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The effect of ethyl methane sulphonate and diethyl sulphate on chilli (*Capsicum annuum* L.) in M_1 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 M_1 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 M_1 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 15^{th} century and spread to rest of the globe. In 17^{th} century Portuguese introduced it into India. It is an indispensable spice essentially used in every Indian cusine 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$_1$ 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 M_1 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 M_1 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 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 of 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.	%
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 increase the impairment of 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 annuum* 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)	Peicarp: 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 M_1 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 lengthy of pods, number of pods and number of clusters

in M_1 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 (M_1) 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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