CHAPTER- III
MATERIALS AND METHODS

The present investigation on “Effect of growing media and GA$_3$ on seed germination and seedling growth of acid lime (Citrus aurantifolia Swingle) cv. Kagzi lime” was conducted at Fruit Research Station Madhdibaug, Department of Horticulture, JAU, Junagadh. The details of the experiment and methods followed during the course of investigation are described under following heads.

3.1 Experimental site

The present investigation was carried out at Fruit Research Station Madhdibaug, Department of Horticulture, college of Agriculture, Junagadh Agricultural University, Junagadh during 2016.

3.2 Geographical Location

The Junagadh in the state of Gujarat, is located at the foothills of the mount Girnar. The geographical coordinates of Junagadh are:
Longitude - 70° 28' East
Latitude - 21° 31' North
Junagadh stands at 107 meters above sea level (MSL).

3.3 Climate and weather conditions

Climatically, Junagadh climate is characterized by fairly hot summer, moderate cold winter and more humid and warm monsoon with heavy rains. The maximum temperature rises up to 43.60 °C in the month of May (2016), minimum temperature recorded 6.50 °C in the month of December (2016). Annual rainfall is about 1271.5 mm. Monsoon starts from second week of June and last up to the first week of October (2016). Most of the rainfall is received from South West monsoon.

The meteorological data on important weather parameters were recorded during the year 2016 from the Instructional Farm, Department of Agronomy, Junagadh Agricultural University, Junagadh.

The weekly average weather data during the period of experiment recorded at the Agro-Meteorological Observatory, located at Instructional Farm, Junagadh Agricultural University, Junagadh are presented in Appendix- I and also graphically depicted in Fig. 3.1.
3.4 Experimental materials

Different growing media were obtained from Madhdibaug Farm, Department of Horticulture, College of Agriculture, Junagadh Agricultural University, Junagadh (Gujarat) and were prepared according to proportion of various soil, cocopeat, compost and vermicompost.

3.5 Preparation of GA$_3$ solution

Seeds were soaked in 50, 100, 150 and 200 ppm GA$_3$ solution for 24 hours which was prepared by dissolving 50, 100, 150 and 200 mg GA$_3$, respectively (GA$_3$ was completely dissolved by addition of small quantity of acetone) in 1 liter of water.

3.6 Experimental details

<table>
<thead>
<tr>
<th>1. Location</th>
<th>Fruit Research Station, Madhdibaug, Dept. of Horticulture, COA, JAU, Junagadh</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Year of experiment</td>
<td>2016</td>
</tr>
<tr>
<td>3. Statistical design</td>
<td>CRD (factorial)</td>
</tr>
<tr>
<td>4. Number of factors</td>
<td>2 Factor A-Media, Factor B-GA$_3$</td>
</tr>
<tr>
<td>5. Number of treatments</td>
<td>20</td>
</tr>
<tr>
<td>6. Number of repetitions</td>
<td>3</td>
</tr>
<tr>
<td>7. Number of plants per treatment</td>
<td>30</td>
</tr>
</tbody>
</table>

3.7 Treatment details:


M$_1$- Soil
M$_2$- Soil + Vermicompost (1:1)
M$_3$- Soil + Vermicompost + Cocopeat (1:1:1)
M$_4$- Soil + Compost + Cocopeat (1:1:1)
M$_5$- Soil + Compost + Vermicompost (1:1:1)


G$_1$- GA$_3$ 50 ppm
G$_2$- GA$_3$ 100 ppm
G$_3$- GA$_3$ 150 ppm
G$_4$- GA$_3$ 200 ppm
3.8 Treatment Combinations:

- **T₁ M₁G₁**: Soil + GA₃ 50 ppm
- **T₂ M₁G₂**: Soil + GA₃ 100 ppm
- **T₃ M₁G₃**: Soil + GA₃ 150 ppm
- **T₄ M₁G₄**: Soil + GA₃ 200 ppm
- **T₅ M₂G₁**: Soil + Vermicompost (1:1) + GA₃ 50 ppm
- **T₆ M₂G₂**: Soil + Vermicompost (1:1) + GA₃ 100 ppm
- **T₇ M₂G₃**: Soil + Vermicompost (1:1) + GA₃ 150 ppm
- **T₈ M₂G₄**: Soil + Vermicompost (1:1) + GA₃ 200 ppm
- **T₉ M₃G₁**: Soil + Vermicompost + Cocopeat (1:1:1) + GA₃ 50 ppm
- **T₁₀ M₃G₂**: Soil + Vermicompost + Cocopeat (1:1:1) + GA₃ 100 ppm
- **T₁₁ M₃G₃**: Soil + Vermicompost + Cocopeat (1:1:1) + GA₃ 150 ppm
- **T₁₂ M₃G₄**: Soil + Vermicompost + Cocopeat (1:1:1) + GA₃ 200 ppm
- **T₁₃ M₄G₁**: Soil + Compost+ Cocopeat (1:1:1) + GA₃ 50 ppm
- **T₁₄ M₄G₂**: Soil + Compost+ Cocopeat (1:1:1) + GA₃ 100 ppm
- **T₁₅ M₄G₃**: Soil + Compost+ Cocopeat (1:1:1) + GA₃ 150 ppm
- **T₁₆ M₄G₄**: Soil + Compost+ Cocopeat (1:1:1) + GA₃ 200 ppm
- **T₁₇ M₅G₁**: Soil + Compost + Vermicompost (1:1:1) + GA₃ 50 ppm
- **T₁₈ M₅G₂**: Soil + Compost + Vermicompost (1:1:1) + GA₃ 100 ppm
- **T₁₉ M₅G₃**: Soil + Compost + Vermicompost (1:1:1) + GA₃ 150 ppm
- **T₂₀ M₅G₄**: Soil + Compost + Vermicompost (1:1:1) + GA₃ 200 ppm

**Note**: Media taken proportion by volume base.

3.9 Cultural Operations

Right from the beginning to the end of experiment various cultural operations were performed time to time as and when required.

3.9.1 Layout of experiment

Layout of the experiment was done according to the experimental design. The marking of lines was done with the help of marker in polythene bags. Later on polythene bags were prepared to sown the seeds of kagzi lime.

3.9.2 Seed extraction

Seeds were carefully extracted from uniform size fully ripened and healthy fruits of acid lime. Extracted seeds were washed in running water and dried under
shade for 1 hour. Before drying of seeds, they were dipped in water to remove the dead floating seeds. Non-viable and dead seeds were generally light in weight; hence, they float on water. Other seeds, which settled at the bottom of the bucket, were considered as for viable and was used for sowing.

### 3.9.3 Sowing of seeds

Treated seed of Kagzi lime were sown after 24 hours in polythene bags of 20 × 10 cm size filled in different mixture and its combinations on 12th May 2016. One seed per poly bag was sown at 1.2 cm depth. Treatment contained 30 polythene bags. 5 poly bags were arranged in each row and column to make 3 repetitions as described earlier.

### 3.10 After cares

#### 3.10.1 Watering

Water was done immediately after sowing of seeds in the polythene bags and repeated depending upon soil moisture status of the plant.

#### 3.10.2 Weeding

For the control of weeds, hand weeding was carried as an when required.

#### 3.10.3 Plant protection

No major pest and diseases were observed in experimental site.

### 3.11 Observations recorded

The following parameters regarding seed germination and growth were recorded.

#### 3.11.1 Seed germination parameters

##### 3.11.1.1 Days to first germination

The days taken for germination were calculated from the date of sowing up to germination of acid lime seeds and average was calculated.

##### 3.11.1.2 Germination percentage

Total number of germinated seeds under each treatment was counted daily, right from the first emergence of the seed up to the period of completion of seed germination. The per cent of seed germination was calculated by following formula:

\[
\text{Germination per cent} = \frac{\text{Number of germinated seeds}}{\text{Number of seed sown}} \times 100
\]

#### 3.11.1.3 Survival percentage (%)
The survival percentage of each treatment was recorded at 120 days after seed sowing. The survival percentage was calculated by using formula as given below:

\[
\text{Survival (\%) = } \frac{\text{No. of survived seedlings}}{\text{Total no. of seedlings}} \times 100
\]

3.11.1.4 Mortality percentage (%)

\[
\text{Mortality percentage (\%) = } \frac{\text{Total no. of seedling mortality}}{\text{Total no of germinated seed}} \times 100
\]

3.11.1.5 Germination span

Germination span was calculated as the difference between initial and final emergence in days.

3.11.1.6 Seedling vigour

The seedling vigour was calculated by formula i.e germination percentage multiply by seedling height at 60, 90 and 120 days after sowing and average value was calculated.

3.11.1.7 Germination index

The Germination index calculated by the following formula:

\[
\text{Germination Index} = \frac{\text{No. of germinating seeds}}{\text{Days of first count}} + \frac{\text{No. of germinating seeds}}{\text{Days of final or last count}}
\]

3.11.1.8 Polyembryony (%)

Five seedlings were randomly selected from each treatment form that embryos were separated and counted.

\[
\text{Polyembryony (\%) = } \frac{\text{No. of seedlings}}{\text{No. of polyembryony germinated plants}} \times 100
\]

3.11.2 Plant Growth parameters

3.11.2.1 Plant height (cm)

The height of five tagged seedlings was measured by metric scale from the base of shoot to the tip of the shoot of the seedling at 60, 90 and 120 days after sowing and average value was calculated.

3.11.2.2 Stem girth (mm)

The stem girth of five seedlings was measured with the help of vernier callipers at the 60, 90 and 120 days after sowing and average value was calculated.
3.11.2.3 Number of leaves

The number of leaves of five seedlings was counted at the 60, 90 and 120 days after sowing and average value was calculated.

3.11.2.4 Leaf area (cm²)

The leaf area was measured by “leaf area meter” of five seedlings at the 60, 90 and 120 days after sowing and average value was calculated.

3.11.2.5 Number of roots

The number of roots of same five randomly selected seedlings in each treatment was counted at the end of experiment and mean number of roots per seedling was calculated.

3.11.2.6 Root length (cm)

The root length of five seedlings was measured by metric scale from the base of shoot to tip of root at the end of experiment and average value was calculated.

3.11.2.7 Fresh weight of shoot (g)

The fresh weight of five seedling shoots were weighed on digital weighing balance and average value was calculated at the end of experiment.

3.11.2.8 Dry weight of shoot (g)

The dry weight of seedling shoots were calculated by drying up rooted seedling under shed for three days and then oven dried at 60°C till the constant weight. The average value was calculated at the end of experiment.

3.11.2.9 Fresh weight of root (g)

The fresh weight of five seedling roots were weighed on digital weighing balance and average value was calculated at the end of experiment.

3.11.2.10 Dry weight of root (g)

The dry weight of seedling roots were calculated by drying up rooted seedling under shed for three days and then oven dried at 60°C till the constant weight. The average value was calculated at the end of experiment.

3.11.2.11 Root-shoot ratio

The shoot: root ratio of five seedling was calculated by dividing shoot dry weight by root dry weight at the end of experiment and average value was calculated.

3.12 Statistical analysis
The experiment was laid out in a Complete Randomized Block Design with Factorial concept. The recorded data were analyzed statistically using various techniques as described by Panse and Sukhatme (1985). The treatment means were compared with C.D. at 5 per cent level.

3.13 Economics

In order to evaluate the effectiveness of different treatments and to ascertain the most remunerative treatment, the expenses incurred on all operations were computed and added.

The gross realization was worked out on the basis of mean of survival of seedlings per each treatment at prevailing market price. The net realization per treatment was calculated by deducting cost of cultivation from the gross realization for each treatment and recorded accordingly. The Cost Benefit Ratio was calculated on the basis of the formula given below.

\[
\text{CBR} = \frac{\text{Gross realization (Rs. /Treat.)}}{\text{Total cost of cultivation (Rs. /Treat.)}}
\]