CHAPTER 3

METERIALS AND METHODS

The present investigation ‘Evaluation of seed, progeny and cutting of Candidate Plus Trees of Bombax ceiba L. in East and West Karbi Anglong Forest Divisions of Assam’ was carried out at Forest Research Institute, Dehradun and Karbi Anglong (Assam). The materials and methods employed during the course of the investigation are given in the chapter.

3.1 Investigation sites

3.1.1 Location

3.1.1.1 Site of work:

All nursery and laboratory studies were carried out at the campus of Forest Research Institute, Dehradun. It is located at 30°20’10.31”N latitude and 77°59’55.32”E longitude.

(i) Collection of seeds and cuttings

Assam is located in the north eastern part of India covering an area of about 78,438 sq. km. Karbi Anglong district has the total geographical area of 10,434 sq. km which accounts for 13.3 per cent of the total geographical area of the State. It mostly consists of undulating and hilly terrain with numerous rivers and streams. As per the State of Forest report 2015 of Forest Survey of India, Dehradun, 76.23 per cent of the geographical area is under forest cover. 564 sq. km. of the district are very dense forest cover; 3,766 sq. km. of the district are under moderate forest cover while 3624 sq. km are under open forest cover. The important forest types found in Karbi Anglong District are: moist semi-evergreen forests, moist mixed deciduous forests, riverain type and miscellaneous type with scattered pure or mixed patches of bamboos (Plate 5).

Following are the forest areas where seeds and cuttings of phenotypically superior trees will be collected:

1. Amlokhi (East Karbi Anglong Forest division) (S1) (Plate 6)
Materials and methods

2. Deopani (Nambor Wildlife Sanctuary in East Forest Division) (S2) (Plate 7)

3. Sarsari (Dhansiri Reserve Forest in West forest division) (S3) (Plate 8)

4. Barlangfer (Marat Longri Wildlife Sanctuary in West forest Division) (S4) (Plate 9)

The selection of Candidate Plus Trees (CPTs) of *Bombax ceiba* L. in East and West Karbi Anglong Forest Divisions of Assam were done by State Forest Dept. (Plate 10) and eight trees from each site were taken for the present study.

(ii) Raising of nursery stock trial:

Nursery trial was established at two sites (one site in north India and the other site in East or West Karbi Anglong Forest divisions in Assam).

3.1.1.2 Prevailing weather conditions

Dehradun is in the influence of sub-tropical climate and experiences about 2200 mm rainfall annually. Major part of the rainfall is received from mid-June to mid-September. Winter showers are mild. The weather data at the campus of Forest Research Institute, Dehradun for the study period are annexed in Appendix A.

Karbi Anglong experiences different climates in different parts due to variation in the topography. The winter begins from October and continues till February. During summer, the atmosphere becomes sultry. The temperature in summer ranges from 23 degree Celsius to 32 degree Celsius, while in winter, it ranges from 6 degree Celsius to 12 degree Celsius. The monsoon starts in mid May and continues till September. Though the average rainfall in the region is 1147 mm but it is not uniformly distributed. Blocks like Lumbajong, Samelangso, Langsomepi and Nilip receives rainfall between 1000-2500 mm. The area between Kheroni to Dhansiri is a main shadow area and receives even less than 1000 mm rainfall.

3.2 Experimental Methodology

3.2.1 Fruits and seed morphology

From each sites in Assam fruits and seed were collected for their morphological studies in April 2013.
3.2.1.1 Observations

The following observations were recorded:

3.2.1.1.1 Fruit fresh weight

Fruit fresh weight was recorded before being dried to extract to seed and expressed in grams (g).

3.2.1.1.2 Fruit dry weight

Fruit dry weight was recorded after air drying it for 6-7 days and expressed in grams (g).

3.2.1.1.3 Fruit length

Observations were made on five fruits from each tree and expressed in cm.

3.2.1.1.4 Fruit diameter

Observations were made on five fruits from each tree and expressed in cm.

3.2.1.1.5 Seed Weight per fruit

Weight of seed per fruit was recorded and expressed in grams (g).

3.2.1.1.6 100-seeds Weight

Weight of 100 seeds per fruit was recorded and expressed in g.

3.2.1.1.7 Pericarp weight

The pericarp weight were calculated and expressed in grams as follows:

Pericarp weight (g) = Fruit dry weight (g) – (seed weight per fruit + floss weight) (g).

3.2.1.1.8 Floss Weight

After extraction of seed, the weight of floss from each fruit was recorded and expressed in grams (g).
3.2.1.9 Seed diameter

Seed diameter from each fruit was recorded and expressed in mm (Plate 11 and 13).

3.2.1.10 Seed: fruit dry weight ratio

The seed: fruit weight ratio was calculated by dividing seed weight per fruit by fruit weight and expressed as ratio up to three decimal places for stand and tree means.

\[
\text{Seed: fruit dry weight ratio} = \frac{\text{Seed weight per fruit (g)}}{\text{Fruit dry weight (g)}}
\]

3.2.1.11 Pericarp: fruit dry weight ratio

The pericarp: fruit weight ratio was calculated as follows:

\[
\text{Pericarp: fruit dry weight ratio} = \frac{\text{Pericarp weight (g)}}{\text{Fruit dry weight (g)}}
\]

3.2.1.12 Floss: fruit dry weight ratio

The floss: fruit dry weight was calculated as follows:

\[
\text{Floss: fruit dry weight ratio} = \frac{\text{Floss weight (g)}}{\text{Fruit dry weight (g)}}
\]

3.2.2 Raising of seedling in nursery

Seeds were sown at two sites: Dehradun and Karbi Anglong in the Month of April-May. Seed were sown in poly house in nursery (Plate 13).

3.2.2.1 Observations

The following observations were made:

3.2.2.1.1 Days to germination

The appearance of bud from the seed was counted as germination. Observations made every single day upto 2 weeks.
3.2.2.1.2 Germination per cent

The seed from each site were sown in poly house in 3 replications and 20 seed per replication. Germination per cent was recorded by the formula:

$$\text{Germination per cent } (\%) = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}}$$

3.2.2.1.3 Germination Value

Germination value (GV) is the index combined speed and completeness of seed germination. Daily germination counts were recorded and calculated as per the method given by Czabator (1962).

$$\text{GV} = \text{PV} \times \text{MDG}$$

Where;

$\text{PV} = \text{Peak value of germination}$

$\text{MDG} = \text{Mean daily germination}$

Peak value

Peak value was calculated as the maximum mean daily germination (MDG) reached at any time during the period of test (Czabator, 1962).

3.2.2.1.4 Germination Energy

Germination energy (GE) was calculated on the basis of percentage of total number of seed that had germinated when germination reached its peak (ISTA, 1985).

$$\text{GE} = \frac{\text{Total number of seeds germinated upto the time of peak germination}}{\text{Total number of seeds sown}}$$

3.2.3 Evaluation of growth traits

3.2.3.1 Seedlings characteristics

After the emergence of leaves in seedlings, they were transferred into poly bags of size 22 x 13 cm containing sand 2 : soil 1 : FYM 1 (v/v).
3.2.3.1 Observations

The following observations were recorded.

3.2.3.1.1 Seedling height

Seedling height were recorded at every 7 days after transplant into poly bag and expressed in cm. The seedling height was measured from ground level to the tip using measuring scale.

3.2.3.1.2 Collar diameter

Collar diameter was measured above the ground using vernier calliper and expressed in mm.

3.2.3.1.3 Number of shoots

Average number of shoots per seedling was recorded.

3.2.3.1.4 Per cent survival

Per cent survival at the end of growing season was recorded.

3.2.4 General procedure used for propagation and aftercare of plant

The general procedure described hereunder was used, unless otherwise stated in respective experiments for propagation and cultivation of *Bombax Ceiba* L. in the present investigation.

3.2.4.1 Collection preparation of branch cutting

Branch cuttings were collected from Candidate Plus Trees (CPTs) from the sites in Karbi Anglong (Assam). The branch cuttings were collected in February 2012. The branches were cut into longer shoots which were waxed to prevent drying and placed in gunny bags and transported to experimental sites. The branches were re-cut with the help of secateurs into cutting and planted immediately. The length of the branch cutting was kept at 16 cm.
3.2.4.2 Preparation of nursery bed

Nursery site had gentle slope and was free from problem of water logging. The soil was loam and was medium in organic carbon, N, P and K. The pH was 7.3. Sand was ploughed in @ 400 ft per 100 m² in nursery area before start of the investigation. Before start of each experiment involving nursery beds, the soil was ploughed with a harrow twice to about 20 cm depth, tilled with a tiller 4 times to about 40-45 cm depth and levelled. Roots and weeds were removed during plugging operation. FYM @ 500 Ft³ per 200 m², de-oiled neem cake @ 10 Kg per 100 m² and 2 Kg Thimet per 100 m² were ploughed in the soil with tiller. Raised beds about 15 cm above ground level were prepared.

3.2.4.3 Construction of non-mist propagation chamber

Non-mist propagation chambers (each of 2.5 m length x 1.25 m width x 0.5 m front to 1.0 m rear height) were constructed as per design suggested by Leakey et al. (1990). The rooting medium was sand which remained saturated with water all the time through capillary action. UV-stabilised plastic sheet that covered the chambers from sides and top, conserved water vapours, thereby maintaining high humidity condition inside the chamber. A green agro-shade net (25 per cent light permeability) was stretched over the non-mist propagation chambers at 3 metre height during April to July in order to reduce the rise in temperature inside the chambers. These low-cost chambers served the function of the expensive mist chamber quite effectively. The temperature and relative humidity measured inside the chamber during the study duration are given in Appendix B. Bavistin solution of 0.025 per cent concentration was sprayed on the branch cuttings and shoot-sprouts every alternate week throughout the experiment which prevented infection of the plant material by any possible disease.

3.2.4.4 Planting of branch cuttings

The branch cuttings were inserted vertically. A gap of 5-6 cm left between adjacent cuttings on both sides. The cuttings were gently pressed into the sand so that one-third part of the cutting protruded out of sand. Water was sprinkled over cuttings with a fine rose can. Lid of the chamber was opened for operation or data collection.
While closing the lid, water was gently and uniformly sprinkled with fine rose can to moisten the sand and plant material.

3.2.4.5 Removal of rooted branch cuttings

The removal of rooted cuttings was started after 9 weeks of planting the cuttings in the non-mist propagation chamber. All the cuttings where callus formation was observed on the day of observation were removed. Unrooted cuttings were left undisturbed so as to form roots in subsequent weeks. Removal of rooted cuttings was carried out repeatedly at weekly intervals and this operation continued up to the end of 12 weeks.

3.2.4.6 Hardening of rooted branch cuttings

The rooted cuttings were removed and planted in polythene bag of size 22 cm x 13 cm having sand 2: soil 1: FYM 1 (v/v). The polythene bags were placed in similar non-mist propagation chambers. The polythene bags were maintained in the non-mist propagation chambers for twenty one (21) days for establishment and acclimatization. After twenty one (21) days, they were removed from the chambers in the evening and placed in shade house made of agro-shade net (25 per cent permeability). After staying in shade house for twenty one (21) days, they were removed in the evening and placed in the open. The plants were now considered as hardened.

3.2.4.7 Maintenance of plants in nursery

The plants were irrigated twice a week during summer and once a week after the end of monsoon rains. Irrigation was done to saturate the soil thoroughly. The spray of insecticides/ fungicides, weeding, hoeing etc was done manually as and when required.

3.2.5 Experimental details

3.2.5.1 Effect of IBA concentration on propagation of Bombax ceiba L. through mature branch cuttings

In this experiment, the effect of different level of growth regulators has been studied on the rooting and sprouting of softwood branch cuttings of B. ceiba L. Cuttings of
Materials and methods

2-3 cm diameter class and 16 cm length classes were taken and planted vertically in non-mist propagation chambers on February, 2012.

The technical details of the experiment are as follows:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Indole-3-butyric acid (IBA) concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5000 ppm</td>
</tr>
<tr>
<td>2</td>
<td>10000 ppm</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
</tr>
</tbody>
</table>

Number of replications: 3

Design: Randomised complete block design

32 plants of *Bombax ceiba* L. from the mentioned sites in Karbi Anglong (Assam) were selected as donor plants for branch cuttings. The selected plants were free of apparent physiological disorders, diseases and insect infestations. Cuttings were severed from the primary axes of the seedling to avoid the plagiotropic growth habitat common to cuttings collected from secondary and tertiary axes. 960 cuttings of 16 cm length and 2-3 cm diameter class were prepared from the branches of the donor plants, collected and transported to Dehradun after proper packaging.

Cuttings were set the day following collection in the non-mist propagation chambers. Before settings, branch cuttings on the basis of application of different types of growth regulators were graded into afore mentioned classes as per technical details. There were 3 replications with ten (10) cuttings per treatment per replication. Different non-mist propagation chambers served as different replications. The cuttings were firstly treated with Bavistin by dipping them in 1 per cent solution of fungicide for 5 minutes. Thereafter, each growth hormone concentration as per technical details was applied in their powder formulations to the basal 1 cm portion of cuttings. Excessive hormone was removed by gently tapping the basal portion of cuttings.

Further observations, maintenance, transplanting into polythene bags, hardening etc were like the experiment described under section 3.2.4.5 and 3.2.4.6.
3.2.5.2 Effect of concentration of IBA on propagation of *Bombax ceiba* L. through softwood branch cuttings

In this experiment, the effect of different concentrations of Indole-3-butyric acid (IBA) on the rooting and sprouting of softwood branch cuttings of young *Bombax ceiba* L. has been studied. Cutting of 2-3 cm diameter class and 16 cm length classes were taken from 6-7 year plants from the selected sites in Karbi Anglong and planted vertically in non-mist propagation chambers on February 2014.

The technical details of the experiment are as follows:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Indole-3-butyric acid (IBA) concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10000 ppm</td>
</tr>
<tr>
<td>2</td>
<td>20000 ppm</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
</tr>
</tbody>
</table>

Number of replications: 3

Design: Randomised complete block design

40 plants of *Bombax ceiba* L. from the mentioned sites in Karbi Anglong (Assam) were selected as donor plants for branch cuttings. The selected plants were free of apparent physiological disorders, diseases and insect infestations. Cuttings were severed from the primary axes of the seedling to avoid the plagiotropic growth habitat common to cuttings collected from secondary and tertiary axes. 960 cuttings of 16 length and 2-3 cm diameter class were prepared from the branches of the donor plants collected and transported to Dehradun after proper packaging.

Cuttings were set the day following collection in the non-mist propagation chambers. Before settings, branch cuttings on the basis of application of different types of growth regulators were graded into afore mentioned classes as per technical details. There were 3 replications with ten (10) cuttings per treatment per replication. Different non-mist propagation chambers served as different replications. The cuttings were firstly treated with Bavistin by dipping them in 1 per cent solution of fungicide for 5 minutes.
Materials and methods

Thereafter each growth hormone as per technical details was applied in their powder formulations to the basal 1 cm portion of cuttings. Excessive hormone was removed by gently tapping the basal portion of cuttings.

Further observations, maintenance, transplanting into polythene bags, hardening etc were like the experiment described under section 3.2.4.5 and 3.2.4.6.

3.2.5.3 Effect of IBA on propagation of *Bombax ceiba* L. through juvenile branch cuttings

Juvenile cuttings of 1 year plants were taken from nursery raised seedlings collected from the sites in Karbi Anglong (Assam). Juvenile cuttings 16 cm length were taken and planted vertically in the non-mist propagation chamber.

The technical details of the experiment are as follows:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Indole-3-butyric acid (IBA) concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6000 ppm</td>
</tr>
<tr>
<td>2</td>
<td>10000 ppm</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
</tr>
</tbody>
</table>

Number of replications: 3

Design: Randomised block design

Cuttings were severed from the primary axes of the seedling to avoid the plagiotropic growth habitat common to cuttings collected from secondary and tertiary axes.

3.2.5.4 Observations

The following observations were recorded:

3.2.5.4.1 Per cent sprouting

The cuttings were observed at 7 days intervals to examine their sprouting. The branch cutting was recorded to have sprouted if leaf primordium was visible. The total sprouted cuttings under each treatment were counted and expressed as per centage of total branch cuttings placed.
3.2.5.4.2 Callus induction per cent

The callus induction was recorded on day 24 after planting. This number was expressed as per cent age of total branch cuttings planted.

3.2.5.4.3 Per cent rooting

On day 45 of planting the cutting, the cuttings were observed for rooting. A cutting was considered to be rooted if a minimum of one root = 1 mm in length was present. The branch cuttings producing root was recorded. This number was expressed as per cent age of total branch cuttings planted.

3.2.5.4.4 Number of shoot

The numbers of shoots produced on each branch cutting was recorded.

3.2.5.4.5 Shoot length

The shoot length of each shoot was measured, recorded and expressed in cm.

3.2.5.4.6 Per cent survival

The branch cuttings surviving for 9 weeks or 12 weeks in the non-mist propagation chamber and developing callus or roots were counted and expressed as per cent age of total branch cuttings in the non-mist propagation chamber.

3.2.6 Physiochemical properties of soil

Composite soil samples were collected from two different depths viz., (i) “upper” (0–15 cm), (ii) “lower” (15–30 cm) for assessing the chemical properties of the soil in all the selected sites. Walkley and Black’s rapid titration method as modified by Walkley (1947) was adopted for organic carbon estimation. The factor of 1.724 was used to convert the organic carbon (per cent) into soil organic matter (per cent). The pH of soil was determined directly with the help of control dynamics digital pH meter. Available phosphorus was determined in the soil by Olsen et al., (1954) method. Potassium was extracted by neutral normal ammonium acetate method (Morwin and Peach, 1951) and was determined by the flame photometer (Evans Electro Selenium Ltd; Holsted Essex,
Materials and methods

England). Total nitrogen was measured by using the standard Kjeldhal procedure (Bremner and Mulvaney, 1982). Total carbon (per cent) was divided by total nitrogen (per cent) to get values of C:N ratio.

3.2.7 Statistical analysis

3.2.7.1 Analysis of variance

The data were statistically analysed using computer software SPSS version 16 and Microsoft excel 2007. The amount of variation i.e. variance and its components were analysed as suggested by Sokal and Rohlf (1969). All the germination data and binary data (i.e. 0 and 1 data) were transformed as described by Gomez and Gomez (1984) to satisfy the condition of the homogeneity of variance for ANOVA (analysis of variance). F value (variance ratio) and CD (Critical Difference) were also calculated to test the best treatment (i.e. stand) when ANOVA was significant at p ≤ 0.05.

3.2.7.2 Variance components

The data were analysed for variance components by two levels nested ANOVA with unequal sample size (i.e. unbalanced) for morphological fruit traits and germination traits suggested by Sokal and Rohlf (1969). The structure of the analysis of variance is given in Table 1.

Table 1: Two level nested analysis of variance with unequal size of fruit traits

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Mean sum of squares</th>
<th>Expected M.S. for a pure Model II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among stands</td>
<td>MS$_S$</td>
<td>$\sigma^2_e$+$\sigma^2_s$</td>
</tr>
<tr>
<td>Among trees within stands</td>
<td>MS$_T$</td>
<td>$\sigma^2_e$+$\sigma^2_s$</td>
</tr>
<tr>
<td>Among fruit within trees or Error</td>
<td>MS$_e$</td>
<td>$\sigma^2_e$</td>
</tr>
</tbody>
</table>
Where,

\[ \sigma_s^2 = \text{variance of among stands} \]

\[ \sigma_{ts}^2 = \text{variance of among trees within stands} \]

\[ \sigma_f^2 = \text{variance among fruits within trees (for two level) or error} \]

\[ n_o' \text{ and } nt = \text{coefficient of variation for among stands} \]

\[ n_o = \text{coefficient of variation for among trees within stands} \]

The data of seed morphological traits were analysed for variance components by three levels nested ANOVA with unequal sample size (i.e. unbalanced). The structure of the analysis of variance is given in Table 2.

**Table 2: Three level nested analysis of variance with unequal size of seed traits**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Mean sum of squares</th>
<th>Expected M.S. for a pure Model II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among stands</td>
<td>MS_s</td>
<td>( \sigma_e^2 + n_o' \sigma_{ts}^2 + (nt) \sigma_s^2 )</td>
</tr>
<tr>
<td>Among trees within stands</td>
<td>MS_T</td>
<td>( \sigma_f^2 + n_o \sigma_{ts}^2 + (nt) \sigma_s^2 )</td>
</tr>
<tr>
<td>Among fruits within trees</td>
<td>MS_F</td>
<td>( \sigma_f^2 + n_o \sigma_f^2 )</td>
</tr>
<tr>
<td>Among seeds within fruits or Error</td>
<td>MS_e</td>
<td>( \sigma_e^2 )</td>
</tr>
</tbody>
</table>

Where,

\[ \sigma_s^2 = \text{variance of among stands} \]

\[ \sigma_{ts}^2 = \text{variance of among trees within stands} \]

\[ \sigma_f^2 = \text{variance of among fruits within trees} \]

\[ \sigma_f^2 = \text{variance among seeds within fruits (for three level) or error} \]
Materials and methods

\(n_o'', nf\) and \(nft = \) coefficient of variation for among stands

\(n_o', nf = \) coefficient of variation for among trees within stands

\(n_o = \) coefficient of variation for among fruits within trees

The analysis of variance was obtained for growth studies as depicted in Table 3.

**Table 3: The analysis of variance for growth studies**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Mean sum of square</th>
<th>Expected M.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stands</td>
<td>MSs</td>
<td>(\sigma^2e + \rho\sigma^2g)</td>
</tr>
<tr>
<td>Within stands or Errors</td>
<td>MSe</td>
<td>(\sigma^2e)</td>
</tr>
</tbody>
</table>

Where,

\[MS_s = \text{mean sum of square}\]
\[MS_e = \text{error mean sum of square}\]
\[\sigma^2e = \text{error or environmental variance}\]
\[\sigma^2g = \text{genotypic variance}\]

**3.2.7.3 Estimation of variability and genetic parameters**

**3.2.7.3.1 Estimation of repeatability**

As genetic effects cannot be separated from environmental effects in natural populations where parental origin and environmental effects are not controlled, the genetic variance between populations and within populations cannot be accurately estimated. In consequence, the heritability at the population and individual tree level cannot be estimated. So repeatability, which can be considered as the upper limit of relation of genetic and phenotypic variance, was calculated with standard error for fruit and seed morphological traits as suggested by Khalil (1984), Becker (1984), Basu (1996) and Falconer and Mackay (1996).
Materials and methods

\[ R = \frac{\sigma^2_d}{\sigma^2_d \sigma^2_e} \]

Where,

- **R** = repeatability
- **\sigma^2_d** = variance of difference among individuals
- **\sigma^2_e** = variance of differences between measurement within the individuals

\[ \text{S.E. (R)} = \left\{ \frac{2(ct - 1)^2(1 - R)^2[1 + (k - 1)R]^2}{ctk^2(ct - t)(t - 1)} \right\}^{1/2} \]

Where,

- **R** = repeatability
- **c** = number of fruit per tree
- **t** = number of tree per stands
- **k** = Coefficient of \( \sigma^2_d \) in E.M.S. (T)

### 3.2.7.3.2 Estimation of heritability

The broad sense heritability is the proportion of the total phenotypic variance caused by all genetic factors, not just additive factors. It measures the extent to which individual differences in a population for germination and growth traits. It was calculated in following steps:

#### 3.2.7.3.2.1 Genetic variance

Phenotypic, genotypic and environmental variances were calculated by the following formula (Singh and Chaudhary, 1996):

\[ \sigma^2_p = \sigma^2_g + \sigma^2_e \]

\[ \sigma^2_g = \frac{(\text{MS}_g - \text{MS}_e)}{r} \]
\[ \sigma^2_e = MS_e \]

Where,
\[ \sigma^2_p = \text{phenotypic variance for character} \]
\[ MS_g = \text{mean sum of square due to genotype} \]
\[ MS_e = \text{mean sum of square due to error} \]
\[ r = \text{number of replications} \]

3.2.7.3.2.2 Genetic coefficient of variability

Genotypic, phenotypic and environmental coefficients of variability was calculated using the formulae (Singh and Chaudhary, 1996).

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

Where,
\[ GCV = \text{Genotypic Coefficient Variation} \]
\[ PCV = \text{Phenotypic Coefficient of Variation} \]
\[ ECV = \text{Environmental Coefficient of Variation} \]
\[ \bar{X} = \text{population mean} \]

Now, heritability (percentage) was calculated by the formula suggested by Johnson et al. (1955a).

\[
H^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100
\]
Materials and methods

Where,

\[ H^2 = \text{heritability in broad sense} \]
\[ \sigma^2_p = \text{phenotypic variance} \]
\[ \sigma^2_g = \text{genotypic variance} \]

3.2.7.3.2.3 Genetic advance

Genetic advance calculated by formula given by Johnson et al. (1955a).

\[ GA = \frac{\sigma^2_e}{\sigma^2_p} \times \sqrt{\sigma^2_p \times X \times K} \]

Where,

\[ K = 2.06, \text{ selection differential at 5 per cent selection intensity} \]

3.2.7.3.2.4 Genetic gain

Genetic gain was expressed in per cent of population mean and was calculated by following method suggested by Johnson et al. (1955b):

\[ \text{Genetic gain (\%)} = \frac{\text{Genetic advance}}{\bar{X}} \times 100 \]

Where,

\[ \bar{X} = \text{population mean} \]

3.2.7.4 Correlation studies

Simple correlation coefficient was calculated for combination of fruit and seed morphological traits, germination traits and climatic and topographic factors using the following standard procedures described by Gomez and Gomez (1984).

\[ r_{(i,j)} = \frac{\text{Covariance between } x_i \text{ and } x_j}{[\text{Variance } x_i \times \text{Variance } x_j]^{1/2}} \]

Where, \( X_i \) and \( X_j \) are variables/ factors
Materials and methods

Plate 5: Map of the study sites

(Source: Google earth)
Plate 6: Marking of tree at natural stand $S_1$

Plate 7: Natural stand at stand $S_2$
Materials and methods

Plate 8: Natural stand at stand $S_3$

Plate 9: Marking of tree in stand $S_4$
Plate 10: With Karbi Anglong Forest Dept. personals
Materials and methods

Plate 11: Seed measurement of *B. ceiba*

Plate 12: Seed measurement

Plate 13: Seed sowing in poly house