CHAPTER 3

MATERIALS AND METHODS
3. MATERIALES AND METHODS

The present investigation was carried out to find out the influence of PGRs on the reversal of reproductive characters of Cucumber and Squash. It was also considered necessary to screen the useful PGR and its most effective concentration. The materials used and methods adopted during the course of investigation are discussed below.

The following plant species were selected for experimentation.

3.1. Description of the cultivars

3.1.1 Cucumber

Cucumber is botanically known as *Cucumis sativus* L. It is a large group comprised of Melons and Cucumber belonging to the family Cucurbitaceae. The Cucumber has been an article of food for hundreds of years. It is a native of Southwestern Asia. Cucumber grows on sprawling, medium length vines clothed with rough, dark green, 3 pointed leaves, which are fuzzy. There are three different types of Cucumber. The first is the greenhouse or English forcing type, which isn’t suitable for outdoor cultivation because of its slow growth. The fruit is long and it has a few black spines that will disappear as the fruits mature. It is usually used for slicing. The second type is grown in gardens. Some kinds have black spines and others have white spines protruding warts when the fruits are
young. The white spined-varieties are more popular. The second type matures in 55 to 60 days and the fruits may be used for pickling or slicing. The third type is also grown outside; they are pickling varieties, which may also be used for salads.

3.1.2 The taxonomic characters of the plant:
The plants are climbing herbs with extra-axillary, simple or branched tendrils, monoecious often with 5-angled stems. Leaves are alternate, simple, often lobed, palmi-veined, exstipulate. Inflorescence is Cymose. Flowers are Unisexual, regular, epigynous, usually yellow in colour, male flowers clustered, calyx without scales. Sepals 5 in number, tubular and imbricate. Petals are 5 inserted on the calyx tube, imbricate. Stamens are 5 in the male flowers and syngenesious. Carpals are 3 in the female flowers and ovary is inferior. Fruits are large, smooth, edible. Seed are Exalbuminous. Embryo is Straight with oily cotyledons.

3.1.3 Squash
The botanical name of Squash is *Sechium edule* L. This is a semi-hardy perennial vine native to South America. The plant produces waxy-green, pear shaped fruits. Some are spiny and some are ridged.
3.1.4 The taxonomic characters of the plant

Plants are climbers with simple or branched tendrils, monoecious hairy stems.

Leaves are simple, alternate, exstipulate and 5-angled. Inflorescence is cymose. Flower is unisexual, regular, epigynous, pale yellow in colour, male flowers clustered. Sepals 5 in number arranged in velvate aestivation. Petals are 5, pale yellow in colour and arranged in velvate aestivation. Stamens 5 in male flowers, syngenecious. Fruits are large, edible, small hairs present over the surface of the fruits.

3.2. Geographical position of the experimental site

The experiment was conducted at Bongaigaon for three successive years 2002, 2003 and 2004. The area of Bongaigaon District is 2510 sq km as per 1991 census. The longitude range is 89° East to 90°96' East, the latitude range is 26°28' North to 26°54' North. The annual rainfall ranges from 250cm to 350cm. The minimum temperature is 13°C and maximum 32°C (National Information Centre, Bongaigaon).

The experimental field is well drained with uniform topography. It is rich in humus and organic matter. The site received free sunshine. The soil of the field is sandy loam with a pH value 5.10.
3.3. **Climatic condition under which the experiment was conducted**

The meteorological data were collected from Central Laboratory, Bongaigaon Refinery Petrochemicals Limited (BRPL), Bongaigaon. The average rainfall, maximum and minimum temperature, relative humidity during 2001, 2002, 2003 and 2004 are presented in appendix table.

3.4. **Soil Analysis**

For chemical and mechanical soil analysis, soil samples from different parts of the experimental field were collected and analysed at the soil testing laboratory, Government of Assam. The results of the analysis are presented in the Table 1.

**Table 1. Results of the analysis of the soil samples**

<table>
<thead>
<tr>
<th>Particulars of analysis</th>
<th>Mechanical analysis(%)</th>
<th>Chemical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth in cm</td>
<td>20 cm</td>
<td></td>
</tr>
<tr>
<td>Coarse sand</td>
<td>15.1 per cent</td>
<td></td>
</tr>
<tr>
<td>Fine sand</td>
<td>39.1 per cent</td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>15 per cent</td>
<td></td>
</tr>
<tr>
<td>Silt</td>
<td>12 per cent</td>
<td></td>
</tr>
<tr>
<td>Textural class</td>
<td>Sandy loam soil</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.10</td>
<td>Acidic</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>2.154</td>
<td>Low</td>
</tr>
<tr>
<td>Organic matter</td>
<td>1.84</td>
<td>High</td>
</tr>
</tbody>
</table>
3.5. Experimental Design

The experiment was conducted in a Randomized Block Design (RBD) with six treatments of each hormone including an untreated control. Each treatment was replicated three times and each containing 3 plants. The treatments were:

\[ T_1 = \text{Ethrel 50 } \mu\text{g/ml} \]
\[ T_2 = \text{Ethrel 100 } \mu\text{g/ml} \]
\[ T_3 = \text{Ethrel 250 } \mu\text{g/ml} \]
\[ T_4 = \text{Ethrel 500 } \mu\text{g/ml} \]
\[ T_5 = \text{Ethrel 1000 } \mu\text{g/ml} \]
\[ T_6 = \text{CCC 50 } \mu\text{g/ml} \]
\[ T_7 = \text{CCC 100 } \mu\text{g/ml} \]
\[ T_8 = \text{CCC 250 } \mu\text{g/ml} \]
\[ T_9 = \text{CCC 500 } \mu\text{g/ml} \]
\[ T_{10} = \text{CCC 1000 } \mu\text{g/ml} \]
\[ T_{11} = \text{CCC Control} \]
3.5.1 Experimental design For Cucumber (Scheme 1)

For Cucumber, equal number of plots for 3 replications were prepared.

Number of replications = 3

Number of treatments = 11

Space between treatments = 1.00 m

Space between replications = 1.20 m

Size of one plot = 1.00 m × 1.00 m

= 1 sq m

Number of plots = 3 × 11

= 33

Area of the experimental field = Length × breadth

= 21.00 m × 5.4 m

= 113.4 m²

Number of plants in a single plot = 1

Number of plants in each experiment = 1 × 33

= 33

In 113.4 m² area, number of plants = 33

In 10,000 m² area, number of plants = 2910

Plant density = 2910 / hectare
Scheme 1: Randomized Block Design for *Cucumis sativus* L.
3.5.2 Experimental design for Squash (Scheme 2)

For Squash equal number of plots for 3 replications were prepared.

Number of replications = 3

Number of treatments = 11

Space between treatments = 1.00 m

Space between replications = 2.00 m

Size of one plot = 1.00 m x 1.00 m

= 1 sq m

Number of plots = 3 x 11

= 33

Area of the experimental field = Length x breadth

= 21.00 m x 7.00

= 147.00 m²

Number of plants in a single plot = 1

Number of plants in each experiment = 1 x 33

= 33

In 147 m² area, number of plants = 33

In 10,000 m² area, number of plants = 2244

Plant density = 2244 / hectare
Scheme 2: Randomized Block Design for *Sechium edule* L
3.5.3 Experimental design For Interactions

For interaction of Ethrel and CCC with \( \text{GA}_3 \) in both Cucumber and Squash, the treatments were

<table>
<thead>
<tr>
<th>Ethrel conc. (( \mu \text{g/ml} ))</th>
<th>( \text{GA}_3 ) conc. (( \mu \text{g/ml} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ( \text{Ethrel 0} ) ( T_1 )</td>
<td>( \text{GA}_3 0 ) ( T_2 )</td>
</tr>
<tr>
<td>50 ( \text{Ethrel 50} ) ( T_6 )</td>
<td>( \text{GA}_3 0 ) ( T_7 )</td>
</tr>
<tr>
<td>100 ( \text{Ethrel 100} ) ( T_{11} )</td>
<td>( \text{GA}<em>3 0 ) ( T</em>{12} )</td>
</tr>
<tr>
<td>250 ( \text{Ethrel 250} ) ( T_{16} )</td>
<td>( \text{GA}<em>3 0 ) ( T</em>{17} )</td>
</tr>
<tr>
<td>1000 ( \text{Ethrel 1000} ) ( T_{21} )</td>
<td>( \text{GA}<em>3 0 ) ( T</em>{22} )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CCC conc. (( \mu \text{g/ml} ))</th>
<th>( \text{GA}_3 ) conc. (( \mu \text{g/ml} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ( \text{CCC 0} ) ( T_1 )</td>
<td>( \text{GA}_3 0 ) ( T_2 )</td>
</tr>
<tr>
<td>50 ( \text{CCC 50} ) ( T_6 )</td>
<td>( \text{GA}_3 0 ) ( T_7 )</td>
</tr>
<tr>
<td>100 ( \text{CCC 100} ) ( T_{11} )</td>
<td>( \text{GA}<em>3 0 ) ( T</em>{12} )</td>
</tr>
<tr>
<td>250 ( \text{CCC 250} ) ( T_{16} )</td>
<td>( \text{GA}<em>3 0 ) ( T</em>{17} )</td>
</tr>
<tr>
<td>1000 ( \text{CCC 1000} ) ( T_{21} )</td>
<td>( \text{GA}<em>3 0 ) ( T</em>{22} )</td>
</tr>
</tbody>
</table>
3.5.3.1 Experimental design for interaction in Cucumber (Scheme 3)

Number of replications = 3
Number of treatments = 25
Number of plots = $3 \times 25$

\[ = 75 \]

Space between replications = 1.20 m
Space between treatments = 1.00 m
Number of plants per plot = 1
Total number of plants in each experiment = 75
Area of the experimental field = $49 \times 5.4$ m

\[ = 264.6 \text{ m}^2 \]
Plant density = 2834/ha

3.5.3.2 Experimental design for interaction in Squash (Scheme 4)

Number of replications = 3
Number of treatments = 25
Number of plots = $3 \times 25$

\[ = 75 \]
Space between replications = 2.00 m
Space between treatments = 1.00 m
Number of plants per plot = 1
Scheme 3: Randomized Block Design for Interaction in *Cucumis sativus* L.
Scheme 4: Randomized Block Design for Interaction in *Sechium edule* L.
Total number of plants in each experiment = 75

Area of the experimental field = 49 m × 7

= 343 m²

Plant density = 2186/hectare

3.6 Sowing of seed

The seeds of cucumber ( *Cucumis sativus* L.cv.Malini ) were collected from Assam Seed Corporation, Guwahati and the seeds of squash were collected from the local market. The experimental field was well prepared before sowing of seed. It was ploughed and divided into 1sq m plots. The seeds of cucumber were soaked overnight in distilled water. The very next day seeds were planted in each plot. For each treatment three replications were made. For cucumber, spacing was maintained at 1.20m between the replications, 1.00m between the treatments. For Squash spacing was maintained at 2.00m between the replications, 1.00m between the treatments. The experiment on cucumber was carried out during the month of February to April and on squash during the month of November to January.

3.7. Manuring

The vermicompost collected from vermiculture centre, Birjhora Mahavidyalaya, Bongaigaon was used for manuring the experimental
field. The chemical fertilizer was totally omitted for manuring the experimental field. Cowdung was used at the time of ploughing the field. The vermicompost was also applied when the plants were at the seedling stage.

3.8. Irrigation

The experimental field was irrigated with tap water. Water supply was started from the day of plantation and continued till the plants were fully matured.

3.9. After care

After planting the seeds it was a major problem to control the weeds. But no chemical was used for the purpose. Weeding and earthing up operations were done manually. Vermicompost was applied at a short interval throughout the life of the plants. The experimental plants were healthy and there was no serious attack of any pests and diseases during the period of experiment.
3.10. Chemical used

The following chemicals were used in the experiment.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Formula</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Gibberellic acid (GA₃)</td>
<td>$C_{19}H_{22}O_6$</td>
<td>LOBA</td>
</tr>
<tr>
<td>2. Ethrel or Ethephon (CEPA)</td>
<td>$C_2H_6ClO_3P$</td>
<td>SISCO</td>
</tr>
<tr>
<td>3. Chlorocholine chloride (CCC)</td>
<td>$\text{CL}-(\text{CH}_2)_2-\text{N}^+-\text{CH}_3-\text{CL}$</td>
<td>LOBA</td>
</tr>
<tr>
<td></td>
<td>$\text{CH}_3$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{CH}_3$</td>
<td></td>
</tr>
</tbody>
</table>

Glasswares

1. Conical flasks,
2. Measuring cylinders,
3. Volumetric flasks,
4. Glass-rods etc were of Borosil brand.

Other

1. Sprayer
3.11. Preparation of Chemical Solutions

3.11.1. Ethephon or Ethrel (2-chloroethyl phosphonic acid)

One ml of ethrel was dissolved in sterile distilled water with stirring in a 1000 ml volumetric flask. The volume was adjusted to 1000ml with sterile distilled water to obtain a stock solution of 1000ml and required concentrations were prepared from the stock solution. Five concentrations of ethrel namely 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml were prepared from the stock solution.

3.11.2. CCC (2-chloroethyl trimethylammonium chloride)

One ml of CCC was taken in a 1000ml volumetric flask and sterile distilled water was added slowly with constant stirring. Then the solution was adjusted to 1000ml by adding sterile distilled water to obtain a 1000 ml stock solution. The required grades were prepared from the stock solution.

3.11.3. Gibberellic Acid

One gram of Gibberellic acid was accurately weighed out and dissolved with small addition of ethyl alcohol in a volumetric flask. Then sterile distilled water was added and heated on a boiling water bath till the
crystals were completely dissolved and the volume was made up to 1000ml. From this stock solution 100 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml of Gibberellic acid were prepared.

3.12. Method Of Application

3.12.1 Foliar Application of Ethrel and CCC

The healthy seeds of *Cucumis sativus* L were collected from Assam Seed Corporation, Guwahati. They were grown in a randomized block design with three replications. After 15 days of sowing the plants were in the seedling stage. First foliar application was done at 5-7 leaved stage. Five concentrations of Ethrel and CCC namely 50 μg/ml, 100 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml were sprayed. The hormones were sprayed on the plants on one sunny morning at the seedling stage. Spraying was done with the help of a hand sprayer fitted with a fine nozzle so as to facilitate uniform wetting of leaves with about 20 ml/plant. The plants started flowering after one month. The number of male and female flowers in each treated plants were counted. Three plants were kept unsprayed and treated as control. The result was noted and male: female ratio of the plant was determined. The whole operation was done once in a year during the month of February to April. This experiment was carried out for three years.
The fruits of *Sechium edule* L were collected from the local market and planted in randomized block design. Each treatment was replicated three times. Five concentration of ethrel and CCC namely 50 μg/ml, 100 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml were prepared and sprayed. The sex ratio of the plant was calculated out.

### 3.12.2 Interaction of GA₃ with Ethrel and CCC

For interaction of Ethrel and CCC with GA₃ was also done on *Cucumis sativus* L and *Sechium edule* L. 100 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml of GA₃ were prepared. When the plants were at seedling stage and about 20 days of emergence of the seedlings the concentrations of GA₃ were applied. For interaction after 30 days of application of the first compound, the second one was sprayed over the leaves of the plants. To see the interaction between the hormones on *Cucumis sativus* L and *Sechium edule* L were grown separately in two fields.

### 3.13. Procedure for observations recorded

To study the effect of different concentrations of ethrel and CCC on cucumber and squash the number of male and female flowers produced in each replication was recorded. The mean of each reading was also
calculated. In this way the mean values of three years experiment were calculated out. Similarly the results of interactions of plant growth regulators on both of these plants were recorded again after 20 days of taking records of the first compound.

3.14. Statistical analysis of data

The experimental data were subjected to statistical analysis for better understanding the effects of PGRs. The data obtained from experiments were analysed statistically by Fishers method of Analysis of Variance (ANOVA). Significance or non significance of the variation due to effect of GA₃, ethrel and CCC was determined by calculating the respective ‘F’ values and ‘CD’s (Panse and Sukhatme 1989).

3.14.1 Critical difference (CD)

The Analysis of Variance gives only an indication of performance of the concentrations and their interactions on sex reversal. To find out the significance or non significance between and amongst treatments the CD was calculated by using the following formula
\[ CD = \sqrt{\frac{\text{Error MSS} \times 2}{n}} \times t \text{ value at 5\% or 1\% at error degrees of} \]

Where \( n \) = The actual number used for calibrating the means.

The calculated \( CD \) was utilized in testing the difference between the two mean values as significant or not.