The effect of ethyl methane sulphonate and diethyl sulphate on chilli (*Capsicum annuum* L.) in M₁ generation

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ABSTRACT

In recent years, the demand of chilli has tremendously increased due to its attractive market price and multifarious used in cooked and processed forms. At present people are much concerned about the fruit quality and yield. Therefore, attention is being paid for development of genotypes having high yield potential with desirable fruit quality characters in a short period of time. For this purpose, seeds of chilli were mutagenised with ethyl methane sulphonate (EMS) and diethyl sulphate (DES) to determine their mutagenic sensitivity in M₁ generation. The increasing concentration of EMS and DES decreased in morphological and yield characters. The spectrum of mutation and induced variability for various quantitative traits were observed in M₁ generation such as germination (%), plant height, primary and secondary branches per plant, days to first flowering, fruit length (cm), fruit girth (cm), total number of fruits per plant, number of seeds per fruit, seed weight per fruit (g), 100 seed weight (g) and pericarp: seed ratio showed variability in chilli with the effect of EMS and DES. The percentage of chromosomal abnormalities in different mitotic stages was significantly higher than that of the control in all the treatment concentrations.

*Keywords*: chilli; EMS; DES; germination; pericarp; mitosis

1. INTRODUCTION

Different definitions of the term “mutation” and this may create the impression, that the term is somewhat woolly. Definitions range from “a sudden phenotypic change in a character of an individual, not due to crossing or segregation” up to “an alteration in the macromolecules in the DNA” (where it remains open, whether the alteration leads to a change in gene function or not). Included under the term “mutation” is also the augmentation of genetic material through nucleotide or gene copies, through additional individual chromosomes, as well as through the multiplication of whole genomes towards polyploidy. In order to speak more clearly about mutations and their potential for crop improvement, it would seen desirable to have different terms at least for (a) the phenotypic alteration and (b) the various underlying molecular and numerical changes. But in any case, a mutation has to be phenotypically expressed to be selectable all other mutations are only of scientific interest (Alexander Micke, 1999).
Mutation methodology has been used to produce many cultivars with improved economic value and study of genetics and plant developmental phenomena (van den Bulk et al., 1990 and Bertagne Sagnard et al., 1996). Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev et al., 2001). It has been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated. Various mutagenic agents are used to induce favourable mutations at high frequency that include ionizing radiation and chemical mutagens (Ahloowalia and Maluszynski, 2001). Chemical mutagens are the one cause of mutations in living organisms. Many of the chemicals have clastogenic effects on plants via reactive oxygen-derived radicals (Yuan and Zhang, 1993).

Chilli (Capsicum annuum L.) belongs to the family Solanaceae. The domestication of chilli first occurred in Central America, most likely in Mexico, with secondary centres in Guatemala and Bulgaria (Salvador, 2002). Chilli was introduced to Europe by Columbus in 15th century and spread to rest of the globe. In 17th century Portuguese introduced it into India. It is an indispensable spice essentially used in every Indian cuisine due to its pungency, taste, colour and aroma. Chilli fruits are rich sources of vitamin C, A and E. In this pivotal vegetable plant Capsicum provides evidence to improve the cytogenetical, morphological and yield parameters with the effect of EMS and DES.

2. MATERIALS AND METHODS

The dried seeds of chilli var. Kovilpatti were treated with different EMS (10, 20, 30, 40 and 50 mM) and DES (5, 10, 15, 20 and 25 mM) concentration. Seeds were presoaked in distilled water for 12hrs to allow uptake of chemical mutagens. After treatment, seeds were thoroughly washed in running tap water for 4h to leach out the residual of chemicals. Untreated seed stock was used as a control. The treated and control seeds were sown in sand beds and watered at least once a day. After 25-30 days, seedlings were shifted to new pots as one plant per pot. The M1 generation (produced directly from mutagen treated seeds) was grown in the pot culture experiment at the Botanical Garden, Department of Botany, Annamalai University. The recommended package of practice for the crop was followed. The M1 plants were harvested on a single plant basis. From each entry, 10 plants were randomly selected for recording observations on important yield attributing characters on plant height, primary and secondary branches per plant, days to first flowering, fruit length (cm), fruit girth (cm), total number of fruits per plant, number of seeds per fruit, seed weight per fruit (g), 100-seed weight (g) and pericarp: seed ratio.

2.1. Cytogenetical analysis

For the cytogenetical analysis, root meristems of chilli (2n = 24) were used. The chilli root tips about 3 cm in length were excised, fixed in glacial acetic acid: alcohol (1:3) solution for 48hr. Then root tip squashes were made by using iron alum, haematoxylin squash technique (Marimuthu and Subramanian, 1960). Cell divisions and cytogenetical abnormalities were observed and photographed under a Nikon image capturing system. The various types of cells with normal and abnormal chromosomal behaviour at various stages were observed and counted.
3. RESULTS AND DISCUSSION

Cytological studies revealed that the use of chemical mutagens stimulated the mitotic activity in the roots of chilli, since the mitotic index increased with the increase in the concentration. The mitotic index value increased up to a certain level concentration. However, EMS treatments induced insignificant mitotic abnormalities compared to control roots (Table 1).

Table 1. Effects of EMS and DES on mitotic index, frequency of mitotic phases and percentage of abnormalities in chilli root tip cells.

| Treatment Conc. (mM) | Total cells divided | Total abnormal cells | Abnormal cells (%) | Mitotic index | Prophase | Metaphase | Anaphase & Telophase |
|----------------------|---------------------|----------------------|-------------------|--------------|----------|----------|--------------------|
|                      | No.     | %     | No.     | %     | No.     | %     | No.     | %     |
| Control              | 507     | 20    | 3.9     | 11.1 | 81      | 15.9 | 163      | 32.1 | 263      | 51.8 |
| EMS 10               | 739     | 82    | 11.1    | 16.6 | 172     | 23.3 | 187      | 25.3 | 380      | 51.4 |
| 20                   | 710     | 85    | 11.9    | 15.6 | 197     | 27.7 | 199      | 28.0 | 314      | 44.2 |
| 30                   | 813     | 146   | 17.9    | 18.0 | 186     | 22.9 | 200      | 24.6 | 427      | 52.52|
| 40                   | 820     | 152   | 18.53   | 18.2 | 195     | 23.8 | 218      | 26.6 | 407      | 49.6 |
| 50                   | 843     | 98    | 11.63   | 18.8 | 223     | 26.4 | 226      | 26.8 | 394      | 46.7 |
| DES 5                | 613     | 53    | 8.65    | 13.6 | 213     | 34.7 | 194      | 31.6 | 206      | 33.6 |
| 10                   | 655     | 45    | 6.9     | 14.5 | 201     | 30.7 | 202      | 30.8 | 252      | 38.5 |
| 15                   | 693     | 130   | 18.8    | 15.4 | 193     | 27.8 | 210      | 30.3 | 290      | 41.8 |
| 20                   | 708     | 113   | 15.96   | 16.9 | 179     | 25.2 | 237      | 33.5 | 292      | 41.2 |
| 25                   | 713     | 100   | 14.03   | 16.2 | 178     | 24.9 | 218      | 30.6 | 317      | 44.5 |

The data showed a significant increase in the percentage of prophase cells 34.7 % with using the 15 mM DES. All the concentrations were capable of inducing various types of chromosomal abnormalities in almost all the stages of mitosis. Sticky chromosomes, precocious movements, bridges, micronucleus, laggards and anaphase with polar deviation were the most common anomalies recorded with the use of EMS and DES in metaphase and anaphase (figures not shown). The percentages of chromosomal abnormalities in different mitotic stages were significantly higher than that of the control and calculated on mitotic index, frequency of phases and percentage of abnormalities in mitosis.

These chromosomal aberrations may consider as indicators of clastogenic effects of their inducers (Badr, 1983). This may indicate an increase in the impairment of the mitotic apparatus, which was not completely inhibited. It is clear from our results that chromosomal stickiness is
the most dominant abnormality produced in different concentrations. Stickiness is a common physiological phenomenon, which may be the result of an action by the chemicals on chromatin fibres (Badr et al., 1987). It has been attributed to an action on the protein of chromosomes (El-Sadek, 1972). Chromosomal bridges may be due to chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation of chromosome segments (Najjar and Soliman, 1980).

Table 2. Mean performance of Capsicum annum in relation to different concentration of EMS and DES.

| Treatment Conc. (mM) | Germination (%) | Plant height (cm) | Primary branches per plant | Secondary branches per plant | Days to first flowering | Fruit length (cm) | Fruit girth (cm) | Total no. of fruits per plant | No. of seeds per fruit (g) | Seed weight per fruit (g) | 100-seed weight (g) | Pecarp: seed ratio |
|---------------------|-----------------|------------------|---------------------------|----------------------------|------------------------|------------------|----------------|-----------------------------|--------------------------|------------------------|---------------------|------------------|
| Control             | 78.54           | 65.60            | 5.96                      | 6.05                       | 48.94                  | 7.14             | 1.95           | 50.14                       | 70.4                     | 0.456                  | 0.501               | 1.7              |
| EMS 10              | 73.50           | 62.12            | 5.93                      | 5.84                       | 50.54                  | 7.30             | 1.78           | 50.56                       | 70.52                    | 0.439                  | 0.503               | 1.3              |
| 20                  | 67.44           | 60.27            | 5.01                      | 6.00                       | 52.12                  | 6.88             | 1.49           | 47.35                       | 64.56                    | 0.400                  | 0.458               | 1.4              |
| 30                  | 54.22           | 63.95            | 5.83                      | 5.84                       | 55.34                  | 6.53             | 1.38           | 52.02                       | 63.02                    | 0.389                  | 0.477               | 1.2              |
| 40                  | 28.04           | 57.30            | 3.05                      | 3.25                       | 61.06                  | 5.53             | 1.05           | 28.44                       | 57.35                    | 0.380                  | 0.376               | 0.6              |
| 50                  | 22.89           | 45.83            | 2.81                      | 3.46                       | 60.56                  | 4.63             | 0.75           | 20.18                       | 53.68                    | 0.301                  | 0.333               | 0.6              |
| DES 5               | 68.93           | 63.12            | 5.80                      | 5.49                       | 50.08                  | 7.16             | 1.62           | 48.22                       | 66.55                    | 0.403                  | 0.488               | 1.4              |
| 10                  | 55.21           | 64.56            | 5.27                      | 5.97                       | 51.01                  | 6.79             | 1.68           | 44.62                       | 63.31                    | 0.382                  | 0.457               | 1.0              |
| 15                  | 49.96           | 61.68            | 5.71                      | 5.53                       | 56.16                  | 6.41             | 1.25           | 47.69                       | 70.18                    | 0.384                  | 0.417               | 1.2              |
| 20                  | 20.53           | 57.38            | 3.50                      | 4.67                       | 59.52                  | 5.32             | 0.93           | 24.18                       | 56.55                    | 0.301                  | 0.378               | 0.9              |
| 25                  | 18.26           | 55.12            | 3.14                      | 4.20                       | 58.38                  | 4.78             | 0.78           | 21.12                       | 40.41                    | 0.301                  | 0.347               | 0.8              |

The seeds are good explants for chemical mutagens to create mutations in a genome of a cell. These mutagens affect the germination process in seeds. The percent of germination in seeds depends on the nature of the mutagen and its treatment dose. After mutagenesis, seeds show the effects of mutagen as modified morphological traits from disturbed physiological processes. The effect of chemical mutagens measured by reduction of germination and growth of seedlings decreased with increase conc. of EMS and DES in chilli. Constantin et al. (1976) observed linear relationship between conc. and reduction survival of field growth of soybean. The effect of mutagens was measured quantitatively by reduction in germination survival (lethality) [Ramasamy, 1973]. Changes in specific activity of enzyme (Endo, 1967) and reduction in productivity of IAA (Miura et al., 1974) and were also causes for reduced growth in the M1 generation.

Observations showed decrease in plant height, number of pods per plant, number of clusters per plant, fruit length and fruit girth with increasing concentration of EMS and DES than control. In the present study, the reduction of these parameters was prominent in EMS and DES, such as inhibitory effects of various mutagens were reported in several other crops (Reddy et al., 1992). Koteswara Rao et al., (1983) reported that irradiation significantly reduced some polygenic characters in length of pods, number of pods and number of clusters...
in M₁ generation. Days to first flowering increased with increasing conc. of EMS and DES. However, number of primary branches per plant, number of pods per plant, decreased mean performance value with increasing dosage. The mutagenic effect was found decreasing in quantitative characters in soybean (Pavadai and Dhanavel, 2004) and cluster bean (Velu et al., 2007). Mutagen treatment causes complex genetic and physiological damages. The first generation (M₁) developed from treated seeds, for example, suffers from growth inhibition, may be partly sterile, and may lose many plants before flowering and seed set (Ojiewo et al., 2006a).

4. CONCLUSION

The cytological studies revealed that EMS and DES induced more aberrations. Chromosomal stickiness was the most common anomaly observed in root tips treated with the chemical mutagens. Magnitude of induced variation was found to depend upon the mutagen used, character under study and the genotypic background of the mutant. These promising mutant lines need to be further utilized in next generations to derive distinct lines with improved agronomic traits.

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