CHAPTER III
MATERIALS AND METHODS

3.1 LOCATION

The present study was taken up with a view to elicit information on the genetic basis of fruit yield and its attributes in ridge gourd. Experiment was conducted at Instructional Farm, Department of Agronomy, Junagadh Agricultural University, Junagadh, during summer 2016. Geographically, Junagadh is situated at 21.5° N latitude and 70.5°E longitudes with an altitude of 60 meters above the mean sea level. The soil of the experimental site was medium black, alluvial in origin and poor in organic matter.

Meteorological data on maximum and minimum temperature and relative humidity for summer 2016 was obtained from the meteorological observatory, Junagadh Agricultural University, Junagadh (Appendix-I).

3.2 EXPERIMENTAL MATERIALS

The seven diverse lines and three testers varieties used in the present investigation were collected from the different states of the country on the basis of morphological variability for growth, maturity, fruit size and shape; fruit yield and yield contributing characters. The origin and salient feature of all the cultivars included in the experiments are given in Table - 3.1.

3.3 EXPERIMENTAL METHODS

3.3.1 Production of Hybrid (F1) Seed

The seeds of inbred lines used as parents were planted at Vegetable Research Station, Junagadh Agricultural University, Junagadh, during kharif 2015. The crosses were made in Line x Tester (Seven x Three) fashion to obtain sufficient quantity of seeds of the 21 hybrids.
Table 3.1 Source and salient features of parental lines of ridge gourd

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parents</th>
<th>Source/Origin</th>
<th>Salient features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>JRG-13-01</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Fruits are medium long, thin &amp; light green color, insect, pest &amp; disease resistance.</td>
</tr>
<tr>
<td>2</td>
<td>JRG-13-02</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Fruits are medium long, thick, light green color, tolerant to powdery mildew and higher yield.</td>
</tr>
<tr>
<td>3</td>
<td>JRG-13-03</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Marketable fruits with good yield, more fruit weight and dark green foliage.</td>
</tr>
<tr>
<td>4</td>
<td>JRG-13-04</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Fruits are downey mildew resistance, medium long &amp; light green color.</td>
</tr>
<tr>
<td>5</td>
<td>JRG-13-05</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Fruits are light green with good length, more girth and weight.</td>
</tr>
<tr>
<td>6</td>
<td>JRG-13-06</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Long, light green fruit. Pest resistance downey mildew and powdery mildew highly tolerant.</td>
</tr>
<tr>
<td>7</td>
<td>JRG-13-07</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Fruit are light green with good length and less fruit girth.</td>
</tr>
<tr>
<td>Testers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Arka Sujat</td>
<td>IIHR, Bangalore.</td>
<td>Fruits lush green, cylindrical, medium long (35-45 cm) and average weight (350g). Yield 63 t/ha in 100 days.</td>
</tr>
<tr>
<td>9</td>
<td>Pusa Nasdar</td>
<td>IARI, New Delhi.</td>
<td>Green long fruit, prominent ribs, good vine length, long crop span, high fruit weight, good bearing, high yielding.</td>
</tr>
<tr>
<td>10</td>
<td>Jaipur Long</td>
<td>Jaipur, (Rajasthan).</td>
<td>Green, long tender fruits, long crop duration, high fruit weight, good bearing, high yielding.</td>
</tr>
<tr>
<td>Standard check</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>GJRGH-1</td>
<td>Vegetable Research Station, JAU, Junagadh. (Gujarat)</td>
<td>Highly resistance to Powdery mildew &amp; downey mildew. Dark green color &amp; pest resistance.</td>
</tr>
</tbody>
</table>
Ridge gourd is a highly cross pollinated crop. Anthesis occurs between 4.00 to 8.00 PM. Pollen fertility is the maximum on the day of anthesis. Stigma is receptive six hrs before to 84 hrs after anthesis (Singh, 1957). The female and male flowers, which open during evening, were all the male will drop in morning. In the evening, the respective female flowers were hand pollinated with the pollens collected from the desired male flowers. The parents were also selfed simultaneously to obtain pure seed of each parental line. The selfed and crossed flowers were labeled properly. Sufficient quantity of selfed and crossed seeds were obtained at the end of kharif 2015. The seeds were dried properly and stored in brown paper bags.

3.3.2 Experimental Details

The experimental material, consisting 32 entries including 10 parents and their resultant 21 hybrids with along one check hybrid, was laid out in randomized block design with three replications at the Instructional Farm, Department of Agronomy, Junagadh Agricultural University, Junagadh during summer 2016. Each entry consisted a single row of ten plants. The plants were spaced at a distance of 2.0 m between rows and 1.0 m within a row. Three seeds were dibbled in each hill, which were later on kept single plant per hill when the crop was twenty days old. Thus, the perfect population of ten plants of each genotype was obtained. The vines were trained to horizontal trellises.

All the recommended cultural practices were adopted during growing season. Before sowing, the experimental plot was prepared by ploughing followed by three harrowing. At the time of last harrowing, FYM at the rate of 25 tonnes/ha was applied. The chemical fertilizers were applied at the rate of 100:50:50, NPK kg/ha in two split doses. Half the dose of N with along full dose of P$_2$O$_5$ and K$_2$O was applied as basal dose before sowing. Remaining half dose of nitrogen was applied one month after sowing. The irrigation was given as and when required to the crop. The systemic insecticide granular carbofuran-3G was applied at the rate of 25 kg/ha after seed germination. Three hand weedings were done during the season. Alternate sprays of insecticides and fungicides viz, Methyl-O-demetone 0.03%, DDVP 0.05%, Carbendazim 0.05 and Mancozeb 0.2% were given as protective measures against leaf minor, fruit fly and downy mildew as and when required.
3.4 OBSERVATIONS RECORDED

Five competitive plants from each entry in each replication were randomly selected before flowering and tagged for the purpose of recording observations on different characters (except days to 50% flowering) and their average values were used in the statistical analysis.

3.4.1. Days to 50 % flowering

The number of days taken from the date of sowing to the date of anthesis of main umbel in 50 % plants was recorded on the plot basis during the flower initiation period.

3.4.2. Days to opening of first female flower

The days required for opening of first female flower from the date of sowing on each five randomly selected plants were recorded and average numbers of days required for opening of female flower was worked out.

3.4.3. Days to opening of first male flower

The days required for opening of first male flower from the date of sowing on each five randomly selected plants were recorded and average numbers of days required for opening of male flower was worked out.

3.4.4. Node number of first female flower

Total numbers of nodes were counted in main shoot from the base of stem to appearance of first female flower in respect of five randomly selected plants and average was worked out.

3.4.5. Node number of first male flower

The node number on which first male flower appears on the main shoot was counted from the base of stem in respect of five randomly selected plants and average was worked out.

3.4.6. Days to first picking

The number of days required for first picking of green tender and marketable fruits in five randomly selected plants from date of sowing was recorded.
3.4.7 Length of main vine (m)

The length of main vine was measured in meter with the help of measuring tape from the base of the vine to the growing tip of main stem at the final harvest for five randomly selected plants.

3.4.8 Number of primary branches per vine

The number of primary branches on main stem was counted at final harvest for five randomly selected plants and mean number of branches per vine was calculated.

3.4.9 Number of fruits per vine

The number of fruits from all pickings, harvested from the five randomly selected plants, was counted and averaged was worked out.

3.4.10 Fruit weight (g)

Ten fruits from five randomly selected plants was selected during peak period of harvesting for recording fruit weight in grams.

3.4.11 Length of fruit (cm)

The length of the ten selected tender edible fruits from five randomly selected plants were recorded in centimeter from peduncle end to the blossom end of the fruit and the average length of fruit was worked out.

3.4.12 Girth of fruit (cm)

The fruits used for recording the length were also used for measuring the girth. Girth was recorded in centimeter by measuring at the mid-portion of the fruit with the help of the measuring tape and average girth of fruit was worked out.

3.4.13 Rind thickness (mm)

The rind thickness of the ten selected tender edible fruits was recorded in millimeter and average rind thickness was worked out.

3.4.14 Flesh thickness (mm)

The flesh thickness of the ten selected tender edible fruits was recorded in millimeter and average flesh thickness was worked out.

3.4.15 Fruit yield per vine (g)

The weight of harvested fruits of all pickings from each randomly selected plant was summed up after the last picking and the average fruit yield per vine was calculated in gram.
3.4.16 Number of seeds per fruit

In each plot, five randomly selected fruits was allowed to mature on the untagged plants. The ripened fruits was harvested and seeds was calculated individually from the five randomly selected fruits and average number of seeds per fruit was worked out.

3.4.17 100-seed weight (g)

Randomly sample of hundred seeds was collected and weighed on an electrical balance to obtain the test weight in grams.

3.5 STATISTICAL PROCEDURES:

The replication wise mean values of each genotype for various characters were used for statistical and genetical analysis. Following statistical procedures were used for analysis.

3.5.1. Analysis of variance for experimental design
3.5.2. Estimation of heterobeltiosis and standard heterosis
3.5.3. Combining ability analysis

3.5.1 Analysis of variance for experimental design

The analysis of variance (Table 3.2) was performed to test the significance of difference among the genotypes for all the characters following fixed effect model as suggested by Panse and Sukhatme (1985).

\[ Y_{ij} = \mu + g_i + r_j + e_{ij} \]

Where,

\[ Y_{ij} = \text{Value of } i^{th} \text{ genotype in } j^{th} \text{ replication} \]
\[ \mu = \text{Population mean} \]
\[ g_i = \text{An effect of } i^{th} \text{ genotype (} i = 1,2,\ldots,g \) \]
\[ r_j = \text{An effect of } j^{th} \text{ replication (} j = 1,2,\ldots,r \) \]
\[ e_{ij} = \text{Uncontrolled variation associated with } i^{th} \text{ genotype and } j^{th} \text{ replication} \]
### Table 3.2. Analysis of variance for experimental design

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Df</th>
<th>MS</th>
<th>EMS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>(r-1)</td>
<td>M₁</td>
<td>$\sigma^2_r + g\sigma^2_r$</td>
<td>$M_1/M_6$</td>
</tr>
<tr>
<td>Genotypes</td>
<td>(g-1)</td>
<td>M₂</td>
<td>$\sigma^2_r + r\sigma^2_g$</td>
<td>$M_2/M_6$</td>
</tr>
<tr>
<td>Parents (P)</td>
<td>(p-1)</td>
<td>M₃</td>
<td>$\sigma^2_r + r\sigma^2_p$</td>
<td>$M_3/M_6$</td>
</tr>
<tr>
<td>Hybrids (H)</td>
<td>(h-1)</td>
<td>M₄</td>
<td>$\sigma^2_r + r\sigma^2_h$</td>
<td>$M_4/M_6$</td>
</tr>
<tr>
<td>P vs H</td>
<td>1</td>
<td>M₅</td>
<td>$\sigma^2_r + r\sigma^2_{pH}$</td>
<td>$M_5/M_6$</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(g-1)</td>
<td>M₆</td>
<td>$\sigma^2_e$</td>
<td>-</td>
</tr>
</tbody>
</table>

Where,

- $r = \text{Number of replications}$
- $g = \text{Number of genotypes}$
- $p = \text{Number of parents}$
- $h = \text{Number of hybrids}$
- $M_1 = \text{Mean sum of square due to replications}$
- $M_2 = \text{Mean sum of square due to genotypes}$
- $M_3 = \text{Mean sum of square due to parents}$
- $M_4 = \text{Mean sum of square due to hybrids}$
- $M_5 = \text{Mean sum of square due to parents vs hybrids}$
- $M_6 = \text{Mean sum of square due to error}$
- $\sigma^2_r = \text{Expected replication variance}$
- $\sigma^2_e = \text{Expected genotypic variance}$

### 3.5.2 Estimation of heterobeltiosis and standard heterosis

#### 3.5.2.1 Heterobeltiosis

It was calculated as the deviation of $F_1$ from the better parent (Fonseca and Patterson, 1968) and was expressed as per cent basis by the following formula;
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\[
\text{Heterobeltiosis (\%)} = \frac{\overline{F_1} - BP}{BP} \times 100
\]

Where,
- \( \overline{F_1} \) = Mean performance of \( F_1 \)
- \( BP \) = Mean performance of better parent of the respective cross

3.5.2.2 Standard Heterosis

It was calculated as the deviation of \( F_1 \) from the standard check (GJRGH-1) and expressed as per cent basis by the following formula;

\[
\text{Standard heterosis (\%)} = \frac{\overline{F_1} - SC}{SC} \times 100
\]

Where,
- \( \overline{F_1} \) = Mean performance of \( F_1 \)
- \( SC \) = Mean performance of standard check (GJRGH-1)

The standard error of difference for heterobeltiosis and standard heterosis was calculated as follows:

\[
\text{S.E. (d)} = \sqrt{\frac{2Me}{r}}
\]

Where,
- \( Me \) = Error mean square
- \( r \) = Number of replications

In above two cases of heterosis, critical difference were computed by multiplying respective standard error of differences with ‘t’ value at given error degrees of freedom at 0.05 or 0.01 probability level as follows:

\[
\text{C.D.} = \text{S.E.}(d) \times t \text{ value at error degrees of freedom}
\]

3.5.3 Combining Ability Analysis

3.5.3.1 Analysis of variance for combining ability (line x tester)

Analysis of variance for combining ability (Table 3.3) was performed according to the model given by Kempthorne (1957), which is related to design-II of
Comstock and Robinson (1952) in terms of covariance of half-sibs (H.S.) and full-sibs (F.S.).

\[ Y_{ijk} = \mu + G_i + G_j + S_{ij} + r_k + \epsilon_{ijk} \]

\[ \sum G_i = \sum G_j = \sum S_{ij} = 0 \]

Where,

- \( Y_{ijk} \): Phenotypic expression of \( ij^{th} \) genotypes in \( k^{th} \) replication
- \( \mu \): General mean
- \( G_i \): General combining ability of \( i^{th} \) female parent (line)
- \( G_j \): General combining ability of \( j^{th} \) male parent (tester)
- \( S_{ij} \): Specific combining ability of cross between \( i^{th} \) line and \( j^{th} \) tester
- \( r_k \): Effect of \( k^{th} \) replication
- \( \epsilon_{ijk} \): Random error associated with \( ij^{th} \) genotype and \( k^{th} \) replication

### Table 3.3. Analysis of variance for combining ability

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Df</th>
<th>MS</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In terms of variance</td>
<td>In terms of full sib and half sib</td>
</tr>
<tr>
<td>Replications</td>
<td>( (r-1) )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hybrids</td>
<td>( (h-1) )</td>
<td>-</td>
<td>( \sigma^2_e + r \sigma^2_{ht} )</td>
</tr>
<tr>
<td>Lines</td>
<td>( (l-1) )</td>
<td>( M_1 \sigma^2_e + r \sigma^2_{ht} + rt \sigma^2_i )</td>
<td>( \sigma^2_e + r \text{Cov. (F.S.)} - 2\text{Cov. (H.S.)} + [\text{trCov. (H.S.)}] )</td>
</tr>
<tr>
<td>Testers</td>
<td>( (t-1) )</td>
<td>( M_2 \sigma^2_e + r \sigma^2_{ht} + rl \sigma^2_i )</td>
<td>( \sigma^2_e + r \text{Cov. (F.S.)} - 2\text{Cov. (H.S.)} + [rl \text{Cov. (H.S.)}] )</td>
</tr>
<tr>
<td>Line x Tester</td>
<td>( (l-1)(t-1) )</td>
<td>( M_3 \sigma^2_e + r \sigma^2_{lt} )</td>
<td>( \sigma^2_e + r \text{Cov. (F.S.)} - 2\text{Cov. (H.S.)} )</td>
</tr>
<tr>
<td>Error</td>
<td>( (r-1)(l-1) )</td>
<td>( M_4 \sigma^2_e )</td>
<td>( \sigma^2_e )</td>
</tr>
</tbody>
</table>

From the expectation of mean squares, covariance of full-sibs and half-sibs were estimated using the sum of squares due to lines \( (M_1) \), testers \( (M_2) \) and lines x testers \( (M_3) \) where,

\[
\text{Cov. (H.S.)} = (M_1-M_3) + (M_2-M_3) / r(l+t)
\]

\[
\text{Cov. (F.S.)} = (M_1-M_4) + (M_2-M_4) + (M_3-M_4) / 3r + 6r \text{ Cov. (H.S.)} - r (l+t) \text{ Cov. (H.S.)} / 3r
\]
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Where,

\[ r = \text{Number of replications} \]
\[ h = \text{Number of hybrids} \]
\[ l = \text{Number of lines} \]
\[ t = \text{Number of testers} \]
\[ lt = \text{Number of hybrids} \]

\[ \sigma^2_t = \text{Variance due to general combining ability of testers} \]
\[ \sigma^2_l = \text{Variance due to general combining ability of lines} \]
\[ \sigma^2_{lt} = \text{Variance due to specific combining ability of hybrids} \]

The estimates of variance components due to lines, testers and hybrids were calculated as under

\[ \sigma^2_t = \frac{(M_1 - M_3)}{rt} \]
\[ \sigma^2_l = \frac{(M_2 - M_3)}{rl} \]
\[ \sigma^2_{lt} = \frac{(M_3 - M_4)}{r} \]

Estimates of covariance for half sib (H.S.) and covariance for full sib (F.S.) would be:

\[ \text{Cov. (H.S.)} = \frac{(l \sigma^2_t + t \sigma^2_l)}{(l + t)} \]
\[ \text{Cov. (F.S.)} = (\sigma^2_{lt} + 2\text{Cov. (H.S.)}) \]

The estimated variances due to gca and sca were computed using covariance of half-sibs and full-sibs:

\[ \sigma^2_{gca} \cong \text{Cov. (H.S.)} \]
\[ \sigma^2_{sca} \cong \text{Cov. (F.S.)} - 2\text{Cov. (H.S.)} \]
\[ \sigma^2_{gca} = \frac{(M_1 + M_2 - 2M_3)}{r (1 + t)} \]
\[ \sigma^2_{sca} = \frac{[(M_1 + M_2 + M_3 - 3M_4) + (6r \text{ Cov. H.S.} - (r (1 + t) \text{ Cov. H.S.}))]}{3r} \]

To judge the significance of estimated variance, following tests were made.

Ho: \( \sigma^2_{lt} = 0 \) was tested by \( F \left(n_3, n_4\right) = M_3/M_4 \). If \( M_3 \) was significant, then test would be \( F \left(n_1, n_3\right) = M_1/M_3 \) for \( \sigma^2_{t} = 0 \) and \( F \left(n_2, n_3\right) = M_2/M_3 \) would be for \( \sigma^2_{l} = 0 \). If, \( M_3 \) was non-significant, then \( M_4 \) was used as denominator, where \( n_1, n_2, n_3 \) and \( n_4 \) were degrees of freedom associated with \( M_1, M_2, M_3 \) and \( M_4 \), respectively.
3.5.3.2 Combining ability effects

The combining ability effects were estimated as under

(i) **General combining ability effects of lines**

\[ \hat{g}_i = (Y_{i..}/tr) - (Y.../ltr) \]

Where,

\[ Y_{...} = \text{Total of all hybrids over replications} \]

\[ Y_{i..} = \text{Total of } i^{th} \text{ line over all testers and replications} \]

(ii) **General combining ability effects of testers**

\[ \hat{g}_j = (Y_{.j}/lr) - (Y.../ltr) \]

Where,

\[ Y_{.j} = \text{Total of } j^{th} \text{ tester over all lines and replications} \]

(iii) **Specific combining ability effects of crosses**

\[ S_{ij} = (Y_{ij}/r) - (Y_{i..}/tr) - (Y_{.j}/lr) + (Y.../ltr) \]

Where,

\[ Y_{ij} = \text{Total of } ij^{th} \text{ cross combination over all replications} \]

The test of significance of general and specific combining ability effects was done as under

\[ \text{SEd (gca for lines)} = \sqrt{\frac{2 \text{ Me}}{r \times t}} \]

\[ \text{SEd (gca for testers)} = \sqrt{\frac{2 \text{ Me}}{r \times l}} \]

\[ \text{SEd (sca for crosses)} = \sqrt{\frac{2 \text{ Me}}{r}} \]

\[ \text{C.D.}(\%) = \text{SEd} \times t_{0.05} (n_e) \]

Where,

\[ \text{Me} = \text{Error mean square in the analysis of combining ability} \]

\[ r, t \text{ and } l = \text{Number of replications, testers and lines, respectively} \]

\[ t_{0.05} (n_e) = \text{Value at error degrees of freedom at 5 \% level of significance.} \]