CHAPTER – III

MATERIAL AND METHODS

The present studies were conducted to trace the path of Heterosis and combining abilities for various quantitative traits of cotton *Gossypium hirsutum* L. at research area of cotton, at Salem District during the years 2010 – 2013. Different experiments were also conducted to know the precise physico-chemical properties of the locality where the field experiments were carried out which are presented in the following table. The soil was clayey loam in texture, slightly calcareous having pH 7.7. Organic matter, N and P contents were found low (0.78, 0.05, 4.98).

Physico-Chemical properties of soil

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay (%)</td>
<td>40</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>38</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>30</td>
</tr>
<tr>
<td>Texture Class</td>
<td>Loam</td>
</tr>
<tr>
<td>pH</td>
<td>7.7</td>
</tr>
<tr>
<td>ECe (mmhos/cm)</td>
<td>3.2</td>
</tr>
<tr>
<td>CaCO₃ %</td>
<td>128</td>
</tr>
<tr>
<td>Organic Matter %</td>
<td>0.80</td>
</tr>
<tr>
<td>Total N %</td>
<td>0.45</td>
</tr>
<tr>
<td>Available P (ppm)</td>
<td>5.98</td>
</tr>
<tr>
<td>Available K (ppm)</td>
<td>131</td>
</tr>
<tr>
<td>Available Zn (ppm)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

The experimental germplasm comprised of Eight genotypes from local varieties of *Gossypium hirsutum* L have been developed and maintained at central...
Institute for cotton research, Coimbatore and the advanced breeding culture KAV 003, KAV 004, KAV 005, KAV 006, KAV 009, were developed at breeding division.

These varieties have been maintained true to type. All of these varieties were grown during the cropping season in 2010, keeping row to row 75 cm and plant to plant 30 cm distance, respectively. All the required agronomic practices and crop protection measures were engaged from sowing till harvesting of the crop.

During the flower initiation, in the month of August and September 2010, all possible crosses, including reciprocals were adopted among the ten cultivars. Hand emasculation and pollination was maintained during crossing and in addition all necessary procedures were applied during crossing operations to avoid contamination of the germplasm used. The F₁ hybrid seed from all the crosses along with their selfs (parents) were sown in the field during May 2010 in a triplicated randomized complete block design.

Each entry was planted in a 3.3 meter long row keeping ten plants experimental while two plants were left as non experimental on either side of the row by keeping plant to plant distance 30 cm and row to row 75 cm. The F₁ hybrid genotypes from each cross along with parents were self pollinated to raise F₂ generation.

The F₂ population was grown during May 2012, in triplicated progeny row trial employing randomized complete block design.

The plot size for each cross was 3.3 x 6 meters. The outer plants from each treatment in F₁ and F₂ population were left as non-experimental. The data with regard to plant height number of bolls per plant, boll weight, and yield of seed cotton per plant, lint percentage, lint, index, seed index, and staple length were recorded in F₁ and F₂ generations during both the crop seasons. The detail of the methods applied is as under.

1. Height of Main Stem

When the growth of the plant had ceased, height of main stem of the plant was measured in centimeters with measuring rod from the first cotyledonary node to apex.
2. Number of bolls per plant

The total number of bolls picked at each picking was noted and recorded from each individual plant. When final picking was over, the pick wise record was summed to calculate the total number of bolls per plant calculated for all the families.

3. Boll weight

Average weight per boll was worked out by dividing the total yield of seed cotton per plant by the total number of bolls picked in two pickings.

4. Yield of seed cotton per plant

Two pickings of seed cotton were arranged with interval of 3 weeks. Pickings were done during daytime of dry seed cotton and seed cotton was put in cotton bags of the individual plants. After cleaning the produce of each plant was weighed on a triple beam balance and yield in grams per plant was obtained.

5. Number of Seeds per boll

From each treatment forty bolls were separated and number of seeds in each boll were calculated and then summed up and divided by forty to take the average number of seeds per boll.

6. Lint percentage

Cleaned and dry samples of the seed cotton were weighed and then ginned separately with a single roller electric gin. The lint obtained from each sample was weighed and lint percentage was calculated by the following formula:

\[
\text{Lint percentage} = \frac{\text{Weight of lint in a sample}}{\text{Weight of seed cotton in a sample}} \times 100
\]

7. Lint Index

It is defined as the absolute weight of lint produced by individual seed or it is the weight of lint obtained from 100 seeds in grams. Lint index of each plant in the progeny was calculated by applying the formula.

\[
\text{Lint Index} = \frac{\text{Seed Index} \times \text{Lint percentage}}{100 - \text{Lint percentage}}
\]
8. Seed index

Seed index is 100 seed weight in grams. 100 seeds were taken from the sample at random and were weighed in grams.

9. Staple length

Staple length was measured from the representative sample of every plant by “Digital Fibro Grph Model – 330”.

STATISTICAL ANALYSIS

The data were subjected to analysis of variance technique as proposed by Steel and Torrie (1980) for all the traits studied to establish the level of significance of differences among various F₁ and F₂ hybrid populations and their parental lines. The significant genotypic differences permit the use of simple additive-dominance model. Regression analysis as proposed by Mather and Jinks (1977)

A. GENETIC MECHANISM ANALYSIS

The diallel technique was adopted for genetic analysis, explored by Hyman (1954 a, b) and applied by Mather and Jinks (1977).

Diallel assumptions and their validity test

A full diallel cross is the set of all possible matings between several genotypes which may be individuals, clones, homozygous lines, etc. and if there are (n) of these, there are \( n^2 \) mating combinations, counting reciprocals separately. A diallel table shows an arrangement of data in a square of \( n^2 \) observations from a set of diallel crosses between \( n \) parental lines. Each row and column of the data table corresponds to the measurements on the offspring with a common parental genotype. The (n) parents form the leading diagonal of the table and each male array (row) has a common male parent, being like the female array (column), which is of the same genotype as its common female parent.

Mather and Jinks (1971) explained that there are two phases of analysis of the diallel. In the first phase formal analysis of variance of the data is done which shows whether there is significant additive and non-additive genetic component variation present. The main items for differences among the same set of genotypes and so, in the absence of complications such as maternal effects, should yield estimates of the same component of variation, which will of course be the additive variation (D). The
item for interaction of female and male parents of the gene effects includes dominance as well as non additiviity of non allelic genes in producing their effects. The mean square for differences between reciprocals can be compared with error variation to provide a test of equalinearity in the genetically determination of the character.

The second phase of diallel analysis is the graphic representation of the variance (Vr) of all components of the rth array and the covariance (Wr) of all the off springs in each parental arrays with the non-recurring parents.

The information about the gene action was achieved by plotting the covariance (Wr) of each array against its variance (Vr). The slope and the position of regression line fitted to the array points within the limited parabola (Wr² = Vp. Vr) exposed the degree of dominance and the presence or absence of gene interaction. The limiting parabola was contracted on the basis of formula, Wr² = Vr x Vp, i.e. by plotting Vr, (Vr x Vp)²/² points. The corresponding values for Wr for all observed Vr were calculated as (Vr x Vp)²/² where Vp = variance of the parents. The different arrays (varieties) were fitted within the limits of the parabola using the individual variance and covariance as their limiting point.

Array nearest to the point of origin possessed most of the dominant genes, while the variety that laid the farthest possessed mot recessive genes, and the intermediate position signified the presence of both dominant and recessive genes in the array. The standard error for the regression line slope was estimated according to Snedecor and Cochran (1962)

The adequacy of the additive-dominance model to the data set and hence fulfillment of assumptions for the model can be estimated with the help of two tests. The consequence of the failure of those assumptions makes model inadequate. For the additive domininance model to be adequate and hence the fulfillment of the assumptions for the model, date should satisfy both tests.

Firstly, a general test of assumptions is provided by regression analysis of Wr on Vr. Of this test means that either genes show non-allelic interaction i.e. are not independent in their action, or show non-random association among the parents, i.e. are non-independent in their distribution.
The second test of adequacy of this diallel analysis is the analysis of variance of $W_r + V_r$. If dominance (or for that matter certain types of non-allelic interaction) is present $W_r + V_r$ must change from array to array and at the same time if there is non-allelic interaction between the alleles, $W_r-V_r$ will vary between arrays. Although if dominance is present, $W_r - V_r$ will not vary more than expected from error variation.

**Assumptions of the diallel analysis**

1. Diploid segregation
2. Homozygous parents
3. No reciprocal effect
4. No epistasis
5. No multiple allelism
6. Independent assortment of the genes

Though *Gossypium hirsutum* L. is a tetraploid (amphiploid) between the two species of A and D genome but it segregates in a diploid manner Endrizzi (1962) and Kimber, 1961), so the research material fulfils the first assumption.

The gene pool of cotton that was obtained being maintained at the Central Institute for Cotton Research, Regional Station Coimbatore and Tamilnadu Agriculture University Coimbatore. Therefore, the parental lines in the crossing program were assumed to be homozygous for all the characters.

The entries in the off diagonal cells of the diallel table were replaced by their means of direct cross and reciprocal prior to analysis for removing the reciprocal differences. The remaining three assumptions of non-allelic interaction, multiple allelism and independent assortment of the genes were satisfied through the analysis of variance of $W_r - V_r$ entities for the arrays of each replicated diallel table. Significant “F values” in the analysis of variance will reveal their heterogeneity, which invalidates any one of these assumptions.

**Estimation of genetic components**

The opinion of genetic components in different plant characters were done by method given by Mather and Jinks (1971 and 1977).
B. COMBINING ABILITY ANALYSIS

Analysis for combining ability effects were performed by using Griffing’s (1956) Method I, model II. Combining ability was computed as under.

i) General Combining Ability (GCA) Effects

\[
g^i = \frac{1}{2P} (X_i + X.i) - \frac{1}{P^2} X.. \]

Where
- \( g^i \) = General combining ability effects for line.
- \( P \) = Number of parents / varieties.
- \( X_i \) = Total of mean values of F\(_1\)’s resulting from crossing j lines with ith lines
- \( X.i \) = Total of mean values of F\(_1\)’s resulting from crossing I lines with jth lines
- \( X.. \) = Grand total of all the mean values in the table

ii) Specific Combining Ability (SCA) Effects.

\[
S^{ij} = \frac{1}{2} (X_{ij} + X_{ji}) - \frac{1}{2P} (X_i + X_j + X_{ij}) + \frac{1}{P^2} X.. \]

Where
- \( S^{ij} \) = Specific combining ability between ith and jth lines.
- \( X^{ij} \) = Mean values of the F resulting from crossing the ith and jth lines with ith lines.
- \( X_{ij} \) = Mean value for the F\(_1\) resulting from crossing the jth and ith inbreds.
- \( X_i \) = Total of mean values of F\(_1\)’s resulting from crossing jth line with ith inbreds.
- \( X_{ij} \) = Reciprocal values of Xi.
- \( X_i \) = Total values for the F\(_1\)’s resulting from the crossing ith line with jth line
- \( X_j. \) = Values of reciprocal F\(_1\)’s of \( X_j. \)
- \( X.. \) = Grand total of the observations.

III. Reciprocal Effects:

\[
r^{ij} = \frac{1}{2} (X_{ij} - X_{ji}) \]
Where

\( r_{ij} = \) Reciprocal effects of ith and jth varieties / lines.
\( X_{ij} = \) Mean values for the F\(_1\) resulting from crossing the ith and jth line.
\( X_{ji} = \) Reciprocal value of F\(_1\) resulting from X\(_{ij}\).

The variance due to crosses was partitioned into the variance due to general combining ability effects, specific combining ability effects and reciprocal effects, by the following formulae for Model I and Model II (Griffing, 1956) Table 1.

### Analysis of variance for Method I giving expectations of mean squares for the assumption of Model II.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
<th>Expectation of mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Combining Ability</td>
<td>( P^{-1} )</td>
<td>( S_g )</td>
<td>( M_g )</td>
<td>( \sigma^2 + \frac{2}{p} \sigma_{s}^2 + 2 \frac{p}{p^2} \sigma_{g}^2 )</td>
</tr>
<tr>
<td>Specific Combining Ability</td>
<td>( P \frac{(p-1)}{2} )</td>
<td>( S_s )</td>
<td>( M_s )</td>
<td>( \sigma^2 + \frac{2}{p^2(p-1)} \sigma_{s}^2 )</td>
</tr>
<tr>
<td>Reciprocal effects</td>
<td>( P \frac{(p-1)}{2} )</td>
<td>( S_r )</td>
<td>( M_r )</td>
<td>( \sigma^2 + 2 \sigma^2 )</td>
</tr>
<tr>
<td>Error</td>
<td>( m )</td>
<td>( S_c )</td>
<td>( M_c )</td>
<td>( \sigma^2 )</td>
</tr>
</tbody>
</table>

Where

\[
S_g = \frac{1}{2p} \sum_{i} (X_{i.} + X_{.j})^2 - 2x_2,
\]

\[
S_s = \frac{1}{2} \sum_{i} \sum_{j} X_{ij} (X_{i.} + X_{.j}) - \frac{1}{2p} \sum_{i} (X_{i.} + X_{i.})^2 + \frac{1}{2p} \sum_{i} x_{i.}^2.
\]

\[
S_r = \frac{1}{2} \sum_{i} \sum_{j} (X_{ij} - X_{.j})^2.
\]

Where

\( P \) = Number of Parents.
$M_c$ = Mean square for error.

$M_g$ = Mean Squares to general combining ability effects.

$M_g$ = Mean squares to general combining ability effects.

$M_s$ = Mean squares due to specific combining ability effects

$M_r$ = Mean squared due to reciprocal effects.

$\sigma^2_g$ = Components of variance for general combining ability.

$\sigma^2_s$ = Components of variance for specific combining ability.

$\sigma^2_r$ = Components of variance for reciprocal effects.

Variance components are estimated by equating the observed and expected mean squares in Table 1. The estimates are computed as follows:

$$\sigma^2_g = \frac{1}{1 + \frac{1}{2p} \left( \frac{M_c^1 + p(p-1)M_s}{M_g} \right)}$$

$$\sigma^2_s = \frac{p^2}{2c} [M_s - M_c]$$

and

$$\sigma^2_r = \frac{1}{2} [M_r - M_c]$$

Where

$$C = p^2 - p + 1.$$