3. MATERIALS AND METHODS

The present investigation was undertaken at the Experimental Farm of Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur from February-August 2010 to 2012. The details of material used and methods employed are presented below:

3.1 EXPERIMENTAL SITE

3.1.1 Location

The Experimental Farm is situated at 32° 6’ N latitude and 76° 3’ E longitude at an elevation of 1290.8 m (a.m.s.l.).

3.1.2 Climate

The place experiences severe winters and mild summers with high rainfall. Agro-climatically, the location represents the mid-hill zone of Himachal Pradesh (zone-II) and is characterized by humid sub-temperate climate with high rainfall (2500 mm) of which 80 per cent is received during June to September. The soil is acidic in nature with pH ranging from 5.0 to 5.6 and soil texture is silty clay loam. The mean weekly meteorological data as recorded at Meteorological Observatory of the Department of Agronomy during the crop growing period of location is given in Appendix-I and Figure 3.1.

3.2 EXPERIMENTAL MATERIALS

Four parents, including two resistant parents (EC-464107 and EC-464115), one moderately resistant (Kandaghat Selection) and one susceptible parent (Sweet Happy-I) to bacterial wilt disease, were involved in a crossing programme to generate the experimental material (Plate 3.1). The details of parents used are given below:
Figure 3.1: Mean weekly meteorological data (March to August, 2012)
Plate 3.1: Parents and standard checks used in the investigation

EC-464107 (R1)
EC-464115 (R2)
California Wonder (Check)
Kandaghat Selection
Indira (Check)
Sweet Happy-I
California Wonder (Check)
Indira (Check)
Table 3.1: Characteristics of the parents involved in the study

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parents</th>
<th>Growth habit</th>
<th>Fruit shape, size and colour</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EC-464107</td>
<td>Indeterminate</td>
<td>Blocky, medium and yellowish green</td>
<td>AVRDC, Taiwan</td>
</tr>
<tr>
<td>2</td>
<td>EC-464115</td>
<td>Indeterminate</td>
<td>Blocky, medium and yellowish green</td>
<td>AVRDC, Taiwan</td>
</tr>
<tr>
<td>3</td>
<td>Kandaghat Selection</td>
<td>Determinate</td>
<td>Blocky, medium and dark green</td>
<td>RRS, Kandaghat, UHF, Solan</td>
</tr>
<tr>
<td>4</td>
<td>Sweet Happy-I</td>
<td>Determinate</td>
<td>Blocky, large and dark green</td>
<td>CSK HPKV, Palampur</td>
</tr>
</tbody>
</table>

3.2.1 Pollination Work

3.2.1.1 Development of six generations to study the genetics of horticultural traits

Two bacterial wilt resistant parents viz., EC-464107 and EC-464115, one moderately resistant (Kandaghat Selection) and one susceptible parent (Sweet Happy-I) were used to conduct the present investigation. The following crosses were made to produce F1 hybrid seed in the polyhouse during summer-rainy and autumn-winter, 2010 [Plate 3.2 (a, b, c & d)].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Crosses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EC-464107 × Kandaghat Selection</td>
</tr>
<tr>
<td>2</td>
<td>EC-464115 × Kandaghat Selection</td>
</tr>
<tr>
<td>3</td>
<td>EC-464107 × EC-464115</td>
</tr>
<tr>
<td>4</td>
<td>EC-464107 × Sweet Happy-I</td>
</tr>
</tbody>
</table>

Each F1 was raised to obtain F2 seed and simultaneously backcrossed to both the parents to produce B1 and B2 seeds in the polyhouse during summer-rainy and autumn-winter seasons, 2011. The details of the seeds obtained are as below:
F₂ seeds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Crosses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EC-464107 × Kandaghat Selection</td>
</tr>
<tr>
<td>2</td>
<td>EC-464115 × Kandaghat Selection</td>
</tr>
<tr>
<td>3</td>
<td>EC-464107 × EC-464115</td>
</tr>
<tr>
<td>4</td>
<td>EC-464107 × Sweet Happy-I</td>
</tr>
</tbody>
</table>

Back cross-1 (B₁) seeds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Backcrosses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(EC-464107 × Kandaghat Selection) × EC-464107</td>
</tr>
<tr>
<td>2</td>
<td>(EC-464115 × Kandaghat Selection) × EC-464115</td>
</tr>
<tr>
<td>3</td>
<td>(EC-464107 × EC-464115) × EC-464107</td>
</tr>
<tr>
<td>4</td>
<td>(EC-464107 × Sweet Happy-I) × EC-464107</td>
</tr>
</tbody>
</table>

Back cross-2 (B₂) seed:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Backcrosses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(EC-464107 × Kandaghat Selection) × Kandaghat Selection</td>
</tr>
<tr>
<td>2</td>
<td>(EC-464115 × Kandaghat Selection) × Kandaghat Selection</td>
</tr>
<tr>
<td>3</td>
<td>(EC-464107 × EC-464115) × EC-464115</td>
</tr>
<tr>
<td>4</td>
<td>(EC-464107 × Sweet Happy-I) × Sweet Happy-I</td>
</tr>
</tbody>
</table>

All the parents were crossed to develop the hybrid seed by hand using the recommended procedure of hand emasculation and pollination in the low tunnel polyhouse.

3.2.1.2 Development of six generations to study the genetics of bacterial wilt resistance

To ascertain the genetics of bacterial wilt resistance, six generations viz., P₁, P₂, F₁, F₂, B₁ and B₂ of four crosses were developed by utilising two resistant parents (EC-464107 and EC-464115), one moderately resistant (Kandaghat Selection) and one susceptible parent (Sweet Happy-I). Details of hybrid combinations are as follows:
Plate 3.2 (a, b c & d): Raising of parents and pollination work for development of various generations
The F₁ seed of the above four crosses were produced in the low tunnel polyhouse during summer-rainy and autumn-winter, 2010. The F₁’s were selfed and back crossed with both the parents (P₁ and P₂) to get F₂, B₁ and B₂ seeds respectively during summer-rainy and autumn-winter seasons in 2011.

3.3 EXPERIMENTAL LAYOUT, NURSERY SOWING AND TRANSPLANTING

3.3.1 Genetics of Horticultural Traits

The seeds of six generations (P₁, P₂, F₁, F₂, B₁ and B₂) of four crosses were raised in growing media in plastic pro-trays contained mixture of Cocopeat, Vermiculite, Perlite in ratio of 3:1:1 (volume basis) in low tunnel polyhouse on February 4, 2012 [Plate 3.3 (a, b, c & d)]. The seeds were treated with the fungicide Diathane M-45 and Bavistin before sowing. Seedlings were transplanted on 11th and 12th April, 2012 in a Randomized Complete Block Design with three replications. The plants were spaced at 60 cm (inter-row) × 45cm (intra-row).

3.3.2 Genetics of Bacterial Wilt Resistance

Seeds of six generations viz., P₁, P₂, F₁, F₂, B₁ and B₂ of four crosses evolved to study the genetics of bacterial wilt resistance were raised in growing media in plastic pro-trays inside the low tunnel polyhouse on February 4, 2012. Seedlings were transplanted in bacterial wilt sick plots on April 10, 2012 crosswise in a Randomized Complete Block Design with three replications. The spacings were kept as 30cm inter row and 20cm intra-row. Each experimental plot consisted of 1 row of 3m length each for P₁, P₂ and F₁, 4 rows for B₁, B₂ and 7 rows for each F₂. A row of each of susceptible check varieties Indira (hybrid) and California Wonder (open pollinated variety) included after every 15th row to ensure uniform distribution of inoculum in the experimental fields.
3.3.3. Cultural Practices

Beside the application of Farm Yard Manure (FYM) @ 20t/ha⁻¹, the chemical fertilizers were applied as per the recommended package of practices (90Kg N, 75Kg P₂O₅ and 50Kg K₂O) ha⁻¹. One -third dose of N and full doses of P₂O₅ and K₂O were applied at the time of field preparation. Remaining two third dose of N was top dressed in equal amounts after 30 and 45 days of transplanting. Except FYM before transplanting and foliar application of urea at an interval of 10-15 days, no fertilizer was added in the experiment material used to ascertain the genetics of bacterial wilt resistance so as to restrict vegetative growth of the plants.

3.4 RECORDING OF DATA

3.4.1 Genetics of Bacterial Wilt Resistance

The observations were recorded on bacterial wilt incidence at weekly interval. Ooze test was carried out 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 Horticultural Traits

3.4.2.1 Quantitative traits

The data were recorded on randomly tagged 5 plants per replication (excluding border plants) in the non-segregating generations (P₁, P₂ and F₁), 30 plants per replication in the back cross generations (B₁ and B₂) and 60 plants per replication in the segregating generation (F₂) on the following traits:

(A) Phenological and structural traits

1. Days to first flower

Days to first flower was recorded from the date of transplanting to the date when first flower on plant in each entry had flowered.
Plate 3.3 (a, b, c & d): Raising of healthy nursery in plastic plug protrays
2. **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.

3. **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.

4. **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.

5. **Primary branches per plant**

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

6. **Harvest duration (days)**

   Total numbers of days from first picking to final picking of marketable fruits for each genotype were recorded.

7. **Fruit position**

   Fruit position was recorded at full mature green stage to classify genotypes as pendent, semi-pendent and erect.

8. **Lobes per fruit**

   The number of lobes were counted at the middle of the fruit and mean values were computed.

(B) **Fruit yield traits**

1. **Gross fruit yield per plant (g)**

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

2. **Total fruits per plant**

   Number of fruits picked in all the harvests were counted and finally added to work out the total fruits per plant.
3. **Marketable fruit yield per plant (g)**

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

4. ** Marketable fruits per plant**

   Number of marketable fruits picked from individual plant were counted at each harvest and finally summed up to work out the marketable fruits per plant.

5. **Fruit length (cm)**

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

6. **Fruit width (cm)**

   After recording the fruit length, the same fruits were also used for measuring the fruit width at stem end, middle of the fruit and blossom end and mean values were computed.

7. **Average fruit weight (g)**

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

(C) **Quality Traits**

1. **Fruit colour**

   The colour of the fruits compared with Royal Horticultural Society (RHS) colour charts and classified into green group (GG) and yellow green group (YGG) categories. Along with this, fruit colour was also recorded on the basis of visual observation and genotypes were categorized as dark green (DG), medium dark green (MDG), medium light green (MLG) and yellow green (YG) fruits.

   The efforts are made for characterization of these genotypes on the basis of their morphology. Hence, these genotypes were critically observed as par the ‘Minimum Descriptor of Vegetable crops’ for bell pepper suggested by Srivastava et al. (2001) for the following characters *viz.*, fruit shape, fruit shape at pedicel attachment and blossom end fruit shape.

2. **Fruit shape**

   This observation was taken at near maturity stage of fruits. On the basis of fruit shape (Figure 3.2), the genotypes were categorized into four groups namely, blocky, triangular, elongate and companulate.
Figure 3.2: Fruit shape
3. **Fruit shape at pedicel attachment**

This character was observed at near maturity stage of fruits (Figure 3.3) and the genotypes were classified into cordate, truncate, lobate, obtuse and acute groups.

![Figure 3.3: Fruit shape at pedicel end](image)

4. **Blossom end fruit shape**

Blossom end fruit shape was recorded at near maturity stage (Figure 3.4) and the genotypes were divided into pointed, blunt, sunken and sunken and pointed groups.

![Figure 3.4: Blossom end fruit shape](image)

5. **Pericarp thickness (mm)**

After recording the fruit length and fruit width, the same fruits were used for measuring the pericarp thickness in millimetres with the help of vernier caliper and mean values were computed.

6. **Ascorbic acid content (mg/ 100 g)**

The ascorbic acid contents were estimated by 2, 6-dichlorophenol Indophenol Visual Titration Method as described by Ranganna (1979).
Reagents:

a) Three per cent metaphosphoric acid (HPO$_3$): Prepared by dissolving the sticks or pellets of HPO$_3$ in 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 distilled water containing 42 mg of sodium bicarbonate. The solution was cooled and diluted with 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 HPO$_3$ 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 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 ascorbic acid content was calculated by using the following formula and expressed as mg of ascorbic acid/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,

\[\text{Titre} = \text{Volume of dye used to titrate the aliquot of extract of a given sample}\]

7. **Capsaicin content (%)**

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

**Reagents**

1. Acetone/ Ethyl acetate
2. Aluminium oxide active basic
3. Folin and ciocalteau’s phenol (FC) reagent (available as 2N, diluted with equal volume of distilled water just before use).
4. 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 Na₂CO₃ 10 H₂O was filtered through glass wool to obtain the mother liquid.
5. Methanol (CH₂O)

**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 Na₂CO₃ 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.
- Ten ml volume was evaporated to dryness at temperature less than 65°C and the colour was developed as for calibration curve.

c) Calculations:

Capsaicin content (%)  
\[
\frac{\text{Standard concentration} \times \text{Volume made up} \times \text{OD of sample}}{\text{OD (standard)} \times \text{weight of sample} \times \text{Volume of sample used}} \times 100
\]

3.5. **BIOMETRICAL ANALYSIS**

3.5.1 **Computation of Generation Means**

Means of various generations were calculated from individual plant data as:

\[
\bar{X} = \frac{\sum x_i}{n}
\]
Where,

\[ \bar{X} = \text{generation mean}, \]
\[ \sum x_i = \text{grand total}, \]
\[ x_i = i^{th} \text{ observation in a particular generation, and} \]
\[ n = \text{number of plants} \]

### 3.5.2 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 means \((V_x)\) was obtained by dividing the variance within generation \((V_x)\):

\[ V_{\bar{X}} = \frac{V_x}{n} \]

Where,

\[ V_x (\text{variance of the generation mean}) = \frac{1}{n-1} \left[ \sum x_i^2 - \left( \sum x_i \right)^2 / n \right] \]
\[ x_i = i^{th} \text{ observation of a population and} \]
\[ n = \text{number of observations within generation} \]

The value thus obtained was used for further analysis.

### 3.5.3 Scaling Tests

#### 3.5.3.1 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:

\[ A = 2\bar{B}_1 - \bar{P}_1 - \bar{F}_1 \]
\[ B = 2\bar{B}_2 - \bar{P}_2 - \bar{F}_1 \]
\[ C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2 \]
\[ D = 2\bar{F}_2 - \bar{B}_1 - \bar{B}_2 \]

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:

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

Where,

\(VB_1, VP_1\) 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:

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

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.

\textit{e.g.} For Scale A \( = 2\overline{B}_1 - \overline{P}_1 - F_1 \)

- Population size for \(B_1= 30\) plants/replication
- Population size for \(P_1= 5\) plants/replication
- Population size for \(F_1= 5\) plants/replication

So total population size for scale A will be equal to 90 + 15 + 15 = 120 and total degree of freedom = 120-1=119 (Table value of ‘t’ at 119 \(df\) (0.05%) = 1.98)

For a population size more than 30, we take a constant value of ‘t’ as 1.96.

When calculated value for the respective scale is more than the tabulated value, the scale will be considered significant. The significant of A and B tests indicates the presence of all three types of epistatic interactions \(\text{viz.,} \) additive \(\times\) additive [i], additive \(\times\)
dominance \([j]\) and dominance \(\times\) dominance \([l]\) whereas C scaling test reveals the presence of dominance \(\times\) dominance \([l]\) type of interaction and D scaling test indicates the significance of additive \(\times\) additive \([i]\) type of gene interaction.

3.5.3.2 Estimation of genetic effects and joint scaling test

Estimation of various genic effects and test of fitness of appropriate genetic model was done according to joint scaling test of Cavalli (1952), as described in detail by Mather and Jinks (1982). Joint scaling test in general consists of estimating genetic parameters \(m\), \([d]\) and \([h]\) by weighted least square technique, followed by comparison of observed means with their expected values derived from the estimates of the parameters. The observed and expected generation means were compared by Chi-square test with the degree of freedom equals to number of generations \((n)\) minus the number of parameters \((p)\) estimated.

In the present study, the estimation of genic effects and chi-square test of goodness of fit were carried out, using three-parameter and six-parameter models. In three-parameter model (additive-dominance model or non-epistatic model), the following genic effects were estimated:

\[
m = \text{Inbred population mean} = \frac{1}{2}\bar{P}_1+\frac{1}{2}\bar{P}_2+4\bar{F}_2-2\bar{B}_1-2\bar{B}_2
\]

\([d]\) = additive gene effects

\[
[d] = \frac{1}{2} \bar{P}_1-\frac{1}{2}\bar{P}_2
\]

\([h]\) = dominance gene effects

\[
[h] = 6\bar{B}_1+6\bar{B}_2-8\bar{F}_2\bar{F}_1-3/2\bar{P}_1-3/2\bar{P}_2
\]

The variances for these estimates are calculated as follows.

\[
V_m = \frac{1}{4}V\bar{P}_1+\frac{1}{4}V\bar{P}_2+16V\bar{F}_2+4V\bar{B}_1+4V\bar{B}_2
\]

\[
V_d = \frac{1}{4}V\bar{P}_1+1/4V\bar{P}_2
\]

\[
V_h = 36V\bar{B}_1+36V\bar{B}_2+64V\bar{F}_2+V\bar{F}_1+9/4V\bar{P}_1+9/4V\bar{P}_2
\]
In six-parameter model (digenic interaction model), following genic effects were estimated:

\[ m = \text{Inbred population mean} = \bar{F}_2 \]
\[ [d] = \text{additive gene effects} = \bar{B}_1 + \bar{B}_2 \]
\[ [h] = \text{dominance gene effects} = \bar{F}_1 - 4\bar{F}_2 - 1/2\bar{P}_1 - 1/2\bar{P}_2 + 2\bar{B}_1 + 2\bar{B}_2 \]
\[ [i] = \text{additive} \times \text{additive gene effects} = 2\bar{B}_1 + 2\bar{B}_2 - 4\bar{F}_2 \]
\[ [j] = \text{additive} \times \text{dominance gene effects} = \bar{B}_1 - 1/2\bar{P}_1 - \bar{B}_2 + 1/2\bar{P}_2 \]
\[ [l] = \text{dominance} \times \text{dominance gene effects} = \bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 + 4\bar{F}_2 - 4\bar{B}_1 - 4\bar{B}_2 \]

The variances for these estimates are calculated as follows.

\[ V_m = \bar{V}\bar{F}_2 \]
\[ V_d = \bar{V}\bar{B}_1 + \bar{V}\bar{B}_2 \]
\[ V_h = \bar{V}\bar{F}_1 + 16\bar{V}\bar{F}_2 + 1/4\bar{V}\bar{P}_1 + 1/4\bar{V}\bar{P}_2 + 4\bar{V}\bar{B}_1 + 4\bar{V}\bar{B}_2 \]
\[ V_i = 4\bar{V}\bar{B}_1 + 1/4\bar{V}\bar{B}_2 + 16\bar{V}\bar{F}_2 \]
\[ V_j = \bar{V}\bar{B}_1 + 1/4\bar{V}\bar{P}_1 + \bar{V}\bar{B}_2 + 1/4\bar{V}\bar{P}_2 \]
\[ V_l = \bar{V}\bar{P}_1 + \bar{V}\bar{P}_2 + 4\bar{V}\bar{F}_1 + 16\bar{V}\bar{F}_2 + 16\bar{V}\bar{B}_1 + 16\bar{V}\bar{B}_2 \]

The genetic expectation of different generation means, used in the present study for the estimation of various genic effects in the presence of digenic interactions, were as follow:
For three-parameter model

\[
\begin{align*}
P_1 &= m + d \\
P_2 &= m - d \\
F_1 &= m + h \\
F_2 &= m + \frac{1}{2} h \\
B_1 &= m + \frac{1}{2} h + \frac{1}{2} d \\
B_2 &= m + \frac{1}{2} h - \frac{1}{2} d
\end{align*}
\]

For six-parameter model

\[
\begin{align*}
P_1 &= m + d + i \\
P_2 &= m - d + I \\
F_1 &= m + h + i \\
F_2 &= m + \frac{1}{2} h + \frac{1}{4} \text{l} \\
B_1 &= m + \frac{1}{2} d + \frac{1}{2} h + \frac{1}{4} \text{l} + \frac{1}{4} \text{j} + \frac{1}{4} \text{l} \\
B_2 &= m - \frac{1}{2} d + \frac{1}{2} h + \frac{1}{4} \text{l} - \frac{1}{4} \text{j} + \frac{1}{4} \text{l}
\end{align*}
\]

The statistical analysis were carried out by using ‘Windostat’ software programme developed by ‘Indostat services, Hydrabad’. The programme first tries to fit m, d and h parameter model and deletes any parameter whose ‘t’ value is less than 2.0 and thereafter it tests the model significance by Chi-square test and if this test is significant then the programme fit six-parameter model (m, d, h, i, j and l) and does a step down for non significant parameters. When all the parameters are significant then it computes Chi-square for joint scaling. The significance of Chi-square were tested at ‘6-p’ degree of freedom where ‘p’ denotes the number of significant parameter. Thus best fit model was indentified with minimum non-significant value of Chi-square and with maximum number of significant parameter as suggested by Mather and Jinks (1982). The worked example in details for marketable fruit yield per plant in the cross EC-464115 × KS to calculate the value of $\chi^2$ in three- parameter model and $\chi^2$ test of significance for three and six- parameter-model for days to first picking are given in Appendices-XXII and XXIII, respectively.
3.5.4 Evaluation of Hybrids

3.5.4.1 Analysis of Variance

For working out the analysis of variance, the data of evaluation of F₁ hybrid trails were analysed by using the following model as suggested by Panse and Sukhatme (1984).

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

Where,

\( y_{ij} \) = phenotypic observation of \( i \)th entry in \( j \)th replication,

\( \mu \) = 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>Mr/ Me</td>
</tr>
<tr>
<td>Entries</td>
<td>(g-1)</td>
<td>Mg</td>
<td>Mg/ Me</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(g-1)</td>
<td>Me</td>
<td></td>
</tr>
</tbody>
</table>

Where,

\( r \) = number of replications,

\( g \) = number of entries

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:

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

Standard error for the difference of treatment mean:

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

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).

\[ CD = SE (d) \times \text{‘t’ value at error degree of freedom at P = 0.05.} \]

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

### 3.5.4.2 Estimation of Heterosis

Heterosis effects were expressed as per cent increase (+) or (-) in the mean values of \( F_1 \) hybrid over the better parent or the standard checks Indira (SC-I) and California Wonder (SC-II) was calculated as reported by Hayman (1957). The magnitude of heterosis was estimated in relation to respective better parent (BP) and the standard check (SC)

\[
\text{Heterosis over better parent (BP) (\%) } = \frac{F_1 - \text{BP}}{\text{BP}} \times 100
\]

\[
\text{Heterosis over the standard checks (SC-I and SC-II) (\%) } = \frac{F_1 - \text{SC}}{\text{SC}} \times 100
\]

**i) Calculation of standard errors:**

1) SE for testing heterosis over BP = \( \pm \sqrt{2Me/r} \) = SE (H₁)

2) SE for testing heterosis over SC = \( \pm \sqrt{2Me/r} \) = SE (H₂)
ii) **Test of significance for heterosis:**

1) Heterosis over BP = \[ \frac{F_1 - \text{BP}}{\text{SE}(H_1)} \] = ‘t\(_1\)’ calculated value

2) Heterosis over SC = \[ \frac{F_1 - \text{SC}}{\text{SE}(H_2)} \] = ‘t\(_2\)’ calculated value

The ‘t’ calculated values (t\(_1\), t\(_2\)) for heterosis over better parent (BP) and standard checks (SCI and SCII), respectively were compared with ‘t’ tabulated values for error degree of freedom at \( P = 0.05 \).