3. MATERIALS AND METHODS

The present investigation on “Heterosis and gene action studies for fruit yield and horticultural traits in chilli (Capsicum annuum var. annuum L.)” was carried out at the Experimental Farms of the Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur and Hill Agricultural Research and Extension Centre, CSK Himachal Pradesh Krishi Vishvavidyalaya, Bajaura, Kullu during Kharif, 2010 and 2011. The details of materials used and methods employed in the present study to understand the nature of combining ability, type of gene action governing the inheritance of economic characters and the nature and extent of heterosis in chilli (Capsicum annuum var. annuum L.) are presented as under:

3.1 Locations

3.1.1 Palampur

The Experimental Farm is located at an elevation of about 1290.8 m above mean sea level with 32° 8’ North latitude and 76° 3’ East longitude, representing mid hill zone of Himachal Pradesh and has a sub-temperate climate with high rainfall (2500 mm)/annum. The soil of this zone is silty clay loam with acidic reaction.

3.1.2 Bajaura

The Experimental Farm is situated at 31° 8’ North latitude and 77° East longitude at an elevation of 1090 m above mean sea level. Bajaura falls under mid-hill, sub-humid zone (Zone-II) of the state and is endowed with mild summer and cool winter with low monsoon rains. The soil of this location is sandy loam with high water-table.

The mean monthly meteorological data, with regard to temperature, relative humidity and rainfall during the cropping period at both the locations are presented in Appendix I and II.
Fig. 1 Mean weekly weather conditions during the cropping season at Palampur
Fig. 2 Mean weekly weather conditions during the cropping season at Bajaura
3.2 Experimental material and layout plan

3.2.1 Experimental material

The experimental material for the present study comprised of $F_1$ population of 33 crosses which were developed by crossing 11 lines of chilli, viz., ‘Jawahar Mirch 283’, ‘Chilli Sonal’, ‘PAU Selection Long’, ‘Arka Lohit’ ‘LCA 436’, ‘Pusa Jwala’, ‘Pusa Sadabahar’, ‘Kashmir Long’, ‘Selection 352’, ‘LCA 443’ and ‘LCA 206’ and with three testers, viz., ‘Pant C 1’, ‘Anugraha’ and ‘Surajmukhi’. Hybrid ‘CH-1’ was used as a standard check. These genotypes were collected from different sources (Table 3.1).

3.2.2 Hybridization programme

All the lines used as female parents were crossed to each of the testers by hand pollination following line × tester model. These genotypes were grown under naturally ventilated polyhouse at the Vegetable Research Farm, Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during summer, 2009 and 2010. The healthy flower buds from new flush, due to open on the next day, were selected for emasculation and pollination. The selected buds were hand emasculated using forceps in the evening hours between 4.00 pm to 6.00 pm. Pollination of the emasculated flowers was done next day morning between 8 am to 12 noon. Well opened flowers with dehisced anthers were collected from the male parents and the stigma of female parent was touched with the respective dehisced anthers of male flowers. The crossed flowers were tagged depicting name of the cross and date of pollination. At maturity, red ripened fruits were harvested and sun dried. Seeds were extracted manually from the fruits, sun dried and stored. Thus, line × tester full-sib crossed true to type seeds were obtained for evaluation in the next season. The self seeds of the parents were also collected during the same seasons.
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Testers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Pant C 1</td>
<td>University of Agricultural Sciences and Technology, Pantnagar</td>
</tr>
<tr>
<td>2.</td>
<td>Anugraha</td>
<td>CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur</td>
</tr>
<tr>
<td>3.</td>
<td>Surajmukhi</td>
<td>CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur</td>
</tr>
<tr>
<td>b) Lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Jawahar Mirch 283</td>
<td>Indian Agriculture Research Institute, New Delhi</td>
</tr>
<tr>
<td>2.</td>
<td>Chilli Sonal</td>
<td>Punjab Agricultural University, Ludhiana</td>
</tr>
<tr>
<td>3.</td>
<td>PAU Selection Long</td>
<td>Punjab Agricultural University, Ludhiana</td>
</tr>
<tr>
<td>4.</td>
<td>Arka Lohit</td>
<td>Indian Institute of Horticultural Research, Hessarghatta, Bangaluroo</td>
</tr>
<tr>
<td>5.</td>
<td>LCA 436</td>
<td>Regional Agricultural Research Station, Lam, Guntur</td>
</tr>
<tr>
<td>6.</td>
<td>Pusa Jwala</td>
<td>Indian Agriculture Research Institute, New Dehli</td>
</tr>
<tr>
<td>7.</td>
<td>Pusa Sadabahar</td>
<td>Indian Agriculture Research Institute, New Dehli</td>
</tr>
<tr>
<td>8.</td>
<td>Kashmir Long</td>
<td>Shere Kashmir University of Agricultural Sciences and Technology, Srinagar</td>
</tr>
<tr>
<td>9.</td>
<td>Selection 352</td>
<td>Indian Agricultural Research Institute, New Dehli</td>
</tr>
<tr>
<td>10.</td>
<td>LCA 443</td>
<td>Regional Agricultural Research Station, Lam, Guntur</td>
</tr>
<tr>
<td>11.</td>
<td>LCA 206</td>
<td>Regional Agricultural Research Station, Lam, Guntur</td>
</tr>
<tr>
<td>c) Standard check</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>CH-1</td>
<td>Punjab Agricultural University, Ludhiana</td>
</tr>
</tbody>
</table>
Contd…/
Plate 1: Parents and standard check used in the investigation
3.2.3 Experimental layout plan

The thirty three $F_1$s, fourteen parents and standard check ‘CH-1’ were grown in a Completely Randomized Block Design with three replications at Palampur and Bajaura for two consecutive summer-rainy seasons during 2010 and 2011. For screening of these crosses and parents for bacterial wilt disease, a separate experiment was laid out simultaneously at Palampur during both the years in bacterial wilt sick plots by planting ten plants of each entry.

3.2.4 Nursery sowing and transplanting

Seeds were sown in nursery beds of size $3 \times 1$ m on 2nd and 6th March at Palampur and Bajaura during 2010, respectively, whereas, seed sowings in the respective environments were carried out on 7th and 10th April during 2011. Twelve plants of 10-15 cm height were transplanted in the field on 20th and 22nd April during 2010 and 4th and 10th May during 2011 at Palampur and Bajaura, respectively, with inter and intra-row spacing of 45 cm each.

3.3 Cultural practices

The experimental fields in the respective environments were ploughed using a 3-disc tractor and twice using a 7-disc tractor followed by power tiller. The recommended farmyard manure @ 20 tonnes/hectare was mixed in the soil at time of field preparation. The fertilizers were applied @ 75: 60: 60 kg N, P$_2$O$_5$ and K$_2$O/hectare with half of recommended N, full P and K at planting time and remaining N in two equal splits at one month interval after planting. Irrigation was provided one week prior to planting and immediately after transplanting for proper establishment of plants in the soil and thereafter, at ten days interval prior to the onset of monsoon. Five hand weeding were carried out at monthly interval to keep the field weed free. Drainage was also provided to keep the fields free from stagnation of water during rainy season. The harvestings were carried out manually.

3.4 Recording of observations

The observations were recorded on randomly taken five competitive plants in each entry and replication followed by computing their means for the following traits.
1) **Days to 50% flowering**

Calculated from the date of planting to the appearance of first flowering in 50 per cent plants of a genotype.

2) **Days to first harvest**

The number of days taken from the date of transplanting to the date of first marketable harvest of green fruits were calculated.

3) **Primary branches/plant**

Number of branches arising from the main stem were counted in randomly taken plants.

4) **Fruit length (cm)**

Polar length of ten randomly taken fresh fruits was measured in centimeters with a scale and average worked out for each of the parents and crosses.

5) **Fruit girth (cm)**

Equatorial length of each of the above fruits was measured in centimeters with vernier caliper at middle of the fruit.

6) **Average fruit weight (g)**

Average fruit weight was calculated by dividing the total marketable fruit yield by total number of marketable fruits/plant.

7) ** Marketable fruits/plant**

The total number of marketable fruits picked from randomly taken plants at each harvest were counted and finally summed-up to work out the total number of fruits/plant.

8) ** Marketable fruit yield/plant (g)**

The number of marketable fruits at each picking were weighed and averaged to get the marketable fruit yield/plant in grams.
9) **Harvest duration (days)**

Total number of days from first picking to the final picking of marketable fruits for each genotype were worked out.

10) **Plant height (cm)**

Plant height was measured in centimeters from the base to the top of the central apical shoot at the time of final harvest in each entry over the replications.

11) **Average dry fruit weight (g)**

Average dry fruit weight was calculated by dividing the total dry fruit yield by total number of dry fruits/plant.

12) **Dry fruit yield/plant (g)**

 Marketable fruits harvested from five randomly selected plants at red ripe stage were dried and weighed to work out the average dry fruit yield/plant in grams.

13) **Ascorbic acid (mg/100g)**

Ascorbic acid content in chilli was estimated by ‘2,6-dichlorophenol-indophenol Visual Titration Method’ as described by Ranganna (1979).

**Reagents:**

a) 3% metaphosphoric acid (HPO$_3$): Prepared by dissolving the sticks or pellets of HPO$_3$ in glass distilled water.

b) Ascorbic acid standard: 100 mg of L-ascorbic acid was weighed accurately and volume made up to 100 ml with 3 per cent HPO$_3$. 10 ml of this solution was further diluted to 100 ml with 3 per cent HPO$_3$. (1 ml = 0.1 mg ascorbic acid)

c) Dye solution: 50 mg of the sodium salt of 2,6-dichlorophenol-indophenol was dissolved in approximately 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate. The solution was cooled and diluted with glass distilled water to 200 ml. Stored in a refrigerator and standardized every day.
Procedure

Standardization of dye

- Five ml of standard ascorbic acid solution was taken in a beaker and 5 ml of HPO₃ was added to it. This solution was titrated with the dye solution to a pink colour which persisted for 15 seconds. Dye factor (mg of ascorbic acid per ml of the dye) was determined by using the formula:

\[
\text{Dye factor} = \frac{0.5}{\text{Titre}}
\]

Here,

i. 0.5 means 0.5 mg of ascorbic acid in 5 ml of 100 ppm standard ascorbic acid solution,

ii. Titre = Volume of dye used to neutralize 5 ml of 100 ppm standard ascorbic acid solution along with 5 ml of metaphosphoric acid.

- Ten grams of macerated sample was blended with 3 per cent metaphosphoric acid and the volume finally made up to 100 ml.

- Out of this 100 ml solution, 10 ml of solution was taken and titrated against 2,6-dichlorophenol-indophenol dye till the appearance of rose pink colour.

- The ascorbic acid content was calculated by using the following formula and were expressed as mg of ascorbic acid/100 g of fresh sample.

\[
\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract taken for estimation} \times \text{Weight of sample taken for estimation}} \times 100
\]

Here,

Titre = Volume of dye used to titrate the aliquot of extract of a given sample.

14) Capsaicin content (%)

The capsaicin content in the fruits was determined by calorimetric method using Folin-Ciocalteau reagent described by Bajaj (1980). The capsaicin
concentration in different samples was noted from the standard capsaicin curve and finally the results were converted into percentage.

Reagents

a) Acetone

b) Aluminium oxide active basic

c) Folin and ciocalteau phenol (FC) reagent (available as 2N; diluted with equal volume of distilled water just before use).

d) Sodium carbonate anhydrous: 35 g of anhydrous sodium carbonate was dissolved in 100 ml of water at 70-80°C, filtered and allowed to cool overnight. Super saturated solution with crystals of Na$_2$CO$_3$ 10 H$_2$O was filtered through glass wool to obtain the mother liquid.

e) Methanol (CH$_2$O)

Procedure

a) **Standard curve:** 0 to 1.5 ml of standard capsaicin were taken in small beakers and evaporated to less than 0-5 ml at room temperature. 0-5 ml FC reagent and 6-5 ml of distilled water were added to beaker and allowed to stand for three minutes. Then 1 ml of Na$_2$CO$_3$ solution was added and mixed well. Whole quantity was transferred to 10 ml volumetric flask and final volume was made up with distilled water. Centrifugation for 10-15 minutes at 10,000 rpm was done. Absorbance was measured at 760 nm after one hour rest at room temperature.

b) **Extraction:**

- 0-5 g of dried powdered capsicum fruits were extracted with 25 ml acetone.

- Mixture was shaken for 10 minutes and allowed to stand for four hours.

- After that mixture was filtered through glass wool plugged in a short stemmed funnel. Volume was made up to 25 ml. Two ml of this extract was passed through basic alumina column. Column is 1.5 g basic
alumina (have layers of glass wool, aluminium oxide and sodium sulphate of 2 fingers height each) in to 10 × 0.9 cm column which is washed with 5 ml of acetone.

- Column was washed with 3 × 5 ml of acetone after loading. These washings were discarded. Pure capsaicin was eluted with acetone; methanol and water mixture in ratio of 75:25:1 and final volume made up to 50 ml.

- 10 ml volume was evaporated to dryness at temperature less than 65°C and the colour was developed as for calibration curve.

c) Calculations: Suppose OD of sample = x. Then from standard curve, concentration of capsaicin against x = y mg. This y mg is in 10 ml which is taken from 50 ml. So, concentration of capsaicin = 5y in 50 ml. Again this 5 y is from 2 ml of extract which is taken from 25 ml of extract made at first step. So, in 25 ml, concentration of capsaicin = (5y × 25 mg)/2. This 25 ml extract was prepared from 0.5 g of sample. Therefore, 0.5 g (500 mg) of sample has 125/2y mg of capsaicin.

- 1g of sample has 125 y mg of capsaicin.
- 100 g of sample has 12500 y mg of capsaicin.
- Therefore, 100 g of sample contains 12500 y mg of capsaicin.
- In per cent capsaicin content will be 12.5 y

15) Capsanthin/ colouring matter (ASTA units)

Capsanthin was determined as per procedure given by A.O.A.C. (1980).

Requirement: Spectrophotometer, Acetone

Procedure

- 100 mg of powdered sample was taken in 100 ml volumetric flask, diluted with acetone and corked tightly.
• The solution prepared was shaken well and allowed to stand in dark for sixteen hours at room temperature.

• The mixture was shaken again and particles were allowed to settle for two minutes.

• A clear portion of the extract was transferred to cell and absorbance was measured at 465 nm using acetone as a blank.

Calculations

\[
\text{ASTA colour value for capsicum} = \frac{(A_{\text{ext}} \text{ at } 465 \text{ nm}) \times (16.4 \text{ I}_{1})}{\text{g sample}}
\]

16) Oleoresin (ASTA units)

Oleoresin was calculated as per procedure given by A.O.A.C. (1980).

Requirements: Spectrophotometer, acetone

Procedure

• 100 mg of powdered sample was transferred to 100 ml volumetric flask.

• The final volume was made up with acetone, shaken well and allowed to stand for two minutes.

• 10 ml extract was pipetted into another 100 ml volumetric flask and final volume made up with acetone and was shaken again.

• Absorbance of this solution was measured at 460 nm against acetone as blank.

Calculations

\[
\text{ASTA colour value for oleoresin} = \frac{(A_{\text{ext}} \text{ at } 460 \text{ nm}) \times (164 \text{ I}_{1})}{\text{g sample}}
\]

Where,

\[
\text{I}_{1} \text{ (correction factor)} = \frac{\text{Declared OD of NBS std. at } 465 \text{ nm}}{\text{Observed OD of NBS std at } 465 \text{ nm}}
\]
Standard of NBS (National Board of Spice) is 1 M Ferrous ammonium sulphate and declared OD is 0.64. In the spectronic, declared OD is equal to observed so, there was no need to multiply with $I_f$.

q) **Bacterial wilt incidence (%)**

Bacterial wilt disease incidence in chilli was recorded as per Sinha *et al.* (1990) scale. Mortality (confirmed by ooze test) in each genotype was recorded and expressed in per cent to categorize the genotypes into resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible as per scale:

**Rating System for Bacterial Wilt Incidence**

<table>
<thead>
<tr>
<th>Bacterial wilt (%)</th>
<th>Reaction category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>Resistant (R)</td>
</tr>
<tr>
<td>11-20</td>
<td>Moderately resistant (MR)</td>
</tr>
<tr>
<td>21-30</td>
<td>Moderately susceptible (MS)</td>
</tr>
<tr>
<td>31-70</td>
<td>Susceptible (S)</td>
</tr>
<tr>
<td>71-100</td>
<td>Highly susceptible (HS)</td>
</tr>
</tbody>
</table>

Incidence of Bacterial wilt (%) = \[ \frac{\text{Number of plants infested}}{\text{Total number of plants}} \times 100 \]

3.5 **Statistical analysis**

The data recorded on 33 crosses along with 14 parents and one standard check were analyzed as per the design for working out the following values.

3.5.1 **Analysis of variance**

For working out the analysis of variance, the data were analyzed by using the following model as suggested by Panse and Sukhatme (1967).

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

\[ Y_{ij} = \text{Phenotypic observation of } i^{\text{th}} \text{ genotype grown in } j^{\text{th}} \text{ replication} \]

\[ \mu = \text{General population mean} \]

\[ g_i = \text{Effect of } i^{\text{th}} \text{ genotype} \]

\[ r_j = \text{Effect of } j^{\text{th}} \text{ replication, and} \]

\[ e_{ij} = \text{Error component} \]

**Analysis of variance**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean sum of square</th>
<th>Expected mean sum of square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>(r-1)</td>
<td>Mr</td>
<td>( \sigma_e^2 + g \sigma_r^2 )</td>
</tr>
<tr>
<td>Entries</td>
<td>(g-1)</td>
<td>Mg</td>
<td>( \sigma_e^2 + r \sigma_g^2 )</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1) (g-1)</td>
<td>Me</td>
<td>( \sigma_e^2 )</td>
</tr>
</tbody>
</table>

where,

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

\[ g = \text{number of entries} \]

\[ \sigma_e^2 = \text{error variance} \]

\[ \sigma_g^2 = \text{variance due to entries} \]

\[ \sigma_r^2 = \text{variance due to replications} \]

The replication and entries mean sum of square were tested against error mean squares by ‘F’ test for \((r-1), (r-1) (g-1)\) and \((g-1), (r-1) (g-1)\) degree of freedom at \(P = 0.05\).

The pooled over environments analysis was done as follows
## Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Mean sum of square</th>
<th>Expected mean sum of square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications (within environments)</td>
<td>E(r-1)</td>
<td>Mr</td>
<td>-</td>
</tr>
<tr>
<td>Environment</td>
<td>(E-1)</td>
<td>ME</td>
<td></td>
</tr>
<tr>
<td>Entries</td>
<td>(g-1)</td>
<td>Mg</td>
<td>(\sigma_e^2 + r \sigma_g^2 \times E + rE\sigma_g^2)</td>
</tr>
<tr>
<td>Entries x Environment</td>
<td>(g-1)(E-1)</td>
<td>Mg x E</td>
<td>(\sigma_e^2 + r \sigma_g^2 \times E)</td>
</tr>
<tr>
<td>Pooled error</td>
<td>E(g-1)(r-1)</td>
<td>Me(C)</td>
<td>(\sigma_e^2)</td>
</tr>
</tbody>
</table>

where,

- \(r\) = number of replicates
- \(E\) = number of environments
- \(g\) = number of entries
- \(\sigma_e^2\) = Error variance
- \(\sigma_g^2 \times E\) = Variance due to entries x environment interactions, and
- \(\sigma_g^2\) = Variance due to entries

\[
Me(C) = \frac{(\text{Error ss at Env. I} + \text{Error ss at Env. II} + \text{Error ss at Env. III})}{(\text{df at Env. I} + \text{df at Env. II} + \text{df at Env. III})}
\]

The replications (within environments), environments, entries, entries x environments mean sum of square were tested against error mean squares by ‘F’ test for \(E\) (r-1), \(E\) (g-1) (r-1), for (E-1), \(E\) (g-1) (r-1), for \(g\) (1), \(E\) (g-1) (r-1) and \((g-1)\) (E-1), \(E\) (g-1) (r-1) degree of freedom at \(P = 0.05\), respectively.

From these analyses, the following standard error were calculated where the ‘F’ test was significant. Standard error for the entry mean:
SE (m) = Individual environment = $\pm (Me/2)^{1/2}$

Pooled environment = $\pm (Me(C)/rE)^{1/2}$

Standard error for the difference of entry means:

SE (d) = Individual environment = $\pm (2Me/r)^{1/2}$

SE (d) = Pooled environment = $\pm (2Me(C)/rE)^{1/2}$

The critical difference (CD) at 5% level of significance was obtained by multiplying SE (d) by the table value of ‘t’ at error degree of freedom and $P = 0.05$.

CD = SE (d) $\times$ ‘t’ value at error degree of freedom and $P = 0.05$

Coefficient of variation (CV) % = $(ME^{1/2}$ or $ME(C)^{1/2}$/general mean) $\times$ 100

3.5.2 Line $\times$ tester analysis

In this case the replication wise mean values of $F_1$ generation of 33 crosses for each trait were subjected to statistical analysis using the following model suggested by Kempthorne (1957) and the solved example given by Dabholkar (1992).

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$

where,

$Y_{ijk}$ = value of the $ijk^{th}$ observation of the cross involving $i^{th}$ line and $j^{th}$ tester in $k^{th}$ replication,

$\mu$ = general mean (an effect common to all hybrids in all replications),

$g_i$ = general combining ability (GCA) effect of $i^{th}$ line,

$g_j$ = general combining ability (GCA) effect of $j^{th}$ tester,
\[ S_{ij} = \text{specific combining ability (SCA) effect of the cross involving } i^{th} \text{ line and } j^{th} \text{ tester}, \]

\[ e_{ijk} = \text{error associated with } ijk^{th} \text{ observation}, \]

\[ i = i^{th} \text{ line (1, 2, 3 …………………..11)} \]

\[ j = j^{th} \text{ tester (12, 13, 14), and} \]

\[ k = k^{th} \text{ replication (1, 2, 3)} \]

**Analysis of variance for crosses and for combining ability**

(partitioning crosses sum of squares)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>(r-1)</td>
<td>( \sum_{k=1}^{r} \frac{(x..k)^2}{fm} - \frac{x^2}{fmr} )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cross</td>
<td>(fm-1)</td>
<td>( \sum_{ij=1}^{fm} \frac{x^2_{ij}}{r} - \frac{x^2}{fmr} )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lines</td>
<td>(f-1)</td>
<td>( \sum_{i=1}^{f} \frac{x^2_i}{mr} - \frac{x^2}{fmr} )</td>
<td>( M(f) )</td>
<td>( \sigma_e^2 + r\sigma_{lm}^2 + rm\sigma_f^2 )</td>
</tr>
<tr>
<td>Testers</td>
<td>(m-1)</td>
<td>( \sum_{j=1}^{m} \frac{x^2_j}{fr} - \frac{x^2}{fmr} )</td>
<td>( M(m) )</td>
<td>( +r\sigma_{lm}^2 + rf\sigma_m^2 )</td>
</tr>
<tr>
<td>Line \times tester</td>
<td>(f-1)</td>
<td>( \sum_{ij=1}^{fm} \frac{x^2_{ij}}{r} - \frac{\sum_{i=1}^{f} x^2_{i}}{mr} - \frac{\sum_{j=1}^{m} x^2_{j}}{fr} + \frac{x^2}{fmr} )</td>
<td>( M(fm) )</td>
<td>( \sigma_e^2 + r\sigma_{lm}^2 )</td>
</tr>
<tr>
<td>Error</td>
<td>(fm-1)</td>
<td>( \text{By difference (for crosses)} )</td>
<td>( Me )</td>
<td>( \sigma_e^2 )</td>
</tr>
<tr>
<td></td>
<td>(r-1)</td>
<td>( \sum_{i=1}^{f} \sum_{j=1}^{m} \sum_{k=1}^{r} x^2_{ijk} - \frac{x^2}{fmr} )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>(fmr-1)</td>
<td>( \sum_{i=1}^{f} \sum_{j=1}^{m} \sum_{k=1}^{r} x^2_{ijk} - \frac{x^2}{fmr} )</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
where,

\[ f = \text{number of lines}, \]
\[ m = \text{number of testers}, \]
\[ x_{..k} = \text{sum of } k^{th} \text{ replication of crosses}, \]
\[ x_{...} = \text{sum of all crosses of all lines and testers over all replications}, \]
\[ x_{ij.} = \text{sum of } ij^{th} \text{ hybrid combination over all replications}, \]
\[ x_{i..} = \text{sum of } i^{th} \text{ line over all testers and replications}, \]
\[ x_{j..} = \text{sum of } j^{th} \text{ tester over all lines and replications}, \]
\[ x_{ijk} = \text{ij}^{th} \text{ observation in } k^{th} \text{ replication}, \]
\[ M(f) = \text{mean squares due to lines}, \]
\[ M(m) = \text{mean squares due to testers}, \]
\[ M(f \times m) = \text{mean squares due to line x tester interactions}, \]
\[ Me = \text{error mean squares}, \]
\[ \sigma_f^2 = \text{variance due to lines/progeny variance arising from differences among female parents/lines}, \]
\[ \sigma_m^2 = \text{variance due to testers/progeny variance arising from differences among male parents/testers}, \]
\[ \sigma_{f \times m}^2 = \text{variance due to lines x testers/progeny variance arising from interaction of the contribution of female and male parents, and} \]
\[ \sigma_e^2 = \text{environmental variance/error variance among individuals from same mating} \]

### 3.5.3 Estimation of general and specific combining ability effects

GCA and SCA effects were obtained from the two way table of female parents vs. male parents in which each figure was total over replications. The individual effects were estimated as follow:
(i) GCA effects of \( i^{th} \) line

\[
\frac{g_i}{mr} = \frac{X_{i..}}{mr} - \frac{X_{...}}{fmr}
\]

where,

\[
X_{...} = \text{sum total of all crosses},
\]

\[
X_{i..} = \text{total of } i^{th} \text{ female parents over all males and replications},
\]

\[
m = \text{number of testers/male parents}
\]

(ii) GCA effects of \( j^{th} \) tester

\[
\frac{g_j}{fr} = \frac{X_{j..}}{fr} - \frac{X_{...}}{fmr}
\]

where,

\[
X_{j..} = \text{total of } j^{th} \text{ male parent over all females and replications}
\]

(iii) SCA effects of \( ij^{th} \) cross

\[
\frac{s_{ij}}{r} = \frac{X_{ij.}}{r} - \frac{X_{i..}}{mr} - \frac{X_{j..}}{fr} + \frac{X_{...}}{fmr}
\]

where,

\[
X_{ij.} = \text{ij}^{th} \text{ combination total over all replications}
\]

(iv) Standard errors for different combining ability effects

(a) \( \text{SE (} g_i \text{ ) lines} = \pm \sqrt{\frac{Me}{mr}} \)

(b) \( \text{SE (} g_j \text{ ) testers} = \pm \sqrt{\frac{Me}{fr}} \)

(c) \( \text{SE (} s_{ij} \text{ ) crosses} = \pm \sqrt{\frac{Me}{r}} \)

(d) \( \text{SE (} g_i \cdot g_j \text{ ) lines} = \pm \sqrt{\frac{2Me}{mr}} = \text{SE (} D_1 \text{)} \)
(e) \[ SE (g_i - g_j) \] testers = \[ \pm \frac{\sqrt{2Me}}{fr} \] = SE (D_2)

(f) \[ SE (s_j - s_k) \] crosses = \[ \pm \frac{\sqrt{2Me}}{r} \] = SE (D_3)

where,

\[ Me = \text{mean sum of squares due to error} \]

**Pooled analysis of variance for combining ability**

Pooled over environments analysis of variance for combining ability was done as per following:

**Analysis of variance**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>Expected MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environments</td>
<td>(E-1) [ \text{df} ]</td>
<td>[ \sum_{n=1}^{E} \frac{x^2 \ldots n}{mfr} - \frac{x^2 \ldots}{mfrE} ]</td>
<td>[ \ldots ]</td>
<td>[ \ldots ]</td>
</tr>
<tr>
<td>Testers</td>
<td>(m-1) [ \text{df} ]</td>
<td>[ \sum_{j=1}^{m} \frac{x^2 \ldots j}{mfrE} - \frac{x^2 \ldots}{mfrE} ]</td>
<td>[ M_1 ]</td>
<td>[ \sigma_e^2 + r\sigma_{fm}^2E + ] [ rE\sigma_{fm}^2 + rE\sigma_m^2 ]</td>
</tr>
<tr>
<td>Lines</td>
<td>(f-1) [ \text{df} ]</td>
<td>[ \sum_{i=1}^{f} \frac{x^2 \ldots i}{mfrE} - \frac{x^2 \ldots}{mfrE} ]</td>
<td>[ M_2 ]</td>
<td>[ \sigma_e^2 + r\sigma_{fm}^2E + ] [ rE\sigma_{mf}^2 + rE\sigma_m^2 ]</td>
</tr>
<tr>
<td>Lines × Testers</td>
<td>(m-1) [ \text{df} ]</td>
<td>[ \sum_{i=1}^{mf} \frac{x^2 \ldots ij}{fr} - \sum_{j=1}^{m} \frac{x^2 \ldots j}{mfr} - \sum_{i=1}^{f} \frac{x^2 \ldots i}{frE} + \frac{x^2 \ldots}{mfrE} ]</td>
<td>[ M_3 ]</td>
<td>[ \sigma_e^2 + r\sigma_{fm}^2E + ] [ rE\sigma_{mf}^2 ]</td>
</tr>
<tr>
<td>Testers × Env.</td>
<td>(m-1) [ \text{df} ]</td>
<td>[ \sum_{j=1}^{m} \frac{x^2 \ldots jn}{fr} - \sum_{n=1}^{E} \frac{x^2 \ldots n}{mfr} - \sum_{j=1}^{m} \frac{x^2 \ldots j}{frE} + \frac{x^2 \ldots}{mfrE} ]</td>
<td>[ M_4 ]</td>
<td>[ \sigma_e^2 + r\sigma_{fm}^2E + ] [ r\sigma_m^2E ]</td>
</tr>
<tr>
<td>Lines × Env.</td>
<td>(f-1) [ \text{df} ]</td>
<td>[ \sum_{i=1}^{f} \frac{x^2 \ldots ij}{mr} - \sum_{j=1}^{m} \frac{x^2 \ldots jn}{mfr} - \sum_{i=1}^{f} \frac{x^2 \ldots i}{frE} + \frac{x^2 \ldots}{mfrE} ]</td>
<td>[ M_5 ]</td>
<td>[ \sigma_e^2 + r\sigma_{fm}^2E + ] [ r\sigma_m^2E ]</td>
</tr>
<tr>
<td>Lines × Testers × Env.)</td>
<td>(m-1) [ \text{df} ]</td>
<td>[ \sum_{i=1}^{mf} \frac{x^2 \ldots ijn}{r} - \sum_{j=1}^{m} \frac{x^2 \ldots ij}{mfr} - \sum_{i=1}^{f} \frac{x^2 \ldots i}{mr} + \frac{x^2 \ldots}{mfrE} ]</td>
<td>[ M_6 ]</td>
<td>[ \sigma_e^2 + r\sigma_{fm}^2E ]</td>
</tr>
<tr>
<td>Pooled error</td>
<td>E(mf-1) [ \text{df} ]</td>
<td>Error as at environment-I + Error as at environment-II +</td>
<td>[ M_e ]</td>
<td>[ \sigma_e^2 ]</td>
</tr>
</tbody>
</table>
where,
\( m \) = number of males,
\( f \) = number of females,
\( E \) = number of environments,
\( r \) = number of replications at each environment,
\( x... \) = sum of all crosses of all lines, testers, replications and over all environments,
\( x...n \) = sum of all crosses of all lines and testers over replications,
\( x_{ij...} \) = sum of \( j^{th} \) testers over all lines, replications and environments,
\( x_{ij} \) = sum of \( j^{th} \) tester over all lines, replications and environments,
\( x_{ij.n} \) = sum of \( j^{th} \) tester over all lines and replications at \( n^{th} \) environment,
\( x_{ij.n} \) = sum of \( j^{th} \) tester over all lines and replications at \( n^{th} \) environment, and
\( M_e \) = Pooled error mean square

Pooled general and specific combining ability effects were estimated as follows:

(i) **Estimation of general mean**
\[
\mu = \frac{x...}{mfre}
\]
where,
\( x... \) = total of all crosses over all replications in all environments

(ii) **gca effects of \( i^{th} \) line**
\[
g_i = \frac{x_{i...}}{mre} - \frac{x...}{mfre}
\]
where,
\( x_{i...} \) = sum of \( i^{th} \) lines over all testers, replications and environments
\( e \) = number of environments

(iii) **gca effects of \( j^{th} \) tester**
\[
g_j = \frac{x_{j...}}{frE} - \frac{x...}{mfrE}
\]
where,
\[ x_{j..} = \text{sum of } j^{th} \text{ testers over all lines, replications and environments} \]

(iv) **Sca effects of ij**th **cross**

\[ S_{ij} = \frac{x_{ij}}{\text{re}} - \frac{x_i}{\text{mre}} - \frac{x_j}{\text{fre}} + \frac{x...}{\text{mfre}} \]

where,
\[ x_{ij..} = \text{ij}^{th} \text{ cross total over all replications and environments.} \]

(v) **Standard error for pooled combining ability effects**

(a) \[ \text{SE pooled (g_i) lines} = (\text{Me}/\text{rme})^{1/2} \]
(b) \[ \text{SE pooled (g_j) testers} = (\text{Me}/\text{rfe})^{1/2} \]
(c) \[ \text{SE pooled (S_{ij}) crosses} = (\text{Me}/\text{re})^{1/2} \]
(d) \[ \text{SE (g_i - g_j) lines} = (2\text{Me}/\text{rme})^{1/2} = \text{SE (D1a)} \]
(e) \[ \text{SE (g_i - g_j) testers} = (2\text{Me}/\text{rfe})^{1/2} = \text{SE (D2a)} \]
(f) \[ \text{SE (S_{ij} - S_{kj}) crosses} = (2\text{Me}/\text{re})^{1/2} = \text{SE (D3a)} \]

(vi) **Test of significance for GCA and SCA effects**

There are two methods

**Method-I**

GCA and SCA effects \( \geq [(\text{SE}_{g_i}/\text{SE}_{g_j}/\text{SE}_{s_{ij}}) \times 't' \text{ tab at error degree of freedom and } P = 0.05] \) were marked significant (*).

**Method-II**

(a) \[ t_i \text{ (cal) for GCA of lines (females)} = (g_i - 0)/\text{SE (g_i)} \]
(b) \[ t_j \text{ (cal for GCA of testers (males)} = (g_j - 0)/\text{SE (g_j)} \]
(c) \[ t_{ij} \text{ (cal) for SCA of crosses} = (S_{ij} - 0)/\text{SE (S_{ij})} \]

where,
\[ t_i \text{ (cal), } t_j \text{ (cal) and } t_{ij} \text{ (cal) are the calculated 't' values,} \]
\[ g_i = \text{GCA effect of } i^{th} \text{ line,} \]
\[ g_j = \text{GCA effect of } j^{th} \text{ tester, and} \]
\[ s_{ij} = \text{SCA effect of } ij^{th} \text{ cross} \]

The GCA effects of lines and testers and SCA effects of crosses were marked significant (*) when the values of \( t_i \text{ (cal)}, t_j \text{ (cal) and } t_{ij} \text{ (cal) were } \geq 't' \)
tabulated value at error degree of freedom of individual environment or pooled over environment and \( P = 0.05 \).

(vii) **Critical differences (CD) for comparing GCA effects of lines/testers and SCA effect of crosses**

(a) CD for GCA (lines) = \( \text{SE} \ (D1a) \times 't' \ \text{tab (error df, P=0.05)} \)

(b) CD for GCA (testers) = \( \text{SE} \ (D2a) \times 't' \ \text{tab (error df, P=0.05)} \)

(c) CD for SCA (crosses) = \( \text{SE} \ (D3a) \times 't' \ \text{tab (error df, P=0.05)} \)

The difference between GCA of any two lines/testers and SCA of any two crosses were considered significant when the differences were \( \geq \) respective CD values.

### 3.5.4 Estimation of variance components

1. **Individual environment**

\[
\text{Cov (HS)} = \sigma_l^2 \ (\text{females}) = \frac{(M_l - M_{fm})}{mr} = \sigma_{GCA}^2 \ (\text{lines})
\]

\[
\text{Cov (HS)} = \sigma_m^2 \ (\text{males}) = \frac{(M_m - M_{fm})}{fr} = \sigma_{GCA}^2 \ (\text{testers})
\]

\[
\text{Cov HS (average)} = \frac{1}{r} (2fm-f-m) [(f-1) (M_l) + (m-1) (M_m)/1+m-2M_{fm}]
\]

\[
\sigma_{fm}^2 \ (\text{females x males}) = \frac{(M_{fm} - Me)}{r} = \sigma_{SCA}^2
\]

(i) **Estimation of Cov HS (average) and Cov (FS)**

These were calculated as:

\[
\text{Cov HS (average)} = \frac{(m\sigma_l^2 + f\sigma_m^2)}{(f+m)}
\]

\[
\text{Cov (FS)} = \sigma_{fm}^2 + 2 \ \text{Cov (HS)}
\]

These can also be calculated from the expectation of mean squares as:

\[
\text{Cov HS (average)} = \frac{(Mf + Mm - 2 M_{fm})}{r} (f + m)
\]

\[
\text{Cov HS (FS)} = \frac{[Mf+Mm + M_{fm} - 3 Me + 6r \ \text{Cov (HS)} - r (f+m) \ \text{Cov (HS)}]}{3r}
\]

2. **Pooled over environments**

\[
\text{Cov (HS)} = \sigma_l^2 \ (\text{females}) = \frac{(Mf - M_{fm})}{mrE}
\]

\[
= \sigma_l^2 \times E \ (\text{female \times environment}) = (MfE - M_{fmE})/mr
\]

\[
\text{Cov (HS)} = \sigma_m^2 \ (\text{males}) = \frac{(Mm - M_{fm})}{frE}
\]

\[
= \sigma_m^2 \times E \ (\text{males \times environments}) = (MmE - m_{fmE})/fr
\]

\[
\sigma_{fm}^2 \times E \ [(\text{females \times males}) \times \text{Environment}] = M_{fmE} - Me/R = \sigma_{SCA}^2 \times E
\]
(I) Estimation of Cov HS (average) and Cov (FS)
These were calculated as:

\[
\text{Cov HS (average)} = \frac{(m \sigma_f^2 + f \sigma_m^2)}{(f + m)}
\]

\[
\text{Cov HS (average) } \times \text{environment} = \frac{(m \sigma_f^2E + f \sigma_m^2 E)}{(f + m)}
\]

\[
\text{Cov (FS)} = \sigma_{fm}^2 + 2 \text{Cov (HS)}
\]

\[
\text{Cov (FS) } \times \text{environment} = \sigma_{fm}^2 e + 2 \text{Cov (HS) } \times \text{Environment}
\]

These also be calculated from the expectation of mean square as Cov HS (average) = \( M_f + M_m - 2 M_{fm} \) / \( rE(f + m) \).

\[
\text{Cov HS (average) } \times \text{environment} = \frac{(M_fE + M_mE - 2M_{gm}E)}{r(f + m)}
\]

\[
\text{Cov (FS)} = \frac{[M_f + M_m + M_m - 3Me + 6rE \text{Cov (HS)} - rE (f+m) \text{Cov (HS)}]/3rE}{r(f+m)}
\]

\[
\text{Cov (HS) } \times \text{Environment} = \frac{(M_fME - Me)}{r}
\]

(ii) Estimation of GCA and SCA variances
From the estimates of Cov (HS) and Cov (FS), variances due to general combining and specific combining ability were calculated as:

\[
\sigma_{GCA}^2 = \text{Cov (HS)} = \frac{(M_f + M_m - 2 M_{fm})}{rE(f + m)}
\]

\[
\sigma_{GCA}^2 \times \text{Environment} = \text{Cov (HS) } \times \text{environment} = \frac{(M_fE + M_mE - 2M_{gm}E)}{r(f + m)}
\]

\[
\sigma_{SCA}^2 = \text{Cov (FS)} - 2 \text{Cov (HS)} = \frac{(mfm - Me)}{r}
\]

\[
\sigma_{SCA}^2 \times \text{Environment} = \frac{\text{Cov (FS) } \times \text{Environment} - 2 \text{Cov (HS) } \times \text{Environment} = (MfME - Me)}{r}
\]

(iii) Estimation of additive (\( \sigma_A^2 \)) and dominance (\( \sigma_D^2 \)) component of variances
For computing the additive and dominance components of variances following formulae have been used Singh and Chaudhary (1977) and Dabholkar (1992).

\[
\sigma_{GCA}^2 = \frac{[(1 + F) / 4]}{\sigma_A^2} = \frac{1}{2} \sigma_A^2
\]

\[
\text{So } \sigma_A^2 = 2 \sigma_{GCA}^2
\]

\[
\sigma_{SCA}^2 = \frac{[(1+ F) / 2]}{\sigma_D^2} = \sigma_D^2
\]

\[
\text{So } \sigma_D^2 = \sigma_{SCA}^2
\]
Where, \( F = \) inbreeding coefficient (\( F = 1.0 \), since the chilli being the often cross pollinated crop does not suffer from significant inbreeding depression).

\[
\sigma_A^2 = \text{additive variance, and} \\
\sigma_D^2 = \text{dominance variance}
\]

### 3.5.5 Per cent contribution of lines, testers and their interactions

These were computed as per the formulae suggested by Singh and Chaudhary (1977).

(i) Per cent contribution of lines

\[
= \left[ \frac{SS (lines)}{SS (crosses)} \right] \times 100
\]

(ii) Per cent contribution to testers

\[
= \left[ \frac{SS (testers)}{SS (crosses)} \right] \times 100
\]

(iii) Per cent contribution of lines \times testers

\[
= \left[ \frac{SS (lines \times testers)}{SS (crosses)} \right] \times 100
\]

### 3.5.6 Estimation of heterosis

The estimates of heterosis were calculated as the deviation of \( F_1 \) mean from the better parent (BP) and standard check [CH-1 (SC)].

1. Heterosis over better parent (%) = \( \left[ \frac{(F_1 - BP)/BP} \right] \times 100 \)

2. Heterosis over standard check (%) = \( \left[ \frac{(F_1 - SC_1)/SC_1} \right] \times 100 \)

#### 1. Calculation of standard errors

(i) SE for testing heterosis over better parents:

- Individual environment \( = \pm (2Me/r)^{1/2} \) = SE (\( H_1 \))
- Pooled environment \( = \pm (2Me/rE)^{1/2} \) = SE (\( H_1 \))

(ii) SE for testing heterosis over Standard check:

- Individual environment \( = \pm (2Me/r)^{1/2} \) = SE (\( H_2 \))
- Pooled environment \( = \pm (2Me/rE)^{1/2} \) = SE (\( H_2 \))
2. Test of significance for heterosis

There are two methods:

\textbf{Method-I}

The difference of \((F_1 - \overline{BP})\) or \((F_1 - \overline{SC})\) ≤ \([SE(H_1) or SE(H_2)] \times 't' \text{ tab, at error degree of freedom of individual environment analysis of variance or at error degree of freedom of pooled over environments analysis and } P=0.05 \text{ were considered significant and the asterisk(*) was put on the per cent values only. This method is relatively less time consuming.}

\textbf{Method-II}

't' calculated values were worked out as follow

1. 't' calculated values for heterosis over BP = \((F_1 - \overline{BP})/SE(H_1)\)

2. 't' calculated value for heterosis over SC = \((F_1 - \overline{SC})/SE(H_2)\)

The 't' calculated values for heterosis over better parent (BP) and standard check (SC) were compared with 't' tabulated values at error degree of freedom and \(P = 0.05\).

't' calculated values > 't' tabulated values were marked as significant and asterisk was put on per cent values only.

\textbf{3.5.7 Stability analysis}

Parameters of phenotypic stability were computed, using the regression approach of Eberhart and Russell (1966).

\textbf{Eberhart and Russell (1966) model}

The parameters are defined with the following model:

\[Y_{ij} = \mu_i + b_i j + \delta_{ij}\]

\((i = 1, \ldots, g)\)

\((j = 1, \ldots, n)\)
where,

\[ Y_{ij} = \text{mean of } i^{th} \text{ variety in the } j^{th} \text{ environment} \]

\[ \mu_i = \text{mean of } i^{th} \text{ genotype over all environments} \]

\[ b_i = \text{regression co-efficient of the } i^{th} \text{ genotype on the environmental index which measures the response of this genotype to varying environments} \]

\[ \delta_{ij} = \text{The deviation from regression of the } i^{th} \text{ genotype at the } j^{th} \text{ environments} \]

\[ I_j = \text{The environmental index which is defined as the deviation of the mean of all the varieties at a given location from the overall mean i.e. mean of all genotypes at the } j^{th} \text{ environment minus grand mean} \]

3.5.7.1 Computation of stability parameters

(i) Mean performance (\( \bar{X} \)) = \( \sum \frac{Y_{ij}}{n} \)

where,

\( \sum \frac{Y_{ij}}{n} = \text{The mean of } i^{th} \text{ genotype over } 'n' \text{ environments} \)

(ii) Regression coefficient \( (b_i) = \frac{\sum Y_i I_j}{\sum I^2_j} \)

where,

\( \sum Y_i I_j = \text{sum of products of } i^{th} \text{ genotype in } j^{th} \text{ environment and } j^{th} \text{ environment index} \)

\( \sum I^2_j = \text{sum of squares due to environmental index} \)

(iii) Deviation from regression \( (S^2_{di}) = [\sum \delta_{ij}^2/(n-2)] - S^2_{e/r} \)

\( (S^2_{di} \text{ is the non linear component of genotype x environment interaction of } i^{th} \text{ genotype}). \)

where,

\[ \sum \delta_{ij}^2 = (\sum Y_i^2 - Y_{i.}^2/n) - [\sum Y_i I_j^2/\sum I^2_j] \]
\[ S^2e = \text{estimate of the pooled error of the variance of genotypic mean of the } i^{th} \text{ environment} \]
\[ r = \text{number of replications} \]

3.5.7.2 Computation of environment index (I\(_j\))

\[ I_j = \frac{\sum Y_{ij}/g - \sum Y_{ij}/g.n}{\frac{\text{Total of all varieties at } j^{th} \text{ location}}{\text{Number of genotypes}} - \frac{\text{Grand total}}{\text{Total number of observations}}} \]

Analysis of variance of multi-environment data when stability parameters are estimated following Eberhart and Russell (1966) model

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>(ng-1)</td>
<td>[ \sum \sum Y_{ij}^2 - C.F.* ]</td>
<td>0</td>
</tr>
<tr>
<td>Genotypes (G)</td>
<td>(g-1)</td>
<td>[ 1/n \sum Y_{i.}^2 - C.F. ]</td>
<td>MS(_1)</td>
</tr>
<tr>
<td>Environment (E)</td>
<td>(n-1)</td>
<td>[ 1/g \sum Y_{.j}^2 - C.F. ]</td>
<td>-</td>
</tr>
<tr>
<td>G \times E</td>
<td>(g-1)(n-1)</td>
<td>[ \sum \sum Y_{ij}^2 - \sum Y_{i.}^2/n-Y_{.j}^2/g + CF ]</td>
<td>-</td>
</tr>
<tr>
<td>E + (G\times E)</td>
<td>g(n-1)</td>
<td>[ \sum \sum Y_{ij}^2 - \sum Y_{i.}^2/r ]</td>
<td>-</td>
</tr>
<tr>
<td>Environment (Linear)</td>
<td>1</td>
<td>[ 1/g(\sum Y_{.j}^2) - C.F. ]</td>
<td>( \sigma^2e )</td>
</tr>
<tr>
<td>G \times E (Linear)</td>
<td>(g-1)</td>
<td>[ \sum \sum (Y_{ij}^2/\sum I_{ij}^2)-\text{Env(L) SS} ]</td>
<td>MS(_2)</td>
</tr>
<tr>
<td>Pooled deviation</td>
<td>g(n-2)</td>
<td>[ \sum \sum \delta_{ij}^2 ]</td>
<td>MS(_3)</td>
</tr>
<tr>
<td>Pooled deviation due to ( j^{th} ) genotype</td>
<td>(n-2)</td>
<td>[ \sum \delta_{ij}^2 ]</td>
<td>-</td>
</tr>
<tr>
<td>Pooled error</td>
<td>n(r-1)(g-1)</td>
<td>[ \text{Me'} ]</td>
<td>S(_2e) or MS(_4)</td>
</tr>
</tbody>
</table>

* C.F. = \[ \sum \sum Y_{ij}^2/gn \]
where,

$$S^2e = \sum_{j} \frac{S^2_j}{(r-1)(g-1)n}/r$$

where,

$$S^2_j = \text{Error sum of squares at the } j^{th} \text{ location}$$

### 3.5.7.3 Test of significance

(i) The significance of pooled deviation was tested against the pooled error mean of squares tested as $\text{MS}_3/\text{MS}_4$

$$F = \frac{\text{MS due to pooled deviation}}{\text{MS due to pooled error}} = \frac{\text{MS}_3}{\text{MS}_4}$$

(ii) The significance of the difference among genotypic means was tested using 'F' test:

$H_0: \mu_1 = \mu_2 = \ldots = \mu_g$

$$F = \frac{\text{MS due to genotypes}}{\text{MS due to pooled deviation}} = \frac{\text{MS}_1}{\text{MS}_3}$$

(iii) The significance of genotype x environment interaction was tested as:

$$F = \frac{\text{MS due to G x E interaction}}{\text{MS due to pooled error}}$$

(iv) To test that there are no differences among genotypes for their regression on the environmental index:

$H_0: b_1 = b_2 = \ldots = b_g$

$$F = \frac{\text{MS due to G x E (Linear)}}{\text{MS due to pooled deviation}} = \frac{\text{MS}_2}{\text{MS}_3}$$
Note: MS\textsubscript{3} was tested against S\textsuperscript{2}e. In case MS\textsubscript{3} was not significant, S\textsuperscript{2}e and MS\textsubscript{3} were pooled to test the remaining sources of variation.

(v) To test that any regression (b\textsubscript{i}) does not differ from unity, 't' test was used as follows:

\[ t = \frac{b_i - 1}{SE (b)} \text{ at } g \text{ (n-2) d.f. at 5% level of significance} \]

where,

\[ SE (b_i) = \pm \frac{(MS \text{ due to pooled deviations})}{\sqrt{\sum_{j=1}^{n} i_j^2}} \]

(vi) The deviation from regression (S\textsuperscript{2}di) for each genotype was tested using 'F'-test:

\[ F = \frac{\left(\sum_{i=1}^{n} \delta_{ij}^2 / n - 2\right)}{\text{pooled error MS}} \]

3.5.7.4 Standard errors and means

(i) Mean of regression coefficient (b) = \[ \frac{\sum_{i} b_i}{g} \]

(ii) Grand mean (\( \overline{X} \)) = \[ \frac{\text{Grand Total}}{\text{Number of observations}} \]

(iii) SE (mean) = \[ \pm \sqrt{\frac{MS \text{ due to pooled deviation}}{(n-1)}} \]

(iv) SE (difference) = \[ \pm \sqrt{\frac{2 \times MS \text{ due to pooled deviation}}{(n-1)}} \]

(v) CD (5%) = SE (d) \times 't' (5%) at pooled error df