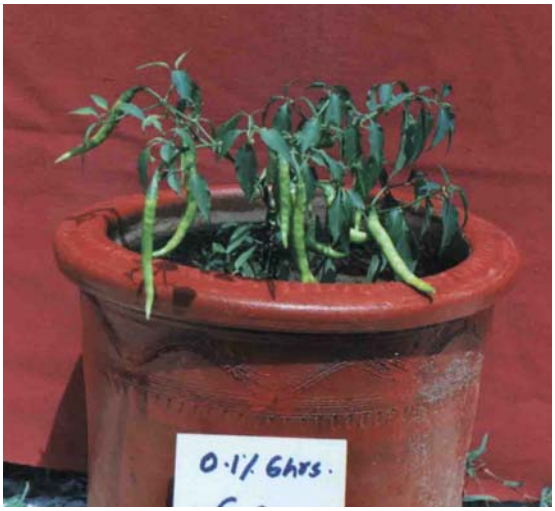
j

k

Fig. 2. (a) Control and mutant 2b (b) mutant 4b (c) control and mutant 4c (d) control and mutant 3

a

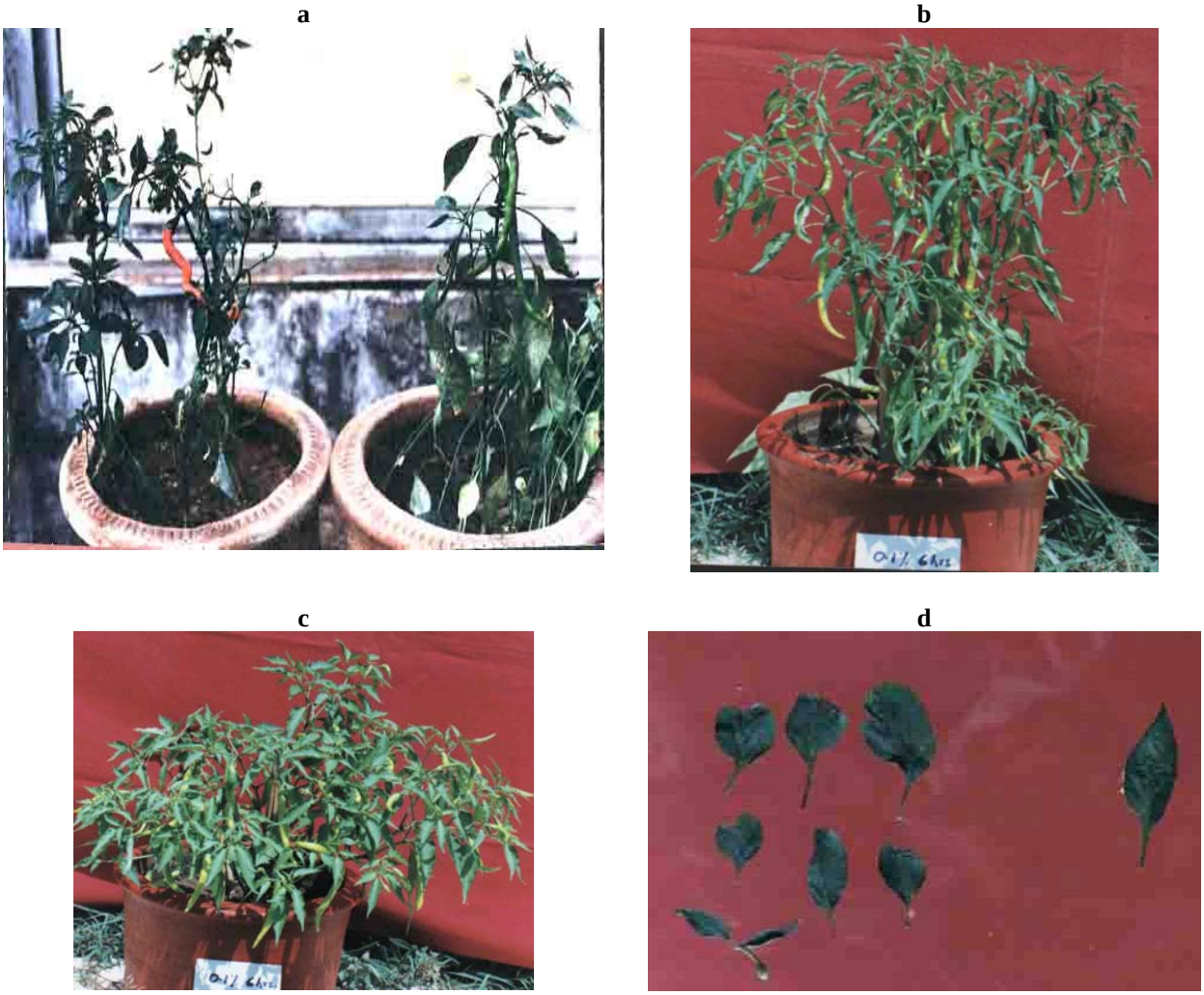
b



c

d

Fig. 3. (a) Comparison of control and early maturing mutant 1b(b) Proliific mutant-4e (c) mutant 4f (d) Leaf variations in mutant 1b



It is known that many plastome mutations interfere with the development of the photosynthetic apparatus (Redei *et al.*, 1984) and can cause male and female sterility.

In this research, total 4 dwarf mutants were isolated (mutant 2b having height 10 cm, mutant 4b having height 14 cm, mutant 4c having height 12 cm and mutant 4d having height 12 cm) (Fig. 2a-d). Among these mutants, 2 dwarfs were sterile (mutant 2b and 3d) while other two dwarfs were fertile (mutant 4b and 4c) indicating that these are different mutants. Mutant 4b had more number of leaves (58) as compared to control plants (ranged from 39-53). These types of dwarf mutants with the height of ~ 10 cm. were also reported earlier (Lippert *et al.*, 1964). The mutants observed by them were flowering, diminutive and glossy. But the mutants isolated in the present study were not diminutive and they were with less chlorophyll content and had seedling like appearance.

In addition to these tall and dwarf mutants, three mutants had normal height with other morphological variations. These were mutant 1b, mutant 4e and mutant 4f (Fig. 3a-c). Among these, mutant 1b was fertile, early maturing with different number and shape of leaves. That mutant had 79 number of leaves while in control number of leaves ranged from 39-53. Leaves of that mutant were different in size and shape from normal plant. Normally leaves were lenceolate with acute tip but in that plant, leaves on the top most were smaller in size, spathulate, that is, lamina broad and rounded at the top and narrow towards the base and some were with notched tip that is obcordate. Leaves had different number of notches. Some had one and some had two notches Fig. 3d. Use of induced mutations for obtaining early maturing cultivars has been a frequent breeding objective (Micke, 1979). Two mutants 4e and 4f were prolific. Former had erect habit with stem originally divided in to three equal branches and number of fruit

produced 22. Later had bushy type of growth habit and number of fruit was 38 while in control number of fruit ranged from 5-17. These prolific mutants indicate that EMS can be used for the improvement of the crop. These types of mutants were also reported earlier (Alcantara *et al*., 1996) but mutants isolated in the present study were different from previously isolated mutants in having vigorous and bushy type of growth habit. Previously isolated mutants had erect growth habit. Further studies of 4e, 4f will be interesting as it is reported that increases in a polygenic character like yield could result from changes in simply inherited traits (Micke *et al.*, 1990).

Results suggest that using a dose of 0.5% EMS concentration for 3hrs can induce morphological mutations. Several unique and interesting mutations were induced in this study. There were some mutants that were completely sterile and cannot be used for further studies. The fertile mutants generated in this study could be valuable for linkage and mapping studies of *Capsicum annuum*. Further more these mutants can also be used to isolate genes involved at different developmental stages of plants. Mutants isolated in this study as well as in many previous studies could serve as genetic markers. This reveals that mutation breeding is a valid and effective crop breeding method for short genome crops like *Capsicum annuum.*

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