MATERIALS & METHODS
CHAPTER – III

MATERIALS AND METHODS

The present investigation entitled ‘Seed vigour of chickpea (*Cicer arietinum* L.) in relation to seed size as selection criterion for yield performance under rainfed and irrigated conditions’ was conducted jointly at Govt. Dodad hari Bajrang Girls P.G. College, Raipur and Indira Gandhi Agriculture University, Raipur Chhattisgarh during 2001-03. Raipur is the capital of Chhattisgarh state and is situated at 21°61' N latitude 81°36' E longitude with an altitude 286.56 meters above the mean sea level. The climatic condition of Raipur is subtropical. Meteorological data of the crop period is given in the appendix I.

3.1 Details of Experiment

The field and laboratory experiments were laid out in three replications in Randomized Complete Block Design at Research Farm, Indira Gandhi Agricultural University, Raipur. The experimental material comprised of 30 chickpea genotypes (15 medium seeded and 15 bold seeded). In field experiment each entry was sown in four rows of four meters length placed at 30 cm apart during winter season of 2002-03. The experiment was sown in heavy soil of pH 7.5. Recommended fertilizer dose of 20 kg N₂, 50 kg P₂O₅ and 20 kg K₂O was applied at the time of sowing. Recommended agronomical practices were adopted to raise the normal crop. The observations were recorded on five randomly selected competitive plants/seedlings. Separate experiments were conducted under irrigated and rainfed situations for bold and medium seeded chickpea groups.
3.2 Experimental Material

The material for the present study comprised of following 15 medium seeded and 15 bold seeded chickpea genotypes.

Medium seeded chickpea genotypes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Genotype</th>
<th>S.No</th>
<th>Genotype</th>
<th>S.No</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>RG 938</td>
<td>13</td>
<td>RG 938</td>
<td>14</td>
<td>RG 938</td>
</tr>
<tr>
<td>4</td>
<td>RG 941</td>
<td>15</td>
<td>RG 941</td>
<td>15</td>
<td>RG 941</td>
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</table>

Bold seeded chickpea genotypes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Genotype</th>
<th>S.No</th>
<th>Genotype</th>
<th>S.No</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RG 2001-1</td>
<td>6</td>
<td>RG 2001-1</td>
<td>11</td>
<td>RG 2001-1</td>
</tr>
<tr>
<td>3</td>
<td>RG 2001-3</td>
<td>8</td>
<td>RG 2001-3</td>
<td>13</td>
<td>RG 2001-3</td>
</tr>
<tr>
<td>5</td>
<td>RG 2001-6</td>
<td>10</td>
<td>RG 2001-6</td>
<td>15</td>
<td>RG 2001-6</td>
</tr>
</tbody>
</table>

3.3 Laboratory observations

Physically sound seeds of the same grade were used for the experimental purpose. Each seed vigour test was carried out in three replications for each genotype separately. Seed and seedling vigour of chickpea were estimated on the basis of following tests during the period from 2001 to 2003.

Direct Tests of Seed Vigour

Those tests which are related to actual conditions prevailing at the time of seed sowing and germination in the field, have been considered as direct tests.
Accelerated Aging Test (Delouche and Baskin, 1973)

The accelerated ageing test has been developed at the seed technology laboratory, Mississippi State University, USA for determining the storage potential of seed lots. The ageing process is accelerated by subjecting the seeds to high temperature and relative humidity in a chamber before standard germination. The seed lots that show high germination in accelerated ageing test are expected to maintain high viability during ambient storage as well. Thus, ageing test gives an indication of the performance of the seed lot during ambient storage.

In the present investigation seed samples were kept in incubater at 45\(^\circ\)C and 100 % relative humidity for 5 days followed by germination count on eighth day after planting at 25\(^\circ\)C temperature.

Controlled Deterioration Test (Mathews and Powel, 1981)

As seeds age, they undergo gradual changes which lower their potential vigour and performance capability. The speed of deterioration depends largely on the environment in which they are stored and to some extent on the environmental conditions which existed during seed development. The initial changes are physiological in nature and can be detected only by severe stress tests. As deterioration proceeds, storability and yield potential are affected. Severe deterioration results in poor germination and plant establishment even under favourable conditions.

The test was performed by placing of 100 seeds on moist blotter paper at 25\(^\circ\)C.
for 12-14 hours to raise the moisture content of the seed to about 22 per cent. The seeds were then placed in sealed polythene tubes for 12 hours at 10°C temperature to allow even distribution of moisture in seeds. After this, seeds were sealed in foil packets and placed in a water bath for 24 hours at 45°C. After removing the seeds from water bath, regular germination test was carried out.

**Brick gravel test**

The test was developed by Hiltner in Germany in 1917. He observed that the seeds of cereal crops affected by *Fusarium* disease were able to germinate in regular test but were not able to emerge from brick gravels of 2-3 mm size. Compared to this, healthy seeds were able to emerge from the brick gravel (Roberts, 1972). The principle is that the weak seedlings are not able to generate enough force to overcome the pressure of brick gravels, so this method can be used to differentiate vigour levels.

In the present study 2-3 mm sand was sieved, moistured and filled in the germination box leaving about 3 cm empty at the top. One hundred seeds were placed in each box. After this 2 – 2.5 cm of porous brick gravel is spread over the seeds. The box is kept in the germinator at 25°C temperature. The box was removed at 8th day and seedlings, which have emerged through the brick gravel, were counted.

**Cold test**

The test aims to differentiate between weak and vigorous seed lots by subjecting them to low temperature prior to germination at optimum temperature.

Hundred seeds of each genotype of each group were used for the test. The test was performed by planting chickpea seeds on the 2 cm thick leveled moist soil. The same
quantity of soil was then placed on top of the seed. Enough cold water of $10^o$ C temperature was added to the soil to bring the medium to approximately 70% of its water holding capacity and then incubated at $10^o$C for 5 days. After 5 days seeds were transferred at temperature $25^o$ C for germination. Seedling counts were made at the 8th day after planting.

**Cool germination test**

The test was conducted in three replications of hundred seeds each. Seeds were planted in 2 cm thick-leveled moist soil and incubated at a constant temperature of $10^o$ C. Seedling counts were made on eighth day after planting.

**Low and high temperature test**

Germination percentage, speed of germination, first count, seedling height at 8th day, seedling growth rate and seedling dry weight were worked out separately for each chickpea genotype at $10^o$ C, $15^o$ C, $20^o$ C, $25^o$ C, $30^o$ C and at $35^o$ C temperature in BOD incubator. Standard procedures were adopted to conduct the tests as described under physiological tests.

**Indirect tests (Physiological tests) for seed and seedling vigour**

The indirect tests are of physiological or biochemical nature related to a particular trait or property of the seed. Physiological tests of seed / seedling vigour for all the chickpea genotypes were conducted as per the following procedures.
Germination

Growth tests are based on the principle that vigorous seeds grow at a faster rate than poor vigorous seeds even under favourable environments. Vigorous seeds rapidly germinate, metabolize and establish in the field. Therefore, any method used to determine the rapidity of growth of the seedling will give an indication of seed vigour level.

Three replicates of hundred seeds were placed between the moist towel papers and germinated at 25°C temperature according to the standard procedure (ISTA, 1985) to determine the percentage of germination on eighth day by counting the normal seedlings (appendix II).

First count

The number of normal seedlings counted at 5th day after planting was considered as the first count.

Speed of germination

After the seeds began to germinate at 25°C in the towel paper, they were evaluated daily at approximately the same time. Normal seedlings were removed and counted daily from the tests. Procedure continued until all the seeds capable of producing a normal seedling germinated till 8th day of planting.

Germination index (G.I) was computed for each genotype separately by using the following formula:

\[ G.I = \sum \frac{n}{d} \]

Where,

- \( n \) = number of seedlings emerging on day 'd'
- \( d \) = day after planting.
Seedling length at 8\textsuperscript{th} day

Length of five normal seedlings grown on moist towel paper and kept at 25°C temperature was measured separately on eighth day and averaged.

Seedlings growth rate

Three replicates of hundred seeds were kept between moist towel papers placed at 25°C temperature in germinator for germination. Lengths of five selected seedling were measured daily from 5\textsuperscript{th} day to 8\textsuperscript{th} day. Seedling growth rate (index) was calculated as follows:

\[
\text{Seedling growth rate (index)} = \frac{SL_1/F_1 + (SL_1 - SL_2)/F_1 + \ldots + (SL_n - SL_{n-1})}{F_n}
\]

\(SL_1\) - mean seedling length at first count.
\(SL_2\) - mean seedling length at second count.
\(SL_1 - SL_2\) = mean increase in length in second count.
\(F_1\) = days to first count.
\(F_n\) = days to final count.

Seedling dry weight

Eight day old five seedlings of each genotype were dried at 110°C temperature for 17 hours in hot air oven. The mean weight of seedlings including cotyledons was measured in gram.

3.4 Field Observations

Observations on metric traits were recorded on single plant basis for each
genotype in each replication of each group under each situation separately. Visual observations viz. days to 50% flowering and days to maturity were recorded on plot basis, as per the descriptors developed by ICRISAT for chickpea.

**Emergence %**

Emergence of seedlings from counted 100 seeds of each genotypes in the field were noted.

**Days to 50 % flowering**

This was noted on plot basis in terms of number of days from the date of sowing to the date on which at least 50 per cent of the plants have at least one flower.

**Days from flowering to maturity**

It was calculated by subtracting the days from first flowering from the days to 80 % pod maturity.

**Days to maturity**

This was recorded on plot basis in days from seedling to 80 per cent pod maturity.

**Plant height**

It was measured in centimeter from the base of the plant to the tip of the main axis.

**Primary branches per plant**

Number of primary branches per plant was counted at the time of harvesting.

**Secondary branches per plant**

Number of secondary branches per plant was counted at the time of harvesting.

**Pods per plant**

The effective numbers of pods were counted at the time of harvesting.
Seeds per pod
Seeds of five pods from each of five plants were counted individually and averaged.

Seeds per plant
Number of seeds of five plants were counted individually and averaged.

100 seed weight
The test weight of counted 100 seeds in gram was recorded for individual genotype.

Biological yield per plant
Total plant dry weight excluding roots was recorded on sensitive balance in gram for each individual plant.

Harvest Index (HI)
Harvest index was calculated as per the following formula and presented in percent.

\[
\text{Harvest index} = \left( \frac{\text{Seed yield (g)}}{\text{Biological yield (g)}} \right) \times 100
\]

Seed yield per plant
Seeds of individual plant were weighed on sensitive balance in gram.

3.5 Statistical analysis
3.5.1 Analysis of variance

The data obtained from the individual plant was statistically analyzed as per the procedure given by Cochran and Cox (1957).
Table: Skeleton of analysis of variance for Randomized Block Design (RBD)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degree of freedom</th>
<th>Mean squares</th>
<th>t, MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>(r-1)</td>
<td>M₂</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>(t-1)</td>
<td>M₁</td>
<td>M₁/M₂</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(t-1)</td>
<td>M₂</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(rt-1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where,
- \( r = \) number of replication
- \( t = \) number of genotype

The significance of treatment differences was evaluated by comparing the calculated \( 't' \) value at one and five per cent level of probability at error degrees of freedom.

**Coefficient of variation**

Genotypic and phenotypic coefficient of variation were calculated by using formula suggest by Burton (1952).

**Genotypic coefficient of variation (GVC)**

\[
GCV = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100
\]

Where,
- \( \sigma^2_g \) = genotypic variance
- \( \bar{X} \) = Mean of character
Phenotypic coefficient variation (PVC)

\[ PCV = \frac{\sqrt{\frac{\sigma^2_p}{\bar{X}}} \times 100}{\bar{X}} \]

Where,

\[ \sigma^2_p = \text{Phenotypic variance} \]

\[ \bar{X} = \text{Mean of character} \]

3.5.2 Parameters of variation

1. Range

The limit of smallest and the largest value of each observation expressed the range of variation.

2. Mean

The mean was calculated as:

\[ \bar{X} = \frac{\sum_{i=1}^{n} X_i}{N} \]

Where,

\[ X_i = \text{character under study} \]

\[ N = \text{number of genotypes} \]

3. Heritability

Heritability in broad sense was calculated as per the formula suggested by Burton, (1952).

\[ h^2 (BS) = \frac{\sigma^2_g}{\sigma^2_p} \times 100 \]
Where,

\[ h^2_{(BS)} = \text{Heritability (broad sense)} \]

\[ \sigma^2_g = \text{Genotypic variance} \]

\[ \sigma^2_p = \text{Phenotypic variance} \]

The broad sense heritability estimates were categorized as low, moderate and high:

- \(< 50\% = \text{Low heritability}\)
- \(50-70\% = \text{Moderate heritability}\)
- \(> 70\% = \text{High heritability}\)

4. **Genetic advance**

Expected Genetic advance (GA) was calculated as per the method suggested by Grafius (1956).

\[ GA = K \sigma_p \cdot h^2 \]

Where,

- \(K = \text{Constant (standard selection differential)}\) having value of 2.06 at 5\% selection intensity.
- \(\sigma_p = \text{Phenotypic standard deviation}\)
- \(h^2 = \text{Heritability estimate}\)

5. **Genetic advance as percentage of mean**

Genetic advance as percentage of means was calculated by following formula.

\[ \frac{GA}{X} \times 100 \]

\[ \text{GA as } \% \text{ of mean} = \frac{GA}{X} \times 100 \]

Where,

- \(GA = \text{Genetic advance}\)

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3.5.3 Coefficient of correlation

Correlation coefficients (r) were calculated in all possible combinations for all the characters of vigour and yield under study at genotypic (r_g), phenotypic (r_p) and environmental (r_e) levels by using the following formula proposed by Miller et al. (1950).

\[ r(xy) = \frac{\text{Cov}(xy)}{\sqrt{\text{Var}(x) \cdot \text{Var}(y)}} \]

Where,

- \( r(xy) \) = Correlation coefficient between x and y
- \( \text{Var}(x) \) = Variance of x variable
- \( \text{Var}(y) \) = Variance of y variable
- \( \text{Cov}(xy) \) = Covariance of characters x and y

Genotypic, phenotypic and environmental correlations were computed by substituting corresponding variance and covariance in the above-mentioned formula.

Significance of correlations

The significance of correlation coefficient (r) was tested by comparing 't' value at 'n-2' degree of freedom using following formula:

\[ t_c = \frac{|r|\sqrt{(n-2)}}{\sqrt{1 - r^2}} \]
If calculated value of ‘tC’ is greater than the tabulated value at (n-2) degrees of
freedom at given probability level, the coefficient of correlation is taken as significant.

**Path coefficient analysis**

The genotypic correlation coefficients were further partitioned into direct and
indirect effects with the help of path coefficient analysis as suggested by Wright
(1921) and further modified by Dewey and Lu (1959).

Path coefficients were estimated using simultaneous equations. The equation
shows a basic relationship between correlation coefficient and path coefficient.

These equations were solved by presenting them in matrix notation

\[ A = B \cdot C. \]

The solution for the vector ‘C’ may be obtained by multiplying both sides by inverse
of ‘B’ Matrix i.e. \( B^{-1} \) thus,

\[ B^{-1} A = C. \]

After calculation the values of path coefficient i.e. ‘C’ vector, it is possible to
obtain the path values for residual (R).

Residual effect was calculated using formula referred from Singh and

\[ R = \sqrt{1 - \sum d_i \times r_{ij}}. \]

Where,

\[ d_i = \text{Direct effect of } i^{th} \text{ character} \]

\[ r_{ij} = \text{Correlation coefficient of } i^{th} \text{ character with } j^{th} \text{ character}. \]
3.5.5 Stability analysis

The replicated mean from five randomly selected plants from each plot was used in the analysis. The data were subjected to stability analysis as per the procedure suggested by Eberhart and Russell (1966).

1. Estimation of Stability parameters

The stability analysis of variance was done on the basis of mean data over replication.

The stability parameters were estimated following the mathematical model.

\[ Y_{ij} = \mu_i + b_i l_j + \delta_y \]

Where,

- \( Y_{ij} \) = Mean value of \( i^{th} \) variety in \( j^{th} \) environment (\( i=1,2,...,T \)) and \( (I=1,2,...,E) \)
- \( \mu_i \) = Mean of \( i^{th} \) variety over all the environments
- \( b_i \) = regression coefficient that measures the responses of the \( i^{th} \) variety of the varying environments.
- \( l_j \) = Environmental index of the \( j^{th} \) environment and is obtained as deviation of mean of all genotypes of the \( j^{th} \) environment from over all mean i.e.

With,

- \( \delta \) = Deviation from regression of the \( i^{th} \) variety at the \( j^{th} \) environment
- \( e_{ij} \) = random error
2. Analysis of variance when stability parameter is estimated according

to Eberhart and Russell (1966) is followed as under

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S. S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>$E_t - 1$</td>
<td>$\sum \sum y_{ij}^2 - CF$</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>$t - 1$</td>
<td>$\sum y_i^2 - CF$</td>
<td>MS$E$</td>
</tr>
<tr>
<td>Environment</td>
<td>$E - 1 + (t - 1)$</td>
<td>$\sum \sum y_{ij}^2 / E$</td>
<td></td>
</tr>
<tr>
<td>(env.) + (Geno. X env.)</td>
<td>$t (E - 1)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environment (Linear)</td>
<td>$1$</td>
<td>$\left( \frac{\sum y_{ij}}{1/\tau} \right)^2 / \left( \sum I_{j}^2 \right)$</td>
<td></td>
</tr>
<tr>
<td>Geno X Env (Linear)</td>
<td>$(t - 1)$</td>
<td>$\sum \left( \frac{\sum y_{ij} I_j}{\sum I_{j}^2} \right)^2 - \text{Env.(lin)S.S.}$</td>
<td>MS$E$</td>
</tr>
<tr>
<td>Pooled deviation</td>
<td>$T (E - 2)$</td>
<td>$\sum \sum \delta_{ij}^2$</td>
<td>MS$E$</td>
</tr>
<tr>
<td>Pooled error</td>
<td>$E(t - 1)(r - 1)$</td>
<td></td>
<td>MS$E$</td>
</tr>
</tbody>
</table>

3. Test of significance

(i) In order to test the significance of the difference among the variety means i.e.

$H_0 : \mu_1 = \mu_2 = \mu_3 \ldots \ldots = \mu_n$

The appropriate 'F' test is defined as

$$F = \frac{MS_1}{MS_3}$$

(ii) To test that the varieties do not differ from their regression on the environmental index i.e., $H_0 : b_1 = b_2 = b_3 \ldots \ldots b_n$

$$F = \frac{MS_2}{MS_3}$$
The deviation of $b_i$ values from zero was tested using 't' test from null hypothesis

$$t = \frac{b_i - 0}{\text{SE}(b)}$$

Where, S.E. $(b) =$ standard error of 'b', if 'b' is significant its significant deviation from unity was tested by following formula.

$$t = \frac{1 - b_i}{\text{SE}(b)}$$

(iii) Pooled deviation

$$F = \frac{\text{MS}_3}{\text{MS}_4}$$

(iv) Individual deviation from linear regressions (i.e. significance of $S^2 d_i$ ) was tested by F test

$$F = \frac{\sum g \delta^2_{g}}{(E - 2) / \text{Pooled error}}$$

Where,

$$\sum g \delta^2_{g} =$variance due to deviation from regression

$E =$Number of environments.