Chapter **3**

Materials and Methods

The details of materials used and methods adopted during the course of investigation are described below:

3.1 Experimental site

The present investigation was carried out at Horticultural Research Station, Mandouri, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal during the year 2014-15 and 2015-16. The research station is located at 23.5° North latitude, 89° East longitude having an average altitude of 9.75m above mean sea level.

Table 1: Soil characteristics of the experimental field

Particulars	Results	Analytical methods
1. Mechanical composition of soil ➢ Sand(%) ➢ Silt (%) ➢ Clay(%)	 48.8 27.1 30.2	International pipette method (Piper, 1966)

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2. Chemical composition of soil ➢ Organic carbon(%) ➢ Total nitrogen(%) ➢ Available phosphorus (Kg/ha) ➢ Available Potassium (Kg/ha)	6.9 0.65 0.07 21.00 78.00	Blackman Ph meter method (Jackson,1973) Walky and Black Method (Jackson, 1973) Modified Kjeldahl's method (Jackson, 1973) Bray and Kurtz method (Jackson, 1973) Flame Photometer method (Jackson, 1973)

3.2 Climatic condition during the experimentation period

Table 2: Meteorological observation during the period of experiment.

2014-15 Months	Temperature (^{0}C)		Rainfall (mm)	Relative humidity (%)		Bright sunshine hour
	Max.	Min		Max.	Min.	
November	29.47	14.98	0.0	95.00	57.78	9.4
December	24.27	11.16	0.0	97.20	63.00	5.7
January	25.16	14.37	1.2	95.65	58.35	8.9
February	28.74	14.42	7.3	86.98	55.58	5.5
March	32.50	18.33	21.2	99.67	63.50	9.7

2015-16 Months	Temperature(°C)		Rainfall (mm)	Relative humidity (%)		Bright sunshine hour
	Max	Min		Max	Min	
November	30.42	16.88	0.0	98.40	55.10	7.27
December	26.00	17.52	0.42	95.61	55.81	3.28
January	25.06	9.85	0.032	97.84	53.97	4.88
February	29.80	16.06	2.76	98	56.97	5.29
March	33.23	19.58	0.85	93.13	51.13	7.14

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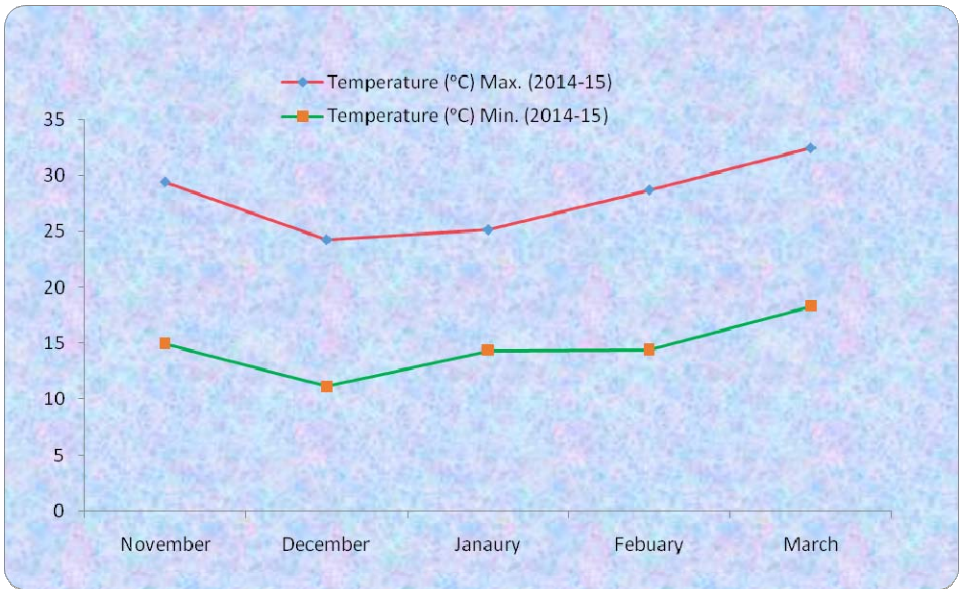

Fig. 2: Monthly minimum and maximum temperature (°C) during 2014-15

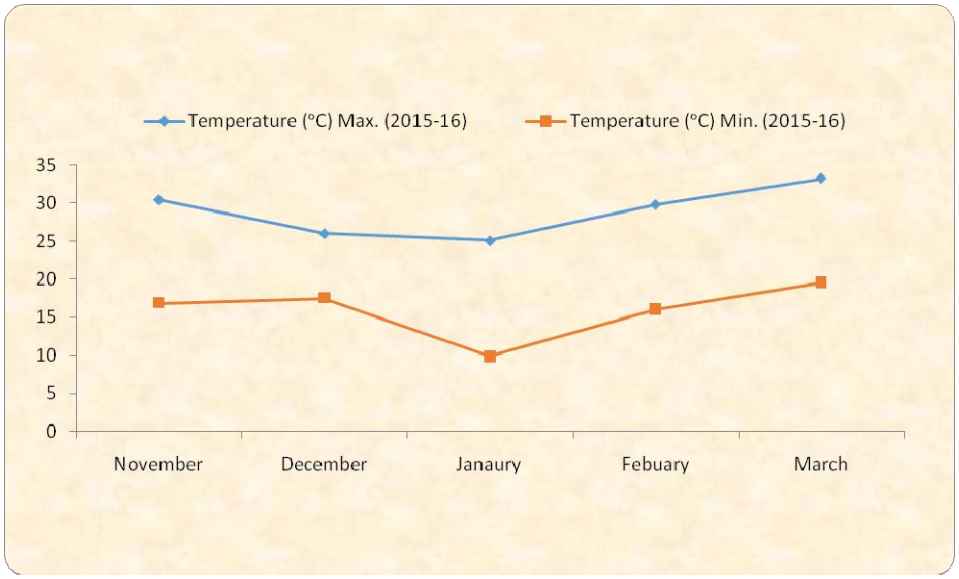

Fig. 3: Monthly maximum and minimum temperature (°C) during 2015-16

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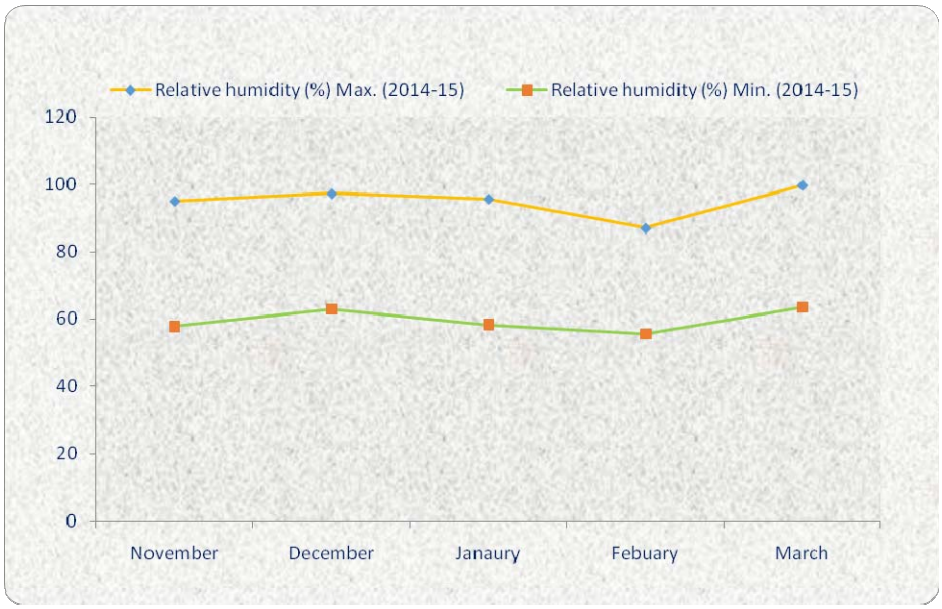

Fig. 4: Monthly maximum and minimum relative humidity (%) during 2014-15

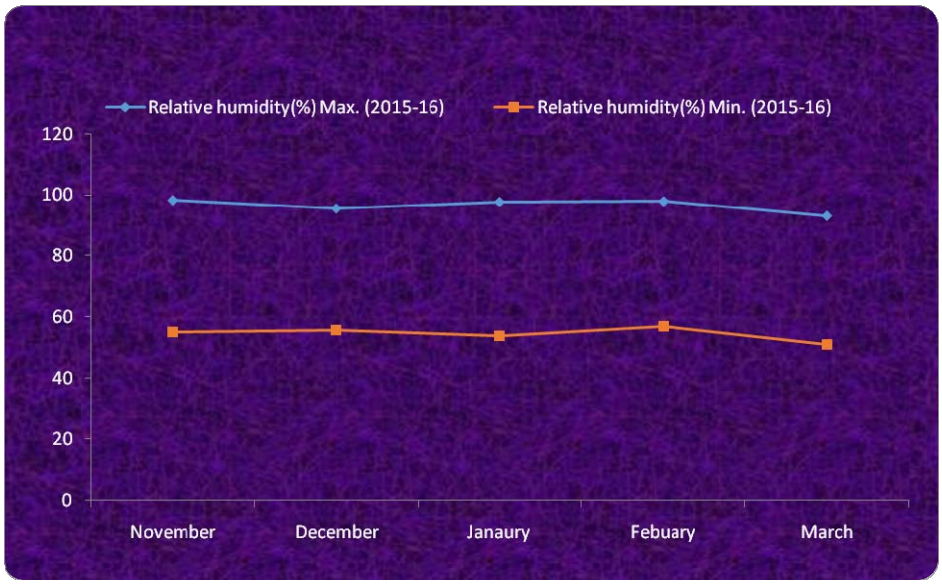

Fig. 5: Monthly maximum and minimum humidity (%) during 2015-16

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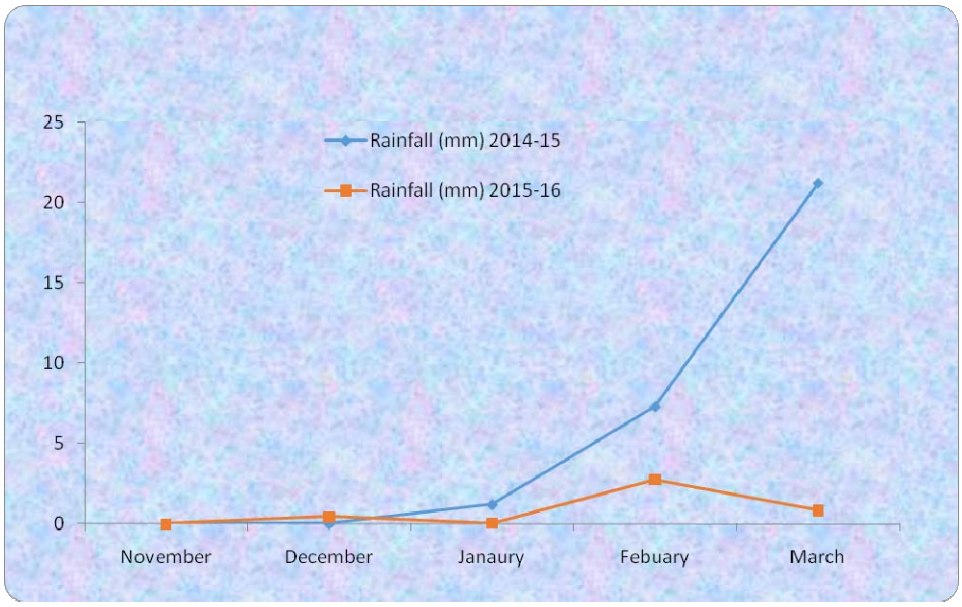

Fig. 6: Monthly rainfall (mm) during period of investigation

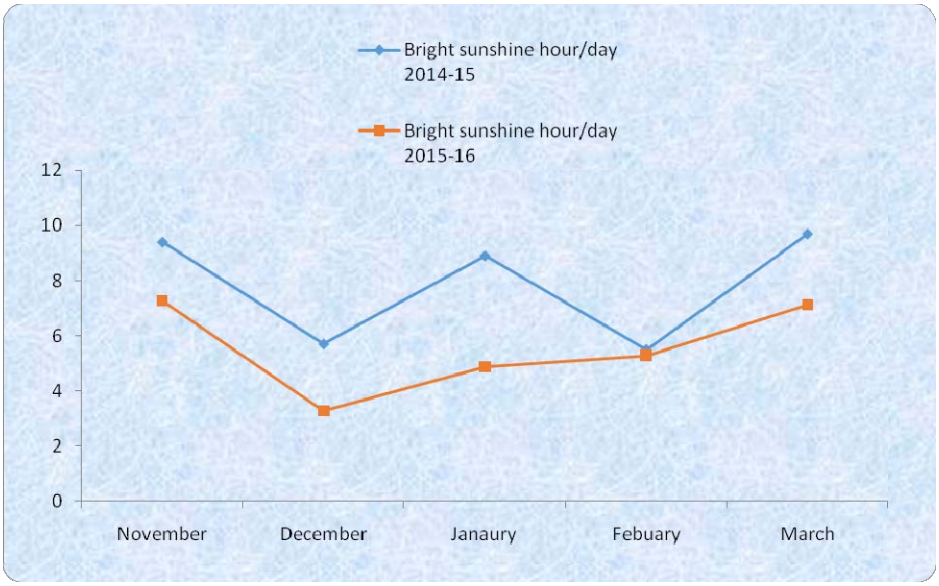

Fig. 7: Monthly bright sunshine hour/day

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3.3. Experimental details

Design of the experiment: Randomized block design (RBD)

Number of treatments: 12

Number of replication: 3

Plot size: 1.8m×1m

Spacing adopted: 25cm×15cm

No. of plants/plot: 48

No. of total plot: 36

Time of planting (2014, 2015): 10th Nov

Table 3: Treatment details

Treatment	Germplasms	Collected from
1.	Arka Isha	IIHR, Bangalore
2.	Manipur collection-1	Imphal, Manipur
3.	Suvashini	Gayespur, West Bengal
4.	West Bengal collection-1	Mohanpur, West Bengal
5.	West Bengal collection-2	Mohanpur, West Bengal
6.	Assam collection	Jorhat, Assam
7.	Five -X	Gayespur, West Bengal
8.	Manipur collection-2	Imphal, Manipur
9.	NRCS A.Cr-1	NRCS, Ajmer
10.	Tripura collection	Tripura
11.	Pant Haritma	GBPUAT, Pantnagar
12.	West Bengal collection-3	Cooch Behar, West Bengal

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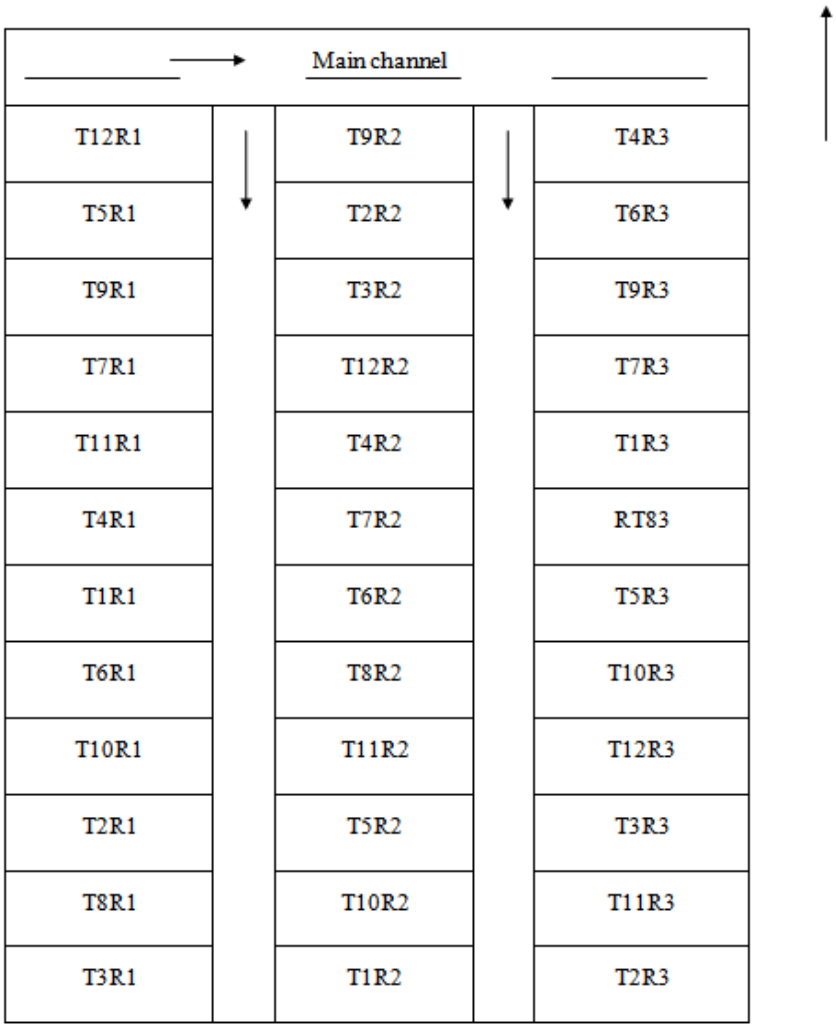

Fig. 1: Layout of experimental plot

Plot size: 1.8m×1m (Irrigation channel: 0.5m, Bund size: 0.30m)

3.4. Cultural operation

Land preparation

One deep ploughing with tractor followed by two ploughings with power tiller was done in order to make soil friable and pulverized. The weeds and stubbles were removed to have a clean seed bed. Levelling was done

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properly as far as practicable with the help of ladder to avoid water stagnation.

Manure and fertilizer

Well rotten Farm Yard Manure (FYM) @ 20t/ha was applied by broadcasting and mixed thoroughly at the time of land preparation. In addition to that the inorganic fertilizers like N, P and K were applied at the rate of 30:40:20 kg N, P_2O_5 and K_2O per ha respectively. Half dose of N and full dose of P and K were applied as basal and remaining half dose of N was applied 30 days after sowing. All the plots received an equal amount of chemical fertilizer. The sources of N, P_2O_5 and K_2O were Urea, single super phosphate and muriate of potash respectively.

Sowing

Seed of coriander were soaked overnight for better and quick germination and dried under shade and were sown in previously prepared plots at depth of 2-3cm and covered with fine layer of soil. The seeds were sown in line with spacing of 25cm (row to row) ×15 cm (plant to plant).

Intercultural operation

Thinning and hoeing was done 30 days after sowing along with first weeding to loosen the soil and maintain the plant spacing as per programme. At the time of thinning, the remaining half dose of N was applied as top dressing. After the first weeding, the experimental plot was kept clean by hand weeding as and when it was required.

Irrigation

A presowing irrigation followed by irrigation a week after sowing was given to enhance uniform seed germination.

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Plate 1: Land Preparation

Harvesting

The crop was harvested when 60 per cent of seeds in main umbels turn yellowish brown in colour. After judging the crop maturity of each plot, plants were harvested by pulling out plants from the soil. The pulled out plants are tied in small bundles and kept upside down in field so that the seed are less exposed to sun and to get uniform colour in seeds.

Drying and threshing

All harvested plants were kept under sun drying for three days and then in shades for two days. Then, threshing was done by beating lightly with

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sticks, followed by winnowing and cleaning. For safe storage, again seeds were dried in bright sun light for two days to bring down the excess moisture content. During drying and threshing, proper care was taken to avoid intermixing of seeds.

3.5. Observations recorded:

Morphological characters

- Plant height (cm)
- Number of basal leaves
- Length of longest basal leaves(cm)
- Number of primary branches
- Number of secondary branches

Phenological characters

- Duration from sowing to flowering (days)
- Duration from sowing to fruit maturity (days)

Yield and yield attributing characters

- Number of umbels per plant
- Number of umbellets per umbel
- Number of seeds per umbel
- Test weight (g)
- Seed yield per plant(g)
- Seed yield per plot(g)
- Estimated seed yield (q/ha)

Quality characters

- Leaf colour
- Basal lobing
- Leaf blade shape

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- Stem colour
- Seed colour
- Seed shape
- Oleoresin content(%)
- Essential oil content (%)

Methods of recording different biometrical data

Crop growth parameters

1. Duration from sowing to first flower initiation

Days to flower initiation were recorded from the date of sowing to flowering.

2. Duration from sowing to fruit maturity

Days to maturity were computed at the time taken from date of sowing to maturity of umbel had turned yellow colour.

3. Plant height (cm)

The heights of plants were measured in coriander from the ground level to the tip of the plant at the time of maturity.

4. Number of basal leaves

Number of basal leaves recorded 30 DAS

5. Length of longest basal leaves

The length of longest basal leaves were measured at 30 DAS

6. Number of Primary branches

Numbers of primary branches per plant were counted at the time of maturity

7. Number of Secondary branches

Numbers of secondary branches were counted at the time of maturity.

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8. Number of umbels per plant

At the time of maturity total number of umbels from each selected plant were picked and counted.

9. Number of umbellets per umbel

Total numbers of umbellets from each umbel were counted at the time of maturity.

10. Number of seeds per umbel

Total numbers of seeds from each umbel were counted at the time of maturity.

11. Test weight (g)

1000-seeds from each of the treatment were counted, sun dried grain and weight recorded through electronic balance.

12. Seed yield per plant(g)

The total seed obtained from the harvest of each of the selected plants was weighed (g) from each treatment separately and recorded as seed yield.

13. Seed yield per plot(g)

Total weight of seed per plot was recorded from each plot and the mean value was taken for analysis.

14. Projected yield per ha(q)

Total amount of seeds from plot of 1×1.8m2 were weighted to obtain the yield per plot. Projected yield per hectare was calculated on the basis of yield per plot and expressed in quintal.

Assay of biochemical constituents

Oleoresin content

Oleoresin content was estimated by Chromatographic column method. For this 10gm of powder sample was taken and placed in chromatographic

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column (bottom of chromatographic column was plugged with cotton plug). The column was filled with acetone and kept overnight. The slurry was collected on a previously weighted beaker and weigh was recorded. This slurry was the heated over a water bath to 40°C. Weight was noted at 15minutes interval and heating was stopped when constant weight was obtained. The the oleoresin content was calculated using the formula given below.

$$\% \text{ Oleoresin} = \frac{\text{weight of oleoresin}}{\text{Weight of sample}} X100$$

Where, weight of oleoresin=(weight of beaker+oleoresin)-weight of beaker

Essential oil content

The essential oil of the sample was estimated using Clevenger type apparatus and the percentage of essential oil was calculated using the following formula:

$$\text{Essential oil content (\%)} = \frac{\text{essential oil extracts (ml)}}{\text{Weight of sample (g)}} X100$$

3.6. Statistical Analysis:

The following statistical analyses were carried out to determine the genetic variability parameters, character associationships and genetic components employing the observations recorded on different growth and fruit characters of chilli.

Analysis of Variance:

The mean value of the characters from each genotypes in each replication was used for statistical analysis.

Model for RBD: $Y_{ij} = \mu + gi + rj + eij$

y_{ij} = Phenotypic observation in its variety and j^{th} replication.

μ = general mean.

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gi = effect of i^{th} varieties.

r_j = effect of j^{th} replication.

eij = Random error effect associated with i^{th} varieties and j^{th} replication.

ANOVA Table:

Sources	d.f.	M.S.	Expected M.S.	F.
Genotypes	(t – 1)	Mt 1.1	δ^2e 1.1 + rδ^2 g 1.1	M + 1.1/Me 1.1
Replications	(r – 1)	Mr 1.1		
Error	(r – 1) (t – 1)	Me 1.1	δ^2 e 1.1	

Where, t = number of genotypes; r = number of replications.

Standard error (S.E.), Standard error of difference between two means (S. Ed.) and Critical difference (C.D.) were calculated by the following formula :

S. E. = $(Mse/r)^{1/2}$

S.Ed = $(2\ Mse/r)^{1/2}$

Where, Mse = Error mean Sum of Square.

r = Number of replications.

C.D. = S. Ed. X t 5% at error d.f.

Co-efficient of Variance (CV%) was calculated as follows

$$CV\ (\%) = \frac{\sqrt{Mse}}{\bar{x}} X100$$

Where, Mse = Error Mean Square

x = Gēneral mean for the character.

Component of variances :

The Expected mean sum of squares for error (Mse) will be partly a random environmental variance.

i.e. Error variance = δ e 1.1 = Mse 1.1

So, Genotype variance = δ^2 g 1.1 = (Mt 1.1 – Me 1.1)/r

The phenotypic variance will consist of variation among genotypes and environmental variation among the individuals of each varieties.

Phenotype variance = δ^2 P 1.1 = (δ^2 g 1.1 + δ^2 e 1.1)

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Where, Mt 1.1 and Me 1.1 stand for mean sum of squares due to genotype and error respectively

The genotype (G.C.V.) and phenotype (P.C.V.) co-efficient of variation were calculated by the following formula given by Burton (1952).

$$GCV = \frac{\text{Genotypic standard deviation}}{\text{Grand Mean}} X100$$

$$PCV = \frac{\text{Phenotypic standard deviation}}{\text{Grand Mean}} X100$$

Heritability in broad sense (H) was estimated by the formula given by Hanson *et al.* (1956).

$$H(\%) = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} X100$$

The expected genetic advance (GA) was calculated by the formula as suggested by Johnson *et al.* (1955) and Lush (1949).

$$GA = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} x\ K\ x\ P = \delta\ H\ x\ K\ x\ \delta\ P$$

Where,

H = Heritability in broad sense.

δ P = Phenotypic Standard deviation.

K = Selection differential, a constant value is 2.06 for 5% selection intensity.

Analysis of Co-variance:

Co-variance components were estimated from co-variance analysis in an analogous manner to the variance components computed from the analysis of variance. The structure of co-variance table was as follows:

ANOVA Table:

Sources	**d.f.**	**M.S.**	**Expected M.S.**	**F.**
Genotypes	(t – 1)	Mt 1.2	δ^2e 1.2 + r δ^2 g 1.1	M + 1.2/Me 1.1
Replications	(r – 1)	Mr 1.2		
Error	(r – 1) (t – 1)	Me 1.2	δ^2 e 1.2	

Where, t = number of genotypes; r = number of replications.

Genotypic co-variance = δ g 1.2 = (Mt 1.2 – Me 1.2)/r

Phenotype co-variance = δ^2 p 1.2 = (Mt 1.2 – δ e 1.2)

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Genetic Correlations were computed from the genetic variance and co-variance by the following formula –

$$r(xy) = \frac{Cov.(xy)}{Var.(X).Var.(Y)}$$

where, r (xy) is the correlation between characters 'x' and 'y',

Cov. (xy) is the Covariance between 'x' and 'y'

Var. (x) is the variance of 'x'

Var. (y) is the variance of y

Path co-efficient was calculated to estimate the direct and indirect effects of the characters as per Dewey and Lu (1959).

D^2 statistic (Mahalanobis, 1936)

D^2 statistic was used for assessing the genetic divergence between populations. The generalized distance between any two populations was defined by-

$D^2 = (\lambda ij)\ \delta i\ \delta j$ where, (λij) was the reciprocal matrix to the common dispersion matrix and δi was the difference between the mean values of the two populations for the i^{th} character. This quantity was estimated by the D^2 statistic as – $D^2 = (S^{ij})\ di\ dj$ where, (S^{ij}) was the sample estimate of (λ^{ij}) and di of δi. Since the formula for computation required the inversion of the matrix, transformation of the original correlated, un-standardized character means of standardized uncorrelated variables was done to simplify the computational procedure. This transformation was effected by pivotal condensation method (Rao, 1952). The grouping of the populations as done by using Tocher's method as described Rao (1952). The criterion used in clustering by this method was that any two entries belonging to the same cluster at least, on an average have shown a smaller D^2 value than those belonging to different clusters.

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