Materials and Methods

The present investigation was undertaken in the Division of Genetics, Indian Agricultural Research Institute, New Delhi, located at an altitude of 228.16 m. Geographical location is 77°10'E latitude and 28°40'N longitude. Annual precipitation rate is around 710 mm and soil is sandy loam. Indian mustard (Brassica juncea Czern. & Coss) is grown mostly during Rabi season from October to March during 2008-2009 and 2009-2010.

Experimental Materials

Eight genetically diverse lines viz. IC 289602, Poorbijiya, IC 199715, Agra Local, Prakash, RH 30, IC 199714 and Pusa Balbir were collected from different sources and study was made for yield related parameters. These parameters of all the selected parents are listed in Table-2.

Crossing Programme

All eight parents were crossed in a half diallel mating design following method 4 as outlined by Griffing (1956b).

Experimental Layout

1) Evaluation for mean performance, combining ability effects, character association analysis and heterosis.

The experimental material consists of eight promising genotypes of Brassica juncea maintaining diversity of their parental forms. The parental types were supplemented by 28 F₁ cross combination generated through half diallel mating system. The material was sown under Randomized Block Design (RBD) with three replications to evaluate for mean performance, combining ability effects, character
association analysis and heterosis. Each plot consisted of single row of 5 m length. The distance between the rows and within the row was kept 45 cm and 15 cm respectively.

2) To study the nature of gene action

Five F₁ were selected from the above experiment on the basis of phonological behaviour and diversity to study the nature of gene action. Back crosses for both parents made in the selected F₁ during Rabi season. Seeds of F₁ form the material for F₂ generation in each cross. Three generations viz. P₁, P₂ and F₁ were evaluated during next Rabi season. The row to row and plant to plant distance was as in experiment 1.

3) To study the inheritance of white rust resistance

Parents showing varying intensities for disease were identified for this experiment. In a few F₁s of first experiment, involving parents with contrasting rust resistance, back crosses were made with both resistance and susceptible parents. The parents, F₁s and back crosses raised creating epiphytotics for scoring of disease and further analysis. The distance between rows and within the row was kept 30 cm and 10 cm respectively.

**Crop Management**

Recommended doses of nutrients @ 80 kg N₂, 60 kg P₂O₅ and 40 kg K₂O/ha were applied. Half of N₂ and entire P₂O₅ and K₂O were applied at the time of field preparation as basal dose. The remaining quantity of nitrogen was given as top dressing. Other operations were undertaken to keep the field free from weeds. Metacystox was sprayed @ 0.01 per cent as prophylactic measure and at the appearance of aphid.
Table 2: Mean of parents for twelve characters of *Brassica juncea* (L.) Czern & Coss.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Genotypes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EC - 289602</td>
<td>30</td>
<td>115</td>
<td>6</td>
<td>32</td>
<td>53.37</td>
<td>145.8</td>
<td>44</td>
<td>1700</td>
<td>175.00</td>
<td>26.33</td>
<td>15.03</td>
<td>1.44</td>
</tr>
<tr>
<td>2</td>
<td>Poorbijaya</td>
<td>45</td>
<td>107</td>
<td>6</td>
<td>20</td>
<td>52.50</td>
<td>181.17</td>
<td>33</td>
<td>1545</td>
<td>153.67</td>
<td>51.00</td>
<td>21.83</td>
<td>3.67</td>
</tr>
<tr>
<td>3</td>
<td>IC - 199715</td>
<td>58</td>
<td>145</td>
<td>6</td>
<td>20</td>
<td>70.43</td>
<td>216.37</td>
<td>55</td>
<td>1328</td>
<td>218.67</td>
<td>58.33</td>
<td>26.68</td>
<td>5.23</td>
</tr>
<tr>
<td>4</td>
<td>Agra Local</td>
<td>59</td>
<td>130</td>
<td>8</td>
<td>21</td>
<td>65.80</td>
<td>168.03</td>
<td>41</td>
<td>1255</td>
<td>221.67</td>
<td>38.00</td>
<td>17.23</td>
<td>3.59</td>
</tr>
<tr>
<td>5</td>
<td>Prakash</td>
<td>45</td>
<td>107</td>
<td>7</td>
<td>25</td>
<td>69.80</td>
<td>193.17</td>
<td>48</td>
<td>1710</td>
<td>325.33</td>
<td>67.33</td>
<td>25.80</td>
<td>4.33</td>
</tr>
<tr>
<td>6</td>
<td>RH - 30</td>
<td>68</td>
<td>148</td>
<td>8</td>
<td>22</td>
<td>67.30</td>
<td>190.53</td>
<td>53</td>
<td>1300</td>
<td>220.00</td>
<td>53.67</td>
<td>24.60</td>
<td>3.77</td>
</tr>
<tr>
<td>7</td>
<td>IC - 199714</td>
<td>66</td>
<td>140</td>
<td>6</td>
<td>20</td>
<td>60.50</td>
<td>191.10</td>
<td>60</td>
<td>1200</td>
<td>194.67</td>
<td>47.00</td>
<td>24.17</td>
<td>5.70</td>
</tr>
<tr>
<td>8</td>
<td>Pusa Bahar</td>
<td>40</td>
<td>105</td>
<td>5</td>
<td>15</td>
<td>59.77</td>
<td>180.00</td>
<td>42</td>
<td>1665</td>
<td>127.33</td>
<td>52.33</td>
<td>24.60</td>
<td>4.53</td>
</tr>
</tbody>
</table>

Where, 1-Days to 50% flowering, 2-Days to maturity, 3-Number of primary branches, 4-No. of secondary branches, 5-Length of main axis (cm), 6-Plant Height (cm), 7-No. of siliquae on main axis, 8-Seed yield per 100 siliquae, 9-Biological yield per plant, 10-Seed yield per plant, 11-Harvest index (%), 12-1000 seed weight.

**Recording of observations**

Observations were recorded for developmental, quantitative and qualitative characters on single plant basis. It was recorded for the following characters:

1. Days to 50% flowering – Days to 50% flowering were taken as the number of days taken from the date of sowing to 50% plants bore flowers.
2. Days to maturity – Days to maturity was recorded as the number of days from the date of sowing of seeds to the date when more than 80% plants matured.

3. Plant height – Height was measured in centimeters from the base of the plant to the apex of the main axis.

4. Length of main axis (cm) – Main axis was measured in centimeters from the base of terminal primary branch to the tip of the main axis.

5. Number of primary branches/ plant – Primary branches, arising from the main axis, were counted.

6. Number of secondary branches/ plant – Branches arising from primary branches were counted.

7. Number of siliquae on the main axis – Number of siliquae on the main axis, at the time of maturity were counted for each of the sampled plant.

8. Seed yield from 100 siliquae – Seeds obtained from the 100-siliquae from five sampled plants were weighed.

9. Biological yield per plant – Average weight of five sampled plant was recorded in grams.

10. Seed yield per plant – Average seed yield of five sampled plants was recorded in grams.
11. 1000 – Seed weight – 1000-seed were counted by electric seed counter (Numigral II) and weight was recorded in grams by mattler electric balance from each plant seed and average weight of five plant’s 1000-seed was taken as 1000-seed weight.

12. Harvest index – This is calculated from the given formula

\[ \text{Harvest Index} = \left( \frac{\text{Economic yield}}{\text{biological yield}} \right) \times 100 \]

Where economic yield is seed yield per plant.

13. White rust infection index – Here infection on leaves were recorded in percentage on each plant of every generation in experiment 3. The data was recorded in two stages and were as follows –
   a. Six leaves were taken from each of the plants from lower position before flowering.
   b. At the full pod stage

The severity of the disease was recorded by using the scale as per Anonymous (1985) in six grades viz., 0,1,2,3,4 and 5 representing 0,3,10,25,40 and more than 40 percent leaf area covered by white rust pustules, respectively. Percent infection index was calculated by the formula outlined by Singh (1984).

\[ \text{Percent Infection Index} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of rating} \times \text{Maximum disease grade}} \times 100 \]

Where, disease rating = grade \times \text{number of leaves in the grade}

The infection index in percentage was then subjected to angular transformation as advocated by Fisher and Yates (1957).

Statistical analysis
The analysis of variance (ANOVA) for mean performance of parental types and their cross combinations were carried out along with character association studies as suggested by Fisher (1954) and Fisher & Yates (1967). Genotypic and phenotypic coefficient of variation were carried out as per Burton and De Vane (1953). Heritability and genetic advance in a broad sense were carried out following Allard (1960). The statistical analysis from the generated data from the experiment was carried out through computer programming by following different approaches as below:

- Gca and sca effects were carried out following Griffing (1956a)
- Generation mean analysis through scaling test was done as per Hayman (1958)
- Heterosis estimation was worked out after Turner (1953) and Hayes et al (1955)

**ANOVA of Experiment 1**

Averages of replication wise data of the treatments were analysed by following methods outlined by Fisher (1958). Treatment variation was further divided into variation due to parents, crosses and parents vs crosses. The partitioning of total variation was done as below.

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.S.</th>
<th>F – Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>(r-1)</td>
<td>Sr</td>
<td>Mr</td>
<td>Mr/Me</td>
</tr>
<tr>
<td>Treatments</td>
<td>(t-1)</td>
<td>St</td>
<td>Mt</td>
<td>Mt/Me</td>
</tr>
<tr>
<td>Parents</td>
<td>(p-1)</td>
<td>Sp</td>
<td>Mp</td>
<td>Mp/Me</td>
</tr>
<tr>
<td>Crosses</td>
<td>p(p-1)/2</td>
<td>Sh</td>
<td>Mh</td>
<td>Mh/Me</td>
</tr>
<tr>
<td>Parents</td>
<td>Vs</td>
<td>1</td>
<td>Sph</td>
<td>Mph/Me</td>
</tr>
<tr>
<td>Crosses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(t-1)</td>
<td>Se</td>
<td>Me</td>
<td></td>
</tr>
</tbody>
</table>

Where; \( r \) = number of replications, \( t \) = number of treatments, \( p \) = number of parents, DF = degree of freedom, SS = Standard deviation squares, MSS = mean of SS, \( e \) = error
Combining Ability Analysis

Combining ability analysis was done as outlined by Griffing (1956 b) for Method 4. The mathematical model for the combining ability was assumed to be.

\[ X_{ij} = \mu + g_i + g_j + s_{ij} + r_j + 1/bckl \Sigma \Sigma e_{ijkl} \]

\[ ij = 1 \ldots \ldots p \]
\[ k = 1 \ldots \ldots b \]
\[ l = 1 \ldots \ldots c \]

Where, \( \mu \) = population mean

\( g_i \) = general combining ability effect for the \( i^{th} \) parent

\( g_j \) = general combining ability effect for the \( j^{th} \) parent

\( s_{ij} \) = specific combining ability effect for the cross between the \( i^{th} \) and \( j^{th} \) parents such that \( r_{ij} = r_{ji} \)

\( e_{ijkl} \) = environmental effect associated with \( e_{ijkl}^{th} \) individual observation

\( p \) = number of parents

\( b \) = number of replications

\( c \) = number of individuals in each replication

The following restrictions are imposed on the combining ability elements

\[ \Sigma g_i = 0 \text{ and } \Sigma s_{ij} = 0 \text{ (for each } j) \]
The analysis of variance for method 4 giving expectations of mean squares for the assumptions of model I and model II are given below.

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>Expectations of M.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Model I</td>
</tr>
<tr>
<td>gca</td>
<td>p-1</td>
<td>S_g</td>
<td>M_g</td>
<td>[\sigma^2 + \frac{1}{\sigma_g^2 + 2(p-1) \frac{\sigma^2}{\sigma_g^2}} (p-1) + 2p]</td>
</tr>
<tr>
<td>Sca</td>
<td>p(p-1)/2</td>
<td>S_s</td>
<td>M_s</td>
<td>[\sigma^2 + \frac{2}{\sum S_j^2} \sum S_j^2 \sigma^2 + \frac{2(p^2 - p + 1) \sigma^2}{p^2}]</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(t-1)</td>
<td>S_e</td>
<td>M_e</td>
<td>[\sigma^2]</td>
</tr>
</tbody>
</table>

Where,

\[S_g = \frac{1}{2P_i} \sum (X_i + X_i)^2 - \frac{2X^2}{p^2}\]

\[S_s = \frac{1}{2ij} \sum \sum (X_{ij} + X_{ji})^2 - \frac{1}{2p_i} \sum (X_i + X_i)^2\]

The error mean square, Me was calculated as Me = Me/r where, Me is the error mean of square from ANOVA for randomized block design in the experiment.

Test of significance for variation due to gca and sca was accomplished by F - test as follows:

To test gca effects

\[F_{[p-1],m} = \frac{M_g}{M_e}\]

To test sca effects

41
Estimation of components of genetic variation

The estimation of components of genetic variation were obtained from the expectation of the mean squares for model I. The notations $\sigma^2_g$ and $\sigma^2_s$ are used for convenience for the components due to gca and sca respectively. The estimates of various components were obtained as given below:

Component due to gca

$$\frac{1}{p-1} \sum g_i^2 = \frac{\sigma^2_g}{2p} = Mg - Me$$

Component due to sca

$$2 \sum \sum s_{ij}^2 = \sigma^2_s = Ms - Me / 2$$

P(p-1)i<j

Estimation of combining ability effects

The general and specific combining ability effects were estimated as under –

Population mean = $\mu - (1/p^2) X$

Gca effect due to i\textsuperscript{th} parent = $g_i = \frac{1}{2p} (X_i + X_j) \frac{1}{p^2} X$

Sca effect due to ij\textsuperscript{th} cross

$$s_{ij} = \frac{1}{2} (X_{ij} + X_{ji}) \frac{1}{2p} (X_i + X_i + X_j + X_j) + \frac{1}{p^2} X$$

F[p(p-1)/2 m] = Ms/Me
Test of significance of estimates of combining ability effects

To test the significance of estimates of gca, sca and reciprocal effects, the respective standard errors were calculated as the square root of the variance of their estimates as below:

\[
\text{var} (g_i) = [(p-1)/2p^2] \sigma^2
\]

\[
\text{var} (S_{ij}) = [(p^2 - 2p + 2)/ 2p^2] \sigma^2 \quad \text{for} (i \neq j)
\]

The various estimates of effects were tested with the help of ‘t’ test as shown below:

\[
t = (g_i - 0) / \text{S.E.}(g_i)
\]

\[
t = (s_{ij} - 0) / \text{S.E.}(s_{ij})
\]

The calculated value of ‘t’ was compared with the tabulated value of ‘t’ at error degrees of freedom.

Test of significance for difference between combining ability effects

The critical differences were calculated as a product of Table value of ‘t’ at error degrees of freedom with the standard error of difference between estimates. The standard error of difference for the estimates were obtained as the square root of their respective variance as given below:

\[
\text{var} (g_i - g_j) = \sigma^2/p \quad \text{for} (i \neq j)
\]

\[
\text{var} (s_{ij} - s_{ik}) = [(p-1) \sigma^2e]/p \quad \text{for} (i \neq j; j \neq k)
\]

\[
\text{var} (s_{ij} - s_{k1}) = [(p-2) \sigma^2e]/p \quad \text{for} (i \neq j; j \neq k; k \neq 1)
\]

Estimation of generation means

This approach is followed for both experiment 2 and experiment 3 data analysis. For the estimation of the magnitude of various main effects and non-allelic interactions were based on three generation means following the perfect fit
solution given by Hayman (1958) as follows:

\[
m = \text{mean} \\
= F_2 \\
[d] = \text{additive effect} \\
= B_1 - B_2 \\
[h] = \text{dominance effect} \\
= 2 B_1 - 2B_2 - 4F_2 + F_1 - 1/2P_1 - 1/2P_2 \\
[i] = \text{additive x additive interaction} \\
= 2 B_1 - 2B_2 - 4F_2 \\
j] = \text{additive x dominance interaction} \\
= 2 B_1 - 1/2P_1 - B_2 + 1/2P_2 \\
[l] = \text{Dominance x dominance interaction} \\
= P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2 \\
\]

Where,

\[P_1, P_2 = \text{mean of parental phenotypes of a cross} \]

\[F_1, F_2 = \text{mean of the progenies of first and second generation of a across.} \]

\[B_1 - B_2 = \text{means of back cross progenies with both parents respectively.} \]

The variances of genetic effects were computed using the following formulae.

\[
V_m = V_{F2} \\
V[d] = V_{B1} - V_{B2} \\
V[h] = 4V_{B1} + 4V_{B2} + 16 \ V_{F2} + V_{F1} + 1/4 \ V_{P1} + 1/4 \ V_{P2} \\
\]
\[ V_{i} = 4V_{B1} + 4V_{B2} + 16V_{F2} \]
\[ V_{j} = V_{B1} + \frac{1}{4}V_{P1} + V_{B2} + \frac{1}{4}V_{P2} \]
\[ V_{1} = V_{P1} + V_{P2} + 4V_{F1} + 16V_{F2} + 16V_{B1} + 16V_{B2} \]

The standard errors of the estimates of gene effects were calculated as the square root of the respective variance estimate.

The test of significance of the estimates were carried out by t-test. The 't' values were calculated as follows:

\[ t_{m} = \frac{[m]}{S.E.m} \]
\[ t_{d} = \frac{[d]}{S.E.d} \]
\[ t_{h} = \frac{[h]}{S.E.h} \]
\[ t_{i} = \frac{[i]}{S.E.i} \]
\[ t_{j} = \frac{[j]}{S.E.j} \]
\[ t_{1} = \frac{[1]}{S.E.1} \]

**Estimation of Heterosis**

The estimation of heterosis was done in the Experiment 1 and 2 in the following way. The average, \(F_{1}\) values over replication were used for estimation of heterosis expressed in percentage over mid parent (MP) and better parent (BP) values.

The computation of heterosis values was carried out according to Turnner (1953) and Hayes et al (1955).

Mid parent (MP) value = \(\frac{P_{1} + P_{2}}{2}\)

Percent heterosis over MP \((H_{1}) = \frac{([F_{1} - MP]/ MP)}{100} \]
Percent heterosis over BP \( (H_2) = \frac{[(F_1 - BP)/BP]}{X 100} \)

The mean deviation of MP and BP from \( F_1 \) were defined as below

\[ H_1 = F_1 - MP \]

\[ H_2 = F_1 - BP \]

The significance of \( F_1 \) heterosis was tested by comparing the mean deviations with critical values (CD) obtained by separately for MP and BP employing the formulae given below:

\[ CD \text{ for heterosis over } MP = \frac{3}{2} \times \frac{\text{EMS}}{r} \times t \text{ value} \]

\[ CD \text{ for heterosis over } BP = 2 \times \frac{\text{EMS}}{r} \times t \text{ value} \]

Where, \( r = \text{number of replications} \)

\( t = \text{tabulated value of 't' at error degrees of freedom} \)

\( \text{EMS} = \text{mean square deviation} \)

**Correlation coefficient**

The correlation coefficients were determined among 12 characters for 28 treatments of a half Diallel cross study (experiment 1) by taking appropriate variances and co-variances. The significance of phenotypic and genotypic correlation coefficient was tested against 't' values in the table of correlation coefficient (Fisher and Yates, 1963) at n-2 degree of freedom at 0.05 and 0.01 levels of probability. Phenotypic and genotypic correlations were computed as per Singh and Chaudhary (1985).

\[ \text{Phenotypic correlation} = r_{xy}(p) \]
= [cov \( xy \) (p)]/ [\( v_x \) (p) \( v_y \) (p)]\(^{1/2}\)

Genotypic correlation = \( r_{xy}(g) \)

= [cov \( xy \) (g)]/[\( v_x \) (g) \( v_y \) (g)]\(^{1/2}\)

Where,

\( Cov\ xy\ (p)\) and \( cov\ xy\ (g)\) = phenotypic and genotypic co-variances between x and y

\( V_x\ (p)\) and \( V_x\ (g)\) = phenotypic and genotypic variance of character x

\( V_y\ (p)\) and \( V_y\ (g)\) = phenotypic and genotypic variance of character y