Chapter-3
Materials and methods
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MATERIALS AND METHODS

3.1. Materials

3.1.1. Varieties used

Two varieties of faba bean (*Vicia faba* L.) namely, 05/249 local and 05/233 HBP were used in the present study. Seeds of both the varieties were obtained from Genetic Section of the Indian Agricultural Research Institute of New Delhi.

3.1.2. Mutagens used

Ethylmethane sulphonate (EMS)-CHO<sub>3</sub> SO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>-, an alkylating agents, manufactured by Sisco Research Laboratories Pvt.Ltd.,Mumbai.

EMS was used alone and in combination with dimethyl sulfoxide(DMSO)-CH<sub>3</sub>SO.CH<sub>3</sub>-manufactured by Ranbaxy Laboratories Pvt.,Ltd., S.A.S Nagar, Punjab.

Hydrazine hydrate (HZ)-NH<sub>2</sub> -NH<sub>2</sub> -H<sub>2</sub>O, a base analogue, is manufactured by Qualigens Fine Chemical, Mumbai.,

3.2. Experimental procedure

3.2.1. Pretreatment

Healthy seeds of uniform size of each variety were used. Seeds were soaked in distilled water for 9 hours prior to the treatment with mutagens.
3.2.2. Mutagens administration

**Concentrations used:** Four different concentrations viz., 0.02, 0.04, 0.06, and 0.08% of EMS, EMS+2%DMSO and HZ were used for treating the presoaked seeds. (2% DMSO was prepared by dissolving 2ml of DMSO in 100 ml of distilled water).

**Treatment time:** The treatments were given at temperature of 22 ±1°C for 6 hours.

**Sample size:** 255 Seeds were used for each treatment and control.

**Controls:** For each variety, 255 pre-soaked seeds were again soaked in phosphate buffer for 6 hours to serve as controls.

3.3. M₁ generation

Three replications of seventy seeds each were sown for every treatment and control in each variety in the field. The remaining lot of forty five seeds of each treatment with their respective controls of both the varieties was spread over moist cotton in petriplates, in order to determine percentage of seed germination and seedling height i.e. root and shoot length. The petriplates were kept in B.O.D. incubator at 22 ±1°C temperature.

3.3.1. Observations recorded in M₁ generation

Following parameters were studied in M₁ generation

**3.3.1.1 Seed germination:** After recording germination counts, the percentage of seed germination was calculated on the basis of total number of seeds sown in
petriplates. Seeds which gave rise to both radical and plumule were considered as germinated.

\[
\text{Germination (\%) } = \frac{\text{No. of seed germinated}}{\text{Total no. of seed sown}} \times 100
\]

3.3.1.2. Seedling height

On the seventh day, the seedling height was estimated in centimeters by measuring the root and shoot lengths from each treatment and control. Seedling injury was calculated in terms of reduction in seedling height with respect to control.

3.3.1.3. Plant survival

The surviving plants in different treatments were counted at the time of maturity and the survival was computed as percentage of the germinated seeds in the field.

3.3.1.4. Pollen fertility

Pollen fertility was estimated from fresh pollen samples. From mature anthers, some amount of pollen was dusted on a slide containing a drop of 1% acetocarmine solution. Pollen grains, which took stain and had regular outline were considered as fertile, while empty and unstained ones as sterile.

The following formula was used to calculate the percentage inhibition or injury or reduction:
Percentage inhibition
Or
Percentage injury = \frac{\text{Control - Treated}}{\text{Control}} \times 100
Or
Percentage reduction

3.3.2. Morphological variants

Some induced morphological variants affecting plant from, plant height and leaf were isolated in M1 generation. The frequency of morphological variants was calculated by the following formula:

\text{Frequency} (\%) = \frac{\text{Number of variants}}{\text{Total number of } M_1} \times 100

3.3.3. Quantitative traits

Observations were also made on 25-30 normal-looking plants in each treatment with their controls.

The following nine quantitative traits were studied in M1 generation.

1. **Plant height**: Plant height was measured at maturity in centimeters from the base up to the apex of the plant.

2. **Days to flowering**: Days to flowering were noted as the number of days taken by the plant from the date of sowing to the date of opening of the first flower bud.

3. **Days to maturity**: Number of days taken by the plant from the date of sowing up to the date of harvesting of the plant.
4. **Number of fertile branches**: Number of fertile branches were counted at maturity as the number for fertile branches, which had more than one pod.

5. **Number of pods**: Number of pods were counted at maturity as the number of pods borne on the whole plant.

6. **Seeds per pod**: Twenty best pods were threshed and number of seeds per pod was counted. The mean was calculated for each plant.

7. **Pod length**: The pods were measured in centimeters and the mean for each selected plant was calculated for pod length.

8. **100-seed weight (g)**: It was the weight of random sample of hundred seeds from each plant.

9. **Total plant yield**: Plant yield was the weight of total number of seeds harvested per plant and the yield of each plant was recorded in gram.

### 3.4. Cytological studies

For meiotic analysis, young flower buds from each treatment and their control in both the varieties were fixed in Carnoy’s fluid (1 part glacial acetic acid: 3 parts chloroform : 6 parts of ethyl alcohol) for 30 minutes. The material was transferred to propionic alcohol saturated with ferric acetate for 24 hours. The flower buds were washed with and preserved in 70% alcohol. Anthers were smeared in 1% propiono aceto carmine solution and pollen mother cells were examined for their be behaviour at various stages of microsporogenesis. Photographs were taken from temporary preparation.
3.4. Statistical analysis

3.4.1. Assessment of variability

An insight into the magnitude of variability present in a crop species is of utmost importance, as it provides the basis of effective selection. The variability present in breeding population was assessed by using simple measures of variability. Data collected for nine quantitative traits in M1 generation were subjected to statistical analysis to find out ranged, mean, standard error, standard deviation and coefficient of variation.

3.4.1. Mean ($\bar{X}$)

The mean was computed by taking the sum of the number of values and ($X_1, X_2, \ldots, X_n$) dividing by the total number of values involved, thus

$$\bar{X} = \frac{X_1 + X_2 + X_3 + \ldots + X_n}{N}$$

Or

$$\bar{X} = \frac{\sum X_n}{N}$$

Where, $X_1, X_2, X_3, \ldots, X_n =$ Observations

$N =$ Total number of observations involve

3.5.1.2. Standard error (S.E.)

It is the measure of the uncontrolled variation present in a sample. It is estimated by dividing the standard deviation by the square root of the number of observations in the sample and is denoted by S.E.

$$S.E = \frac{S.D.\ of\ the\ sample}{\sqrt{N}}$$
Where, S.D. = Standard deviation

\[ N \] = Number of observations

3.5.1.3. **Standard deviation (S.D.)**

The Standard deviation was calculated by the following formula for each parameter of study.

\[
S.D. = \sqrt{\frac{(X-X_1)^2 + (X-X_2)^2 + \ldots + (X-X_n)^2}{N}}
\]

Where, \((X)\) = Mean of the observations involved

\[ X_1, X_2, \ldots, X_n = \text{observation} \]

\[ N = \text{Total number of observations} \]

3.5.1.4. **Coefficient of variability (C.V.)**

It measures the relative magnitude of variation present in observations relative to magnitude of their arithmetic mean. It is defined as the ratio of standard deviation to the arithmetic mean expressed as percentage and is a unit less number. The following formula was applied to compute coefficient of variability (C.V).

\[
\text{C.V. (\%)} = \frac{\text{Standard deviation}}{\bar{X}} \times 100
\]

Or

\[
= \frac{\text{S.D}}{\bar{X}} \times 100
\]

Where, S.D. = Standard deviation of sample

\( \bar{X} \) = Arithmetic mean

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