3. MATERIAL AND METHOD

In present investigation, the research work was carried out to study heterosis, combining ability, mode of gene action and stability of okra hybrids. Exploitation of hybrid vigour is important tool for crop improvement. The extent of hybrid vigour is depends on genetic variability and diversity among selected parental lines. Lines used in breeding programme have prime importance. Therefore, materials used and methods adopted for conducting the research work is discussed here.

The present research work was carried out on Research Farm, Nimbkar seeds Pvt. Ltd. Phaltan Dist. Satara, Maharashtra at its geographical coordinates are 17.98° North, 74.43° East, altitude of 568 meter above sea level, This area receives much less rainfall from June to September, and it has been declared as a drought-prone place by the government and sometimes gets nil rainfall during the rainy season.

3.1. Material for research work

The germplasm lines for conducting research work were collected from IIVR (Indian Institute of Vegetable Research) Varanasi, were evaluated for their *per se* performance for eight quantitative traits including seed yield per plant during *kharif* 2009 (Table 3.1). Analysis of variances for dispersion indicates significant differences among the genotypes. Twenty five genotypes were evaluated on the basis of *per se* performance (Shaikh *et al*.,

2013) and divergence analysis (Shaikh *et al*. 2013). On the basis of *per se* performance, twelve genotypes *viz.*, BO-2, 35, 447, EC-316053, Vaibhav, VRO-4, IIVR-11, HRB-55, 162, 315, 364 and 410 were selected. Out of these 12 lines, on the basis of morphological characters, 5 lines were used as females and 7 lines as males. Five females and seven males were crossed in Line x Tester manner during summer 2010 season to produce the 35 hybrids by hand emasculation and pollination method.

Table No. 3.1. List of okra germplasm lines evaluated during *k*harif 2009.

Sr. no.	Name of the genotype	Source of genotype
1	BO-2	IIVR (Indian Institute of Vegetable Research) Varanasi.
2	VRO-4	IIVR (Indian Institute of Vegetable Research) Varanasi.
3	VRO-6	IIVR (Indian Institute of Vegetable Research) Varanasi.
4	IIVR-11	IIVR (Indian Institute of Vegetable Research) Varanasi.
5	35	IIVR (Indian Institute of Vegetable Research) Varanasi.
6	HRB-55	IIVR (Indian Institute of Vegetable Research) Varanasi.
7	DOV-91-4	IIVR (Indian Institute of Vegetable Research) Varanasi.
8	134	IIVR (Indian Institute of Vegetable Research) Varanasi.
9	148	IIVR (Indian Institute of Vegetable Research) Varanasi.
10	156	IIVR (Indian Institute of Vegetable Research) Varanasi.
11	162	IIVR (Indian Institute of Vegetable Research) Varanasi.
12	EC-171	IIVR (Indian Institute of Vegetable Research) Varanasi.
13	173	IIVR (Indian Institute of Vegetable Research) Varanasi.
14	198	IIVR (Indian Institute of Vegetable Research) Varanasi.
15	315	IIVR (Indian Institute of Vegetable Research) Varanasi.
16	320	IIVR (Indian Institute of Vegetable Research) Varanasi.
17	337	IIVR (Indian Institute of Vegetable Research) Varanasi.
18	364	IIVR (Indian Institute of Vegetable Research) Varanasi.
19	391	IIVR (Indian Institute of Vegetable Research) Varanasi.
20	403	IIVR (Indian Institute of Vegetable Research) Varanasi.
21	410	IIVR (Indian Institute of Vegetable Research) Varanasi.
22	447	IIVR (Indian Institute of Vegetable Research) Varanasi.
23	EC-316053	IIVR (Indian Institute of Vegetable Research) Varanasi.
24	Vaibhav	IIVR (Indian Institute of Vegetable Research) Varanasi.
25	Parbhani Kranti	IIVR (Indian Institute of Vegetable Research) Varanasi.

3.2. Crossing Program

Twelve selected germplasm lines were distributed in 5 as lines and 7 as tester and planted in summer 2010 at 60 x 30 cm row to row and plant to plant spacing respectively, at the ratio of 4 rows of female and one row of male, crossed in line x tester fashion to develop 35 F_1 hybrids, by adopting the method of hand emasculation and pollination.

Table no. 3.2. List of parents selected as lines and testers for crossing.

Sr.No.	Genotype	Parent	Source
1	BO-2	Line	IIVR (Indian Institute of Vegetable Research) Varanasi.
2	35	Line	
3	447	Line	
4	EC-316053	Line	
5	Vaibhav	Line	
6	VRO-4	Tester	IIVR (Indian Institute of Vegetable Research) Varanasi.
7	IIVR-11	Tester	
8	HRB-55	Tester	
9	162	Tester	
10	315	Tester	
11	364	Tester	
12	410	Tester	

Emasculation was carried out in evening between 5-7 PM of flower buds having light green and pollinated next day morning with pollen from opened flower on same day morning between 7-10 AM. Followed the all

recommended agronomic practices and plant protection measures to obtain healthy and disease free seeds of hybrids. Seeds harvested at proper stage of maturity, cleaned, dried and labelled for conducting the trials.

Table no. 3.3: List of crosses developed by Line x tester design.

Sr. No.	CROSS	Sr. No.	CROSS
1	BO-2 x VRO-4	19	447 x 315
2	BO-2 x IIVR-11	20	447 x 364
3	BO-2 x HRB-55	21	447 x 410
4	BO-2 x 162	22	EC-316053 x VRO-4
5	BO-2 x 315	23	EC-316053 x IIVR-11
6	BO-2 x 364	24	EC-316053 x HRB-55
7	BO-2 x 410	25	EC-316053 x 162
8	35 x VRO-4	26	EC-316053 x 315
9	35 x IIVR-11	27	EC-316053 x 364
10	35 x HRB-55	28	EC-316053 x 410
11	35 x 162	29	Vaibhav x VRO-4
12	35 x 315	30	Vaibhav x IIVR-11
13	35 x 364	31	Vaibhav x HRB-55
14	35 x 410	32	Vaibhav x 162
15	447 x VRO-4	33	Vaibhav x 315
16	447 x IIVR-11	34	Vaibhav x 364
17	447 x HRB-55	35	Vaibhav x 410
18	447 x 162		

3.3. Layout of experiment

Thirty five okra hybrids along with their 12 parents and three standard checks namely, Parbhani Kranti (OPV), Syngenta–152(Hybrid) and Avantika (Hybrid) were evaluated in Randomized block design (RBD) with three replications during five seasons i.e. *kharif* 2010, summer 2011, *kharif* 2011, summer 2012 and *kharif* 2012. The trials were planted at the spacing of 60 cm x 30 cm i.e. at row to row and plant to plant distance maintain 60 cm and 30 cm respectively. Each plot consists of three rows of four meter length. The cultural, agronomic and plant protection stanadard recommended practices were adopted. Observations were recorded on morphological, yield and yield contributing characters at appropriate stage.

3.4. Recording of Observations

Observations were recorded on randomly selected five competent plants from each plot for different traits and averaged for statistical analysis. Eight characters, viz., days to first flowering, days to 50% flowering, number of branches per plant, plant height, tender fruit length, number of seeds per fruit, number of fruits per plant and weight of fruits per plant were studied and observations were recorded as under.

3.4.1 Days to first flowering

The number of days taken from the date of sowing to onset of first flower appear on the plant.

3.4.2. Days to 50 % flowering

The number of days taken from the date of sowing to the day on which 50 percent of the population flowered was recorded.

3.4.3. Number of branches

The total number of branches on each plant was counted after the final harvest.

3.4.4. Plant height (cm)

The height of the plant from the ground level to the tip was measured at the time of final harvest**.**

3.4.5 Tender fruit length (cm)

The length of tender fruit was measured in centimeters (cm) from the base of calyx to tip of the fruit.

3.4.6. Number of seeds per fruit

Fully matured and dried fruits were harvested and the numbers of seeds per fruit were recorded.

3.4.7. Number of fruits per plant

The total number of fruits per plant at of all the picking was counted and recorded as number of fruits per plant.

3.4.8. Weight of fruits per plant (g)

Tender fruit from each harvest were weighed using sensitive balance and recorded in grams (g).

3.5. Statistical analysis and interpretation

Data recorded for yield and yield contributing characters analyzed using WINDOSTAT software.

3.5.1. Heterosis

The magnitude of heterosis was estimated in relation to mid-parent (MP), better parent (BP), standard variety (SH) as percentage increase or decrease of F_1 over respective parental values. Average values over replications were used for estimating the heterosis over mid, better, and check parent. Emphasis to say heterotic cross depended upon the trait that may be desired in any one of the two sides higher or lower. The estimation of heterosis was carried out following the methods suggested by Turner (1953) and Hayes et al. (1955).

$$\textbf{a. Heterosis over mid-parent (M P)} = \frac{\bar{F1} - \bar{MP}}{\bar{MP}} \times 100$$

$$MP = \frac{P1 + P2}{2}$$

$$\textbf{b. Heterosis over better parent (BP)} = \frac{\overline{F1} - \overline{BP}}{\overline{BP}} \times 100$$

$$\textbf{C. Heterosis over standard check (SC)} = \frac{\overline{F1} - \overline{SC}}{\overline{SC}} \times 100$$

Where,

$\overline{F1}$, $\overline{BP}$, and $\overline{SC}$ are mean values of F_1 hybrids, better parent and standard check respectively.

The significance of F_1 heterosis value was tested by comparing them with CD values obtained separately for MP, BP and SC employing the formula given below.

$$\text{CD for MP} = \sqrt{\frac{3/2 \times MSSe}{r}} \times \text{'t' value}$$

$$\text{CD for BP and SC} = \sqrt{\frac{2 \times MSSe}{r}} \times \text{'t' value}$$

Where,

MSSe = Error mean sum of squares

r = Number of replications

't' = Table 't' value at error degrees of freedom

3.5.2. Analysis of variance (ANOVA)

Analysis of variance for individual character was done on the basis of the mean value for Randomized Complete Block Design. The model of analysis of variance table adopted is given below.

3.5.2.1 Analysis of variance (ANOVA)

Source	**d.f.**	**S.S.**	**M.S.S.**	**F**
Replication	r-1	RSS	MSSr(M1)	(M1/M3)
Treatments	t-1	TSS	MSSt(M2)	(M2/M3)
Error	(r-1)(t-1)	ESS	MSSe(M3)	

Where,

r = Number of replications

t = Number of treatments

MSSr = Mean sum of squares due to replication

MSSt = Mean sum of squares due to treatments

MSSe = Mean sum of squares due to error

3.5.2.2 Analysis of Variance for parent and crosses

Source of variation	**d.f.**	**SS**	**M.S.**	**F**
Replication	(r-1)	A	V1	V1/V6
Treatment	(t-1)	B	V2	V2/V6
Parents	(p-1)	C	V3	V3/V6
Crosses	(c-1)	D	V4	V4/V6
PVC	1	E	V5	V5/V6
Error	(t-1)(r-1)	F	V6	
Total	(rt-1)	G	V7	

Where,

r, t, p and c were replication, treatments, parents, and crosses respectively, while A, B, C, D, E, F, G were SS due to replication, treatments Parents, Crosses, PVC, error and total respectively, where as V1, V2, V3,V4, V5, V6 and V7 were SS due to M.S.S respectively.

3.5.2.3 Analysis of Variance for combining ability

Source of variatrion	**d.f.**	**SS**	**MSS**	**F**
Replication	(r-1)	A	Mr	Mr/Me
Males	(m-1)	B	Mm	Mm/Mmxf
Females	(f-1)	C	Mf	Mf/Mmxf
Males x Females	(m-1)(f-1)	D	Mmxf	Mmxf/Me
GCA	(n-1)	E	Mg	$\sigma^2_{e+}\sigma^2_s+(n+2)\ \sigma^2_g$
SCA	n(n-1)/2	F	Ms	$\sigma^2_{e+}\sigma^2_s$
Error	(r-1)(mf-1)	G	Me	
Total	rmf-1	H		

Where,

r, n, m and f where number of replication, parents, males and females respectively, while A, B, C, D, E, F, G and H where SS due to replication, males, females, male x female, *GCA* ,*SCA*, error and total, where as Mr, Mm, Mf, M(m x f) and Me where MSS due to replication, males, females, males x females, error and total respectively.

Mean sum of square due to males x females interaction was used to test the significance of males and females. Whereas, Me, was used to test the significance of m x f interaction.

3.5.3.1. The *GCA* variance (additive) due to males and females and *SCA* variance (non –additive) were calculated as follows.

$$\sigma^2 \mathbf{M\ (variance\ due\ to\ males)} = \frac{Mm \times F}{F \times r}$$

Where,

Mm = MSS due to males

Mm x F = MSS due to male x female

F = Number of females

r = Number of replication

$$\sigma^2 \mathbf{f\ (variance\ due\ to\ females)} = \frac{Mf - Mm \times F}{M \times f}$$

Where,

MF = MSS due to females

Mm x f = MSS due to male x female

$$\sigma^2 gca\ \mathbf{(additive\ variance)} = \frac{\sigma^2 2m \times \sigma^2}{2}$$

$$\sigma^2 \textbf{ M x F (non additive variance)} = \frac{Mm \times F - Me}{r}$$

Where,

Mm x F = MSS due to male x female interaction

Me = Error Variance

r = Number of replication

σ^2 ***sca*** **(dominance variance)** = σ^2 x f

These effects were tested at 5 % and 1 % level of significance. The ration of additive variance to dominance variance calculated as follows.

$$\textbf{Ration} = \frac{\sigma^2 gca}{\sigma^2 sca}$$

3.5.3.2 Estimation of combining ability

Estimation of general combining ability effects of testers and lines are calculated from two way tables, as:

a) Males or tersters *gca* effect for males were calculated by using the following formula.

$$gca \textbf{ (males)} = \frac{Y_i}{r\,f} - \frac{Y_{...}}{r\,m\,f}$$

b) Female or line *gca* effect for female were calculated by using the following formula.

$$gca \textbf{ (female)} = \frac{Y_j}{rm} - \frac{Y_{...}}{rm\,f}$$

Where,

r = Number of replication

f = Number of females

m = Number of males

Y_i = Total over i^{th} female

Y_j = Total over j^{th} male

3.5.3.3 Estimation of specific combining ability effects

Specific combing ability effects for individual crosses were worked out by using following formula,

$$S_{ij} = \frac{Y_{ij.}}{r} - \frac{Y_{i..}}{rf\,m} - \frac{Y_{\bullet i.}}{rm} + \frac{Y_{..}}{rmf}$$

Where,

Yij = Individual value of cross

Yi = Total of crosses with i^{th} males

Yj = Total of crosses having j^{th} females

Y = Grand total

M = Number of males

f = Number of females

r = Number of replications

3.5.3.4. The standard error and critical difference were calculated to test the significance of GCA and SCA effects as follows.

Standard error for i^{th} male is calculate as below

$$S.E.\ gi = \left(\frac{M_4}{rm} \right)^{\frac{1}{2}} \quad .i = 1,.,.,.,.,.,.,\ m$$

To identify the significantly superior tester, standard error is calculated as

$$S.E.(gi - gj)\ tester = \left(\frac{2\ M_4}{rm} \right)^{\frac{1}{2}} .$$

Standard error for j^{th} female is calculate as below

$$S.E.\ gi = \left(\frac{M_4}{rf} \right)^{\frac{1}{2}} \quad .j = 1,.,.,.,.,.,.,f$$

Significance of *gca* is tested by‘t’ test as below

$$tg = \frac{g - 0}{S.E._{g}}$$

To identify the significantly superior line, standard error is calculated as

$$S.E.(gi - gj)\ lines = \left(\frac{2\ M_4}{r\ f} \right)^{\frac{1}{2}} .$$

To identify significantly differing lines or tester for *gca* effect calculated as

$$t\,(gi - gj) = \frac{g_i - g_j}{S.E.(gi - gj)}$$

Standard error for SCA effect is calculated as below,

$$S.E._{Sij} = \left(\frac{\text{Error mean square}}{r} \right)^{\frac{1}{2}}$$

To identify the significantly different combination, Standard error calculated as,

$$S.E.\,(sij - sbj) = \left(\frac{2\text{Error mean square}}{r} \right)^{\frac{1}{2}}$$

Significance of SCA effects is tested by‘t’ test as following,

$$t = \frac{S_{ij}}{S.E.\ S_{ij}}$$

Significant difference between SCA effects is tested by‘t’ test as following,

$$t = \frac{s_{ij} - s_{bj}}{S.E.(s_{ij} - s_{bj})}$$

Critical difference between *gca* effects of testers or lines calculated as below:

If the difference between *gca* effect of two testers or lines is more than S.E. x t value at 5 % and 1 %, level of significance, tester or line with large *gca* effect could be regarded as significantly superior to other. Similarly the two tester or lines can be tested, weather they differ significantly from each other or not by using the relevant S.E.(gi- gj).

Critical difference between to SCA effect is calculated as below:

If SCA effects of any cross exceeds than $S.E.S_{ij}$ x t value at 5 % and 1 %, level of significance, SCA effect could be regarded as significantly superior to other. Similarly the two SCA effects of crosses can be tested, weather they differ significantly from each other or not by using the relevant S.E. (sij- sbj).

3.5.4. Statistical Analysis for stability

3.5.4.1 Analysis of Variance:

Replication wise mean values were subjected to conventional RBD analysis. Linear model was used to represent the mean performance of a treatment in any plot was:

$$X_{ij} = \mu + t_i + b_j + e_{ij}$$

Where,

X_{ij} = mean performance of i^{th} genotype in j^{th} replication.

(i = 1,2,…….; j = 1,2,……)

μ = population mean value

t_i= effect of i^{th} treatment

b_j = effect of j^{th} replication

e_{ij} = random error component

Partitioning of the total variation due to genotype, replication and error along with expected values of mean squares (MS) is given below in the table:

3.5.4.1 Analysis of variance

Source of variance	**Degree of freedom**	**Mean sum of squares**	**Expected mean square**
Replication	(r-1)		
Genotype	(g-1)	M_g	$\sigma^2_e + r\sigma^2_g$
Error	(r-1) (g-1)	M_e	σ^2_e
Total	(rg-1)		

Where,

r = number of replications

g = number of genotypes

M_g = mean squares of genotypes

M_e = mean squares of error

σ^2_e = error variance, and

σ^2_g = genetic variance

The overall significance of differences among mean of genotypes was tested by 'F' test.

3.5.4.2 Phenotypic Stability Analysis:

The statistical technique proposed by **Eberhart and Russel (1966)** was utilized to estimate the stability parameters and G x E interactions with respect to different characters.

Eberhart and Russel (1966) suggested three parameters to measure the stability of genotypes as mentioned below:

1. Mean.
2. Regression of individual mean performance on environmental index,
3. Deviation from regression.

These parameters are defined in the following model:

$$y_{ij} = \mu_i + b_i I_j + \delta_{ij}$$

Where,

y_{ij} = mean performance of i^{th} genotype in j^{th} environment.

(i = 1,2,……..g; j = 1,2,……n)

μ_i = mean performance of i^{th} genotype over all the environment.

b_i = regression coefficient of i^{th} individual mean performance on environmental index I_j.

δ_{ij} = deviation from regression of i^{th} genotype at j^{th} environment.

The environmental index I_j is obtained as the mean of j^{th} environment minus grand mean. This can be expressed as:

$$I_j = \left[\left(\sum_{i=1}^{g} Y_{ij}/g\right) - \left(\sum_{i=1}^{g}\sum_{j=1}^{n} Y_{ij}/gn\right)\right]$$

g = number of genotypes

n = number of environments

and $\sum_{j=1}^{n} I_j = 0$

The first stability parameter, regression coefficient (b_i), was estimated using the following formula:

$$b_i = \frac{\sum_{j=1}^{n} Y_{ij} I_j}{\sum I_j}$$

The second stability parameter, the mean square deviation from linear regression ($S^2 d_i$), was estimated using the following formula:

$$S^2 d_i = \frac{[\sum_{j=1}^{n} \delta^2_{ij}]}{(n-2)} - S^2_e / r$$

Where, S^2_e/r is the estimate of pooled error (or the variance of a variety mean of the j^{th} location), and r = number of replications.

$$\sum_{j=1}^{n} \delta^2_{ij} = \left(\sum_{j=1}^{n} Y^2_{ij} - \frac{Y^2_i}{n}\right) - \frac{\left(\sum_{j=1}^{n} Y_{ij} I_j\right)^2}{\sum I^2_j}$$

The estimate of pooled error was done by taking the average of error mean square over all the environments. The detailed analysis of variance for estimation of stability parameter is presented below:

The following tests of significance were performed:

1. The test of significance for difference among the mean performance of genotype was done using F-test:

$$F = \frac{MS_1}{MS_4}$$

2. Genotype-environment interaction was tested using F-test:

$$F = \frac{MS_2}{MS_5}$$

3. The genetic differences among genotypes for their regression of environment index were tested using F-test:

$$F = \frac{MS_3}{MS_4}$$

4. The deviation of b_i values from unity was tested using t-test:

$$t = \frac{b_i - 1}{SE(b_i)} \text{ at } n-2 \text{ d.f.}$$

where,

$$SE(b_i) = \frac{\sqrt{\text{Pooled deviation MS}}}{\sum_{j=1}^{n} I_j^2}$$

5. Deviation from regression for each genotype was tested using F-test.

$$F = \frac{\frac{\sum_{j=1}^{n} \partial_{ij}^2}{n-2}}{MS_5}$$

on n-2 and pooled error d.f. at 5% and 1% level of significance.

3.5.4.2 Analysis of variance for stability analysis (Eberhart and Russell, 1966)

Source	**Degree of freedom**	**Sum of squares**	**Mean squares**
Total	(gn – 1)	$\Sigma_i \Sigma_j Y_{ij}^2 - CF$ = Total SS	
Genotype (G)	(g – 1)	$(1/n)s \Sigma_i Y_i^2 - CF$ = Genotype SS	MS_1
Environment (E)	(n – 1)	$(1/g)s \Sigma_j Y_j^2 - CF$ = Environment SS	
G x E	(n – 1)(g – 1)	Total SS – (Genotype SS + Env SS)	
E + (G x E)	g (n – 1)	$\Sigma_i \Sigma j Y_{ij}^2 - (1/n) \Sigma_i Y_i^2$	
E (linear)	1	$(1/g)(\Sigma_j Y_j I_j)^2 / \Sigma_j I_j^2$ = Env (linear) SS	
G x E (linear)	(g – 1)	$\Sigma_i [\Sigma_j Y_{ij} I_j]^2$ – Env (linear) SS	
Pooled deviation	g (n – 2)	$\Sigma_i \Sigma_j \delta_{ij} = \Sigma_i [\Sigma_j Y_{ij}^2 - (Y_i^2/n) - (\Sigma_j Y_{ij} I_j^2/\Sigma_j I_j^2)]$	MS_4
Genotype 1	(n – 2)	$\Sigma_j Y_{ij}^2 - (Y_1^2/n) - (\Sigma_j Y_{ij} I_j)^2/\Sigma_j I_j^2 = \Sigma_j \delta_{ij}^2$	
Genotype g	(n – 2)	$[\Sigma_j Y_{gj}^2 - (Y_g^2/n) - (\Sigma_j Y_{gj} I_j)^2]/\Sigma_j I_j^2 = \Sigma_j \delta_{gj}^2$	
Pooled error	n(n – 1)(g – 1)	$\Sigma_j S_{ej}^2/r$	MS_5

Where,

g = number of genotypes

n = number of environments

r = number of replications

S_{ej}^2 = estimate of error mean square at each environment, and

CF = correction factor