CHAPTER III

MATERIALS AND METHODS

The details of the materials used and techniques adopted during the course of investigation are described below.

3.1. Experimental site and Climate:

The present investigation was conducted during the seasons 2007-2008, 2008-2009 and 2009-2010 at a paddy growing field of Dhemaji district, Assam (North Latitude 27°15' to 28°00' and East Longitude 94°05' to 95°30'). The soil of the experimental field was alluvial sandy loamy with pH 5.5. The mean humidity during 07-08 season has been recorded as 82.42%, average temperature as 24.75°C with maximum 39.6°C in October and May and minimum 6.2°C in January. The average rainfall during season 07-08 was 2419 mm. The mean humidity during 08-09 season has been recorded as 79.80%, average temperature as 24.1°C with maximum 33°C in September and minimum 10.2°C in December. The average rainfall during season 08-09 was 2343 mm. Again the average humidity during 09-10 season has been recorded as 84.55%, average temperature as 23.9°C with maximum 34°C in September and minimum 6.5°C in January. The average rainfall during 09-10 season was 2493 mm (Table: 3.1 Source: Meteorological station 423090 (VELR) and E and D Office, Dhemaji).

3.2. Experimental materials:

The experimental material of the study comprised of 68 rice cultivars collected from different pockets of Dhemaji district. This material includes 46 sali seasonal
Table 3.1 Meteorological data for the experimental period

<table>
<thead>
<tr>
<th>Year</th>
<th>Temperature °C</th>
<th>Mean Humidity (%)</th>
<th>Total Rainfall (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td></td>
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<td>80</td>
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<td>30.2</td>
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</tr>
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<td>23</td>
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<tr>
<td>June</td>
<td>21.8</td>
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<tr>
<td>July</td>
<td>24.3</td>
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<td>Average</td>
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<td>295.69</td>
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(Source: Meteorological station 423090 (VELR) and E and D Office, Dhemaji)
Table 3.2.1: Name and site of collection of indigenous *sali* cultivars.

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Name of the cultivars</th>
<th>Cultivar code</th>
<th>Site of collection</th>
<th>Sl no</th>
<th>Name of the cultivars</th>
<th>Cultivar code</th>
<th>Site of collection</th>
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<td>Silapathar</td>
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<td>Khoiron</td>
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<td>C6</td>
<td>Betonipam</td>
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<td>Laudubi</td>
<td>C29</td>
<td>Moridhal</td>
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<td>Malbhug</td>
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<td>C22</td>
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<td>Sowagmoni</td>
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Table 3.2.2. Name and site of collection of indigenous *bao* cultivars

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<th>Cultivar code</th>
<th>Site of collection</th>
<th>Sl No</th>
<th>Name of cultivars</th>
<th>Cultivar code</th>
<th>Site of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td><em>Maguribao</em></td>
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<td><em>Miabao</em></td>
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<td>Bordoloni</td>
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<td>3</td>
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Table 3.2.3. Name and site of collection of indigenous *ahu* cultivars

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<th>Cultivar code</th>
<th>Site of collection</th>
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<th>Name of cultivars</th>
<th>Cultivar code</th>
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<td><em>Maiguni</em></td>
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Table 3.2.4. Name and site of collection of high yielding varieties.

<table>
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<tbody>
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<td><em>Mala</em></td>
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<td><em>Ronjit</em></td>
<td>C4</td>
<td>Assam Agricultural University</td>
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indigenous cultivars (Table: 3.2.1), 10 bao seasonal indigenous cultivars (Table: 3.2.2), 8 ahu seasonal indigenous cultivars (Table: 3.2.3) and 4 high yielding checks viz. Basdhan, Ijong, Ronjit and Mala. (Table: 3.2.4).

3.3. Layout plan:

The experiment was laid in Randomized Block Design replicated thrice. 45 days old seedlings were transplanted into the experimental site with 20 X 15 cm spacing. Row to row and hill (plant) to hill distance was 20 cm and 15 cm respectively.

3.4. Observations recorded:

The data were recorded five randomly selected plants from each replication leaving the first two border rows from all the four sides, in order to avoid the sampling error. The observations were recorded as per the following procedure. Readings from five plants were averaged replication wise and the mean data was used for statistical analysis for the characters as follow:

3.4.1. Agro-morphological traits:

3.4.1.1. Plant height:

Plant height was measured in centimetre from the ground level to the top of the panicle (excluding awn) at the time of maturity.

3.4.1.2. Number of tillers/plant:

The total numbers of tillers were counted as per plant basis.

3.4.1.3. Days to 50% flowering

The number of days from date of sowing to the 50 per cent of the plants in the plot initiated flowering was recorded as days to 50 per cent flowering (Yosida, 1981).
3.4.1.4. Leaf area (LA):

Leaf area was estimated by multiplying length by its breadth and again multiplies by 0.75 score based on standard evaluation system for rice (IRRI, 1996).

3.4.1.5. Flag-leaf length:

Flag leaf length was measured from its base of attachment to the sheath to the tip of leaf blade and expressed in centimetre at 40 days after transplanting.

3.4.1.6. Flag-leaf breadth:

The measurement of flag leaf breadth was taken in the widest portion of the leaf blade and expressed in centimeter.

3.4.1.8. Grain length:

Length of un-hulled grains was measured with Slide calliper and expressed in milimeter.

3.4.1.9. Grain breadth:

Breadth of un-hulled grains was measured as the distance across the fertile lemma and palea at the widest point with Slide calliper and expressed in milimeter.

3.4.1.10. Length-breadth ratio of grain (L/B ratio):

Grain length-breadth ratio was measured by dividing the length of grain by breadth.

3.4.1.11. Harvest index:

Dry weight of both straw and grains of the sampled plants were weighed separately after harvest. By straw the above ground portions of the plant devoid of grains was meant. According to formula given by Yoshida (1981), harvest index was computed as the fraction of grain yield to the total biomass including straw and grain.
yields, as

\[ HI = \left(\frac{\text{grain yield}}{\text{grain yield} + \text{straw yield}}\right) \times 100 \]

3.4.1.12. Length of panicle:

The average panicle length of five plants on the main culm from the base of the panicle to the top of the last spikelet excluding awns was recorded in centimeter.

3.4.1.13. Test (1000 seeds) weight:

A sample of 100 well developed fresh whole seeds was collected and weighed in grams and computed to 1000 grains weight.

3.4.1.14. Spikelet’s per panicle:

Total number of spikelets on main panicle was counted and recorded at the time of maturity.

3.4.1.15. Percentage of viable seeds:

Counted the number of well filled grains of five randomly selected panicles for each cultivar and expressed in percentage.

3.4.1.16. Spikelet density:

Spikelet density was calculated by dividing total number of spikelets by length of panicle for five randomly selected replicates for each cultivar (according to Chang et al., 1965) and expressed in number/cm.

3.4.1.17. Spikelet’s per plant:

Spikelets per plant were determined by counting the number of both well filled and aborted spikelets of five randomly selected replicates.
3.4.1.18. Panicles per plant:

The total numbers of panicles per plant were recorded in five replications for each cultivar.

3.4.1.19. Grain yield/plant:

Grain yield/plant was computed according to Datta, 1981 with slight modification as

\[ \text{GY/P} = [(\text{Number of panicles/ plant}) \times (\text{number of spikelets/plant}) \times (\% \text{ of Viable seeds}) \times (1000 \text{ seeds wt.}) \times 10^{-5}] \]

3.4.1.20. Germination percentage:

Estimation of germination percentage was done in petridishes and cotton was used as germination media. The petridishes were lined with filter paper and filled with cotton leaving 2 cm from the top. Adequate moisture level was maintained in the germination media. Germination test for each cultivar was carried out taking 20 seeds at every sampling time in five replications, were left for 7 days, counted germinating seeds and recorded.

3.4.1.21. Length of flag-leaf sheath:

The average flag-leaf sheath length of five plants on the main culm from the base of the flag-leaf to the attachment on the main culm was recorded in centimeter.

3.4.1.22. Length of root:

Length of root was measured in centimetre from the ground level to the top of the healthy root (excluding fibrous roots) at the time of maturity.
3.4.1.23. Percentage of pollen viability:

Pollen culture media for estimation of germination percentage of pollen were prepared according to standard method of Brewbaker & Kwack (1963). Boric acid, calcium nitrate, magnesium sulphate and potassium nitrate were added in 1:3:2:1 proportion to distilled water as ppm /ml unit and the solution was used as culture medium for pollen germination. Germination test for each cultivar was carried by taking pollens from freshly opened flower in five replications for each cultivar and were left for 1 hour in germinating media. Numbers of germinating pollens were than counted and expressed in percentage.

3.4.1.24: Test for pre-harvest sprouting:

Five randomly selected plants were gently bent to submerged panicles into water at 40 days after flowering for 24 hours and kept within a water soaked cloth bag and again enclosed in a polyethylene bag for 6 days (total 7 days of water soaked condition). Counted the germinating spikelets per panicle and converted to percentage (Mahbub et al., 2005).

3.4.1.25. Grain type:

Grain type was determined according to Systematic classification of rice grain, Ramaiah Committee 1969, (as referred in Rice Research in India, ICAR Publication, 1985) as follows,
Table 3.3. Systematic classification of rice grain

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Grain length</th>
<th>Grain L/B ratio</th>
<th>Grain Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.00 mm and above</td>
<td>3.00 mm and above</td>
<td>Long slender (LS)</td>
</tr>
<tr>
<td>2</td>
<td>Less than 6.00 mm</td>
<td>3.00 mm and above</td>
<td>Short slender (SS)</td>
</tr>
<tr>
<td>3</td>
<td>Less than 6.00 mm</td>
<td>2.50 to 3.00 mm</td>
<td>Medium slender (MS)</td>
</tr>
<tr>
<td>4</td>
<td>6.00 mm and above</td>
<td>Less than 3.00mm</td>
<td>Long bold (LB)</td>
</tr>
<tr>
<td>5</td>
<td>Less than 6.00 mm</td>
<td>Less than 3.00mm</td>
<td>Short bold (SB)</td>
</tr>
</tbody>
</table>

3.4.2. Anatomical traits:

3.4.2.1. Number of xylem vessels:

Permanent slides of finely sectioned root by double stained method were prepared and counted the number of xylem vessels under light microscope.

3.4.2.2. Number of stomata (both upper and lower surface) (1.417 mm²):

Slides were prepared by impression method (suggested by Bruce and Itzick, 2004) for counting number of stomata. Numbers of stomata were counted for each microscopic field with five replications under 10X x 40X magnification and the area of microscopic field was calculated using stage and ocular scale.

3.4.3: Biochemical analysis:

3.4.3.1. Total carbohydrate content:

The total carbohydrate content was estimated by Anthrone method proposed by Hedge and Hofreiter, 1962 as explained by Sadasivam and Manickam, 1991. For extraction of total carbohydrate, 100 gm of fresh dehulled rice kernels were ground and extracted with 2.5 N HCl by keeping in a boiling water bath for three hours.
Extract was than cooled to room temperature and neutralised with solid sodium carbonate and centrifuged after making up the volume to 100 ml. 0.5 ml aliquot of the supernatant was used as stock solution. 4 ml of Anthrone reagent was added slowly by the side of the test tube containing 1 ml of aliquot of the properly diluted carbohydrate extract and heated for 8 minutes in boiling water bath. The tubes were than cooled rapidly and the intensity of the colour was measured in a spectrophotometer (Systronic UV-VIS spectrophotometer 118) at 630 nm against a reagent blank. The carbohydrate content was estimated from a standard curve, prepared with known concentration of glucose. The values obtained from standard curve were then converted to percentage concentration.

3.4.3.2. Total starch content:

The starch content was estimated by the method as proposed Hedge and Hofreiter, 1962 and Thayumanavan and Sadasivam, 1984 and as explained by Sadasivam and Manickam (1991). For extraction of starch, 100 gm of fresh rice kernels were grinded and extracted with 80% hot ethanol to make the sample free of sugars. Starch was extracted with 52% perchloric acid from the sugar free samples by constant stirring for 30 minutes. This was than centrifuged and the supernatant was collected in a volumetric flask of 50 ml. The process was repeated for 2-3 times and the volume of the combined extract was made up to the mark of the flask. 4 ml of Anthrone reagent was added slowly by the side of the test tube containing 2 ml of aliquot of the properly diluted starch extract under cool condition. The tubes were then placed in boiling water bath and kept for 10 minutes. Then tubes were cooled immediately into room temperature. The intensity of the colour was measured in a
spectrophotometer (Systronic UV-VIS spectrophotometer 118) at 630 nm against a reagent blank. The sugar content was estimated from a standard curve, prepared with known concentration of glucose. The value obtained from standard curve was multiplied by a factor 0.9 to get the starch content. The estimation was carried out with five replicates and the mean was expressed as g of starch per 100g fresh sample.

3.4.3.3. Amylose content:

Amylose content was estimated by the method complied by Sadasivam and Manickam, 1991; following McCready et al., 1950, Juliano, 1971 and Thayumanavan and Sadasivam, 1984. Dehulled fresh powered sample (100 mg) was taken, added 1ml of distilled ethanol and 10 ml of 1 N NaOH gently by the side and leaved for overnight. Than the volume was made up to 100 ml with distilled water and 2.5 ml of extract was taken for evaluation. 20 ml of distilled water and three drops of phenolphthalein were added to the extract solution and neutralised with 0.1 N HCl. 1 ml of iodine reagent was added, volume was made to 50 ml and the intensity of the colour was measured in a spectrophotometer (Systronic UV-VIS spectrophotometer 118) at 590 nm against a reagent blank. 1 ml of standard amylose (100mg/100 ml) solution was taken and treated using the same procedure. Calculated the amylose content using the standard graph of amylose and expressed in percentage. The estimation was done with five replications and their mean was recorded.

3.4.3.4. Amylopectin content:

Amylopectin content was determined by subtracting the amylose content from that of starch as suggested by Sadasivam and Manickam, 1991.
3.4.3.5. Protein content:

Protein content was estimated by Lowry’s method as explained by Sadasivam and Manickam, 1991. Extraction of protein was done by washing 100 mg grinded dehulled fresh kernels with ethanol: petroleum ether in 2:1 proportion with constant stirring for 5 minutes and washed again with ether. Solvent was than decanted and dried residue was dissolved in 10% TCA. This was than cooled at 4°C for 4 hours, centrifuged and dissolved the precipitate in 0.1N NaOH. Solution was used as sample extraction. 1 ml aliquot of properly diluted protein extract was taken and 5 ml of reagent C was added to it. After 10 minutes 0.5 ml of reagent D was added and incubated in room temperature for 30 minutes. Colour was measured in a spectrophotometer (Systronic UV-VIS spectrophotometer 118) at 660 nm against a reagent blank (1ml distilled water + 5ml reagent C + 0.5 ml of reagent D). Calculated the protein content using the standard graph of protein and expressed in percentage. The estimation was done with five replications and their mean was recorded.

3.4.3.6. Estimation of chlorophyll content (mg/g):

Fresh leaf sample was collected from 5 plants at random at booting to heading stage. The photosynthetic pigment was chemically extracted in 80% acetone (prechilled). The absorbance of the solution of each entry under test was read at 663 nm and 645 nm in a spectrophotometer (Systronic UV-VIS spectrophotometer 118). Using the absorption coefficients, the amount of total chlorophyll pigment, chlorophyll a pigment and chlorophyll b pigment were calculated with the following formula outlined by Sadasivam and Manickam (1991).

\[
\text{chlorophyll a (mg/g) = [12.7 (A663) – 2.69 (A645)]x V/(1000xW)}
\]
Chlorophyll b (mg/g) = \[22.9 \times (A_{645}) - 4.68 \times (A_{663})\] \times \frac{V}{1000 \times W}

Total chlorophyll content (mg/g) = \[20.2 \times (A_{645}) + 8.02 \times (A_{663})\] \times \frac{V}{1000 \times W}

Where,

- \( A = \) absorbance at specific wavelengths
- \( V = \) final volume of chlorophyll extract in 80% acetone
- \( W = \) Fresh weight of leaf tissue taken for the extraction of pigments.

3.5. Statistical analysis:

The mean data recorded on the above observations were subjected to following biometrical analyses:

3.5.1. Standard Error:

The standard error of the mean difference (SEM±) was calculated according to the expression suggested by Snedecor and Cochran, 1976.

\[
SEM = \sqrt{\frac{2 \times EMS}{r}}
\]

Where,

- \( EMS = \) Error mean square
- \( r = \) Number of replication

3.5.2. Critical difference:

The significance or otherwise the differences between individual means were ascertained by the comparison with appropriate critical difference as described by Panse and Sukhatme, (1978).

\[
C.D. = SEM \times t_{0.05}
\]
Where,

\[ t = \text{tabulated value at error degree of freedom at 5 percent level of significance} \]

\[ \text{SEM} = \text{standard error of the mean difference} \]

Significant ‘F-value’ indicates that there is significant difference among the treatments. But to compare any two particular treatments, it is tested against C.D. value.

3.5.3: Analysis of variance:

The analysis of variance was worked out to test the differences among genotypes by F-test. It was carried out according to the procedure of Randomized Block Design for each character as per methodology advocated by Panse and Sukhatme, (1978). ANOVA helps in partitioning the total variance into three components viz. replication, treatment and error. To test the significance of the varietal mean difference, the following analysis of variance table was drawn up.

Table 3.4: Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.S.</th>
<th>F-value (Cal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Due to replications</td>
<td>(r-1)</td>
<td>SSr</td>
<td>SSr/(r-1)=MSr</td>
<td>MSr/EMS</td>
</tr>
<tr>
<td>Due to treatments (genotype)</td>
<td>(t-1)</td>
<td>SSt</td>
<td>SSt/(t-1)=Mst</td>
<td>Mst/EMS</td>
</tr>
<tr>
<td>Due to error</td>
<td>(r-1)(t-1)</td>
<td>SSE</td>
<td>SSE/(r-1)(t-1)</td>
<td>=EMS</td>
</tr>
</tbody>
</table>

Where,

\[ r = \text{Number of replications} \]

\[ t = \text{Number of treatments} \]
SSr = sum of squares due to replications
SSt = sum of squares due to treatments
SSe = sum of squares due to error
MSr = Mean sum of square due to replications
MSt = Mean sum of squares due to treatments
EMS = Error mean sum of squares

Test of significance:

If the variance ratio (or) F-calculated value for treatment was greater than the f-table value at 5% and 1% level of significance, the variance between treatments was considered to be significant. If the F-calculated value is less than F-tabulated value, the difference between treatments was considered to be non-significant (Fisher and Yates, 1963).

3.5.4. Genetic variability:

3.5.4.1. Range:

It was taken as the minimum and maximum values for each trait within population.

Range = X1 to Xn.

Where,

X1 = Lowest mean value of the character
Xn = Highest mean value of the character

3.5.4.2. Components of variance:

These were calculated according to the formula given by Lush (1949) and Chaudhary and Prasad (1968).
3.5.4.2.1. Genotypic variance:

The genotypic variance ($\sigma^2_g$) is the variance due to the genotypes present in the population. The formula used for calculation of genotypic variance was

\[
\text{Genotypic variance } (\sigma^2_g) = \frac{\text{MSt} - \text{EMS}}{r}
\]

Where,

- $\text{MSt} =$ Mean sum of squares due to treatments
- $\text{EMS} =$ Error mean sum of squares
- $r =$ number of replications.

3.5.4.2.2. Environmental or error variance ($\sigma^2_e$):

The environmental or error variance ($\sigma^2_e$) is the variance due to environmental deviation.

\[
\text{Error variance } (\sigma^2_e) = \text{EMS}
\]

Where,

- $\text{EMS} =$ Error mean sum of squares

3.5.4.2.3. Phenotypic variance ($\sigma^2_p$):

Phenotypic variance ($\sigma^2_p$) denotes the total variance present in a population for particular character and is calculated by following formula

\[
\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \sigma^2_e.
\]

Where,

- $\sigma^2_g =$ genotypic variance
- $\sigma^2_e =$ environmental variance

3.5.4.3. Coefficient of variation (C.V):
It is the measure of variability evolved. Coefficient of variation is the ratio of standard deviation of a sample to its mean and expressed in percentage.

\[
CV (%) = \frac{\text{Standard Deviation}}{\text{Mean}}
\]

In the present investigation three types of coefficient of variations were estimated viz., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and error/environmental coefficient of variation (ECV). The formulae used to calculate PCV, GCV and ECV were given by Burton and Devane (1952).

3.5.4.3.1. Phenotypic coefficient of variation (PCV):

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

Where,

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

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

3.5.4.3.2. Genotypic coefficient of variation (GCV):

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

Where,

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

\[
\bar{X} = \text{Mean}
\]
ECV\% = \frac{\sigma^2_e}{\bar{X}} \times 100

Where,

\sigma^2_e = \text{Error standard deviation}

\bar{X} = \text{Mean}

GCV and PCV values were categorized as low, moderate and high values as indicated by Sivasubramanian and Menon (1973). It is as follow:

1-10\% : Low
11-20\% : Moderate
21\% and above : High

3.5.4.4. Heritability in broad sense (h^2):

Heritability in broad sense is the ratio of genotypic variance to the total variance. It is that portion of total variability or phenotypic variability which is heritable and due to the genotype. Broad sense heritability was estimated as the ratio of genotypic variance to the phenotypic variance and was expressed in percentage (Hanson et al., 1956).

h^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100

Where,

\sigma^2_g = \text{genotypic variance}

\sigma^2_e = \text{phenotypic variance}

The heritability percentage was categorized as low, moderate and high as suggested by Robinson et al., (1949).

0-30\% : Low
31-60% : Moderate
61% and above : High

3.5.4.5. Expected Genetic advance

Genetic advance is the improvement in mean genotypic value of selected plants over the parental population. The extent of genetic advance to be expected from selecting five per cent of the superior progeny was calculated by using the following formula given by Johnson et al. (1955).

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

Where,

\( K = \) Constant selection differential at 5% level intensity (= 2.06)
\( \sigma_p = \) Phenotypic standard deviation
\( h^2 = \) Heritability in broad sense

3.5.4.6. Genetic advance as percent of mean (GAM):

The formula of Genetic advance as percent of mean was as follow:

\[ GAM = \frac{GA}{\bar{X}} \times 100 \]

Where,

GA = genetic advance
\( \bar{X} = \) Grand mean

Genetic advance as per cent of mean was categorized as low, moderate and high by following Johnson et al. (1955). It is as follows.
0-10% : Low
11-20% : Moderate
Above 20% : High

3.5.5. Correlation studies:

The correlation coefficients were calculated to determine the degree of association of the yield attributes with yield and also among yield attributes themselves. The analysis of Covariance was conducted by following the method designed by Singh and Chaudhary (1977). The genotypic and phenotypic correlation coefficients between two characters say x and y under study were calculated.

3.5.5.1. Genotypic correlation coefficient ($r_{gxy}$):

$$r_{gxy} = \frac{\sigma_{gxy}}{\sqrt{\sigma^2_{gx}} \times \sqrt{\sigma^2_{gy}}}$$

Where,

$r_{gxy} =$ Genotypic correlation coefficient between x and y.

$\sigma^2_{gx} =$ Genotypic variance of x

$\sigma^2_{gy} =$ Genotypic variance of y

$\sigma_{gxy} =$ Genotypic covariance between x and y and it is given by

$$\sigma_{gxy} = \frac{\text{MSP}_g - \text{MSP}_e}{r}$$

Where,

MSP$_g =$ Genotypic mean sum of product

MSP$_e =$ Error mean sum of product

$r =$ replication

3.5.5.1. Phenotypic correlation coefficient ($r_{gxy}$):
\[ r_{pxy} = \frac{\sigma_{pxy}}{\sqrt{\sigma^2_{px} \times \sigma^2_{py}}} \]

Where,

- \( r_{pxy} \) = Phenotypic correlation coefficient between \( x \) and \( y \).
- \( \sigma^2_{px} \) = Phenotypic variance of \( x \)
- \( \sigma^2_{py} \) = Phenotypic variance of \( y \)
- \( \sigma_{pxy} \) = Phenotypic covariance between \( x \) and \( y \) and is given by

\[ \sigma_{pxy} = \sigma_{gxy} + \sigma_{exy} \]

The significance of correlation coefficient was tested by comparing the observed value of correlation coefficient with the table value at 5 per cent probability level with \( (n-2) \) degrees of freedom for its significance, where ‘\( n \)’ is the number of cultivars.

Correlation coefficient analysis was done by computer software package INDOSTAT.

3.5.6. Genetic divergence analysis

3.5.6.1 Mahalanobis \( D^2 \) analysis:

Mahalanobis (1936) \( D^2 \) statistic was used for assessing the genetic divergence between populations. The generalized distance between any two populations is given by formula.

\[ D^2 = \sum \lambda_{ij} \sigma_{ai} \sigma_{aj} \]

Where,

- \( D^2 \) = Square of generalized distance
- \( \lambda_{ij} \) = Reciprocal of the common dispersal matrix
\[ \sigma_{ai} = \mu_{i1} - \mu_{i2}. \] (The difference between the two mean values of the two populations for ith character)

\[ \sigma_{aj} = \mu_{j1} - \mu_{j2}. \] (The difference between the two mean values of the two populations for jth character)

\[ \mu = \text{General mean} \]

Since, the formula for computation requires inversion of higher order determinant, transformation of the original correlated un-standardized character mean (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computation procedure. The \( D^2 \) values were obtained as the corresponding uncorrelated (Ys) values of any two uncorrelated genotype (Rao, 1952).

3.5.6.2 Clustering of \( D^2 \) values:

All the \((n-1)/2\) \( D^2 \) values were clustered using Tocher’s method as described by Rao (1952).

3.5.6.2.1 Intra cluster distance:

The intra cluster distances were calculated by formula given by Singh and Chaudhary (1977).

Square of the intra cluster distance = \( \frac{\sum D^2_i}{n} \)

Where,

\( \Sigma D^2_i \) is the sum of distances between all possible combinations of the entries included in a cluster.

\( N = \text{Number of possible combinations} \)
3.5.6.2.2 Inter cluster distance:

The inter cluster distances were calculated by the formulae described by Singh and Chaudhary (1977).

Square of the intra cluster distance = \( \frac{\Sigma \Delta_i^2}{n_in_j} \)

Where,

\( \Sigma \Delta_i^2 \) is the sum of distance between all possible combinations \( (n_in_j) \) of the entries included in the cluster study.

\( N_i \) = Number of entries in cluster i

\( N_j \) = Number of entries in cluster j

3.5.6.2.3. Contribution of individual characters towards divergence

In the combinations each character was ranked. Rank 1 was given to highest mean difference and ranked P to the lowest mean difference, where P is the total number of characters.

Genetic divergence analysis was done by computer software package INDOSTAT.