MATERIALS AND METHODS

The present investigation was carried out to obtain information on the status of quality seeds and other related studies on seed and seedling vigor in pea. The studies were conducted at two different agro climatic zones viz. Mountain Research Centre for Field Crops (MRCFC) Khudwani Anantnag, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir (Latitude: 33° 44` North and Longitude: 75° 09` East) i.e. location I and Kisan (PG) College Simbhaoli, Hapur (KPGCS) Uttar Pradesh, (Latitude: 28 40` North and Longitude: 77° 25` East) i.e. location II. Seed was increased at both the above mentioned sites. The objective behind production of seed at two different ecologies was to ascertain genotypic stability for vigor traits. Seed increased at both the sites was evaluated for vigor traits in the Seed Technology Laboratory of Department of Seed Science and Technology, Ch Charan Singh University, Meerut. On the basis of these laboratory observations parents (Line and Testers) were selected. Crosses were made and later F1 generation was raised at Kisan (PG) College Simbhaoli, Hapur (KPGC), Uttar Pradesh.

3.1 SEEDS FOR EXPERIMENT

3.1.1 Procurement of seed

Forty local and exotic genotypes of pea were procured from Division of Germplasm Collection and Exploration, National Bureau of Plant Genetic Resources Pusa, New Delhi.

3.1.2 Seed Increase

During Rabi crop season 2009 (November, 2009-April 2010), forty pea genotypes (Table 3.1) procured from above sources mentioned above were planted in the experimental fields of Mountain Research Centre for Field Crops (MRCFC) Khudwani Anantnag, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir i.e. location I, and Department of Agri. Botany Kisan (PG) College Simbhaoli, Hapur (KPGC), Uttar Pradesh i.e. location II, to obtain fresh seeds for conducting different studies planned on seed and seedling vigor. Seed increase was done at two sites in order to obtain fresh seed from different growing ecologies.

3.1.2.1 Seed increase at two experimental sites

All the forty genotypes (Table 3.1) were planted at the above mentioned sites. Land was prepared using conventional methods i.e. disking, rolling and harrowing. It was tilled to a depth 25 cm. Wakil, a formulated mixture of Metalaxyl, Fludioxonil and Cymoxanil for the control of *Peronospora* spp (downy mildew), *Pythium* spp and *Ascochyta* spp, was applied to all seeds before sowing @ 2gm/kg. Seed was sown with hand at a depth of 5 cm; in 15 cm rows with varying inter row spacing to achieve the required pea populations. Irrigation was applied to the trial based on crop requirement. The peas were sprayed with various insecticides and pesticides to protect them from insect pests and diseases.

Table 3.1: Genotypes used during the present study and their sources

S.No	Name of accession	Designation/ Name of genotype	Botanical Name	Biological status	Source	Origin/Country
1	**IC-424886**	Field Pea	*Pisum sativum*	Landrace	NBPGR, New Delhi	India (Bihar)
2	**EC-342007**	244219-B	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
3	**IC-417876**	Purbisem/Balka	*Pisum sativum*	Landrace	NBPGR, New Delhi	India (Bihar)
4	**IC-424896**	Pea	*Pisum sativum*	Landrace	NBPGR, New Delhi	India (Bihar)
5	**IC-208368**	Unknown	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
6	**IC-267151**	Matar	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
7	**EC-538004**	X 78123	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
8	**DMR-11**	DMR-11	*Pisum sativum*	Variety	NBPGR, New Delhi	India
9	**EC-389377**	ACC 21	*Pisum sativum*	Landrace	NBPGR, New Delhi	Syrian Arab Republic
10	**IC-208364**	Unknown	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
11	**IC-417967**	NA*	*Pisum sativum*	NA	NBPGR, New Delhi	NA
12	**IC-417586**	NA	*Pisum sativum*	NA	NBPGR, New Delhi	NA
13	**EC-538005**	X-78125	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
14	**IC-267181**	Matar	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
15	**IC-208385**	Unknown	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
16	**EC-50777**	Var-0297	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
17	**IC-424895**	NA	*Pisum sativum*	NA	NBPGR, New Delhi	NA
18	**IC-417880**	Deshila Matar	*Pisum sativum*	Landrace	NBPGR, New Delhi	India (Bihar)
19	**EC-6620**	Kelvendon Wonder	*Pisum sativum*	Landrace	NBPGR, New Delhi	United Kingdom
20	**EC-538010**	OSU 667	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
21	**EC-398599**	PI 206794	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
22	**IC-208366**	Unknown	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
23	**EC-398602**	PI 358617	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
24	**EC-501259**	BR-144	*Pisum sativum*	Other	NBPGR, New Delhi	South Africa
25	**DMR-7**	DMR-7	*Pisum sativum*	Variety	NBPGR, New Delhi	India
26	**IC-417878**	Deshila Matar	*Pisum sativum*	Landrace	NBPGR, New Delhi	India (Bihar)
27	**IC-417585**	NA	*Pisum sativum*	NA	NBPGR, New Delhi	NA
28	**EC-381866-1**	Pea	*Pisum sativum*	Unknown	NBPGR, New Delhi	Unknown
29	**IC-267127**	Matar	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
30	**EC-538008**	OSU-663	*Pisum sativum*	Others	NBPGR, New Delhi	United States of America
31	**EC-499762**	Pea	*Pisum sativum*	Landrace	NBPGR, New Delhi	United States of America
32	**IC-208375**	Unknown	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
33	**IC-208369**	Unknown	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
34	**IC-267162**	Matar	*Pisum sativum*	Others	NBPGR, New Delhi	India (U.P.)
35	**NBP-72**	NBP-72	*Pisum sativum*	Variety	NBPGR, New Delhi	India
36	**NBP-61**	NBP-61	*Pisum sativum*	Variety	NBPGR, New Delhi	India
37	**NBP-82**	NBP-82	*Pisum sativum*	Variety	NBPGR, New Delhi	India
38	**NBP-60**	NBP-60	*Pisum sativum*	Variety	NBPGR, New Delhi	India
39	**EC-397113**	NA	*Pisum sativum*	NA	NBPGR, New Delhi	NA
40	**IC-418020**	Matar	*Pisum sativum*	Landrace	NBPGR, New Delhi	India (Jharkhand)

*, (NA) : Data not available.

3.1.3 Screening for Seed and Seedling Vigor

The seeds were collected from two different growing ecologies at harvestable maturity and stored for two months (60 days) at ambient storage conditions so that they may be least affected during storage. The seeds were kept in cloth bags and then stored in laboratory at an average temperature of 32^0C and 60% RH for two months to overcome the dormancy if any. The vigor tests were conducted shortly after storage period at Seed Technology Laboratory of Department of Seed Science and Technology, Ch Charan Singh University, Meerut from July-August 2010. The seeds were subjected to following vigor tests. These tests were conducted as per recommendations of International Seed Testing Association (ISTA, 1985). Each treatment was replicated three times.

3.1.3.1 Hundred Seed Weight

Three random samples of 100 well filled seeds each from the bulk produce of each accession were counted and weighed in grams.

3.1.3.2 Germination percentage and Speed of Germination (GSI)

Seed germination tests were carried out according to ISTA (1993) methods, and performed with three replications of 25 seeds each. The germination test was conducted using between paper (BP) method of germination and 25 seeds per replication were sown on paper towel (22 x 23mm), victory band, Shin bash paper company, Shizuoka Japan. Seeds were placed on the surface of double sheets of paper towel which were moistened with distilled water. The seeds were covered with another sheet of paper towel. The sheets were rolled and placed vertically in a plastic beaker, covered with polythene bag and placed at 25^oC in a germinator. 10ml of distilled water was added to each beaker every alternate day while inside the germinator to keep uniform moisture content of paper towels. No light was provided during germination, as light is not necessary for germination of pea seeds (I.S.T.A., 1985). Seeds were considered germinated when the emerging radical was at least 2 mm long (Murillo Amador et.al, 2002). Germination data was recorded from day one (D1) to day eighth (D8). Final count was made on eighth (8^{th}) day. The germination percentage was calculated by counting the total number of seeds germinated from each replication at final count.

To calculate speed of germination the number of normal seedlings were calculated from D1 to D8 and the speed of germination index (GSI) was calculated using following formula (Maguire,1962).

$$SGI = \frac{\text{Number of normal seedlings}}{\text{Days of Ist count}} + \ldots\ldots + \frac{\text{Number of normal seedlings}}{\text{Days of final count}}$$

3.1.3.3 Seedling Root Length

Between paper (BP) germination test was used. Three replications of 25 seeds were germinated between germination paper towels at 25 C in the dark germinator. Each replication consisted of three paper towels, two below the seeds and one covering the seeds. The seeds were placed at the center of the paper towels in a straight line with the radicle end towards the bottom of the towels. Once the seeds were covered with the third towel, the paper towels were folded from both sides towards the middle in a rectangle shape. The seedling root length of only normal seedlings was measured in cm at 8^{th} day after planting. Means of seedling root length of ten normal seedlings were then calculated in cm per seedling (ISTA, 1995).

3.1.3.4 Seedling Shoot Length

The same method used in the determination of seedling root length as described in 3.1.3.3 was used to measure seedling shoot length. Only normal seedlings were measured for seedling shoot length in cm. Means of seedling shoot length of ten normal seedlings were then were calculated in cm per seedling**.**

3.1.3.5 Total Seedling Length

Length of ten normal seedlings from each replicate of every accession grown in moist towel paper kept at optimum temperature of 25± ^{o}C was measured in cms on the day of final count (8^{th}) day. The diseased and deformed seedlings were discarded and not taken into consideration (ISTA, 1995). The shoot and root of each of seedlings was measured separately. The readings were then averaged.

3.1.3.6 Fresh Seedling Weight

The seedlings used for the measurement of seedling length were then used for the assessment of fresh weight. Ten normal seedlings from each replicate of every accession were weighed on the automatic digital balance along with the cotyledons and data was recorded. The readings were then averaged.

3.1.3.7 Dry Seedling Weight

The seedlings which were earlier used for the measurement of fresh seedling weight were then used for assessment of dry seedling weight. Therefore, ten seedlings from each replicate of every accession were placed in paper bags and transferred to an

oven, then dried at 80^0C for 48 hours. These were then weighed on automatic digital balance for obtaining seedling dry weight in milligrams. The readings were then averaged.

3.1.3.8 Vigor Index I and Vigor Index II

The germination percentage obtained in the germination test was used to calculate vigor index. The vigor index was calculated adopting the method of Abdul Baki and Anderson (1973).

Vigor Index I = Germination percentage x average seedling length

Vigor Index I = Germination percentage x seedling dry weight

3.1.3.9 Conductivity Test

Three replications of 25 uninjured seeds of each accession were weighed to 2 decimal places. The seeds of each replication were placed in a 200 ml beaker and 75 ml of deionized water was added. The seeds were gently stirred by a stirring rod to ensure that all seeds were completely immersed and evenly distributed. All beakers were covered by aluminum foil to reduce contaminations. The beakers were placed at the constant temperature of 20°C for 24 hours. The electrical conductivity of leachates of each replication was measured by using portable conductivity meter (Model LT-17) and $^{-1}g^{-1}$) and recorded as per the AOSA (1983) seed vigor testing handbook.

3.1.3.10 Viability Test

In this test the seeds were soaked in water for 6 hours to fully imbibe the seed and soften it for cutting. A moistened seed takes up the stain more rapidly. With the help of forceps and sharp single-edged razor blades individual seeds were cut to open the seeds. The solution that is used to make a tetrazolium (TZ) test is colorless. It was made by dissolving 5 gms of 2,3,5-triphenol tetrazolium chloride powder in 1000 ml of distilled water to make 0.5% solution.

This colorless solution was then added to every petri dish containing 50 seeds each (3 replications in each accession).The Petri dishes were then kept at a dark place in laboratory for 4 hours and then taken out for evaluation. The TZ solution reacts with respiratory enzymes (i.e., dehydrogenase) to form an insoluble light pink (magenta) precipitant called formazan. Following that, tetrazolium solution was removed, and seeds were washed with distilled water. Vigor evaluation was made on the basis of color intensity, location, missing embryo parts, fractures, and location of imperfect embryo parts within the essential embryonic structures. The viable seeds

were evaluated more critically into categories of high, medium and low vigor. a). Staining is uniform and even, tissue is firm and bright. (high vigor) b). Embryo completely stained or embryonic axis stained and extremities unstained, some over stained/less firm areas exist, (medium vigor) c). Large areas of non-essential structures unstained; extreme tip of radicle unstained (dicots) and the tissue are milky, flaccid and over stained, (low vigor).

3.1.4 Statistical Analysis

The data recorded for each character on the basis of test parameters were averaged and the mean values obtained were used for statistical analysis. The data from different test parameters was subjected to analysis of variance as a randomized complete block design, to calculate genotypic and phenotypic coefficient of variation, heritability and genetic advance using software INDOSTAT 7.5 version and SPAR developed by Indian Agricultural Statistical Research Institute, New Delhi. The simple correlation studies were carried out among different parameters to know the relationship among seed and seedling traits. These parameters were calculated as per following procedure.

3.1.4.1 Analysis of variance

Analysis of variance for individual character was done on the basis of mean values as suggested by Panse and Sukhatme (1942). The model of analysis of variance adopted is given below.

Source	**d.f.**	**SS**	**MSS**	**F ratio**
Replication	(r-1)	RSS	Mr (M1)	M1/M3
Treatment	(t-1)	TSS	Mt (M2)	M2/M3
Error	(r-1) (t-1)	ESS	E (M3)	-

r = Number of replications

t = Number of treatments

Significance of treatment was tested at five and one per cent probability levels.

3.1.4.2 Components of variance

Genotypic and phenotypic variance was computed from ANOVA table based on the expected mean sum of squares as follows.

The treatment sum of square is made up of environmental variation along with

= number of replications).

$$\text{Genotype variance (Vg)} = \frac{M_2 - M_3}{r}$$

$$\text{Phenotype variance (Vp)} = \frac{M_2 - M_3}{r} + M$$

or

$$V_p = v_g + v_e$$

Where v_e = Environmental variance

3.1.4.3 Coefficient of variation (Cv)

Genotypic and phenotypic coefficients of variation were computed according to Burton Devane (1953).

$$\text{Genotypic cofficient of variance (GCV)} = \frac{\sqrt{Vg}}{\bar{x}} \times 100$$

$$\text{Phenotypic coefficien t of variance (PCV)} = \frac{\sqrt{Vp}}{\bar{x}} \times 100$$

Where,

Vg = Genotypic variance.
Vp = Phenotypic variance.
$\bar{x}$ = General mean of the character.

3.1.4.4 Heritability

Broad sense heritability was estimated as the ratio of genotypic variance to the phenotypic variance and was expressed in percentage (Hanson et al., 1956).

$$\text{Heritabili ty } (h^2) = \frac{Vg}{Vp} \times 100$$

Where,

Vg = Genotypic variance
Vp = Phenotypic variance

3.1.4.5 Genetic advance (GA)

Genetic advance (GA) was computed according to the formula given by Johnson et al. (1955).

$$\text{Genetic advance (GA)} = h^2 \times i \times \sigma p$$

Where,

h^2 = Broad sense heritability

i = Selection differential (2.06) at 5% selection intensity

p = Phenotypic standard deviation

3.1.4.6 Phenotypic and genotypic coefficient of correlations

Correlation coefficients were worked out to determine the degree of association among the various vigor characters. This was done according to the formula given by Al- Jibouri et al. (1958).

$$r(xy) = \frac{Cov(xy)}{\sqrt{Var\,(x) \times Var\,(y)}}$$

Where,

r (xy) = Correlation coefficient between characters x and y

Cov (xy) = Covariance of characters x and y

Var x = Variance of character x

Var y = Variance of character y

Test of significance of correlation was done by comparing the computed values against table 'r' values given by Fisher and Yates (1963).

3.1.4.7 Path coefficient analysis

The estimates of direct and indirect effects were calculated by the path coefficient analysis method as suggested by Wright (1921) and illustrated by Dewey and Lu (1959). The direct path coefficient was calculated by solving the formula sets of 'P' simultaneous equations by the abbreviated "Doo-Little Technique" as described by **Goulden (1959).**

$P_{Y1}r_{12} + P_{y2}r_{12} + \ldots\ldots\ldots\ldots P_{yp}r_{1p} = r_{y1}$

$P_{Y1}r_{21} + P_{y2}r_{22} + \ldots\ldots\ldots\ldots P_{yp}r_{2p} = r_{y2}$

$P_{Y1}r_{p1} + P_{y2}r_{p2} + \ldots\ldots\ldots\ldots P_{yp}r_{pp} = r_{yp}$

Where,

$P_{y1}, P_{y2} \ldots\ldots\ldots P_{yp}$ are direct path effects of independent variables 1, 2, ... P on the dependent variable 'y'.

r11, r12 . . . . . r1p . . . . rpp are correlation coefficients between various independent variables.

$r_{11}, r_{12} \ldots\ldots r_{1p} \ldots\ldots r_{pp}$ are correlation coefficients of independent variables with dependent variable.

The indirect eff $(P_{yj} \times r_{ij})$.

Residual effect (Pry) is calculated as $1 = P_{ry} + P_{y1} + r_{y1} + P_{y2}.r_{y2} + \ldots P_{yp}r_{yp}$.

3.2 SELECTION OF PARENTS AS LINES AND TESTERS

Speed of germination was used as a measure of seed and seedling vigor for selection of parents to be used for crosses (Maguire, 1962; Seshu et al., 1987). The accessions possessing high value for speed of germination were classified as high vigor types and those showing low speed of germination as low vigorous seeds. The three accessions showing high vigor values were selected as testers and twenty accessions showing low vigor values were selected as lines (Table no. 3.2). Further studies were based on these crosses.

Table No: 3.2 Characteristics of twenty three genotypes used in the line x tester crosses.

S.No	Genotype	Speed of germination	Germination Percentage	100 Seed weight (gm)	Total seedling length (cm)	Seedling dry weight (mg)	Vigor index I	Vigor index II	Conductivity Value	Viability percentage	Remarks	Selected Parene
1	IC-208375	15.25	87.0	16.24	22.91	566.66	1984.12	49246.66	5.63	95.11	High	Tester
2	EC-398602	14.65	89.0	15.07	23.30	470.66	2065.36	41963.33	9.50	91.73	High	Tester
3	NBP-82	14.17	88.0	14.99	32.35	320.00	2837.46	32226.66	6.23	94.11	High	Tester
4	EC-538008	10.73	86.0	12.60	18.86	320.00	1627.53	27570.00	20.80	96.40	Low	Line
5	IC-417878	10.50	67.0	10.62	9.20	440.00	621.11	29710.00	25.26	76.07	Low	Line
6	IC-208366	10.49	70.0	12.60	8.78	266.66	614.98	18666.66	10.96	77.29	Low	Line
7	IC-267127	10.45	73.0	11.84	9.30	300.00	674.98	21840.00	14.80	83.73	Low	Line
8	DMR-7	10.43	77.0	12.45	15.60	380.00	1207.90	29430.00	24.76	93.11	Low	Line
9	IC-424886	10.35	71.0	11.56	4.01	291.00	286.02	27823.33	23.36	79.18	Low	Line
10	IC-267162	10.34	89.0	13.82	17.24	386.66	1529.32	34580.00	18.76	81.33	Low	Line
11	IC-417586	10.17	69.0	11.84	8.46	423.33	557.00	29056.66	15.76	76.51	Low	Line
12	IC-267151	10.16	76.0	11.86	8.21	264.00	623.36	20073.33	15.26	91.22	Low	Line
13	IC-208364	10.01	75.0	11.28	11.29	306.66	849.65	23173.33	12.20	93.69	Low	Line
14	EC-398599	9.84	86.0	13.87	11.18	300.00	958.40	25706.66	21.53	78.18	Low	Line
15	IC-424895	9.63	82.0	11.05	6.26	183.33	512.36	14936.66	23.70	73.73	Low	Line
16	DMR-11	9.43	60.0	11.89	6.32	296.66	382.74	32583.33	13.56	77.14	Low	Line
17	IC-208385	9.39	80.0	13.73	11.85	255.33	942.02	20380.00	32.96	86.29	Low	Line
18	EC-342007	9.33	65.0	12.85	7.0	370.00	459.93	24163.33	23.30	84.82	Low	Line
19	EC-538004	9.27	62.0	9.51	8.90	296.66	550.83	18320.00	12.73	81.48	Low	Line
20	IC-424896	9.06	84.0	10.23	6.16	448.33	474.71	37816.66	24.26	83.49	Low	Line
21	IC-267181	8.97	53.0	12.06	6.92	320.00	386.18	16786.66	22.76	83.22	Low	Line
22	IC-208368	8.00	63.0	13.25	9.10	454.00	611.40	28460.00	23.03	90.03	Low	Line
23	EC-538005	6.92	54.0	10.67	8.34	510.00	481.05	27580.00	20.43	85.07	Low	Line

3.3 LINE x TESTER CROSSES

The twenty three selected accessions (Table 3.2) were crossed during Rabi 2010 (2010-2011) at experimental field of Department of Agri. Botany Kisan (PG) College Simbhaoli Hapur (KPGCS), Uttar Pradesh in a line x tester design to raise the hybrid seed.

3.3.1 Crossing Procedure

Seeds of all selected parents were sown in the field. The row to row and plant of plant distances was kept at 50cm and 10cm respectively. Effective measures were taken to grow the normal crop.

The parents used as females were hand emasculated at an early blooming stage in the morning. If more than one flower was present on peduncle, only one was retained and another was snipped off. The sepal in front of keel was torned away with the help of forcep. By positioning the forefinger behind the flower and the thumb in front of flower, a light squeezing pressure was applied to spread the standard and wings to expose the keel. The keel was spread apart with the help of forcep, and its both halves were hold down by the thumb and finger to spread it. The ten anthers were removed by grasping the filaments with the forcep and were pulled gently.

The emasculated flowers were covered with butter paper to prevent incidences of cross pollinations if any. For pollination, the anthers of the respective testers at a stage just prior or just after to their opening were collected and transferred on the tips of forceps or toothpick to the stigma of emasculated flower at about 4:30 PM in the afternoon. Extensive care was taken to avoid any injury to the style. After the pollination, the petals were fold back to their original position to protect the stigma from desiccation and reduce the exposure to foreign pollen. The pollinated flowers were identified with a tag indicating the names of male and female parents, and the date of pollination. F1 seeds were collected at the harvestable maturity. The seeds were dried to 10 % moisture and stored in cloth bags under ambient conditions.

3.4 GENETIC STUDIES

3.4.1 Laboratory Studies

Half of the hybrid seeds were used for laboratory studies carried out at Seed Technology Laboratory of Department of Seed Science and Technology, Ch Charan Singh University, Meerut from May-June 2011. The seeds were subjected to following laboratory studies.

(3.4.1.1) **Hundred Seed Weight……....** (See 3.1.3.1)

(3.4.1.2) **Speed of Germination** (**GSI**)……**....** (See 3.1.3.2)

(3.4.1.3) **Seedling Root Length…….**(See 3.1.3.3)

(3.4.1.4) **Seedling Shoot Length……...** (See 3.1.3.4)

(3.4.1.5) **Total Seedling Length** ..(See 3.1.3.5)

(3.4.1.6) **Fresh seedling weight……....** (See 3.1.3.6)

(3.4.1.7) **Dry Seedling Weight…….** (See 3.1.3.7)

(3.4.1.8) **Vigor Index I and Vigor Index II…….** (See 3.1.3.8)

(3.4.1.9) **Conductivity Test……....** (See 3.1.3.9)

(3.4.1.10) **Viability Test** (See 3.1.3.10)

3.4.2 Field Studies

The remaining hybrids and twenty three parents involved in the line x tester crosses were raised at the experimental field of Department of Agri. Botany Kisan (PG) College Simbhaoli, Hapur (KPGCS), Uttar Pradesh during Rabi 2011 (2011-2012) field in a randomized block design (RBD) with three replications. A replication comprised of 83 entries (60 F_1s+23 parents). Each entry was assigned a single row/plot of three meter length in every replication. The row to row and plant of plant distances was kept at 50cm and 10cm respectively. Effective measures were taken to grow the normal crop. Five randomly selected consecutive normal plants from each plot of each replication were subjected to following observation.

3.4.2.1 Plant height

Height of the plant was noted with the help of measuring tape at maturity. For this purpose ten plants from each treatment were selected randomly, their height was taken and averages were computed.

3.4.2.2 Pods per plant

Pods were harvested when they ripened. Ten plants from each treatment were selected randomly and number of productive pods was counted and average was calculated.

3.4.2.3 Pod length

Length of pods was measured with the help of measuring tape after harvesting pods and ten pods were selected from each treatment. Length of pod was measured from the base (the point it is connected to plant) up to its extreme tip.

3.4.2.4 Seeds per pod

The pods which were selected for measuring the length were threshed separately, seeds were counted and numbers of seeds per pod were obtained.

3.4.2.5 Seed yield per plant

From each replication ten plants were selected randomly. Seeds from plants were threshed individually, seeds were weighed in grams and average was obtained.

3.5 STATISTICAL ANALYSIS

The data recorded on the 60 F1 hybrids and 23 parents were subjected to the following statistical analysis.

3.5.1 Line x Tester Analysis

3.5.1.1 Analysis of variance

The first step in the Line x Tester analysis is to perform analysis of variance for Randomized Block Design to test the significance of difference among genotypes including crosses and parents. The analysis of variance was done for all the characters as per the method given by Kempthorne (1957). The variance was estimated as under.

S.No	Source of variation	d.f.	SS	MS	F value calculated
1	Replication	(r-1)	RSS	RSS/(r-1)	RMSS/EMSS
2	Treatment	(n-1)	TrSS	TrSS/(n-1)	TrMSS/EMSS
3	Error	(r-1) (n-1)	ESS	ESS/(r-1) (n-1)	
Total		(m-1)	Total SS		

1. C.F. = (G.T.) ²/r x n
2. Total S.S. = Total sum of square of all the observations – C.F.
3. $\text{Replicatio n S.S.} = \frac{\text{SS for all replicatio n total}}{\text{r}} - \text{C.F.}$
4. $\text{Treatment S.S.} = \frac{\text{SS for all replicatio n total}}{\text{r}} - \text{C.F.}$
5. Error S.S. = Total S.S. (Replication S.S. + Treatment S.S.)

Where,

r = Number of replications

n = Number of treatments

The metric traits which appeared to be significant were further subjected to line x tester analysis.

In order to test the significance of the crosses and parents individually, further partitioning of treatment S.S. was done for males, females, males Vs females, hybrids and hybrids Vs parents as follows.

S.No	Source of variation	d.f.	Means of squares	F value calculated
1	Replication	(r-1)		
2	Treatment	(n-1)		
3	Parents	(p-1)		
4	Hybrids	(h-1)		
5	Parents vs hybrids	(1)		
6	Males	(m-1)		
7	Females	(f-1)		
8	Males vs females	(1)		
9	Error	(r-1) (n-1)		
Total				

Where,

r = number of replications
n = number of treatments
h = number of hybrids
m = number of males
f = number of females

3.5.1.2 Heterosis

Heterosis over mid parent (MP)

The heterosis expressed as percentage increase or decrease in the mean value of hybrids over its mid parental value.

$$\text{Percent heterosis over better parent (BP)} = \frac{\overline{F_1} - \overline{MP}}{MP} \times 100$$

Where,

$\overline{F_1}$ = Mean of the F_1 hybrid

$\overline{MP}$ = mean of the two parents of that particular F1 cross

Heterosis over better parent (BP)

The heterosis expressed as percentage increase or decrease in the mean of F_1 hybrids over its better parent.

$$\text{Percent heterosis over better parent (BP)} = \frac{\overline{F_1} - \overline{B}}{BP} \times 100$$

Where,

□□□□□□□□□□□□□□□□□□□□ $\overline{F_1}$ = Mean of the F_1 hybrid

$\overline{B}$ = Mean of the better parent of that particular cross

Calculation of critical difference for heterosis over BP and MP.

i) C.D. for MP = $\sqrt{\frac{3Me}{2r}} \times t$ value at 5% and 1%

ii) C.D. for BP = $\sqrt{\frac{2Me}{2r}} \times t$ value at 5% and 1%

Where,

Me = Error mean sum of squares

r = Number of replications

3.5.1.3 Combining ability

Combining ability analysis for the F1 hybrids was based on the procedure developed by Kempthorne (1957). The sum of squares due to different factors was partitioned as shown below.

S.No	Source	d.f.	SS	MS	Expected
1	Replication	(r-1)	$\frac{\Sigma x2\ldots k}{Mf} - \frac{X2\ldots.}{mfr}$		
2	Hybrids	(mf-1)	$\frac{\Sigma x2ij}{r} - \frac{X2\ldots.}{mfr}$		
3	Males	(m-1)	$\frac{\Sigma x2i}{fr} - \frac{X2\ldots.}{mfr}$	M1	σ^2e+r (Cov (F.S.)-2 COV (H.S.) + (fr Cov (H.S.))
	Females	(f-1)	$\frac{\Sigma x2j}{mr} - \frac{X2\ldots.}{mfr}$	M2	σ^2e +r ((Cov(F.S.)-2 Cov (H.S.)) – (mrCOV (H.S.))
	Males x Females	(m-1)	$\frac{\Sigma x2ij}{r} - \frac{X2\ldots.}{mfr}$	M3	σ^2e + r ((Cov. (F.S.)-2 Cov. (H.S.))
	Error	(r-1) (mf-1)	By subtraction	M4	σ^2e
	Total	(mfr-1)	$\Sigma x2ijk - \frac{X2\ldots.}{mfr}$		

Where,

m = Number of male parents

f = Number of female parents

r = Number of replications

x = grand total

x . . . k = Sum of the kth replications

=Sum of the jth female parents overall male parents and replications

x(ij) = Sum of the ijth hybrid combination overall replications

xi = Sum of the ith male parents overall females and replications

xijk = (ij)th observation in kth replication

M_1 = Mean sum of square of males

M_2 = Mean sum of square of females

M_3 = Mean sum of square of males and females interaction

M_4 = Error mean sum of squares

From the expectation of mean square Co-variance of full sibs (Cov (F.S.)) and covariance of half sib (Cov. (H.S.)) were estimated as follows.

$$\text{Cov (H.S.)} = \frac{M_1 - M_3) + (M_2 - \quad _3)}{r\ m + f)}$$

$$\text{Cov (F.S.)} = \frac{M_1 - M_4) + (M_3 - M_4)}{3r} + \frac{\sigma r\ \text{Cov. (H.S.)} - r(m+f)}{3}$$

After estimating the Cov (H.S.) and Cov (F.S.) by above equations, variance due to general combining ability (s^2gca) and variance due to specific combining ability (s^2sca) were estimated as:

s^2gca = Cov (H.S.) here breeding coefficient =1

s^2sca = (Cov (F.S.) – 2 Cov. (H.S.))

3.5.1.3.1 Estimation of general and specific combining ability effects

The model used to estimate the general and specific combining ability effects of the ijk observations was;

$$Xijk = \mu + gi + gj + Sij + eijk$$

Where,

μ = Population mean

gi = gca effects of the ith male parent

gj = gca effects of the jth female parent

sij = sca effects of the ijth combination

eijk = Error associated with the observation xijk

The individual effects were estimated as follows:

a) μ = x . . . / mfr x . . . . = grand total

b) gi = xi . . . . /fr x . . . ./mfr

xi. . . = Total of ith male parents overall females and replications

c) $$gi = \frac{xj}{mr} - \frac{x...}{mfr}$$

xj = Total of jth female parent overall male parents and replication

d) $$Sij \quad \frac{xij}{r} - \frac{xi...}{fr} \quad \frac{xj}{mr} + \frac{x...}{mfr}$$

Where,

xij = (ij)th combination total overall replications

m = Number of male parents

f = Number of female parents

r = Number of replications

3.5.1.3.2 Calculation of S.E. of differences between general and specific combining ability effect of two parents

i) S.E. (gi) = $\sqrt{M(e)/rt}$

ii) S.E. (gt) = $\sqrt{M(e)/rl}$

iii) S.E. (sij) = $\sqrt{M(e)/r}$

iv) S.E. (gi – gj) for lines = $\sqrt{2M(e)/rl}$

v) S.E. (gi – gj) for testers = $\sqrt{2M(e)/rt}$

vi) S.E. (sij – skl) for other hybrid = $\sqrt{}$

Where;

Me = Mean square due to error

r = Number of replications

t = Number of testers

l = Number of lines