CHAPTER III

MATERIALS AND METHODS

The present investigations were carried out at the Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during Feb-August 2004 - 2006 and at the Experimental Farm of Hill Agricultural Research and Extension Centre (HAREC), Bajaura during February-August, 2006. The details of material used and methods employed for the present investigations are discussed in detail below:

3.1 EXPERIMENTAL SITE

3.1.1 Palampur

The Experimental Vegetable Farm of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is located at an elevation of about 1290.8m above mean sea level with 32°06’ North latitude and 76°32’ East longitude, representing mid hill zone (Zone 2.2) of Himachal Pradesh and has a sub-temperate climate with high rainfall (2500mm). The soil of this zone is silty clay loam with acidic reaction.

3.1.2 Bajaura

The Experimental Farm of HAREC, Bajaura is situated at an elevation of about 1090 m above mean sea level with 31°08’ North latitude and 77° East longitude. Bajaura falls under mid-hills, sub-humid zone (Zone 2.1) of the state and is endowed with mild summers and cool winters with low monsoon rains (975mm). The soil of the location is sandy loam with acidic to neutral in reaction along with high water table.
The weather data (February-August, 2007) of Palampur and Bajaura collected from the Meteorological Observatory, Department of Agronomy, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur are presented in Appendix-I and II, respectively.

### 3.2 Experimental Material

Four parents, including two resistant (PBC-631 and IHR-546) and two susceptible (California Wonder and Yolo Wonder) to bacterial wilt disease, were involved in a crossing programme to generate the experimental material. The important characteristics of the parents involved in crossing plan along with their sources of procurement are given below:

**Table 3.1: Characteristics of the parents involved**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parents</th>
<th>Fruit shape, size and colour</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PBC-631</td>
<td>Paprika type, long and light green</td>
<td>AVRDC, Taiwan</td>
</tr>
<tr>
<td>2.</td>
<td>IHR-546</td>
<td>Triangular, medium and dark green</td>
<td>IIHR, Bangalore</td>
</tr>
<tr>
<td>3.</td>
<td>California Wonder (CW)</td>
<td>Blocky, large and green</td>
<td>IARI, Katrain</td>
</tr>
<tr>
<td>4.</td>
<td>Yolo Wonder (YW)</td>
<td>Blocky, large and green</td>
<td>IARI, Katrain</td>
</tr>
</tbody>
</table>

**3.2.1 Crossing programme**

To ascertain the genetics of bacterial wilt resistance and various morphological and biochemical traits, six generations viz., P₁, P₂, F₁, F₂, B₁ and B₂ of six crosses were developed by utilizing the four diverse parents. Details of F₁ hybrid combinations are given below:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Hybrid combination (Cross)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PBC-631 x California Wonder</td>
</tr>
</tbody>
</table>
2. PBC-631 x Yolo Wonder
3. IHR-546 x California Wonder
4. IHR-546 x Yolo Wonder
5. Yolo Wonder x California Wonder
6. IHR-546 x PBC-631

The $F_1$ seed of the above crosses was produced at Palampur in the polyhouse during summer-rainy season, 2004. $F_1$s were then selfed and backcrossed with both the parents ($P_1$ and $P_2$) to get $F_2$, $B_1$ and $B_2$ seeds, respectively at Palampur in the polyhouse during summer-rainy season in 2005. Simultaneously, crosses were also attempted in the second year to generate $F_1$s to have sufficient seed for final evaluation.

### 3.4 Recording of Data

#### 3.4.1 Genetics of bacterial wilt resistance

The observations were recorded on bacterial wilt incidence at weekly intervals. Ooze test was carried to ensure the death of plants due to bacterial wilt. All the plants showing wilting symptoms were subjected to ooze test up to final count (90 days after transplanting (DAT)). The plant survival data as on 90 DAT were utilized to ascertain the genetics of bacterial wilt disease. Plant survival (%) was calculated as:

\[
\text{Plant survival (%) = } \frac{\text{Number of healthy plants in the last recording}}{\text{Number of plants established}} \times 100
\]

#### 3.4.2 Genetics of morphological traits

The data were recorded on randomly tagged 5 plants per replication in the non-segregating generations ($P_1$, $P_2$ and $F_1$), 20 plants per replication in the back cross
generations (B₁ and B₂) and 40 plants per replication in the segregating generation (F₂) on the following traits:

1) **Days to 50 per cent flowering**
   Days to 50 per cent flowering were recorded from the date of transplanting to the date when fifty per cent plants in each entry had flowered.

2) **Days to first picking**
   Days to first picking were recorded from the date of transplanting to the date when at least one fruit was harvested.

3) **Fruit length (cm)**
   Fruit length (polar distance) was measured from the stem end to the blossom end.

4) **Fruit diameter (cm)**
   After recording the fruit length, the same fruits were also used for measuring the fruit width at pedicel end, middle of the fruit and near apex. These three observations were then averaged to calculate fruit diameter.

5) **Fruit pedicel length (cm)**
   The same fruits were then used to measure the pedicel length with the help of scale from free end to the other where it was attached with the fruit.

6) **Pericarp thickness (cm)**
   The same fruits were then again used to measure the pericarp thickness with the help of vernier caliper.

7) **Number of fruits per plant**
   Number of fruits picked in all the harvests were counted and finally added to work out the total number of fruits per plant.
8) **Fruit yield per plant (kg)**

The weight of fruits harvested in each picking was added to calculate the fruit yield per plant.

9) **Average fruit weight (g)**

Average fruit weight was worked out by dividing the total yield with total number of fruits.

10) **Number of pickings**

Number of pickings were counted and finally added to calculate the number of pickings per plant.

11) **Number of branches per plant**

Number of branches arising from the main stem were calculated at the end of final picking.

12) **Plant height (cm)**

It was also taken at the end of final picking and measured from soil level to the top of the central apical shoot.

### 3.4.3 Genetics of biochemical traits:

For total soluble solids, ascorbic acid content, peroxidase activity and polyphenol oxidase activity, fresh fruits were used and these characters were expressed on fresh weight basis, whereas total sugars, reducing sugars, non-reducing sugars, total phenols and ortho dihydroxy phenols were expressed on dry weight basis of fruits.

1) **Total soluble solids (%)**
Total soluble solids (TSS) of the fruits were observed under room temperature with the help of ‘ERMA Hand Refractometer’ by putting 2-3 drops of juice on prism and the values expressed as per cent of juice (A.O.A.C., 1970).

2) **Ascorbic acid content (mg/100g)**

The ascorbic acid contents were estimated by 2,6-dichlorophenol Indophenol Visual Titration Method as described by Ranganna (1979). The standard ascorbic acid solution was prepared by dissolving 100 mg of L-ascorbic acid in 100 ml of metaphosphoric acid (3%). 10 ml of this solution was diluted to 100 ml (100 ppm) with 3 per cent metaphosphoric acid.

The metaphosphoric acid (3%) solution was prepared by dissolving 15g of metaphosphoric acid in 500 ml glass distilled water. The dye was prepared by dissolving 50 mg of sodium salt of 2,6-dichlorophenol indophenol in about 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate. This was cooled and volume made upto 200 ml. To determine the dye factor, 5 ml each of standard ascorbic acid and metaphosphoric acid (3%) solution were taken in a flask and titrated against the dye to a pink colour which persisted for atleast 15 seconds. Dye factor (mg of ascorbic acid neutralized by one ml of dye) was calculated by using the following formula:

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

Here,

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

titre = volume of dye used to neutralize 5 ml of 100 ppm standard ascorbic acid solution along with 5 ml of metaphosphoric acid.
10 g of macerated sample was blended with 3 per cent metaphosphoric acid to make up the volume to 100 ml. Out of this 100 ml solution, 10 ml solution was taken and titrated against 2,6-dichlorophenol indophenol dye. The end point was determined by the appearance of rose pink colour which persisted for at least 15 seconds. The results thus obtained were expressed in terms of mg of ascorbic acid per 100 g of pulp. The ascorbic acid contents were calculated by using the following formula:

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

Here,

- \(\text{Titre} = \text{Volume of dye used to titrate the aliquot of extract of a given sample}\)

3) **Total phenols (mg/100g)**

Total phenols were determined by Folin-Ciocalteau method by Sadasivam and Manickam (1992).

**Materials:** 80 per cent ethanol, Folin-Ciocalteau Reagent (FCR), \(\text{Na}_2\text{CO}_3\) 20 per cent, standard (100 mg catechol in 100 ml water)- diluted 10 times for a working standard.

**Extraction:** Weighed 0.5 g of the sample and ground it with a pestle and mortar in 10 time volume of 80 per cent ethanol. Centrifuged the extract for 20 minutes. Collected the supernatant and re-extracted the residue with five times the volume of 80 per cent ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in 5 ml of distilled water.

**Procedure:** Pipetted out 0.1 ml aliquots of different samples into test tubes and made the volume in each tube to 3 ml with distilled water. Added 0.5 ml of FCR and after three minutes, added 2 ml of 20 per cent \(\text{Na}_2\text{CO}_3\) solution to each tube. Mixed thoroughly and
placed the tubes in a boiling water bath for exactly one minute, cooled and measured the absorbance at 650 nm against the reagent blank. Prepared a standard curve using different concentrations of catechol. From the standard curve found out the concentrations of total phenols and expressed as mg total phenols per 100 g of sample.

4) **Ortho dihydroxy phenols (mg/100g)**

Ortho dihydroxy phenols were determined by Arnow’s method (Mahadevan and Sridhar, 1986).

**Reagents:** 80 per cent ethanol, 0.5 N HCl, 1 N NaOH, Arnow’s reagent (Dissolved 10 g of sodium nitrite (NaNO₂) and 10 g of sodium molybdate (NaMoO₂) in 100 ml of water and stored in a brown bottle) and standard (100 mg catechol in 100 ml water)-diluted 10 times for a working standard.

**Procedure:** Extraction was carried out as in total phenols. Pipetted out 0.1 ml aliquots of different samples into test tubes and made the volume in each tube to 1 ml with water. Added 0.5 N HCl, 1ml of Arnow’s reagent, 10 ml of distilled water and 2 ml of 1 N NaOH and mixed thoroughly (pink colour appeared). Maintained the reagent blank without extract and measured the absorbance at 515 nm. Calculated the amount of ortho-dihydric phenols present in the sample using the standard curve prepared from working standard catechol solution at different concentrations and expressed as mg/100 g.

5) **Total free amino acids (mg/100g)**

Estimation of amino acids was done by ninhydrin method suggested by Jayaraman (1981).

**Reagents:** Ninhydrin solution (Prepared by dissolving 2 g of ninhydrin in 25 ml of methyl cellulose, to this solution 25 ml of 0.2M acetate buffer (pH 5.5) was added), 80 per cent ethanol in distilled water and glycine as standard.
**Extraction:** 0.2 g of powdered sample was extracted with 80 per cent (ethanol in water) solvent. Heated the mixture to 70-80°C during extraction. The cooled extracts were centrifuged and clear extract was concentrated.

**Procedure:** 0.1 ml of aliquot was taken and the final volume of 4.0 ml was made with distilled water. 1 ml of ninhydrin reagent was added and mixed well. The tubes were kept in boiling water bath for 15 minutes. The tubes were cooled and 1ml of 50 per cent ethanol was added to the tubes. The pink colour developed was measured at 550nm in spectrophotometer. The concentration of total free amino acids was then calculated from the standard curve prepared from glycine (0.9 mg/ml).

6) **Total sugars (mg/g)**

Total sugars were estimated by the method given by Dubois et al. (1956).

**Reagents:** 80 per cent ethanol, lead acetate, sodium acetate, 5 per cent phenol and 95.5 per cent sulphuric acid.

**Extraction:** 0.5 g of sample (dried fruit) was macerated in 50 ml of ethanol (80%) and transferred to a conical flask. The contents of the flask were then boiled on boiling water bath up to half of the volume (25 ml). The contents were filtered and filtrate was made to 98 ml with distilled water. 1 ml of saturated lead acetate solution was added to it. To remove the lead ions a pinch of sodium oxalate crystals was added and the volume was made to 100 ml with distilled water.

**Procedure:** 0.2 ml aliquot was taken in the test tube and 1 ml of 5 per cent phenol (freshly prepared) and 5 ml of 95.5 per cent of concentrated sulphuric acid was added from the top, not from the side of test tube in ice cold solution. The intensity of pink colour was read at 490 nm. The amount of sugars present in the extract was then
calculated using a standard curve from glucose (0.1 mg ml$^{-1}$).

7) **Reducing sugars (mg/g)**

Reducing sugars were estimated by the method given by Miller (1972).

**Reagents:** Dinitrosalicylic acid reagent (DNS reagent) (Prepared by dissolving 1 g dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 ml 1 per cent NaOH and stored in 4°C) and 40 per cent Rochelle salt solution (Potassium sodium tartarate).

**Procedure:** Extraction was carried out as in total sugars. Pipetted out 0.5 ml of the extract in test tubes and equalized the volume to 3 ml with distilled water in all the tubes. Added 3 ml of DNS reagent. Heated the contents in boiling water bath for 5 minutes. When the contents of the tubes were still warm, added 1ml of 40 per cent Rochelle salt solution. Cooled and read the intensity of dark red colour at 510 nm. Ran a series of standards using glucose (0.1 mg ml$^{-1}$) and calculated the amount of reducing sugars present in the sample using the standard graph.

8) **Non-reducing sugars (mg/g)**

Calculated by subtracting reducing sugars from total sugars.

9) **Peroxidase activity (change in OD/min/g)**

Peroxidase activity was calculated by the method given by Mahadevan and Sridhar (1986).

**Reagents:** 0.05 M pyrogallol dissolved in phosphate buffer at pH 6.0 and 1 per cent H$_2$O$_2$.

**Extraction:** Took 1 gm of fresh fruit tissue and placed it in pre-cooled pestle and mortar, added chilled phosphate buffer of pH 6.6 and ground it. Squeezed the extracts through 3
layers of muslin cloth to remove the pulp and centrifuged the extracts for 20 minutes at 4°C. Decanted the supernatant and used the clear extract as enzyme source.

**Procedure:** Pipetted 3 ml of pyrogallol solution and 0.1 ml of tissue extract into colorimeter tube and adjusted the absorbance to zero at 420 nm in the colorimeter. Added 0.5 ml of H$_2$O$_2$ to the tube and inverted the tube immediately to mix the contents and replaced it in the spectrophotometer. Changes in absorbance at every 30 seconds up to 3 minutes were recorded.

**10) Polyphenol oxidase activity (change in OD/min/g)**

It was also calculated by the method given by Mahadevan and Sridhar (1986).

**Reagents:** 0.01 M catechol dissolved in phosphate buffer at pH 6.0.

**Procedure:** Extraction was carried out as in peroxidase. Pipetted 2 ml of the extract and 3 ml of phosphate buffer to a colorimeter tube, mixed the contents by inverting, placed in the spectrophotometer at 495 nm and adjusted the absorbance to zero. Removed the tube and added 1 ml of catechol solution and mixed again. Placed the tube immediately in the spectrophotometer and recorded the changes for every 30 seconds up to 3 minutes.

### 3.4 Statistical Analysis

Statistical analysis for the characters studied was done at the Department of Statistics, Mathematics and Physics of College of Basic Science, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur by using the Windowstat 8.0 programme of Indostat Services, Hyderabad.

**3.4.1 The computation of generation means**

Means of various generations were calculated as:
\( \bar{X} = \frac{\sum x_i}{n} \)

where,

\( \bar{X} \) = generation mean,

\( \sum x_i \) = grand total,

\( x_i \) = \( i \)th observation in a particular generation, and

\( n \) = number of plants

### 3.4.2 Estimation of variance of generation means (\( V_x \))

The generation means were subjected to sampling variation which can be estimated by normal statistical procedure. The estimate of variance of generation mean (\( V_X \)) was obtained by dividing the variance within generation (\( V_x \)):

\[ V\bar{X} = V_x / n \]

where,

\( V_x \) (variance of the generation mean) = \( 1/(n - 1) [\sum x_i^2 - (\sum x_i)^2 / n] \)

\( x_i \) = \( i \)th observation of a population and

\( n \) = number of observations within generation

The value thus obtained was used for further analysis.

### 3.4.3 Simple scaling tests

To test the adequacy of additive-dominance model following scaling tests given by Mather (1949) and Hayman and Mather (1955) were used:

\[ A = 2B_1 - \overline{P}_1 - \overline{F}_1 \]

\[ B = 2B_2 - \overline{P}_2 - \overline{F}_1 \]

\[ C = 2F_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2 \]

\[ D = 2F_2 - B_1 - B_2 \]
The deviation of these scaling tests from zero was tested using the respective standard errors. The deviations from zero of any of these quantities indicated the inadequacy of additive-dominance model. The standard errors of the above scaling tests were calculated as follows:

\[
\begin{align*}
SE(A) &= \pm \left( 4VB_1 + VP_1 + VF_1 \right)^{1/2} \\
SE(B) &= \pm \left( 4VB_2 + VP_2 + VF_1 \right)^{1/2} \\
SE(C) &= \pm \left( 16VF_2 + VF_1 + VP_1 + VP_2 \right)^{1/2} \\
SE(D) &= \pm \left( 4VF_2 + VB_1 + VB_2 \right)^{1/2}
\end{align*}
\]

where, 

VB₁, VP₁ etc. are variances of the respective generation means. The deviations of A, B, C and D from zero, were tested using their respective standard errors (C test) as follows:

\[
\begin{align*}
C(A) &= A/SE(A), \\
C(B) &= B/SE(B), \\
C(C) &= C/SE(C), \text{ and} \\
C(D) &= D/SE(D),
\end{align*}
\]

The significance of A, B, C and D tests were tested against the value of ‘t’ tabulated by comparing the values of C(A), C(B), C(C) and C(D) at a degree of freedom which was calculated by summing up the degree of freedom appropriate to the sampling variance of each generation involved in a particular test. The significant deviation of any of the scaling tests A, B, C and D from zero, indicates the failure of additive-dominance model. The significant deviation of A and B tests from zero indicate the presence of [j] type of interaction (additive x dominance), C scaling test reveals the presence of [l] type of interaction (dominance x dominance), and D scaling test indicates the significance of additive x additive [i] type of gene interaction.
3.4.4 Estimation of genic effects

Estimation of various gene effects and test of fitness of appropriate genetic model was done following ‘joint scaling test’ of Cavalli (1952), as described in detail by Jinks and Jones (1958). Joint scaling test involves estimation of various genetic parameters by using the observed means of different generations. Estimates of these parameters obtained through appropriate weighted least square analysis were used to calculate expected means and were then compared with observed means. The estimation of genic effects and Chi-square test of goodness of fit were carried out first for fitting of a 3-parameter model. In case of three parameter model (additive-dominance model) the following genic effects were estimated:

\[ m = \text{general mean} \]
\[ [d] = \text{additive} \]
\[ [h] = \text{dominance} \]

where, additive-dominance model was a failure, a 6 parameter model was used and following genic effects were estimated:

\[ m = \frac{1}{2} P_1 + P_2 + 4F_2 - 2B_1 - 2B_2 \]
\[ [d] = \frac{1}{2} P_1 - \frac{1}{2} P_2 \]
\[ [h] = 6B_1 + 6B_2 - 8F_2 - F_1 - (3/2) P_1 - (3/2) P_2 \]
\[ [i] = 2B_1 + 2B_2 - 4F_2 \]
\[ [j] = 2B_1 - P_1 - 2B_2 + P_2 \]
\[ [l] = P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2 \]
Since the number of estimated parameters are equal to the number of
generations used, no degree of freedom was left for testing adequacy of the model.
However, standard errors of the parameters were obtained in the usual way as
suggested by Mather and Jinks (1982). The standard errors were calculated as follows:

\[
\begin{align*}
\text{SE}[m] &= \pm \left( \frac{1}{4}V_{P1} + \frac{1}{4}V_{P2} + 16V_{F2} + 4V_{B1} + 4V_{B2} \right)^{1/2} \\
\text{SE}[d] &= \pm \left( \frac{1}{4}V_{P1} + \frac{1}{4}V_{P2} \right)^{1/2} \\
\text{SE}[h] &= \pm \left( 36V_{B1} + 36V_{B2} + 64V_{F2} + V_{F1} + \frac{9}{4}V_{P1} + \frac{9}{4}V_{P2} \right)^{1/2} \\
\text{SE}[i] &= \pm \left( 4V_{B1} + 4V_{B2} + 16V_{F2} \right)^{1/2} \\
\text{SE}[j] &= \pm \left( 4V_{B1} + V_{P1} + 4V_{B2} + V_{P2} \right)^{1/2} \\
\text{SE}[l] &= \pm \left( V_{P1} + V_{P2} + 4V_{F1} + 16V_{F2} + 16V_{B1} + 16V_{B2} \right)^{1/2}
\end{align*}
\]

The significance of \([m], [d], [h], [i], [j]\) and \([l]\) was tested using their respective standard
errors (t-test). The significance of these parameters was tested against the table value of
\('t'\) at a degree of freedom obtained by summing up the degree of freedom appropriate to
the sampling variance of each generation involved in computing a particular parameter.

3.4.5 Analysis of variance

For working out the analysis of variance, the data were analysed by using the following
model as suggested by Panse and Sukhatme (1984).

\[ y_{ij} = u + g_i + r_j + e_{ij} \]

where,

- \(y_{ij}\) = phenotypic observation of \(i\)th entry in \(j\)th replication,
- \(u\) = general mean,
- \(g_i\) = effect of \(i\)th entry,
- \(r_j\) = effect of \(j\)th replication, and
- \(e_{ij}\) = error component.
Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean squares</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>(r-1)</td>
<td>Mr</td>
<td>$\sigma^2_e + g\sigma^2_r$</td>
</tr>
<tr>
<td>Entries</td>
<td>(g-1)</td>
<td>Mg</td>
<td>$\sigma^2_e + r\sigma^2_g$</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(g-1)</td>
<td>Me</td>
<td>$\sigma^2_e$</td>
</tr>
</tbody>
</table>

where,

\[ r = \text{number of replications}, \]
\[ g = \text{number of entries}, \]
\[ \sigma^2_g = \text{variance due to entries}, \]
\[ \sigma^2_r = \text{variance due to replications}, \]
\[ \sigma^2_e = \text{error variance} \]

The replications and treatments mean squares were tested against error mean squares by ‘F’ test for (r-1), (r-1)(g-1) and (g-1), (r-1)(g-1) degrees of freedom at 5% level of significance ($P \leq 0.05$). From this analysis, the following standard errors were calculated where the ‘F’ test was significant.

Standard error for the treatment mean:

\[ \text{SE} (m) = \pm (Me/r)^{\frac{1}{2}} \]

Standard error for the difference of treatment mean:

\[ \text{SE} (d) = \pm (2Me/r)^{\frac{1}{2}} \]

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

Coefficient of variation (CV)% = \frac{(Me)^{1/2}}{\text{General mean}} x 100

### 3.4.6 Estimation of correlation at phenotypic and genotypic levels

For computing phenotypic and genotypic coefficients of correlation, analysis of co-variance was carried out.

#### Analysis of co-variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Mean sum of square</th>
<th>Expected mean sum of product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>(r−1)</td>
<td>Mr_{xy}</td>
<td>σ_{e_{xy}} + \nu.σ_{r_{xy}}</td>
</tr>
<tr>
<td>Genotypes</td>
<td>(v−1)</td>
<td>Mv_{xy}</td>
<td>σ_{e_{xy}} + r.σ_{v_{xy}}</td>
</tr>
<tr>
<td>Error</td>
<td>(r−1)(v−1)</td>
<td>Me_{xy}</td>
<td>σ_{e_{xy}}</td>
</tr>
</tbody>
</table>

where,

Error co-variance (σ_{e_{xy}}) = Me_{xy}

Genotypic co-variance (σ_{v_{xy}}) = \frac{(Mv_{xy} - Me_{xy})}{r}

Phenotypic covariance (σ_{p_{xy}}) = σ_{v_{xy}} + σ_{e_{xy}}

The phenotypic and genotypic coefficients of correlation were computed following Al-Jibouri et al. (1958):

Phenotypic coefficient of correlation (r_{p_{xy}}):

\[ r_{p_{xy}} = \frac{σ_{p_{xy}}}{(σ^2_{p_x} \cdot σ^2_{p_y})^{1/2}} \]

Genotypic coefficient of correlation (r_{v_{xy}}):
\[ r_{v_{xy}} = \frac{\sigma v_{xy}}{(\sigma^2 v_x \cdot \sigma^2 v_y)^{1/2}} \]

where,

\[ \sigma p_{xy} = \text{Phenotypic covariance between two traits, x and y} \]
\[ \sigma v_{xy} = \text{Genotypic covariance between two traits, x and y} \]
\[ \sigma^2 p_x \text{ and } \sigma^2 p_y = \text{Phenotypic variance of traits, x and y, respectively} \]
\[ \sigma^2 v_x \text{ and } \sigma^2 v_y = \text{Genotypic variance of traits, x and y, respectively} \]

The significance of phenotypic coefficient of correlation was tested against ‘r’ values as given by Fisher and Yates (1963) at \( n-2 \) degree of freedom, where ‘n’ denotes number of genotypes.

### 3.4.7 Estimates of direct and indirect effects

Path-coefficient is a standardized partial regression coefficient. It permits the partitioning of coefficients of correlation into direct and indirect effects. The path coefficient analysis of component traits with plant survival were carried out by following Dewey and Lu (1959) as under:

\[
\begin{align*}
Py_1 + Py_2.r_{12} + Py_3.r_{13} + \ldots \ldots + Py_n.r_{1n} &= ry_1 \\
Py_1.r_{12} + Py_2 + Py_3.r_{23} + \ldots \ldots + Py_n.r_{2n} &= ry_2 \\
Py_1.r_{13} + Py_2.r_{23} + Py_3 + \ldots \ldots + Py_n.r_{3n} &= ry_3 \\
&\vdots \;
Py_1.r_{n1} + Py_2.r_{n2} + Py_3.r_{n3} + \ldots \ldots + Py_n &= ry_n
\end{align*}
\]

where,

\[ Py_1, Py_2, Py_3 \ldots \ldots Py_n \text{ are the direct path effects of 1, 2, 3, \ldots \ldots, n variables on the dependent variable ‘y’.} \]
\[ r_{12}, r_{13}, \ldots, r_{(n-1)n} \] are the possible coefficients of correlation between various independent variables and \[ r_{y1}, r_{y2}, r_{y3}, \ldots, r_{yn} \] are the correlation coefficients of independent variables with dependent variable ‘y’.

The variation in the dependent variable which remained undetermined by including the given variables was assumed to be due to variable(s) not included in the present investigation. The degree of determination \((P^2 \times R)\) of such variable(s) on the dependent variable was calculated as follows:

\[
\text{Residual effect (P X R)} = (1 - R^2)^{1/2}
\]

\[
R^2 = P_{y1}r_{y1} + P_{y2}r_{y2} + \ldots + P_{yn}r_{yn}
\]

where, \(R^2\) is the square multiple correlation coefficient and is the amount of variation in yield that can be accounted for by the yield component characters.

### 3.4.8 Estimation of heterosis

The estimates of heterosis were calculated as the deviation of F\(_1\) mean \((\bar{F}_1)\) from the better parent (BP) and standard check.

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

2. Heterosis over the standard check (SC) % \[ \frac{F_1 - \bar{SC}}{\bar{SC}} \times 100 \]

### 3.4.9 Calculation of standard errors

1) SE for testing heterosis over BP = \(\pm \sqrt{2Me/r} = SE (H_1)\)

2) SE for testing heterosis over SC = \(\pm \sqrt{2Me/r} = SE (H_2)\)

### 3.4.10 Test of significance for heterosis
\[
\frac{F_1 - \text{BP}}{\text{SE}(H_1)} = \text{'t'}_1 \text{ calculated value}
\]

\[
\frac{F_1 - \text{SC}}{\text{SE}(H_2)} = \text{'t'}_2 \text{ calculated value}
\]

The 't' calculated values (t₁, t₂) for heterosis over better parent (BP) and standard check (SC), respectively were compared with 't' tabulated values for error degree of freedom at \( P = 0.05 \).