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**Research Article**

# EMS induced mutagenicity in pollen mother cells of Sesbania Pea (*Sesbania cannabina* Poir.)

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**Abstract:**
Mutagenic effect of EMS on pollen mother cells of *Sesbania cannabina* has been investigated in the present cytogenetical observation. The progenies were obtained from seeds treated with four different time durations (1, 3, 5, and 7 hours) of 0.5% solution of ethyl methane sulphonate (EMS). Study revealed various types of chromosomal aberrations at different doses of EMS. Increase in chromosomal aberrations was dose dependent manner. Pollen mother cells of *Sesbania cannabina* exhibited an increased incidence of chromosomal bridges, unorientation, laggards and precocious movement etc. at each dose of treatment. Anaphasic and telophasic bridges were major abnormalities and found to be highest at 3 hr dose of treatment. Anaphasic bridges were more prominent than telophasic bridges and percentages of different types of bridges at anaphase and telophase were also studied and scored.

**Key words:**
*Sesbania cannabina,* ethyl methane sulphonate (EMS), chromosomal aberrations, bridges.

## Introduction

Study of chromosome biology is an important source of information regarding the genotoxic and mitotoxic behavior of any mutagen. Cytological studies provide important information for plant breeding program for induction of mutation in genetic composition and to select beneficial one. The normal meiosis ensures gamete viability, which if altered has a significant effect on the genotypic and phenotypic characters of plants. The technology of mutation induction has become an established tool in plant improvement program in order to supplement existing germplasm and to improve cultivars in specific traits.

A number of physical and chemical agents is known with their mutagenic properties; relatively few of them are applied commonly for practical mutation breeding programs. Ethyl methanesulfonate (EMS) has recently received much attention as a most effective and efficient mutagen in higher plants (Zohary, 1984). EMS is most effective mutagen for induction of genetic variability in many crops (Jabeen & Mirza, 2002; Kumar & Rai, 2005**)**. EMS is an alkylating agent and induces chemical modification of nucleotides, which results in impairing and base changes **(**Kim et al., 2006**)** and it also induces a higher proportion of point mutation **(**Minocha & Arnason, 1962; Hajra, 1979**)** but loss of a chromosomal segment or deletion can also occur **(**Jabeen & mirza, 2004**)**. In majority of cases, EMS induces -C to -T changes resulting in C/G to T/A substitution whereas methane sulfonate produces T/A to G/C transversion and A/T to G/C transition **(**Krieg, 1963; Kovalchuk et al., 2000; Greene et al., 2003).

Cytological study of mitosis and meiosis is considered to be one of the most dependable indices to estimate the potency of mutagen **(**Siddiqui et al., 1982**)**. Results of many investigations revealed that use of chemically induced mutants can also provide useful information for understanding the function of essential genes by generating weak nonlethal alleles.EMS induces relatively few strand breaks in comparison to physical irradiation mutagen and it leads to inversion or deletion mutation **(**Koornneef et al., 1982).

*Sesbania cannabina* is a multipurpose leguminous crop with high growth rate and high biomass production. It is widely used as a green manure crop for wheat, rice, maize, etc. It is widely adaptable to climatic conditions such as water logging, drought, heavy metal toxicity and high alkalinity. They can also grow and adapt to varied soil environments,

control soil erosion and enhance soil fertility where they are grown. It is also used for firewood, for making crops and nets and in some instances its leaves and flowers are used for human consumptions. They have been used in the application because agriculturists have been impressed by its special qualities of vigorous growth, adaptation to various soils and environmental condition and enhancement of soil fertility **(**Hossain & Becker, 2001**)**. Because of these valuable characterstics, it is a promising multipurpose plant resource. Objective of present cytogenetical study is to investigate the effect of EMS on pollen mother cells of *Sesbania cannabina* and to evaluate the feasibility of using this mutagen for induction of desired mutation in this genus which could be further utilized for plant breeding program to obtain *Sesbania cannabina* plants with better phenotypic and genotypic characters.

**Material and Methods:**

Treatment and sowing**:** Seeds of *Sesbania cannabina* variety ND-1 obtained from Sunn Hemp Research Station, Pratapgarh, India were soaked in water for 14 hr. Soaked seeds were then treated with 0.5% solution of EMS for different time durations (1, 3, 5 and 7 hr). After treatment, the seeds were washed in running water to remove EMS. After that, the treated seeds as well as control seeds were sown in triplicate to raise M1 generation.

Field work**:** On the onset of budding the young floral buds were fixed in Carnoy's fixative (1:3, glacial acetic acid: abs. Alcohol) in their respective bottles for 24 hr and then stored in 70% ethyl alcohol in refrigerator and were used for cytogenetical analysis.

Cytological Studies**:** Slides were prepared using anther squash technique. Anthers were squashed in 2% acetocarmine. Slides were observed under microscope to observe different meiotic stages and various chromosomal aberrations and data were taken for chromosomal aberrations of each treated set as well as for control.

**Results and Discussion**

The meiotic study of pollen mother cells of control exhibited a normal diploid status with chromosome number (n = 12) i.e. 12 bivalents at metaphase I and diakinesis (Fig.1-a, b) and normal separation (12:12) at anaphase I. In treated sets however total abnormality percentage increased with increase in doses of EMS. During different meiotic stages, pollen mother cells, displayed various types of aberrations such as unoriented metaphase II with precocious movement (Fig.1-c), laggards at anaphase I (Fig.1-d), precocious movement at metaphase I (Fig.1-e), unequal separation at anaphase I (Fig.1-f), disturbed polarity at anaphase II (Fig.1-g), multivalent formation at metaphase (Fig.1-h), scattering at metaphase (Fig.1-i) and different types of chromosomal bridges (Fig.2 a-i). Percentages of different types of chromosomal aberrations have been shown in table-1. Pollen mother cells observed at anaphase exhibited abnormalities such as bridges, laggards, disturbed polarity etc. However, pollen mother cells at telophase exhibited only bridges. Chromosomal bridges were found to be most prevalent aberrations among anaphase and telophase stages of meiosis. The percentage of bridges observed during anaphase of 3 hr dose was 5.47% while it was only 3.94% at 7 hr dose of treatment. Similar results were obtained in case of telophasic bridges as shown in Table-1. The frequency and spectrum of different types of anaphasic and telophasic bridges were also scored and it was found that anaphasic bridges were more prevalent than telophasic bridges. Pollen mother cells at anaphase I exhibited 8 different types of variations in chromosomal bridges viz. single bridge, incomplete bridge, diagonal bridge, bridges with laggard, lateral single bridge, double bridge, lateral double bridge and multiple bridges, while, pollen mother cells at telophase I exhibited only two types of bridge variations i.e. single bridge and incomplete bridge. Different types of anaphasic and telophasic bridge variations have been shown in table-2 and Fig.2a-i. Present study indicated that the 3hr dose of EMS is most effective and efficient for induction of chromosomal bridge variations at anaphasic and telophasic stages of pollen mother cells of *Sesbania cannabina.*

The most prevalent abnormality at 7 hr dose was also the bridges (3.94%) followed by stickiness at metaphase (2.63%), laggard at anaphase (2.63%), precocious movement at metaphase (1.97%) etc. The highest frequency of laggards (2.63%) was observed at 7 hr doses of treatment. Unorientation at metaphase was also observed to be 1.97% at 7 hr dose of treatment. Disturbed polarity was found to be highest at 7 hr dose of treatment (1.97%). The other observed abnormalities were asynchronous division and unequal separation at anaphase etc.

Mutagenic treatment may lead into genetic changes in an organism, break the linkages and produce many new promising economic traits for improvement of treated plants **(**Shah et al., 2006), hence mutation breeding program has proved to be a successful tool in bringing amelioration in self-pollinated crops.EMS is reported to be the most effective and powerful

mutagen (Girija & Dhanvel, 2009). Khatri et al (2005) reported that EMS could be fruitfully applied to develop new varieties with high yield and other improved organic traits. Increase in meiotic aberrations was found alongwith increase in doses of treatment of EMS. Different types of metaphasic, anaphasic and telophasic aberrations were scored. Similar types of aberrations were also reported by many workers in different plant materials after treatment with different mutagens. During the cytological observations of different treated sets, it was found that bridges were the most prominent aberrations at all the doses and it was highest at 3hr dose of treatment. Sinha and Godward (1972) suggested that paracentric inversions may lead to formation of chromatin bridges at anaphase and telophase stages of meiosis. Bridge formation may occur due to the failure of chiasmata in a bivalent to terminalize and the chromosome stretched between the poles (Saylor & Smith, 1996). Single and multiple chromosome bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage fusion bridge cycles (McClintok, 1941; Kumar & Singh, 2002).

Chromosomal stickiness may be due to genetic or environmental factors (Consolaro et al., 1996) and several agents have been reported to cause stickiness of chromosome (Souza & Pagliarini, 1996). Laggards observed during the cytological studies might have originated due to delayed terminalization, stickiness of chromosomal ends or because of failure of the chromosomal movements (Jayabalan & Rao, 1987; Soheir et al., 1989). Disturbed polarity observed during anaphase may be due to the spindle disturbance (Bhat et al., 2007). Jayabalan and Rao (1987) suggested that stickiness might be due to disturbance in the cytochemically balanced reaction. Tarar and Dnyansagar (1980) have reported that stickiness at meiosis was due to depolymerisation of nucleic acid caused by mutagenic treatment or it may be due to chemically induced stickiness to direct action of mutagen on the histone proteins leading to improper folding of DNA (Gaulden, 1987). Bridges are also induced by many other mutagens such as methyl methane sulphonate (Khan et al, 2009), sodium azide (Srivastava & Kapoor, 2008) etc. Precocious movement of chromosomes may be due to the spindle dysfunction. Kumar and Rai (2007) reported that precocious chromosome migration to the poles may be due to the formation of univalent chromosome at the end of prophase I or precocious chaisma terminalization at diakinesis or metaphase. Unorientation at metaphase and scattering of chromosome may be due to inhibition of spindle formation or destruction of spindle fiber formed (Kumar & Rai, 2007).

During the present investigation, through EMS, many chromosomal anomalies were induced. The genetic structure of present study material was highly affected by EMS treatment, favoring new genetic changes in the following generations. From the present study, it is quite evident that EMS is very efficient mutagen for creating genetic variability in the natural gene pool of *Sesbania cannabina.*

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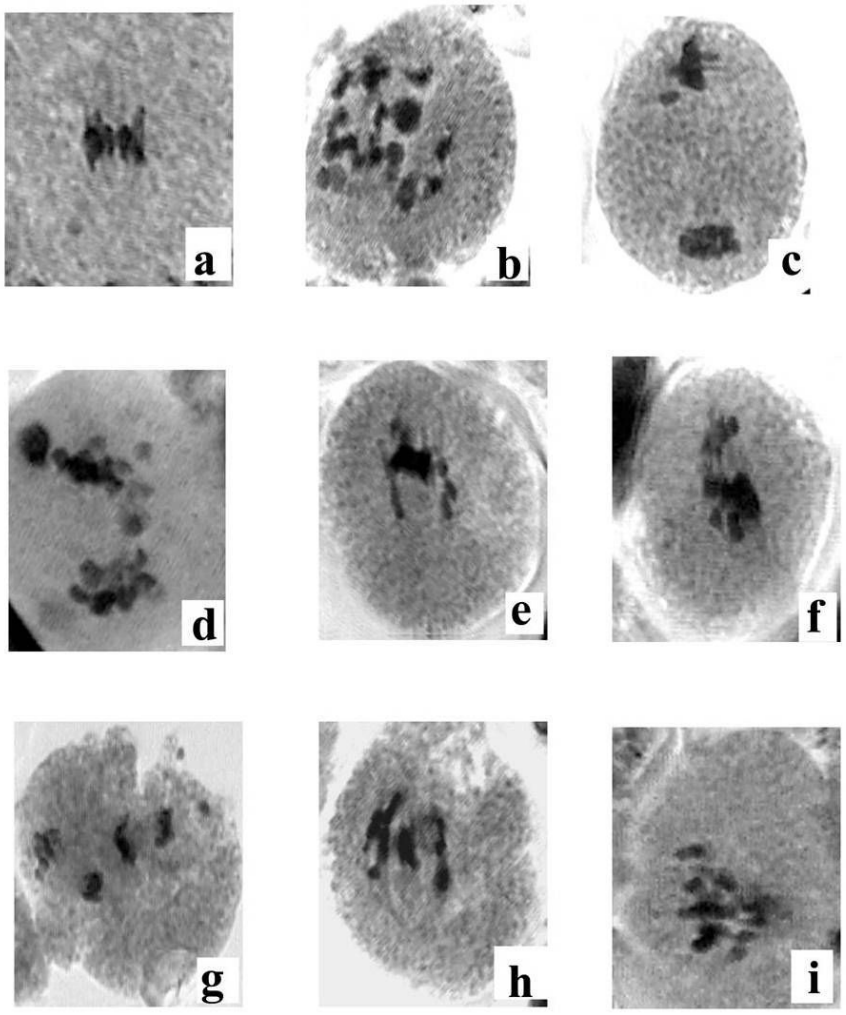


Figure 1.

a. Pollen mother cell (PMC) at metaphase I (n = 12)
b. PMC showing Diakinesis (n = 12)
c. PMC showing unoriented metaphase II with precocious movement
d. PMC showing laggard at anaphase I
e. PMC showing Precocious movement at metaphase I
f. PMC showing Unequal separation at anaphase I
g. PMC showing disturbed polarity at anaphase II
h. PMC at metaphase I showing multivalent formation
i. PMC showing scattering at metaphase I

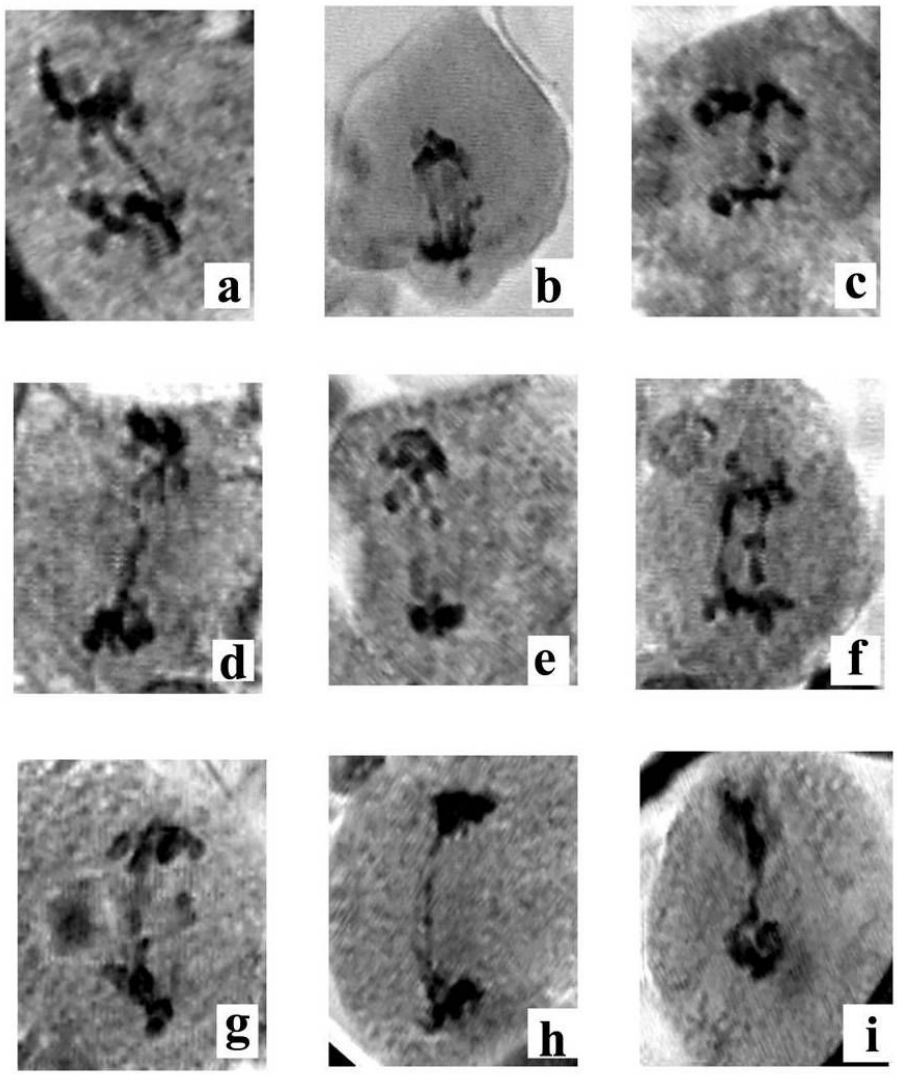


Figure 2.

a. Diagonal bridge
b. Multiple bridge
c. Unequal separation at anaphase with bridge formation
d. Single bridge
e. Incomplete bridge
f. Lateral double bridge
g. Bridge with laggard
h. Lateral single bridge
i. Telophasic single bridge

**Table 1. Effect of EMS on different meiotic phases of pollen mother cells of *Sesbania cannabina*.**

| Mutagen | Doses | Total no of PMCs scored | Total no of abnormal PMCs | Metaphasic abnormalities (%) | | | | Anaphasic abnormalities (%) | | | | Telophasic abnormalities (%) | Other abnormalities (%) | Total abnorm-ality (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Un | St | Sc | Pr | St | Br | Dis pol | Lg | Br | | |
| | Control | 310 | - | - | - | - | - | - | - | - | - | - | - | - |
| | 1hr | 303 | 24 | 0.99 | 0.33 | 0.99 | 1.32 | - | 1.32 | 0.66 | 0.66 | 0.99 | 0.66 | 7.92 |
| EMS | 3hr | 201 | 25 | 0.99 | 0.49 | 0.49 | 0.49 | - | 5.47 | 0.99 | 0.49 | 1.98 | 0.99 | 12.43 |
| (0.5%) | 5hr | 195 | 35 | 2.05 | 2.05 | 1.53 | 1.53 | 1.02 | 3.07 | 1.53 | 2.05 | 1.02 | 2.05 | 17.94 |
| | 7hr | 152 | 32 | 1.97 | 2.63 | 1.31 | 1.97 | 1.31 | 3.94 | 1.97 | 2.63 | 1.97 | 1.31 | 21.05 |

Un- Unorientation, St- Stickiness, Sc- Scattering, Pr- Precocious movement, Br- Bridges, Dis pol- Disturbed polarity, Lg- Laggards

**Table 2. Percentage of different types of bridge variations during anaphase and telophase stages.**

| Doses of Treatment (0.5% EMS) | % of anaphasic bridge variations | Types of anaphasic bridge variations (%) | | | | | | | | % of telophasic bridge variations | Types of telophasic bridge variations (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Single bridge | Incomplete bridge | Diagonal bridge | Bridge with laggard | Lateral single bridge | Double bridge | Lateral double bridge | Multiple bridge | | Single bridge | Incomplete bridge |
| 1 hr | 1.32 | 0.66 | - | - | - | 0.33 | 0.33 | - | - | 0.99 | 0.66 | 0.33 |
| 3hr | 5.47 | 1.48 | 0.49 | 0.49 | 0.99 | - | 0.99 | 0.99 | - | 1.98 | 0.99 | 0.99 |
| 5 hr | 3.07 | 0.51 | 0.51 | - | 0.51 | 0.51 | - | - | 1.02 | 1.02 | 0.51 | 0.51 |
| 7 hr | 3.94 | 0.65 | - | 1.31 | - | 1.31 | - | - | 0.65 | 1.97 | 0.65 | 1.31 |