CHAPTER III

MATERIALS AND METHODS

The present investigation on "Heterosis and combining ability analysis for seed yield and its components in sesame (*Sesamum indicum* L.)" was carried out at Sagadividi Farm, Department of Seed Science & Technology, College of Agriculture, Junagadh Agricultural University, Junagadh during Summer 2016. Geographically, Junagadh is situated at 21^0 N Latitude and 70.5^0 E Longitude with an altitude of 60 meters above the mean sea level. Details of weather parameters of the crop season during which the experiment was conducted are presented in Appendix-I.

3.1 EXPERIMENTAL MATERIALS

The experimental material used in the present study consisted of 54 test entries comprising of 40 hybrids developed from 8 female lines and 5 male parents, and one standard check (Guj.Til-2). The crosses were made during *kharif* 2015 at Sagadividi farm, Department of Seed Science & Technology, College of Agriculture, Junagadh Agricultural University, Junagadh through line x tester mating design. The genotypes used as parental lines and their sources are listed in Table 3.1.

3.2 CROSSING PROGRAMME

The crosses were made during *Kharif* 2015. The well developed closed flower buds about to open next morning were selected and emasculated for crossing purpose. The corolla was removed gently without injuring gynoecium instead of removing each anther individually. Damaged buds and opened flowers were removed every day and thrown away from crossing block. Pollination was done in next morning between 8.00 and 10.00 AM. For that, mature unopened buds collected from desired male plants were kept in sunlight to allow pollen liberation. These pollen grains were dusted on the stigmas of emasculated flower buds of the female plants. The pollinated stigmas were then covered with straw tube to prevent contamination from foreign pollen grains and labeled properly. The mature crossed capsules were harvested separately. At the same time few closed flower buds of each parent were selected and selfed for collection of selfed seeds to be used as parental seeds in the next season. The selfed and crossed capsules were harvested separately at the time of maturity. The capsules were dried under sunlight and seeds were collected separately.

Table 3.1. Pedigree, source and salient features of parental lines used in study

Sr. No.	Parents	Pedigree	Source	Features
Lines				
1	AT 351	G.Til-1 x AT-206	Gujarat	Brown seeds
2	AT 355	AT-177 x G.Til-2	Gujarat	Black seeds
3	TC 66	Not available	Indigenous	Brown seeds
4	DPI 1484	Not available	Indigenous	Brown seeds
5	IC 96128	Not available	Indigenous	Black seeds
6	RT 33	Not available	Gujarat	White seeds
7	LIMDI 9	Local collection Gujarat	Gujarat	White seeds
8	RSE 3	Not available	Indigenous	White seeds
Testers				
9	Guj.Til-1	Selection from Mt-6752	Gujarat	Multi opposite, glabrous capsules, creamy white seeds, branching type
10	Guj.Til-2 (C)	G.Til-1 x TC-25	Gujarat	Hairy stem, more branched, glabrous capsule, multi opposite, white seeded.
11	Guj.Til-3	G.Til-1 x AHT-85	Gujarat	Single opposite, glabrous capsules, white and bold seeds, branching type
12	Guj.Til-4	G.Til-1 x RT-125	Gujarat	Multi alternate, glabrous capsules, white seeds, branching type, early Maturing
13	Guj.Til-10	Selection from TNAU-17	Gujarat	Glabrous stem, profuse branching, glabrous capsule, late maturing, single alternate capsule arrangement, black seeded.

3.3 EXPERIMENTAL DETAILS

The complete set of 54 genotypes comprising 40 hybrids, 13 parents and one standard check (Guj.Til-2) were evaluated in a Randomized Block Design with three replications at Sagadividi Farm, Department of Seed Science & Technology, College of Agriculture, Junagadh Agricultural University, Junagadh during summer 2016. Each entry was accommodated in single row plot of 3 meter length with row to row and plant to plant distances of 45 cm and 15 cm, respectively. All the recommended agronomical practices and plant protection measures were followed for raising a normal crop.

3.4 CHARACTERS STUDIED

Five randomly competitive plants from each entry excluding border plants in each replication were selected to record the observations on different characters and their averages were used for statistical analysis. The observations on days to 50 per cent flowering and days to maturity were recorded on plot basis.

1) Days to 50 per cent flowering

The number of days taken from the date of sowing to appearance of flowering in about 50 per cent of plants in a plot were counted as days to 50 per cent flowering.

2) Days to maturity

The number of days taken from sowing to physiological maturity were considered as days to maturity.

3) Plant height (cm)

The height of plant was measured in centimeters from ground level to the tip of main shoot at the time of maturity.

4) Height to first capsule (cm)

Height was measured in centimeters from base of plant to the first capsule appearance on the main stem.

5) Number of branches per plant

The total number of capsule bearing branches per plant were counted at the time of maturity.

6) Number of internodes per plant

The total number of internodes on main stem of selected plants were counted at the time of maturity.

7) Length of capsule (cm)

Length from base to the tip of capsule was recorded in centimeters for five capsules per plant from each of the five selected plants.

8) Width of capsule (cm)

Capsule width was measured in centimeters from middle portion of the five capsules per plant from each of the five selected plants.

9) Number of capsules per plant

Total number of capsules from each five randomly selected plants were counted and recorded.

10) Number of capsules per leaf axil

The number of capsules per leaf axil of the main shoot were recorded for each five randomly selected plants and average was worked out.

11) Number of seeds per capsule

Number of seeds of ten capsules from each randomly selected plants in a plot were counted manually and the average value was recorded.

12) 1000-seed weight (g)

This was recorded by taking random sample of the well mixed bulk product from each genotype. One thousand seeds were counted manually and weighed in grams on an electronic balance.

13) Seed yield per plant (g)

Seeds harvested from five selected plants were dried, cleaned and weighed in grams on an electronic balance.

14) Oil content (%)

Seed samples were drawn randomly from the bulk produce of each genotype on plot basis and oil content was determined by Nuclear Magnetic Resonance (N.M.R analyzer) as suggested by Tiwari *et al.* (1974).

3.5 STATISTICAL PROCEDURES:

The replication wise mean values of each genotype for various characters were used for statistical and genetical analysis. Following statistical procedures were used for analysis.

3.5.1. Analysis of variance for experimental design

3.5.2. Estimation of heterobeltiosis and standard heterosis

3.5.3. Combining ability analysis

3.5.1 Analysis of variance for experimental design

The analysis of variance (Table 3.2) was performed to test the significance of difference among the genotypes for all the characters following fixed effect model as suggested by Panse and Sukhatme (1985).

$$Y_{ij} = \mu + g_i + r_j + e_{ij}$$

Where,

Y_{ij} = Value of ith genotype in j^{th} replication,

μ = Population mean,

g_i = An effect of ith genotype (i = 1,2,….g),

r_j = An effect of jth replication (j = 1,2,….r), and

e_{ij} = Uncontrolled variation associated with i^{th} genotype and j^{th} replication

Table 3.2. Analysis of variance for experimental design

Source of variation	**d. f.**	**M.S.**	**E.M.S.**	**F**
Replications	(r-1)	M_1	$\sigma_e^2 + g\sigma_r^2$	M_1/M_6
Genotypes	(g-1)	M_2	$\sigma_e^2 + r\sigma_g^2$	M_2/M_6
Parents (P)	(p-1)	M_3	$\sigma_e^2 + r\sigma_p^2$	M_3/M_6
Hybrids (H)	(h-1)	M_4	$\sigma_e^2 + r\sigma_h^2$	M_4/M_6
P *vs* H	1	M_5	$\sigma_e^2 + r\sigma_{ph}^2$	M_5/M_6
Error	(r-1) (g-1)	M_6	σ_e^2	-

Where,

r = Number of replications,

g = Number of genotypes,

p = Number of parents,

h = Number of hybrids,

M_1 = Mean sum of square due to replications,

M_2 = Mean sum of square due to genotypes,

M_3 = Mean sum of square due to parents,

M_4 = Mean sum of square due to hybrids,

M_5 = Mean sum of square due to parents *vs* hybrids,

M_6 = Mean sum of square due to error,

σ^2_r = Expected replication variance,

σ^2_g = Expected genotypic variance,

σ^2_p = Expected variance due to parents,

σ^2_h = Expected variance due to hybrids,

σ^2_{ph} = Expected variance due to parents vs. hybrids, and

σ^2_e = Expected error variance.

3.5.2 Estimation of heterobeltiosis and standard heterosis

3.5.2.1 Heterobeltiosis

It was calculated as the deviation of F_1 from the better parent (Fonseca and Patterson, 1968) and was expressed as per cent basis by the following formula;

$$\text{Heterobeltiosis (\%)} = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

Where,

$\overline{F_1}$ = Mean performance of F_1

$\overline{BP}$ = Mean performance of better parent of the respective cross

3.5.2.2 Standard Heterosis

It was calculated as the deviation of F_1 from the standard check (Guj.Til-2) and expressed as per cent basis by the following formula;

$$\text{Standard heterosis (\%)} = \frac{\overline{F_1} - \overline{SC}}{\overline{SC}} \times 100$$

Where,

$\overline{F_1}$ = Mean performance of F_1

$\overline{SC}$ = Mean performance of standard check (Guj.Til-2)

The standard error of difference for heterobeltiosis and standard heterosis was calculated as follows:

$$S.E.\ (d) = \sqrt{\frac{2Me}{r}}$$

Where,

Me = Error mean square

r = Number of replications

In above two cases of heterosis, critical difference were computed by multiplying respective standard error of differences with 't' value at given error degrees of freedom at 0.05 or 0.01 probability level as follows:

C.D. = S.E.(d) x t value at error degrees of freedom

3.5.3 Combining Ability Analysis

3.5.3.1 Analysis of variance for combining ability (line x tester)

Analysis of variance for combining ability (Table 3.3) was performed according to the model given by Kempthorne (1957), which is related to design-II of Comstock and Robinson (1952) in terms of covariance of half-sibs (H.S.) and full-sibs (F.S.).

$$Y_{ijk} = \mu + G_{i.} + G_{.j} + S_{ij} + r_k + \in_{ijk}$$

$$\sum G_{i.} = \sum G_{.j} = \sum S_{ij} = 0$$

Where,

Y_{ijk} = Phenotypic expression of ijth genotypes in kth replication

μ = General mean

$G_{i.}$ = General combining ability of ith female parent (line)

G_j = General combining ability of jth male parent (tester)

S_{ij} = Specific combining ability of cross between ith line and jth tester

r_k = Effect of kth replication

$\in_{ijk}$ = Random error associated with ijth genotype and kth replication

Table 3.3. Analysis of variance for combining ability

Sources of variation	d. f.	MS	Expected mean squares	
			In terms of variance	In terms of full sib and half sib
Replications	(r-1)	-		-
Hybrids	(h-1)	-	$\sigma^2_e + r\,\sigma^2_{lt}$	-
Lines	(l-1)	M_1	$\sigma^2_e + r\,\sigma^2_{lt} + rt\,\sigma^2_l$	σ^2_e + r Cov. (F.S.) - 2(Cov.(H.S.) + [trCov. (H.S.)]
Testers	(t-1)	M_2	$\sigma^2_e + r\,\sigma^2_{lt} + rl\,\sigma^2_t$	σ^2_e + rCov. (F.S.)-2Cov.(H.S.) + [lr Cov. (H.S.)]
Line x Tester	(l-1)(t-1)	M_3	$\sigma^2_e + r\,\sigma^2_{lt}$	σ^2_e + rCov.(F.S.)- 2Cov. (H.S.)
Error	(r-1)(lt-1)	M_4	σ^2_e	σ^2_e

From the expectation of mean squares, covariance of full-sibs and half-sibs were estimated using the sum of squares due to lines (M_1), testers (M_2) and lines x testers (M_3) where,

$$Cov.(H.S.) = (M_1-M_3) + (M_2-M_3) / r(l + t)$$

$$Cov.(F.S.) = (M_1-M_4) + (M_2-M_4) + (M_3-M_4) / 3r + 6r\ Cov.(H.S.) – r (l + t)\ Cov.(H.S.) /3r$$

Where,

r = Number of replications

h = Number of hybrids

l = Number of lines

t = Number of testers

lt = Number of hybrids

σ^2_t = Variance due to general combining ability of testers

σ^2_l = Variance due to general combining ability of lines

σ^2_{lt} = Variance due to specific combining ability of hybrids

The estimates of variance components due to lines, testers and hybrids were calculated as under

$$\sigma^2_l = (M_1 - M_3)/rt$$

$$\sigma^2_t = (M_2 - M_3)/rl$$

$$\sigma^2_{lt} = (M_3 - M_4)/r$$

Estimates of covariance for half sib (H.S.) and covariance for full sib (F.S.) would be:

$$Cov.(H.S.) = (l\ \sigma^2_t + t\ \sigma^2_l)/(l +t)$$

$$Cov.(F.S.) = [\sigma^2_{lt} + 2Cov.(H.S.)]$$

The estimated variances due to gca and sca were computed using covariance of half-sibs and full-sibs:

$$\sigma^2_{gca} \cong Cov.\ (H.S.)$$

$$\sigma^2_{sca} \cong Cov.\ (F.S.) - 2\ Cov.\ (H.S.)$$

$$\sigma^2_{gca} = (M_1 + M_2 – 2M_3)/r\ (l + t)$$

$$\sigma^2_{sca} = [(M_1 + M_2 + M_3 – 3M_4) + (6r\ Cov.\ H.S. – (r\ (l + t)\ Cov.\ H.S.))]/3r$$

To judge the significance of estimated variance, following tests were made.

Ho: $\sigma^2_{lt} = 0$ was tested by F (n_3, n_4) = M_3/M_4. If M_3 was significant, then test would be F (n_1, n_3) = M_1/M_3 for $\sigma^2_f = 0$ and F (n_2, n_3) = M_2/M_3 would be for $\sigma^2_t = 0$. If, M_3 was non-significant, then M_4 was used as denominator, where n_1, n_2, n_3 and n_4 were degrees of freedom associated with M_1, M_2, M_3 and M_4, respectively.

3.5.3.2 Combining ability effects

The combining ability effects were estimated as under

(i) General combining ability effects of lines

$\hat{g}_i = (Y_{i..}/tr) - (Y.../ltr)$

Where,

Y... = Total of all hybrids over replications

$Y_{i..}$ = Total of ith line over all testers and replications

(ii) General combining ability effects of testers

$\hat{g}_j = (Y_{.j.}/lr) - (Y.../ltr)$

Where,

$Y_{.j.}$ = Total of jth tester over all lines and replications

(iii) Specific combining ability effects of crosses

$S_{ij} = (Y_{ij.}/r) - (Y_{i..}/tr) - (Y_{.j.}/lr) + (Y.../ltr)$

Where,

Y_{ij} = Total of ij^{th} cross combination over all replications

The test of significance of general and specific combining ability effects was done as under

SEd (gca for lines) $= \sqrt{2\ Me / r \times t}$

SEd (gca for testers) $= \sqrt{2\ Me / r \times l}$

SEd (sca for crosses) $= \sqrt{2\ Me / r}$

C.D.(%) = SEd x $t_{0.05}$ (n_e)

Where,

Me = Error mean square in the analysis of combining ability

r, t and l = Number of replications, testers and lines, respectively

$t_{0.05}$ (n_e) = Value at error degrees of freedom at 5 % level of significance.