CHAPTER 3
MATERIAL AND METHODS
3.1 EXPERIMENTAL GENOTYPE

Experimental genotype selected for the present investigation was *Coriandrum sativum* Linn. variety CS-287 commonly known as coriander. The experimental seed material of *Coriandrum sativum* Linn. variety CS-287 was released by Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu and same variety was used for the present investigation.

![Experimental genotype](image)

**Fig. No.: 3.1: Experimental genotype**

3.2 MUTAGENS USED

- **Chemical Mutagen** – Ethyl Methanesulphonate (EMS)
- **Physical Mutagen** – Gamma Rays

**Ethyl Methanesulphonate (EMS)**

Ethyl Methanesulphonate (EMS) is a mutagenic and carcinogenic organic compound with the chemical formula CH$_3$SO$_3$C$_2$H$_5$. It can produce random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation. EMS typically produces only point mutations (Wikipedia). EMS is a clear, colorless liquid with density 1.1452 g/cm$^3$ at (22°C) and its Molecular formula is CH$_3$SO$_3$C$_2$H$_5$.

**Table No. 3.1: Properties of EMS**

<table>
<thead>
<tr>
<th>Properties of EMS</th>
<th>CH$_3$SO$_3$C$_2$H$_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Formula</td>
<td>CH$_3$SO$_3$C$_2$H$_5$</td>
</tr>
<tr>
<td>Molar Mass</td>
<td>124.16 g/mol</td>
</tr>
<tr>
<td>Appearance</td>
<td>Clear colorless liquid</td>
</tr>
<tr>
<td>Density</td>
<td>1.1452 g/cm$^3$ (22°C)</td>
</tr>
<tr>
<td>Melting Point</td>
<td>&lt; 25°C</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>213-213.5°C, 486.2-486.7 K</td>
</tr>
</tbody>
</table>
SOURCE OF SEED MATERIAL (*Coriandrum sativum* L.)

PHOTO PLATE NO. 1: SOURCE OF SEED MATERIAL
Gamma Rays

Gamma rays are emitted by certain radioactive elements such as cobalt and electron beams are produced by accelerating electrons to high energies in special machines. Gamma rays have shorter wavelength than the wavelength of ultraviolet light. X- Rays, which have wavelength of 0.1 to 40 nm, and gamma rays, have even shorter wavelength, which forms of ionizing radiation. Longer wavelengths comprise non-ionizing radiation. These forms of radiation also kill microorganisms and viruses and ionizing radiation damages DNA and produces peroxides, which act as powerful oxidizing agents in cells. This radiation can cause mutations in human cells if it reaches them. The principal effect of ionizing radiation is the ionization of water, which forms highly reactive hydroxyl radicals. These radicals react with organic cellular components, especially DNA. One, or a few, hits may only cause nonlethal mutations, some of them conceivably useful. More hits are likely to cause sufficient mutations to kill the microbe.

(Source: Wikipedia online 2012)

3.3 MODE OF TREATMENT

1. Ethyl Methanesulphonate (EMS)

Ethyl Methanesulphonate (EMS) was obtained from Spectrochem Pvt. Ltd. Mumbai (India) with a molecular weight 124.16 g/mol and density 1.20 g/cm³. To determine the lethal dose (LD₅₀) and suitable concentrations of mutagens for the further studies, chemical mutagenic treatments were administered at room temperature of 25 ± 2°C. Healthy and dry seeds of the Coriandrum sativum Linn. variety CS- 287 having uniform size were selected for the treatment. Seeds were surface sterilized with 0.1% Mercuric Chloride solution for about one to two minutes than washed thoroughly and soaked in distilled water for 6 hours for pre -soaking of seeds, which were made the seed coat permeable for the mutagenic treatment.

The fresh, aqueous solutions of the mutagen were prepared prior to treatments. The different concentrations used for the chemical mutagenic treatments were 0.050%, 0.075%, 0.10% and 0.125%. After the pre - soaking seeds were immersed in the mutagenic solution for 4 hours with continuous shaking. The volume of the chemical solution used was five times more than that of the seeds to facilitate uniform absorption. Seeds soaked in distilled water for 6 hours served as control. Immediately after the completion of treatment, the seeds were washed thoroughly under running
PHOTO PLATE NO. 2: MUTAGENIC TREATMENT
tap water for 3 to 4 times. Later on they were subjected to post-soaking in distilled water for 4 hours.

2. Gamma radiation

Healthy, uniform size and dry seeds of the *Coriandrum sativum* Linn. variety CS-287 was packed in polythene bags and sealed them for the Gamma radiation. Electromagnetic, ionizing radiations were applied from Co$^{60}$ source of irradiation. Facility was availed from by Department of Nuclear Chemistry, Department of Chemistry, University of Pune. - 411007. The seed samples were exposed to doses of 10kR, 20kR, 30kR and 40kR of Gamma rays according to LD$_{50}$.

For each treatment 500 seeds were used, 300 seeds from each treatment were sown in the field according to Complete Randomized Block design (CRBD) with 3 replications along with control to rise M$_1$ generation.

3.4 STUDIED IN M$_1$ GENERATION

1. Germination percentage

Germination was studied in different conditions of light and temperatures. Due to hard seed coat germination, study was carried out in tray with sterile soil. The number of seeds showing emergence of radical were observed and recorded for calculating germination percentage.

2. Seedling height and seedling injury

The seedling height was recorded at the end of first week and the percentage of seedling injury was calculated from the data of seedling height. The methodology by (Konzak *et al.*, 1965) was followed.

3. Survival of plants:

The percent plants survival was recorded at the time of maturity of plants.

4. Pollen sterility:

Pollen sterility was determined in 10 randomly selected plants belonging to each treatment. The pollen grains from freshly dehisced anthers were stained with 1% acetocarmine. Fully stained pollens were counted as fertile and the empty, partially stained and shriveled ones were considered as sterile.
GERMINATION OF SEEDS TREATED WITH EMS

GERMINATION OF SEEDS TREATED WITH GAMMA RAYS

PHOTO PLATE NO.3 : SEED GERMINATION IN TRAYS
EMS TREATMENT

GAMMA RAYS TREATMENT

PHOTO PLATE NO.4: SEEDLING HEIGHT AND SEEDLING INJURY
PHOTO PLATE NO. 5: COMPLETE RANDOMIZED BLOCK DESIGN
FIELD PREPARATION

FIELD VIEW

PHOTO PLATE NO. 6: FIELD PREPARATION
3.5 STUDIES IN M₂ AND M₃ GENERATIONS

From each concentration/dose of mutagen about 25 plants from the progeny were randomly selected, harvested and collected seeds for further generations. The observations were recorded in M₂ and M₃ generations. The Frequency and spectrum of chlorophyll mutants and viable mutations, quantitative characters and biochemical studies were carried out.

Chlorophyll mutations

The chlorophyll mutations were screened and recorded in the field when the seedlings were 10-15 days old. The types of chlorophyll mutations scored like *albina*, *xantha*, *chlorina* and *viridis*. These are classified according to the terminology of Gustafsson (1940). The frequency of chlorophyll mutants was calculated according to Gaul (1960) i.e. number of mutants / 100 M₂ plants.

Estimation of mutagenic effectiveness and efficiency

Mutagenic effectiveness and efficiency of different mutagens were calculated according to the formulae suggested by Konzak et al. (1965). The mutagenic effectiveness can be a measure of the frequency of mutations induced by a unit dose of mutagen (kR or time × concentration) while mutagenic efficiency gives an idea of the proportion of mutations in relation to biological damage such as lethality, seedling injury, pollen sterility and chromosomal aberrations.

**Mutagenic effectiveness**

\[
\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Dose or (Time X Concentration)}} = \frac{\text{MF}}{\text{kR or MF/TC}}
\]

Where,

\begin{align*}
\text{MF} &= \% \text{ of chlorophyll mutations in M₂ generation.} \\
T &= \text{Period of treatment with chemical mutagen.} \\
C &= \text{Concentration of chemical mutagens,} \\
kR &= \text{unit of gamma radiation.}
\end{align*}

**Mutagenic efficiency**

\[
\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological damage}} = \frac{\text{MF/L, MF/I, MF/S, MF/MI}}
\]
Where,

L = % of lethality in M1 generation.
I = % of seeding injury in M1 generation.
S = % of pollen sterility in M1 generation.
MI = % of mitotic abnormalities in M1 generation.

**Mutation rate**

The mutation rate was calculated by the following formula:

$$MR = \frac{\text{Number of treatments of that particular mutagen}}{\text{Some of values of effectiveness or efficiency of a particular mutagen}}$$

This gives an idea of mutations induced by a particular mutagen irrespective of dose.

**Data on Quantitative characters**

From each chemical and physical mutagenic treatment, 25 plants were randomly selected for studies and collection of the data on different quantitative characters in M2 and M3 generations. Similarly 25 plants were selected from the control for comparative studies. The data of quantitative characters like plant height, number of branches, number of umbellate per umbel, number of umbels per plant, days to flowering, number of fruits per umbel, number of fruits per plant, days to maturity, size (diameter) of fruit and weight of 100 fruits.

**3.6 SCORING OF VARIANTS AND MUTANTS**

**In M1 Generation**

The various leaf abnormalities and chlorophyll deficient sectors in the leaves were noted. The frequency of plants carrying the chlorophyll deficient sectors of different types (viz., *xantha*, *viridis* and *chlorina*, etc.) was calculated mutagen-wise separately.

**In M2 and M3 Generations**

**Macromutations (viable mutations)**

Mutations that can be scored externally and which affect the morphological characters of the plant were considered as macromutations. They were scored during the entire life cycle of the plant in M2 and M3 generations. All such plants were
harvested separately in M\textsubscript{2} and M\textsubscript{3} generations. Their true breeding nature has been ascertained during subsequent generations.

**Micromutations**

These are the mutations, which cannot be detected visually but need biostatistical analysis for their detection. Such plants were subjected to biostatistical analysis and screened accordingly. The parameters were the same for M\textsubscript{2} and M\textsubscript{3} generation.

### 3.7 Statistical Analysis

Statistical data was analyzed using following formulae.

Mean: \[ \frac{\sum x}{n} \]

Variance: \[ \frac{\sum x^2}{n} - \bar{x}^2 \]

Standard Deviation (S.D.): \[ \sqrt{\text{Variance}} \]

Standard error (SE): \[ \frac{\text{S.D.}}{\sqrt{N}} \]

Coefficient of variation (CV): \[ \frac{\text{S.D.}}{\text{Mean}} \times 100 \]

Critical difference (CD): \[ \text{SE (d)} \times t \text{ e. d. f.} \]

Where, SE (d) = SE (difference) = SE (Mean) \times 2

\[ \text{SE (m)} = \sqrt{\frac{\text{Mean error sum of squares}}{r}} \]

\[ r = \text{Number of replications} \]

The ANNOVA was calculated as per Panse and Sukhatme (1976) and the following abbreviations were used.

- S.V. = Source of Variation
- D.F. = Degree of freedom
- S.S. = Sum of squares
Components of variation

The phenotypic and genotypic variances were calculated using the respective mean squares from variance table (Johnson et al., 1955) as below.

Environmental variance \( \sigma^2_e = \text{EMS} \)

Genotypic variance \( \sigma^2_g = \frac{\text{GMS} - \text{EMS}}{r} \)

Phenotypic variance \( \sigma^2_P = \sigma^2_g + \sigma^2_e \)

Where,

GMS = Genotypic mean sum of square
EMS = Error mean sum of square
r = Number of replications.

Coefficient of variation

The genotypic and phenotypic coefficient of variations was calculated following the formulae given by Burton and De Vane (1953).

\[
\text{GCV} = \frac{\sigma_g}{\bar{X}} \times 100
\]

\[
\text{PCV} = \frac{\sigma_p}{\bar{X}} \times 100
\]

Where,

\( \sigma^2_g = \) Genotypic variance for the character
\( \sigma^2_p = \) Phenotypic variance for the character
\( \bar{X} = \) Mean for the character

Broad sense heritability

Heritability in broad sense was estimated for various characters as suggested by Hanson et al. (1956).

\[
h^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100
\]

Where,
\( \sigma^2_g = \text{Genotypic variance} \)

\( \sigma^2_p = \text{Phenotypic variance} \)

**Genetic Advance (GA)**

Genetic advance (at 5% selection intensity) was calculated by the formula suggested by the Johnson *et al.* (1955).

\[
\text{G.A} = K \frac{\sigma^2_g}{\sigma^2_p} \times \sigma^2_p
\]

Where,

\( \sigma^2_g = \text{Genotypic variance} \)

\( \sigma^2_p = \text{Phenotypic variance} \)

\( K = \text{Selection differential as defined by Lush (1949),} \)

(At 5% selection intensity, the value of \( K = 2.06 \))