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Effect of chemical mutagenesis in *Capsicum annuum* L. CvJwalamukhi

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ABSTRACT

Ethyl Methane Sulphonate (EMS)is a powerful chemical mutagen that is capable of inducing mutations at a faster rate. Specific effects of this mutagen on the chromosomes of the plant *Capsicum annuum* L. cvJwalamukhi was the subject of this study. Different treatments (0.1-0.4% EMS) were done and seedlings from each treatment were grown under uniform environmental conditions. A set of control plants was also maintained. EMS was found to affect the floral structure and alter behaviour of chromosomes during meiosis. There are some flowers with more than the usual number of petals and stamens and also the normal size of gynoecium. At the same time, flowers with reduced petals and stamens were also noticed. Abnormally enlarged gynoecium was noticed in flowers treated with 0.4 % EMS. Cytological preparations showed various kinds of abnormalities such as chromosome stickiness, laggards, bridges, micronuclei, spindle disturbances etc. in meiosis. The study proved potent mutagenic effects of EMS on the plant *C. annuum*.

KEY WORDS: Capsicum annuum, EMS, mutagenesis, chromosomes

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INTRODUCTION

Induced mutation has become an effective tool to improve a crop through creation of variability. The chemical mutagens produce either mutagenic changes or inactivating alterations on DNA molecule. Ethyl Methane Sulphonate (EMS) is found to be a powerful mutagen, teratogen and carcinogen. It is an organic compound with chemical formula $C_3H_8O_3S$. It induces mutations at a rate of 5×10^{-4} to 5×10^{-2} per gene without substantial killing. EMS can replace any purine from DNA by any of the four bases A, T, G, and C. In most of the cases EMS induce C to T changes resulting in C/G to T/A substitution. Mutations produced by EMS can be studied in genetic screening or other assays. EMS induces a high rate of mutation at the regions near replicating forks because these regions are single stranded and EMS is more effective on single stranded DNA. EMS is also known as methane sulfonic acid, ethyl ether, ethyl methane sulfonic acid or ethyl metylate. Among the chemical mutagens, EMS mostly induces higher proportion of point mutations (Minocha and Arnason, 1962; Hajra, 1979).

EMS produce broad range of effects on plants. EMS induced seed mutagenesis in rice (Nair and Ninan, 1973), male sterility in wheat (Maan and Williams, 1984), herbicide tolerance in soybean (Sebastian et al., 1989), early flowering in spring rape (Thurling and Depittayanan, 1992), phenotypic variations like potato shaped leaves and reduced fruit size and disease resistance in tomato (Yudhvir, 1995)and increased pollen viability and fruit rot resistance in bell pepper (Ashok et al., 1995).

Capsicum annuum L. cv. Jwalamukhi is a high yielding, short-lived perennial herb with white solitary flowers. The plant is cultivated as a rain fed crop and also as an irrigated crop. The fruits are persistent, pendent and seeds are cream or bright yellow coloured. The present study was done to analyse the effect of EMS on meiosis and pollen viability of *C. annuum.*

MATERIALS AND METHODS

Induction of mutation

Seeds purchased from Kerala Agricultural University, Vellayani, chemical mutagen Ethyl Methane Sulphonate (EMS), phosphate buffer and sodium thiosulphate were used for chemical mutagenesis.

The dry seeds of *C. annuum* were soaked in distilled water for six hours prior to the treatment. Different concentrations of EMS viz. 0.1%, 0.2%, 0.3% and 0.4% were prepared in phosphate buffer (pH 7.4) and fifty seeds were treated in each concentration. Following the treatment, the seeds were dipped in 5% aqueous solution of sodium thiosulphate for 10 minutes to stop the action of the mutagen. Then the seeds were washed thoroughly with running water. Seeds

soaked in distilled water for 6 hours were used as control. The seeds from each treatment were sowed in separate pots.

Floral characters

Flowers from the treatments were examined regularly to record the variations from the control plants.

Cytological Studies

Meiotic studies have been made from the flower buds collected from M_1 generation. The young flower buds were collected from treated and control plants between 9.30 am and 10 am and fixed separately in Carnoy's fluid (3 alcohol: 1 acetic acid mixture). Smear preparation was done in 2% acetocarmine solution prepared in 45% acetic acid. Microphotographs of aberrant as well as normal meiotic stages were taken.

The pollen grains from mature flower buds just before anthesis were mounted in a drop of glycerine: acetocarmine (1:1) on a clean glass slide. The slides were kept for 30 minutes and then observed under a microscope. The round and completely stained pollengrains were counted as fertile and partially stained and irregularly shaped pollengrains were counted as sterile. The pollengrains were counted from 10 randomly selected fields from the slide.

From this data, the percentage of pollen sterility was calculated using the formula,

$$\text{Percentage of pollen sterility} = \frac{\text{Number of sterile pollengrains}}{\text{Total number of pollengrains}} \times 100$$

The data obtained from different observations were subjected to statistical analysis.

RESULTS

Various types of floral and cytological abnormalities were observed during the study. Increased number of floral parts such as petals and stamen and enlarged ovary, presence of rudimentary petals and stamens are the major abnormalities observed in the flower (Plate 1). Cytological preparations showed micronuclei at interphase and telophase;vacuole formation in prophase; fragmentation, unequal segregation, bridges and laggards, stickiness, multipolar segregation, diagonal anaphase and polyads (Figures 1-12). The percentage of aberrations increased proportionately with the increase in concentration of EMS. In 0.1% EMS treatment, very few abnormalities were recorded. Among all treatments 0.4% EMS treatment showed maximum percentage of abnormalities (Table 2).The percentage of pollen fertility was found to be decreased with increasing concentration of EMS (Table 3). Compared to control plants, all the treated plants exhibited higher degree of pollen sterility.

Table 1: Preparation of EMS solution

Concentration of EMS solutions (10 ml)	Volume of EMS (ml)	Volume of Phosphate Buffer (ml.)
0.1%	0.01	9.99
0.2%	0.02	9.98
0.3%	0.03	9.97
0.4%	0.04	9.96

Table 2: Effect of EMS on cytological characters (chromosomal abnormality in percentage)

Treatment	No. of PMC examined	Stickiness at anaphase	Disturbed polarity at anaphase	Laggards at anaphase	Non – Disjunction at anaphase	Bivalent association	Micronuclei at telophase	Percentage abnormal cells
Control	110	-	-	-	1.25	-	-	1.25
0.1 % EMS	110	-	-	-	2.75	-	0.5	3.25
0.2 % EMS	110	1.75	-	-	-	-	2.0	3.75
0.3 % EMS	110	-	-	1.25	-	0.5	2.75	4.5
0.4 % EMS	110	0.30	4.25	6.5	6.25	3.0	4.5	24.8
Total							**37.55 %**	

Table 3: Effect of EMS on pollen fertility

Treatment	Percentage of pollen sterility
Control	45.70
0.1%EMS	48.84
0.2%EMS	69.00
0.3%EMS	72.00
0.4%EMS	72.00

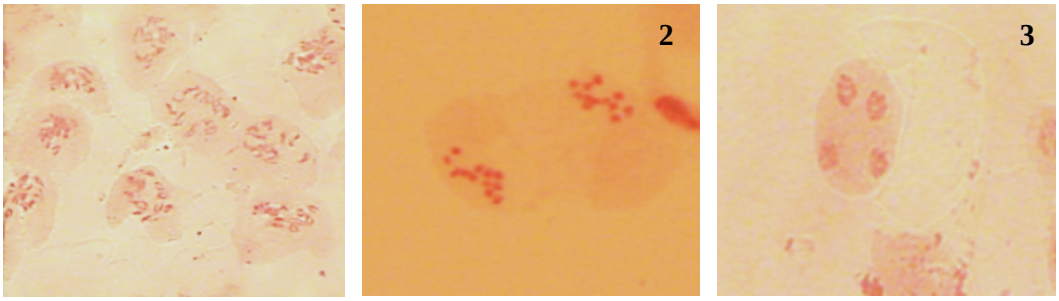
Plate 1: Abnormalities observed in the flowers of treated plants

1 **2** **3**

4 **7** **8**

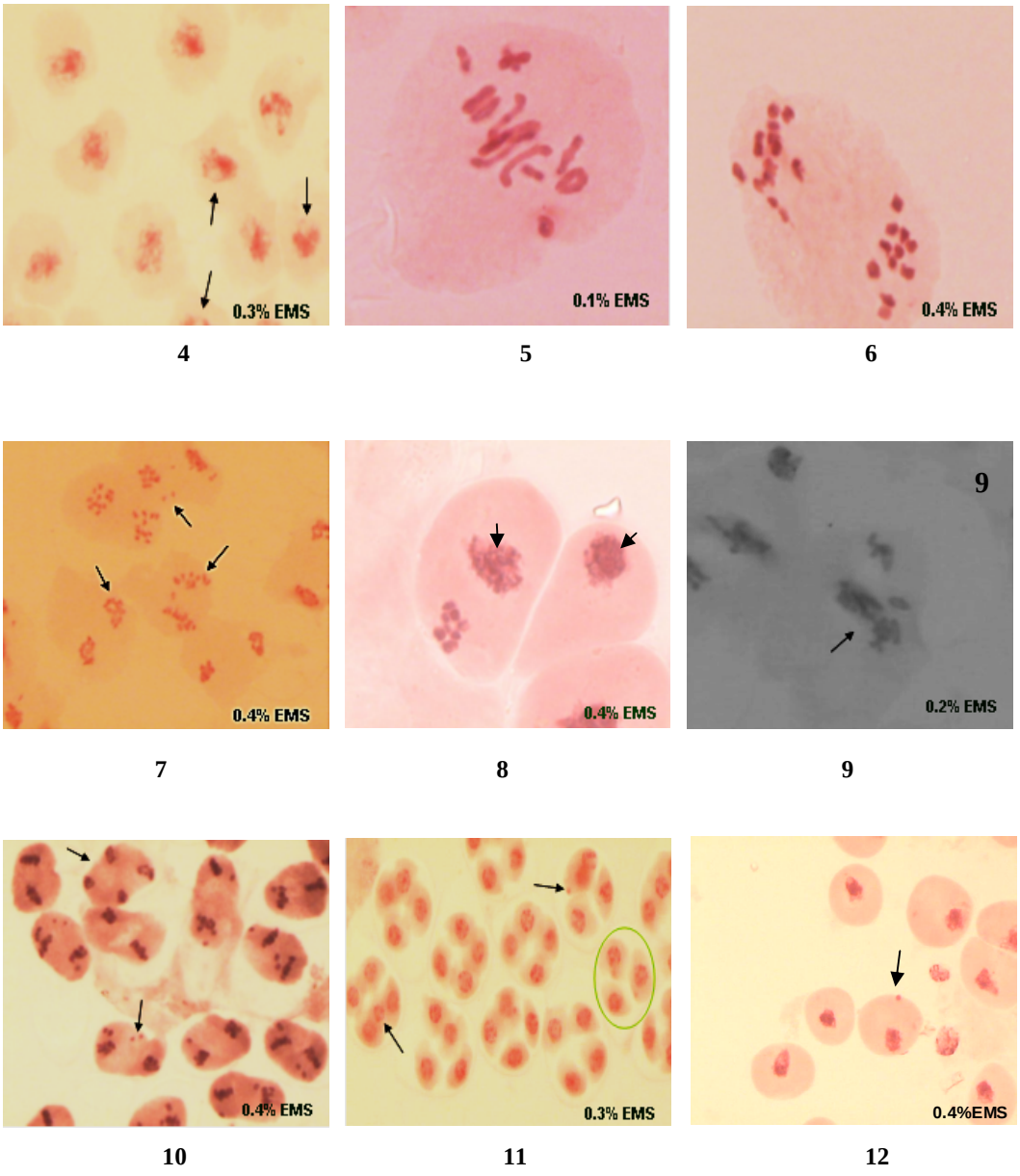
Plate 1: 1- An abnormal flower with 6 petals and 6 stamens; 2 & 3- Flowers of different size; 4- a flower with unusual ovary; 5- A flower showing reduced petal; 6- Rudimentary stamens in a flower; 7- Enlarged ovary with outer whorls reduced; 8- Abnormal ovary with style

Figures 1-3: Normal prophase I, anaphase I and telophase II in control plants



1 **2** **3**

Figures 4-12 :Abnoralities observed in Meiosis I and II with various treatments



Figs. 4-12:4- Vacuole formation in prophase; **5-** MetaphaseI showing fragment; **6-** Unequal segregation in anaphaseI; **7-** Laggards, sticky chromosomes and unpaired chromosomes; **8-** chromosome dissolution at one end; **9-** anaphase bridge;**10-** diagonal anaphases with laggards and unpaired chromosomes; **11-** triads and polyads in telophaseII; **12-** Microspore with micronuclei

DISCUSSIONS

The chromosomal behaviour during meiosis is considered to be one of the most reliable indices for estimating the potency of mutagens and the response of a genotype to mutation. In control

plants, meiosis had been normal with 12 bivalents (2n = 12) at metaphase. The EMS treated plants showed a wide range of chromosomal aberrations. The percentage of abnormalities increased with increasing concentration of EMS. The chromosomal behaviour during meiosis is considered to be one of the most reliable indices for estimating the potency of mutagens and the response of a genotype to mutation. The treated populations exhibited micronuclei, unequal segregation, multipolar segregation, bivalent association, laggards, stickiness and univalents.

The major type of abnormality observed in the prophase nuclei was nuclear lesions or vacuole formation. Nuclear lesions are areas of chromosome disintegration (Busch, 1973). According to him the chemical reacts with basic protein of chromatin resulting in the breakdown of chromatin packing and subsequent disruption. These vacuolated nuclei or nuclear lesions were areas of chromatin disintegration (Mercy Kutty and Stephen, 1980).

EMS was found to increase stickiness of the metaphase chromosomes (Sreekrishna, 2006).According to him stickiness is due to heterochromatinization of chromosomes resulting in denaturaion of nucleic acids, which makes the chromosomes hazy and adhesive.The laggards observed in anaphaseI may be the reflection of gene mutations, chromosome breakage, disturbances during pairing and influence of environmental conditions (Nerkar, 1977; Dnyansagar and Thengane, 1977).The chromosomes that fail to attach to the spindle form lagging chromosome and move to either one of the two poles. These lagging chromosomes are called laggards. Univalent are produced due to desynapsis or asynapsis (Sinha and Godward,1972; Kalloo, 1972). The presence of anaphase bridges indicated the union of centric fragments of broken chromosomes (Yagua and Morris, 1957).

Multipolar segregation noticed at anaphaseI may be either due to the shifting of the poles of the mitotic apparatus (Kihlman, 1966) or due to inhibition of protein synthesis and binding of chemical to DNA to prevent unwinding for transcriptions of spindle messengers (Mercy Kutty and Stephen, 1980).Dissolution of spindle attached to the chromosomes might result in unsynchronization and disturbed telophase and which in turn reduce pollen fertility. The increased pollen sterility with the increased EMS concentration is the cumulative effect of various chromosomal aberrations[19] (Khan et al., 1998).

Micronuclei were also present in telophase II and pollens. Micronuclei lead to the loss of genetic material. These are regarded to be formed from acentric fragments or isolated entire chromosomes. Auerbach (1962) had reported that micronuclei may be originated from a lagging chromosome or from a chromosome fragment. EMS has localized action on centromere and it either inactivates chromosome or cause chromosome breakage.

CONCLUSIONS

EMS treatment produced various types of meiotic abnormalities in *Capsicum annuum* cultivar variety Jwalamukhi. The frequency of abnormalities increased with increase in concentration of the mutagen. The major abnormalities observed was univalents at anaphase, stickiness at anaphase, association of bivalents, non-disjunction at anaphase, laggards at anaphase and micronuclei at telophase and in microspores. The percentage of abnormalities was found to be higher in 0.4% than other concentrations of EMS.Pollen fertility decreases with increase in concentration of EMS.

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